2012

The Dynamic Streptococcus Pyogenes Transcriptome in the Host Cell Environment and Contributions by Phage

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THE DYNAMIC *STREPTOCOCCUS PYOGENES* TRANSCRIPTOME IN THE HOST CELL ENVIRONMENT AND CONTRIBUTIONS BY PHAGE

A Thesis Presented to the Faculty of

The Rockefeller University

in Partial Fulfillment of the Requirements for

the degree of Doctor of Philosophy

by

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June 2012

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The Rockefeller University 2012

This thesis investigates the transcriptional responses of *Streptococcus pyogenes* in the pharyngeal environment and characterizes two transcriptional regulators involved in the adaptive response to the host. Furthermore, this thesis explores the potential role of integrated prophage on the streptococcal transcriptome. We are specifically interested in global regulatory systems in group A streptococci and how they impact virulence regulation. With this work, we hoped to identify new regulatory elements involved in the infection process.

The first objective of this thesis was to determine the transcriptional shift induced in streptococci upon introduction into the *in vitro* host environment. Using three time points, we examined the dynamic transcriptome remodeling program that streptococci undergo following exposure to cell-free pharyngeal culture supernatants or during co-culture with intact pharyngeal monolayers. These studies highlighted that streptococci modulate expression of virulence factors in the host environment using a combination of stand-alone regulators (most of which are uncharacterized) and two-component regulatory systems.

The next phase of this work involved characterizing two of the stand-alone regulators identified in our transcriptome screen. The first *spy1215* encodes a sirtuin-like deacetylase that is homologous to a repressor of virulence in the malarial parasite *Plasmodium falciparum*. Our work presents the first evidence of a direct link between a bacterial sirtuin (Spy1215) and virulence regulation. Interestingly, Spy1215-mediated virulence repression was determined to be dependent on signals from pharyngeal cells in *S. pyogenes.* The second regulator *spy1755* was found to be an activator of fatty acid biosynthesis in group A streptococci and required for normal growth rates in laboratory media.

Finally, we endeavored to elucidate the role of integrated prophage in the regulation of streptococcal gene expression. Using the first *S. pyogenes* strain cured of all phage, we explored the effect of phage deletion on the streptococcal transcriptome during early and late exponential growth in laboratory media. We found a limited effect on gene expression in the absence of integrated phage. The genes whose expression was most affected by phage deletion were found to lie downstream of phage insertion sites. Overall, this work supports observations thus far that the streptococcal strain cured of all phage displays a limited phenotype via numerous measures *in vitro* and *in vivo*.

To my father Emilio Ramon Juncosa: your fearlessness in life continues to inspire all who knew you. Your example will forever fuel my insatiable quest for knowledge, enlightenment and personal achievement.

I miss you!

ACKNOWLEDGEMENTS

Creating this thesis truly took a village, and I am forever grateful to those that contributed to my efforts in big and small ways.

I would first like to thank my adviser Dr. Vincent Fischetti for welcoming me into the laboratory and supporting each endeavor with full faith in my knowledge and abilities. I would also like to thank Dr. Patricia Ryan, whose expertise and knowledge in the field of microarrays made this work possible. I have also been fortunate to develop a close friendship with Patti, and her guidance has been pivotal to my development as a scientist.

The work in this volume would not have been possible without the assistance of Dr. Chad Euler, whose hands are truly magical when it comes to creating deletion mutants in *S. pyogenes*. Finally, I must acknowledge Mrs. Clara Eastby, whose dedication to fulfilling the laboratory's cell culture needs allowed me to focus on my experiments and data analysis.

I would also like to thank my committee members Dr. Tom Muir, Dr. Alexander Tomasz, and Dr. Joseph Ferretti for their helpful comments and insightful discussions.

I also wish to thank all the members of the Fischetti lab (past and present) and my friends for their help, encouragement, and support. Often times, the best remedy to a failed experiment is good company, and I have had it in spades.

My family have truly been a source of continual inspiration throughout my life, and my time in graduate school has been no different. Although my father Emilio Juncosa did not see the end of this journey, he was instrumental in encouraging me to pursue my current path. My mother Nelcy Juncosa and sister Cynthia Juncosa have been amazing cheerleaders on the sidelines throughout the Ph.D. process. I am blessed to have a family that is so close and supportive.

Finally, I want to express my deepest gratitude to my husband Raul Velazquez. He has helped me through this process in innumerable ways, from freezing down bacterial cultures to cooking and cleaning as I wrote this volume. I will never forget his dedication, kindness, generosity, and friendship through this difficult journey. He, too, shares in my accomplishments.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

1.1 Genus *Streptococcus*

Streptococci are Gram-positive, non-motile bacteria that are spherical or oval in shape and grow in chains or pairs. They belong to the phylum *Firmicutes* and produce lactic acid as the major metabolic end product of carbohydrate fermentation. Most streptococci are oxidase- and catalase-negative, and many are facultative anaerobes.

For clinical purposes streptococci are further delineated by their ability to lyse erythrocytes when grown on agar plates containing 5% sheep or horse blood. Hemolytic patterns range from gamma (no lysis) to alpha (greenish zone of partial lysis) and beta (zone of complete lysis) (Brown 1919). With the exception of the alpha-hemolytic pathogen *Streptococcus pneumoniae,* most gamma- and alpha-hemolytic streptococci are associated with the normal flora of the upper respiratory and gastrointestinal tracts.

Beta-hemolytic streptococci, however, include both human and animal pathogens. The Lancefield serological grouping system for beta-hemolytic streptococci relies on differences in immunoreactivity to their cell wall polysaccharides (groups A, B, C, F, and G) or lipoteichoic acids (group D) (Lancefield 1933; Koneman, *et al.* 1997). While Lancefield groups B, C, D, and G are associated with a number of animal and human infections, the group A streptococci cause the majority and widest range of human infections amongst the streptococcal species (Fischetti, *et al.* 2006).

1.2 Group A *Streptococcus*

Group A *Streptococcus* (GrAS), or *Streptococcus pyogenes,* is an obligate human pathogen with no known environmental reservoir. The organism can colonize the human nose and throat, and estimates indicate that up to 30% of the population asymptomatically carry group A streptococci in the upper respiratory tract (Fischetti, *et al.* 2006). Under the Lancefield classification scheme, *S. pyogenes* is identified by its group A carbohydrate containing *N*-acetyl-glucosamine linked to a rhamnose polymer backbone (McCarty 1956).

Group A streptococci can further be subdivided based on M protein, the dominant streptococcal surface antigen. Originally, M protein classification was based on serological tests that could distinguish 80 serotypes (Lancefield 1928; Facklam 1997). Immunoreactivity was determined to stem from the N-terminal half of M protein, which is antigenically variable (Fischetti 1989). Since the 1990s, PCR and sequencing technologies have allowed a molecular approach to *S. pyogenes* classification using polymorphisms in the *emm* gene, which encodes M protein (Beall, *et al.* 1995). Currently, there are over 125 recognized M types, and infection results in type-specific immunity (Fischetti, *et al.* 2006).

1.3 Group A Streptococcal Disease

S. pyogenes is responsible for a broad range of acute suppurative infections and non-suppurative sequelae (Table 1.1; reviewed in Cunningham 2000). In the United States, more than 25 million cases of GrAS infections occur

annually (Fischetti, *et al.* 2006). Group A streptococci are the predominant bacterial cause of acute pharyngitis, accounting for 15-36% of all pharyngitis cases in children and 4-10% of cases in adults worldwide (Carapetis, *et al.* 2005; Pfoh, *et al.* 2008). Other acute infections associated with *S. pyogenes* include pyoderma, scarlet fever, toxic shock syndrome, necrotizing fasciitis, and septicemia. Post-streptococcal sequelae range from acute rheumatic fever to acute glomerulonephritis and reactive arthritis (Cunningham 2000).

1.3.1 Streptococcal Pharyngitis

GrAS pharyngitis is generally self-limiting, resolving within two weeks without antibiotic therapy. The prevalence of streptococcal pharyngitis is highest during the winter months and preferentially affects children ages 5 to 15 (Fischetti, *et al.* 2006). At its peak incidence, 50% of all children ages 5 to 7 experience one streptococcal infection each year (Fischetti, *et al.* 2006).

Over 600 million cases of symptomatic GrAS pharyngitis occur annually worldwide among individuals over the age of four (Carapetis, *et al.* 2005). In the United States alone, medical and nonmedical costs associated with GrAS pharyngitis in children and adolescents can reach $539 million annually (Pfoh, *et al.* 2008). Group A streptococci remain susceptible to penicillin treatment, although resistance to the second drug of choice erythromycin has been observed (Fischetti, *et al.* 2006).

Table 1.1 Spectrum of clinical diseases associated with *S. pyogenes*

Asymptomatic colonization
Throat
Skin (immediately preceding infection)
Also vagina, anus, scalp

Suppurative respiratory disease
Peritonsillar abscess
Retropharyngeal abscess
Cervical lymphadenitis
Sinusitis
Otitis media
Pneumonia
Empyema

Genitourinary
Urinary tract infection

Central nervous system
Meningitis
Brain abscess

Cardiac
Endocarditis

Gastrointestinal
Peritonitis
Hepatic
Liver abscess

Superficial infection
Pharyngitis and pharyngotonsillitis
Pyoderma

Invasive disease
Bacteremia/septicemia
Skin/soft tissue suppurative disease
Erysipelas
Cellulitis (including perianal cellulitis)
Wound infection
Varicella superinfection
Necrotizing fasciitis
Pyomyositis
Puerperal sepsis
Neonatal omphalitis

Musculoskeletal
Osteomyelitis
Septic arthritis

Post-infectious sequelae
Rheumatic fever
Acute post-streptococcal glomeruolnephritis
Reactive arthritis
Erythema nodusum
P.A.N.D.A.S. Pediatric autoimmune neuropsychiatric disorder

Toxin-mediated disease
Scarlet fever
Streptococcal toxic shock syndrome

Adapted from (Euler 2010) with permission.

1.3.2 Acute Rheumatic Fever and Rheumatic Heart Disease

Although streptococcal pharyngitis is a relatively innocuous infection, acute rheumatic fever (ARF) manifests in 3% of untreated or improperly treated cases (Fischetti, *et al.* 2006). ARF is an autoimmune condition that involves inflammation of the joints (arthritis), heart valves (carditis), central nervous system (involuntary movements; Sydenham's chorea), skin (erythema marginatum), and/or subcutaneous nodules (reviewed in Cunningham 2000). While migratory arthritis is the most common symptom of ARF, carditis is the most serious and can lead to permanent damage of the heart valves (Cunningham 2000).

Carditis, or rheumatic heart disease (RHD), is estimated to affect 60% of ARF patients (Carapetis, *et al.* 2005). RHD presents as mitral and/or aortic regurgitation by Doppler echocardiography (Minich, *et al.* 1997). RHD disproportionately affects children in the developing world, particularly in sub-Saharan Africa, South Central Asia, and the Pacific, where access to medical care and antibiotics can be limited (Figure 1.1; Jackson, *et al.* 2011). An estimated 19.6 million people (2.4 million children aged 5-14 years) worldwide are affected by RHD, which accounts for nearly 500,000 deaths annually (Carapetis, *et al.* 2005).

In less developed countries, RHD is the leading cause of acquired heart disease in children. Valvular damage stemming from RHD can also underlie infective endocarditis and stroke (Carapetis, *et al.* 2005). Current treatments

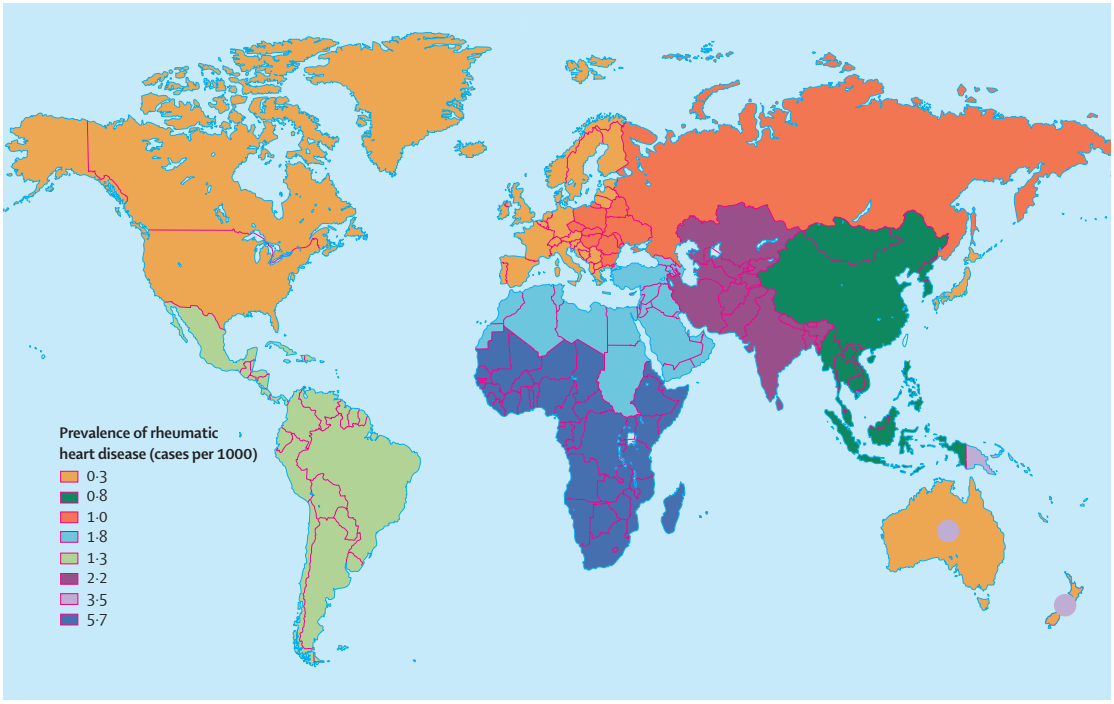


Figure 1.1 Prevalence of rheumatic heart disease in children aged 5-14 years

Circles within Australia and New Zealand represent indigenous populations.

Reprinted from (Carapetis, *et al.* 2005).

include costly long-term antibiotic prophylaxis to prevent streptococcal pharyngitis recurrence and heart valve replacement, highlighting the need for a group A streptococcal vaccine in the developing world (Carapetis, *et al.* 2005).

The mechanism of ARF and RHD involves both antibody- and T-cell mediated immune responses to group A streptococci that cross-react with antigens in the heart myocardium (RHD) and other body tissues (ARF) (Fischetti, *et al.* 2006). Particular M types of *S. pyogenes*, including types 1, 3, 5, 6, 14, 18, 19, and 24, have been associated with pharyngitis and ARF (Cunningham 2000).

1.3.3 Glomerulonephritis

Acute post-streptococcal glomerulonephritis may develop following pharyngitis or impetigo, although the M types underlying the disease differ between the two types of acute streptococcal infection (Cunningham 2000). Clinical manifestations include discolored urine due to hematuria, edema of the face and extremities, and circulatory congestion from renal impairment (Cunningham 2000). Although the disease usually resolves, ~1% of children suffer irreversible renal failure (Cunningham 2000; Carapetis, *et al.* 2005).

Autoimmune mechanisms are involved, but it remains unclear if the renal damage is due to deposition of immune complexes, antibodies to streptococcal antigens that cross-react with kidney epitopes, alteration of glomerular tissues by streptococcal exoproteins, or complement activation by streptococcal antigens in the kidneys (Cunningham 2000). Each year, 470,000 acute glomerulonephritis

cases arise worldwide resulting in an estimated 5,000 deaths, mostly in developing nations (Carapetis, *et al.* 2005).

1.3.4 Streptococcal Invasive Disease

Group A streptococci can gain access to normally sterile sites and cause invasive disease. Invasive GrAS infections, including necrotizing fasciits ('flesh-eating' disease), septicemia, and toxic shock syndrome, affect 660,000 individuals annually, translating into over 160,000 deaths worldwide (Carapetis, *et al.* 2005). Community-based studies have indicated that school-aged children may serve as a reservoir for streptococcal strains that cause invasive disease in their area, adding to the urgency for an effective group A streptococcal vaccine (Pfoh, *et al.* 2008).

Since the 1980s, there has been a global resurgence of group A streptococcal invasive disease (reviewed in Aziz & Kotb 2008). Hypotheses vary on the underlying reason for the surge in infections from an increase in the virulence potential of specific strains to an overall rise in GrAS transmission rates and prevalence (Rogers, *et al.* 2007; Aziz & Kotb 2008). In the United States and other developed nations, the dominant strains associated with streptococcal pharyngitis and invasive disease have been serotype M1 isolates (Shulman, *et al.* 2004; Steer, *et al.* 2009).

In particular, one globally disseminated subclone designated M1T1 has emerged as a leading cause of streptococcal invasive disease (Aziz & Kotb

2008). Genetic analyses revealed that the acquisition of new prophages encoding highly pathogenic virulence factors correlated with the reemergence of GrAS invasive disease (Cleary, *et al.* 1998; Sumby, *et al.* 2005; Aziz & Kotb 2008). Furthermore, *in vivo* mutations of a virulence transcriptional repressor have also been associated with hypervirulence in M1T1 invasive isolates (Sumby, *et al.* 2006; Aziz & Kotb 2008).

1.4 Group A Streptococcal Genomics

Genome sequences are currently available for 14 *S. pyogenes* strains (10 M types) representing 70% of the serotypes that most commonly cause streptococcal pharyngitis and invasive disease in the western hemisphere (Table 1.2; Ferretti, *et al.* 2001; Beres, *et al.* 2002; Smoot, *et al.* 2002; Nakagawa, *et al.* 2003; Banks, *et al.* 2004; Green, *et al.* 2005; Sumby, *et al.* 2005; Beres, *et al.* 2006; Beres & Musser 2007; Holden, *et al.* 2007; McShan, *et al.* 2008; Bessen, *et al.* 2011).

The pool of sequenced genomes include serotypes primarily recognized as underlying pharyngitis and rheumatic fever (M1, M3, M5, M6, M12, and M18), those associated with skin infections and glomerulonephritis (M2, M4, and M49), and serotypes isolated from invasive disease cases (M1, M3, M12, and M28) (Cunningham 2000; Beres & Musser 2007; McShan, *et al.* 2008).

Group A streptococci have a singular circular chromosome averaging ~1.9 Mb in length, with a percent G+C content of 38.5%. Nearly 85% of each

Table 1.2 Sequenced *S. pyogenes* genomes

S. pyogenes Strain	M-type	Genome Size (bp)	CDS Count	% of CDS with No Assigned Function	No. of Prophages	Notes on Strain Isolation	Reference
SF370	1	1,852,441	1,697	37.1	4	Wound infection (1985)	Ferretti, *et al.* 2001
MGAS5005	1	1,838,554	1,865	16.6	3	Invasive disease (Canada, 1996)	Sumby, *et al.* 2005
MGAS10270	2	1,928,252	1,987	17.2	5	Pharyngitis case (Texas, late 1990s)	Beres, *et al.* 2006
MGAS315	3	1,900,521	1,865	35.5	6	Toxic shock syndrome (Texas, late 1980s)	Beres, *et al.* 2002
SSI-1	3	1,894,275	1,861	39.7	6	Toxic shock syndrome (Japan, 1994)	Nakagawa, *et al*. 2003
MGAS10750	4	1,937,111	1,979	17.2	4	Pharyngitis case (Florida, 2001)	Beres, *et al.* 2006
Manfredo	5	1,841,271	1,803	28.4	5	Acute rheumatic fever case (USA, 1952)	Holden, *et al.* 2007
MGAS10394	6	1,899,877	1,886	22.7	8	Childhood pharyngitis (Pennsylvania, 2001)	Banks, *et al.* 2004
MGAS2096	12	1,860,355	1,898	17.3	2	Acute glomerulonephritis (Trinidad, 1960)	Beres, *et al.* 2006
MGAS9429	12	1,836,467	1,878	16.7	3	Pediatric pharyngitis (Texas, 2001)	Beres, *et al.* 2006
MGAS8232	18	1,895,017	1,845	38.6	5	Acute rheumatic fever case (Utah, 1987)	Smoot, *et al.* 2002
MGAS6180	28	1,897,573	1,894	18	4	Invasive disease (Texas, 1998)	Green, *et al.* 2005
NZ131	49	1,815,724	1,699	25.7	3	Acute glomerulonephritis (New Zealand, 1991)	Mcshan, *et al.* 2008
Alab49	53	1,827,308	1,773		4	Impetigo (Alabama,1986)	Bessen, *et al.* 2011

genome (the streptococcal 'core' metagenome) is highly conserved in terms of gene content and context across strains with over 98% nucleotide identity (Beres & Musser, 2007). Integrated prophage sequences constitute 10% of the *S. pyogenes* genome in all sequenced strains and are the major contributor to variation in gene content (Ferretti, *et al.* 2001; Banks, *et al.* 2002; Beres & Musser 2007; McShan, *et al.* 2008).

All of the sequenced genomes are polylysogenic, each strain carrying 2 to 8 prophages that encode for important streptococcal virulence factors. Significant diversity in prophage content has been observed both between and within serotypes (Smoot, *et al.* 2002; Beres & Musser 2007; McShan, *et al.* 2008). Notably, strains of one particular M type are not necessarily clonally related and can vary significantly in their pathogenicity and interactions with host cells (Beres & Musser 2007).

1.4.1 Strain SF370

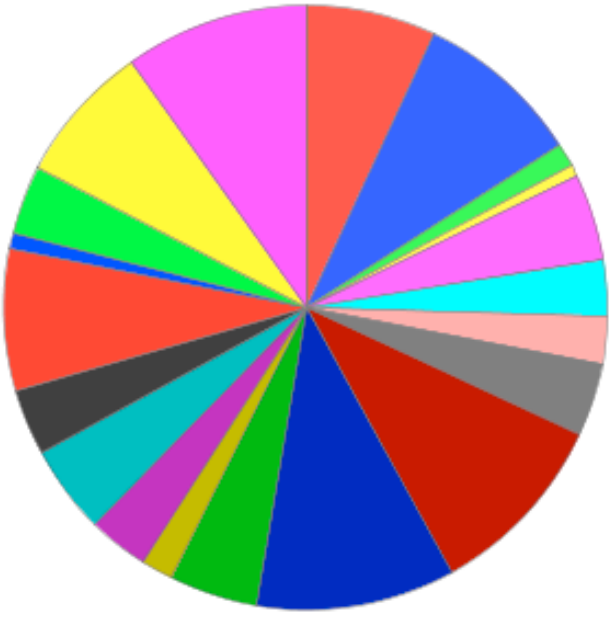
The strain used exclusively for this work is serotype M1 SF370. Originally isolated from a wound infection in the mid-1980s, strain SF370 was the first *S. pyogenes* genome sequenced (Ferretti, *et al.* 2001). The genome contains 4 integrated prophages: 370.1, 370.2, 370.3, and 370.4. The element 370.1 represents the only intact prophage in SF370 and is inducible by mitomycin C (Ferretti, *et al.* 2001). The contribution of these prophages to streptococcal gene regulation will be considered in Section 7 of this thesis. Overall, the SF370

genome encodes over 50 virulence factors, some of which are located within these prohpage sequences (Table 1.3; Ferretti, *et al.* 2001).

The SF370 genome includes a total of 1,697 recognized protein coding regions, which can be classified into clusters of orthologous groups (COGs) (Tatusov, *et al.* 1997; Tatusov, *et al.* 1999). The COG database was designed to extract maximum information from genomes by classifying conserved genes using homologous relationships (Tatusov, *et al.* 2000). Orthologs within a group usually share the same function, thus providing functional information (Tatusov, *et al.* 1997). Figure 1.2 provides a visual breakdown of the distribution of SF370 proteins among the functional categories. COG designations will be utilized throughout this thesis for examining the SF370 transcriptome in response to the host cell environment and to genetic manipulations of the streptococcal genome.

1.5 Virulence Factors

Streptococcus pyogenes utilizes a multitude of secreted and surface-bound proteins to adhere to epithelial cells, evade the human immune response, invade deeper tissues, and secure nutrients from the host environment. DNA microarray analyses and sequencing of diverse strains has revealed that the virulence factor compliment varies even between strains of the same serotype (McMillan, *et al.* 2007; Beres & Musser 2007; McShan, *et al.* 2008).
As discussed above, integrated prophages are a significant contributor to this diversity, but the presence of chromosomally encoded virulence factors



Functional category	ORFs
Amino acid transport and metabolism	101
Carbohydrate transport and metabolism	130
Cell cycle control, cell division, chromosome partitioning	18
Cell motility	9
Cell wall/membrane/envelope biogenesis	68
Coenzyme transport and metabolism	43
Defense mechanisms	37
Energy production and conversion	57
Function unknown	146
General function prediction only	155
Inorganic ion transport and metabolism	68
Intracellular trafficking, secretion, and vesicular transport	25
Lipid transport and metabolism	47
Nucleotide transport and metabolism	68
Posttranslational modification, protein turnover, chaperones	52
Replication, recombination and repair	110
Secondary metabolites biosynthesis, transport and catabolism	12
Signal transduction mechanisms	53
Transcription	109
Translation, ribosomal structure and biogenesis	143
Not in COGs	489

Figure 1.2 Distribution of SF370 proteins among functional categories (COGs) Some ORFs have been assigned to multiple COGs based on protein homology. Pie chart adapted from the U.S. Department of Energy Integrated Microbial Genomes database (http://img.jgi.doe.gov/).

(particularly adhesins) can also vary (McMillan, *et al.* 2007). For example, skin-tropic M type strains are often distinguished from throat-tropic M type isolates by the presence of the chromosomally encoded serum opacity factor (*sof*) on the former (Widdowson, *et al.* 1970; Cunningham 2000; McShan, *et al.* 2008).

Overall, studies continue to highlight that a strain's pathogenicity and tissue tropism are closely linked to the virulence factors it encodes. Table 1.3 provides a list of the known virulence factors (both chromosomally encoded and phage encoded) in the SF370 genome. Below a few of the major characterized virulence factors are further described.

1.5.1 Chromosomally Encoded Virulence Factors

Streptococci express a number of proteins firmly or loosely associated with the bacterial surface (M protein, capsule, pilus, C5a peptidase, fibronectin-binding proteins). These factors play a role in adhesion to host cells and/or immune evasion (reviewed in Hynes 2004). Secreted proteins can include degradative enzymes (SpeB, DNases, hyaluronidases), toxins (streptolysins and superantigens), and proteins for immune evasion (antibody-degrading factors) (Hynes 2004).

1.5.1.1 M Protein

First described in 1927 by Rebecca Lancefield, the M protein is the dominant protein on the surface of *S. pyogenes* (Lancefield 1928). As previously

Table 1.3 Virulence factors encoded by the SF370 genome (Pages 15-19)

Chromosomally Encoded

Locus Tag[1]	Gene Name	Protein Function and/or Recognized Role in Virulence	Reference[2]
spy0019	*sibA*	Secreted immunoglobulin binding protein; Binds IgG, IgA, IgM	Fagan, *et al.* 2001
spy0125[3]	*cpa.1*	Minor pilin subunit protein (AP1); Binds collagen	Ferretti, *et al.* 2001; Smith, *et al.* 2010
spy0127	*sipA1*	Pilin-specific signal peptidase; Plays chaperon-like function in pilus polymerization	Zahner & Scott 2008
spy0128		Major pilin subunit protein (Lancefield T antigen); Responsible for pilus length and stability	Mora, *et al.* 2005; Abbot, *et al.* 2007
spy0129	*srtC1*	Sortase; Polymerizes the pilus structure	Barnett, *et al.* 2004; Mora, *et al.* 2005
spy0130		Minor pilin subunit protein; Links pilus to cell wall (AP2)	Abbot, *et al.* 2007; Smith, *et al.* 2010
spy0165	*nga*	NAD-glycohydrolase; Reduces host cell energy stores	Ferretti, *et al.* 2001
spy0167	*slo*	Streptolysin O; Cytolysin that forms pores in host cell membranes and induces apoptosis in phagocytes	Ferretti, *et al.* 2001
spy0212	*speG*	Pyrogenic exotoxin; Nonspecifically stimulates T cells	Ferretti, *et al.* 2001
spy0274	*sdh/plr*	Surface dehydrogenase (GAPDH); Binds to plasminogen and fibronectin; Regulates host cell signaling	Pancholi & Fischetti 1992
spy0378	*hlyX*	Hemolysin	Ferretti, *et al.* 2001
spy0380		Exopolyphosphatase; Possible adhesin	Ferretti, *et al.* 2001
spy0390	*ideS*	Secreted cysteine protease; Cleaves the IgG heavy chain	von Pawel-Rammingen, *et al.* 2002
spy0416	*scpC/spyCEP*	Secreted protease; Degrades IL-8 and other host chemokines	Hidalgo-Grass, *et al.* 2006
spy0428	*spyA*	Surface-bound ADP-ribosyltransferase; Function in virulence not understood	Coye & Collins 2004
spy0436	*speJ*	Pyrogenic exotoxin; Nonspecifically stimulates T cells	Ferretti, *et al.* 2001

Table 1.3 Chromosomally encoded virulence factors

spy0470	*mcrA*	Myosin cross-reactive antigen; Antigenic mimicry	Ferretti, *et al.* 2001
spy0591		Possible proteinase (collagenase)	Ferretti, *et al.* 2001
spy0605	*bsa/gpoA*	Glutathione peroxidase; Involved in antioxidant defense during suppurative disease	Brenot, *et al.* 2004
spy0731	*eno*	Alpha-enolase; Binds plasminogen	Pancholi & Fischetti 1998
spy0737	*epf*	Surface protein; Binds plasminogen	Ferretti, *et al.* 2001
spy0738-0746	*sagA-sagI*	Streptolysin S; Hemolysin that kills neutrophils and interrrupts host intercellular junctions for dissemination	Ferretti, *et al.* 2001
spy0747	*spnA*	Cell-surface nuclease; Destroys neutrophil extracellular traps	Hasegawa, *et al.* 2010
spy0861	*sib35/mac*	Secreted protein that mimics a host cell receptor; Binds & degrades IgM, IgA, IgG; Blocks phagocytosis and ROS production by PMNs	Lei, *et al.* 2001; Kawabata, *et al.* 2002
spy1013	*fbp/fbp54*	Fibronectin-binding protein	Courtney, *et al.* 1994
spy1032	*hylA*	Hyaluronidase	Ferretti, *et al.* 2001
spy1054	*sclB/scl2*	Collagen-like protein	Ferretti, *et al.* 2001
spy1159	*hlyIII*	Hemolysin III	Ferretti, *et al.* 2001
spy1273	*cfa*	CAMP factor; Helps lyse erythrocytes; Binds Fc regions of Ig	Ferretti, *et al.* 2001
spy1302	*cdg/amyA*	Amylase/cyclomaltodextrin glucanotransferase; Important for epithelial translocation	Ferretti, *et al.* 2001
spy1312-1309	*dltA, dltB, dltC, dltD*	D-alanylation of surface lipoteichoic acids (LTA); Insertion of positively charged residues protects against cationic antimicrobial peptides and lysozyme killing; Enhances host cell adherence & invasion	Kristian, *et al.* 2005

Table 1.3 Chromosomally encoded virulence factors

spy1357	*grab*	G-related α-2-binding protein; Binds a human protease inhibitor, which prevents degradation of bacterial surface-bound adhesins & recruits SpeB to surface for defense against antimicrobial peptides	Ferretti, *et al.* 2001
spy1361	*slr*	Histidine triad protein (surface-bound lipoprotein); Recruits collagen type I to surface	Reid, *et al.* 2003
spy1497	*hylA1*	hemolysin	Ferretti, *et al.* 2001
spy1600	*hyl*	O-GlcNAse; Used on eukaryotic modified glycoproteins (role possibly limited to sugar foraging)	Ferretti, *et al.* 2001
spy1798	*shr*	Heme acquistion and adhesin; Binds fibronectin and laminin	Fisher, *et al.* 2008
spy1801	*isp2*	Immunogenic secreted protein; Function unknown	Ferretti, *et al.* 2001
spy1813	*endoS*	Secreted IgG glycan hydrolase; Inhibits function of IgG Fc region	Collin & Olsen 2001
spy1851		C3-degrading proteinase; Inhibition of complement	Ferretti, *et al.* 2001
spy1896	*ropA/tig*	Trigger factor; Chaperone essential for SpeB secretion and maturation	Ferretti, *et al.* 2001
spy1911	*salY*	ABC transporter permease; Intracellular survival in macrophages	Phelps & Neely 2007
spy1915	*salA*	Salivaricin A; Lantibiotic	Ferretti, *et al.* 2001
spy1972	*pulA*	Pullulanase; Binding to carbohydrates on host cells	Shelburne, *et al.* 2011
spy1979	*ska*	Streptokinase; Activates plasmin to degrade blood clots and ECM components	Ferretti, *et al.* 2001
spy1983	*sclA/scl1*	Collagen-like protein; Binds to host LDL (lipoprotein in blood plasma), collagen, and laminin	Ferretti, *et al.* 2001

Table 1.3 Chromosomally encoded virulence factors

spy1998	*smeZ*	Pyrogenic exotoxin; Nonspecifically stimulates T cells	Ferretti, *et al.* 2001
spy2006	*htpA*	Surface-bound histidine triad protein; Immunogenic in mice	Kunitomo, *et al.* 2007
spy2007	*lmb/lsp*	Suface lipoprotein; Zn acquisition from host; Binds to human basal lamina glycoprotein laminin	Ferretti, *et al.* 2001
spy2009	*fbaA*	Fibronectin-binding adhesin; Controlled by Mga; Required for adherence and invasion	Terao, *et al.* 2001
spy2010	*scpA*	C5a peptidase; Inhibits complement	Ferretti, *et al.* 2001
spy2016	*sic*	Streptococcal inhibitor of complement; Inactivates lysozyme, LL-37, and host defensins	Ferretti, *et al.* 2001
spy2018	*emm1*	M protein; Dominant GrAS virulence factor; Binds fibronectin; Mediates phagocytosis resistance	Ferretti, *et al.* 2001
spy2025	*isp*	Immunogenic secreted protein; Function unknown	Ferretti, *et al.* 2001
spy2039	*speB*	Exracellular cysteine protease; Mediates immune modulation, ECM hydrolysis, and release of GrAS surface antigens	Ferretti, *et al.* 2001
spy2043	*speF/spd*	Pyrogenic exotoxin; Nonspecifically stimulates T cells; DNase	Ferretti, *et al.* 2001
spy2200-2202	*hasA, hasB, hasC*	Hyaluronate capsule synthetase; Mediates phagocytosis resistance and cytoskeletal rearrangements in host cells for intercellular translocation	Ferretti, *et al.* 2001
spy2216	*degP/htrA*	Membrane associated protease; Role in defenses against oxidative stress & maturation of SpeB	Jones, *et al.* 2001

Table 1.3 Virulence factors encoded by the SF370 genome

Phage Encoded

Locus Tag	Gene Name	Protein Function and/or Recognized Role in Virulence	Reference[2]
spy0701	*hylP1*	Hyaluronidase	Ferretti, *et al.* 2001
spy0711	*speC*	Pyrogenic exotoxin; Nonspecifically stimulates T cells	Ferretti, *et al.* 2001
spy0712	*spd1/mf2*	Extracellular DNase	Ferretti, *et al.* 2001
spy0997	*hylP2*	Hyaluronidase	Ferretti, *et al.* 2001
spy1007	*speI*	Pyrogenic exotoxin; Nonspecifically stimulates T cells	Ferretti, *et al.* 2001
spy1008	*speH*	Pyrogenic exotoxin; Nonspecifically stimulates T cells	Ferretti, *et al.* 2001
spy1436	*spd3/mf3*	Extracellular DNase	Ferretti, *et al.* 2001
spy1445	*hylP3*	Hyaluronidase	Ferretti, *et al.* 2001

[1] Locus tag in the SF370 genome.

[2] For genes identified as virulence factors during the initial SF370 sequencing project, the reference indicates the original publication of the genome (Ferretti, *et al.* 2001). For genes not annotated as virulence factors at the time of publication for the genome, the reference signifies the initial description of the virulence gene or the first association of a previously characterized ORF with a function in streptococcal virulence.

[3] ORF not represented on the SF370 microarray used for this work.

described, classification of *S. pyogenes* strains is based on M protein—traditionally through serological assays and more recently via molecular analyses of the *emm* gene. As antibody responses to M protein are protective, development of vaccine strategies targeting the antigenically variable M protein has long been a focus for researchers and actively continues today (Lancefield 1962; Fischetti 1989; reviewed in Fischetti, *et al.* 2006).

M protein is a long coiled-coil protein that is anchored at its C-terminus to the bacterial membrane and extends up to 60 nm from the bacterial surface (Figure 1.3; Fischetti 1989). It is composed of three sections of tandem repeat sequences, with the A- and B-repeats in the N-terminal half exhibiting high variability and the C-repeats closer to the bacterial surface being conserved across M types (Jones, *et al.* 1985).

M protein has long been recognized as a primary virulence factor for GrAS. The antigenic diversity of the molecule offers protection to particular strains when infecting hosts that are still immunologically naïve to its respective serotype. M protein also offers active protection against phagocytosis by host cells (Fischetti 1989). In addition, the M1 protein binds fibronectin, mediating adherence to and internalization by epithelial cells (Ellen & Gibbons 1972; Courtney, *et al.* 1986; Sanford, *et al.* 1982; Cue, *et al.* 2001; Rezcallah, *et al.* 2005). M protein may also mediate host cell adherence by binding to glycosaminoglycans on cell surfaces or the extracellular matrix (Frick, *et al.* 2003b).

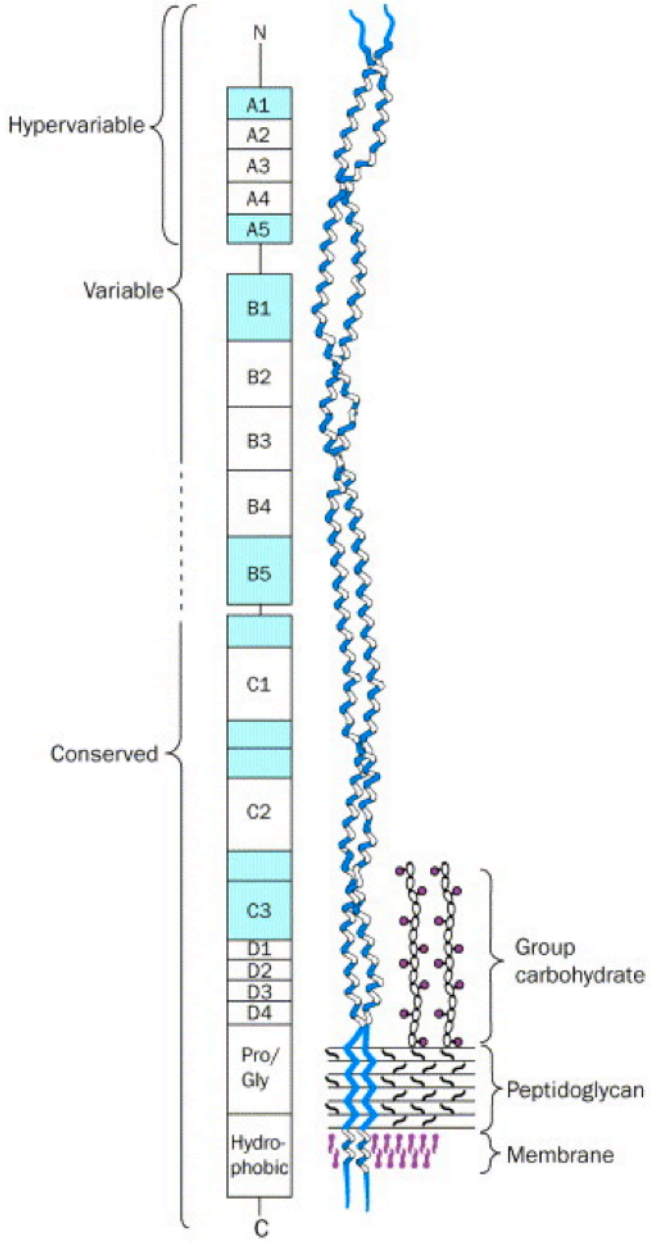


Figure 1.3 Characteristics of the complete M6 protein sequence

Blocks A, B, C, and D designate the locations of the sequence repeat blocks. Blocks highlighted in blue have a sequence that diverges from the central consensus sequence. Pro/Gly denotes the proline- and glycine-rich regions likely located in the peptidoglycan. The C-terminal end is anchored within the cell wall and membrane. Adapted with permission from (Fischetti, *et al.* 1996).

1.5.1.2 Pilus

The elaboration of pili by GrAS was only recently confirmed (Mora, *et al.* 2005; reviewed in Kreikemeyer, *et al.* 2011). The pilus structure is comprised of a major pilin protein (*spy0128*) and may include one or more accessory proteins that can serve as an adhesive pilus cap (*spy0125*) or the cell wall anchor (*spy0130*) (Figure 1.4, Part B; Mora, *et al.* 2005; Smith, *et al.* 2010). A pilus-specific sortase (*spy0129*) catalyzes transpeptidase reactions to trigger polymerization of pilin subunits, which can be aided in some strains by a putative signal peptidase (*spy0127*) with chaperon-like functions (Barnett, *et al.* 2004; Mora, *et al.* 2005; Zahner & Scott 2008).

Inactivation of pili components has demonstrated the importance of streptococcal pili for initial host cell adherence and the formation of biofilms (Manetti, *et al.* 2007). Studies in our laboratory and others have shown that without intact pili GrAS adherence to cultured pharyngeal cells, human tonsillar epithelium, and primary human keratinocytes is significantly comprised (Abbot, *et al.* 2007; Manetti, *et al.* 2007; Ryan, *et al*. 2007). The human host appears to have capitalized on the GrAS pilus for innate defenses. The salivary glycoprotein gp340 binds to pili leading to bacterial aggregation, which could be important for pathogen clearance (Liljemark, *et al.* 1981; Edwards, *et al.* 2008).

The pilus genes are encoded in the highly variable FCT region of the genome with one pilus variant expressed per strain (Mora, *et al.* 2005; reviewed in Kreikemeyer, *et al.* 2011). The region was previously associated with the T

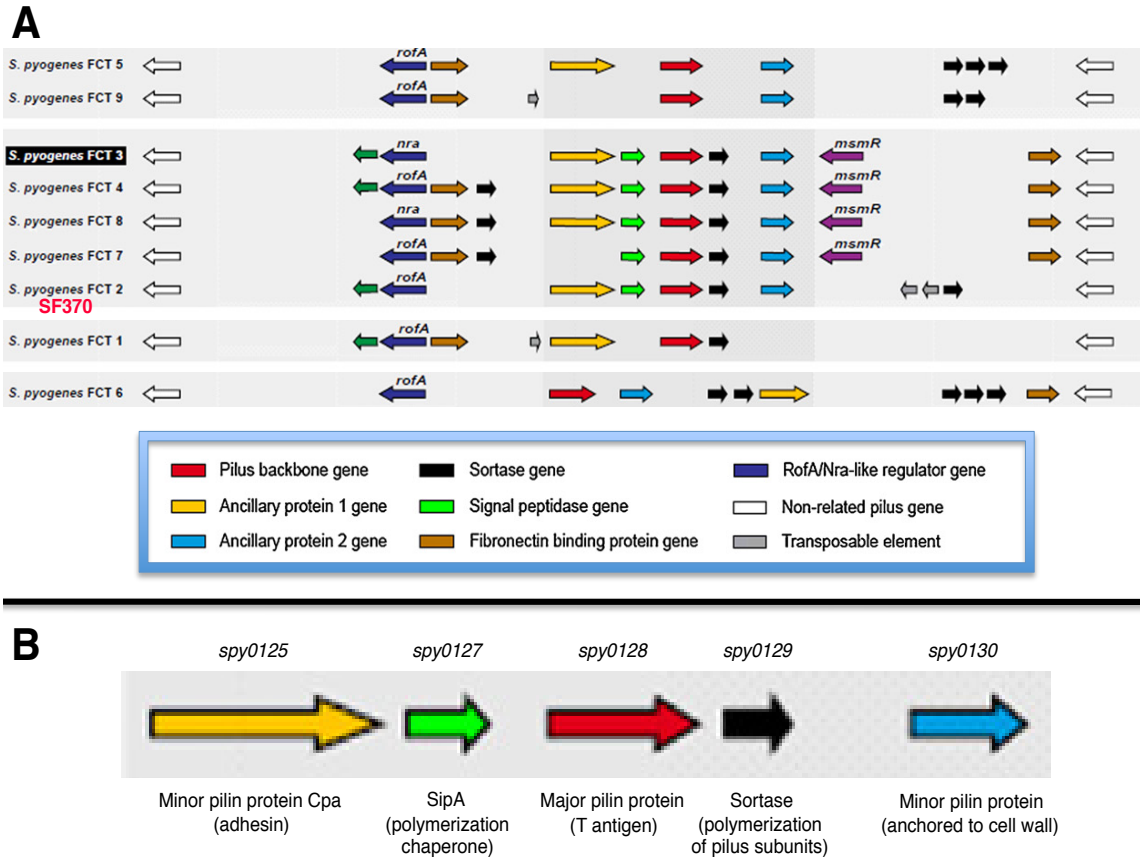


Figure 1.4 Genetic organization of the FCT loci in *S. pyogenes*

A. Comparison of the 9 FCT loci identified by PCR and sequencing analysis of 57 strains in Falugi, *et al.* 2008.

B. Examination of the pilus genes and their orientation in strain SF370. Characterized functions appear below each arrow designating individual ORFs.

Figure adapted from (Kreikemeyer, *et al.* 2011).

antigen, which has been used for serological typing in conjunction with the M protein and is now recognized to be the major pilin protein (Lancefield and Dole 1946; Mora, *et al.* 2005; Falugi, *et al.* 2008). At least 9 different FCT loci—varying in the number of genes present and/or their relative positions—encode GrAS pili with SF370 harboring the FCT-2 locus (Figure 1.4, Part A; Falugi, *et al.* 2008). The FCT-3 and FCT-4 are loci are the most common among GrAS isolates and share the greatest similarity of all FCT loci (Figure 1.5; Falugi, *et al.* 2008).

Vaccines against the T antigen show promise since fewer variants are required to cover the most common strains in circulation when compared to M type-dependent strategies (Figure 1.6). Although 27 M types were found to be in circulation in the United States and Europe in the early 2000s, only 12 T types would be needed to confer immunity to 24 of those M types (~90%) (Shulman, *et al.* 2004; Creti, *et al.* 2007; Falugi, *et al.* 2008). Furthermore, immunization of mice with recombinant pilus proteins has been demonstrated to confer protection against GrAS mucosal challenge, and anti-pilin antibodies have been detected in sera from human pharyngitis cases (Mora, *et al.* 2005; Manetti, *et al.* 2007).

1.5.1.3 Capsule

Observations at the beginning of the 20th century demonstrated that *S. pyogenes* isolated from the blood of septic patients elaborated a hyaluronate capsule (Bordet 1909; Kendall, *et al.* 1937; reviewed in Stollerman & Dale 2008).

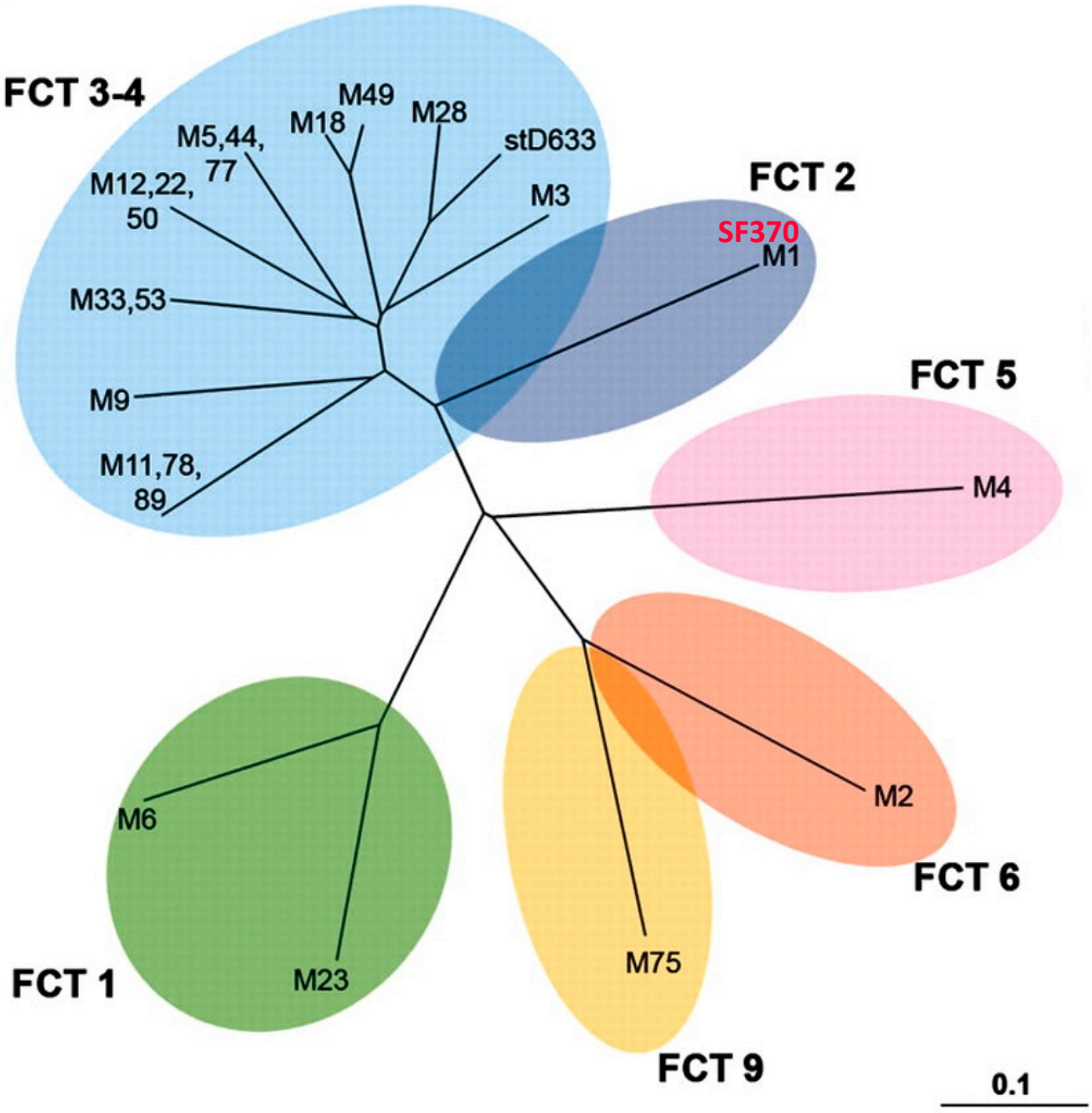


Figure 1.5 Phylogenetic clustering of M serotypes based on their FCT loci

Different colors indicate the clusters into which the variants group.

Figure adapted from (Falugi, *et al.* 2008).

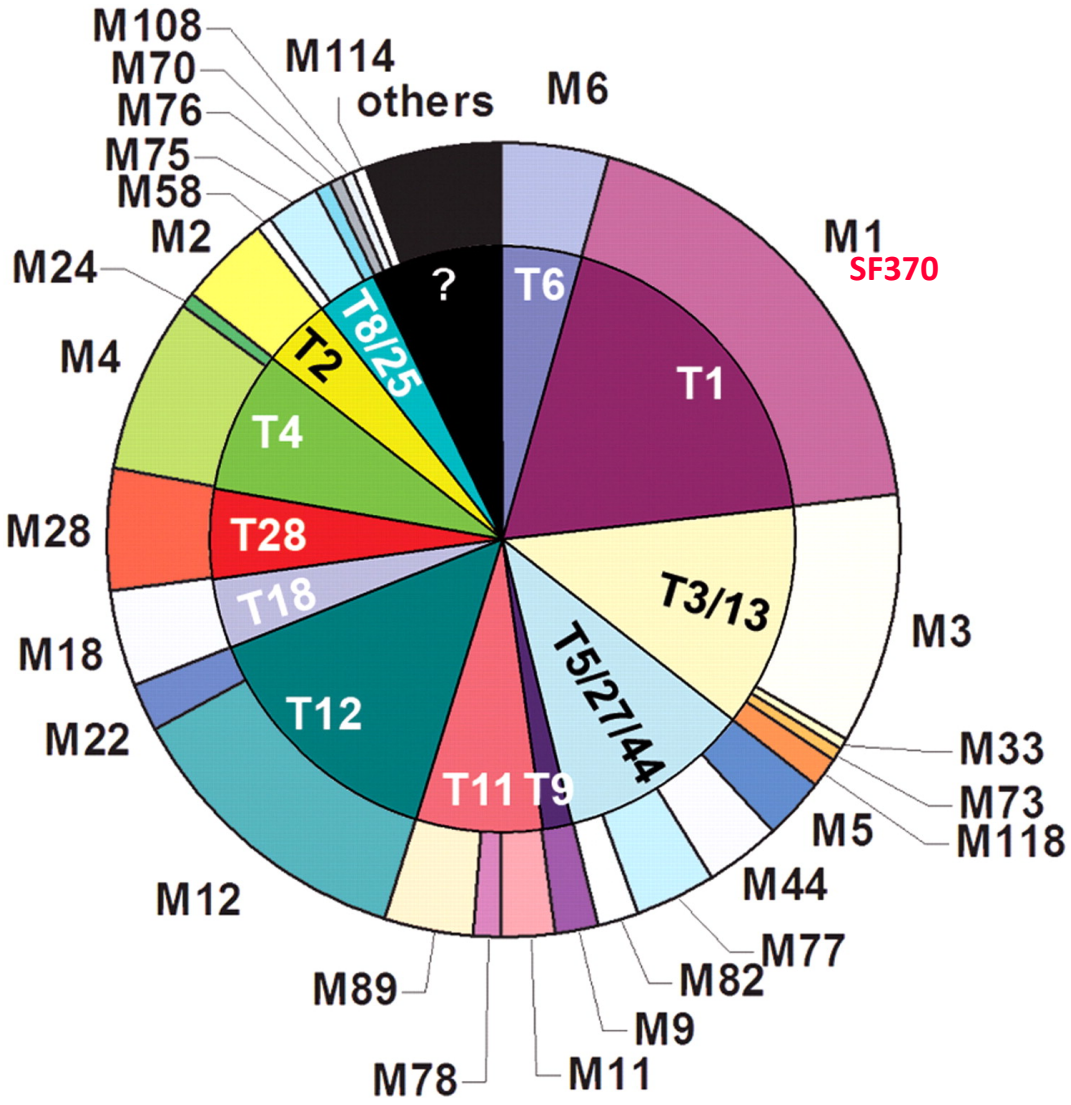


Figure 1.6 M type and T type distribution among clinically relevant *S. pyogenes* strains

The outer circle demonstrates the distribution of M types among circulating strains. The inner circle depicts the T type most frequently associated with a given M type.

Figure reprinted from (Falugi, *et al.* 2008).

Standard laboratory culturing, however, eliminated expression of the carbohydrate capsule within hours, resulting in loss of the mucoid phenotype on blood agar plates (Wilson 1959). Within a few decades, it became clear that the streptococcal capsule mediated phagocytosis resistance (Ward & Lyons 1935). Clinical surveys have indicated that capsule is often correlated with rheumatic fever outbreaks (Stollerman & Dale 2008).

Capsule generated by three hyaluronate producing genes (*hasA, hasB,* and *hasC*) is an adhesin that promotes GrAS binding to human epithelial cells via the hyaluronic-acid-binding-protein CD44 (Dougherty & van de Rijn 1994; Schrager, *et al.* 1998; Cywes, *et al.* 2000). This interaction results in the induction of cytoskeletal rearrangements in epithelial cells that disrupts intercellular junctions allowing the streptococci to translocate through the epithelium without entering host cells (Cywes & Wessels 2001). In addition, capsule has been implicated in the aggregation of collagen by M3 and M18 streptococci associated with rheumatic fever outbreaks in the United States during the 1980s (Dinkla, *et al.* 2003).

1.5.1.4 C5a Peptidase

Surface-associated C5a peptidase (*scpA*) is an endopeptidase specific for complement component C5a, and may function to eliminate immune signaling by the C5a molecule and protect streptococci from host defenses (Cleary, *et al.* 1992; Ji, *et al.* 1996). C5a peptidase is induced in response to culture in human

serum (mRNA transcription) and plasma (protein detection) (Gleich-Theurer, *et al.* 2009; Johansson, *et al.* 2005). Strong antibody responses to C5a peptidase have been detected in sera from adults, but not in uninfected children (O'Connor, *et al.* 1991). At least one study has suggested that C5a peptidase may not a virulence factor in all strains, since inactivation of the protein did not affect murine throat colonization in an M50 strain (Husmann, *et al.* 1997).

1.5.1.5 SpeB – Cysteine Protease

First identified in the 1940s, SpeB is the major secreted protein of GrAS (Elliott 1945). The cysteine protease is responsible for cleavage of host proteins for immune modulation (Table 1.4) and the processing and release of streptococcal proteins, including M protein, from the bacterial surface (Table 1.5) (reviewed in Nelson, *et al.* 2011). SpeB is first produced as a 40 kDa zymogen that is autocatalytically processed into the 28 kDa active form (Elliott & Dole 1947; Liu & Elliott 1965; Doran, *et al.* 1999). The virulence protein Trigger factor (*ropA*) is an essential chaperone for SpeB secretion and maturation, and the surface-associated serine proteinase HtrA is also necessary for SpeB maturation (Lyon & Caparon 2003; Lyon & Caparon 2004).

SpeB degrades IgA, IgM, IgD, and IgE into small fragments, and cleaves IgG in the hinge region to produce Fc and Fab fragments (Collin & Olsen 2001; Collin & Olsen 2001b). SpeB can also hydrolyze IgG bound to antigens on the bacterial surface through its Fab portion, but not IgG bound by the non-specific

Table 1.4 SpeB activity on host proteins/systems

Protein/system	Activity	Cleavage site (P3, P2, P1–P1′) or region	Potential/shown effect	References
Immune proteins/systems				
IgG	Cleavage	LLG-G in γ-chain	Decreased opsonophagocytosis	Collin and Olsén (2001a); Collin et al. (2002); Eriksson and Norgren (2003)
IgA, IgM, IgD, IgE	Degradation	Heavy chains	Immune evasion	Collin and Olsén (2001b)
C3b	Cleavage/degradation	RTL-D, NHK-L, LAR-S, VEL-I	Reduced phagocytosis	Kuo et al. (2008); Terao et al. (2008)
Properdin	Degradation	Several unknown sites	Inhibition of alternative complement activation	Tsao et al. (2006)
Chemokines	Degradation/processing	NQG-T, VSQ-K (CXCL9)	Reduced signaling and/or antibacterial activity	Egesten et al. (2009)
IL-1β	Activation	YVH-D	Proinflammatory	Kapur et al. (1993a)
H-kininogen	Bradykinin release	LMK-R, PFR-S	Vasodilation, pain	Herwald et al. (1996)
Mast cells	Activation	Unknown	Histamine release, degranulation	Watanabe et al. (2002)
Other host proteins/systems				
Fibrinogen	Degradation	C-terminally in Aα-chain	Inhibition of hemostasis	Elliott (1945); Matsuka et al. (1999)
Plasminogen	Degradation	Several unknown sites	Reduction of plasmin on bacterial surface	Cole et al. (2006)
Vitronectin	Degraded	Several unknown sites	Facilitating tissue invasion and spread	Kapur et al. (1993b)
Fibronectin	Fragmented	Several distinct unknown sites	Facilitating tissue invasion and spread	Kapur et al. (1993b)
Urokinase receptor	Cleavage	Close to the GPI anchor	Inhibition of pericellular plasmin activity and cell migration	Wolf et al. (1994)
MMPs	Activation	Removal of MMP proregions	Facilitating tissue invasion and spread and apoptotic killing of cells	Burns et al. (1996); Tamura et al. (2004)
Decorin	Degradation	Several unknown sites	Dermatan sulfate release→protection against AMPs	Schmidtchen et al. (2001)
Integrins	Binding	RGD in SpeBm binds to integrins	Cellular targeting of SpeBm activity	Stockbauer et al. (1999)
Laminin	Binding	Unknown site of interaction	Binding of laminin to bacterial surface	Hytönen et al. (2001)
A1AT	Binding	Interaction with inactive and active A1AT	Contact system activation	Meinert Niclasen et al. (2011)
A2M	Cleavage and entrapment	Bait region	Protection against AMP LL-37	Nyberg et al. (2004a)
Apoptosis	Induction of caspase mediated apoptosis	Unknown	Killing of epithelial cells	Tsai et al. (1999)

Table reprinted from (Nelson, *et al.* 2011).

Fc region (Eriksson & Norgren 2003). Hydrolysis of IgG by SpeB results in a reduction of opsonophagocytosis (Collin, *et al.* 2002).

Furthermore, SpeB can inhibit the complement pathway by degrading C3b, a potent opsonin for phagocytic cells (Kuo, *et al.* 2008). The innate defenses of epithelial cells include the elaboration of antimicrobials (LL-37) and proinflammatory chemokines, which SpeB also degrades (Nyberg, *et al.* 2004; Egesten, *et al.* 2009). For hydrolysis of LL-37, enzymatically active SpeB is held at the bacterial surface by a complex formed from the streptococcal surface-associated virulence protein GRAB and the human protease inhibitor α2-macroglobulin (Nyberg, *et al.* 2004).

SpeB degradation of host extracellular matrix components, including vitronectin and fibronectin, may play a role in tissue invasion and spreading within the host (Kapur, *et al.* 1993). In addition, SpeB has been proposed to induce apoptosis of epithelial cells directly through proteolysis or indirectly via activation of host matrix metalloproteases (Tsai, *et al.* 1999; Burns, *et al.* 1996; Tamura, *et al.* 2004). SpeB also appears to be an adhesin independent of its proteolytic functions as it binds to laminin (Hytonen, *et al.* 2001).

On the bacterial surface, SpeB can exert posttranslational regulation and virulence modulation by releasing active proteins from the cell wall, processing proteins to alter their function, or degrading proteins (Nelson, *et al.* 2011). SpeB can release numerous adhesins, including M protein and fibronectin-binding proteins, changing the affinity of IgG to the bacterial cell and modulating

Table 1.5 SpeB activity on bacterial proteins/systems

Protein/system	Activity	Cleavage site(s) (P3, P2, P1–P1′) or region	Potential/shown effect	References
Surface proteins				
M protein	N-terminal and C-terminal cleavage	NIR-L and S region	Release from surface/altered IgG/plasma protein binding	Elliott (1945); Berge and Björck (1995); Raeder et al. (1998)
Protein H	Cleavage	D region	Release from surface/complement activation in solution	Berge et al. (1997)
Protein F1	Degradation	Sites unknown	Reduced internalization into human cells	Nyberg et al. (2004b)
Fba	Degradation	Sites unknown	Reduced binding to factor H and factor H-like protein 1	Wei et al. (2005)
C5a peptidase	Cleavage	APA-K, AVI-D, SGTS, and C-terminally	Release from surface/inhibition of neutrophil recruitment	Berge and Björck (1995)
Slr	Cleavage	Sites unknown	Altered collagen binding	Collin (unpublished laboratory data)
Secreted proteins				
SpeBz	Cleavage/processing	AIK-A, KVN-L, QIK-E, TYA-G, EIK-Q	Generation of SpeBm	Liu and Elliott (1965b); Doran et al. (1999)
Streptokinase	Degradation	Sites unknown	Loss of plasminogen activation	Svensson et al. (2002)
EndoS	Processing	VML-K	Loss of IgG glycan hydrolyzing activity	Allhorn et al. (2008)
Sda1	Degradation	Sites unknown	Decreased extracellular trap clearance	Walker et al. (2007)
Streptolysin O	Processing	MIK-L	No effect on cytolytic activity	Pinkney et al. (1995)
SmeZ	Degradation/processing	Sites unknown	Loss of superantigen activity	Nooh et al. (2006)
Other systems				
Biofilm	Dispersal	Unknown	Increased lesion size	Connolly et al. (2011)
Hyaluronic acid capsule	Inhibition of synthesis	Unknown	Altered surface properties	Woischnik et al. (2000)

Table reprinted from (Nelson, *et al.* 2011).

internalization by host cells (Berge & Bjorck 1995; Raeder, *et al.* 1998; Nyberg, *et al.* 2004b). SpeB can also release C5a peptidase from the cell surface reducing recruitment of neutrophils to the infection site (Wexler & Cleary 1985; Berge & Bjorck 1995).

SpeB is known to degrade streptokinase (Ska) and numerous streptococcal superantigens, with SmeZ being the most sensitive to SpeB action (Cole, *et al.* 2006; Nooh, *et al.* 2006). Virulence factor processing also appears to be a function of SpeB in relation to streptolysin O and the antibody glycan-hydrolyzing enzyme EndoS (Pinkney, *et al.* 1995; Allhorn, *et al.* 2008). Recently, SpeB has been implicated in the dispersal of *S. pyogenes* biofilms, leading to increased dissemination in a mouse model of infection (Connolly, *et al.* 2011).

The *speB* gene is highly conserved in group A streptococci (Nelson, *et al.* 2011). Expression of SpeB *in vivo* is supported by seroconversion studies, and the presence of low anti-SpeB Ig titers appears to correlate with severe disease (Holm, *et al.* 1992; Gubba, *et al.* 1998). Furthermore, injection of anti-SpeB antibodies in mice is protective (Kapur, *et al.* 1994). Arguments have been made on both sides, however, regarding the importance of high SpeB activity (immune evasion) versus low SpeB activity (maintenance of GrAS surface adhesins) for invasive infection (Nelson, *et al.* 2011). At least *ex vivo,* SpeB appears to be repressed in human blood (Raeder, *et al.* 2000).

1.5.1.6 Streptokinase (Ska)

Using a non-enzymatic mechanism, secreted streptokinase (*ska*) converts plasminogen to its active form plasmin (Reddy & Markus 1972; Schick & Castellino 1974). The Ska-plasmin complex can then degrade blood clots as well as components of the extracellular matrix to enhance spread of the streptococci or its toxins (reviewed in McArthur, *et al.* 2012). Streptococci can also recruit the complex on to their surface, but the function for immune evasion has not been examined (Lottenberg, *et al.* 1992; McArthur, *et al.* 2012). Deposition of streptokinase in the glomeruli of the kidneys has been implicated in the activation of complement and initiation of acute glomerulonephritis (Holm 1988; Nordstrand, *et al.* 1998).

1.5.1.7 Streptococcal Inhibitor of Complement (SIC)

The streptococcal inhibitor of complement can interact with numerous host proteins and helps to evade innate immune defenses (Hynes 2004). SIC can inhibit complement-mediated lysis by binding to the C5b-C7 component of the membrane attack complex (Fernie-King, *et al.* 2001). In addition, SIC can inactivate the antimicrobial peptide LL-37 and the human neutrophil alpha-defensin (Frick, *et al.* 2003). SIC can also bind to and neutralize the antibacterial activities of lysozyme and secretory leukocyte proteinase inhibitor (Fernie-King, *et al.* 2002). Recently, researchers have demonstrated that SIC is highly

expressed in the hypervirulent M1T1 clone and contributes to the virulence phenotype in a murine model of invasive disease (Pence, *et al.* 2010).

1.5.1.8 Streptolysin O (SLO) and NADase (SPN)

Streptolysin O is a secreted, oxygen-sensitive hemolysin of group A streptococci (Hynes 2004). SLO interacts with host cell cholesterol forming 25-30 nm pores following aggregation of the toxin in the host membrane (Shatursky, *et al.* 1999). Target cells include erythrocytes, macrophages, leukocytes, platelets, and numerous cell culture types (Hynes 2004). The toxic effect of SLO can also be observed by injection into animals, and SLO deletion mutants are attenuated in a murine skin infection model (Ginsburg 1972; Limbago, *et al.* 2000).

Similar to the type III secretory pathway of Gram-negative bacteria, SLO has been shown to deliver proteins from the bacterial cytoplasm directly into the cytosol of a host cell. The effector molecule NAD glycohydrolase (NADase or SPN) encoded by *nga* is translocated via the SLO pore (Madden, *et al.* 2001). Once in the host cell, NADase depletes the intracellular energy stores (NAD+ and ATP), possibly reducing the ability of host cells to combat or repair the SLO-mediate membrane damage (Michos, *et al.* 2006).

An invasive variant of the virulent M1T1 clone appears to upregulate transcription of the *slo-nga* operon and produce more active SLO and NADase (Sumby, *et al.* 2006). SF370 has been observed to be lacking a functional

extracellular NADase activity (Ajdic, *et al.* 2000). Sequence analyses of the *nga* gene from a number of strains indicate that this gene is under positive selection. Thus, it is hypothesized that SPN may have NADase-independent virulence functions not yet recognized (Riddle, *et al.* 2010).

Recent studies have indicated that SLO-mediated host membrane damage *in vivo* may contribute to pathogenesis by inhibiting internalization of GrAS by pharyngeal cells and preventing intracellular killing by lysosomal fusion (Hakansson, *et al.* 2005; Logsdon, *et al.* 2010). In addition, SLO can induce apoptosis in macrophages by permeabilizing the mitochondrial outer membranes of host cells. The rapid apoptosis blunts the macrophage-dependent inflammatory cytokine responses (Timmer, *et al.* 2009).

1.5.1.9 Streptolysin S (SLS)

Streptolysin S is an oxygen-stable, non-immunogenic hemolysin that can be found intracellularly, on the bacterial cell surface, or in the extracellular space (Hynes 2004). SLS and SLO are the enzymes responsible for the beta-hemolytic phenotype of *S. pyogenes* on blood agar plates. Targets for SLS include lymphocytes, neutrophils, platelets, and subcellular organelles (Ginsburd 1972; Hryniewicz & Pryjma 1977). Similar to the process of bacterial lysis by complement, SLS lysis involves insertion of a lysin complex into the host cell membrane resulting in the formation of transmembrane pores (Carr, *et al.* 2001). SLS is produced from a nine-gene operon, where the first gene *sagA* encodes

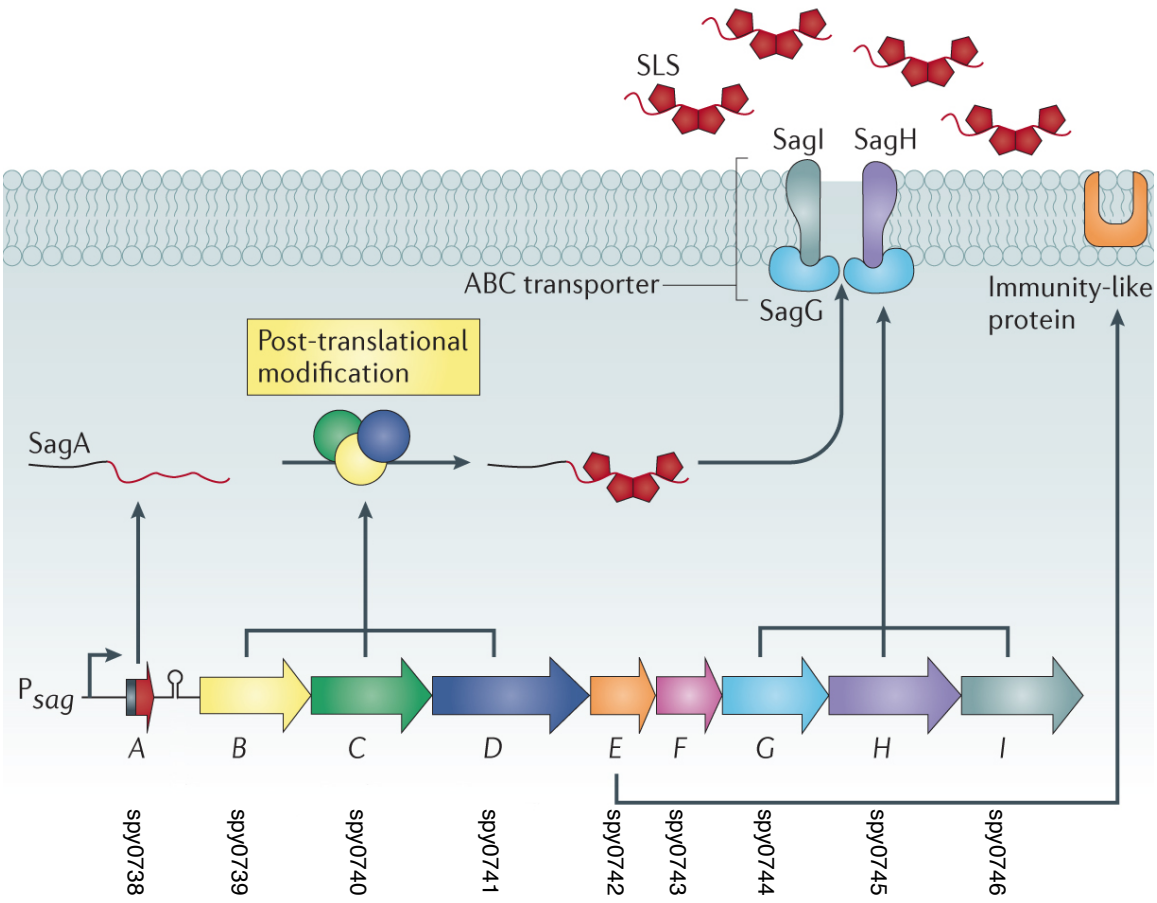


Figure 1.7 Production, processing, and export of streptolysin S by the *sag* operon

The carboxy-terminal core peptides of SLS-associated gene A protein (SagA) are post-translationally modified by the SagBCD complex to form biologically active SLS. The amino-terminal leader sequence (black) is cleaved from the mature core peptide following modification, resulting in mature peptide product. SagGHI form an ABC-type transporter for SLS export. The function of SagF is unclear, and SagE appears to be an immunity protein required for viability.

Figure adapted from (Molloy, *et al.* 2011).

the precursor peptide that is processed and exported by the other gene products in the locus (Figure 1.7; reviewed in Molloy, *et al.* 2011).

SLS has been implicated in the development of necrotizing fasciitis in group G streptococci. SLS can directly lysis cells of deep soft tissues and feeding vessels, leading to recruitment of neutrophils that add host-derived oxidants and proteases to the toxic mix (Humar, *et al.* 2002). SLS-mediated killing of neutrophils is considered an important mechanism of immune evasion (Miyoshi-Akiyama, *et al.* 2005). In addition, SLS can activate an inflammatory apoptotic pathway in macrophages, though the contribution of SLS to phagocytic resistance appears to be strain specific (Molloy, *et al.* 2011). A role for SLS in systemic dissemination of GrAS has recently been described (Sumitomo, *et al.* 2011). By an uncharacterized mechanism, SLS recruits the host Cys protease calpain to the cell membrane and induces proteolytic degradation of the intercellular junctions allowing invasion through a paracellular route (Sumitomo, *et al.* 2011).

1.5.1.10 CAMP Factor

The CAMP factor is involved in erythrocyte lysis and Fc binding of IgM and IgG in group B *Streptococcus* (Christie, *et al.* 1944; Jurgens, *et al.* 1987). GrAS was long considered to be CAMP negative, but sequencing of the SF370 genome revealed that *S. pyogenes* does encode the secreted toxin (Ferretti, *et al.* 2001). Analyses revealed that CAMP factor from GrAS is fully functional and its gene *cfa*

is maximally transcribed during the exponential phase of growth (Gase, *et al.* 1999). The role of CAMP in GrAS pathogenesis has not been investigated.

1.5.2 Phage Encoded Virulence Factors

Integrated phages not only contribute to the genetic diversity of GrAS strains, but they can also introduce virulence factors that alter the phenotype of a particular strain in the infection setting. Although strain SF370 encodes for extracellular DNases, hyaluronidases, and superantigens within its bacterial chromosome, additional proteins from each category have been introduced by phage transduction as evidenced by the four integrated prophage sequences in the SF370 genome (Ferretti, *et al.* 2001).

1.5.2.1 Superantigens

The streptococcal superantigens (both chromosomally and phage encoded) are monomeric proteins, whose secreted mature forms have molecular weights in the range of 23.6-27.4 kDa (reviewed in Proft & Fraser 2007). The term 'superantigen' refers to the ability of these proteins to stimulate large numbers of CD4 and CD8 T cells (White, *et al.* 1989). Superantigens (SAgs) bind to and crosslink the major histocompatibility complex (MHC) class II of an antigen-presenting cell to the T cell receptor of CD4 and CD8 lymphocytes independent of the antigen-binding site. This interaction can nonspecifically activate up to 25% of the body's T cell population resulting in a massive release

of proinflammatory cytokines leading to fever and shock (Jupin, *et al.* 1988; Choi, *et al.* 1990; Herman, *et al.* 1991; Mueller-Alouf, *et al.* 1996).

The action of SAgs can occur at very low concentrations making them the most powerful T cell mitogens ever discovered (Fraser, *et al.* 2000). It is unclear why GrAS would target T cells for activation. One hypothesis is that SAgs may corrupt the host immune system by skewing cytokine responses away from induction of T cells that promote the production of high-affinity cytotoxic antibodies (Proft & Fraser 2007). Alternatively, SAgs may act by inducing T cell anergy, which is a nonresponsive state marked by reduced cytokine production (Proft & Fraser 2007).

Although the production of SAgs is generally low during *in vitro* growth, induction of phage encoded SAgs has been observed during murine subcutaneous infections (SpeA) and in response to a soluble human factor present in pharyngeal cell culture supernatants and human saliva (SpeC) (Kazmi, *et al.* 2001; Broudy, *et al.* 2001). Furthermore, transcriptome analyses have revealed that SAg expression is upregulated during *ex vivo* growth in blood (Graham, *et al.* 2003). Epidemiological and clinical observations combined with experimental studies strongly suggest that SAgs play an important role in streptococcal toxic shock syndrome (Proft & Fraswer 2007).

The SF370 phage encoded superantigens include SpeC, SpeH, and SpeI. Chromosomally encoded superantigens in SF370 are SmeZ, SpeG, and SpeJ.

In addition, the SF370 genome contains a superantigen pseudogene designated *speK* (Ferretti, *et al.* 2001).

1.5.2.2 DNases

GrAS encode a number of functional DNases both within the bacterial chromosome and in integrated prophages, which can be antigenically distinct (Hynes 2004; Sumby, *et al.* 2005b). These DNase genes are upregulated in response to streptococcal interactions with human pharyngeal cells, polymorphonuclear leukocytes, and the oropharynx (Broudy, *et al.* 2002; Banks, *et al.* 2003; *Voyich*, *et al.* 2003; Virtaneva, *et al.* 2003). Furthermore, the presence of anti-DNase immunoglobulin in patient sera indicates that they are produced and secreted *in vivo* (Kaplan & Huew 1980; Hostetler, *et al.* 1988).

Encoded in the SF370 chromosome, superantigen *speF* has exonuclease activity and is also known as *spd* (DNase B), although the epitopes responsible for T cell stimulation and DNA degradation are separate (Eriksson, *et al.* 1999). SF370 also carries genes for the phage DNases *spd1* and *spd3* (Ferretti, *et al.* 2001). Protein expression of Spd1 (in combination with SpeC) is induced by the soluble factor present in human saliva and pharyngeal cell supernatants (Broudy, *et al.* 2002).

The role of extracellular DNases in virulence was investigated in an M1 strain. Systematic elimination of *spd* and two phage-encoded DNases in that strain highlighted that DNases elaborated by streptococci are critical for virulence

in a non-human primate model of GrAS pharyngitis (Sumby, *et al.* 2005b). DNases were determined to be important for defenses against extracellular killings by polymorphonuclear leukocytes, likely through the degradation of neutrophil extracellular traps (NETs). It was also suggested that DNases help initially dissolve mucus in the oropharynx providing access to the underlying epithelium (Sumby, *et al.* 2005b). As the infection progesses, DNases may then decrease the viscosity of purulent exudates and break up bacterial aggregates, allowing for bacterial spread from the initial infection site (Sumby, *et al.* 2005b).

1.5.2.3 Hyaluronidases

Streptococci encode genes for secreted hyaluronidases—enzymes that digest hyaluronic acid (HA)—both within the bacterial chromosome and in prophage sequences (Ferretti, *et al.* 2001). Phage encoded hyaluronidases, however, are not homologous to the chromosomal gene *hylA* at the level of DNA or amino acid sequence. SF370 carries three phage encoded hyaluronidases: *hlyP1*, *hlyP2*, and *hlyP3* (Ferretti, *et al.* 2001). The hyaluronic acid of the streptococcal capsule is identical to human HA, and both serve as targets for streptococcal hyaluronidases (Sandson, *et al.* 1968; Starr & Engleberg 2006).

Experiments using an isogenic mutant of the chromosomal hyaluronidase *hlyA* indicated that activity of the enzyme against the streptococcal capsule is negligible during exponential growth (Starr & Engleberg 2006). The function of hyaluronidases in virulence remains unclear, but data from the *hylA* mutant

support a role for hyaluronidases in digesting host HA for nutritional purposes and to allow the spread of macromolecules, which could potentially include superantigens. It has also been proposed that hyaluronidase-mediated breakdown of the host extracellular matrix could allow for spread of streptococci in soft tissues, but this was not supported by *in vivo* murine infections with the *hylA* mutant (Hynes 2004; Starr & Engleberg 2006).

1.6 Virulence Factor Regulation

Group A streptococci regulate the production of virulence factors in response to their growth phase, changing environmental conditions (temperature, pH, oxygen), and specific cues from the host (secreted host factors, interactions with phagocytic cells, adherence to epithelial cells) (reviewed in Fiedler, *et al.* 2010). Regulation of virulence in *S. pyogenes* is widely considered to be executed at the transcriptional level. Studies that have examined the correlation between mRNA levels and protein production in *S. pyogenes* have found good agreement (Lyon, *et al.* 2001; Chaussee, *et al.* 2002; Shelburne III, *et al.* 2005b; Kreikemeyer, *et al.* 2007; Wen, *et al.* 2011; Shelburne III, *et al.* 2011b).

Transcriptional regulation is accomplished via a complex web of transcription factors and two-component regulatory systems. Nearly 100 genes have been identified through the GrAS genome sequencing projects that have homology to transcriptional regulators (Hynes 2004). SF370 encodes over 40 stand-alone regulators (Table 1.6) and 13 two-component systems (Table 1.7).

1.6.1 Stand-alone Regulators

Expression of multigene virulence regulons in group A streptococci is under the control of two-component systems (detailed below) and 'stand-alone' transcriptional regulators (Table 1.6). These stand-alone regulators can positively or negatively affect virulence factor expression in response to environmental signals communicated by unknown sensory elements (Kreikemeyer, *et al.* 2003). The best characterized of these regulators is *mga*, but we are beginning to understand the role of additional stand-alone regulators, including *rofA*, *nra, ralp3, msmR, rivR, rgg, ccpA*, *lacD.1, mtsR,* and *perR.*

The main insight garnered from recent studies is that these regulators are part of a complex web by which streptococci sense and respond to their environment. Regulators often exhibit growth phase-dependent expression and regulatory activity (Figure 1.8; McIver 2009). The dependence of transcriptional alterations on growth phase could simply be a proxy for streptococcal responses to the changing metabolic environment and nutrient depletion, cell cycle status, or population density signals (Steiner & Malke 2000; Steiner & Malke 2001; Ryan & Shapiro 2003; Waters & Bassler 2005). Increasing evidence highlights, however, that care should be taken in drawing generalities regarding their regulons, as the actions of these regulators can be serotype- and strain-specific.

Table 1.6 Putative and Characterized Stand-alone Regulators of SF370 (Pages 44-46)

Recognized Virulence Regulators

Locus Tag	Gene Name	Regulatory Targets
spy0124	*rofA*	Activation of FCT region (pilus)
spy0188	*vlg*	Unknown
spy0216	*rivR*	Activation of Mga regulon genes (via *mga*)
spy0450	*mtsR*	Metal transport systems regulation; Repression of *ska* (streptokinase); Activation of *mga, emm*
spy0514	*ccpA*	Mga regulon (*mga* activation); Carbohydrate utilization genes (repression); *speB* (activation)
spy0797	*amrA*	Activation of Mga regulon (via Mga); Mechanism of action unknown
spy0887	*vfr*	Repression of *speB* via RopB; No DNA binding activity
spy1293	*malR*	Repression of maltodextrin transport and pullulanase operon; Phage gene repression
spy1531	*perR*	Regulation of metal homeostasis, oxidative stress response; Activation of phage DNases
spy1605	*rocA*	CovRS regulon by activation of *covRS*
spy1642	*luxS*	Quorum-sensing?; Activation of *speB*; Repression of *sagA* (SLS)
spy1704	*lacD.1*	*speB* (repression), SLO, SLS (*sagH*); Action likely through protein-protein interactions
spy1777	*codY*	Repression of CovRS regulon (via *covRS*), grab; Activation of Mga regulon (via *mga*)
spy1857	*srv*	Activation of *mga, covRS, speG, ska* (streptokinase), *scpA* (C5a peptidase), *hasA* (capsule)
spy1981	*relA*	Stringent response to amino acid starvation; *grab* and *speH* (repression), *ska* (activation)
spy2019	*mga*	Activation of *emm* (M protein), *scpA* (C5a peptidase), *sic*, *fbaA* (fibronectin-binding), *sclA* (collagen-like), *slo, opp/dpp* (small peptide transport)
spy2042	*ropB/rgg*	Activation of *speB*; Regulation of metabolic and stress response genes

Table 1.6 Putative and Characterized Stand-alone Regulators of SF370

Putative and/or Uncharacterized Regulators

Locus Tag	Gene Name	Functional Domain Information
spy0092	*adcR*	MarR family repressor
spy0164	*nusG*	Transcription antiterminator
spy0181		BglG family antiterminator
spy0259		RpiR family regulator
spy0338	*nrdR*	Putative regulator
spy0544		Putative regulator
spy0571	*licT*	BglG family antiterminator
spy0627	*regR*	LacI family regulator
spy0715		GntR family regulator
spy0824		LysR family regulator
spy0846		TetR family regulator
spy0853		Fructose represssor
spy0898	*cpsY*	Putative regulator
spy1081	*srtR*	Putative DNA-binding protein
spy1088		Putative repressor protein
spy1179		GntR family regulator
spy1198		Putative repressor protein
spy1202		GntR family regulator
spy1210		GntR family regulator
spy1215		SIR2 family regulator
spy1258		TetR family regulator
spy1285		GntR family regulator
spy1297		LacI family regulator
spy1325		BglG family antiterminator
spy1350	*psr*	Repressor of Penicillin-binding-protein 5
spy1386		Putative regulator
spy1496		ArgR family regulator
spy1535		Ribose transport operon regulator

Table 1.6 Putative/Uncharacterized Regulators

spy1549	*ahrC.2*	Arginine repressor
spy1596		Putative regulator
spy1602		GntR family regulator
spy1634		LysR family regulator
spy1699		Putative regulator
spy1712	*lacR.1*	DeoR family regulator
spy1717	*copY*	Cooper transport operon repressor
spy1724	*nusA*	Transcription antiterminator
spy1733		Cell envelope-related repressor
spy1755		MarR family regulator
spy1763	*hrcA*	Repression of heat shock operons
spy1817	*scrR*	LacI family regulator
spy1818	*nusB*	Transcription antiterminator
spy1834		Putative regulator
spy1861		Putative repressor protein
spy1863		MerR family activator
spy1870		GntR family regulator
spy1878		MerR family regulator
spy1924	*lacR.2*	Lactose phosphotransferase system repressor
spy1934		Putative regulator
spy1952		BglG family antiterminator
spy1960		MarR family regulator
spy2053		Putative regulator
spy2054		DeoR family regulator
spy2074	*ctsR*	Heat and stress response regulator
spy2099		GntR family regulator
spy2150	*ahrC*	Arginine repressor
spy2163	*cadC*	Cadmium efflux pump regulator
spy2172		PadR family regulator
spy2177		TetR family regulator

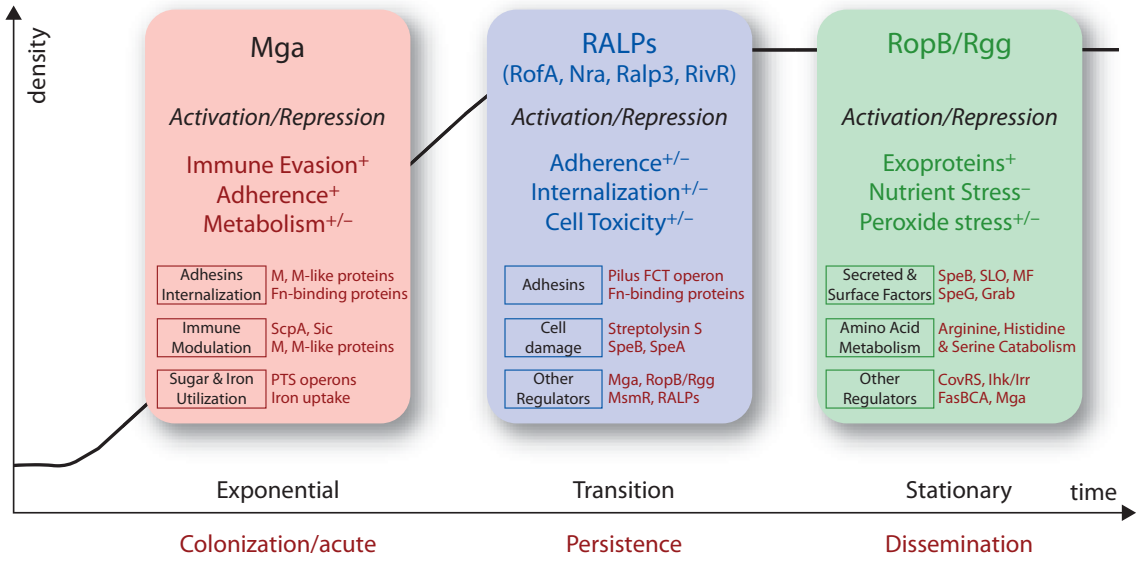


Figure 1.8 Growth, metabolism, and stand-alone regulatory systems of GrAS

In vitro growth curve is shown in background with phase of growth indicated. Boxes representing each stand-alone network are placed at the time during growth when they exhibit maximal expression and the relevant infection stage is given at the bottom. Prevalence of activation versus repression of the various virulence functions is indicated by the superscript: activated (+) or repressed (-). Overall processes and specific molecules regulated by each are shown. Figure adapted from (McIver 2009).

1.6.1.1 Mga

The *mga* gene lies immediately upstream of *emm*, which encodes M protein. The gene was first identified as *virR* (virulence regulator) by researchers examining the genetic basis of spontaneous M protein loss in an M12 isolate (Spanier, *et al.* 1984). Following the realization that small deletions in this upstream ORF could eliminate M protein production, it was deemed that this locus encoded a regulatory switch (Spanier, *et al.* 1984).

Subsequent studies using insertional mutagenesis in an M6 strain defined the gene *mry* (M protein RNA yield), a locus upstream of the M6 gene (*emm6*) that is required for M protein transcription (Caparon & Scott 1987). Furthermore, *mry* was found to act in *trans* as a positive regulator of *emm6* and include helix-turn-helix domains for DNA binding (Perez-Casal, *et al.* 1991). Upon recognition that *virR* and *mry* were the same gene using DNA sequence comparisons and PCR analyses, researchers agreed to rename the positive regulator *mga* (multiple gene regulator of group A streptococcus) (Chen, *et al.* 1993; Scott, *et al.* 1995).

A survey of 36 GrAS serotypes revealed that *mga* was present in all examined strains (Podbielski 1992). In strain SF370, *spy2019* encodes Mga (Ferretti, *et al.* 2001). Sequence analysis of *mga* from an additional 114 GrAS strains indicated that there are two major alleles: *mga-1* and *mga-2* (Bessen, *et al.* 2005). Mga-1 and Mga-2 DNA-binding sites exhibit few or no amino acid replacements indicating that the regulators act on the same target genes. Little

recombination between alleles of the divergent *mga* lineages supports the hypothesis that the *mga* alleles have evolved to take advantage of separate host niches. Mga-1 is associated with throat-tropic strains, while Mga-2 is present in skin-tropic and generalist strains (Bessen, *et al.* 2005).

Expression of *mga* is maximal during the exponential phase of growth indicating that the Mga regulon is required for rapid expansion into new tissue sites (McIver & Scott 1997). The *mga* gene is also known to respond to environmental conditions, including elevated carbon dioxide levels, increased temperature, and iron-limiting growth conditions (Pines & Reeves 1978; Caparon, *et al.* 1992; Podbielski, *et al.* 1992; McIver, *et al.* 1995). Mga is autoregulated and thus can further amplify the expression of its regulon (McIver, *et al.* 1999). Regulation of *mga* also appears to involve other stand-alone regulators, including activation by CcpA and RivR (discussed below) (Almengor, *et al.* 2007; Roberts & Scott 2007).

Recent transcriptional analyses of the Mga regulon in three GrAS serotypes indicated that Mga activates or represses expression of more than 10% of the genome during exponential growth (Ribardo & McIver 2006). Beyond activation of M protein expression, Mga positively regulates a core group of virulence genes important for adherence to and internalization by host cells, as well as immune evasion (Figure 1.9; reviewed in Hondrop & McIver 2007). Most of these virulence genes are located within the *mga* locus, but in some strains, Mga can also regulate the unlinked *speB* gene (Podbielski, *et al.* 1995; Ribardo &

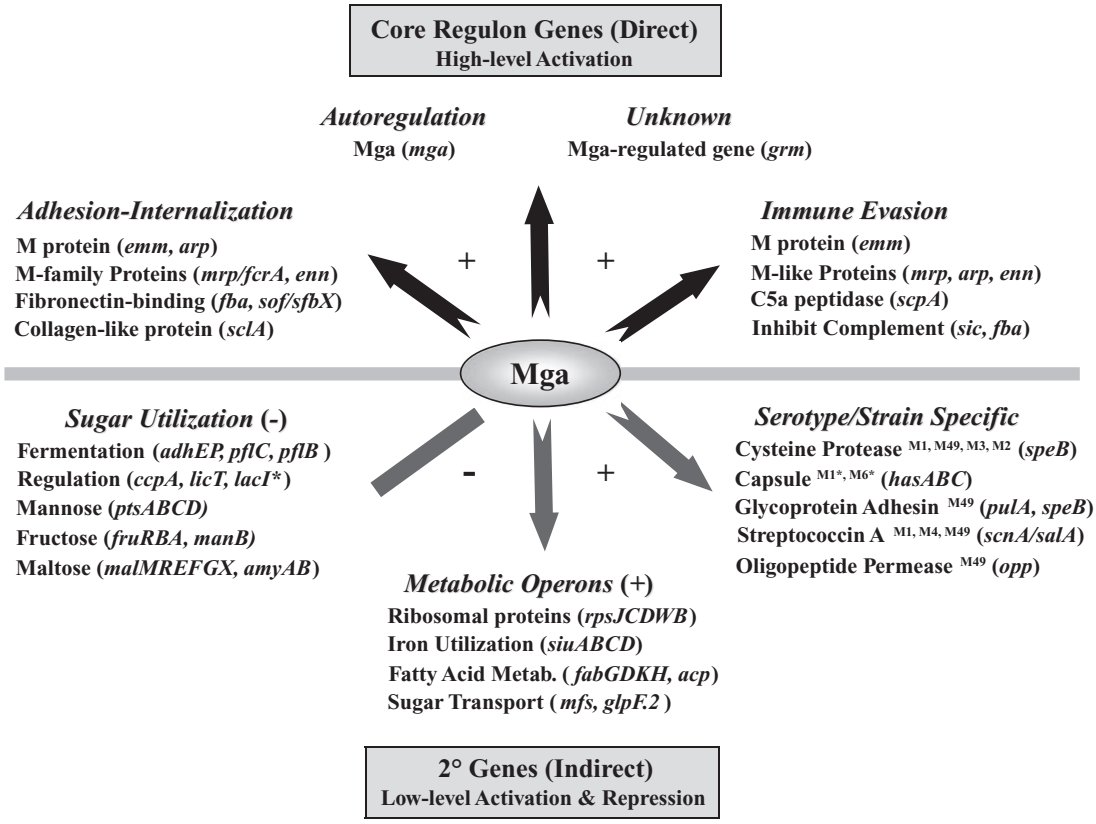


Figure 1.9 Overview of the Mga regulon in GrAS

A compilation of Mga-regulated genes and their gene products based on their known or predicted function in GrAS. An arrow indicates transcriptional activation, while repression is designated by a bar. Core Mga regulon genes that show high-level activation and reflect direct binding of Mga to their respective promoters are listed at the top. The secondary regulon genes that show low-level regulation and likely are indirectly influenced by Mga are shown at the bottom.

Figure adapted from (Hondrop & McIver 2007).

McIver 2006). Notably, comparison of the Mga regulon in two M6 strains indicated that the target genes for Mga regulation can vary even within the same serotype (Ribardo & McIver 2006).

Mga also enacts low-level repression (and some activation) of gene expression for proteins involved in transport and utilization of carbohydrates, iron, and amino acids (Ribardo & McIver 2006). It remains to be determined how Mga may act directly or indirectly in regulating these metabolic genes. Loss of Mga altered the ability of streptococci to grow in chemically defined media with sugar sources corresponding to the repressed operons strengthening the connection between Mga regulation and carbon utilization (Ribardo & McIver 2006).

Genome-wide transcriptional analyses of GrAS during growth *ex vivo* and *in vivo* support the role of Mga regulation in virulence. During growth in whole human blood, *mga* expression peaked at 30 minutes after inoculation and returned to steady-state levels by 90 minutes (Graham, *et al.* 2005). Researchers postulated that Mga regulation is critical for GrAS survival in blood as Mga activates the antiphagocytic M protein and Sic (Graham, *et al.* 2005). In a longitudinal study of experimental pharyngitis in cynomolgus macaques, expression of Mga reached its maximum during the early acute phase of infection (Virtaneva, *et al.* 2005). At this point, streptococci actively grow in the animals, and symptoms are most severe. Expression of carbohydrate utilization genes was also high during this infection phase (Virtaneva, *et al.* 2005).

1.6.1.2 Introduction to RALPs

RALPs (RofA-like proteins) are regulatory proteins important for virulence modulation in Group A streptococci. Four RALPs have been identified in GrAS: *rofA, nra* (*ralp2*), *ralp3*, and *rivR* (*ralp4*) (Granok, *et al.* 2000). GrAS strains possess at least *rofA* or *nra*, but can include several RALPs (Kreikemeyer, *et al.* 2002). Strain SF370 encodes *rofA* (*spy0124*), *ralp3* (*spy0735*), and *rivR* (*spy0216*), which are discussed further below (Ferretti, *et al.* 2001).

The RALPs are similar in size (approximately 58 kDa) with 52% similarity and 29% identity to each other (Granok, *et al.* 2000). The helix-turn-helix region of the RALPs is not well-conserved suggesting that these regulators recognize unique DNA sequences (Granok, *et al.* 2000). In general, RALPs have been associated with regulating genes important for host-cell interactions and minimizing host cell damage. These regulators appear to act primarily during transition to the stationary phase of growth (as reviewed in McIver, 2009).

The connection between RALP gene patterns and particular GrAS serotypes associated with streptococcal disease remains unclear. One study examining isolates from 61 serotypes indicated that no link exists between the presence of RALP genes (*rofA* and *nra*), RALP-dependent adhesins (fibronectin- and collagen-binding proteins), and GrAS serotypes (Kreikemeyer, *et al.* 2002). A follow-up study examining 114 GrAS isolates concluded that *rofA* is more likely to be encoded with the *mga-1* allele in throat-tropic GrAS strains, while *nra* is associated with the *mga-2* allele in skin specialist strains (Bessen, *et al.* 2005).

1.6.1.3 Regulators of the FCT Region: RofA, Nra, MsmR

As previously discussed, the FCT region is a section of high recombination within the GrAS genome that contains genes encoding fibronectin- and collagen-binding proteins and the pilus (Bessen & Kalia, 2002; Mora*, et al.* 2005). Expression of these virulence factors is influenced by three global regulators residing in the FCT region: the RALPs RofA and Nra, and the regulator MsmR (Besson & Kalia, 2002). GrAS strains vary in the virulence and regulator gene composition of the FCT region as demonstrated in Figure 1.4 (Beres & Musser 2007). Strain SF370 encodes *rofA* for regulation and *cpa* for binding to collagen (Bessen & Kalia, 2002).

1.6.1.4 RofA

RofA (regulator of F) was first identified as a *trans*-acting positive regulator of *prtF1* (fibronectin-binding protein F) in a serotype M6 strain during anaerobic growth (Fogg, *et al.* 1994). The 1.5 kb ORF for *rofA* was found to reside directly upstream of *prtF1*, but to be divergently transcribed from the virulence gene (Fogg, *et al.* 1994). A subsequent survey of 62 isolates representing 61 serotypes found that 83% of tested strains contained *rofA* (Kreikemeyer, *et al.* 2002). Additional analysis of the M6 strain revealed that the *rofA* gene is subject to positive autoregulation, and RofA binds to two sites in the intergenic region containing both the *rofA* and *prtF1* promoters (Fogg & Caparon 1997; Granok, *et*

al. 2000). The larger RofA binding site also plays a role in RofA-independent activation of *rofA* and *prfF1* (Granok, *et al.* 2000).

Detailed analysis of the two RofA binding sites resulted in the characterization of a preliminary consensus sequence: TTTTCACCAAAAANCAT (Granok, *et al.* 2000). Examining the SF370 genome, two putative binding sites for RofA were identified. The first was adjacent to the gene for the collagen-binding protein *cpa.1* (*spy0125*) (Granok, *et al.* 2000). This was an obvious candidate as *cpa.1* resides immediately upstream of *rofA* (*spy0124*) in this strain, which represents the same relative position to *rofA* as the *prtF1* ORF in M6.

The second RofA binding site in SF370 was found in the *clpX* gene (*spy0885*) (Granok, *et al.* 2000). In Gram-positive organisms, *clpX* and *clpP* reside on unlinked loci and encode the ATP-binding and proteolytic subunit, respectively, of a stress-induced ATP-dependent protease (Gerth, *et al.* 1996). In *Bacillus subtilis*, the heat shock gene *clpP* is directly repressed by the stress regulator CtsR (Derre, *et al.* 1999). A consensus CtsR binding sequence was identified via *in silico* analysis that is upstream of the streptococcal *clpP* gene (*spy0395*) in SF370. The CtsR homolog of SF370 encoded by *ctsR* (*spy2074*) shares 45% amino acid identity to the CtsR protein of *B. subtilis* (Derre, *et al.* 1999). If RofA does regulate *clpX*, it would provide a link between virulence modulation and stress responses in group A streptococci.

As expected, an isogenic M6 *rofA* mutant that encodes *prtF1* exhibited reduced transcription of the protein F ORF (Beckert, *et al.* 2001). On the other

hand, *speA* (phage-encoded superantigen), *sagA* (streptolysin S), *emm6* (M6 protein) and *mga* were upregulated in comparison to wild-type M6 (Beckert, *et al.* 2001). These results indicate that *rofA* normally acts as a transcriptional repressor for these key virulence factors. The RofA consensus binding sequence is absent from the upstream regions of these genes, however, indicating that either RofA can bind alternative DNA sequences or RofA acts on the expression of these genes in an indirect fashion.

Examining the extracellular matrix-binding capabilities of the *rofA* mutant, Beckert *et al.* observed reduced binding to soluble fibronectin in the mutant when incubated in a CO_2-enriched environment. The difference in binding was most pronounced at stationary phase (Beckert, *et al.* 2001). The M6 *rofA* mutant also exhibited reduced attachment to Hep2 cells in comparison to wild-type streptococci. Host cell viability, however, was markedly reduced in the presence of the *rofA* mutant, which could result from the overproduction of the virulence factors SpeA and streptolysin S in the mutant (Beckert, *et al.* 2001).

An isogenic *rofA* mutant in the M2 serotype yielded similar results. Fibronectin-binding was greatly diminished at late exponential and stationary phases in the mutant despite the absence of genes for fibronectin- or collagen-binding proteins in the region immediately upstream of *rofA* (Kreikemeyer, *et al.* 2002). In contrast to wild-type M6 RofA, the wild-type M2 regulator positively affects *emm2* (M2 protein) expression. In addition, RofA also activates the capsule synthesis enzyme *hasB* and the ribosomal small subunit protein L12

rpsL in the M2 strain. Genes under negative RofA control included *mga*, *ska* (streptokinase), and *sagA* (streptolysin S). Expression of the superantigen *speA* was unaffected in the M2 strain (Kreikemeyer, *et al.* 2002).

Contrary to the M6 mutant, the M2 *rofA* mutant adhered to cultured Hep2 cells more readily than wild-type streptococci (Kreikemeyer, *et al.* 2002). Downregulation of the *hasB* gene in the *rofA* mutant could play a role in increased adherence as inactivation of the *has* operon in the M2 serotype was previously observed to boost streptococcal binding to keratinocytes (Darmstadt, *et al.* 2000). Overall, limited transcriptional studies of *rofA* mutants have revealed that RofA can both activate and repress gene expression in a strain-specific manner.

With the recent observation that *S. pyogenes* can elaborate pili, genetic linkage analysis was performed on FCT region genes and indicated that the *rofA* regulator may play a role in expression of the sortase gene *srtB* (Kratovac, *et al.* 2007; Mora, *et al.* 2005). RofA activation of pilus expression was directly tested by introducing the throat specialist M6 *rofA* gene into a skin-tropic M53 *nra* (*ralp2*) deletion mutant. Pilus synthesis was restored by RofA in the M53 mutant as measured by transcript level and protein generation (Lizano, *et al.* 2008).

1.6.1.5 Regulator of the ERES Region: RALP3

The ERES region of the streptococcal genome contains four genes: *eno, ralp3, epf* (also known as *lsa*), and *sagA* (Kreikemeyer, *et al.* 2007). Although

the virulence factors *eno* (enolase) and *sagA* (streptolysin S) are encoded by all GrAS serotypes, the presence of *epf/lsa* and the *ralp3* regulator is a serotype-specific trait. In 2007, *in silico* inspection of available GrAS genomes combined with Southern blot and site-specific PCR surveys of clinical isolates revealed that *ralp3/epf* is present in all tested isolates of serotypes M1, M4, M12, M28, and M49, but absent in M2, M3, M5, M6, and M18 serotype strains (Figure 1.10; Kreikemeyer, *et al.* 2007).

Encoded by the largest ORF in GrAS, Epf/LSA is a cell surface-anchored protein with plasminogen-binding activity and a predicted molecular mass of 224 kDa in SF370 (Kreikemeyer, *et al.* 2007). An isogenic *epf/lsa* mutant in an M1T1 strain exhibited decreased virulence in a murine intraperitoneal challenge model (Kwinn, *et al.* 2007). Considering that the four-gene region encodes three virulence factors surrounding a regulator gene, researchers have proposed that the ERES region is a pathogenicity island. Further evidence supporting this hypothesis stems from the presence of ORF remnants (*spy0732* and *spy0733*) in the center of the ERES region with homology to transposase genes (Kreikemeyer, *et al.* 2007).

The *ralp3* regulatory gene (*spy0735*) was originally identified by homology searches to RofA in the SF370 genome (Granok, *et al.* 2000). Sequence analysis during genome assembly classified *spy0735* as inactive in SF370 due to a frame shift mutation, so its regulatory function (if any) in this strain is not known (Ferretti, *et al.* 2001). Much like RofA, RALP3 regulates the gene (*epf/lsa*)

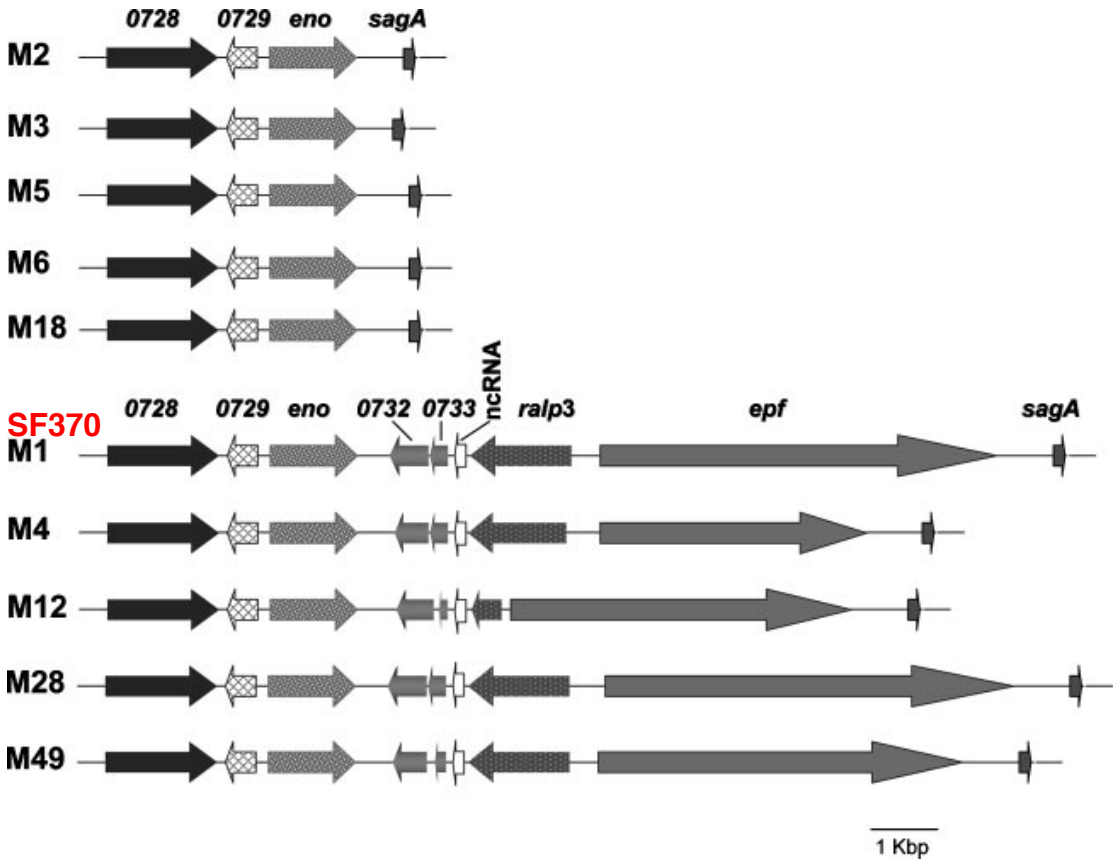


Figure 1.10 Presence and organization of ERES gene block in GrAS serotypes The data were extracted from genome sequences accessible from NCBI. The gene locus tags refer to strain SF370. ncRNA indicates a putative untranslated small RNA species.

Figure adapted from (Kreikemeyer 2007).

immediately downstream, but divergently transcribed from its own coding region (Kreikemeyer, *et al.* 2007). Expression of *ralp3* is highest during the transition phase of growth (Kreikemeyer, *et al.* 2007). Inactivation of *ralp3* in the invasive M1T1 clone and an M49 strain revealed the strain-specific nature of RALP3 regulation (Kreikemeyer, *et al.* 2007; Kwinn, *et al.* 2007).

In the M1T1 clone, RALP3 is a transcriptional repressor of virulence factors, including hyaluronic acid capsule (*has*), cysteine protease (*speB*), streptococcal inhibitor of complement (*sic*), and the global two-component regulator *covR*. The *ralp3* M1T1 mutant exhibited decreased virulence when injected intraperitoneally into mice (Kwinn, *et al* 2007). Although RALP3 represses *epf/lsa* in the M1T1 clone, the regulator activated *epf/lsa* expression in the M49 strain (Kwinn, *et al.* 2007; Kreikemeyer, *et al.* 2007).

1.6.1.6 RivR (Ralp4) and RivX

The gene *rivR* (RALP-IV, or RALP4) is a member of the RofA-like protein family of transcriptional regulators (Granok, *et al.* 2000). Experiments in M1 and M6 strains indicated that *rivR* (*spy0216*) is directly repressed by the two-component regulator CovR (Roberts, *et al.* 2007; Ferretti, *et al.* 2001). Repression is accomplished by direct binding of phosphorylated CovR to conserved regions downstream of the *rivR* promoter that inhibit elongation of the transcript by RNA polymerase (Roberts, *et al.* 2007).

Downstream of *rivR* lies a small non-coding regulatory RNA known as *rivX.* Transcriptome analysis of an isogenic mutant lacking *rivR* and *rivX* indicated that these elements have positive and negative regulatory effects (Roberts & Scott 2007). Both RivR and *rivX* were found to upregulate the Mga regulon but via different mechanisms. RivR directly enhanced transcriptional activation by Mga, while *rivX* had no direct interactions with the Mga protein. Instead, *rivX* may enhance the translation of Mga or affect *mga* expression indirectly through another regulator. In murine invasive skin infections, the mutant lacking *rivR/X* was attenuated for virulence (Roberts & Scott 2007).

RivR and *rivX* provide the first direct evidence that GrAS integrates environmental cues through a complex web of interconnected stand-alone transcriptional regulators and two-component systems. Although Mga can directly respond to environmental conditions, including extracellular iron and carbon dioxide levels, RivR and *rivX* provide a link to the CovR/S regulatory network. Under stress conditions, CovR is inactivated relieving the repression on *rivR/X,* thereby allowing RivR and the small RNA *rivX* to exert their positive influence on Mga and the Mga regulon (Roberts & Scott 2007).

1.6.1.7 Rgg/RopB

The *rgg* (also known as *ropB,* regulator of proteinase B) stand-alone regulator is responsible for growth phase-dependent transcriptional activation of the cysteine protease *speB* (Chaussee, *et al.* 1999; Lyon, *et al.* 1998). The gene

for *rgg/ropB* (*spy2042*) is close to, but divergently transcribed from *speB* (*spy2039*) (Ferretti, *et al.* 2001). Rgg/RopB is a 33 kDa protein with DNA-binding capabilities (Neely, *et al.* 2003; Anbalagan, *et al.* 2011). RopB shares similarities with phage-encoded regulators and the G+C content of *ropB* is only 32%, much lower than the genome average of 39%, indicating that the gene may have been acquired by horizontal gene transfer (Dmitriev, *et al.* 2006). In addition, RopB can bind to promoters and affect transcription of prophage genes in an M49 strain (Anbalagan, *et al.* 2011).

Transcriptome analysis of the *ropB* mutant in the M49 strain NZ131 demonstrated that RopB modulates the expression of >30% of the GrAS genome in all growth phases, with its greatest effect during stationary phase (Dmitriev, *et al.* 2006). In general, RopB represses gene expression during exponential growth and activates transcription in stationary phase (Dmitriev, *et al.* 2006). RopB is essential for downregulating genes responsive to oxidative and thermal stress, as well as genes involved in amino acid catabolism and alternative sugar utilization during growth in high glucose environments (reviewed in McIver 2009). RopB was not only important for virulence regulation, but also appeared to play a role in the induction of phage NZ131.1 in the M49 strain (Dmitriev, *et al.* 2006).

A subsequent study examined *ropB* mutants in M1 strains (including SF370) and a different M49 strain at stationary phase (Dmitriev, *et al.* 2008). Across all strains, the core RopB regulon was determined to include only *speB* and its co-transcribed neighboring gene *spy2040* (Dmitriev, *et al.* 2008). In

SF370, *ropB* inactivation had a minor effect on growth in Todd-Hewitt broth or chemically defined media with a slightly shorter lag period and different growth yield. Expression of 45 loci in SF370 was affected by *ropB* inactivation, with the most prominent effect on the activation of purine metabolism genes (*spy0025-spy0028*) (Dmitriev, *et al.* 2008).

Two uncharacterized stand-alone regulators in SF370 were affected by mutation in the *ropB* gene: *spy0627* (*regR*) and *spy1202* (*gntR*) (Dmitriev, *et al.* 2008). This is in sharp contrast to the large effect of *ropB* inactivation on other regulators (20 genes), including repression of *mga* and activation of *covR/S*, in the M49 NZ131 strain (Chaussee, *et al.* 2002; Dmitriev, *et al.* 2006). Overall, this study illustrated that RopB regulation varies among serotypes and even between strains within a single serotype. Furthermore, this regulatory variation was not due to sequence variation in the *ropB* ORF or to differing *ropB* transcript levels (Dmitriev, *et al.* 2008).

Examinations of clinical isolates from patients with streptococcal toxic shock syndrome (STSS) indicate that mutations in *ropB* are important for disease progression (Ikebe, *et al.* 2010). Inactivation of RopB resulted in increased lethality in murine models and precluded streptococcal killing by human neutrophils. This effect seems to rely on upregulation of virulence factors, including strreptolysin O, in the absence of RopB repression (Ikebe, *et al.* 2010).

SpeB Regulation by a Pheromone-like Circuit: Vfr

A novel locus *vfr* (virulence factor related; *spy0887*) unique to *S. pyogenes* has recently been found to negatively regulate *speB* transcription and relieve its growth phase dependency (Ma, *et al.* 2009). Transcription of *vfr* is highest during exponential growth when *speB* expression is low (Ma, *et al.* 2009; Shelburne III, *et al.* 2011b). Overexpression of this locus, however, dramatically reduces *speB* transcription even at stationary phase and leads to virulence attenuation in two mouse models of invasive disease (Shelburne III, *et al.* 2011b).

Vfr lacks common DNA-binding domains, but contains a putative amino-terminal secretion signal sequence, which is sufficient for Vfr repression of *speB* during exponential growth (Ma, *et al.* 2009; Shelburne III, *et al.* 2011b). Data support a model whereby the Vfr peptide is secreted into the extracellular space. The pheromone peptide can then be imported by streptococci to exert its regulatory function on *speB* expression (Shelburne III, *et al.* 2011b). The exact mechanism by which Vfr represses *speB* transcription is thought to involve Vfr peptide binding to RopB (Shelburne III, *et al.* 2011b).

Computer modeling of RopB determined that RopB has structural homology to Gram-positive quorum-sensing regulators, which use secreted peptides as signals and alter gene expression in response to bacterial cell density (Shelburne III, *et al.* 2011b). Although RopB directly binds to the *speB* promoter and activates transcription, this regulator is not responsible for growth phase-dependent SpeB expression (Neely, *et al.* 2003). Thus, growth phase-

dependent repression of *speB* expression likely stems from binding of the Vfr peptide to RopB, altering the regulator's ability to interact with the *speB* promoter (Shelburne III, *et al.* 2011b).

1.6.1.8 Regulators of Carbon Catabolite Repression

Carbon catabolite repression is a global regulatory strategy employed by bacteria to conserve energy normally spent on alternate sugar catabolism pathways when glucose is readily available (Titgemeyer & Hillen 2002). Recent studies (discussed below) have highlighted that group A streptococci also use CCR regulators to modulate expression of virulence factors in response to changing nutrient conditions, particularly low glucose levels. In GrAS, *ccpA*, *lacD.1,* and *malR* are the best-characterized CCR regulators.

1.6.1.9 CcpA

The regulator CcpA (catabolite control protein A; *spy0514*) predominantly represses metabolic pathways and virulence factors in group A streptococci during growth in nutrient-rich environments (Shelburne III, *et al.* 2008). First studied in *Bacillus subtilis*, CcpA binds directly to conserved *cre* sequences (TGWAANCGNTNWCA) in the promoters of target genes (Moreno, *et al.* 2001). During active glucose transport into the bacterial cell, the phosphocarrier protein HPr (*spy1373*) is phosphorylated enhancing CcpA promoter binding and gene repression (reviewed in Deutscher, *et al.* 2006).

Under nutrient-limited conditions as would be found in human saliva and the oropharynx, HPr is dephosphorylated causing disassociation of the HPr-CcpA-*cre* complex. With CcpA repression relieved, alternative carbon utilization pathways are activated along with expression of virulence factors important for infection, such as the extracellular DNase *spd*, *cfa* (CAMP factor), and *sagA* (streptolysin S) (Shelburne III, *et al.* 2008; Kietzman & Caparon 2010). In addition, CcpA positively regulates the cysteine protease *speB* in human saliva (Shelburne III, *et al.* 2008). Thus, CcpA provides a direct link between nutrient availability in the extracellular environment and virulence regulation in GrAS.

Contradictory observations were noted from isogenic *ccpA* mutants in the same M1 serotype background (strain MGAS5005). Although one group found that the mutant was less virulent when injected intraperitoneally into mice (Shelburne III, *et al.* 2008), another team discovered that the mutant was hypervirulent in the same murine invasive infection model (Kinkel & McIver 2008). The latter group subsequently linked increased streptolysin S expression in the *ccpA* mutant to the hypervirulent phenotype. It has been proposed that the attenuation phenotype observed previously may have resulted from subtle differences in execution of the i.p. infection assays (Kinkel & McIver 2008).

Transcriptome analyses of the two isogenic *ccpA* mutants grown in Todd-Hewitt broth indicated differing levels of CcpA gene regulation. Shelburne III, *et al.* determined that CcpA affected expression of 20% of all ORFs in early-exponential phase growth and 10% of ORFs in late-exponential phase

(Shelburne III, *et al.* 2008). Only 6% of the ORFs were found to be regulated in the *ccpA* mutant during exponential growth by Kinkel and McIver (2008). As expected from its role in *B. subtilis*, the largest number of transcripts repressed by CcpA in both data sets were involved in the metabolism and transport of carbohydrates, including lactose, fructose, mannose, and maltose. Virulence factor repression by CcpA, including the genes *spd* and *sagA,* was noted by both groups (Shelburne III, *et al.* 2008; Kinkel & McIver 2008).

A bioinformatics survey of the SF370 genome indicated that *cre* sites are present in the promoter or coding regions of 98 ORFs, including *mga*, *speB*, and *sagA* (Almengor, *et al.* 2007). Although *mga* was not affected by CcpA regulation in the two M1 studies discussed above, CcpA can directly bind the *cre* site upstream of *mga* in an M6 strain. An isogenic *ccpA* mutant in the M6 background exhibited reduced *mga* expression during exponential growth in Todd-Hewitt broth (Almengor, *et al.* 2007). Thus, in the M6 strain, CcpA is required for maximal expression of Mga and provides an additional link between carbohydrate levels and virulence regulation.

1.6.1.10 LacD.1

The gene *lacD.1* (*spy1704*) encodes a class I tagatose-1,6-bisphosphate aldolase that resembles enzymes involved in galactose metabolism in other Gram-positive bacteria (Loughman & Caparon 2006). At the amino acid level, LacD.1 is 73% identical to another GrAS aldolase LacD.2 (*spy1919*), which is

present in a secondary, functional Lac locus. Although they are highly homologous, only LacD.1 exhibits regulatory activity, while LacD.2 is solely responsible for growth on galactose (Loughman & Caparon 2007). The regulatory function of LacD.1 is independent of aldolase activity but dependent on substrate binding (Loughman & Caparon 2006).

Unlike CcpA, LacD.1 negatively regulates *speB* expression (Loughman & Caparon 2006). Regulation by CcpA and LacD.1 are mediated through independent pathways (Loughman & Caparon 2006; Kietzman & Caparon 2010). In addition, LacD.1 regulates a subset of genes, including the virulence factors *slo* (streptolysin O) and *sagH* (streptolysin S biogenesis), in response to changes in pH and NaCl (Loughman & Caparon 2006).

Researchers have determined that the presence of glucose alters the expression of up to 15% of the streptococcal genome in an M5 strain. Nearly 60% of the transcriptional response to glucose could be attributed to the combined regulatory efforts of LacD.1 and CcpA (Kietzman & Caparon 2011). Most of the genes regulated were involved in metabolic processes and experienced repression. A subset of genes was regulated by both CcpA and LacD.1, but the regulators acted in an antagonistic fashion. Furthermore, the regulators act at different points during mouse soft tissue infection. CcpA regulation corresponded to maximum lesion size, while LacD.1 influenced gene expression early and late in infection (Kietzman & Caparon 2011).

1.6.1.11 MalR

Maltodextrins (carbohydrates with 2 to 7 glucose monomers) are present in high quantities in the human oropharynx where they can serve as key nutrients for GrAS (Kaczmarek & Rosenmund 1977; Mormann & Muhlemann 1981; Scannapieco, *et al.* 1993). Genes involved in maltodextrin transport and utilization are upregulated during GrAS incubation in human saliva. Two maltodextrin transport genes *malE* (*spy1294*) and *malT* (*spy1986*) are expressed in human pharyngitis and have been shown to play a role in persistence in human saliva and colonization of the murine oropharynx (Shelburne III, *et al.* 2006; Shelburne III, *et al.* 2007).

The *malR* regulator is highly conserved in group A streptococci and has been identified in 35 strains representing 18 serotypes (Shelburne III, *et al.* 2007). The *malR* gene (*spy1293*) is encoded within the maltodextrin transport and metabolism gene region (*spy1291-1296*). MalR binds to highly conserved *cre* sites and negatively regulates the maltodextrin genes, as well as the pullulanase gene region (*spy1972-1976*) involved in the production of maltodextrin from polysaccharides (Shelburne III, *et al.* 2007; Shelburne III, *et al.* 2008b). Notably, CcpA also binds to these *cre* sites, but results in maltodextrin gene activation (Shelburne III, *et al.* 2008b).

Expression of *malR* increases during streptococcal incubation in pooled adult human saliva, and MalR plays a key role in bacterial persistence in saliva and the murine oropharynx (Shelburne III, *et al.* 2007; Shelburne III, *et al.* 2011).

The MalR regulon was explored in an M1 strain yielding the observation that the regulator modulates expression of only 25 genes during the mid-exponential phase of growth (Shelburne III, *et al.* 2011). This stands in stark contrast to CcpA, which is estimated to affect hundreds of genes (Kinkel & McIver 2008; Shelburne III, *et al.* 2008). The majority of affected genes were repressed by MalR and functioned in polysaccharide transport and metabolism. Notably, MalR also repressed a number of phage genes (Shelburne III, *et al.* 2011).

1.6.1.12 Regulators of Responses to Amino Acid Starvation: RelA and CodY

Group A streptococci rely on the host environment for the acquisition of amino acids to fuel growth. Although host tissues, including human blood, are rich in protein, these niches tend to be deprived of free amino acids (Steiner & Malke 2000). The stand-alone regulator *relA* (*spy1981*) controls the stringent response by which bacteria can greatly diminish RNA synthesis when amino acids become scarce (Steiner & Malke 2000). Examination of gene expression in an M49 strain during amino acid starvation indicated that RelA represses metabolic enzymes, transporter proteins, and the virulence factors *grab* and *speH* (superantigen). RelA was found to activate *ska* (streptokinase) under these conditions (Malke, *et al.* 2006).

CodY (*spy1777*) is a highly conserved *trans*-acting regulator in Gram-positive pathogens with low G+C genome content (Guedon, *et al.* 2001; Ratnayake-Lecamwasam, *et al.* 2001). When branched-chain amino acids are

available, CodY generally represses gene expression, but the regulator has also been found to activate transcription of certain genes. Like RelA, CodY regulates metabolic enzymes, transporter proteins, and virulence factors in response to amino acid starvation (Malke, *et al.* 2006).

Microarray analysis of a *codY* deficient mutant revealed that CodY affects expression of about 17% of genes in an M49 strain during exponential growth in laboratory media (Kreth, *et al.* 2011). CodY plays an important role in integrating regulatory networks in response to nutrient status by repressing the two-component systems *covR/S* (*spy0336/0337*) and *sptR/S* (*spy0874/0875*) and activating *mga, rofA, rivR* and *fasX* in human blood and/or laboratory media using the same M49 strain (Malke & Ferretti 2007; Kreth, *et al.* 2011). It was proposed that CodY regulation may fine-tune the transition from a colonization or invasive phenotype to a GrAS persistence state within the host (Kreth, *et al.* 2011).

Genes in the Mga regulon, including *emm49* (M protein), *sclA* (collagen-like protein), and *scpA* (c5a peptidase), were all upregulated in human blood (Malke & Ferretti 2007). Furthermore, *ska* (streptokinase) and *ides* (IgG-degrading enzyme) were activated as one might expect when their direct repressor CovR is itself downregulated by CodY. Strong repression by CodY in blood was also observed for *grab* and *braB* (branched-chain amino acid transporter; *spy0323*) (Malke & Ferretti 2007). The action of CodY on other regulators in human blood was further evidenced by expression changes in their downstream virulence factors (Malke & Ferretti 2007).

Interestingly, while CodY and RelA both autoregulate, neither regulator affects the expression of the other (Malke, *et al.* 2006). In addition, the regulons of CodY and RelA in response to amino acid starvation do not overlap (Malke, *et al.* 2006).

1.6.1.13 Regulators of Metal Homeostasis: PerR and MtsR

Iron homeostasis is crucial for bacterial survival. Although iron is important for diverse metabolic functions, excess intracellular iron can lead to the generation of oxygen radicals by the Fenton reaction. Thus, metal-dependent transcriptional regulators repress iron transporters in the presence of the metal. In pathogenic bacteria, such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*, regulators of iron acquisition and oxidative stress often control virulence factor expression as well (Barton, *et al.* 1996; Ando, *et al.* 2003). The metal-dependent regulators PerR (*spy1531*) and MtsR (*spy0450*) have both been implicated in maintaining the delicate balance of iron levels in *S. pyogenes.*

Group A streptococci encode three ATP-binding cassette type (ABC) transporters for nutrient metal ions. MtsABC (*spy0453-0456*), FtsABCD (*spy0383-0386*), and HtsABC (*spy1787-1796*) are important for uptake of iron and manganese, ferric ferrichrome, and heme, respectively (Hanks, *et al.* 2005; Janulczyk, *et al.* 2003; Bates, *et al.* 2003). Inactivation of these transporters leads to virulence attenuation highlighting the importance of metal uptake for

streptococci in the host environment (Janulczyk, *et al.* 2003; Montanez, *et al.* 2005; Fisher, *et al.* 2008).

The *mtsR* gene is divergently transcribed from the adjacent *mtsABC* locus (Bates, *et al.* 2005). MtsR modulates the expression of *mtsABC* and *htsABC* in response to both iron and manganese, but does not affect expression of *ftsABCD* (Hanks, *et al.* 2006). The regulation by MtsR is strain-specific, however, as the regulator does not affect *mtsABC* expression in an M49 strain (Toukoki, *et al.* 2010). On the other hand, PerR is not directly involved in the regulation of these transport systems in response to iron, manganese, or zinc (Hanks, *et al.* 2006).

The MtsR regulon was recently examined in an M49 strain revealing that the regulator modulates expression of 64 genes during growth in iron-rich media (Toukoki, *et al.* 2010). The majority of these genes had no assigned function. MtsR-repressed genes included a wider spectrum of functions, such as protein uptake, amino acid biosynthesis, and the virulence factor *ska* (streptokinase). MtsR activated transcription of genes important for nucleotide metabolism, transport, cell envelope, and regulation. Notably, MtsR was shown to directly upregulate both *mga* and *emm* in M1, M6, and M49 strains linking extracellular metal availability to virulence regulation (Toukoki, *et al.* 2010). High manganese levels in saliva may prompt MtsR activation of *emm* during early stages of pharyngitis (Chicarro, *et al.* 1998; Toukoki, *et al.* 2010).

The effect of *mtsR* mutations on virulence has not been clear. In a zebrafish model of intramuscular infection, an isogenic M49 *mtsR* mutant

exhibited reduced virulence (Bates, *et al.* 2005). A subsequent study with an M1 *mtsR* mutant, however, failed to identify any difference in virulence from the wild-type in a mouse subcutaneous infection model (Hanks, *et al.* 2006). More recently, an M3 isolate associated with necrotizing fasciitis was discovered to harbor a point mutation in the *mtsR* coding region (Olsen, *et al.* 2010). Further analysis indicated that attenuation in mouse and primate models resulted when MtsR inactivation led to decreased SpeB activity through a secondary regulatory network (Olsen, *et al.* 2010).

The *perR* regulator was first identified by its role in managing oxidative stress and metal homeostasis (King, *et al.* 2000; Ricci, *et al.* 2002). Although early transcriptome analysis in an M5 strain revealed a limited PerR regulon of only 6 genes during mid-exponential growth in Todd-Hewitt broth, a subsequent study in an M3 strain determined that PerR regulates 42 genes mostly during late-exponential growth in the same media (Brenot, *et al.* 2007; Gryllos, *et al.* 2008). PerR was later found to reach maximal expression during early stationary phase in an M1 strain when its regulatory effects would be most pronounced (Wen, *et al.* 2011). Some strain-specific variation in PerR regulation may also play a role in the discordant findings (Gryllos, *et al.* 2008).

Proteomic and transcriptional analyses have determined that PerR both activates and represses gene expression (Gryllos, *et al.* 2008; Wen, *et al.* 2011; Grifantini, *et al.* 2011). Examining the PerR regulon during peroxide stress, researchers found that PerR represses genes involved in DNA metabolism, metal

homeostasis, and amino acid transport late in the exponential growth phase (Grifantini, *et al.* 2011). The decrease in these pathways may allow for DNA and protein repair before normal cellular functions are restored (Gryllos, *et al.* 2008).

Independent studies of streptococci grown without oxidative stress revealed that PerR regulation is quite different in this environment and point to a role for PerR in activating carbohydrate metabolic pathways (Gryllos, *et al.* 2008; Wen, *et al.* 2011; Grifantini, *et al.* 2011). Utilization of alternate sugars may be important for colonization of the oropharynx and help the bacteria meet energy demands associated with oxidative stress (Gryllos, *et al.* 2008). Interestingly, phage encoded secreted DNases, including *mf3* in an M1 strain and *mf4* in an M3 isolate, that may play a role in virulence also appear to be activated by PerR during growth *in vitro* (Gryllos, *et al.* 2008; Wen, *et al.* 2011).

Isogenic mutants of *perR* in M1 and M5 strains exhibited reduced virulence following subcutaneous and intraperitoneal inoculation in mice (King, *et al.* 2000; Ricci, *et al.* 2002; Brenot, *et al.* 2005). An isogenic mutant in an M3 strain revealed that PerR plays a role in resistance to phagocytic killing by macrophages *in vitro* and survival in human blood. PerR was also important for pharyngeal colonization by GrAS in a baboon model (Gryllos, *et al.* 2008).

1.6.1.14 Additional regulators – LuxS, Srv, RocA, AmrA, HrcA, SigX

LuxS

Quorum-sensing (QS), by which bacteria detect their cell density and adjust their gene expression accordingly, has been found to rely on the secretion and sensing of small peptides in Gram-positive bacteria (reviewed in Miller & Bassler 2001). The autoinducer II pathway (AI-2) dependent on the gene *luxS* (*spy1642*) is shared by diverse bacterial species (Bassler, *et al.* 1997; Surrette, *et al.* 1999). Considering the universality of this QS pathway, some researchers have postulated that LuxS is used for general metabolic regulation with virulence modulation occurring as a side effect (Kreikemeyer, *et al.* 2003).

Inactivation of *luxS* in an M6 strain resulted in decreased SpeB activity, but increased *sagA* transcription and streptolysin S production by the mutant (Lyon, *et al.* 2001). In an M3 invasive strain, *luxS* inactivation led to reduced transcription of *speB*, but induction of *emm3* expression. In addition, the mutant could be recovered from HEp-2 epithelial cells at higher numbers than wild-type (Marouni & Sela 2003). A broader analysis in 2008 revealed that repression of *luxS* is important for cellular responses to acidic conditions, and that *luxS* inactivation leads to increased survival within HEp-2 cells and murine macrophages (Siller, *et al.* 2008).

Srv

The *srv* gene (streptococcal regulator of virulence; *spy1857*) was first identified as a homolog to the virulence regulator PrfA in *Listeria monocytogenes* (Reid, *et al.* 2001). The gene is highly conserved in GrAS strains that are geographically and phylogenetically diverse (Reid, *et al.* 2001). Srv has a DNA-binding motif, and the gene is maximally expressed *in vitro* during early growth and late exponential phase. Inactivation of *srv* leads to reduced virulence when inoculated intraperitoneally into mice (Reid, *et al.* 2004).

Transcriptome analysis in an M1 strain revealed that Srv directly or indirectly activates over 300 genes and represses only 4 genes during exponential growth in Todd-Hewitt broth (Reid, *et al.* 2006). Genes activated by Srv included those involved in the metabolism, transport, and/or catabolism of nutrients, as well as genes involved in translation and posttranslational control (Reid, *et al.* 2006). In addition, Srv induced expression of 21 transcriptional regulators, such as *covRS*, *rofA,* and *mga*, and 18 virulence factors, including *speG, cfa, mf3, and scpA*. The production of many secreted proteins was also affected by Srv with extracellular Sic levels dramatically reduced when *srv* was inactivated (Reid, *et al.* 2006).

RocA

The *rocA* regulator (regulation of *covR*; *spy1605*) was first identified by its ability to repress capsule synthesis via CovR expression (Biswas & Scott 2003).

The *covRS* two-component system (detailed below) is integral for virulence regulation and directly represses the capsule synthesis operon in *S. pyogenes* (Federle, *et al.* 1999). RocA activates transcription from the *covRS* promoter and represses its own expression. The *rocA* gene appears to be unique to *S. pyogenes* (Biswas & Scott 2003). The greater regulon of RocA has not been explored.

AmrA

The integral membrane protein encoded by *amrA* (activation of Mga regulon; *spy0797*) shares similarity to transporters involved in cell wall biosynthesis. The protein was found to be required for maximal activation of the Mga regulon in an M6 strain (Ribardo & McIver 2003). It is unclear how AmrA functions to upregulate the Mga regulon, but the protein may interact with Mga directly or provide activation signals via protein intermediates. Either way, AmrA provides a link between growth phase monitoring and virulence regulation by Mga (Ribardo & McIver 2003).

HrcA

The *hrcA* regulator (*spy1763*) represses expression of the streptococcal heat shock operons; *dnaK* (*spy1759-1761*) and *groEL* (*spy2070-2072*) (Woodbury & Haldenwang 2003). Following heat shock, HrcA repression is relieved increasing transcription and translation of the molecular chaperones

within 5 minutes of temperature alteration. Increased *groEL* and *dnaK* transcripts and protein were detectable for 30 minutes following heat shock (Laport, *et al.* 2001; Woodbury & Haldenwang 2003). Antibodies against GroEL and DnaK have been identified in patients with streptococcal infections, but their role in pathogenesis remains unexplored (Lemos, *et al.* 1998; Lemos, *et al.* 2000).

SigX (Secondary Sigma Factor)

Streptococcus pyogenes encodes two identical copies of the secondary sigma factor σ^X: *sigX1* (*spy0300*) and *sigX2* (*spy1902*) (Opdyke, *et al.* 2001). The proteins encoded by these ORFs are 40% identical at the amino acid level to the *Streptococcus pneumoniae* RNA polymerase sigma factor ComX, which is essential for competence and possibly virulence (Campbell, *et al.* 1998; Lee & Morrison, 1999; Opdyke, *et al.* 2001). Although competence has not been observed in *Streptococcus pyogenes*, SigX in an M6 strain can induce transcription from genes encoding DNA uptake and recombination machinery important for competence (Woodbury, *et al.* 2006).

Studies have yet to reveal a role for SigX in virulence, stress tolerance, or biofilm formation in GrAS (Woodbury, *et al.* 2006). When examined *in vitro*, SigX activates transcription of *cinA* (competence-inducible protein; SF370 *spy2117*) and *femB* (peptidoglycan crosslinking; SF370 *spy1205*). Both ORFs include a sequence in the -10 region in their promoters referred to as a cin-box (TACGAATA), which is also required for transcription by *S. pneumoniae* ComX *in*

vivo (Lee & Morrison, 1999; Opdyke, *et al.* 2001). A total of 15 ORFs in SF370 contain a cin-box in their promoters (Woodbury, *et al.* 2006). Using an M6 strain, SigX activity was found to be limited *in vivo* at two levels: transcription from the *sigX* ORFs was low during growth in Todd-Hewitt broth and the protease ClpP inhibited intracellular accumulation of SigX (Opdyke, *et al.* 2003).

1.6.2 Two-Component Systems

Working in conjunction with stand-alone regulators, two-component systems (TCS) offer GrAS the ability to integrate extracellular signals directly into regulatory circuits (reviewed in Kreikemeyer, *et al.* 2003). A sensor histidine kinase sits in the cell membrane, awaiting its appropriate signal via its extracellular domain. Upon binding of the extracellular signal, these kinases often dimerize and become autophosphorylated at conserved histidine residues in their cytoplasmic transducer domains. The phosphorylated transducers can then transfer the phosphoryl group to an aspartyl residue in the cytoplasmic response regulator, altering its affinity for DNA targets (reviewed in Koch 2000).

The phosphorylation of response regulators in GrAS can lead to activation or repression of downstream genes, including virulence factors (Kreikemeyer, *et al.* 2003). The sequenced genomes of *S. pyogenes* have been found to encode 13 two-component systems (Table 1.7). Regarding virulence regulation, the most studied systems in GrAS are *covRS, sptRS, trxRS,* and *fasABCX.*

Table 1.7 Two-component Systems of SF370

Locus Tags	Gene Names	Regulatory Targets and/or Role in Virulence
spy0242-0245	*fasB-fasC-fasA*	Repression of *fbp54* (fibronectin-binding adhesin); Activation of *ska* (streptokinase) and *sagA* (streptolysin S)
spy0336-0337	*covR-covS*	Repression of *has* (capsule), *sag* (streptolysin S), *ska* (streptokinase), *speB, grab, spd* (DNase); Repression of Mga regulon (via *rivR* and *trxSR* repression)
spy0528-0529	*vicRK/sycFG*	Only TCS (response regulator) essential for GrAS growth; Possible role in nutrient uptake and osmotic stress response
spy0874-0875	*sptR-sptS*	Important for persistence in human saliva; Activation of *spd*, *speB, sic, has,* and complex carbohydrate utilization pathways
spy1061-1062		Activation of mannose/fructose-PTS system
spy1081-1082	*srtR-srtK*	Not yet examined
spy1106-1107		Activation of malate metabolism
spy1236-1237	*ciaH-ciaR*	Activation of metabolic genes; Little effect on virulence regulation
spy1553-1556		Responsible for large transcriptional shift (~475 genes) upon entering stationary phase
spy1587-1588	*trxR-trxS*	Activation of Mga regulon (via *mga* activation)
spy1621-1622		Response to acid stimuli; Resistance to neutrophil killing
spy1908-1909	*salR-salS*	Regulation of the lantibiotic salivaricin; SF370 lacks key genes for salivaricin peptide modifications and export
spy2026-2027	*ihk-irr*	Evasion of neutrophil killing; Activation of genes important for redox balance, cell wall biosynthesis, and antimicrobial peptide resistance

1.6.2.1 CovRS

The CovRS two-component system was first described as a repressor of capsule (*hasABC*) gene expression in serotypes M1, M2, M3, M6, and M49 (Levin & Wessels 1998; Bernish & van de Rijn 1999; Wessels 1999). Subsequent studies revealed that CovRS can directly or indirectly affect the expression of 15% of the GrAS genome late in growth (Graham, *et al.* 2002; Dalton, *et al.* 2006). In addition to the *has* operon, the DNA-binding response regulator CovR can directly repress expression of *sagA* (streptolysin S), *ska* (streptokinase), and *speB* (cysteine protease) (Federle, *et al.* 1999; Heath, *et al.* 1999; Graham, *et al.* 2002). CovR directly represses its own transcription, as well as that of the two-component system *trxRS* and stand-alone virulence regulator *rivR*—both activators of the Mga regulon (Roberts, *et al.* 2007; Leday, *et al.* 2008).

CovR predominantly represses the genes under its direct control (Bernish & van de Rijn 1999; Federle, *et al.* 1999; Miller, *et al.* 2001; Federle & Scott 2002; Gao, *et al.* 2005; Gusa & Scott 2005; Gusa, *et al.* 2006; Roberts, *et al.* 2007). CovR binds to an ATTARA motif in the promoter of target genes blocking transcription. The number of ATTARA recognition sequences in the GrAS genome is at least double the number of available CovR molecules in streptococci indicating that CovR must be able to bind preferentially at specific loci (Federle & Scott 2002; Churchward 2007). Two upstream activators of CovR

repression are the stand-alone regulators RocA and Srv (Reid, *et al.* 2006; Biswas & Scott 2007).

As previously mentioned, *covRS* is repressed by the stand-alone regulator CodY (Malke & Ferretti 2007). In addition, CodY and CovR share numerous gene targets for direct or indirect regulation, including the *has* operon (capsule) and *ska* (streptokinase) (Malke, *et al.* 2006; Malke & Ferretti 2007). Microarray analyses of *codY* and *covRS* mutants in an M49 strain indicated that the two regulators act in opposition. While CovR represses gene expression, CodY serves as a transcriptional activator for shared targets (Kreth, *et al.* 2011).

Although the sensor kinase CovS is believed to be the primary activator of CovR via phosphorylation (particularly in the presence of extracellular Mg^{2+}), *covS* mutants have demonstrated that CovS is not essential for CovR-mediated repression of the *has* and *sag* operons (Dalton & Scott 2004; Churchward 2007; Gryllos, *et al.* 2007). CovS can also act as a phosphatase under stress conditions, dephosphorylating CovR to relieve CovR virulence repression. Stressors that stimulate CovS phosphatase activity include low iron concentrations, the host antimicrobial peptide LL-37, acidic pH, and an elevated growth temperature of 39°C—all conditions that the pathogen could face *in vivo* (Dalton & Scott 2004; Dalton, *et al.* 2006; Froehlich, *et al.* 2009).

Inactivation of *covR* or *covS* can significantly affect the pathogen's virulence in mouse models of infection (Gryllos, *et al.* 2001; Engleberg, *et al.* 2004; Dalton, *et al.* 2006b; Graham, *et al.* 2006). Transcriptional analyses

revealed that expression of *covRS* in murine soft tissue infectious is repressed allowing the production of virulence factors (Graham, *et al.* 2006). Recent studies indicate that *in vivo* spontaneous mutations inactivate *covS* and act as a switch to a more virulent invasive phenotype (Engleberg, *et al.* 2001; Sumby, *et al.* 2006; Walker, *et al.* 2007; Garcia, *et al.* 2010).

During *ex vivo* growth, *covRS* expression rapidly increased in the first 30 minutes after introduction into whole blood from Todd-Hewitt broth, but returned to pre-existing levels by 60 minutes (Graham, *et al.* 2005). Derepression of the *covRS* loci was quickly followed by derepression of known CovR gene targets, including *sagA* and *ska*. Overall, inactivation of *covR* during growth in blood resulted in altered expression of 56% of GrAS genes when compared to wild-type, indicating that CovR plays an important role in metabolic adaptation of the pathogen to human blood (Graham, *et al.* 2005).

In experimental pharyngitis infections in macaques, the production of CovRS was found to vary during the course of colonization and infection. The highest *covRS* transcriptional rates correlated to a dramatic reduction in expression of the Mga regulon (Virtaneva, *et al.* 2005). CovRS is also important for biofilm formation (Cho & Caparon 2005).

1.6.2.2 TrxRS

The TrxRS two-component system shares homology with the *hk07-rr07* TCS essential for full virulence in *S. pneumoniae* (Hava & Camilli 2002; Throup,

et al. 2000). Inactivation of *trxR* in an M1 serotype resulted in attenuated virulence using a murine soft tissue infection model (Leday, *et al.* 2008). Transcriptome analyses performed on the *trxR* mutant grown to late exponential phase in Todd-Hewitt broth indicated that TrxR regulates expression of a small subset of genes (~29 ORFs). TrxR was found to both activate and repress gene expression, but TrxR does not autoregulate (Leday, *et al.* 2008).

Notably, TrxR positively regulates the Mga regulon through *mga* activation, and represses genes involved in general metabolism and stress responses (Leday, *et al.* 2008). Activation of the Mga regulon by TrxR was not observed in SF370, however, indicating that this action of TrxR on virulence regulation may be strain-specific even within a serotype. TrxRS is repressed by CovRS in two different serotypes of GrAS (Dalton, *et al.* 2006; Graham, *et al.* 2002; Leday, *et al.* 2008). Figure 1.11 illustrates the interplay of TrxRS, Mga, CovRS, and RivR on virulence factor expression.

1.6.2.3 SptRS

Expression of *sptRS* was first examined in a microarray screen of GrAS gene expression during growth in pooled adult human saliva. The *sptRS* genes had the highest transcripts following 16 hours of saliva incubation and the greatest increase in transcripts between 4 hours and 16 hours (Shelburne III, *et al.* 2005). Furthermore, *sptR* was found to be expressed 3-fold higher than a

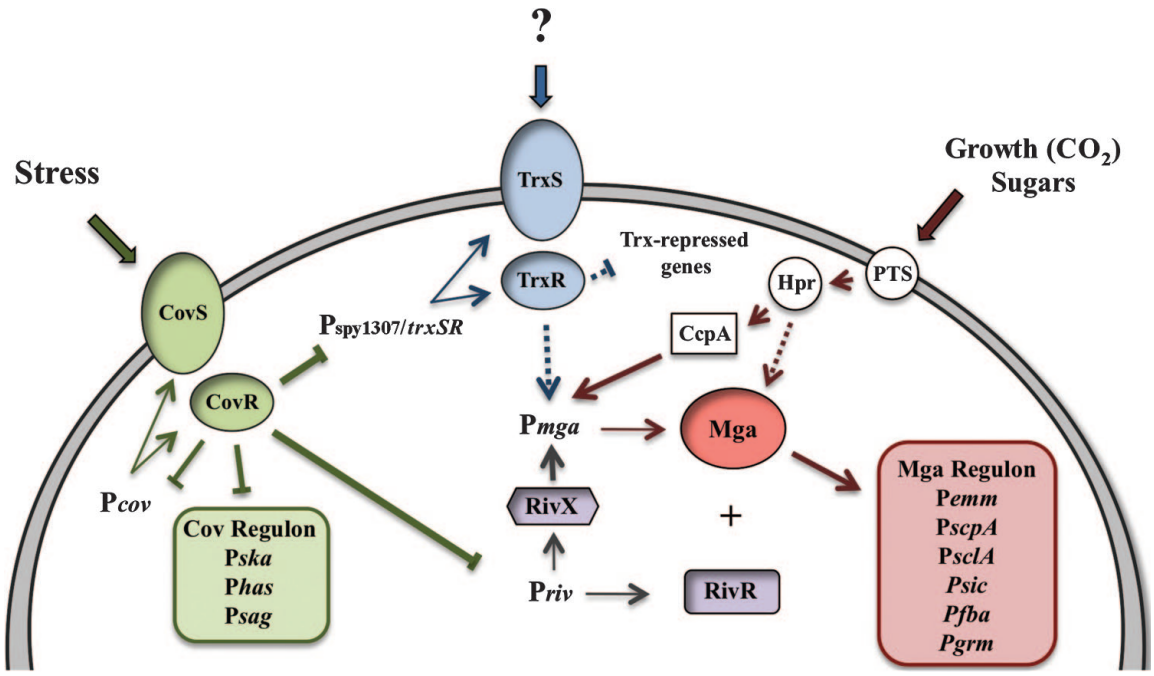


Figure 1.11 Model for interacting virulence regulation in GrAS

Schematic representation of components for the TCSs CovRS (green) and TrxRS (blue), as well as stand-alone regulators Mga (red) and RivR/RivX (purple), are shown within the context of a GrAS cell. Thin arrows show production of gene products from the indicated promoter. Thick arrowheads indicate activation, and thick flat ends reflect repression by the connected regulator. Solid lines indicate direct regulation, while dashed lines indicate either indirect or unknown regulation. Known or predicated external signals for the regulatory pathways are shown.

Figured reprinted from (Leday, *et al.* 2008).

control gene in streptococci isolated from patients with active pharyngitis (Shelburne III, *et al.* 2005).

An isogenic *sptR* mutant in an M1 serotype exhibited reduced viability in saliva, indicating that SptR is important for persistence in human saliva. Culturing the *sptR* mutant in saliva for 16 hours revealed that SptR regulates 20% of GrAS ORFs in this setting. SptR activates transcription of *spd* (DNase)*, spd3* (DNase)*, sic, speB,* and *hasA* (capsule) (Shelburne III, *et al.* 2008). These genes have been associated with colonization and infection of the oropharynx (Ashbaugh, *et al.* 2000; Lukomski, *et al.* 2000; Sumby, *et al.* 2005b). In particular, SpeB has been recognized as a key effector for successful persistence in human saliva (14,24,30, Shelburne III, *et al.* 2005b). SptR is also an important activator of complex carbohydrate utilization pathways that are necessary for persistence in the nutrient-limited environment of saliva (Shelburne III, *et al.* 2005).

1.6.2.4 Ihk/Irr

The *ihk/irr* two-component system was found to be upregulated during GrAS interactions with neutrophils and in isolates from pharyngitis patients (Voyich, *et al.* 2003). Inactivation of *irr* in an M6 strain resulted in attenuated virulence in murine models of soft tissue infection and bacteremia. Irr appears to be most important for evasion of innate defenses, including neutrophil-derived

reactive oxygen species and antibacterial peptides, such as LL-37 (Voyich, *et al.* 2004).

Examining the transcriptome during early and late exponential growth in Todd-Hewitt broth, Irr was found to regulate 20% of the GrAS genome (Voyich, *et al.* 2004). Irr upregulates the virulence factors *sagA* (streptolysin S)*, fbp* (fibronectin-binding protein)*,* and the DNases *mf* and *mf3*. Irr is also responsible for activating a number of other regultators, including *rocA* (activator of CovRS) and *codY.* Furthermore, Ihk/Irr regulate expression of genes important for cell redox status modulation and cell wall biosynthesis (Voyich, *et al.* 2004).

1.6.2.5 CiaHR

The *ciaHR* system is maximally expressed during the transition phase of growth in Todd-Hewitt broth (Riani, *et al.* 2007). Transcriptome analysis of an isogenic *ciaH* mutant in an M49 serotype revealed a regulon of ~65 genes, 40% of which encoded hypothetical and phage proteins (Riani, *et al.* 2007). Genes activated by CiaH encode ribosomal proteins and sugar transport systems. Downregulated genes functioned in ion transport and stress responses. The only virulence factors found to be regulated by CiaH were a hemolysin (repressed) and a DNase (activated) (Riani, *et al.* 2007).

1.6.2.6 Additional TCS

The *fas* operon encodes two sensor kinases (FasB and FasC) and one response regulator (FasA) (Kreikemeyer, *et al*. 2001). The main effector of the FasBCA system is a small, non-coding RNA *fasX*. The activity of FasBCA is growth-phase dependent and includes downregulation of *fbp54* (fibronectin-binding protein), as well as upregulation of *sagA* (streptolysin S) and *ska* (streptokinase) (Kreikemeyer, *et al*. 2001).

The *spy1621-1622* system is upregulated during GrAS incubation in amniotic fluid (Sitkiewicz, *et al.* 2010). Analysis of a sensor kinase mutant for the Spy1621-1622 system in an M1 strain revealed that the TCS is important for responses to acid stimuli and resistance to neutrophil killing in human blood (Ichikawa, *et al.* 2011). In a murine soft tissue infection model, Spy1621-1622 was also critical for virulence as measured by lethality and lesion size (Ichikawa, *et al.* 2011).

Analyses of response regulator mutants for Spy1061-1062, Spy1106-1107, and Spy1553-1556 in an M1 strain revealed that the regulons of these two-components systems during *in vitro* growth is growth-phase dependent and can vary from 12% to 41% of GrAS genes (Sitkiewicz & Musser 2006). The mutants did not exhibit changes in virulence potential using murine bacteremia and soft tissue infection models (Sitkiewicz & Musser 2008). VicRK appears to be essential for GrAS growth in SF370 (Ribardo, *et al.* 2004).

1.6.2.7 Unconventional TCS: Eukaryotic-type Serine/Threonine Signaling

Traditionally, prokaryotes have been thought to use histidine kinases (two-component systems), while eukaryotes utilize serine/threonine or tyrosine kinases. Recent analyses of prokaryotic genomes, however, have revealed that bacteria also harbor genes encoding eukaryotic-type serine/threonine kinases and phosphatases (Shi, *et al.* 1998; Bakal & Davies 2000; Kennelly 2002; Deutscher & Saier 2005). Some bacteria can possess up to 50 genes encoding serine/threonine kinases (Petrickova & Petricek 2003).

In *S. pyogenes,* genome sequencing of multiple serotypes revealed the presence of one putative eukaryotic-type serine/threonine kinase (SP-STK) and its protein phosphatase (SP-STP). In SF370, the genes *sp-stk* (*spy1625*) and *sp-stp* (*spy1626*) lie next to each other in the bacterial chromosome and are co-transcribed (Jin & Pancholi 2006).

SP-STK is a transmembrane protein with an N-terminal manganese-dependent threonine kinase domain and a C-terminus with three extracellular PASTA domains. PASTA (penicillin-binding and serine/threonine kinase-associated) domains bind to peptidoglycan and β-lactam antibiotics (Yates, *et al.* 2002). SP-STK exhibits autophosphorylation activity and was found to act on a histone-like protein (SP-HLP) encoded by *spy1489*. The SP-STP phosphatase is highly secreted and dephosphorylates both SP-STK and SP-HLP (Jin & Pancholi 2006).

Mutants were constructed in SF370 to examine the functions of various SP-STK domains. Cells missing the entire *sp-stk* gene exhibit severe morphological abnormalities with the bacteria appearing enlarged and elongated (Jin & Pancholi 2006; Bugrysheva, *et al.* 2011). These studies also revealed that SP-STK is likely involved in regulating the expression of many surface proteins, including M protein and the hyaluronic acid capsule (Jin & Pancholi 2006). One mechanism of action for SP-STK gene regulation is phosphorylation of the global regulator CovR (Agarwal, *et al.* 2011). In addition, the intracellular concentration of SP-STK is crucial for normal signaling events in the bacterium, as overexpression, no expression, or abnormally localized SP-STK leads to misregulation with significant consequences on the expression of certain proteins (Jin & Pancholi 2006).

Mutants lacking either the entire SP-STK protein or one of its domains were found to display distinct phenotypes regarding cell growth, colony morphology, cell division/separation, adherence potential for host cells, and resistance to phagocytosis by human neutrophils (Jin & Pancholi 2006). In a murine myositis model of infection, *sp-stk* deletion mutants in a different M1 strain were greatly attenuated in their virulence potential (Bugrysheva, *et al.* 2011). Transcriptome analyses of this M1 strain and the *sp-stk* mutant revealed that SP-STK is an activator of gene expression. Genes activated by SP-STK function in virulence, glucan metabolism, fatty acid biosynthesis, osmoregulation, cell division/separation, and penicillin tolerance (Bugrysheva, *et al.* 2011).

The phosphatase SP-STP is not essential for GrAS survival as once proposed, but does play a crucial role in virulence expression (Agarwal, *et al.* 2011). Inactivation of the *sp-stp* gene did not affect the growth pattern and cell morphology of the mutants, but did result in the formation of long chains due to upregulation of the cell wall hydrolase CdhA (*spy0019*) (Pancholi, *et al.* 2010; Agarwal, *et al.* 2011). SP-STP inactivation also resulted in loss of anti-phagocytic activity by GrAS attributable to a reduction in capsule synthesis. As with SP-STK, changing the levels of SP-STP expression even in wild-type streptococci had deleterious effects on the cells (Agarwal, *et al.* 2011).

Overall, virulence regulation by SP-STP was found to be strain-specific, with *sp-stp* inactivation leading to activation or repression of various virulence determinants, including *speB, sic, sclA,* and the pilus (Agarwal, *et al.* 2011). SP-STP regulation of the *sagA* (streptolysin S) operon was strain-specific with activation in SF370 and no effect in another M1 strain. Effects of SP-STP inactivation on GrAS adherence to and internalization by pharyngeal cells was also strain specific, with the mutant in the SF370 background exhibiting reduced adherence and increased internalization and the mutant in another M1 background unaltered for adherence and only slightly less invasive (Agarwal, *et al.* 2011).

Most recently, *sp-stp* was found to be highly regulated in SF370 following internalization by pharyngeal cells *in vitro*. SP-STP was shown to be secreted by intracellular GrAS and traverse the host cell nuclear membrane. Both in the host

cytoplasm and nucleus, SP-STP activated pro-apoptotic signaling cascades resulting in mitochondrial dysfunction and perturbations in the phosphorylation states of eukaryotic histones. (Agarwal, *et al.* 2012).

Histone-like proteins in bacteria resemble eukaryotic histones in their biochemical properties (small basic proteins that bind DNA) and are highly conserved among Gram-positive cocci (Drlica & Rouviere-Yaniv 1987; Jin & Pancholi 2006; Rajagopal, *et al.* 2006; Saskova, *et al.* 2007). In bacteria, histone-like proteins have been associated with gene regulation, DNA replication, and organization of the bacterial nucleoid (Dorman & Deighan 2003; Robinow & Kellenberger 1994; Rimsky 2004). Histone-like protein in *S. pyogenes* (SP-HLP) is required for growth and has been reported to bind host cells and induce cytokine release (Zhang, *et al.* 1999; Stinson, *et al.* 1998; Bugrysheva, *et al.* 2011). It is not yet clear, however, if histone-like SP-HLP can act as a transcriptional regulator in GrAS.

1.7 Role of RNases in Expression Regulation

Regulation of gene expression in *S. pyogenes* is widely considered to be executed at the transcriptional level. As previously mentioned, studies that have examined the correlation between mRNA levels and protein production have found good agreement (Lyon, *et al.* 2001; Chaussee, *et al.* 2002; Shelburne III, *et al.* 2005b; Kreikemeyer, *et al.* 2007; Wen, *et al.* 2011; Shelburne III, *et al.* 2011b). The amount of mRNA in a cell, however, is not only affected by the rate of

transcription, but also by mRNA decay. Although most work has focused on finding the regulators that activate or repress mRNA production, researchers are only beginning to examine how streptococcal RNases affect mRNA stability. Notably, recent evidence indicates that mRNA decay may play a significant role in growth phase-dependent gene regulation (Barnett, *et al.* 2007).

1.7.1 CvfA (RNase Y)

Although the stand-alone regulators detailed above work by binding DNA or other regulators and directly influencing transcription rates, the regulator *cvfA* (*spy1633*) exerts its influence on gene expression at the posttranscriptional level in response to growth phase and nutritional stress (Kang, *et al.* 2010). CvfA orthologs in *Bacillus subtilis* and *Staphylococcus aureus* have RNase activity, and it has been proposed that CvfA-dependent regulation in *S. pyogenes* occurs via directed mRNA degradation (Nagata, *et al.* 2008; Shahbabian, *et al.* 2009; Kang, *et al.* 2010). Furthermore, CvfA interacts directly with the glycolytic enzyme enolase, which may serve to transmit information of the nutritional status of the bacterium for virulence regulation (Kang, *et al.* 2010).

Genome-wide transcriptional analyses of an isogenic *cvfA* mutant during growth in C medium (rich in peptides, but poor in glucose) revealed that CvfA regulates expression of nearly 30% of all ORFs (447 total) at stationary phase in an M5 strain (Kang, *et al.* 2010). Genes under CvfA repression included those involved in energy production and conversion, as well as genes functioning in the

transport and metabolism of amino acids, carbohydrates, and lipids. Meanwhile, *cvfA* transcription induced virulence factor expression, including *speB, sagA,* and the DNase *mf*, perhaps through mRNA degradation of secondary virulence repressors and/or activators (Kang, *et al.* 2010). As *in vitro* gene expression in stationary phase is directly correlated to *in vivo* transcription during murine soft tissue infections, it follows that an isogenic *cvfA* mutant was attenuated for virulence in the subcutaneous infection model (Loughman & Caparon 2006b; Kang, *et al.* 2010).

Recently, researchers determined that *speB* transcripts levels increase during late logarithmic and early stationary phases (Chen, *et al.* 2012). The rise in transcript abundance was linked to enhanced mRNA stability and was correlated with lower culture pH. In *cvfA* deletion mutants, the abundance of *speB* was significantly reduced. Furthermore, the long *speB* transcript was present in higher levels than the more stable short form (Chen, *et al.* 2012). The researchers hypothesized that CvfA may be important for production of the short form by endonucleolytic cleavage of the long transcript. Thus, processing of the long transcript into the short form by CvfA may lead to an accumulation of the stable short transcript and increased SpeB protein production (Chen, *et al.* 2012).

1.7.2 PNPase

The 3'-to-5' exoribonuclease *pnpA* (*spy1946*) also appears to play a role in growth-phase dependent virulence regulation by degrading mRNAs (Barnett, *et*

al. 2007). During stationary phase, most GrAS mRNAs are not easily detectable under standard laboratory conditions and have been termed Class I messages (Hollingshead, *et al.* 1987, Bugrysheva & Scott 2010). In an M3 strain, however, transcripts for the virulence factors streptolysin S (*spy0738, spy0746*) and streptodornase (*spy2043*) account for a greater fraction of the total streptococcal mRNA at stationary phase than in exponential growth and have been labeled Class II messages (Barnett, *et al.* 2007; Bugrysheva & Scott 2010).

Analyses revealed that the abundance of Class II transcripts could be partially explained by their enhanced stability in stationary phase (Barnett, *et al.* 2007). As PNPase degrades these transcripts in exponential phase, the increased stability late in growth could be attributed to reduced PNPase activity, which is supported by dramatic downregulation of *pnpA* expression in stationary phase (Barnett, *et al.* 2007; Bugrysheva & Scott 2010b).

1.7.3 J RNases

Two endoribonucleases *rnjA* (RNase J1; *spy1876*) and *rnjB* (RNase J2; *spy1020*) were recently described in GrAS that are essential for growth (Bugrysheva & Scott 2010). Endonucleolytic cleavage of Class II messages by the J RNases appears to be required for subsequent PNPase digestion (Bugrysheva & Scott 2010b). Experiments suggest that RNase J1 and J2 have preferred affinity for Class I messages, delaying the degradation of Class II messages by PNPase and resulting in their enhanced stability (Bugrysheva &

Scott 2010). Overall, our understanding of mRNA decay as a mechanism of gene regulation and the environment signals that affect RNase activity remains largely incomplete.

1.8 Small Regulatory RNAs

Small RNAs (80 to 500 base pairs) that exert regulatory functions comprise 10 to 20% of genes in bacteria (reviewd in Romby & Charpentier 2010). Studies have indicated that small RNAs may play a role in regulating bacterial virulence (Romby, *et al.* 2006; Toledo-Arana, *et al.* 2007). Regulatory RNAs can act through different mechanisms, including mRNA degradation, transcription termination, or translation inhibition through antisense base-pairing; sequestration of regulatory proteins; resistance to phage through DNA targeting by CRISPRs; or responses to environmental signals via *cis*-acting regions of mRNAs that attenuate downstream transcription or control translation (Romby & Charpentier 2010; Nozawa, *et al.* 2011).

Bioinformatics and microarray-based genome-wide searches in an M1 strain have led researchers to estimate that *S. pyogenes* could encode 75 small RNAs (Livny, *et al.* 2006; Perez, *et al.* 2009). This estimate includes at least 7 riboswitches—structures residing in 5' mRNA regions that can bind intracellular metabolites and affect downstream mRNA transcription and translation (Vitreschak, *et al.* 2004). Thus far, only three sRNAs have been examined: non-coding *fasX* RNA, the processed *rivX* RNA, and the protein coding *pel* RNA. All

of these small RNAs have been associated with virulence factor regulation, although the exact mechanisms of action have not been fully elucidated (Romby & Charpentier 2010).

The *fasX* RNA is a 300-nucleotide transcript and the main effector molecule of the FasABC two-component system important for virulence regulation in *S. pyogenes* (Kreikemeyer, *et al.* 2001). The *fasX* RNA controls expression of extracellular matrix protein-binding adhesins (*fasX* repression) and the secreted virulence factors streptokinase and streptolysin S (*fasX* activation) in an M49 strain (Kreikemeyer, *et al.* 2001). Subsequent studies revealed that *fasX* exerts its influence on streptokinase levels by binding to the 5' end of *ska* mRNA. Stabilization of the *ska* transcript by *fasX* was observed to increase streptokinase activity 10-fold (Ramirez-Pena, *et al.* 2010).

The *rivX* RNA (discussed previously) enhances abundance of transcripts for the virulence factors under Mga control (Roberts & Scott 2007). The *rivX* RNA appears to be a processed form of a larger transcript that also codes for the RivR regulator protein and may be a *trans*-acting sRNA (Roberts & Scott 2007).

The *pel* RNA (pleiotropic effect locus) is 500 nucleotides in length and enhances virulence factor expression in a strain-specific manner. In an M49 strain, *pel* activates expression of *emm* and *speB*, and enhances secretion of streptokinase (Li, *et al.* 1999). In an M1 strain, *pel* positively affects transcription of *emm* and *sic*, while exerting posttranscriptional control over *speB* (Mangold, *et al.* 2004). The *pel* transcript is not translated into protein, but contains a portion

that codes for *sagA*—the first gene in the streptolysin S operon (Mangold, *et al.* 2004). Thus, the *pel* locus is involved in sRNA-mediated regulation and virulence factor production.

CRISPRs represent a novel defense system employed by archaea and bacteria targeting bacteriophage and plasmid DNA (reviewed in Marraffini & Sontheimer 2010). The CRISPR loci are comprised of tightly clustered, highly conserved repeat sequences (23 to 50 base pairs) that are separated by non-repetitive spacers of similar length, but which share sequence identity to DNA from phage or plasmids. The number of repeat-spacer units per CRISPR locus can be in the hundreds, but 66 is the average. An AT-rich leader sequence lies upstream of the repeats. Conserved CRISPR-associated (*cas*) genes are encoded adjacent to the repeats and provide important protein machinery for CRISPR activity (Marraffini & Sontheimer 2010). Surveys have identified CRISPR loci in 40% of bacteria and up to 90% of archaea (Grissa, *et al.* 2007).

In 2007, researchers studying a CRISPR locus in *Streptococcus thermophilus* provided the first experimental evidence that CRISPRs drive immunity to phage infection. CRISPR spacer sequences were found to match a section of DNA in the targeted phage (Barrangou, *et al.* 2007). In addition, bacteria acquire new repeat-spacer units during phage challenge that provide 'genetic memory' of previous infections (Barrangou, *et al.* 2007). The repeat-spacer units are transcribed as a long RNA precursor that is processed by Cas proteins into short fragments representing individual spacer units (crRNAs).

When foreign DNA enters the bacterial cell, the crRNA binds by base-pairing to the DNA and activates an effector complex of Cas proteins to block DNA invasion via an unknown mechanism (Marraffini & Sontheimer 2010).

In *S. pyogenes*, two CRISPR loci have been identified: CRISPR1 and CRISPR2 (Nozawa, *et al.* 2011). Examination of multiple strains (representing numerous serotypes) revealed that CRISPR loci are not always present, but their positions within the genome are conserved across CRISPR-containing strains. The average number of repeats across *S. pyogenes* strains was only 6.6, significantly lower than for other streptococcal species and perhaps signifying reduced GrAS resistance to phage (Nozawa, *et al.* 2011).

In SF370, CRISPR1 (found between *spy1049* and *spy1050*) has 7 repeat-spacer units and CRISPR2 (located between *spy1559* and *spy1561*) has 4 repeat-spacer units (Nozawa, *et al.* 2011). Spacers in SF370 matched the sequences of prophage regions in other *S. pyogenes* genomes, including MGAS5005.3 (a prophage in another M1 strain). None of the SF370 spacer sequences matched prophage regions in its own genome, indicating that these sequences are critical for restricting DNA insertions by new phage (Nozawa, *et al.* 2011).

1.9 Streptococcal Transcriptome Studies

The availability of multiple genome sequences over the past decade has allowed the design and use of microarray technology for *S. pyogenes*

transcriptome analyses. These studies have provided the field with a window into GrAS virulence regulation under a variety of conditions (reviewed in Fiedler, *et al.* 2010b). Most microarray work has been conducted using three strains: SF370 (M1), MGAS5005 (M1T1 clone representative), and NZ131 (M49).

1.9.1 GrAS in Media

The transcriptome of GrAS in response to a variety of standard laboratory growth conditions has been thoroughly explored. In general, growth phase-dependent studies have revealed a reduction in transcription upon entry into stationary phase (Beyer-Sehlmeyer, *et al.* 2005; Chaussee, *et al.* 2008; Barnett, *et al.* 2007). Virulence factor expression indicated that the suboptimal growth conditions experienced during transition phase *in vitro* most closely mimics *in vivo* infection conditions (Beyer-Sehlmeyer, *et al.* 2005). Transcriptional responses have also been characterized for GrAS upon temperature change (29 to 37°C), growth under varying pH levels, and the addition of NaCl (Smoot, *et al.* 2001; Loughman & Caparon 2006b).

1.9.2 GrAS in Contact with Host Cells *in vitro* and *in vivo*

Numerous studies have examined the transcriptional responses of GrAS to human bodily fluids, including adult saliva, blood, and amniotic fluid (Graham, *et al.* 2005; Shelburne III, *et al.* 2005; Malke & Ferretti 2007; Sitkiewicz, *et al.* 2010). In the acidic, nutrient-limited environment of saliva, the TCS SptRS was

crucial for persistence. In addition, genes involved in virulence, virulence regulation, carbohydrate utilization pathways, and oxidative stress responses were upregulated (Shelburne III, *et al.* 2005). Streptococci undergo a large transcriptional shift (~75% of genes) during 90 minutes of *ex vivo* growth in blood (Graham, *et al.* 2005). Genes involved in glycolysis were shut down, while amino acid-fermenting pathways were activated. Virulence factors were also upregulated (Graham, *et al.* 2005).

Interactions of GrAS with host cells *in vitro* have been a focus in our laboratory and others. The transcriptome of *S. pyogenes* strain SF370 upon adherence to human pharyngeal cells was elucidated in our laboratory (Ryan, *et al.* 2007). Approximately 79 genes were regulated upon binding of streptococci to host cells (as compared to associated streptococci), including genes involved in virulence, pilus assembly, metabolism, regulation, and the production of phage proteins, such as the superantigen *speH* (Ryan, *et al.* 2007).

In the past month, the transcriptional responses of SF370 internalized by human pharyngeal cells were reported (Agarwal, *et al.* 2012). A total of 432 genes were found to be regulated as a result of internalization, in comparison to control bacteria in media. Over 80% of the genes were upregulated, including those involved in virulence, translation, DNA replication, cell division, and amino acid transport. Downregulated genes included a significant number of phage genes (Agarwal, *et al.* 2012).

During phagocytosis by polymorphonuclear neutrophil granulocytes (PMNs), streptococci were found to upregulate virulence regulators, virulence factors that modulate host immune defenses, and genes involved in detoxifying reactive oxygen species. Notably, 20 phage-encoded genes were also upregulated (Voyich, *et al.* 2003).

Studies of GrAS transcriptional responses *in vivo* have included non-human primates and mouse models of disease, as well as analysis of GrAS gene expression in humans with pharyngitis (Virtaneva, *et al.* 2005; Graham, *et al.* 2006; Shea, *et al.* 2010; Olsen, *et al.* 2010; Livezey, *et al.* 2011). Concerted activation of gene groups was observed during the different stages of experimental pharyngitis infection in cynomolgus macaques (Virtaneva, *et al.* 2005). Initial colonization was marked by activation of carbohydrate metabolism pathways. During the acute-phase of infection denoted by its high bacterial load in the pharynx, induction of prophage genes, SptRS, and a second wave of carbohydrate metabolism genes was observed. Finally, the asympomatic phase was characterized by low levels of streptococci in the pharynx and induction of transporter genes and CovRS (Virtaneva, *et al.* 2005).

An examination of GrAS gene expression during pharyngitis in 11 patients revealed two distinct transcriptional clusters that differed significantly in the genes that were highly expressed (Livezey, *et al.* 2011). Although no one serotype could account for the clustering, isolates of each particular M type were all present in the same gene expression cluster. The authors postulated that the

two clusters may simply reflect differences in environmental conditions within the throat swab area or timing of the sample collection between patients during the symptomatic phase, but a number of other factors were considered (Livezey, *et al.* 2011).

Both clusters exhibited induction of genes involved in streptococcal adherence to host cells, including M protein and fibronectin-binding protein. (Livezey, *et al.* 2011). Genes for complex carbohydrate utilization pathways were also upregulated, including the stand-alone regulator MalR. There was a distinct regulation of genes related to amino acid starvation in one cluster. Genes involved in oxidative stress responses were highly expressed in both clusters and appeared to be dependent on PerR regulation (Livezey, *et al.* 2011). Notably, one cluster exhibited dramatic induction of phage-encoded genes, while the other had no significant prophage gene upregulation. The former cluster also exhibited a greater number of highly expressed virulence genes and bacteriocin-related genes, possibly indicating that these streptococci were more virulent or in competition with other oral microbial flora (Livezey, *et al.* 2011).

2. OBJECTIVES

Upon introduction into the oral cavity, streptococci come into contact with a variety of host factors, including antimicrobial peptides and host metabolites. Environmental cues, such as temperature, pH, and glucose levels, also help signal to the bacterium that it has entered its target niche. Until recently, researchers did not fully appreciate the role these environmental signals play in inducing large-scale transcriptional remodeling within the pathogen. Microarray studies have elucidated that incubation in blood and adult saliva activates regulatory pathways that significantly reshape the streptococcal transcriptome in short periods of time (Graham, *et al.* 2005; Shelburne III, *et al.* 2005).

In our laboratory, previous studies had demonstrated that streptococci also react to a soluble factor elaborated by human pharyngeal cell cultures (Broudy, *et al.* 2001; Broudy, *et al.* 2002). Two phage-encoded factors (superantigen *speC* and DNase *spd1*) were found to be transcriptionally induced in an M76 variant by incubation with pharyngeal supernatants and co-culture with intact pharyngeal monolayers. Furthermore, the prophage that harbors these virulence factor genes could itself be induced to form fully functional phage particles capable of lysogenizing naïve streptococci in the immediate vicinity (Broudy and Fischetti 2003).

At the inception of this work, our understanding of the streptococcal adaptive response to pharyngeal cell environments was limited to these early lysogenic phage studies. Thus, we endeavored to describe the global

transcriptional events that accompany exposure to pharyngeal cultures. By capitalizing on the sequencing of the SF370 genome and the increasing availability of microarray technology, we sought to capture snapshots of the SF370 transcriptome during incubation with secreted host factors in pharyngeal supernatants and resulting from transient host cell contact during co-culture with intact pharyngeal monolayers. To obtain a better understanding of the timing associated with transcriptome remodeling, we sampled transcriptome profiles at 30 minutes, 1.5 hours, and 2.5 hours during the culture period.

Our microarray analyses revealed a large program of transcriptional remodeling in response to both secreted host factors and potential transient cell contact. Surprisingly, the expression of well-characterized virulence regulators (discussed at length in the introduction to this thesis) were largely unaffected by the host environment. Two stand-alone regulators of unknown function that were upregulated in the host cell environment were selected for further study. Isogenic mutants of *spy1215* and *spy1755* were generated and characterized in the SF370 background.

Comparison of the *spy1215* deletion mutant and wild-type SF370 in the host cell environment revealed that this sirtuin-like regulator represses expression of virulence factors, but only in the presence of intact pharyngeal monolayers. During incubation with pharyngeal supernatants, however, this regulator appears to be important for supporting active translation. To our knowledge, this is the first time that a sirtuin-like regulator has been linked to

virulence in an animal bacterial pathogen. Growth curve analyses immediately highlighted that *spy1755* plays an important role in streptococcal growth. Supplementation of culture broth with Tween-80 restored a wild-type growth pattern, indicating that Spy1755 is necessary for activating streptococcal fatty acid biosynthesis.

Finally, we sought to elucidate the role (if any) of lysogenic phage in the regulation of virulence factors and the greater adaptive response of streptococci to the host environment. To this point, it had been demonstrated that streptococcal transcriptional regulators, including RofA, RopB, MalR, PerR, and the TCS CiaHR, could affect the expression of phage-encoded genes, but it was not known if phage regulators could affect chromosomally encoded gene expression (Becket, *et al.* 2001; Riani, *et al.* 2007; Gryllos, *et al.* 2008; Anbalagan, *et al.* 2011; Shelburne III, *et al.* 2011; Wen, *et al.* 2011).

Our laboratory recently generated the first streptococcal strain completely cured of all lysogenic phage using the SF370 background (Euler 2010). Surprisingly, transcriptome analyses comparing the mutant to wild-type SF370 during growth in THY revealed a limited role for phage elements in overall streptococcal gene expression. The largest effects were observed for genes directly adjacent to phage insertion sites, whose expression was altered following deletion of the prophage region. It remains to be determined, however, if curing of lysogenic phage would have a broader impact on the streptococcal transcriptome during growth in host environments *in vitro* and/or *in vivo.*

3. MATERIALS AND METHODS

3.1 Bacterial strains and growth conditions

S. pyogenes strain SF370 (an M1 serotype) was originally isolated from a wound infection and kindly provided by J. Ferretti, University of Oklahoma Health Sciences Center. Isogenic KO mutants used throughout these studies were derived from the SF370 background (Table 3.1). *E. coli* strain One Shot DH5α (Invitrogen) was used as the host strain for plasmid construction and vector propagation.

SF370 was grown at 37°C in Brain Heart Infusion (BHI) broth or Todd Hewitt broth + 0.2% Yeast Extract (THY) and on Proteose Peptone Blood Agar (i.e. supplemented with 5% defibrinated sheep blood (Cleveland Scientific) or Colombia Blood Agar plates (BD, Becton, Dickinson and Company). *E. coli* was cultured in Luria-Bertani (LB) broth and on LB agar at 37°C.

Growth of SF370Δ1755 in section 6 of this thesis was supplemented with 0.1% Tween-80 (Sigma) where indicated. When required, media was supplemented with antibiotics at the following concentrations: erythromycin at 200 µg/ml for *E. coli* and 15 µg/ml for *S. pyogenes*; kanamycin at 50 µg/ml for *E. coli* and 250 µg/ml for *S. pyogenes*; streptomycin at 200 µg/ml for *S. pyogenes*. All antibiotics were supplied by Sigma, and all media was supplied by Difco (BD, Becton, Dickinson and Company) unless stated otherwise.

For microarray assays in sections 4 and 5 of this thesis, strains SF370 and SF370Δ1215 were grown to late log-phase ($O.D._{600}$ 0.7) in THY.

Table 3.1 SF370 and isogenic mutant strains

S. pyogenes strain[a]	Bacteriophage and/or gene deleted	Antibiotic resistance phenotype[b]	Reference or source
SF370	None	Sensitive	Ferretti, *et al.* 2001
SF370Δ1215	*spy1215*	Erm^R	This study
SF370Δ1755	*spy1755*	Erm^R	This study
CEM1ΔΦ[c]	370.1, 370.2, 370.3, 370.4	Kan^S Sm^R	Euler 2010

[a] Either all phage (marked by Φ) or the gene (designated by a number) to the right of the Δ symbol have been deleted

[b] Abbreviations used: Erm^R, erythromycin-resistant; Kan^S, kanamycin-sensitive; Sm^R, streptomycin-resistant

[c] Spontaneous streptomycin-resistant derivative of SF370 was utilized for isogenic phage-KO mutant creation

Bacterial cells were washed in 0.1 M phosphate-buffered saline (PBS, pH 7.4), resuspended in minimal essential medium (MEM, Invitrogen, Carlsbad, CA), and incubated for 1h at 37°C. Glycerol (10% v/v) was added and cultures were flash frozen in liquid N_2 and stored at -80°C. To minimize culture-to-culture variability, these stock cultures were used for all subsequent microarray experiments examining transcriptional responses to the host environment.

For microarray assays in section 7 of this thesis, strains SF370 and CEM1$\Delta\Phi$ were grown overnight to stationary phase. Glycerol (20%v/v) was added to 5 ml aliquots of overnight cultures. The cultures were then flash frozen in liquid N_2 and stored at -80°C. To minimize culture-to-culture variability, these stocks were used for subsequent *in vitro* growth phase-dependent transcriptome studies.

3.2 Growth conditions for pharyngeal cultures

The human pharyngeal cell line Detroit 562 (ATCC# CCL 138) was grown in MEM (Invitrogen, Carlsbad, CA) containing 10% fetal bovine serum. Cells were cultured in Falcon® 6-well plates (35 mm diameter) at 37°C under 5% CO_2.

3.3 DNA manipulations

Streptococcal genomic DNA was isolated with either the DNeasy Tissue Kit or the Blood & Cell Culture DNA Kit (Qiagen) following the manufacturer's protocols, except for the substitution of a modified lysis buffer (50 mM Tris-Cl

pH 6.6, 50 mM EDTA, 0.5% Tween-20, 0.5% TritonX-100) supplemented with 500U of the amidase enzyme lysin PlyC (Nelson, *et al.* 2001), and 250 ng/ml of RNase A (Qiagen). Plasmid DNA was isolated from *E.coli* using the QIAprep Spin Miniprep Kit or HiSpeed Plasmid Midi Kit (Qiagen). DNA fragments were gel purified from 1% Agarose gels using the QIAquick Gel Extraction Kit (Qiagen). T4 DNA ligase and all restriction enzymes were purchased from New England Biolabs and used according to the manufacturer's instructions, unless otherwise stated.

Oligonucleotides were obtained from Fisher Scientific-Operon. PCR was performed using AmpliTaq Gold DNA polymerase, Gold Buffer, 1.5 mM $MgCl_2$, and 200 µM dNTPs (Applied Biosystems) following standard protocols with the Eppendorf Mastercycler. DNA sequencing was performed by GENEWIZ, Inc. (North Brunswick, NJ). DNA and RNA sequence analysis, comparison, and manipulation required Lasergene software modules (DNASTAR Inc.). DNA primers were designed with MacVector software (Accelrys Inc.).

3.4 Spotted oligonucleotide microarrays

Sense strand oligonucleotides (primarily 55-mers), representing the 1769 open reading frames (ORF) in the genome of *Streptococcus pyogenes* strain SF370 were designed by Invitrogen and produced by Eurofins MWG Operon (Huntsville, AL) (Table A.1 in appendix). Oligonucleotides were spotted onto Corning Epoxide Coated Slides (Corning Inc, Life Sciences, Acton, MA) by

Microarrays Inc (Huntsville, AL). Slides were post-processed by Microarrays Inc, and adequate oligonucleotide deposition was verified using their quantitative Veriprobe QC assay (http://www.microarrays.com/mi-quality.php). Each oligonucleotide was spotted six times in a well-spaced configuration to generate in-slide replicates.

3.5 Pharyngeal supernatant and cell association assay

Assays on streptococcal association with the human pharyngeal cell line Detroit 562 were performed, as described previously (Ryan, *et al.* 2001), with the following modifications. Pharyngeal cells were grown to confluence (5×10^6 cells/well) in 6-well plates. Intact monolayers were washed three times with 2 ml PBS to remove serum. Approximately 4.5 ml of serum-free MEM was then added to each well, and the pharyngeal monolayers were incubated for 16-18 hours at 37°C with 5% CO_2.

Pharyngeal supernatants containing secreted host factors were subsequently harvested from the monolayers and filtered through a 0.2 μm membrane to remove large debris and detached pharyngeal cells. For the association assay, 2 ml of filtered pharyngeal supernatant were added back to the confluent monolayers after a final PBS wash. In addition, 2 ml of filtered supernatant per well were reserved for use in parallel with empty 6-well plates to examine streptococcal responses elicited solely by secreted host factors.

Streptococcal stock cultures in MEM and glycerol were pre-incubated at 37°C for 1 hour, and aliquots (~50 μl) were added to each well (2 ml volume) of 6-well plates containing either confluent monolayers or filtered pharyngeal supernatants. To serve as a control for studies examining wild-type SF370 responses to the host environment (section 4), aliquots of streptococci were added to fresh MEM in 50 ml Falcon® tubes. Tubes were used because the bacteria were found to adhere in great numbers to the surface of 6-well plates in the absence of pharyngeal supernatants or confluent monolayers (unpublished observations).

Co-culture plates containing streptococci and confluent pharyngeal monolayers were gently centrifuged (200 x *g*) for 5 minutes at room temperature, as previously described (Cue & Cleary 1997). The centrifugation step was added to ensure that all streptococci could potentially begin interacting with the monolayer at the start of the incubation period. Cultures of streptococci in fresh MEM and in the presence of pharyngeal supernatants or pharyngeal monolayers were incubated at 37°C with 5% CO_2.

Assays examining time-dependent transcriptional changes in section 4 of this thesis were performed at a multiplicity of infection (MOI) of 80 (4×10^8 CFU/well). Control streptococci were resuspended in fresh MEM to achieve equivalent CFU/ml concentrations. Streptococci exposed to pharyngeal supernatants or in co-culture with pharyngeal monolayers and their respective control samples in MEM were incubated for 30 minutes, 1.5 hours, and 2.5 hours.

Assays for comparison of SF370 and SF370Δ1215 in the presence of pharyngeal supernatants or confluent monolayers detailed in section 5 were performed using an MOI of 40 (2 x 10^8 CFU/well).

Associated (non-adherent) streptococci were harvested from the wells containing pharyngeal supernatants and confluent monolayers by aspiration. One PBS wash (1 ml volume) was performed to ensure collection of all associated bacteria. Control streptococci suspended in MEM were recovered by centrifugation.

3.6 Scanning electron microscopy

Pharyngeal monolayers were grown to 80% confluence on coverslips placed at the bottom of wells in Falcon 6-well plates. After preincubation for 1 hour at 37°C, aliquots of SF370 (~50 μl) were added to each well (total volume of 2 ml). Plates were subjected to gentle centrifugation to place all streptococci on the monolayer surface (as detailed above).

Following 2.5 hours at 37°C in 5% CO_2, one plate was selected for visualization of associated (non-adherent) bacteria. The 2 ml media volume was gently siphoned out of each well to prevent removal of loosely associated streptococci and replaced with 2 ml of sterile PBS. A separate plate was prepared for visualization of only adherent bacteria that had tightly bound to the epithelial cells. The 2 ml co-culture volume was aspirated, and the monolayers

were vigorously washed three times using 2 ml volumes of PBS to remove the associated population. Finally, 2 ml of PBS were left on the monolayers.

The streptococci and pharyngeal monolayers were fixed in 2.5% glutaraldehyde at 4°C overnight. The co-cultures were then treated with 1% osmium tetroxide in 0.1M cacodylate buffer pH 7.4 for an hour, dehydrated using graded ethanol solutions, and critical point dried. The slides were coated with a thin gold-palladium layer using a Desk IV coater (Denton Vacuum). Images were obtained using a LEO 1550 scanning electron microscope, with field-emission electron gun. Images were captured to qualitatively compare the relative numbers of associated and adherent streptococci on the pharyngeal monolayer following 2.5 hours of co-incubation.

3.7 *In vitro* growth assay for CEM1ΔΦ

The 5 ml frozen aliquots of SF370 and CEM1ΔΦ were used to inoculate 1000 ml culture volumes of THY. Cultures were incubated at 37°C until early exponential growth ($O.D._{600}$ 0.35) or late exponential growth ($O.D._{600}$ 0.85). Streptococci growing in THY were recovered by centrifugation.

3.8 RNA isolation

Streptococci from the pharyngeal association assays and the CEM1ΔΦ *in vitro* growth assay were washed twice in PBS, resuspended in 0.1X TE (pH 6.0), and flash frozen in a bath of ethanol and dry ice. Bacteria were lysed with the

amidase enzyme lysin PlyC (Nelson, *et al.* 2001). Lysin was added to the bacterial samples (2 U/10^8 cfu) and incubated for 1 minute at room temperature, which was determined to be optimum for complete streptococcal lysis in preliminary experiments. RNA was isolated immediately after lysis with a modified phenol-chloroform protocol, as described previously (Philbrick, *et al.* 1991), substituting acid phenol (Invitrogen) to reduce genomic DNA contamination.

RNA was digested with DNase I (TURBO DNA-*free*, Invitrogen). Removal of contaminating genomic DNA was confirmed by the absence of any PCR product using ethidium-bromide gel visualization following 40 cycles of PCR. Primers specific for the ORF *spy0930* (Table A.2 in appendix) were used as this gene was previously reported in our laboratory to be constitutively expressed in MEM and the host cell environment (unpublished observations). RNA quantity was determined with the NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE), and RNA quality was assessed with the Nucleic Acid Bioanalyzer 2100 (Agilent Tech., Palo Alto, CA).

3.9 Synthesis of cDNA and labeling

DNase-treated streptococcal total RNA (2.5 µg) was reverse transcribed using the Superscript Indirect cDNA Labeling System (Invitrogen). Random hexamers (Invitrogen) primed the reverse transcription reaction that incorporated 5-(3-aminoallyl)-dUTP into the first synthesized cDNA strand. cDNAs from

experimental streptococci and control streptococci were indirectly labeled with N-hydroxysuccinimide activated fluorescent dyes cyanine 3 (Cy3) and cyanine 5 (Cy5), respectively, as outlined in the Superscript kit. Amersham CyDyes were purchased for this use from GE Healthcare Life Sciences (Piscataway, NJ). Labeled cDNA samples were purified following Superscript kit instructions. cDNA yield and dye incorporation rates were assessed using the NanoDrop 1000 spectrophotometer to ensure high-quality labeled probes.

3.10 Microarray hybridization and image acquisition

Biological replicate experiments incorporating dye swaps were performed to account for both biological and technical variability (Yang & Speed 2002). For the time-dependent transcriptome studies in section 4, eight biological replicate experiments were prepared for each time point (0.5 h, 1.5 h, 2.5 h) under the two experimental conditions: exposure to pharyngeal monolayers and incubation in pharyngeal supernatants. For microarray analysis, 4 replicates for each data set were labeled in the standard dye orientation, and 4 replicates were labeled in the flip orientation (total of 48 separate hybridizations).

The SF370Δ1215 studies in section 5 involved six biological replicates (3 in the standard dye configuration and 3 in the flip orientation) for each experimental condition: 2.5 h exposure to pharyngeal supernatants and 2.5 h co-culture with pharyngeal monolayers (total of 12 hybridizations). The growth phase-dependent transcriptional studies of CEM1ΔΦ in section 7 were performed

with four biological replicates each at early and late exponential growth phases (all replicates hybridized in the standard and the flip orientations for a total of 16 hybridizations).

Microarray slides were blocked with a prehybridization solution containing 10 mg/ml bovine serum albumin (Sigma). Labeled cDNA samples were hybridized to the arrays under standard glass microscope coverslips in a hybridization buffer containing 50% deionized formamide (Sigma), 10X SSC, and 0.2% SDS for 16 h at 55°C in a stationary hybridization oven. Slides were washed with agitation as follows: one wash with 0.2X SSC, 0.1% SDS at 55°C for 15 minutes and two washes with 0.1X SSC at room temperature for 15 minutes each.

Slides were dried via centrifugation (1000 rpm, 3 minutes) and then scanned with the Agilent High-Resolution C Microarray Scanner (Agilent Technologies, Santa Clara, CA) at 5 μm per pixel resolution. The resulting images were processed using the GenePix Pro program (version 4.0, Axon, Union City, CA).

3.11 Data filtering, normalization, statistical significance analysis, and calculation of *P* values for individual genes

Following image analysis, low level processing of microarray data included probe and array quality filtering to remove probes that were saturated, displayed a low signal to noise ratio, and/or produced signal in only one dye channel.

Lowess standardization was performed for data normalization, and a modified *t*-test (based on CyberT software; Baldi & Long 2001) was implemented to calculate a *P* value for the $\log_2$-fold change (expression ratio in the experimental sample to the appropriate control) of each gene, as previously described (Ryan, *et al.* 2007).

The *t*-test statistics and *P* values generated in this analysis were used to rank genes undergoing statistically significant changes in expression ($P < 0.05$) during association with the host cell environment (pharyngeal supernatants or confluent monolayers) as compared to control streptococci in MEM. For transcriptome analyses of the isogenic mutants SF370ΔΔ1215 and CEM1ΔΔΦ, significant changes in gene expression refer to the ratio of expression in the mutant to wild-type SF370 under the same host (SF370ΔΔ1215) or growth (CEM1ΔΔΦ) conditions.

No cutoff values were set for the magnitude of the expression change necessary to be included in the set of genes undergoing statistically significant fold changes. Although researchers often disregard expression changes that are less than 2-fold ($\log_2$ value of ±1) between the experimental and control conditions, gene expression changes as low as 1.5-fold ($\log_2$ value of ±0.6) have been demonstrated to be physiologically relevant in *S. cerevisiae* (Hughes, *et al.* 2000).

The GenomeCrawler algorithm, written in the statistical language R (http://www.R-project.org), provided a method to view the microarray output data

and visualize genes undergoing significant fold changes in the context of the entire genome (Ryan, *et al.* 2007).

3.12 Real-time quantitative RT-PCR primers, probes and plasmid standards

Real-time quantitative RT-PCR analysis (TaqMan) was used to verify the fold-change in gene expression estimated by microarray analysis. The list of genes, as well as the oligonucleotide primers and fluorogenic (TaqMan) probes designed by and purchased from Sigma-Genosys (The Woodlands, TX), are provided in the appendix (Table A.3). Endogenous controls used for QRT-PCR normalization were carefully selected for each microarray data set. Criteria for selecting a reference/control gene for a given data set included: *P* value close to 1, log-fold change approaching 0, and a mid-range signal intensity on arrays (Table 3.2). Each of the experimental and control genes examined by QRT-PCR was amplified in its entirety from SF370 genomic DNA by PCR and cloned into pCR-TOPO plasmids (Invitrogen). Cloning primers are listed in Table A.4.

3.13 Quantitative real time RT-PCR and TaqMan analysis

A two-step QRT-PCR procedure was used to convert total RNA from 2 biological replicates (2 experimental samples and 2 controls) that were prepared following the microarray assay protocols detailed above. Using SuperScript II First Strand Synthesis System for RT-PCR (Invitrogen), DNase I-treated RNA preparations (2 μg each) were separately converted to cDNA preparations with

Table 3.2 SF370 genes selected as references for QRT-PCR microarray validation

Reference Genes

Locus Tag	Corresponding Dataset[a]
spy0764	2.5h Co-culture
spy1309	1.5h Co-culture; 0.5h Supernatant
spy1390	1.5h Co-culture; 2.5h Supernatant
spy1877	1.5h Co-culture; 1.5h Supernatant
spy2189	0.5h Co-culture
spy1912	CEM1ΔΦ *in vitro* growth

Validation Targets

Locus Tag	Corresponding Dataset[a]	Expression Ratio (sample:control)
spy0946	All time points: Co-culture & Supernatant	Downregulated
spy1215	All time points: Co-culture & Supernatant	Upregulated
spy0028	CEM1ΔΦ *in vitro* growth	Upregulated
spy1357	CEM1ΔΦ *in vitro* growth	Downregulated
spy1432	CEM1ΔΦ *in vitro* growth	Downregulated

[a] Datasets refer to time-dependent transcriptional responses to host cell environment in section 4, or the phage KO studies in section 7 (marked as CEM1ΔΦ *in vitro* growth).

50 ng random hexamers (Invitrogen) (45°C, 50 min, 20 μl reactions) according to manufacturer instructions.

RNA samples were reverse transcribed in separate reactions and no pooling of samples occurred. Control reactions without reverse transcriptase were included to confirm that genomic DNA was not present. TaqMan analysis was performed (in duplicate) with an ABI Prism 7900 sequence detection system (Applied Biosystems) using Platinum Quantitative PCR SuperMix-UDG (Invitrogen) (according to manufacturer instructions) and primer-probe pairs listed in Table A.3 of the appendix. No-template negative controls were included. Cycling conditions, optimized with plasmid standards, were as follows: 50°C for 2 min and 95°C for 2 min, followed by 45 cycles at 95°C for 15 sec and 60°C for 45 sec.

Standard curves for threshold cycle (C_T) versus copy number for each gene were constructed using known concentrations of plasmid DNA standards (10-fold dilutions ranging from 10^8 copies to 10 copies) that were subjected to the same reaction and cycling conditions and included on each reaction plate. Results were normalized with C_T values for the appropriate reference gene. Data from duplicate reactions were averaged and $\log_2$-transformed to produce a single value for each gene representing the fold difference in the number of cDNA molecules present in experimental streptococcal samples relative to control streptococcal samples.

3.14 Allelic replacement of the *spy1215* and *spy1755* genes in SF370

The strategy for allelic replacement of *spy1215 and spy1755* genes was followed as previously described (Euler, *et al* 2007). Briefly, upstream and downstream DNA regions flanking both genes were separately amplified using the primer sets listed in Table A.5 in the appendix. PCR products were treated with the appropriate restriction enzymes (New England Biolabs) and used according to manufacturer instructions. Fragments were gel purified, and the respective upstream and downstream regions for either *spy1215* or *spy1755* were ligated together into the allelic replacement vector pFW15 (Podbielski, *et al.* 1996), creating plasmids pFW15-*spy1215* and pFW15-*spy1755*.

To construct deletion mutants of the *spy1215* and *spy1755* genes, the vectors were separately electroporated into *S. pyogenes* SF370, and transformants were selected on proteose peptone blood agar supplemented with erythromycin (300 µg/ml). Allelic replacement was confirmed by PCR and DNA sequencing, as well as RT-PCR analyses of total RNA extracted from both late-logarithmic ($O.D._{600}$ 0.7) and stationary phase ($O.D._{600}$ 1) bacterial cultures using gene specific primers (Table A.6). Total RNA from strain SF370 served as a control.

3.15 Light microscopy

SF370, SF370Δ1215, and SF370Δ1755 were grown overnight to stationary phase in THY at 37°C with 5% CO_2. SF370Δ1215 and SF370Δ1755

were grown in the presence of 0.15 μg/ml erythromycin. Cultures of SF370 and SF370Δ1755 supplemented with 0.1% Tween-80 were also included. Overnight cultures were visualized using an Eclipse E400 microscope (Nikon) under a 100X oil immersion lens. Image capture was performed with QCapture Pro software (version 5.1).

3.16 RT-PCR of *spy1215*, *spy1755,* and downstream genes

The sequences of forward (F) and reverse (R) primers for each of the examined genes (*spy1215, spy1755,* and their respective downstream genes) are provided in Table A.6 of the appendix. RT-PCR generation of amplicons was performed with the SuperScript III One-Step RT-PCR system with Platinum *Taq* DNA polymerase (Invitrogen) in reaction mixtures (50 *μ*l) containing 0.2 *μ*M of each gene specific forward and reverse primers and 0.1 *μ*g of DNase-treated, purified total RNA from late-log ($O.D._{600}$ 0.7) and stationary ($O.D._{600}$ 1) phase cultures of strains SF370, SF370Δ1215, and SF370Δ1755.

All remaining components were added as per manufacturer specifications. Control reactions, in which *Taq* DNA polymerase was substituted for the reverse transcription enzyme mixture, were included to confirm that genomic DNA was not present in the RNA preparations. RNA was converted to cDNA (50°C for 30 min), which was then PCR amplified in the same tube (45 cycles of the following conditions: 94°C for 15 sec, 52°C for 30 sec, and 68°C for 2 min). Resulting DNA

fragments were separated on 1% agarose gels in TAE buffer and visualized by ethidium bromide staining.

3.17 Biological assay: *in vivo* murine model of infection

The Rockefeller University's Institutional Animal Care and Use Committee approved all *in vivo* protocols. A systemic infection model, previously described in (Daniel, *et al.* 2010), was used to test virulence potential of SF370Δ1215 as compared to wild-type SF370. Briefly, 4-5 week old female FVB/NJ mice (weight range 15 to 20 g) were obtained from The Jackson Laboratory (Bar Harbor, ME). After a period of acclimation, mice were injected intraperitoneally (IP) with 0.5 ml of mid log-phase ($O.D._{600}$ 0.5) bacteria, that was concentrated and then serially diluted in saline to the desired concentration. The survival rate for each experimental group was monitored every 12 hours for the first 24 hours then every 24 hours up to 5 days post infection.

3.18 Generation of phage deletion mutant CEM1ΔΦ

The deletion of all lysogenic phage from SF370 was previously described (Euler 2010). Briefly, the technique relies on the allelic replacement of an individual bacteriophage gene with a two-gene cassette containing: (1) the gene responsible for kanamycin resistance (Kan^R) (Podbielski, *et al.* 1996), and (2) the wild-type ribosomal subunit *rpsL* gene, which is targeted by the antibiotic streptomycin (Reyrat, *et al.* 1998). While mutations in the chromosomal *rpsL*

gene provide a high level of resistance to streptomycin (Sm^R), this resistance is recessive if a wild-type copy of the *rpsL* gene (such as on the inserted cassette) is also expressed in the same cell (Lederberg 1951; Reyrat, *et al.* 1998).

Spontaneous *rpsL* mutants that are streptomycin resistant (Sm^R) in SF370 were selected for by serial passage of strain SF370 in brain heart infusion broth (BHI) containing increasing concentrations of the antibiotic (0 – 200 μg/ml). Working to cure one lysogenic phage at a time, allelic replacement of a target bacteriophage gene in each progphage was accomplished, as evidenced by the gain of kanamycin resistance. Following overnight growth at 37°C in antibiotic-free BHI, streptococci that had lost the targeted phage could be identified by streaking on proteose peptone plates with 200 μg/ml streptomycin. As streptococci cured of that bacteriophage no long harbor the two-gene cassette with the wild-type *rpsL* gene, they revert to Sm^R and form colonies.

This procedure was repeated four times to remove the SF370 lysogenic phage in a step-wise fashion: Φ370.2, Φ370.1, Φ370.4, and Φ370.3. The resulting mutant deemed CEM1ΔΦ was analyzed by pulse-field gel electrophoresis, Southern blot hydrizidation, PCR, and DNA sequence analysis to confirm that all integrated prophage had been eliminated from the genome (~10% of the bacterial chromosome). The transcriptomes of CEM1ΔΦ and wild-type SF370 were compared during growth in THY as detailed above.

4. STREPTOCOCCAL TRANSCRIPTOME IN THE HOST ENVIRONMENT

4.1 Introduction

Initiation of streptococcal pharyngitis is dependent upon the ability of *S. pyogenes* to adhere to the oropharyngeal epithelium before host clearance. Barriers to streptococcal adherence in the human oropharynx can include entrapment by oral mucus, removal by the ciliary action of the upper respiratory tract epithelium, aggregation by salivary components, and blockage of streptococcal surface adhesins by host immunoglobulin binding (reviewed in Courtney, *et al.* 2002). To combat these challenges, streptococci elaborate a diverse array of surface molecules and secreted proteins to promote host cell binding and subsequent infection (recently reviewed in Nobbs, *et al.* 2009).

It has been proposed that attachment of streptococci to host cells is a two-step process (Figure 4.1; Hasty, *et al.* 1992). In the first step, weak association between streptococci and the host epithelium brings the bacteria into closer contact with target cells. This weak association is mediated by lipotechoic acid in the bacterial cell wall, which serves to overcome electrostatic repulsion between the pathogen and host surface. Alternatively, it has been suggested that first attachment events could be mediated by streptococcal pili that are long enough to penetrate the mucus layer (Nobbs, *et al.* 2009). Overall, this initial interaction is dynamic and reversible.

A second step utilizes a number of streptococcal surface adhesins to achieve firm, functionally irreversible binding to the host epithelium (Courtney, *et*

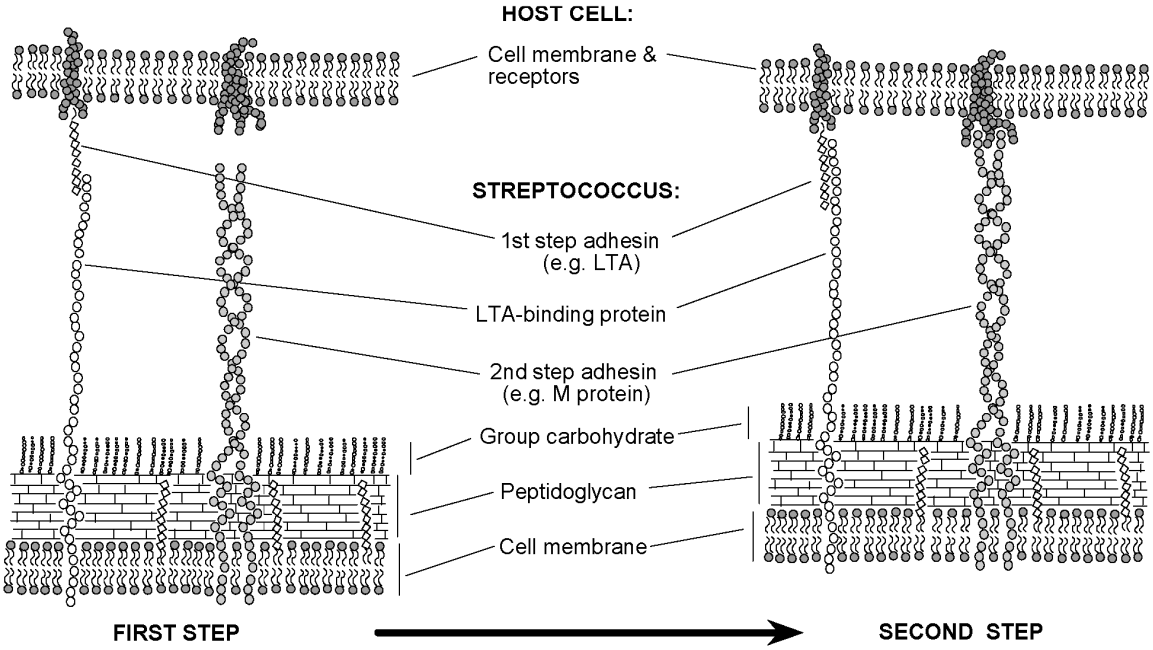


Figure 4.1 Two-step model for *S. pyogenes* adhesion to host cells

It has been proposed that *S. pyogenes* adhesion occurs in two distinct steps. The first step serves to overcome electrostatic repulsion that occurs between the bacteria and host cells. LTA mediates this first step by hydrophobic interactions between its lipid moiety and receptors on host surfaces. The second step leads to high affinity adhesion and relies on other adhesins, including M protein and fibronectin-binding proteins.

Figure adapted from (Courtney, *et al.* 2002).

al. 2002). This binding step confers tissue specificity and involves a number of characterized virulence determinants, including M protein, fibronectin-binding proteins, and laminin-binding proteins. In general, streptococci appear to use multiple adhesin-host receptor interactions with varying affinities to bind to the host epithelium for colonization and infection (Nobbs, *et al.* 2009).

It is now widely recognized that group A streptococci (once considered to be strictly extracellular pathogens) can be internalized by epithelial cells and survive intracellularly for days (LaPenta, *et al.* 1994; Osterlund, *et al.* 1997; Cue, *et al.* 1998). It has been suggested that streptococci may benefit from epithelial cell internalization, as intracellular bacteria are protected from the action of antibiotics and may serve as a reservoir for recurrent infections (Osterlund, *et al.* 1997). Internalization of streptococci by host epithelial cells via two mechanisms has been described (Nobbs, *et al.* 2009; Nitsche-Schmitz, *et al.* 2007).

Interactions between M protein, fibronectin, and integrins on the host cell surface can induce actin polymerization in the host and uptake of streptococci through the fusion of microvilli around the bacterial cells with a zipper-like mechanism (Dombek, *et al.* 1999). Alternatively, internalization by the fusion of host cell caveolae beneath adherent bacteria can result from interactions of the streptococcal adhesin SfbI with fibronectin and host surface integrins (Ozeri, *et al.* 2001). In addition, streptococci can invade deeper tissues by paracellular translocation. Binding of the streptococcal hyaluronic acid capsule to CD44 on

human keratinocytes can induce cytoskeletal changes that disrupt intercellular junctions and allow for streptococcal passage (Cywes & Wessels 2001).

Our laboratory has focused on the infection process of streptococci *in vitro* using human pharyngeal cell cultures. The Detroit 562 cell line originated from a human pharyngeal carcinoma (ATCC-CCL138), and has been used extensively in group A streptococcal research to examine host cell adherence and internalization processes. Streptococcal interactions with cultured pharyngeal cells are generally divided into 3 stages (Figure 4.2).

First, non-adherent bacteria settle within the culture dish and become loosely associated with the monolayer. These associated streptococci actively sense environmental cues, including carbon source availability and host secreted factors, but also potentially interact with pharyngeal cells via weak, reversible binding. The second stage is characterized by tight, irreversible adherence to the host cell surface, and requires trypsin treatment for dissociation. Finally, some adherent streptococci can be internalized by the epithelial cells.

Previous work in our laboratory had examined the transcriptional responses of streptococci following tight adherence to host cells, using the associated bacterial population as a control (Ryan*, et al.* 2007). This analysis provided an intriguing window into the transcriptional events affecting 4% of the genome that accompany host cell adherence. Little information existed, however, on the gene expression changes occurring during the association stage to prepare streptococci for host cell binding. Prior studies in our laboratory had

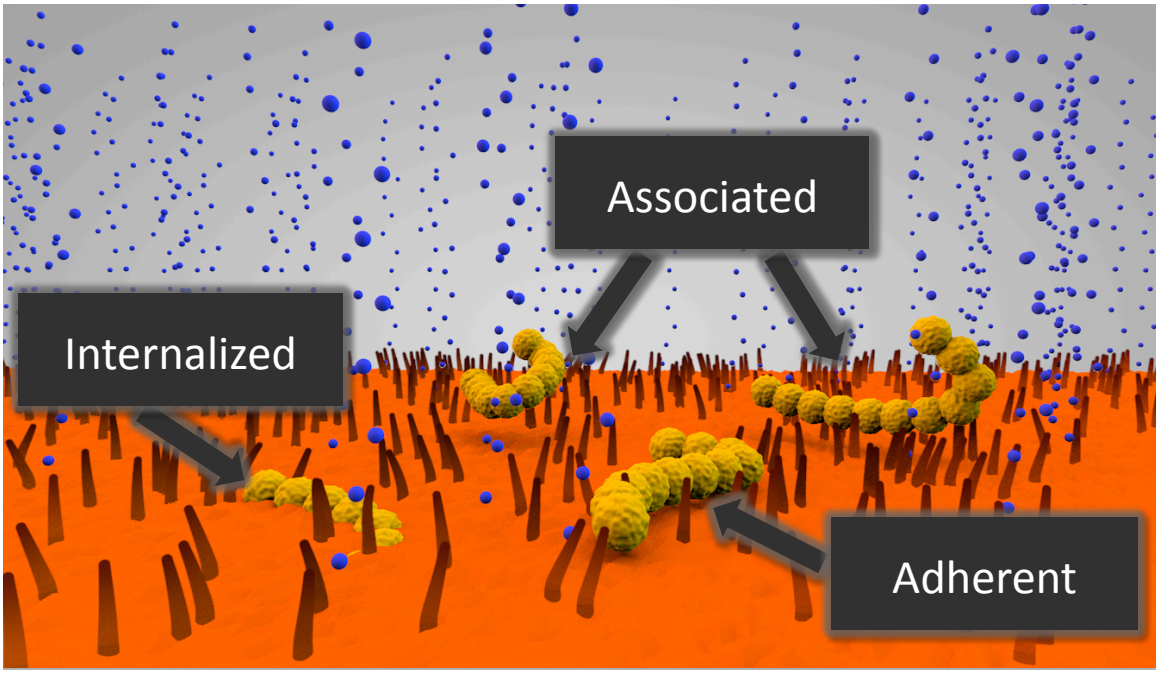


Figure 4.2 Stages of *in vitro* streptococcal infection of pharyngeal cells

Experimental infection of cultured pharyngeal monolayers (orange) by streptococci (yellow) *in vitro* follows three successive stages. During association, streptococci can sense extracellular host signals (blue) and transiently interact with the host cell surface (orange plane and projections). Adherent bacteria then become irreversibly bound to host cell receptors. Finally, some streptococci can be internalized by the epithelial cells (illustrated mid-process above).

indicated that streptococci do actively respond to the host cell environment during loose association, at least at the level of phage induction and phage encoded virulence expression (Broudy, *et al.* 2001; Broudy, *et al.* 2002).

Pharyngeal cell cultures were found to secrete a soluble factor into the surrounding growth media that could induce phage production and gene expression (Broudy, *et al.* 2001). This factor (termed SPIF) was later determined to be present in human saliva (unpublished observations). Upon incubation of streptococci with pharyngeal culture supernatants, full phage particles could be induced in an M6 strain and an M76 variant (Broudy, *et al.* 2001).

Furthermore, phage induction was accompanied by an increase in transcript and protein levels for two virulence factors encoded by that phage: superantigen *speC* and DNase *spd1* (Broudy, *et al.* 2001; Broudy, *et al.* 2002). Experiments in a murine model of nasopharyngeal colonization indicated that *in vivo* induction of this phage also occurred and could result in transduction of a phage-naïve streptococcal strain (Broudy, *et al.* 2003). It remained unclear, however, what other effects SPIF might have on the broader streptococcal transcriptome in the host cell environment.

Another host secreted factor linked to virulence induction in *S. pyogenes* is the human antimicrobial peptide LL-37 (Gryllos, *et al.* 2008b). Antimicrobial peptides (AMPs) are small peptides (2-5 kDa) that can be expressed constitutively by epithelial cells or upon induction by microbial products or cell injury (reviewed in Hancock & Diamond 2000; Otto 2009). These cationic

molecules exert their antimicrobial function by damaging bacterial cell membranes (reviewed in Peschel & Sahl 2006).

Subinhibitory concentrations of peptide LL-37 have also been shown to act as a signal for upregulation of *S. pyogenes* virulence determinants, including the hyaluronic acid capsule, the IgG protease IdeS, and an IL-8 protease, through signal cascades and regulation by CovRS (Gryllos, *et al.* 2008). Furthermore, LL-37-mediated virulence induction conferred enhanced resistance to phagocytic killing when pre-treated streptococci were incubated with human peripheral blood leukocytes (Gryllos, *et al.* 2008). LL-37 is present in human saliva and can be secreted by pharyngeal cultures *in vitro* (Tao, *et al.* 2005; Sharpe, *et al.* 2011).

Combining the evidence that streptococci sense and respond to host-derived factors (SPIF and LL-37) with research highlighting virulence induction following introduction into low-glucose environments (e.g. human saliva and the oropharynx), it became clear that the association stage is critical for the streptococcal adaptive response in the host (link between carbon utilization and virulence induction reviewed in Shelburne III, *et al.* 2008c). We sought to understand how the streptococcal transcriptome is remodeled during association using two experimental conditions.

Streptococcal responses limited to extracellular nutrient status and secreted host factors were probed by exposing streptococci to filtered supernatants from overnight growth of confluent pharyngeal monolayers. The same filtered supernatant was also used in co-culture experiments aimed at

examining the streptococcal transcriptome in response to secreted factors, the nutrient environment, and potential transient interactions with host cells. Furthermore, we were interested in the time-dependent nature of streptococcal transcriptional responses.

Our initial end-point of 2.5 hours for streptococcal cultures in the two host-derived environments was selected based on our previous transcriptome analysis of adherent streptococci. Recognizing that streptococcal adherence to primary tonsillar tissue *in vitro* increases rapidly following a lag phase of 30 minutes (Abbot, *et al.* 2007), we hypothesized that transcriptional changes elaborated during the first 30 minutes of exposure to the host environment were crucial for promoting bacterial adherence. Thus, we chose to perform our experiments with additional RNA sampling points at 30 minutes and 1.5 hours (for an intermediate time point).

The following chapter details the results of our transcriptional screen of *S. pyogenes* during exposure to cell-free pharyngeal supernatants and incubation with confluent pharyngeal monolayers. Each time point selected provides a snapshot of the genes differentially expressed in the host environments as compared to streptococci incubated with MEM (media used for pharyngeal cell culture) for the corresponding length of time. Taken together, these data allow us to begin to tweeze apart responses to secreted factors versus those adaptive changes that require contact or close spatial association with host cells.

4.2 Results

4.2.1 Visualization of associated and adherent SF370 populations by SEM

Associated streptococci incubated with pharyngeal monolayers were imaged using a scanning electron microscope (Figure 4.3). Since the co-culture actually yields a mixed population of associated and adherent bacteria, we created a second set of images where associated streptococci were removed by vigorous washing (Figure 4.4).

Comparison of the two image sets indicated that the adherent population after 2.5 hours of co-culture is a fraction of the total population identified in our images of associated streptococci. This is in agreement with observations of the low percentage of streptococci that bind to host cells within the 2.5 hour co-culture period (calculated in relation to the total inoculum added to each well). Notably, gentle centrifugation yielded roughly equivalent associated populations (by visual qualitative comparison) to co-cultures in which the streptococci were allowed to settle by gravity (data not shown).

Interestingly, we observed that streptococci do not interact with and adhere to pharyngeal cells in a uniform manner (Figure 4.5). Instead, the bacteria tend to aggregate on particular pharyngeal cells, leaving neighboring cells unscathed. It is unclear why (or even if) some host cells are favored over others, and what impact this might have on streptococci and the overall infection process.

Figure 4.3 SEM Visualization of streptococci associated with pharyngeal monolayers

SF370 was added to pharyngeal cell cultures *in vitro* and allowed to interact for 2.5 hours. Media was then carefully siphoned off to prevent removal of streptococci in close association with the epithelial cells. The co-culture samples were fixed, post-processed, and imaged via SEM (as described in the methods section).

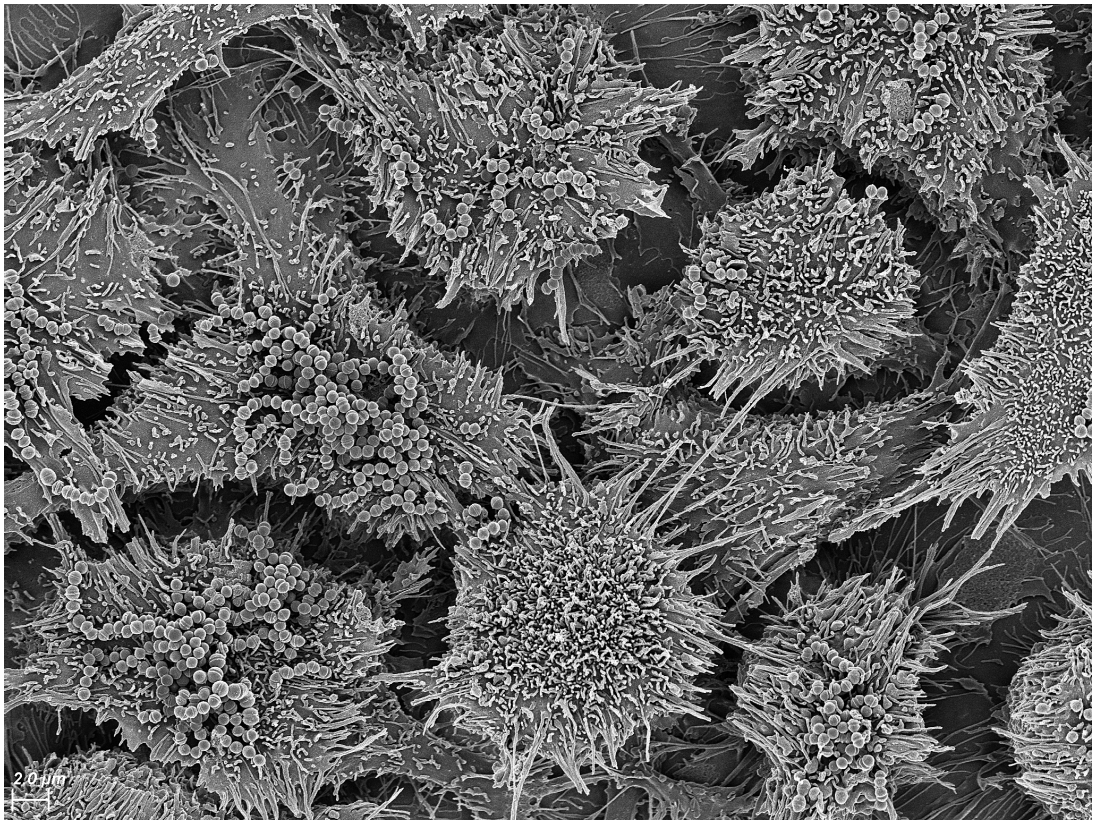
Associated SF370
100 µm

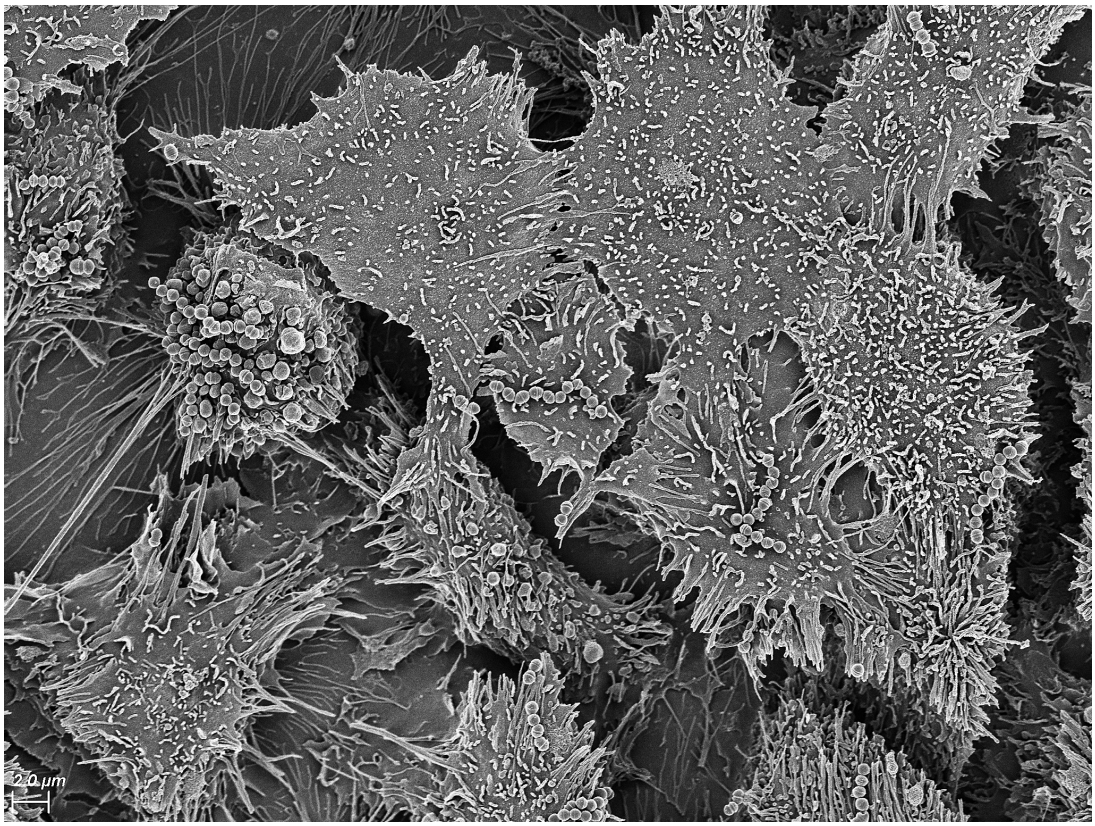
Figure 4.4 SEM Visualization of adherent streptococci on pharyngeal monolayers SF370 was added to pharyngeal cell cultures *in vitro* and allowed to interact for 2.5 hours. Associated streptococci were removed by vigorous washing with PBS. The co-culture samples were fixed, post-processed, and imaged via SEM (as described in the methods section).

Adherent SF370
100 µm

Figure 4.5 SEM images of streptococci preferentially binding to particular pharyngeal cells

Close-up images derived from the same adherent streptococcal samples used to generate the image in Figure 4.4. These images were selected to highlight the observation that streptococci can aggregate on one pharyngeal cell leaving neighboring cells relatively untouched.


2.0 µm


2.0 µm

4.2.2 Overview of the dynamic GrAS transcriptome in pharyngeal environments

Overall, streptococci undergo large-scale transcriptome remodeling upon introduction into the host cell milieu (Table 4.1). Approximately one-third of all the *S. pyogenes* ORFs were regulated in response to one or both of the pharyngeal environments for each time point. A roughly equivalent number of genes were upregulated as were repressed in the host milieu, when compared to control streptococci in MEM media alone. Microarray results (reported as $\log_2$-fold change ratios with their corresponding *P* values) for all time points and both experimental conditions are listed in Table A.7 of the appendix.

Table 4.1 Microarray results summary for transcriptional responses to *in vitro* pharyngeal environments

30 minutes **601 unique genes total (34% of genome)**

Experimental Condition	Upregulated Genes: Number (% of genome)	Downregulated Genes: Number (% of genome)	Total Genes: Number (% of genome)
Pharyngeal Supernatants	280 (15.8%)	198 (11.2%)	478 (27.0%)
Pharyngeal Monolayers	276 (15.6%)	194 (10.9%)	470 (26.6%)

1.5 hours **688 unique genes total (38.9% of genome)**

Experimental Condition	Upregulated Genes: Number (% of genome)	Downregulated Genes: Number (% of genome)	Total Genes: Number (% of genome)
Pharyngeal Supernatants	308 (17.4%)	278 (15.7%)	586 (33.1%)
Pharyngeal Monolayers	273 (15.4%)	258 (14.6%)	531 (30.0%)

2.5 hours **587 unique genes total (33.2% of genome)**

Experimental Condition	Upregulated Genes: Number (% of genome)	Downregulated Genes: Number (% of genome)	Total Genes: Number (% of genome)
Pharyngeal Supernatants	212 (12.0%)	220 (12.4%)	432 (24.4%)
Pharyngeal Monolayers	244 (13.8%)	248 (14.0%)	492 (27.8%)

4.2.3 Comparison of transcriptional responses by gene function across all experimental conditions and time points

For each time point, we classified genes that were differentially expressed in the pharyngeal supernatants and during co-culture into functional categories using the COG system described in the introduction of this thesis. The profiles obtained by functional categorization of genes regulated in our analyses were strikingly similar both between experimental conditions at the same time point and across time points.

Following 30 minutes of exposure to pharyngeal supernatants (Figure 4.6) or pharyngeal monolayers (Figure 4.7), the greatest induction in gene expression was observed for genes operating in metabolic processes and protein translation. Genes involved in metabolism were also a target for repression, as were phage genes. Notably, a large percentage of regulated genes in both data sets were of unknown function.

At 1.5 hours post-exposure to host cell environments *in vitro*, the breakdown of gene functions affected was similar (Figure 4.8 pharyngeal supernatants; Figure 4.9 pharyngeal monolayers). Again, metabolic genes were highly regulated, but the ratio of activated to repressed genes decreased slightly at this later time point. Translation remained a top category for upregulated genes, and a number of phage genes were repressed as seen with the 30-minute data.

This overall functional profile persisted at 2.5 hours post-incubation with pharyngeal supernatants (Figure 4.10) and pharyngeal monolayers (Figure 4.11). Again, metabolic genes were a prime target for active regulation. In the pharyngeal supernatants, roughly equivalent numbers of metabolic genes were activated and repressed, while regulation was still skewed toward activation in the co-culture setting. Translation genes were again upregulated, and phage genes were predominantly downregulated as seen at the previous time points. In addition, we observed a general downregulation of genes involved in DNA replication and cell envelope biogenesis under both experimental conditions that began at the 1.5-hour time point.

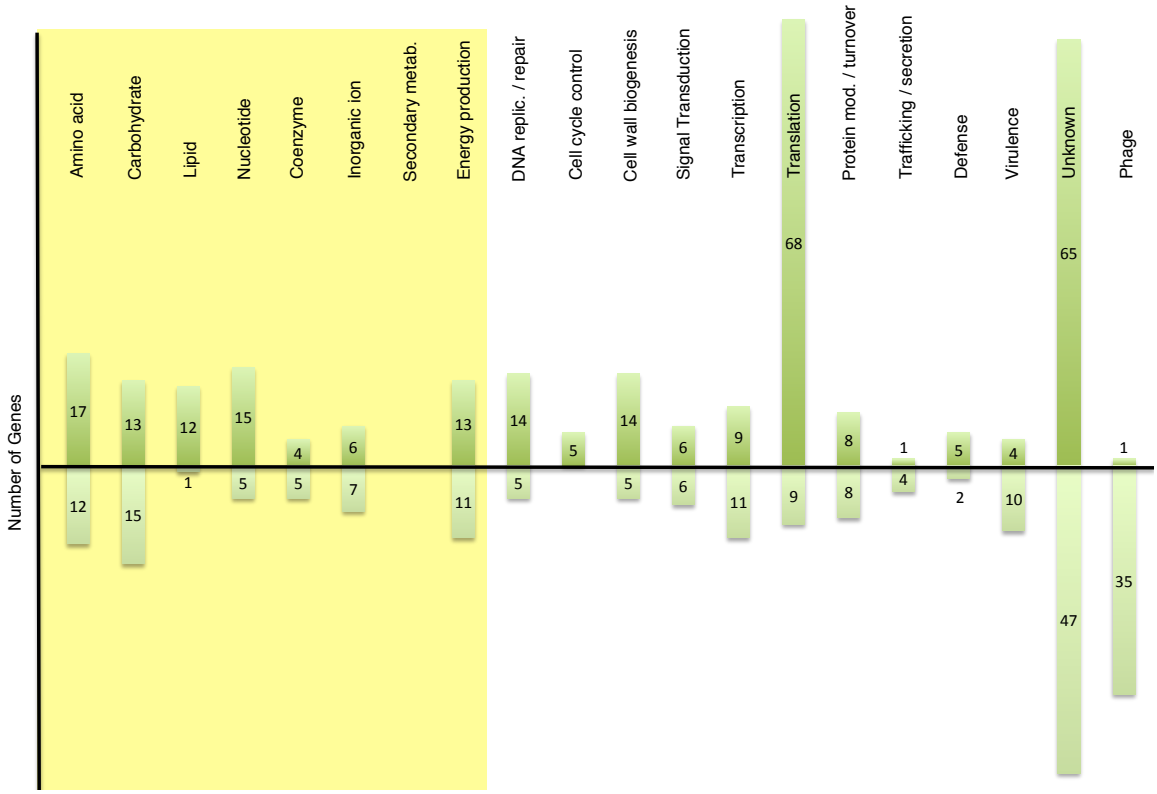


Figure 4.6 COG classification of genes differentially expressed in SF370 following a 30-minute exposure to pharyngeal supernatants

Genes are classified into functional groups based on protein homology to characterized genes. Each bar represents the total number of genes (indicated within the bars) that were differentially upregulated (dark green; positive value relative to x-axis) or downregulated (light green; negative value relative to x-axis) in SF370 exposed to filtered pharyngeal supernatants, as compared to control streptococci in MEM. The area of the graph shaded yellow highlights all functional categories involved in cellular metabolism. Labels for columns within the yellow highlighted area indicate the type of macromolecule transported, synthesized, or hydrolyzed by genes in that category.

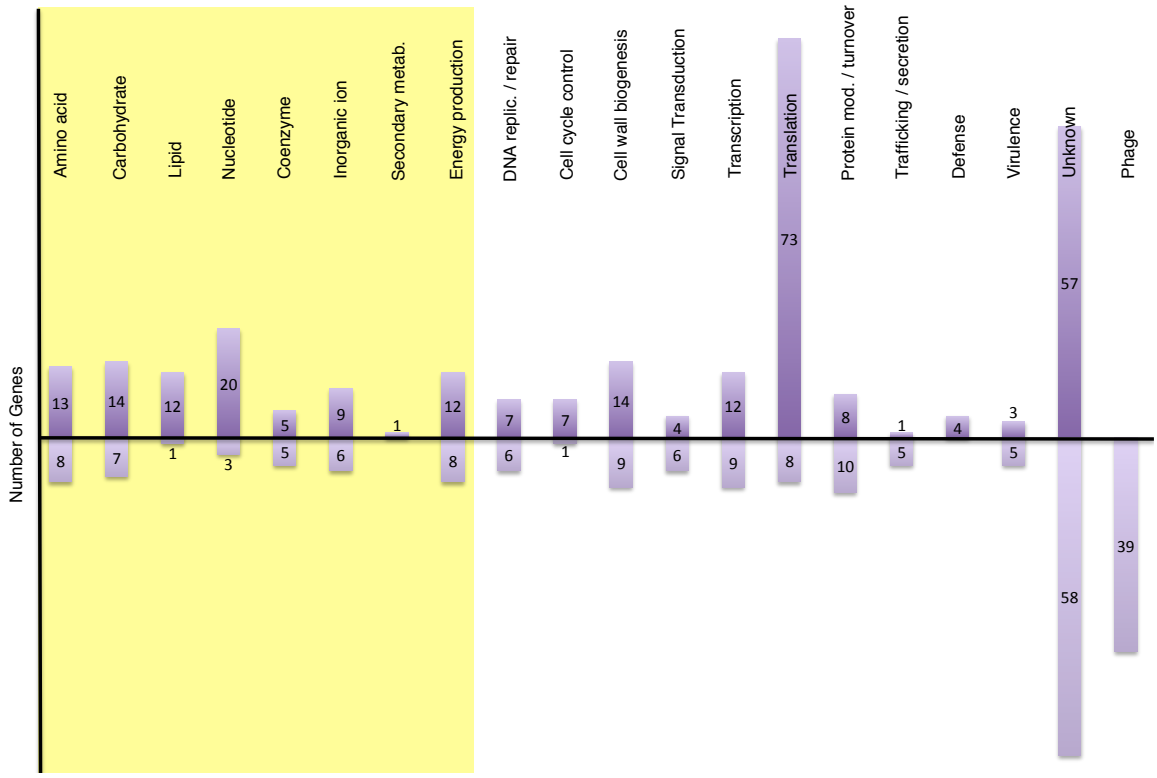


Figure 4.7 COG classification of genes differentially expressed in SF370 following a 30-minute co-culture period with pharyngeal monolayers

Genes are classified into functional groups based on protein homology to characterized genes. Each bar represents the total number of genes (indicated within the bars) that were differentially upregulated (dark purple; positive value relative to x-axis) or downregulated (light purple; negative value relative to x-axis) in SF370 co-cultured with pharyngeal monolayers, as compared to control streptococci in MEM. The area of the graph shaded yellow highlights all functional categories involved in cellular metabolism. Labels for columns within the yellow highlighted area indicate the type of macromolecule transported, synthesized, or hydrolyzed by genes in that category.

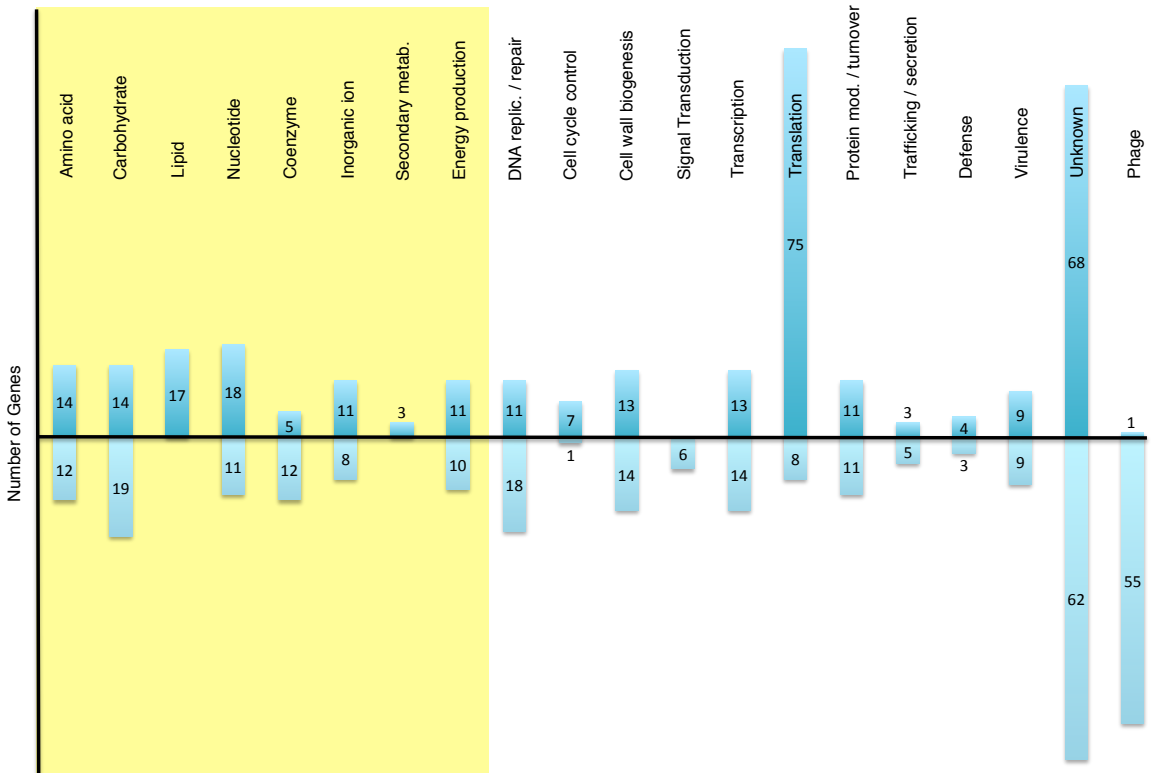


Figure 4.8 COG classification of genes differentially expressed in SF370 following a 1.5-hour exposure to pharyngeal supernatants

Genes are classified into functional groups based on protein homology to characterized genes. Each bar represents the total number of genes (indicated within the bars) that were differentially upregulated (dark turquoise; positive value relative to x-axis) or downregulated (light turquoise; negative value relative to x-axis) in SF370 exposed to filtered pharyngeal supernatants, as compared to control streptococci in MEM. The area of the graph shaded yellow highlights all functional categories involved in cellular metabolism. Labels for columns within the yellow highlighted area indicate the type of macromolecule transported, synthesized, or hydrolyzed by genes in that category.

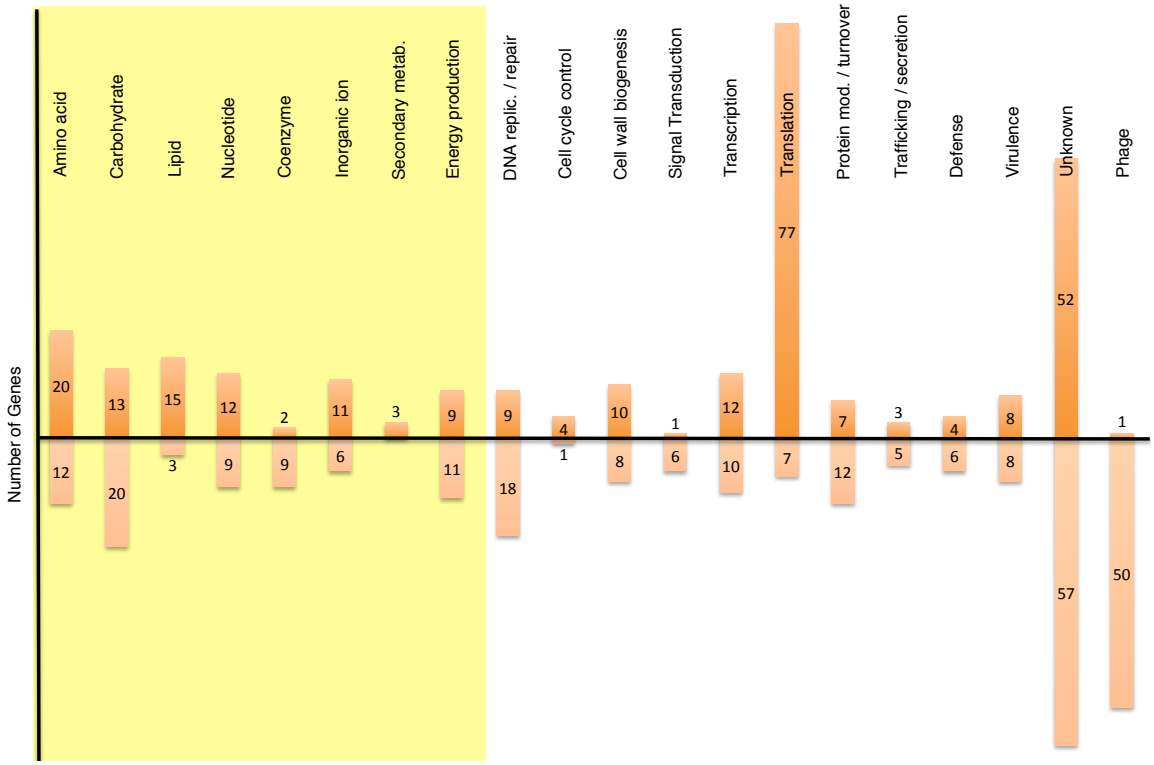


Figure 4.9 COG classification of genes differentially expressed in SF370 following a 1.5-hour co-culture period with pharyngeal monolayers

Genes are classified into functional groups based on protein homology to characterized genes. Each bar represents the total number of genes (indicated within the bars) that were differentially upregulated (dark orange; positive value relative to x-axis) or downregulated (light orange; negative value relative to x-axis) in SF370 co-cultured with pharyngeal monolayers, as compared to control streptococci in MEM. The area of the graph shaded yellow highlights all functional categories involved in cellular metabolism. Labels for columns within the yellow highlighted area indicate the type of macromolecule transported, synthesized, or hydrolyzed by genes in that category.

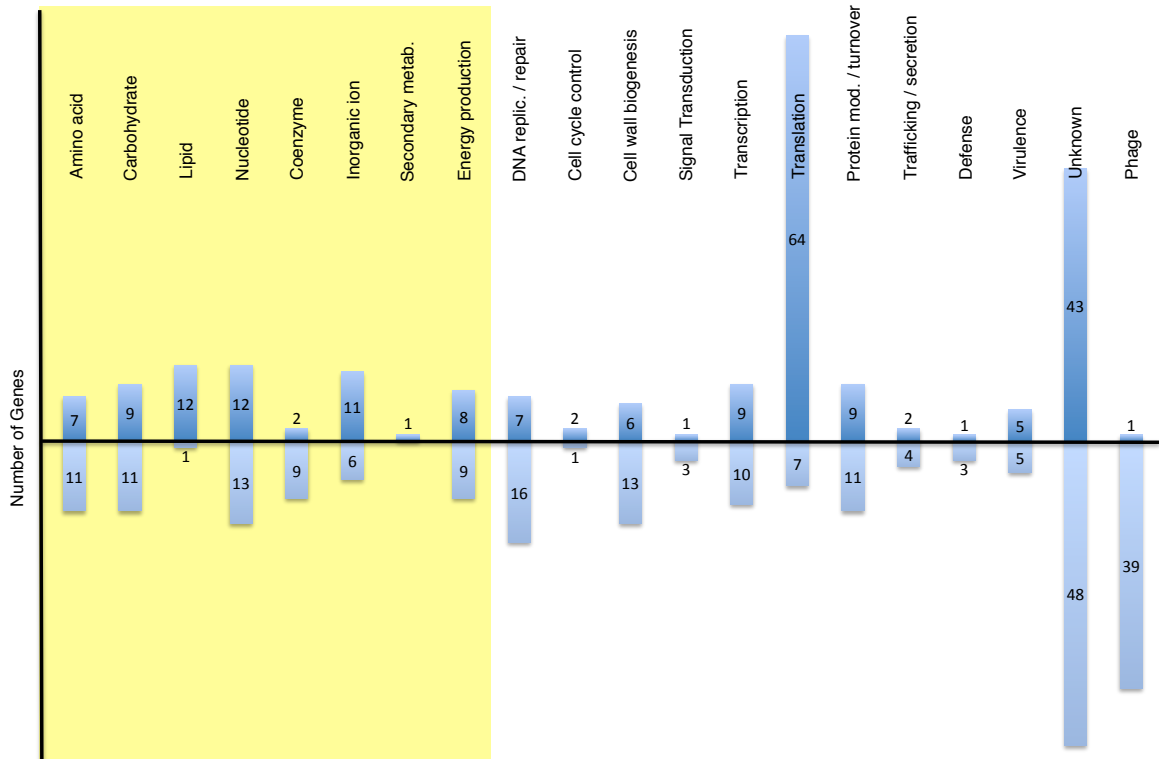


Figure 4.10 COG classification of genes differentially expressed in SF370 following a 2.5-hour exposure to pharyngeal supernatants

Genes are classified into functional groups based on protein homology to characterized genes. Each bar represents the total number of genes (indicated within the bars) that were differentially upregulated (dark blue; positive value relative to x-axis) or downregulated (light blue; negative value relative to x-axis) in SF370 exposed to filtered pharyngeal supernatants, as compared to control streptococci in MEM. The area of the graph shaded yellow highlights all functional categories involved in cellular metabolism. Labels for columns within the yellow highlighted area indicate the type of macromolecule transported, synthesized, or hydrolyzed by genes in that category.

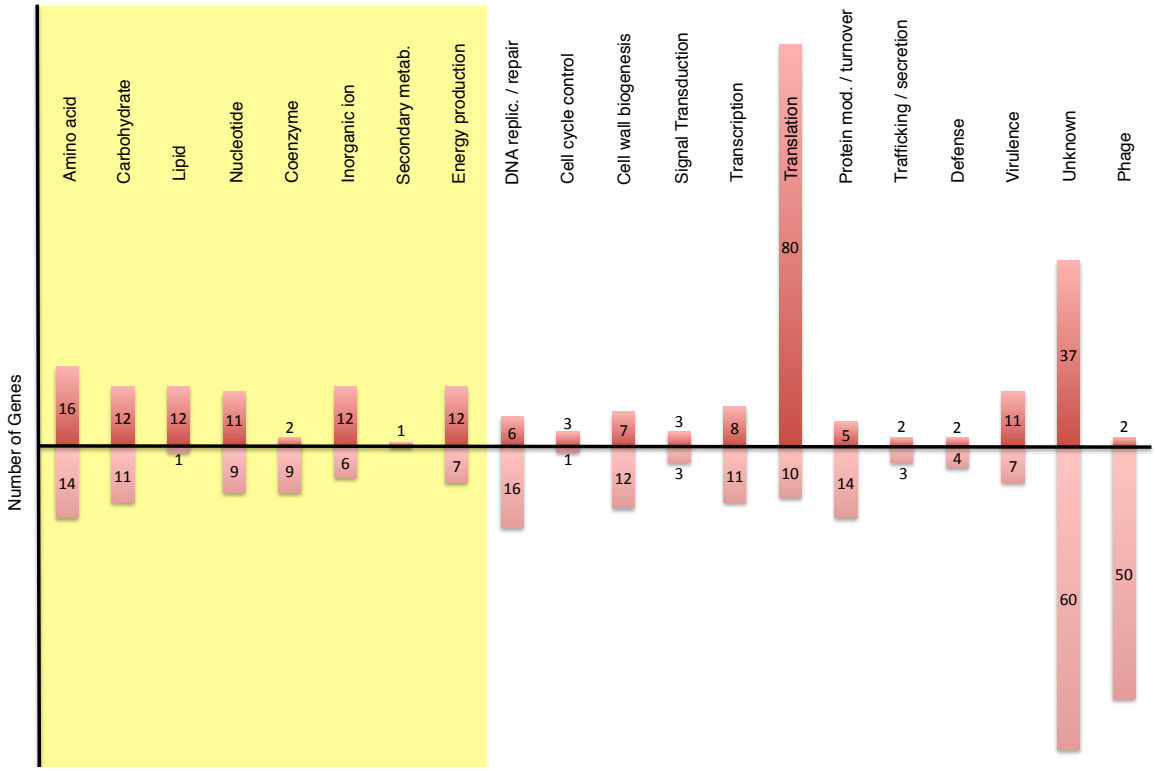


Figure 4.11 COG classification of genes differentially expressed in SF370 following a 2.5-hour co-culture period with pharyngeal monolayers

Genes are classified into functional groups based on protein homology to characterized genes. Each bar represents the total number of genes (indicated within the bars) that were differentially upregulated (dark red; positive value relative to x-axis) or downregulated (light red; negative value relative to x-axis) in SF370 co-cultured with pharyngeal monolayers, as compared to control streptococci in MEM. The area of the graph shaded yellow highlights all functional categories involved in cellular metabolism. Labels for columns within the yellow highlighted area indicate the type of macromolecule transported, synthesized, or hydrolyzed by genes in that category.

4.2.4 Examination of putative host cell contact-dependent transcriptional responses

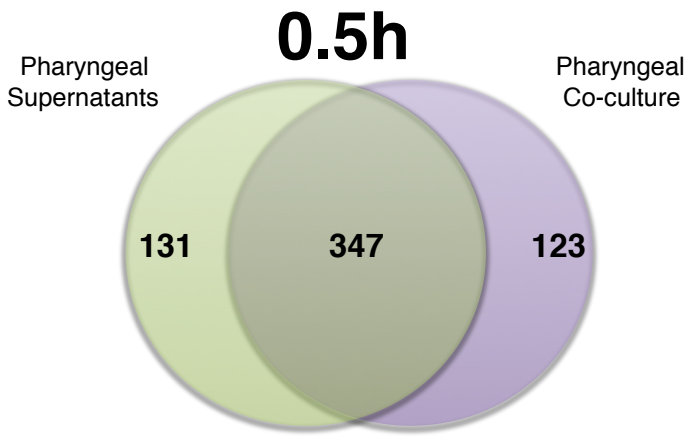
Comparison of genes regulated following exposure to pharyngeal supernatants and during co-culture with pharyngeal monolayers at each time point revealed that there were a number of differentially regulated genes specific to each host environment (Figure 4.12). The Venn diagrams in Figure 4.12 highlight, however, that the majority of genes regulated at each time point were modulated in both host environments.

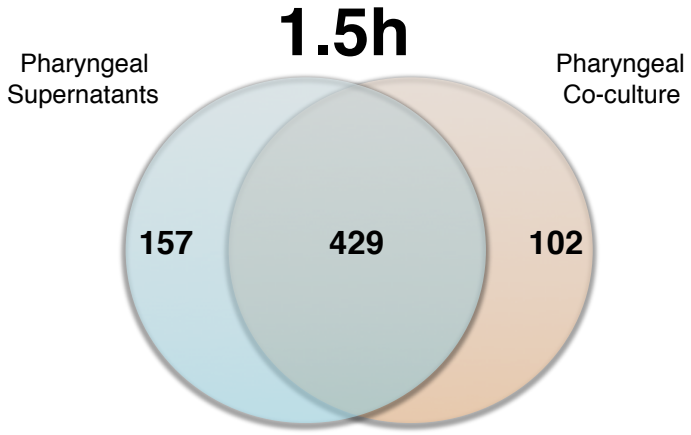
Genes identified as regulated only in the presence of pharyngeal cells were categorized by functional group and compared over the time course (Figure 4.13). In general, the functional categories with the greatest number of differentially expressed genes in response to pharyngeal monolayers mirrored the broader effect of the host environment on streptococcal gene expression. Most genes upregulated within proximity of host cells were involved in metabolism and translation, while genes downregulated were mostly phage encoded or of unknown function.

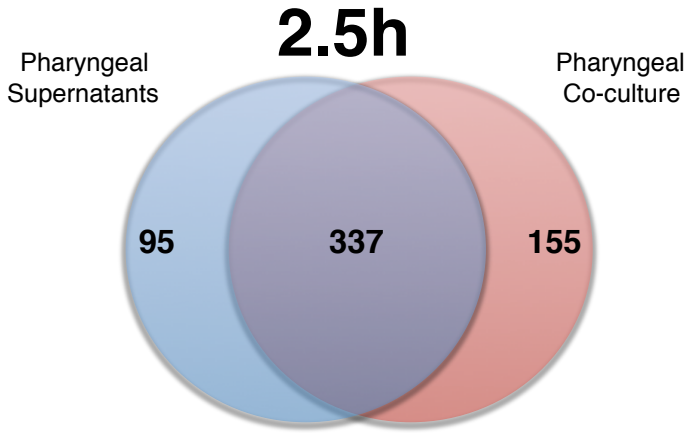
Interestingly, transcription genes regulated in response to host cell monolayers were mostly upregulated at 30 minutes, but subsequently downregulated at 2.5 hours. In addition, virulence factors were upregulated progressively over the time course when streptococci were in co-culture with host cells.

Figure 4.12 Examination of overlap between differentially expressed genes in the two *in vitro* pharyngeal environments

Venn diagrams were created to highlight the high level of overlap between genes exhibiting regulation in response to pharyngeal supernatants and pharyngeal monolayers. Each diagram represents the microarray results from one time point (0.5h, 1.5h, 2.5h) sampled during the experiment.


0.5h
Pharyngeal Supernatants
Pharyngeal Co-culture
131
347
123


1.5h
Pharyngeal Supernatants
Pharyngeal Co-culture
157
429
102


2.5h
Pharyngeal Supernatants
Pharyngeal Co-culture
95
337
155

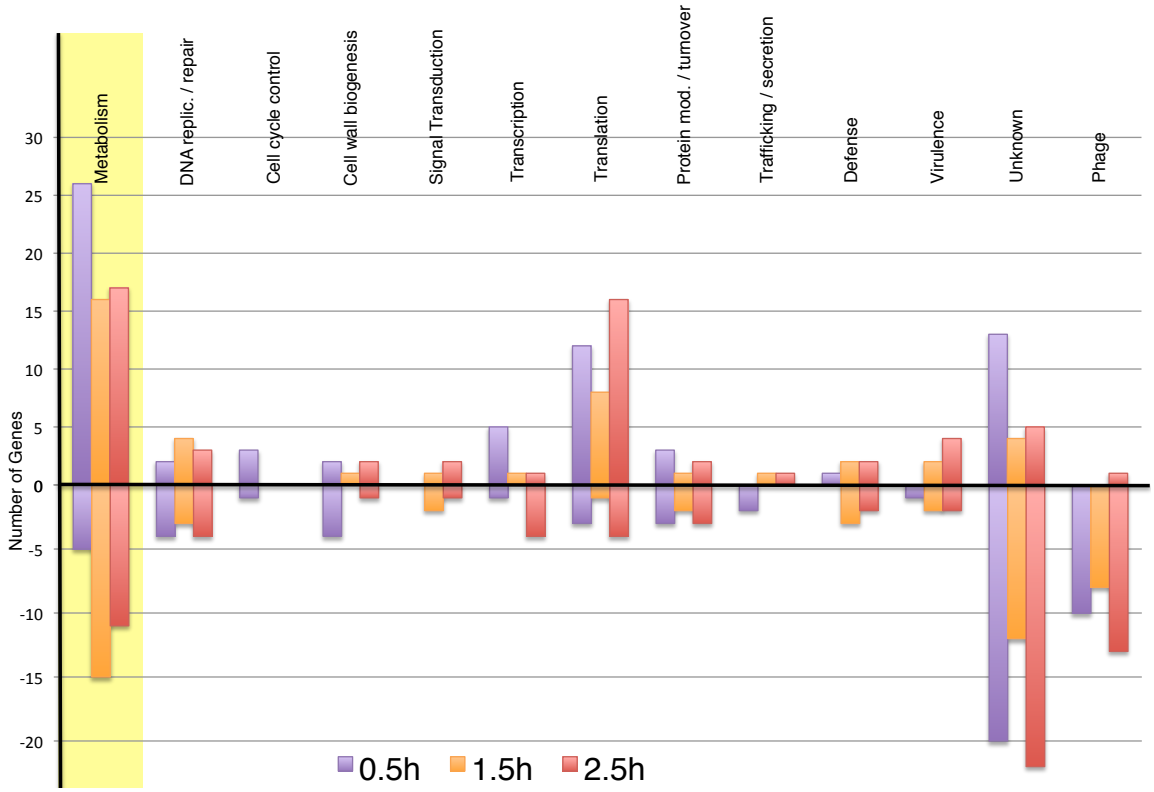


Figure 4.13 COG classification of genes differentially expressed only in the presence of pharyngeal monolayers

Genes are classified into functional groups based on protein homology to characterized genes. Each bar represents the total number of genes that were differentially upregulated (positive value relative to x-axis) or downregulated (negative value relative to x-axis) in SF370 only when co-cultured with pharyngeal monolayers for 0.5h (purple), 1.5h (orange), and 2.5h (red). The area of the graph shaded yellow includes all functional categories involved in cellular metabolism.

4.2.5 Comparison of the streptococcal transcriptome over time for each *in vitro* host environment

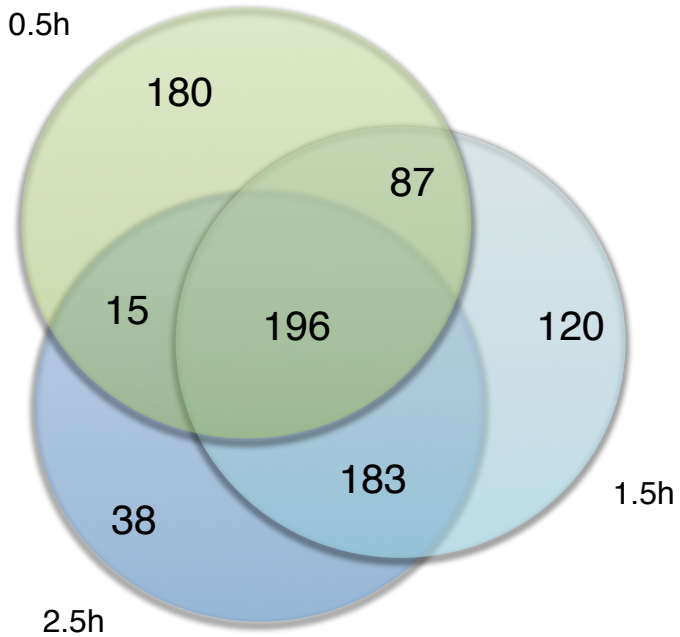
Genes differentially expressed under each experimental condition were examined for temporal patterns in regulation. Using Venn diagrams to visualize the degree of overlap in differentially regulated genes between the various time points for each pharyngeal environment, it became clear that streptococci modulate expression of a core set of genes throughout the incubation period (Figure 4.14). This was true for streptococci exposed to pharyngeal supernatants (196 genes), as well as streptococci co-cultured with monolayers (226 genes). The core set of regulated genes accounts for one third to one half of all genes differentially expressed at any one time point.

Examining genes regulated at two of the three time points, it became clear that streptococci have similar transcriptomes at 1.5 and 2.5 hours post-exposure to pharyngeal supernatants or in co-culture with pharyngeal monolayers. Streptococci studied early in these interactions at 30 minutes exhibited the greatest number of unique differentially expressed genes in response to both *in vitro* host environments. As one might expect, the least amount of overlap was observed between genes regulated at the earliest (30 minutes) and latest (2.5 hours) time points.

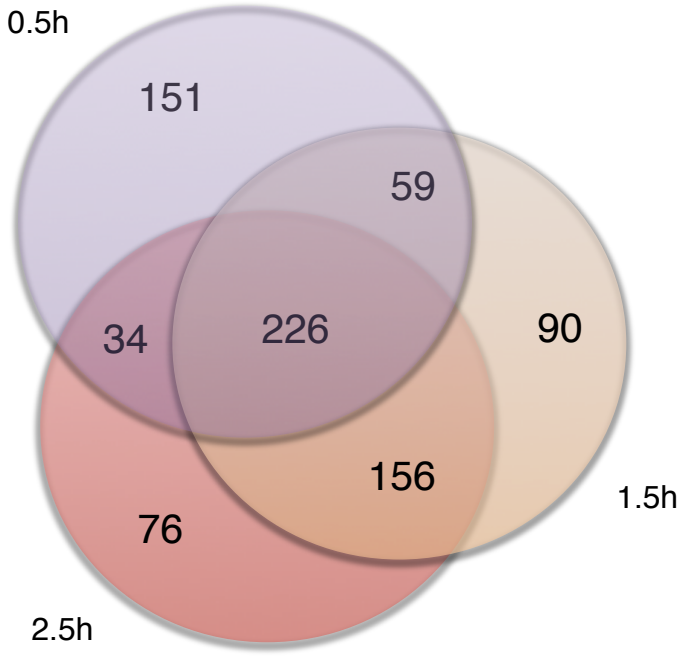
Figure 4.14 Comparison of genes differentially regulated at each time point during exposure to host supernatants or host cells

Venn diagrams were created to highlight the distribution of differentially regulated genes over the three time periods (0.5h, 1.5h, 2.5h) in response to either pharyngeal supernatants (top) or pharyngeal monolayers (bottom). For each diagram, the numbers correspond to genes regulated at all time points ('core' genes in that host environment), as well as genes regulated in only one or two of the three sampled time points.

Pharyngeal supernatants



Pharyngeal monolayers



4.2.6 Identification of genes undergoing divergent regulation over time or across experimental conditions

Despite the large-scale transcriptional responses of streptococci to the host environment, it was striking that few genes were divergently regulated from one time point to another, or between *in vitro* host environments. In fact, of the hundreds of genes differentially expressed at multiple time points and/or in response to both pharyngeal supernatants and monolayers, only 21 genes exhibited a change in the direction of their regulation (Table 4.2).

The *pyrR* regulator of pyrimidine metabolism was upregulated 2-fold over the control condition at 30 minutes in both host environments. By the 1.5-hour mark, the regulator was repressed 2.3-fold below the control under both experimental conditions. In addition, the putative transcriptional regulator *spy1427* was upregulated after 30 minutes in pharyngeal supernatants, but was downregulated in the presence of pharyngeal cells after 2.5 hours. Another interesting change was the phosphotransferase system gene *spy1373*, which was upregulated within 30 minutes and then downregulated by 2.5 hours in both pharyngeal environments.

One of the genes encoding a protein involved in Streptolysin S production was downregulated in both host environments within the first 30 minutes. In the absence of pharyngeal monolayers, this gene was subsequently upregulated at 2.5 hours. Finally, the putative adhesin *adcA* was upregulated after 30 minutes exposure to both pharyngeal supernatants and pharyngeal monolayers. In the

absence of host cells, however, the surface protein was downregulated at 2.5 hours.

Table 4.2 Genes exhibiting changes in direction of regulation over time or across experimental conditions (Pages 160-162)

		Pharyngeal Supernatants						Pharyngeal Monolayers					
		0.5 h		1.5 h		2.5 h		0.5 h		1.5 h		2.5 h	
Locus Tag	Gene Name	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change
spy0224		<0.001	1.04	*ns*		0.015	-0.90	0.004	0.75	*ns*		0.009	-0.92
spy0503		0.044	0.68	*ns*		*ns*		*ns*		*ns*		0.005	-0.81
spy0505		0.001	1.04	*ns*		*ns*		*ns*		*ns*		0.002	-0.96
spy0512		0.001	0.83	*ns*		0.043	-0.98	*ns*		0.006	-1.31	0.005	-1.44
spy0513		0.001	0.93	*ns*		*ns*		*ns*		*ns*		0.004	-1.17
spy0646		*ns*		0.036	0.69	*ns*		0.014	-0.83	*ns*		*ns*	
spy0714	*adcA*	0.001	1.08	*ns*		0.013	-1.24	<0.001	1.37	*ns*		*ns*	
spy0739	*sagB*	0.003	-1.21	*ns*		0.009	1.31	0.030	-0.71	*ns*		*ns*	
spy0830	*pyrR*	0.001	1.40	0.007	-1.24	0.001	-2.35	0.008	0.94	0.001	-1.36	*ns*	
spy0835	*carB*	0.015	1.50	*ns*		0.008	-2.00	0.004	1.61	*ns*		*ns*	
spy0870	*fms*	0.006	-0.73	0.037	0.93	*ns*		*ns*		*ns*		*ns*	
spy0885	*clpX*	0.009	0.66	*ns*		0.009	-1.01	*ns*		*ns*		*ns*	
spy0944		<0.001	-0.83	0.002	-0.85	*ns*		<0.001	-1.25	*ns*		0.042	0.82
spy1131		0.027	-0.74	*ns*		0.004	1.15	0.025	-0.82	*ns*		*ns*	
spy1373		0.016	0.82	*ns*		0.002	-0.91	0.018	0.87	*ns*		0.033	-0.88
spy1425		0.001	0.85	*ns*		*ns*		*ns*		*ns*		0.008	-1.38
spy1427		<0.001	1.28	*ns*		*ns*		*ns*		*ns*		0.005	-1.29
spy1849	*pfl*	0.001	-1.56	*ns*		*ns*		*ns*		<0.001	2.23	<0.001	2.37

Table 4.2 Genes exhibiting changes in direction of regulation over time or across conditions

		Pharyngeal Supernatants						Pharyngeal Monolayers					
		0.5 h		1.5 h		2.5 h		0.5 h		1.5 h		2.5 h	
spy1871	*rpsN2*	0.007	1.57	0.007	-1.31	<0.001	-3.07	0.003	1.77	*ns*		0.026	-1.50
spy2034		0.021	0.76	0.004	-0.96	*ns*		*ns*		<0.001	-2.13	0.002	-2.28
spy2205		0.002	-0.83	0.026	0.84	0.003	1.05	*ns*		*ns*		*ns*	

ns - not significant

Table 4.2 Genes exhibiting changes in direction of regulation over time or across conditions

Locus Tag	Gene Name	COG	Known or proposed function
spy0224		Cell wall biogenesis	Putative UDP-glucose pyrophosphorylase
spy0503		Transcription	Putative RNase
spy0505		Posttranslational modication / protein turnover	Putative glutamine cyclotransferase
spy0512		Energy production / conversion	Putative NAD(P)H-flavin oxidoreductase
spy0513		Amino acid metabolism	Putative dipeptidase
spy0646		Function unknown	Putative hydrolase
spy0714	*adcA*	Inorganic ion metabolism	Zinc transport system; putative adhesin
spy0739	*sagB*	Virulence	Streptolysin S posttranslational modification
spy0830	*pyrR*	Nucleotide metabolism	Pyrimidine regulatory protein
spy0835	*carB*	Amino acid metabolism	Carbamoyl-phosphate synthase (large subunit)
spy0870	*fms*	Translation	Peptide deformylase
spy0885	*clpX*	Posttranslational modication / protein turnover	ATP-dependent protease
spy0944		Function unknown	Hypothetical protein
spy1131		Function unknown	Hypothetical protein
spy1373		Carbohydrate metabolism	Phosphotransferase system
spy1425		Function unknown	Hypothetical protein (transmembrane)
spy1427		Function unknown	Putative transcriptional regulator
spy1849	*pfl*	Energy production / conversion	Pyruvate formate-lyase
spy1871	*rpsN2*	Translation	Ribosomal protein S14
spy2034		Function unknown	Hypothetical protein
spy2205		Carbohydrate metabolism	Glucose uptake protein

4.2.7 Impact of *in vitro* pharyngeal environments on virulence factor expression

Regulation of virulence factor expression in response to both pharyngeal environments was a mosaic of condition-dependent and/or time-dependent events (Table 4.3). Overall, there were more virulence genes repressed in the host environment. A number of virulence factors were only regulated in the absence of pharyngeal cells. The gene for the sortase protein responsible for assembly of the streptococcal pilus was downregulated after 30 minutes in filtered supernatants, as were the genes for the O-GlcNase *hyl*, and hyaluroindase (*hylA*).

The bacteria restricted to pharyngeal supernatants also specifically modulated expression of genes involved in interactions with the immune system. At 30 minutes post-exposure to pharyngeal supernatants, streptococci upregulated *isp*, which encodes a secreted immunogenic protein, and downregulated *sibA*, which is responsible for a secreted Ig-binding protein. At 1.5 hours, superantigen *speJ* was downregulated, but the cell-surface nuclease *spnA* was upregulated.

In streptococci co-cultured with pharyngeal monolayers, the presence of host cells specifically stimulated activation of *emm* (M protein) and the phage-encoded DNase *spd1* at 2.5 hours. In addition, bacteria interacting with host cell monolayers downregulated the phage-encoded superantigen *speI* after 30 minutes. The largest effects of gene activation were exhibited by the secreted protease *speB* (64-fold), the surface-bound histidine triad protein *htpA* (10-fold),

and the C5a peptidase (8-fold). Data were not available on the expression of these genes in all conditions, however, so it is not possible to assert whether these large upregulations were truly confined to the co-culture setting.

Other genes were modulated in both *in vitro* host environments, but exhibited time-dependent regulation. The hemolysin *hlyX* and secreted Ig protease *ideS* were upregulated at various time points in both experimental conditions. Genes for CAMP factor (*cfa*), hemolysin (*hlyIII*), the salvaricin lantibiotic (*salA*), another secreted immunogenic protein (*isp2*), streptokinase (*ska*), the chromosomally encoded DNase and superantigen *speF* (or *spd*), and streptococcal inhibitor of complement (*sic*) were all downregulated in response to both pharyngeal environments. Phage encoded virulence determinants were similarly repressed, including the superantigen *speH*, the DNase *spd3*, and the hyaluronidase *hylP3*. Greatest repression was exhibited by *speF* (up to 10-fold), *spd3* (nearly 5-fold), and *salA* (over 4-fold).

The genes of the streptolysin S operon (*spy0738-0746*) were highly regulated in both pharyngeal environments. Most genes were upregulated at the later time points (1.5 hours and 2.5 hours), with only *sagB* exhibiting downregulation (confined to 30 minutes and reversed by 2.5 hours in pharyngeal supernatants). Although the genes for processing and secreting the streptolysin S propeptide were upregulated up to 10-fold (*sagH* at 2.5 hour in co-culture) late in the experiment, the gene actually encoding the propeptide *sagA* was only

modestly upregulated (1.5-fold), and this response was limited to pharyngeal supernatants.

Table 4.3 Differential expression of virulence factors in SF370 during exposure to *in vitro* host environments (Pages 166-170)

Chromosomally Encoded

		Pharyngeal Supernatants						Pharyngeal Monolayers					
		0.5 h		1.5 h		2.5 h		0.5 h		1.5 h		2.5 h	
Locus Tag[1]	Gene Name	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change
spy0019	*sibA*	0.009	-0.74	*ns*		*ns*		*ns*		*ns*		*ns*	
spy0127	*sipA1*	*ns*		*ns*		*ns*		*ns*		*ns*		*ns*	
spy0128		**		**		**		**		**		**	
spy0129	*srtC1*	0.016	-0.76	*ns*		*ns*		*ns*		*ns*		*ns*	
spy0130		**		**		**		**		**		**	
spy0165	*nga*	*ns*		**		**		**		**		**	
spy0167	*slo*	*ns*		*ns*		*ns*		*ns*		*ns*		*ns*	
spy0212	*speG*	*ns*		*ns*		*ns*		*ns*		*ns*		*ns*	
spy0274	*sdh/plr*	**		**		**		**		**		**	
spy0378	*hlyX*	*ns*		0.050	0.68	*ns*		*ns*		0.049	0.80	0.043	0.75
spy0380		*ns*		0.002	-0.92	0.002	-0.92	*ns*		0.037	-0.71	*ns*	
spy0390	*ideS*	0.020	0.86	0.001	1.72	0.013	1.44	*ns*		*ns*		< 0.001	2.08
spy0416	*spyCEP*	**		**		**		**		**		**	
spy0428	*spyA*	< 0.001	-1.15	0.024	-1.26	*ns*		0.005	-1.39	*ns*		*ns*	
spy0436	*speJ*	*ns*		0.010	-1.55	*ns*		*ns*		*ns*		*ns*	
spy0470	*mcrA*	*ns*		*ns*		*ns*		*ns*		*ns*		*ns*	
spy0591		*ns*		*ns*		*ns*		*ns*		*ns*		*ns*	
spy0605	*bsa/gpoA*	< 0.001	1.21	*ns*		*ns*		< 0.001	1.36	*ns*		*ns*	

Table 4.3 Differential expression of virulence factors in SF370 during exposure to *in vitro* host environments

		Pharyngeal Supernatants						Pharyngeal Monolayers					
		0.5 h		1.5 h		2.5 h		0.5 h		1.5 h		2.5 h	
spy0737	*epf*	**		**		**		**		**		**	
spy0738	*sagA*	**		0.037	0.57	*ns*		**		*ns*		*ns*	
spy0739	*sagB*	0.003	-1.21	*ns*		0.009	1.31	0.030	-0.71	*ns*		*ns*	
spy0740	*sagC*	*ns*		0.049	0.82	*ns*		*ns*		*ns*		0.026	2.10
spy0741	*sagD*	*ns*		0.044	1.11	*ns*		*ns*		0.020	1.65	*ns*	
spy0742	*sagE*	*ns*		*ns*		*ns*		*ns*		*ns*		*ns*	
spy0743	*sagF*	*ns*		*ns*		**		*ns*		0.007	2.42	*ns*	
spy0744	*sagG*	*ns*		< 0.001	2.23	**		*ns*		0.05	2.72	0.003	2.94
spy0745	*sagH*	*ns*		*ns*		**		*ns*		**		0.005	3.44
spy0746	*sagI*	*ns*		*ns*		0.029	1.65	*ns*		0.042	1.19	0.008	2.07
spy0747	*spnA*	*ns*		0.027	1.07	*ns*		*ns*		*ns*		*ns*	
spy0861	*sib35*	**		**		**		**		**		**	
spy1013	*fbp/fbp54*	*ns*		*ns*		*ns*		*ns*		*ns*		*ns*	
spy1032	*hylA*	0.027	-0.63	*ns*		*ns*		*ns*		*ns*		*ns*	
spy1054	*sclB/scl2*	*ns*		*ns*		*ns*		*ns*		*ns*		*ns*	
spy1159	*hlyIII*	*ns*		*ns*		< 0.001	-1.61	*ns*		0.001	-1.35	< 0.001	-1.58
spy1273	*cfa*	0.022	-0.88	**		**		0.004	-1.38	0.013	-0.79	**	
spy1302	*amyA*	*ns*		*ns*		*ns*		*ns*		*ns*		*ns*	
spy1309	*dltD*	*ns*		*ns*		*ns*		*ns*		*ns*		*ns*	
spy1310	*dltC*	*ns*		*ns*		*ns*		*ns*		*ns*		*ns*	

Table 4.3 Differential expression of virulence factors in SF370 during exposure to *in vitro* host environments

		Pharyngeal Supernatants						Pharyngeal Monolayers					
		0.5 h		1.5 h		2.5 h		0.5 h		1.5 h		2.5 h	
spy1311	*dltB*	*ns*		*ns*		*ns*		*ns*		*ns*		*ns*	
spy1312	*dltA*	*ns*		*ns*		*ns*		*ns*		*ns*		*ns*	
spy1357	*grab*	**		**		**		**		*ns*		**	
spy1361	*slr*	**		**		**		**		**		**	
spy1497	*hylA1*	*ns*		*ns*		*ns*		*ns*		*ns*		*ns*	
spy1600	*hyl*	0.004	-1.18	*ns*		*ns*		*ns*		*ns*		*ns*	
spy1798	*shr*	*ns*		**		**		**		*ns*		**	
spy1801	*isp2*	*ns*		0.004	-0.94	*ns*		*ns*		< 0.001	-1.29	0.007	-1.24
spy1813	*endoS*	*ns*		**		**		*ns*		**		**	
spy1851		*ns*		*ns*		*ns*		*ns*		*ns*		*ns*	
spy1896	*ropA/tig*	0.001	0.75	< 0.001	1.10	0.006	1.01	0.009	0.87	0.001	1.10	0.003	1.25
spy1911	*salY*	*ns*		*ns*		*ns*		*ns*		*ns*		*ns*	
spy1915	*salA*	0.025	-0.85	< 0.001	-1.67	< 0.001	-1.92	*ns*		0.00	-2.11	< 0.001	-1.97
spy1972	*pulA*	**		**		**		**		**		**	
spy1979	*ska*	0.027	-0.97	< 0.001	-1.81	*ns*		0.001	-1.38	0.001	-1.79	*ns*	
spy1983	*sclA/scl1*	**		**		**		**		**		**	
spy1998	*smeZ*	**		**		**		**		**		**	
spy2006	*htpA*	**		**		**		< 0.001	3.37	**		*ns*	
spy2007	*lmb/lsp*	**		**		**		**		**		**	
spy2009	*fbaA*	**		**		**		**		**		**	

Table 4.3 Differential expression of virulence factors in SF370 during exposure to *in vitro* host environments

		Pharyngeal Supernatants						Pharyngeal Monolayers					
		0.5 h		1.5 h		2.5 h		0.5 h		1.5 h		2.5 h	
spy2010	*scpA*	*ns*		**		**		*ns*		0.03	3.07	**	
spy2016	*sic*	0.008	-0.58	0.004	-0.72	*ns*		0.020	-0.67	*ns*		*ns*	
spy2018	*emm1*	*ns*		*ns*		*ns*		*ns*		*ns*		0.027	0.69
spy2025	*isp*	0.028	0.73	*ns*		*ns*		*ns*		*ns*		*ns*	
spy2039	*speB*	**		**		**		**		**		< 0.001	6.02
spy2043	*speF/spd*	*ns*		< 0.001	-2.80	< 0.001	-3.31	*ns*		< 0.001	-2.11	0.001	-1.25
spy2200	*hasA*	**		**		**		**		**		**	
spy2201	*hasB*	**		**		**		**		**		**	
spy2202	*hasC*	*ns*		*ns*		**		*ns*		*ns*		*ns*	
spy2216	*htrA*	*ns*		*ns*		*ns*		*ns*		*ns*		*ns*	

ns - not significant

** - raw intensity data from microarrays did not pass quality filters for inclusion in statistical analysis

Table 4.3 Differential expression of virulence factors in SF370 during exposure to *in vitro* host environments

Phage Encoded

		Pharyngeal Supernatants						Pharyngeal Monolayers					
		0.5 h		1.5 h		2.5 h		0.5 h		1.5 h		2.5 h	
Locus Tag[1]	Gene Name	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change
spy0701	*hylP1*		*ns*		*ns*		**		*ns*		**		**
spy0711	*speC*		*ns*		*ns*		*ns*		*ns*		*ns*		*ns*
spy0712	*spd1/mf2*		*ns*		*ns*		*ns*		*ns*		*ns*	0.04	1.25
spy0997	*hylP2*		**		**		**		**		**		**
spy1007	*speI*		*ns*		*ns*		*ns*	0.019	-0.89		*ns*		*ns*
spy1008	*speH*	0.001	-0.85	0.005	-1.12		*ns*	< 0.001	-1.26	0.005	-1.30	0.009	-1.02
spy1436	*spd3/mf3*	0.010	-0.83	< 0.001	-2.00	0.00	-1.90	0.102	-1.17	< 0.001	-2.29	< 0.001	-1.84
spy1445	*hylP3*		*ns*	0.015	-1.15		*ns*		*ns*	0.003	-1.48	0.006	-1.43

ns - not significant

** - raw intensity data from microarrays did not pass quality filters for inclusion in statistical analysis

4.2.8 Impact of *in vitro* pharyngeal environments on expression of streptococcal transcriptional regulators

The well-characterized SF370 stand-alone regulators were largely unaffected at the transcriptional level by either host environment (Table 4.4). The pilus region activator *rofA* was the only transcription factor affected under all conditions and at all time points (repressed up to 2.8-fold). Repression of additional characterized regulators was only evident at 30 minutes. The metal homeostasis regulator *mtsR* was downregulated in both experimental conditions, while the RALP *rivR* was only downregulated in the co-culture setting.

Activation of regulator expression was exhibited *amrA, vfr,* and *relA* in both host environments. The gene for *ropB* (activator of *speB* expression) was only significantly upregulated in the pharyngeal supernatants at 2.5 hours. Noticeably absent were any effects on expression of *mga*, the streptococcal global virulence regulator.

In contrast, nearly two dozen uncharacterized stand-alone regulators were modulated in response to one or both host environments (Table 4.4). Expression of these genes was consistent over time, such that a transcription factor upregulated at 30 minutes would also be upregulated at later time points. The regulator *spy0715* was only repressed in pharyngeal supernatants, while *spy1535* was activated in this setting alone. The only regulator whose modulation was confined to the co-culture setting was *spy1818* (repressed at 2.5 hours).

Various two-component systems were identified in the transcriptional screen (Table 4.5). Three systems were only regulated early during host environment interactions (30 minutes). The *covRS* and *ciaHR* regulators were activated in response to both pharyngeal environments, while *ihk-irr* was only upregulated following exposure to pharyngeal supernatants.

Regulation of two-component systems at the later time points (1.5 and 2.5 hours) was confined to repression. The *salRS* system was downregulated in both environments. The TCS encoded by *spy1621-1622* was only downregulated at 1.5 hours in the co-culture setting. Alternatively, *sptRS* and *srtRK* were only downregulated at 1.5 hours following exposure to pharyngeal supernatants.

Table 4.4 Differential expression of stand-alone regulators in SF370 during exposure to *in vitro* host environments (Pages 173-176)

Recognized Virulence Regulators

		Pharyngeal Supernatants						Pharyngeal Monolayers					
		0.5 h		1.5 h		2.5 h		0.5 h		1.5 h		2.5 h	
Locus Tag	Gene Name	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change
spy0124	*rofA*	<0.001	-0.77	0.005	-0.84	0.0023	-1.18	0.002	-0.85	0.023	-1.16	<0.001	-1.49
spy0188	*vlg*	*ns*		*ns*		*ns*		*ns*		*ns*		*ns*	
spy0216	*rivR*	*ns*		*ns*		**		0.030	-1.03	*ns*		*ns*	
spy0450	*mtsR*	0.026	-0.87	*ns*		*ns*		<0.001	-1.05	*ns*		*ns*	
spy0514	*ccpA*	*ns*		*ns*		*ns*		*ns*		*ns*		*ns*	
spy0797	*amrA*	0.028	0.75	0.020	1.23	*ns*		0.011	1.57	*ns*		*ns*	
spy0887	*vfr*	*ns*		0.028	0.78	*ns*		*ns*		0.023	1.01	*ns*	
spy1293	*malR*	**		**		**		**		**		**	
spy1531	*perR*	**		**		**		**		*ns*		**	
spy1605	*rocA*	*ns*		*ns*		*ns*		*ns*		*ns*		*ns*	
spy1642	*luxS*	*ns*		*ns*		*ns*		*ns*		*ns*		*ns*	
spy1704	*lacD.1*	*ns*		**		**		**		**		**	
spy1777	*codY*	*ns*		*ns*		*ns*		*ns*		*ns*		*ns*	
spy1857	*srv*	*ns*		*ns*		*ns*		*ns*		*ns*		*ns*	
spy1981	*relA*	*ns*		0.002	0.92	0.0497	0.94	*ns*		0.013	1.01	**	
spy2019	*mga*	*ns*		*ns*		*ns*		*ns*		*ns*		*ns*	
spy2042	*ropB/rgg*	*ns*		*ns*		0.048	0.64	*ns*		*ns*		*ns*	

ns - not significant

** - raw intensity data from microarrays did not pass quality filters for inclusion in statistical analysis

Table 4.4 Differential expression of stand-alone regulators in SF370 during exposure to *in vitro* host environments

Putative and/or Uncharacterized Regulators

		Pharyngeal Supernatants						Pharyngeal Monolayers					
		0.5 h		1.5 h		2.5 h		0.5 h		1.5 h		2.5 h	
Locus Tag	Gene Name	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change
spy0164	*nusG*	<0.001	1.14	0.034	1.40	*ns*		0.004	1.71	0.009	1.65	*ns*	
spy0181		*ns*		0.003	-1.34	*ns*		*ns*		0.009	-1.36	0.006	-1.27
spy0338	*nrdR*	0.001	-0.73	*ns*		*ns*		0.018	-0.79	*ns*		*ns*	
spy0627	*regR*	0.007	-0.84	0.005	-0.75	0.005	-0.99	<0.001	-1.20	<0.001	-1.46	<0.001	-1.52
spy0715		*ns*		0.004	-1.13	*ns*		*ns*		*ns*		*ns*	
spy0853		**		**		**		**		**		0.021	2.72
spy1081	*srtR*	**		0.003	-1.06	0.029	-0.88	**		0.034	-1.03	**	
spy1198		**		0.034	-0.96	0.005	-1.24	**		0.007	-1.19	0.006	-1.34
spy1215		*ns*		0.001	1.23	<0.001	1.58	0.001	0.96	<0.001	1.75	0.005	1.61
spy1325		*ns*		0.001	-2.18	0.045	-2.50	*ns*		0.007	-1.94	*ns*	
spy1535		*ns*		0.037	0.78	*ns*		*ns*		*ns*		*ns*	
spy1596		**		0.001	-1.72	0.002	-1.91	**		0.002	-1.66	0.031	-1.76
spy1602		0.001	-0.79	*ns*		*ns*		0.022	-0.74	*ns*		*ns*	
spy1699		0.005	-1.03	*ns*		*ns*		<0.001	-1.24	*ns*		*ns*	
spy1717	*copY*	<0.001	1.76	*ns*		*ns*		*ns*		*ns*		*ns*	
spy1733		*ns*		0.001	1.12	<0.001	1.52	*ns*		0.005	1.93	0.003	1.89
spy1755		*ns*		0.001	1.24	0.013	0.78	*ns*		0.011	1.27	0.034	1.01

Table 4.4 Differential expression of stand-alone regulators

		Pharyngeal Supernatants						Pharyngeal Monolayers					
		0.5 h		1.5 h		2.5 h		0.5 h		1.5 h		2.5 h	
spy1763	*hrcA*	0.018	-0.67	<0.001	-1.99	0.003	-2.00	<0.001	-0.96	<0.001	-2.17	<0.001	-2.40
spy1818	*nusB*	*ns*		*ns*		*ns*		*ns*		*ns*		0.038	-0.84
spy2053		*ns*		0.001	-1.57	0.001	-1.94	*ns*		0.008	-1.40	0.016	-1.99
spy2054		0.001	-1.03	0.002	-1.36	0.002	-1.46	0.003	-1.15	*ns*		*ns*	
spy2074	*ctsR*	*ns*		0.001	0.91	*ns*		*ns*		0.007	0.87	0.042	0.75
spy2172		0.009	-0.82	0.002	-1.33	0.011	-1.47	*ns*		<0.001	-1.72	0.020	-1.59

ns - not significant

** - raw intensity data from microarrays did not pass quality filters for inclusion in statistical analysis

Table 4.5 Differential expression of two-component regulatory systems in SF370 during exposure to *in vitro* host environments

		Pharyngeal Supernatants						Pharyngeal Monolayers					
		0.5 h		1.5 h		2.5 h		0.5 h		1.5 h		2.5 h	
Locus Tag	Gene Name	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change
spy0242-0245	*fasB-fasC-fasA*	*ns*		*ns*		*ns*		*ns*		*ns*		*ns*	
spy0336-0337	*covR-covS*	<0.001	1.55	*ns*		*ns*		<0.001	1.45	*ns*		*ns*	
spy0528-0529	*vicRK/sycFG*	*ns*		*ns*		*ns*		*ns*		*ns*		*ns*	
spy0874-0875	*sptR-sptS*	*ns*		0.002	-0.86	*ns*		*ns*		*ns*		*ns*	
spy1061-1062		*ns*		*ns*		*ns*		*ns*		*ns*		*ns*	
spy1081-1082	*srtRK*	*ns*		0.003	-1.06	*ns*		*ns*		*ns*		*ns*	
spy1106-1107		*ns*		**		**		*ns*		**		**	
spy1236-1237	*ciaHR*	0.002	0.89	**		**		<0.001	1.38	**		**	
spy1553-1556		*ns*		*ns*		*ns*		*ns*		*ns*		*ns*	
spy1587-1588	*trxR-trxS*	*ns*		*ns*		*ns*		*ns*		*ns*		*ns*	
spy1621-1622		*ns*		*ns*		*ns*		*ns*		0.036	-0.74	*ns*	
spy1908-1909	*salR-salS*	*ns*		0.001	-1.20	0.0028	-1.24	*ns*		<0.001	-1.48	0.001	-1.54
spy2026-2027	*ih-irr*	0.002	0.75	*ns*		*ns*		*ns*		*ns*		*ns*	

ns - not significant

** - raw intensity data from microarrays did not pass quality filters for inclusion in statistical analysis

4.2.9 Comparison of differentially regulated genes during association to genes regulated in adherence

Of the 79 genes previously identified in our laboratory as being differentially expressed upon adherence to pharyngeal cells (Ryan, *et al.* 2007), 55 genes were also differentially regulated during association with the pharyngeal environments (Table 4.6). In general, these genes could be separated into two groups: ORFs exhibiting regulation in the same direction during association and adherence (33 genes), and those exhibiting divergent expression changes in association as compared to adherence (22 genes).

The virulence genes identified as differentially regulated in the two infection stages were all divergently regulated. The pilus sortase gene was upregulated in adherent bacteria, but downregulated in streptococci exposed to pharyngeal supernatants for 30 minutes. The secreted protease *speB* was downregulated in adherent bacteria, but highly upregulated in streptococci associated with pharyngeal monolayers. Finally, the phage encoded superantigen *speH* was upregulated in adherence, but downregulated during association.

Numerous stand-alone regulators and two-component systems were modulated in response to both infection stages. The regulator *ropB*, the TCS *ciaHR*, and the putative regulator *spy1215* were all upregulated in both adherent and associated streptococci. For *spy0583, spy2115* (*spx*), and the two-

component histidine kinase *spy1622*, expression was repressed during streptococcal association, but activated in adherent bacteria.

Table 4.6 Genes differentially expressed in associated and adherent streptococci (Pages 179-185)

Genes regulated in the same direction at both infection stages

	Pharyngeal supernatants			Pharyngeal monolayers			Adherent
Locus Tag	0.5h	1.5h	2.5h	0.5h	1.5h	2.5h	2.5h
spy0015		1.74	1.55	1.23	2.18	2.03	1.68
spy0226	1.53			1.06			1.40
spy0475	0.99			0.76			1.09
spy0646		0.69		-0.83			1.32
spy0757		0.99				1.27	1.80
spy0759	1.00	1.72	2.31	1.29	2.31	2.13	1.66
spy0760	1.01	1.69	2.00	1.41	2.09	1.80	1.62
spy0761	1.09	1.82	2.17	1.52	2.27	2.10	1.88
spy0880	0.95	2.33	2.73	1.76	2.77		1.26
spy0881				0.91			1.32
spy0901		-1.11	-1.66		-0.95		-1.95
spy0940	-1.17	-2.40	-2.44	-1.61	-2.21	-2.79	-2.88
spy0947	-1.14	-2.07	-1.92	-1.46	-1.92	-2.40	-2.90
spy0952	-0.97	-2.01	-1.83	-1.40	-1.84	-2.34	-3.36
spy0956	-0.78	-1.92	-1.73	-1.28	-1.75	-2.21	-3.40
spy0958	-0.72	-1.71	-1.69		-1.45	-1.84	-3.12
spy0961		-1.49				-1.68	-2.76
spy0962	-0.86	-1.82	-1.75	-1.23	-1.65	-2.22	-2.57

Table 4.6 Genes differentially expressed in associated and adherent streptococci

	Pharyngeal supernatants			Pharyngeal monolayers			Adherent
Locus Tag	0.5h	1.5h	2.5h	0.5h	1.5h	2.5h	2.5h
spy0963	-0.99	-1.78	-1.86	-1.25		-1.94	-3.53
spy0965	-0.88	-1.92	-1.87	-1.13		-1.94	-3.56
spy0967	-0.88	-1.97	-2.20	-1.34		-2.78	-2.32
spy1180			-1.31				-1.51
spy1214		0.96	1.01		1.15	1.17	2.18
spy1215		1.23	1.58	0.96	1.75	1.61	1.99
spy1236	0.85			1.38			2.48
spy1237	0.89			1.44			2.06
spy1277	-0.74			-0.95			-2.45
spy1639		0.98			1.31		1.14
spy1936	-0.63						-1.97
spy1958	0.60		1.75			1.83	1.88
spy2042			0.64				1.85
spy2047	-0.55	-1.64	-2.01		-1.58	-2.13	-1.62
spy2048		-1.61	-2.10	-0.79	-1.65		-1.54

Table 4.6 Genes differentially expressed in associated and adherent streptococci

Genes regulated in opposite directions in response to each infection stage

	Pharyngeal supernatants			Pharyngeal monolayers			Adherent
Locus Tag	0.5h	1.5h	2.5h	0.5h	1.5h	2.5h	2.5h
spy0129	-0.76						2.60
spy0160	1.70			1.26			-1.35
spy0251	-0.59			-0.87			1.17
spy0583	-0.86	-2.19	-2.38	-1.07	-2.47	-1.71	2.28
spy0840	0.92	1.44	1.74	1.06	2.09	2.27	-1.17
spy1008	-0.85	-1.12		-1.26	-1.30	-1.02	1.45
spy1097		-0.90	-0.92		-1.02	-1.00	1.71
spy1098		-0.95	-1.06		-1.16	-1.30	1.43
spy1099		-1.11	-1.36		-1.28	-1.31	1.52
spy1622					-0.69		1.15
spy1708		-1.57			-1.86		3.52
spy1710					-1.30		3.86
spy1711	-0.92	-1.69	-1.73	-1.33	-2.12	-1.78	3.63
spy1743	1.73	3.02	1.88	2.70	3.07	1.84	-2.47
spy1745	1.66	3.26	2.35	2.77	3.47	2.44	-2.36
spy1754		1.35		0.81	1.36	1.06	-2.40
spy1916	-0.88		-2.42		-2.20	-2.66	1.96
spy1923					-1.05		1.73
spy2039						6.02	-2.33

Table 4.6 Genes differentially expressed in associated and adherent streptococci

	Pharyngeal supernatants			Pharyngeal monolayers			Adherent
Locus Tag	0.5h	1.5h	2.5h	0.5h	1.5h	2.5h	2.5h
spy2059		-1.30				-1.41	1.41
spy2115			-1.42				2.23
spy2215				-0.75			1.18

Table 4.6 Genes differentially expressed in associated and adherent streptococci

Locus Tag	Gene Function	COG Designation
spy0015	ftsH, cell division protein	Cell cycle control
spy0129	Sortase for pilus	Virulence
spy0160	purA, adenylsuccinate synthetase	Nucleotide metabolism
spy0226	gpsA, glycerol-3-P dehydrogenase	Energy production/conversion
spy0251	N-acetylmannosamine-6-P epimerase	Carbohydrate metabolism
spy0475	dgk, diacylglycerol kinase	Cell envelope biogenesis
spy0583	Hypothetical protein	Transcription
spy0646	Hypothetical protein	Function unknown
spy0757	atpH, proton-translocating ATPase	Energy production/conversion
spy0759	atpG, proton-translocating ATPase	Energy production/conversion
spy0760	atpD, proton-translocating ATPase	Energy production/conversion
spy0761	atpC, proton-translocating ATPase	Energy production/conversion
spy0840	rpsP, 30S ribosomal protein S16	Translation
spy0880	mvaS.1, HMG-CoA	Lipid metabolism
spy0881	mvaS.2, HMG-CoA synthase	Lipid metabolism
spy0901	pyrE, orotate phosphoribosyltransferase	Nucleotide metabolism
spy0940	Phage protein	Function unknown
spy0947	Phage protein	Function unknown
spy0952	Phage protein	Function unknown
spy0956	Phage protein	Function unknown
spy0958	Phage protein	Function unknown

Table 4.6 Genes differentially expressed in associated and adherent streptococci

Locus Tag	Gene Function	COG Designation
spy0961	Phage protein	Function unknown
spy0962	Phage protein	Function unknown
spy0963	Phage protein	Function unknown
spy0965	Phage protein	Function unknown
spy0967	Phage protein	Function unknown
spy1008	speH, superantigen	Virulence
spy1097	folE, GTP cyclohydrolase	Coenzyme metabolism
spy1098	folP, dihydropteroate synthase	Coenzyme metabolism
spy1099	folQ, dihydroneopterin aldolase	Coenzyme metabolism
spy1180	Mg^{2+}/citrate complex transporter	Energy production/conversion
spy1214	lplA, lipoate-protein ligase	Coenzyme metabolism
spy1215	sir2 regulator	Transcription
spy1236	ciaH, histidine kinase	Signal transduction
spy1237	ciaR, response regulator	Signal transduction
spy1277	phnA, alkylphosphonate uptake	Inorganic ion metabolism
spy1622	Histidine kinase	Signal transduction
spy1639	atoA, acetoacetyl-CoA transferase	Lipid metabolism
spy1708	lacA.1, gal-6-P isomerase	Carbohydrate metabolism
spy1710	PTS system, enzyme IIB component	Carbohydrate metabolism
spy1711	PTS system, enzyme IIA component	Carbohydrate metabolism

Table 4.6 Genes differentially expressed in associated and adherent streptococci

Locus Tag	Gene Function	COG Designation
spy1743	accA, acetyl-CoA carboxylase subunit	Lipid metabolism
spy1745	accC, acetyl-CoA carboxylase subunit	Lipid metabolism
spy1754	fabH, beta ketoacyl-ACP synthase III	Lipid metabolism
spy1916	lacG, phospho-beta-D-galactosidase	Carbohydrate metabolism
spy1923	lacA.2, galactosidase acetyltransferase	Carbohydrate metabolism
spy1936	Hypothetical protein	Function unknown
spy1958	def, polypeptide deformylase	Translation
spy2039	speB, secreted cysteine protease	Virulence
spy2042	ropB, transcription regulator	Transcription
spy2047	gldA, glycerol dehydrogenase	Energy production/conversion
spy2048	mipB, transaldolase-like protein	Carbohydrate metabolism
spy2059	pbp2A, penicillin-binding protein	Cell envelope biogenesis
spy2115	spx, transcriptional regulator	Transcription
spy2215	Hypothetical protein	Function unknown

4.2.10 Validation of microarray hits by quantitative real-time RT-PCR

Confirmation of microarray results is currently in progress. Primers and fluorogenic probes have been designed to sections of genes that were differentially regulated (both activated and repressed) at the various time points in both experimental conditions. Genes that were unaltered by the host environment (as indicated by our microarray analysis) were selected as controls for normalization. The QRT-PCR results thus far have validated the upregulation of the putative transcriptional regulator *spy1215* and the downregulation of the phage encoded gene *spy0946* (Table 4.7).

Notably, the *gyrA* and *proS* genes traditionally used for QRT-PCR normalization in *S. pyogenes* were both differentially regulated (with *P* values < 0.05) in our expression analyses (data not shown). These results stress that care should be taken when selecting control genes for QRT-PCR analyses.

Table 4.7 Preliminary validation of microarray data by real-time QRT-PCR

spy0946

	Pharyngeal Supernatants		Pharyngeal Co-culture	
Exposure Time	Microarray Log_2 Fold Change	QRT-PCR Log_2 Fold Change	Microarray Log_2 Fold Change	QRT-PCR Log_2 Fold Change
0.5 hours	-1.33	-1.73	-1.62	-1.95
1.5 hours	-2.31	-2.3	-2.11	-2.16
2.5 hours	-2.02	-1.99	-2.4	-1.31

spy1215

	Pharyngeal Supernatants		Pharyngeal Co-culture	
Exposure Time	Microarray Log_2 Fold Change	QRT-PCR Log_2 Fold Change	Microarray Log_2 Fold Change	QRT-PCR Log_2 Fold Change
0.5 hours	*ns*	0.62	0.96	0.36
1.5 hours	1.23	1.2	1.75	0.84
2.5 hours	1.58	0.7	1.61	0.85

ns - not statistically significant

4.3 Discussion

The transcriptional responses of streptococci to the host *in vitro* exhibit a large-scale, complex regulatory pattern that can be both time-dependent and specific to the particular host environment (pharyngeal supernatants versus monolayers). In general, streptococci in pharyngeal environments actively modulate numerous metabolic pathways and upregulate their translational machinery, as compared to the control in MEM. In combination with our unpublished observations that streptococci proliferate in these pharyngeal environments, the data suggest that exposure to the host-modified nutrient environment, secreted host factors, and host cell receptors induces an adaptive response aimed at colonizing the new host niche.

This adaptive response is most pronounced early in the host-bacterium interaction (30 minutes) when we observed the greatest number of unique genes being regulated. The GrAS transcriptomes at the later time points (1.5 and 2.5 hours) had a greater degree of overlap, perhaps indicating that the streptococci have entered a more steady-state phase of coexistence with host cells. Notably, M protein was only activated after 2.5 hours of exposure to host cells, indicating that there is a lag in the response to the host environment for this major virulence determinant.

Interestingly, few genes were regulated in one direction early during interactions with the pharyngeal environment and in the opposite direction later in time. This indicates that streptococci generally commit to either activating or

repressing individual genes in the host environment over this short period. There were two interesting genes, however, whose expression displayed a more fine-tuned profile. The *spy1373* gene encoding part of a streptococcal phosphotransferase system was initially upregulated in both host environments (30 minutes) and then subsequently downregulated under both experimental conditions (2.5 hours). These systems have been linked to cytoplasmic signaling of extracellular carbon source availability that affects virulence regulation pathways via the stand-alone carbon catabolite repressor CcpA (Leday, *et al.* 2008). Thus, *spy1373* may represent an important environmental sensor in the pharyngeal environment.

The putative adhesin *adcA* was upregulated following a 30-minute exposure to both host environments, but was later repressed only in the absence of host cells. This suggests that streptococci in pharyngeal supernatants actively reduce expression of at least one surface adhesin in the absence of potential host cell binding partners. One could hypothesize that this is a response aimed at lowering the cellular metabolic burden, or it could be a method employed to reduce immunogenicity when host cells are not available for adherence.

In the absence of host cells, the pilus sortase gene was also downregulated. Furthermore, the *rofA* regulator responsible for activating the pilus operon was significantly downregulated at all time points and in both host environments. Although pili have been suggested as mediators of initial host cell interactions (Nobbs, *et al.* 2009), our data indicate that this may not be the case,

since streptococci do not activate pilus production in the host cell milieu. The activation of pilus genes during adherence, however, strongly supports a role for pilus at this second stage of infection in SF370 (Ryan, *et al.* 2007).

Overall, streptococci initiated a program of virulence factor repression in response to the pharyngeal environments. The superantigens *speJ, speI,* and *speH* were all downregulated, perhaps again suggesting that streptococci are actively avoiding induction of host immunity. The virulence determinant exhibiting the greatest activation was the secreted protease *speB*, which was expressed at levels 64-fold greater during co-culture than in the control condition (MEM media).

The dramatic induction of *speB* expression was particularly striking, as *speB* is subsequently downregulated upon adherence to host cells (Ryan, *et al.* 2007). This could indicate that SpeB elaboration is initially advantageous to the bacterium (perhaps for scavenging host peptides, degrading antimicrobial peptides, or releasing surface-bound streptococcal proteins), but is no longer beneficial once the streptococci have bound to the host surface where protease activity could cleave off bound adhesins. Notably, the *speB* activator *ropB* was upregulated in both associated and adherent streptococci (despite the divergence observed for *speB* regulation) further supporting previous observations that *speB* regulation is quite complex (reviewed in Carroll & Musser 2011).

The vast majority of phage genes and their encoded virulence factors were downregulated. Induction of phage encoded genes has been observed in streptococci exposed to professional phagocytic cells, during streptococcal adherence to pharyngeal cells, and in experimental pharyngitis using macaques (Voyich, *et al.* 2003; Virtaneva, *et al.* 2005; Ryan, *et al*. 2007). Phage gene downregulation, however, was recently observed for SF370 internalized by pharyngeal cells when compared to streptococci in minimal media (Agarwal, *et al.* 2012). These studies combined with our analysis indicate that phage gene regulation in the host environment is complex and potentially strain-specific.

Although our laboratory previously reported that the soluble factor SPIF derived from pharyngeal cultures could induce expression of the phage encoded virulence factors *speC* and *spd1*, we did not see comparable induction in response to pharyngeal supernatants (Broudy, *et al.* 2001; Broudy, *et al.* 2003). There could be several explanations for this discrepancy. First of all, the strain SF370 was not used in the original studies, although Φ370.1 is the same element induced in the M6 and M76 strains. This could indicate that phage induction is a strain-specific event, which unpublished observations in our laboratory seem to suggest.

Secondly, phage induction was previously achieved in the M6 and M76 strains using pharyngeal supernatants harvested after only 3 hours of exposure to actively dividing pharyngeal monolayers. In contrast, the supernatants utilized for this analysis were exposed to confluent pharyngeal monolayers for 16 to 18

hours. Consequently, differences in the length of time pharyngeal cells are allowed to use nutrients from the media and secrete waste and metabolites into the MEM may affect the concentration and/or activity of SPIF. Evaluation of this alternative is currently being investigated in our laboratory.

Turning to the mediators of streptococcal transcriptome remodeling, we observed that few of the well-characterized stand-alone regulators were differentially expressed in response to the pharyngeal environments. Noticeably absent were effects on *mga* expression, which is an important regulator of numerous virulence factors in streptococci. The regulator *rivR*, which can enhance gene activation by Mga, was downregulated in the co-culture setting at 1.5 hours (Roberts & Scott 2007). Another gene associated with Mga regulon activation *amrA* was upregulated at 30 minutes in both host settings (Ribardo & McIver 2003). Overall, two virulence factors under positive Mga control (*scpA* and *emm*) were both upregulated in the co-culture environment, highlighting that virulence regulation in the host is multifaceted.

Two stand-alone regulators that exhibited interesting expression patterns were *spy0583* and *spy1215*. The gene *spy0583* has been associated with stress response regulation and is downregulated following a 4-hour incubation in pooled adult saliva from humans (Shelburne III, *et al.* 2005). In associated streptococci, *spy0583* was also found to be downregulated, but the transcription-related gene is upregulated in adherent streptococci (Ryan, *et al.* 2007). This indicates that *spy0583* may provide a functional switch for gene expression upon binding to

host cells. On the other hand, *spy1215* is activated in both associated and adherent bacteria (Ryan, *et al.* 2007).

Examining the two-component systems, there was a well-defined temporal pattern of regulation. Early in interactions with the pharyngeal environments (30 minutes), *covRS, ciaHR,* and *ihk/irr* were upregulated, as compared to control streptococci. The *ciaHR* system is also upregulated in adherent bacteria, and appears to be important for metabolic gene activation (Ryan, *et al.* 2007; Riani, *et al.* 2007). Thus, it follows that this TCS would be upregulated when streptococci first enter the pharyngeal environment. Perhaps, a second round of upregulation upon streptococcal binding to host cells indicates that the bacteria readjust their metabolic program when in contact with the host epithelium.

Activation of *covRS* in response to both pharyngeal environments is particularly interesting as this TCS is central to virulence regulation. The CovR response regulator represses expression of *rivR* (as seen in our analysis), as well as expression of *ska*, which was also repressed in both settings at 30 minutes and 1.5 hours. The temporal pattern of *covRS* expression in our study directly mirrored that observed during streptococcal growth in human blood (Graham, *et al.* 2005). Thus, it appears that *covRS* is a critical sensor for streptococci upon initial introduction into the host environment.

The upregulation of *ihk/irr* is notable in that this TCS has been associated with resistance to antimicrobial peptides (Voyich, *et al.* 2003; Voyich, *et al.* 2004). As Detroit 562 pharyngeal cells can elaborate the antimicrobial peptide LL-37,

one might suspect that *ihk/irr* activation may result from LL-37 secretion by host cells (Sharpe, *et al.* 2011). The broader transcriptional picture, however, does not necessarily support this assertion. Although LL-37 also activates *covRS* as seen in our analysis, the known downstream effects of this host peptide on streptococcal gene expression include activation of capsule and various proteases not observed in our data set (Gryllos, *et al.* 2008b).

Furthermore, virulence factors suspected of being important for LL-37 defenses, including the streptococcal inhibitor of complement (*sic*) and the *dlt* operon, which inserts positively charged residues into LTA, were either downregulated (*sic*) or unaffected (*dlt*) by the pharyngeal environment (Otto 2009). The original observation that Detroit cells secrete LL-37 resulted from experiments exposing pharyngeal monolayers to outer membrane vesicles from the Gram-negative bacterium *Haemophilus influenzae* (Sharpe, *et al.* 2011). No studies have examined LL-37 secretion by pharyngeal cells in response to group A streptococci, so it remains an open question if this antimicrobial peptide plays any role in our system.

Downregulation of two-component systems in response to pharyngeal environments was confined to the later time points. The *sptRS* TCS was only downregulated in response to pharyngeal supernatants. This was striking as *sptRS* is also repressed during short exposures (4 hours) to human saliva (Shelburne III, *et al.* 2005). Following long periods in human saliva (16 hours), this TCS is induced and coordinates the transcriptional program required for

long-term persistence in saliva when the bacteria no longer divide in great numbers (Shelburne, *et al.* 2005). Not much is known about the other two-component systems repressed in the pharyngeal environments, although *spy1621-1622* has been linked to virulence (Ichikawa, *et al.* 2011).

Overall, our microarray analyses revealed that streptococci undergo significant remodeling of their transcriptome in a time-dependent manner upon introduction into the pharyngeal environment. We must keep in mind, however, that mRNA levels are dependent not only on the rate of transcription, but are also affected by their rate of decay. Thus, it would be insightful to pursue a similar strategy of transcriptional profiling in mutants deficient for streptococcal RNases. In particular, CvfA (RNaseY) would be a prime candidate for deletion in future work as this RNA degradative enzyme has been demonstrated to play a key role in *speB* transcript levels (Chen, *et al.* 2012).

No doubt continued data mining of our results in light of other published transcriptome studies will yield additional insights. For the purposes of this thesis, we were most struck by the number of uncharacterized stand-alone regulators differentially expressed in response to pharyngeal supernatants and pharyngeal monolayers. Two of these regulators *spy1215* and *spy1755* were selected for further characterization and will be explored in sections 5 (*spy1215*) and 6 (*spy1755*) of this thesis.

5. REGULATION BY SPY1215

5.1 Introduction

Sir2 (silent information regulator) proteins, or sirtuins, are NAD^+-dependent deacetylases first identified in yeast due to their ability to silence gene expression by removing acetyl groups from histone tails inducing a more compact, inaccessible nucleosomal structure (Imai, *et al.* 2000; reviewed in Blander & Guarente 2004). In eukaryotes, Sir2 activity has subsequently been found to impact gene expression, metabolism, cancer, stress responses, and life span control (Brachmann, *et al.* 1995; North, *et al.* 2003; Starai, *et al.* 2003; Guarente 2007; Kwon & Ott 2008). Ongoing work on eukaryotic Sir2 proteins indicates that they can be regulated at the transcriptional, posttranscriptional, and posttranslational levels (Smith, *et al.* 2008).

Homologs of Sir2 proteins are highly conserved from archea and bacteria to humans (Brachmann, *et al.* 1995). Acetylation of proteins at lysine residues is a reversible posttranslational modification performed by acetylases (such as GNATs: Gcn5-like protein *N*-acetyltransferases) and their partner deacetylases (including the Sir2 family) (Neuwald & Landsman 1997; Berndsen & Denu 2008; Brandi, *et al.* 2009). Traditionally, phosphorylation by kinases has been viewed as the dominant posttranslational modification in bacteria. Recent inventories of acetylation sites in *E. coli* and *Salmonella enterica*, however, indicate that the number of target proteins for acetylation and phosphorylation may be similar, with more acetylation sites available overall (reviewed in Jones & O'Connor 2011).

The first Sir2 gene studied in bacteria was *cobB* of *Salmonella enterica*. Research on the late steps in cobalamin biosynthesis revealed that the CobB acts as an ADP-ribosyltransferase, directly catalyzing the synthesis of a cobalamin biosynthetic intermediate (Tsang & Escalante-Semerena 1998). CobB was later found to act primarily as a deacetylase, activating the metabolic enzymes acetyl-CoA synthetase and propionyl-CoA synthetase by deacylation of a lysine residue in their catalytic domains (Starai, *et al.* 2002; Starai, *et al.* 2003; Garrity, *et al.* 2007). These studies provided the first insights into the use of sirtuins by prokaryotes to posttranslationally modify key metabolic enzymes in a reversible fashion (reviewed in Thao & Escalante-Semerena 2011).

A recent proetomic analysis revealed that 90% of the *S. enterica* enzymes involved in central metabolism are acetylated, including glyceraldehyde-3-phosphate dehydrogenase, isocitrate lyase, and isocitrate dehydrogenase kinase/phosphatase (Wang, *et al.* 2010). Following growth on different carbon sources, changes in the acetylation states of these enzymes were noted that directly correlated to alterations in their enzymatic activities *in vitro*, indicating that actions of the *S. enterica* GNAT acetylase (Pat) and sirtuin deacetylase (CobB) are critical for metabolic regulation (Wang, *et al.* 2010). The levels of transcript and protein for Pat and CobB were also found to be regulated in response to growth phase and carbon source. In all, over 191 proteins (50% of which are involved in metabolism) were found to be targets of acetylation in the *S. enterica* molecular screen (Wang, *et al.* 2010).

Similar proteomic analyses of lysine-acetylated proteins in two strains of *E. coli* indicated that nearly half of the proteins affected are involved in metabolism. Enzymes found to undergo acetylation acted in the metabolism of energy, fatty acids, and nucleotides (Yu, *et al.* 2008; Zhang, *et al.* 2009). Furthermore, the action of Sir2 proteins on metabolic enzymes (acyl-CoA synthetases) has been linked to short-chain fatty acid metabolism in *Bacillus subtilis* (Gardner & Escalante-Semerena 2009) and catabolism of aromatic and allicyclic acids in *Rhodopseudomonas palustris* (Crosby, *et al.* 2010). Thus, it appears that lysine acetylation is a key mechanism for regulating the metabolic fates of carbon and energy sources in prokaryotes (Thao & Escalante-Semerena 2011).

In mycobacteria, the Sir2 homolog not only functions to activate acetyl-CoA synthetase (like CobB), but it has also been linked to enhancing recruitment of key proteins to double-stranded DNA breaks for the nonhomologous end-joining repair pathway (Gu, *et al.* 2009; Xu, *et al.* 2011; Li, *et al.* 2011). In *E. coli,* CobB is also involved in regulating chemotaxis (Li, *et al.* 2010). The *E. coli* response regulator CheY transmits sensory signals from chemoreceptors to the flagellar motor by binding to a switch component at the flagellum's base and triggering a change from counterclockwise to clockwise flagellar rotation (Silversmith & Bourret 1999; Sourjik 2004). CheY undergoes auto-acetylation and requires the action of CobB deacetylase for proper chemotactic responses to attractants and repellents (Li, *et al.* 2010).

Generally, lysine acetylation of transcriptional regulators can affect downstream gene expression (reviewed in Kouzarides 2000). Within eukaryotes, reversible acetylation of transcription factors has been found to positively or negatively affect DNA binding affinity depending on where within the protein structure acetylation occurs (Kouzarides 2000). Thus, the action of Sir2 deacetylases can serve to activate or repress gene expression via the removal of acetyl moieties from specific lysine sites in regulators.

In addition, acetylation state can affect the interactions of transcription factors with co-activators (Kouzarides 2000). Finally, acetylation has been found to stabilize at least one transcription factor increasing its half-life in the cell (Martinez-Balbas, *et al.* 2000). Although the majority of these studies have been conducted in eukaryotic systems, a role for Sir2 deacetylases in bacterial gene expression is starting to emerge.

The first clue that Sir2 proteins may be involved in bacterial transcriptional regulation came from the proteomic screens of acetylation protein targets discussed above (Yu, *et al.* 2008; Zhang, *et al.* 2009; Wang, *et al.* 2010). These analyses indicated that proteins involved in the control of transcription and translation undergo lysine acetylation *in vivo.* Two studies in *E. coli* have now confirmed a role for CobB deacetylation in repression of gene expression either by increasing the DNA-binding affinity of one transcriptional repressor (Thao, *et al.* 2010), or reducing transcription from one bacterial promoter by removing key acetyl moieties from the RNA polymerase pore complex (Lima, *et al.* 2011).

Using an *E. coli* proteome array, researchers reported that the bacterial GNAT (acetylase) from *S. enterica* and *E. coli* acetylated several bacterial transcription factors, including RcsB (Thao, *et al.* 2010). The Rcs two-component system, comprised of the sensor kinase RcsA and the response regulator RcsB, is found in a number of enteric bacteria and modulates expression of capsule biosynthesis, cell division, motility, and osmotic responses (Huang, *et al.* 2006; Majdalani & Gottesman 2005). RcsB is acetylated by the *S. enterica* GNAT at a site in its helix-turn-helix, inhibiting RcsB DNA-binding affinity. CobB deacetylation of RscB was demonstrated *in vitro*, and elimination of *cobB* had the effect of derepressing an operon under RcsB negative regulation (Thao, *et al.* 2010). Thus, CobB can repress expression of at least one operon by enhancing RcsB promoter binding through direct modulation of the RcsB acetylation state.

In *E. coli*, the two-component system CpxAR regulates the expression of at least 50 genes (De Wulf, *et al.* 2002; Price & Raivio 2009). In response to certain signals, including alkaline pH, surface attachment, or accumulation of misfolded periplasmic proteins, CpxA autophosphorylates and transfers the phosphoryl group to CpxR promoting CpxR-driven transcription (Danese, *et al.* 1995; Pogliano, *et al.* 1997; Raivio & Silhavy 1997; Danese & Silhavy 1998; Otto & Silhavy 2002; Wolfe, *et al.* 2008). In the absence of such signals, CpxR can still activate transcription in a CpxA-independent fashion (Danese & Silhavy 1998). Experiments revealed that acetylation of the RNA polymerase core complex (RNAP) was required for CpxA-independent transcriptional activation by

CpxR, and that CobB could reduce expression by removing acetyl groups from lysines in the RNAP (Lima, *et al.* 2011).

The studies in *E. coli* serve to highlight that acetylation and phosphorylation pathways can intersect through the actions of kinases/phosphateases and acetylases/deacetylases on two-component response regulators and RNAP complexes that include those regulators. Our understanding of the action of Sir2 deacetylases in bacteria is truly at its infancy, however, as only one homolog of CobB has been examined to any extent. As researchers continue to explore the role of Sir2 proteins in bacterial regulation, it will no doubt become more apparent that acetylation status is critical for gene expression modulation in prokaryotes as has been already widely established for eukaryotic cells.

Our interest in the *S. pyogenes* Sir2 homolog encoded by *spy1215* began with the observation that this gene is upregulated during streptococcal interactions with pharyngeal supernatants and intact pharyngeal monolayers. Previous work in our laboratory had also established that *spy1215* is upregulated even further following adherence to pharyngeal cells (Ryan, *et al.* 2007). Examining the expression data from streptococci in other host environments revealed that the streptococcal Sir2 homolog is induced across a range of conditions. Growth in human blood and interactions with polymorphonuclear leukocytes activated *spy1215* within 60 minutes—the same time scale observed in our analysis (Voyich, *et al.* 2003; Graham, *et al.* 2005). In addition, growth in

amniotic fluid also led to induction of *spy1215* expression (Sitkiewicz, *et al.* 2010).

Taken together, these data suggested that *spy1215* activity may play an important role in streptococcal adaptive responses to host environments. Considering that CobB in *E. coli* can repress gene expression by acting on the global regulator RcsB and the RNAP complex, we hypothesized that Spy1215 deacetylation of transcription factors in *S. pyogenes* could help shape the transcriptome by repressing expression of genes that would be detrimental to its survival in the host and/or ensuring the proper regulation of virulence factors over the course of an infection.

Along this line of inquiry, epigenetic silencing of genes by sirtuins has been demonstrated to be crucial for virulence regulation in the eukaryotic parasite *Plasmodium falciparum*. To help evade the human immune response, the malarial parasite varies its primary surface virulence factor, switching among 60 antigenically variable *var* genes. Malarial sirtuins orchestrate the antigenic switch and ensure expression for only one *var* gene at a time by direct binding of sirtuin-containing protein complexes to *var* promoters (Tonkin, *et al.* 2009; Merrick, *et al.* 2010). The following chapter details our work to elucidate the role of Spy1215 in streptococcal gene regulation within the host environment using an isogenic *spy1215* knockout in the SF370 background.

5.2 Results

5.2.1 Real-time quantitative RT-PCR validation of microarray hit for *spy1215*

Primers and a fluorogenic probe were designed to a section of the *spy1215* ORF to quantify mRNA levels in streptococci exposed to either pharyngeal supernatants or pharyngeal monolayers using streptococci in MEM as a control. Normalization of data was performed using genes identified in the microarray data whose expression was unaltered by the host environment. The QRT-PCR results validated the microarray data indicating that *spy1215* is indeed upregulated at 1.5 and 2.5 hours post-exposure to both host cell environments (Table 5.1).

Table 5.1 Comparison of statistically significant *spy1215* $\log_2$ fold changes generated by microarray and real-time QRT-PCR

	Pharyngeal Supernatants		Pharyngeal Co-culture	
Exposure Time	Microarray $\log_2$ Fold Change	QRT-PCR $\log_2$ Fold Change	Microarray $\log_2$ Fold Change	QRT-PCR $\log_2$ Fold Change
1.5 hours	1.23	1.2	1.75	0.84
2.5 hours	1.58	0.7	1.61	0.85

5.2.2 Confirmation of *spy1215* deletion

An allelic replacement strategy was employed to delete the *spy1215* ORF using a cassette encoding for erythromycin resistance. Following electroporation of SF370, Erm^R colonies were screened for double recombination events and the resulting loss of *spy1215*. PCR amplification of an internal *spy1215* region, as well as the upstream and downstream junctions of the plasmid with the bacterial chromosome confirmed that *spy1215* had been eliminated from the genome of SF370Δ1215 (Figure 5.1).

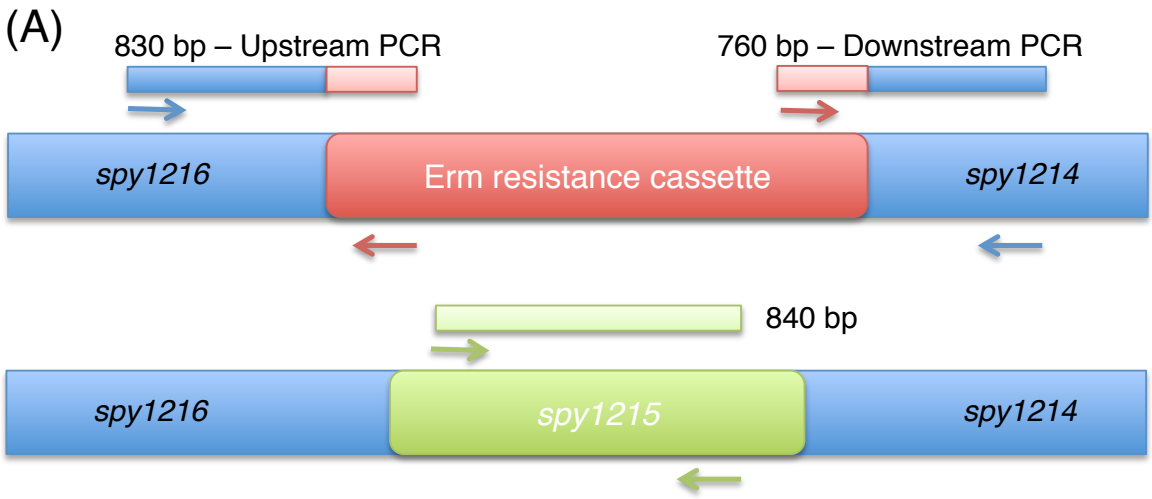
DNA sequencing provided an additional line of supporting evidence for the generation of the *spy1215* isogenic mutant (data not shown). Sequencing analysis revealed, however, that one point mutation had been introduced into the stop codon of the upstream gene *spy1216*. It is possible that transcription of this upstream ORF can now read through the stop codon into the Erm cassette adding up to 10 amino acids to the C-terminus of the protein.

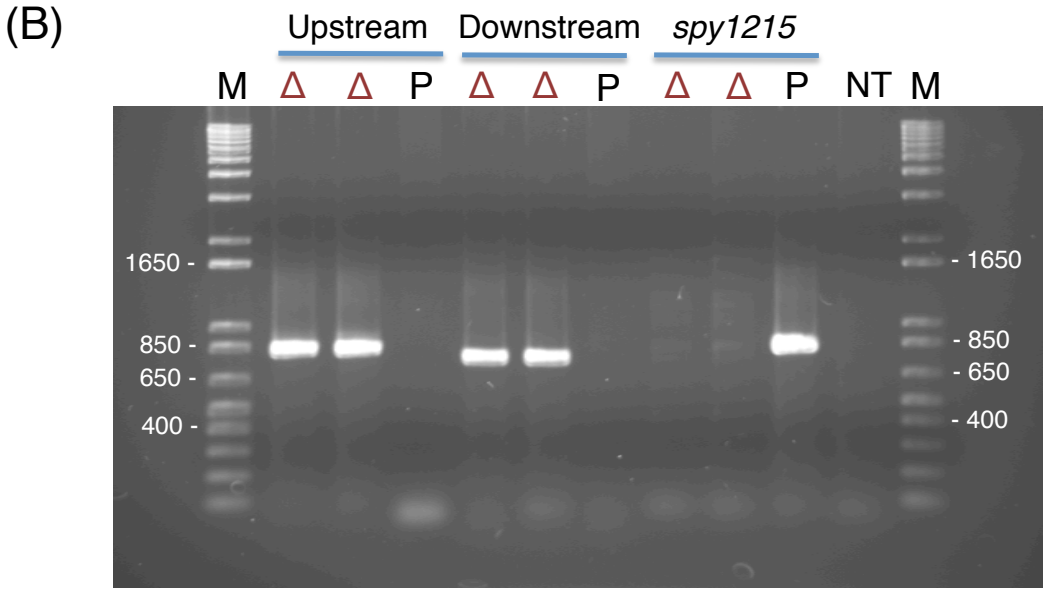
As *spy1216* is of unknown function, we cannot ascertain the potential impact of this mutation *in silico*. Analysis of the *spy1216* sequence, however, did not reveal any conserved domains characteristic of transcription factors, kinases/phosphatases, or acetyltransferases/deacetylases. A corrected SF370Δ1215 mutant has been generated restoring the *spy1216* stop codon, but we have not had the opportunity to repeat the transcriptome analyses as yet. Therefore, all conclusions on Spy1215 function should be considered preliminary until confirmed with the corrected deletion mutant.

Figure 5.1 PCR analysis to confirm *spy1215* deletion from SF370

Panel (A) depicts the PCR scheme. Primers internal to the Erm cassette (red) were used in combination with primers internal to genes upstream and downstream of *spy1215* (blue) to confirm plasmid integration. Expected product sizes resulting from plasmid integration are 830 bp for the upstream region and 760 bp for the downstream region. Primers internal to *spy1215* (green) were used to specifically probe for the presence of the ORF (expected product size is 840 bp).

Panel (B) shows the results of the PCR analysis on genomic DNA from WT SF370 (P) and two different SF370Δ1215 mutants (Δ). Control PCR reactions with primers but no template (NT) were also included. Lane (M) contains 1-kb Plus DNA ladder (1 μg, Invitrogen). PCR products were separated on a 1% agarose gel and visualized by ethidium bromide staining.


(A)
830 bp – Upstream PCR
760 bp – Downstream PCR
spy1216
Erm resistance cassette
spy1214
840 bp
spy1216
spy1215
spy1214


(B)
Upstream
Downstream
spy1215
M Δ Δ P Δ Δ P Δ Δ P NT M
1650 -
850 -
650 -
400 -
- 1650
- 850
- 650
- 400

5.2.3 Growth curve comparison and light microscopy of SF370 and SF370Δ1215

Examination of the growth curves of wild-type SF370 and SF370Δ1215 in BHI broth revealed that growth of the deletion mutant was unaffected by the genetic manipulation (Figure 5.2). Observations of SF370Δ1215 using light microscopy revealed that the mutant did not suffer any significant cell morphology changes when compared to wild-type SF370 (Figure 5.3). SF370Δ1215 colonies appeared as beta-hemolytic and were comparable to wild-type colonies on blood agar plates (data not shown).

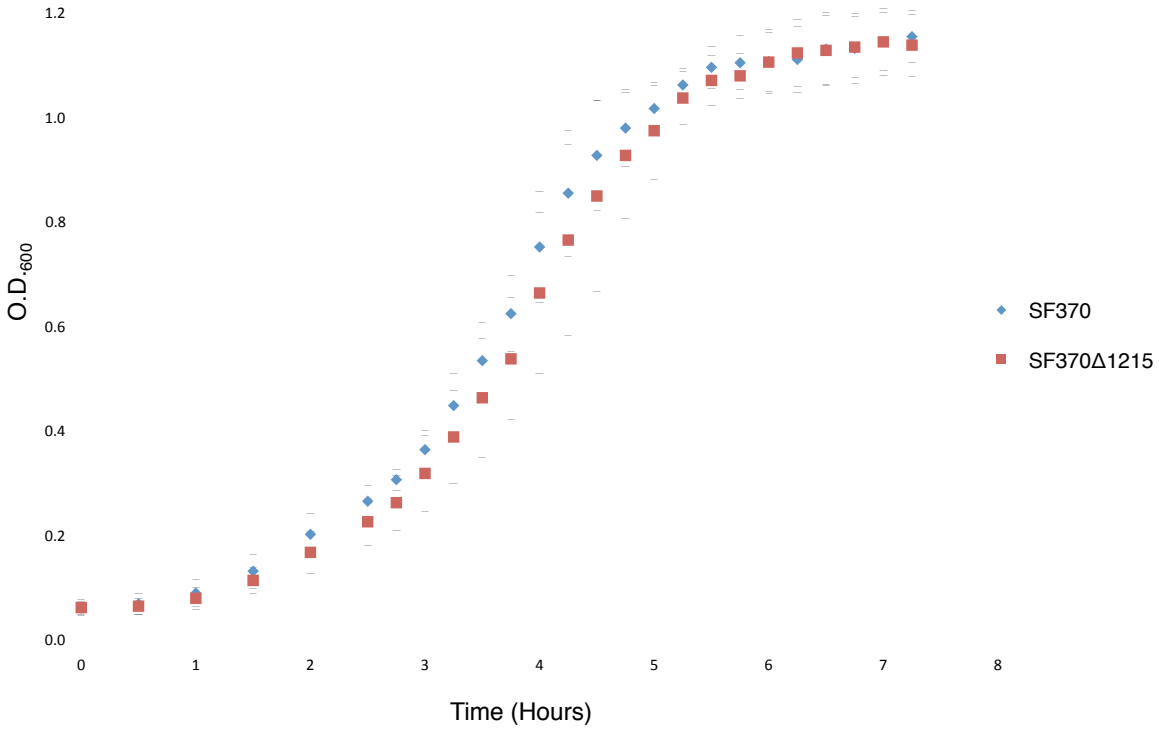


Figure 5.2 Growth comparison of wild-type SF370 and the deletion mutant SF370Δ1215

Overnight cultures were diluted 1:50 in 10 ml of pre-warmed BHI and incubated at 37°C. Absorbance of cultures ($O.D._{600}$) was measured at different time points using a spectronic 20D spectrophotometer (Thermo Electron Corporation) and the results were plotted. Each curve represents an average of four experiments performed on different days. Differences in absorbance readings were not statistically significant at any point along the curve as ascertained by the Student's *t*-test.

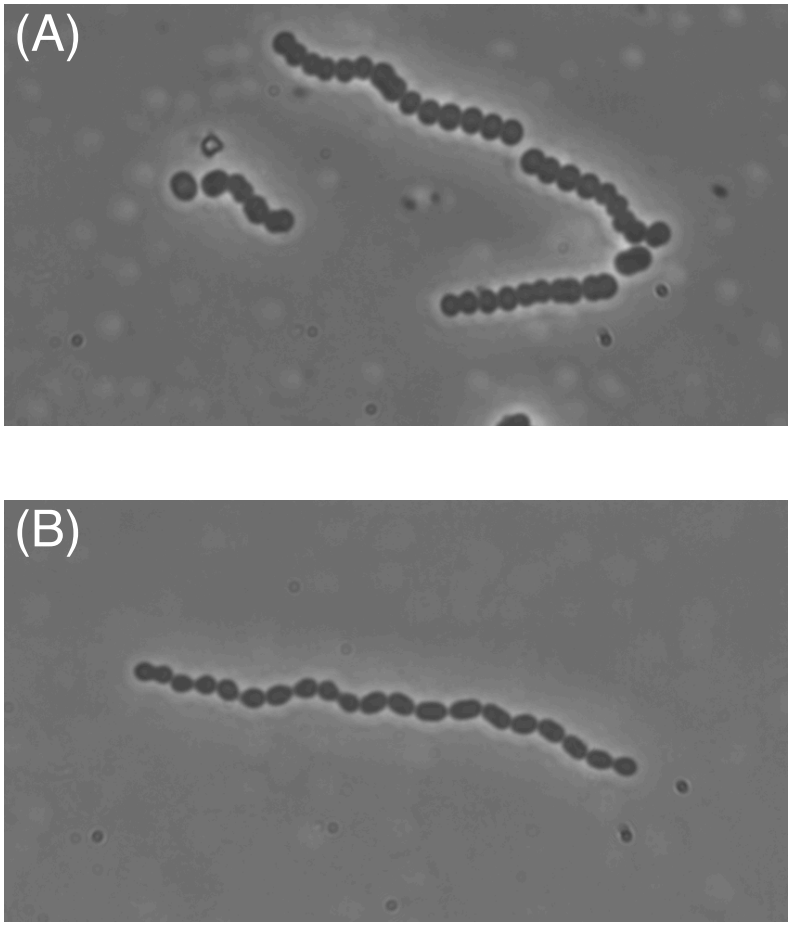


Figure 5.3 Visualization of SF370 and SF370Δ1215 by light microscopy

Panel (A) shows that SF370Δ1215 cell morphology and chain length is largely unaffected, although the cells looked slightly shortened or more compacted. Panel (B) demonstrates wild-type SF370 cell shape and chain length. Panels are representative samples taken from cultures grown to stationary phase overnight in THY broth. Images captured under 100X magnification in conjunction with a 1.5X optovar.

5.2.4 Examination of polar effects on downstream gene expression

Reverse-transcription PCR was utilized to examine if the deletion of *spy1215* had polar effects on the expression of downstream genes. Researchers had not previously investigated if *spy1215* was part of an operon in *S. pyogenes*. Examination of the genomic region surrounding the *spy1215* locus revealed that the gene resides between ORFs transcribed in the same direction (Figure 5.4). Thus, it was possible that deletion of *spy1215* could eliminate expression of *spy1215* as well as downstream genes.

RT-PCR using primers internal to *spy1214*, *spy1213,* and *spy1212* revealed that the deletion of *spy1215* did not affect expression of these genes at late exponential or stationary phase growth in THY (Figure 5.5). Furthermore, RT-PCR of *spy1215* in the isogenic mutant again confirmed the loss of *spy1215* at the level of transcription.

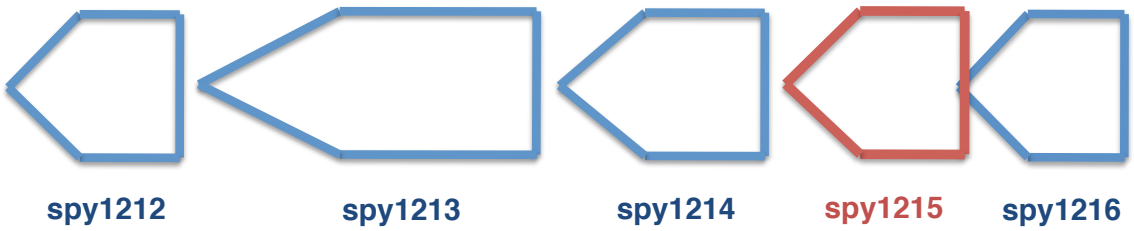


Figure 5.4 Schematic of the *spy1215* genomic region

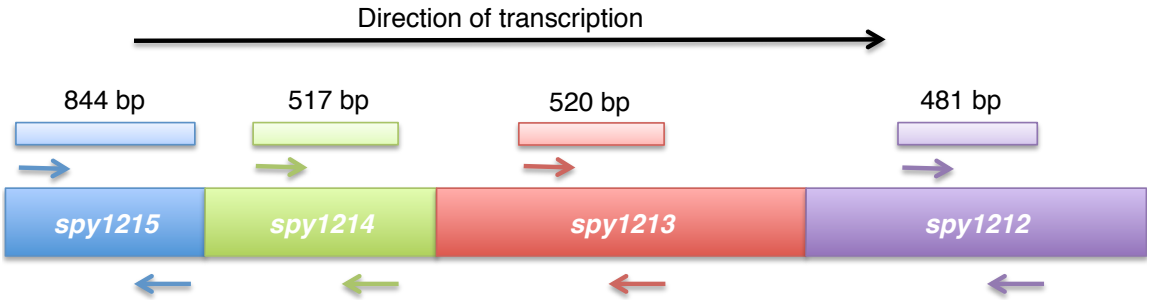
The figure depicts the open reading frames (blue) directly adjacent to *spy1215* (red). All genes are transcribed from the reverse strand, such that *spy1215* lies upstream of *spy1214* for transcription (indicated by direction of arrowheads). ORFs have been drawn in proportion to their nucleotide length and intergenic spaces.

Figure 5.5 RT-PCR analysis of expression from *spy1215* and its downstream genes in SF370Δ1215 and SF370

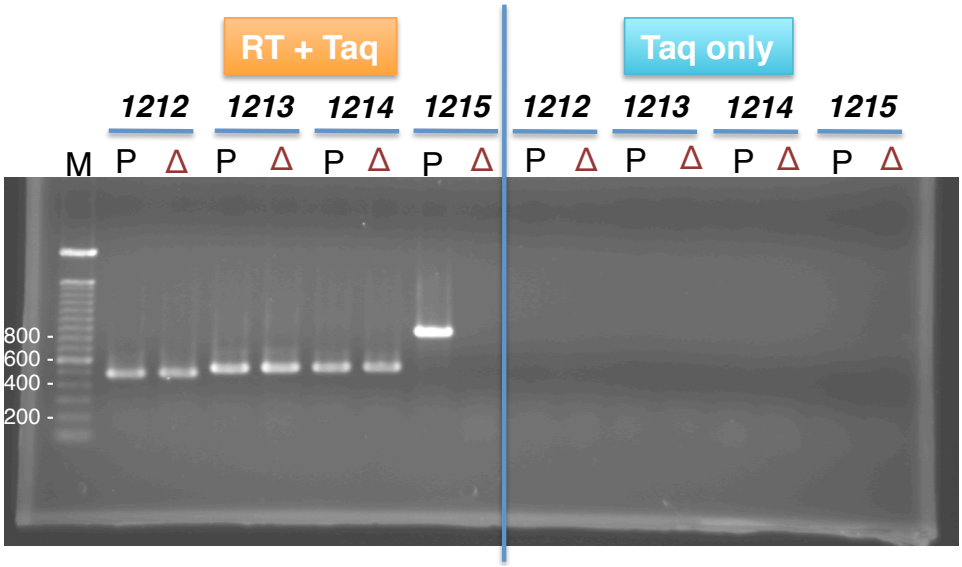
Panel (A) depicts the RT-PCR strategy. Primers were designed to amplify segments of mRNA generated from *spy1215* (blue), *spy1214* (green), *spy1213* (red), and *spy1212* (purple). Expected product sizes are 844 bp, 517 bp, 520 bp, and 481 bp, respectively.

Panel (B) shows the results of the RT-PCR analysis on total RNA from WT SF370 (P) and the mutant SF370Δ1215 (Δ) grown to late exponential phase in THY broth. Lane (M) contains 100 bp DNA ladder (1 μg, Invitrogen). PCR products were separated on a 1% agarose gel and visualized by ethidium bromide staining. Control reactions performed with Taq polymerase (but no RT enzyme) indicated that the RNA samples were free of contaminating DNA (right half of image). Identical results were obtained from total RNA isolated from SF370 and SF370Δ1215 at stationary phase (data not shown).

(A)


Direction of transcription
844 bp
517 bp
520 bp
481 bp
spy1215
spy1214
spy1213
spy1212

(B)


RT + Taq
Taq only
1212
1213
1214
1215
1212
1213
1214
1215
M
P
Δ
P
Δ
P
Δ
P
Δ
P
Δ
P
Δ
P
Δ
P
Δ
800 -
600 -
400 -
200 -

5.2.5 Spy1215 regulon in response to pharyngeal supernatants

We first examined Spy1215-dependent regulation driven by the presence of secreted host factors and changes to the nutrient environment via pharyngeal cell metabolic processes. Comparing the transcriptomes of SF370 and the isogenic mutant SF370Δ1215 following 2.5 hours exposure to filtered pharyngeal supernatants indicated that 91 genes were differentially regulated in the absence of Spy1215 (Table 5.2). No phage genes were identified in the analysis. Overall, the lack of Spy1215 deacetylation led to downregulation of gene expression in the mutant, indicating that sirtuin activity is necessary for gene activation (or derepression) in response to pharyngeal supernatants in the wild-type.

Of the differentially expressed genes, 65 ORFs were downregulated in cells lacking Spy1215-dependent deacetylation. Categorizing these genes by functional groups using the COG system, we observed that the most significant effect of *spy1215* deletion was on expression of genes involved in translation, including 20 genes encoding ribosomal subunit proteins (Figure 5.6). Genes encoding enzymes in the tRNA and inosine monophosphate biosynthesis pathways were also downregulated in the mutant. Inosine is commonly bound to tRNAs and ensures proper translation of codons.

In addition, genes involved in cell cycle control, cell division, and the formation of the septum were downregulated in the absence of *spy1215.* Genes involved in pyrimidine biosynthesis experienced reduced expression in the mutant, as did genes involved in amino acid scavenging. The *spy1214* gene,

which lies immediately downstream of *spy1215*, experienced the greatest deregulation in the mutant with a 16-fold reduction in expression. Notably, expression of M protein was downregulated, and represented the only virulence factor affected negatively by the absence of *spy1215* in response to pharyngeal supernatants.

Twenty-six genes were upregulated in the absence of *spy1215*. The majority of these genes were either of unknown function or involved in protein modifications and turnover. The greatest induction of transcription in the mutant (nearly 5-fold over wild-type levels) was observed for genes encoding enzymes for coenzyme A biosynthesis. Two virulence factors were identified as being upregulated in SF370Δ1215: myosin cross-reactive antigen and *dltB*, an enzyme involved in the insertion of positively charged residues into surface lipotechoic acids. Two proteins involved in peptidoglycan biosynthesis (including a penicillin-binding protein) were also upregulated. Expression induction was also observed for genes involved in oxidative stress responses.

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Table 5.2 Microarray data comparing the transcriptome of SF370Δ1215 to SF370 following 2.5 hours exposure to pharyngeal supernatants (Pages 216-221)

65 Downregulated genes (Higher expression in wild-type SF370)

Locus Tag	Gene Name	*P* Value	Log_2 Fold Change[a]	COG	Known or Proposed Function
M1_Spy_0009		< 0.001	-1.53	Translation	Ribosome-associated heat shock protein
M1_Spy_0010	*divIC*	< 0.001	-2.85	Cell cycle control, mitosis	Septum formation initiator
M1_Spy_0012		0.005	-1.94	Defense mechanism	Beta-lactamase class A
M1_Spy_0013		< 0.001	-1.89	Cell cycle control, mitosis	tRNA(Ile)-lysidine synthase; Implicated in cell cycle control
M1_Spy_0015	*ftsH*	0.007	-0.85	Posttranslational modification	Cell division protease; ATP-dependent metalloprotease
M1_Spy_0024	*purC*	< 0.001	-1.09	Nucleotide metabolism	Phosphoribosylaminoimidazole-succinocarboxamide synthase; Inosine monophosphate biosynthesis
M1_Spy_0032	*purD*	0.011	-0.82	Nucleotide metabolism	Phosphoribosylamine-glycine ligase; Inosine monophosphate biosynthesis
M1_Spy_0033	*purE*	0.004	-1.09	Nucleotide metabolism	Phosphoribosylaminoimidazole carboxylase catalytic subunit; Inosine monophosphate biosynthesis
M1_Spy_0036	*purB*	0.011	-1.04	Nucleotide metabolism	Adenylosuccinate lyase; Inosine monophosphate biosynthesis
M1_Spy_0050	*rplD*	0.025	-0.78	Translation	50S ribosomal protein L4
M1_Spy_0051	*rplW*	0.006	-0.84	Translation	50S ribosomal protein L23
M1_Spy_0052	*rplB*	0.015	-0.80	Translation	50S ribosomal protein L2
M1_Spy_0055	*rplV*	0.039	-0.85	Translation	50S ribosomal protein L22
M1_Spy_0056	*rpsC*	0.012	-0.73	Translation	30S ribosomal protein S3

Table 5.2 Microarray data comparing SF370Δ1215 to SF370 after exposure to host supernatants

M1_Spy_0057	*rplP*	0.004	-0.84	Translation	50S ribosomal protein L16
M1_Spy_0060	*rpsQ*	0.019	-0.85	Translation	30S ribosomal protein S17
M1_Spy_0062	*rplX*	0.001	-0.90	Translation	50S ribosomal protein L24
M1_Spy_0063	*rplE*	0.021	-0.71	Translation	50S ribosomal protein L5
M1_Spy_0065	*rpsH*	0.011	-0.67	Translation	30S ribosomal protein S8
M1_Spy_0066	*rplF*	0.031	-0.72	Translation	50S ribosomal protein L6
M1_Spy_0067	*rplR*	0.014	-0.79	Translation	50S ribosomal protein L18
M1_Spy_0069	*rpsE*	0.023	-0.79	Translation	30S ribosomal protein S5
M1_Spy_0072	*rplO*	0.025	-0.90	Translation	50S ribosomal protein L15
M1_Spy_0073	*secY*	0.013	-0.66	Intracellular trafficking and secretion	Preprotein translocase subunit; Secretion system
M1_Spy_0077	*rpsM*	0.014	-0.80	Translation	30S ribosomal protein S13
M1_Spy_0116		0.039	-0.88	Function unknown	Hypothetical protein (transmembrane)
M1_Spy_0207		0.022	-1.66	Function unknown	Biotin synthase; BioY family protein
M1_Spy_0235	*dut*	0.001	-1.28	Nucleotide metabolism	Deoxyuridine 5'-triphosphate nucleotidohydrolase; Pyrimidine deoxyribonucleotide synthesis
M1_Spy_0236	*sms*	0.038	-1.06	Posttranslational modification	DNA repair protein RadA
M1_Spy_0294	*oppB*	0.003	-1.20	Amino acid metabolism	Oligopeptidepermease
M1_Spy_0295	*oppC*	0.028	-0.93	Amino acid metabolism	Oligopeptidepermease
M1_Spy_0296	*oppD*	0.002	-1.08	Amino acid metabolism	Oligopeptidepermease ATP-binding protein

Table 5.2 Microarray data comparing SF370Δ1215 to SF370 after exposure to host supernatants

M1_Spy_0342	*snf*	0.020	-1.01	Transcription / Replication, recombination, repair	Superfamily II DNA/RNA helicase
M1_Spy_0371		0.002	-1.07	Translation	23S rRNA methyltransferase
M1_Spy_0427	*nrdE.1*	0.034	-1.33	Nucleotide metabolism	Ribonucleotide-diphosphate reductase subunit alpha; Pyrimidine deoxyribonucleotide biosynthesis
M1_Spy_0453	*mtsA*	0.014	-0.81	Inorganic ion metabolism	ABC transporter metal binding protein; Iron, zinc, copper transport
M1_Spy_0454	*mtsB*	0.036	-0.69	Inorganic ion metabolism	ABC transporter ATP-binding protein; Iron, zinc, copper transport
M1_Spy_0461	*rplA*	0.003	-0.99	Translation	50S ribosomal protein L1
M1_Spy_0464		0.027	-0.69	Function unknown	hypothetical protein
M1_Spy_0535		0.019	-1.25	Carbohydrate metabolism	Putative sugar phosphate isomerase
M1_Spy_0558		< 0.001	-1.74	Function unknown	Putative cytoplasmic protein
M1_Spy_0559		0.015	-1.74	Translation	Asparagine synthetase A; Aminoacyl-tRNA biosynthesis
M1_Spy_0560		< 0.001	-1.92	Lipid metabolism	Putative biotin carboxylase
M1_Spy_0600		0.006	-1.57	Function unknown	Putative proton-coupled thiamine transporter
M1_Spy_0724	*rplS*	0.028	-0.65	Translation	50S ribosomal protein L19
M1_Spy_1072	*rplJ*	< 0.001	-1.75	Translation	50S ribosomal protein L10
M1_Spy_1073	*rplL*	0.003	-1.36	Translation	50S ribosomal protein L7/L12
M1_Spy_1214		< 0.001	-4.02	Coenzyme metabolism	Lipoate-protein ligase; Lipoate salvage and modification
M1_Spy_1217		0.010	-1.25	Amino acid metabolism	Glycine cleavage system H protein

Table 5.2 Microarray data comparing SF370Δ1215 to SF370 after exposure to host supernatants

M1_Spy_1234	*rpsT*	0.008	-0.88	Translation	30S ribosomal protein S20
M1_Spy_1372	*pstI*	0.049	-0.88	Carbohydrate metabolism	Phosphoenolpyruvate:sugar phosphotransferase system enzyme I
M1_Spy_1390	*prsA*	0.005	-1.33	Posttranslational modification	Foldase
M1_Spy_1419		0.043	-0.97	Function unknown	Hypothetical protein (transmembrane)
M1_Spy_1420	*murF*	0.006	-0.82	Cell wall/membrane biogenesis	D-Ala-D-Ala adding enzyme; Peptidoglycan biosynthesis
M1_Spy_1569		0.002	-1.35	Amino acid metabolism	Putative shikimate kinase
M1_Spy_1571		0.032	-1.10	Function unknown	Hypothetical protein
M1_Spy_1854	*glpF.2*	< 0.001	-1.88	Carbohydrate metabolism	Glycerol uptake facilitator protein
M1_Spy_1888	*rpmB*	0.010	-0.78	Translation	50S ribosomal protein L28
M1_Spy_1980		0.037	-0.94	Translation	D-tyrosyl-tRNA(Tyr) deacylase
M1_Spy_2005		0.037	-1.07	Function unknown	Hypothetical protein
M1_Spy_2018	*emm1*	< 0.001	-1.42	Virulence	M-protein type I
M1_Spy_2092	*rpsB*	0.017	-0.83	Translation	30S ribosomal protein S2
M1_Spy_2178	*rpsD*	0.020	-0.90	Translation	30S ribosomal protein S4
M1_Spy_2209		0.016	-2.08	Function unknown	Hypothetical protein (transmembrane)
M1_Spy_2210		< 0.001	-1.11	Function unknown	ABC transporter ATP-binding protein

[a] Log fold change indicates the ratio of SF370Δ1215 expression to wild-type SF370

Table 5.2 Microarray data comparing the transcriptome of SF370Δ1215 to SF370 following 2.5 hours exposure to pharyngeal supernatants

26 Upregulated genes (Lower expression in wild-type SF370)

Locus Tag	Gene Name	*P* Value	Log_2 Fold Change[a]	COG	Known or Proposed Function
M1_Spy_0285		0.022	0.85	Posttranslational modification	ABC transporter ATP-binding protein; Iron transport
M1_Spy_0470		< 0.001	0.94	Virulence	Myosin-cross-reactive antigen
M1_Spy_0517	*thrS*	< 0.001	1.35	Translation	Threonyl-tRNA synthetase; Aminoacyl-tRNA biosynthesis
M1_Spy_0577		0.040	0.84	Function unknown	Hypothetical protein
M1_Spy_0813	*gor*	0.035	0.65	Energy production and conversion	Glutathione reductase; Glutathione redox reaction
M1_Spy_0890	*deoB*	0.017	0.76	Carbohydrate metabolism	Phosphopentomutase; Pyrimidine ribonucleoside degradation
M1_Spy_1055	*csrA*	0.031	0.74	Posttranslational modification	Methionine sulfoxide reductase B
M1_Spy_1056		0.004	0.79	Function unknown	Hypothetical protein (transmembrane)
M1_Spy_1093		< 0.001	1.04	Cell wall/membrane biogenesis	D,D-carboxypeptidase, penicillin-binding protein; Peptidoglycan biosynthesis
M1_Spy_1221	*dpfB*	< 0.001	2.32	Coenzyme metabolism	Phosphopantothenate-cysteine ligase; Coenzyme A biosynthesis
M1_Spy_1222	*dfp*	< 0.001	2.34	Coenzyme metabolism	Phosphopantothenoylcysteine decarboxylase; Coenzyme A biosynthesis
M1_Spy_1223		< 0.001	1.96	Function unknown	Hypothetical protein (transmembrane)
M1_Spy_1224	*pgmA*	< 0.001	1.06	Carbohydrate metabolism	Phosphoglucomutase; UDP glucose from glucose-6-pohsphate
M1_Spy_1311	*dltB*	0.010	1.02	Virulence	D-alanylation of surface lipotechoic acid (LTA)

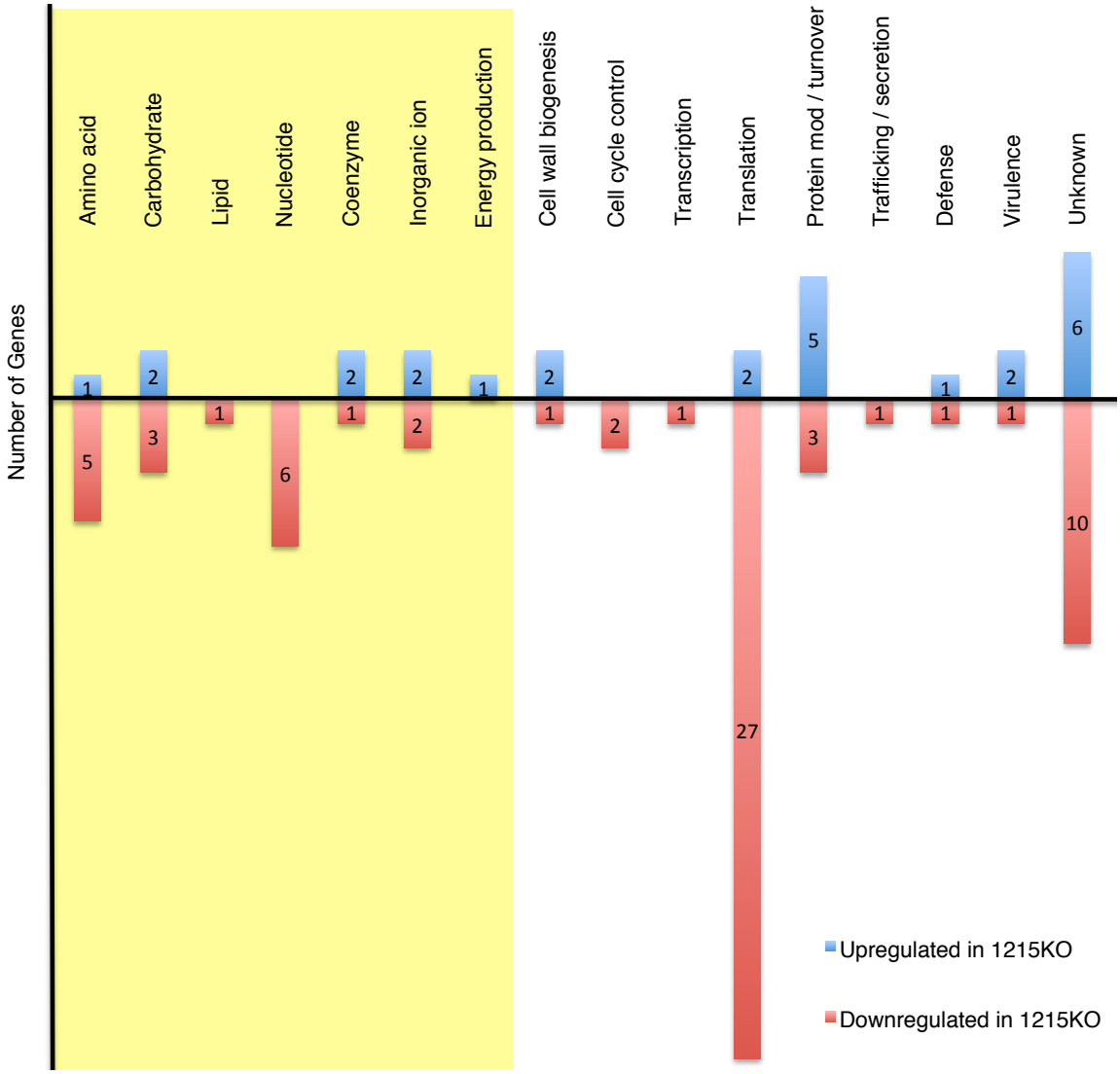
Table 5.2 Microarray data comparing SF370Δ1215 to SF370 after exposure to host supernatants

M1_Spy_1402		0.043	2.10	Cell wall/membrane biogenesis	Putative D-alanyl-D-alanine carboxypeptidase
M1_Spy_1404		0.004	2.02	Function unknown	Hypothetical protein
M1_Spy_1513	*ileS*	0.004	0.94	Translation	Isoleucyl-tRNA synthetase; Aminoacyl-tRNA biosynthesis
M1_Spy_1543		0.033	1.25	Function unknown	Putative C4-dicarboxylate anaerobic carrier protein
M1_Spy_1544	*arcB*	0.002	1.10	Amino acid metabolism	Ornithine carbamoyltransferase; Arginine biosynthesis
M1_Spy_1647		0.012	0.94	Function unknown	Hypothetical protein
M1_Spy_1714	*copZ*	0.013	1.07	Inorganic ion metabolism	Copper chaperone
M1_Spy_1788		0.030	0.99	Inorganic ion metabolism	Cobalt transport protein
M1_Spy_2029		< 0.001	1.40	Defense mechanism	ABC-type antimicrobial peptide transport system, permease component
M1_Spy_2070	*groEL*	< 0.001	1.05	Posttranslational modification	Molecular chaperone GroEL
M1_Spy_2072	*groES*	< 0.001	1.10	Posttranslational modification	Co-chaperonin GroES
M1_Spy_2080	*nox1*	0.026	0.98	Posttranslational modification	NADH oxidase/alkyl hydroperoxidase reductase

[a] Log fold change indicates the ratio of SF370Δ1215 expression to wild-type SF370

Figure 5.6 COG classification of genes differentially expressed in SF370Δ1215 during exposure to pharyngeal supernatants

Genes are classified into functional groups based on protein homology to characterized genes. Each bar represents the total number of genes differentially upregulated (blue) or downregulated (red) in SF370Δ1215 as compared to wild-type SF370. The area of the graph shaded yellow highlights all functional categories involved in cellular metabolic processes. Labels for columns within the yellow highlighted area indicate the type of macromolecule transported, synthesized, or hydrolyzed by genes in that category.


Number of Genes
Amino acid
Carbohydrate
Lipid
Nucleotide
Coenzyme
Inorganic ion
Energy production
Cell wall biogenesis
Cell cycle control
Transcription
Translation
Protein mod / turnover
Trafficking / secretion
Defense
Virulence
Unknown
1
5
2
3
1
6
2
1
2
2
1
2
1
2
1
1
2
1
5
3
1
1
1
2
1
6
10
Upregulated in 1215KO
Downregulated in 1215KO

5.2.6 Spy1215 regulon in response to pharyngeal monolayers

During incubation with pharyngeal monolayers for 2.5 hours, SF370Δ1215 differentially regulated 115 genes compared to wild-type SF370 in the same host cell environment (Table 5.3). Only one phage gene was identified in the analysis. The *spy1437* phage encoded ORF has not been assigned a function, but lies at the 3' end of the Φ370.3 prophage adjacent to the *spd3* DNase gene. Expression of the phage gene was induced 5.4-fold over wild-type levels. Overall, differential regulation of gene expression in the absence of Spy1215 deacetylation yielded roughly equal numbers of upregulated and downregulated genes during incubation with intact host cells and their milieu.

A total of 56 genes were downregulated in the *spy1215* deletion mutant compared to SF370. Grouping the genes by their COG classifications, it is immediately apparent that the greatest reduction in gene expression was exhibited by genes of unknown function and genes involved in nucleotide metabolism (Figure 5.7). The inosine monophosphate biosynthesis pathway was once again a target of downregulation in the mutant, as were genes involved in nucleotide degradation and salvage pathways. Expression of genes involved in amino acid and carbohydrate metabolism were also negatively affected in the mutant.

Five virulence factors were downregulated in SF370Δ1215: GRAB protein (which captures a host protease inhibitor for use by the bacteria), the anti-complement CAMP factor, hemolysin, hyaluronidase, and Trigger factor (which

plays a role in folding of the cysteine protease SpeB as well as other cellular proteins). In addition, three genes important for transcriptional regulation were downregulated in the mutant following exposure to host cells. These included: the transcriptional regulator Spx possibly involved in modulating the oxidative stress response, a GntR family regulator that may respond to oxidized substrates of amino acid metabolism, and the RNase J1. The downstream gene *spy1214* was once again the gene with the greatest reduction in expression (10-fold compared to SF370).

Upregulation of 58 genes was observed in the absence of Spy1215 activity, with most of these genes encoding virulence determinants or proteins of unknown function. The genes exhibiting the most statistically significant increases in expression encode an array of important virulence factors, including streptolysin O, the pilus assembly operon, the secreted protease *spyCEP*, fibronectin-binding protein *fbaA*, and the complement inhibitor *sic.* Upregulation of virulence determinants over wild-type expression ranged from 1.4-fold for the *dlt* enzymes that insert positive residues into surface LTA to 7.3-fold for streptolysin O.

Other genes upregulated in SF370Δ1215 in response to incubation with host cells included another GntR regulator with a domain that indicates it may modulate expression in response to the cellular redox state. Two additional putative DNA-binding proteins were also upregulated. Various metabolic genes were induced in the mutant, such as the maltodextrose utilization protein *malA.*

Table 5.3 Microarray data comparing the transcriptome of SF370Δ1215 to SF370 following 2.5 hours incubation with pharyngeal monolayers (Pages 226-233)

56 Downregulated genes (Higher expression in wild-type SF370)

Locus Tag	Gene Name	*P* Value	Log_2 Fold Change[a]	COG	Known or Proposed Function
M1_Spy_0020	*prsA.2*	0.014	-0.53	Amino acid metabolism	Ribose-phosphate pyrophosphokinase
M1_Spy_0024	*purC*	0.019	-0.97	Nucleotide metabolism	Phosphoribosylaminoimidazole-succinocarboxamide synthase; Inosine monophosphate biosyntheis
M1_Spy_0026	*purF*	0.042	-0.65	Nucleotide metabolism	Amidophosphoribosyltransferase; Inosine monophosphate biosynthesis
M1_Spy_0027	*purM*	0.001	-0.94	Nucleotide metabolism	Phosphoribosylaminoimidazole synthetase; Inosine monophosphate biosynthesis
M1_Spy_0028	*purN*	0.019	-0.86	Nucleotide metabolism	Phosphoribosylglycinamide formyltransferase; Inosine monophosphate biosynthesis
M1_Spy_0032	*purD*	0.001	-0.91	Nucleotide metabolism	Phosphoribosylamine-glycine ligase; inosine monophosphate biosynthesis
M1_Spy_0035	*abiR*	0.012	-0.64	Defense mechanisms	Abortive infection phage resistance protein
M1_Spy_0036	*purB*	0.002	-0.61	Nucleotide metabolism	Adenylosuccinate lyase; Inosine monophosphate biosynthesis
M1_Spy_0146	*pfoR*	0.040	-1.16	Carbohydrate metabolism	Phosphotransferase system transcriptional regulator
M1_Spy_0160	*purA*	0.001	-1.01	Nucleotide metabolism	Adenylosuccinate synthetase; Adenine nucleotide biosynthesis
M1_Spy_0163		< 0.001	-0.78	Function unknown	Putative simple sugar transport system
M1_Spy_0172	*metB*	0.003	-1.63	Amino acid metabolism	Cystathionine beta-lyase; Methionine biosynthesis
M1_Spy_0183	*opuAA*	< 0.001	-1.56	Amino acid metabolism	Glycine betaine/proline ABC transporter ATP-binding protein

Table 5.3 Microarray data comparing SF370Δ1215 to SF370 after incubation with host cells

M1_Spy_0184	*opuABC*	< 0.001	-1.42	Amino acid metabolism	Glycine-betaine binding permease
M1_Spy_0306	*yqeH*	0.028	-0.41	Function unknown	Putative ribosome biogenesis GTPase
M1_Spy_0330	*lemA*	0.007	-0.49	Function unknown	Hypothetical protein (transmembrane)
M1_Spy_0559		0.025	-0.78	Translation	Asparagine synthetase A; Aminoacyl-tRNA biosynthesis
M1_Spy_0601		0.001	-0.86	Cell wall/membrane biogenesis	N-acetylmuramoyl-L-alanine amidase
M1_Spy_0604		0.045	-0.38	Function unknown	Hypothetical protein (signal peptide)
M1_Spy_0608	*ppc*	< 0.001	-1.34	Energy production and conversion	Phosphoenolpyruvate carboxylase; Oxaloacetate synthesis
M1_Spy_0714	*adcA*	0.001	-0.65	Inorganic ion metabolism	Zinc transport system
M1_Spy_0716	*agaS*	0.004	-0.60	Cell wall/membrane biogenesis	Tagatose-6-phosphate aldose/ketose isomerase
M1_Spy_0720		0.009	-0.52	Function unknown	Hypothetical protein
M1_Spy_0814	*folC.2*	0.006	-0.80	Coenzyme metabolism	Folyl-polyglutamate synthetase; Tetrahydrofolate biosynthesis
M1_Spy_0815		0.004	-0.97	Function unknown	Hypothetical protein (transmembrane)
M1_Spy_0855		0.002	-0.78	Carbohydrate metabolism	PTS system fructose-specific transporter subunit IIABC
M1_Spy_0872		0.010	-0.78	Nucleotide metabolism	5'-nucleotidase; Nucleotide degradation
M1_Spy_1135		0.001	-1.69	Nucleotide metabolism	Guanosine 5'-monophosphate oxidoreductase
M1_Spy_1136	*xpt*	0.012	-0.73	Nucleotide metabolism	Xanthine phosphoribosyltransferase; Purine salvage pathway
M1_Spy_1137		0.012	-0.52	Nucleotide metabolism	Purine permease (xanthine and uracil)

Table 5.3 Microarray data comparing SF370Δ1215 to SF370 after incubation with host cells

M1_Spy_1159	*hlyIII*	0.013	-0.45	Virulence	Hemolysin III
M1_Spy_1160		0.041	-0.59	Function unknown	Hypothetical protein
M1_Spy_1214	*lplA*	< 0.001	-3.35	Coenzyme metabolism	Lipoate-protein ligase
M1_Spy_1273	*cfa*	< 0.001	-1.04	Virulence	CAMP factor
M1_Spy_1290		0.002	-0.77	Function unknown	Hypothetical protein (signal peptide)
M1_Spy_1357	*grab*	0.009	-0.78	Virulence	GRAB; G-like alpha 2M-binding protein
M1_Spy_1373	*ptsH*	0.006	-0.49	Carbohydrate metabolism	Phosphocarrier protein HPr
M1_Spy_1402		0.033	-1.09	Cell wall/membrane biogenesis	Putative D-alanyl-D-alanine carboxypeptidase
M1_Spy_1405		0.040	-1.04	Function unknown	Hypothetical protein (transmembrane)
M1_Spy_1569		0.034	-0.69	Nucleotide metabolism	Putative shikimate kinase
M1_Spy_1570		0.001	-0.68	Function unknown	Putative GNAT acetyltransferase
M1_Spy_1571		0.004	-0.66	Function unknown	Hypothetical protein
M1_Spy_1599		0.019	-0.97	Carbohydrate metabolism	Beta-glucosidase
M1_Spy_1600		0.004	-0.81	Virulence	Hyaluronidase
M1_Spy_1602		0.008	-0.71	Transcription	GntR transcriptional regulator (family responsive to oxidized substrates of amino acid metabolism)
M1_Spy_1676	*tkt*	0.002	-0.70	Carbohydrate metabolism	Transketolase; Pentose phosphate pathway
M1_Spy_1827		< 0.001	-1.47	Inorganic ion metabolism	Divalent cation transport protein
M1_Spy_1828		0.002	-1.02	Function unknown	Hypothetical protein (transmembrane)
M1_Spy_1865		0.033	-0.48	Function unknown	Hypothetical protein
M1_Spy_1876	*rnjA*	0.028	-0.44	Transcription	Zn-dependent hydrolase; RNase J1

Table 5.3 Microarray data comparing SF370Δ1215 to SF370 after incubation with host cells

M1_Spy_1889	*fba*	0.034	-0.46	Carbohydrate metabolism	Fructose-bisphosphate aldolase; glycolysis
M1_Spy_1896	*tig*	0.026	-0.54	Virulence	Trigger factor; Protein folding
M1_Spy_2034		0.021	-0.76	Function unknown	Hypothetical protein
M1_Spy_2040		0.002	-1.86	Function unknown	Hypothetical protein
M1_Spy_2115	*spxA*	0.043	-0.59	Transcription	Transcriptional regulator Spx (global regulator of oxidative stress responses in *Bacillus*)
M1_Spy_2189	*sdhB*	0.011	-0.70	Amino acid metabolism	L-serine dehydratase subunit beta

[a] Log fold change indicates the ratio of SF370Δ1215 expression to wild-type SF370

Table 5.3 Microarray data comparing the transcriptome of SF370Δ1215 to SF370 following 2.5 hours incubation with pharyngeal monolayers

58 Upregulated genes (Lower expression in wild-type SF370)

Locus Tag	Gene Name	*P* Value	Log_2 Fold Change[a]	COG	Known or Proposed Function
M1_Spy_0045		0.037	0.50	Defense mechanisms	Na^+ driven multidrug efflux pump
M1_Spy_0100		0.001	0.89	Function unknown	Putative DNA binding protein
M1_Spy_0127		0.005	1.12	Virulence	Signal peptidase I; Pilus synthesis
M1_Spy_0128		0.018	1.24	Virulence	Major pilin protein; Pilus synthesis
M1_Spy_0129		0.001	0.97	Virulence	Sortase B; Pilus synthesis
M1_Spy_0130		< 0.001	1.31	Virulence	Minor pilin protein; Pilus synthesis
M1_Spy_0165	*nga*	0.018	2.50	Virulence	NAD-glycohydrolase
M1_Spy_0166		< 0.001	2.62	Defense mechanisms	Streptococcal NAD-glycohydrolase inhibitor
M1_Spy_0167	*slo*	< 0.001	2.86	Virulence	Streptolysin O
M1_Spy_0170		< 0.001	2.92	Function unknown	Hypothetical protein (transmembrane)
M1_Spy_0205		0.001	0.76	Function unknown	Hypothetical protein
M1_Spy_0207		< 0.001	0.89	Function unknown	Putative biotin synthase; BioY family protein
M1_Spy_0250	*rpmH*	0.050	0.54	Translation	50S ribosomal protein L34
M1_Spy_0287		0.049	0.38	Posttranslational modification	Iron-sulfur cluster assembly protein SufD
M1_Spy_0289	*nifU*	0.007	0.49	Energy production and conversion	Iron-sulfur cluster scaffold-like proteins
M1_Spy_0416	*spyCEP*	< 0.001	2.05	Virulence	Secreted protease
M1_Spy_0428	*spyA*	< 0.001	2.14	Virulence	Surface-bound ADP-ribosyltransferase
M1_Spy_0467		0.001	0.74	Function unknown	Hypothetical protein

Table 5.3 Microarray data comparing SF370Δ1215 to SF370 after incubation with host cells

M1_Spy_0469		< 0.001	0.84	Function unknown	Hypothetical protein (signal peptide)
M1_Spy_0492		< 0.001	0.87	Function unknown	Hypothetical protein (transmembrane)
M1_Spy_0739	*sagB*	0.004	0.99	Virulence	Streptolysin S propeptide modification
M1_Spy_0740	*sagC*	0.037	0.63	Virulence	Streptolysin S propeptide modification
M1_Spy_0746	*sagI*	0.011	0.64	Virulence	Streptolysin S export; ABC transporter ATP-binding protein
M1_Spy_0747	*spnA*	0.004	0.59	Virulence	Cell-surface nuclease
M1_Spy_0757	*atpH*	0.034	0.42	Energy production and conversion	ATP synthase F0F1 subunit delta
M1_Spy_0760	*atpD*	0.010	0.63	Energy production and conversion	ATP synthase F0F1 subunit beta
M1_Spy_0761	*atpC*	0.010	0.50	Energy production and conversion	ATP synthase F0F1 subunit epsilon
M1_Spy_0779	*rpsU*	0.002	0.61	Translation	30S ribosomal protein S21
M1_Spy_0878	*mvaK2*	0.010	0.51	Lipid metabolism	Phosphomevalonate kinase
M1_Spy_0884		0.003	0.66	Function unknown	Hypothetical protein (transmembrane)
M1_Spy_1055	*csrA*	0.047	0.52	Posttranslational modification	Methionine sulfoxide reductase B
M1_Spy_1056		0.003	0.62	Function unknown	Hypothetical protein (transmembrane)
M1_Spy_1204	*guaA*	0.033	0.58	Nucleotide metabolism	GMP synthase; Purine nucleotide biosynthesis
M1_Spy_1259		0.003	0.54	Transcription	GntR family transcriptional regulator; Ligand-binding protein related to C-terminal domains of K^+ channels (regulate in response to redox state)
M1_Spy_1284	*dnaE*	0.014	0.46	Replication, recombination, repair	DNA polymerase III DnaE
M1_Spy_1298	*malA*	0.006	1.02	Carbohydrate metabolism	Surface maltodextrose utilization protein

Table 5.3 Microarray data comparing SF370Δ1215 to SF370 after incubation with host cells

M1_Spy_1308		0.004	0.60	Lipid metabolism	Putative esterase
M1_Spy_1309	*dltD*	< 0.001	0.74	Virulence	D-alanyl-lipoteichoic acid biosynthesis protein; D-alanylation of surface LTA
M1_Spy_1310	*dltC*	0.007	0.50	Virulence	D-alanine-poly(phosphoribitol) ligase subunit 2; D-alanylation of surface LTA
M1_Spy_1389	*alaS*	< 0.001	0.68	Translation	Alanyl-tRNA synthetase; Aminoacyl-tRNA biosynthesis
M1_Spy_1437		0.001	2.42	Function unknown	Phage-encoded (Φ370.3)
M1_Spy_1513	*ileS*	< 0.001	0.91	Translation	Isoleucyl-tRNA synthetase; Aminoacyl-tRNA biosynthesis
M1_Spy_1673		0.046	0.43	Signal transduction	ABC transporter permease
M1_Spy_1681		0.042	0.48	Function unknown	Putative NADH peroxidase
M1_Spy_1832		0.006	0.85	Function unknown	Hypothetical protein (transmembrane)
M1_Spy_1834		0.042	0.82	Transcription	Putative transcriptional regulator
M1_Spy_1961	*polC*	0.021	0.50	Replication, recombination, repair	DNA polymerase III PolC
M1_Spy_1979	*ska*	< 0.001	1.67	Virulence	Streptokinase A
M1_Spy_2000	*dppA*	< 0.001	0.94	Amino acid metabolism	Surface lipoprotein; Peptide/nickel transport
M1_Spy_2003	*dppD*	< 0.001	1.08	Amino acid / inorganic ion metabolism	ABC-type dipeptide/oligopeptide/nickel transport system, ATPase component
M1_Spy_2004	*dppE*	0.022	0.96	Amino acid / inorganic ion metabolism	ABC-type dipeptide/oligopeptide/nickel transport system, ATPase component
M1_Spy_2009	*fbaA*	< 0.001	2.03	Virulence	Fibronectin-binding adhesin
M1_Spy_2010	*scpA*	< 0.001	1.85	Virulence	C5A peptidase
M1_Spy_2016	*sic*	< 0.001	2.27	Virulence	Streptococcal inhibitor of complement

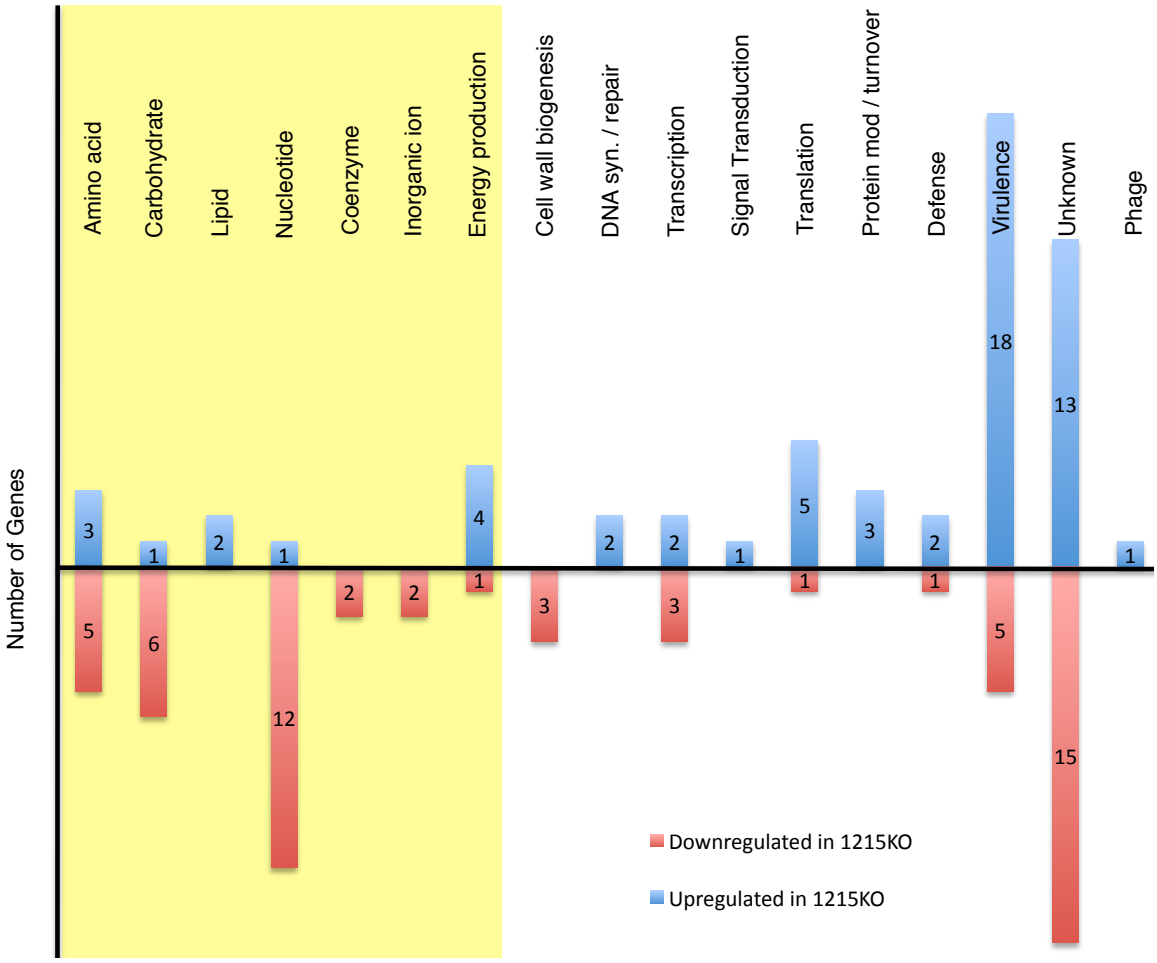
Table 5.3 Microarray data comparing SF370Δ1215 to SF370 after incubation with host cells

M1_Spy_2095	*pepO*	0.008	0.57	Posttranslational modification	Endopeptidase O; Peptide hydrolase
M1_Spy_2154		0.031	0.52	Function unknown	Hypothetical protein (transmembrane)
M1_Spy_2155		0.009	0.64	Function unknown	Hypothetical protein (transmembrane)
M1_Spy_2156	*aspS*	0.001	0.93	Translation	Aspartyl-tRNA synthetase; Aminoacyl-tRNA biosynthesis

[a] Log fold change indicates the ratio of SF370Δ1215 expression to wild-type SF370

Figure 5.7 COG classification of genes differentially expressed in SF370Δ1215 during incubation with pharyngeal monolayers

Genes are classified into functional groups based on protein homology to characterized genes. Each bar represents the total number of genes differentially upregulated (blue) or downregulated (red) in SF370Δ1215 as compared to wild-type SF370. The area of the graph shaded yellow highlights all functional categories involved in cellular metabolic processes. Labels for columns within the yellow highlighted area indicate the type of macromolecule transported, synthesized, or hydrolyzed by genes in that category.


Number of Genes
Amino acid
Carbohydrate
Lipid
Nucleotide
Coenzyme
Inorganic ion
Energy production
Cell wall biogenesis
DNA syn. / repair
Transcription
Signal Transduction
Translation
Protein mod / turnover
Defense
Virulence
Unknown
Phage
3
5
1
6
2
1
12
2
2
4
1
3
2
2
3
1
5
1
3
2
1
18
5
13
15
1
Downregulated in 1215KO
Upregulated in 1215KO

5.2.7 Comparison of SF370Δ1215 transcriptomes in the *in vitro* host environments

Examining the transcriptional profiles of SF370Δ1215 (in comparison to wild-type) following exposure to host cell environments *in vitro* indicated that deletion of *spy1215* had dramatically different consequences for streptococcal gene expression in the two environments tested. While both environments (pharyngeal supernatants and intact monolayers) induced differential gene expression in roughly 5-7% of the SF370Δ1215 genome, there was very little overlap between the genes modulated in each environment (Figure 5.8).

As previously described, the absence of Spy1215 in bacteria exposed to pharyngeal supernatants predominantly resulted in downregulation of genes involved in translation. On the other hand, upregulation of virulence factors was the dominant modulatory effect following incubation with host cell monolayers in the *spy1215* deletion mutant. In both host environments, however, there were a number of genes with unknown function that were differentially regulated (though the specific genes regulated in each environment did not overlap). Thus, it appears that Spy1215 deacetylation activity has very different effects depending on the ability of streptococci to transiently interact with target host cells.

Taking a closer look at the 12 genes regulated in both host environments in the *spy1215* deletion mutant, most genes exhibited either upregulation or downregulation under both experimental conditions (Table 5.4). These genes were involved in translation, posttranslational modifications, and metabolic

processes linked to amino acid availability and translation. Two genes were identified that were upregulated in one experimental setting, but downregulated in the other. The *spy1402* gene encodes a transpeptidase enzyme that crosslinks cell wall peptidoglycan. Expression of this enzyme in the mutant was induced 4-fold during exposure to secreted host factors, but 2-fold reduced in response to intact host cells. Meanwhile, a putative biotin synthase (*spy0207*) was 3-fold reduced in the pharyngeal supernatants and nearly 2-fold upregulated when in the presence of host cells.

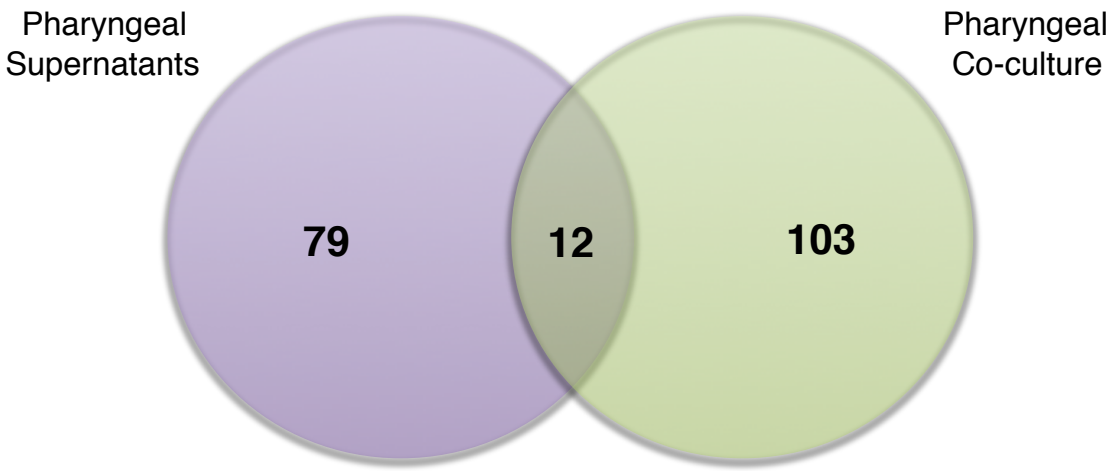


Figure 5.8 Comparison of genes regulated in SF370Δ1215 during exposure to pharyngeal supernatants and intact host cells

The Venn diagram depicts the limited overlap of genes differentially regulated in SF370Δ1215 during culture in the two host environments. Only 12 genes were shared by the two data sets, and 2 of these genes were regulated in opposite directions. Deletion of *spy1215* affected expression of 79 unique genes following exposure to pharyngeal supernatants, while a different set of 103 genes were modulated during co-culture with pharyngeal cells.

Table 5.4 Genes differentially expressed in SF370Δ1215 in response to both host environments (as compared to wild-type SF370)

		Pharyngeal Supernatants		Pharyngeal Monolayers	
Locus Tag	Gene Name	*P* Value	Log_2 Fold Change[a]	*P* Value	Log_2 Fold Change[a]
M1_Spy_0024	*purC*	< 0.001	-1.09	0.019	-0.97
M1_Spy_0032	*purD*	0.011	-0.82	0.001	-0.91
M1_Spy_0036	*purB*	0.011	-1.04	0.002	-0.61
M1_Spy_0207		**0.022**	**-1.66**	**< 0.001**	**0.89**
M1_Spy_0559		0.015	-1.74	0.025	-0.78
M1_Spy_1055	*csrA*	0.031	0.74	0.047	0.52
M1_Spy_1056		0.004	0.79	0.003	0.62
M1_Spy_1214		< 0.001	-4.02	< 0.001	-3.35
M1_Spy_1402		**0.043**	**2.10**	**0.033**	**-1.09**
M1_Spy_1513	*ileS*	0.004	0.94	< 0.001	0.91
M1_Spy_1569		0.002	-1.35	0.034	-0.69
M1_Spy_1571		0.032	-1.10	0.004	-0.66

Note: Genes in bold red font were regulated in opposite directions in SF370Δ1215 following *in vitro* exposure to the two host environments.

5.2.8 Examination of *spy1214-spy1215* co-transcription

Although we had already established that the lipoate ligase gene *spy1214* was transcriptionally active in SF370Δ1215 during growth in THY, our transcriptome data indicated that expression of this ORF was greatly reduced in the deletion mutant following exposure to the two host environments (Table 5.4). Upstream of *spy1214*, we identified a -35 promoter region with the sequence TTGATT along with a ribosomal binding site (GGAG), but no obvious -10 promoter sequence via *in silico* analysis. On the other hand, we found all of the elements of a strong streptococcal promoter upstream of *spy1215* (-35 sequence: TTACAT; -10 promoter: TTAT; ribosomal binding site: AGGAG), with the -35 and -10 sequences separated by 14 nucleotides.

Given the incomplete promoter upstream of *spy1214* and the strong sequences identified adjacent to *spy1215*, we hypothesized that *spy1214* transcription could originate from both promoters with wild-type transcription predominantly originating from the transcription start site upstream of *spy1215*. If the *spy1215* promoter region is responsible for driving most *spy1214* transcription in the wild-type, this would explain the dramatic loss in *spy1214* transcript observed for SF370Δ1215.

To determine if co-transcription with *spy1215* occurs, we performed RT-PCR analysis on RNA isolated from late exponential and stationary phase wild-type cultures grown in THY. Analysis revealed that *spy1214* could indeed be co-transcribed with *spy1215* in both growth phases (Figure 5.9). Thus, it is possible

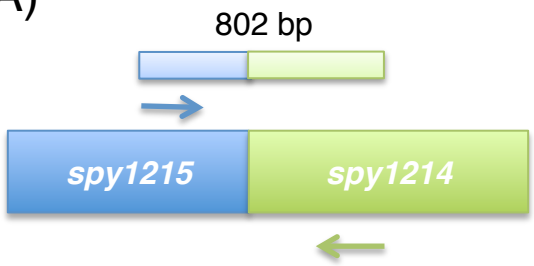
that the drastic downregulation of *spy1214* observed in our transcriptome analyses may be the direct result of eliminating the preferred promoter for *spy1214* expression rather than any Spy1215 deacetylase activity. Further analysis will be required to confirm this hypothesis.

Figure 5.9 RT-PCR analysis of *spy1214-1215* co-transcription

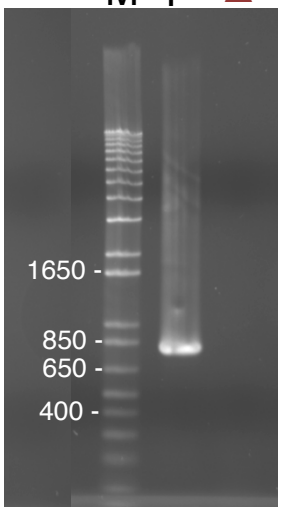
Panel (A) depicts the RT-PCR strategy. Primers were designed to amplify any mRNA resulting from co-transcription of *spy1215* (blue) and *spy1214* (green). If these genes are co-transcribed, the expected product size is 802 bp.

Panel (B) shows the results of the RT-PCR analysis on total RNA from WT SF370 (P) and the mutant SF370Δ1215 (Δ) grown to late exponential phase in THY broth. Lane (M) contains 1 kb plus DNA ladder (1 μg, Invitrogen). PCR products were separated on a 1% agarose gel and visualized by ethidium bromide staining. Control reactions performed with Taq polymerase (but no RT enzyme) indicated that the RNA samples were free of contaminating DNA (data not shown). Identical results were obtained from total RNA isolated from SF370 and SF370Δ1215 at stationary phase (data not shown).

(A)



(B)



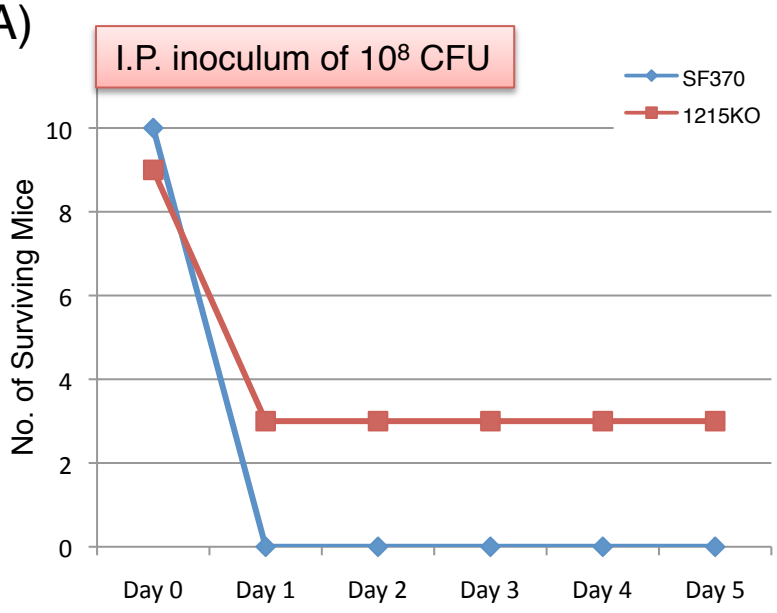
5.2.9 Comparison of SF370 and SF370Δ1215 virulence *in vivo*

As our transcriptome data indicated that SF370Δ1215 upregulates numerous virulence determinants in response to pharyngeal cells, we hypothesized that the *spy1215* deletion mutant may be more virulent than wild-type SF370. To test this theory, we injected mice intraperitoneally with SF370 and SF370Δ1215 using a range of CFU inoculums. Mice were then monitored over the course of five days for lethality. Infected mice were found to die within the first 24 hours or survive until the experiment was terminated at day 5 (Figure 5.10). Overall, there appears to be no significant difference in the virulence potential of SF370 and SF370Δ1215 using this murine infection model.

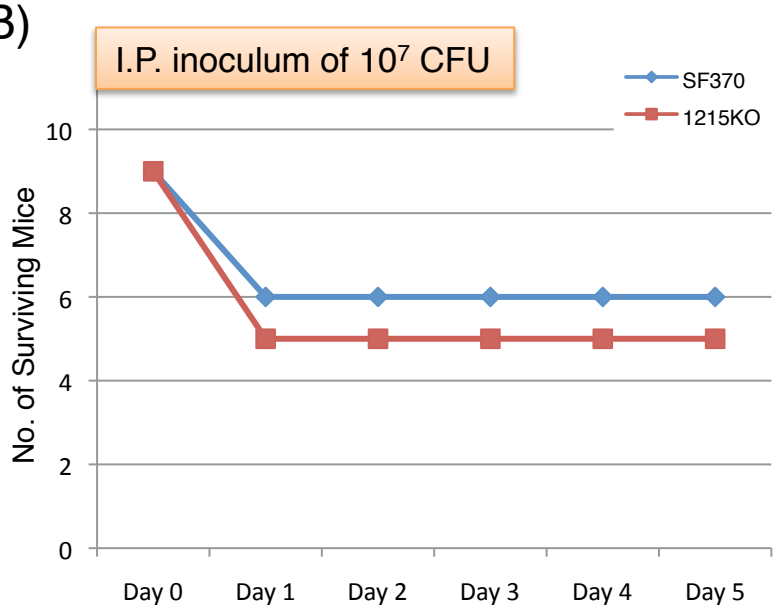
Figure 5.10 Comparison of SF370 and SF370Δ1215 virulence potential using a murine IP infection model

FVB/NJ mice were intraperitoneally (IP) injected with ~10^8 CFU (A) of ~10^7 CFU (B) of SF370 (blue) or SF370Δ1215 (red). Mice were monitored for survival over 5 days. Each graph depicts the number of mice alive on each day post-injection. Results from two independent experiments were combined for this analysis. Nine mice were injected for each experimental condition, with the exception of SF370-injected mice in Panel (A) for which we inoculated 10 mice total.

(A)


I.P. inoculum of 10^8 CFU
SF370
1215KO
No. of Surviving Mice
10
8
6
4
2
0
Day 0
Day 1
Day 2
Day 3
Day 4
Day 5

(B)


I.P. inoculum of 10^7 CFU
SF370
1215KO
No. of Surviving Mice
10
8
6
4
2
0
Day 0
Day 1
Day 2
Day 3
Day 4
Day 5

5.3 Discussion

Although our understanding of sirtuin function in prokaryotes is rudimentary at best (and non-existent regarding group A streptococci), the transcriptome analyses described in this chapter provide preliminary evidence that deacetylases may not only be involved in metabolic regulation, but can also contribute to bacterial virulence regulation.

When we embarked on this project, a few publications indicated that the sirtuin homolog CobB present in *S. enterica* and *E. coli* was important for activating metabolic enzymes by deacetylating key lysine residues at their catalytic sites (Starai, *et al.* 2002; Starai, *et al.* 2003; Garrity, *et al.* 2007). CobB expression and activity was found to change depending on carbon source availability and growth phase (Wang, *et al.* 2010). Furthermore, initial screens of protein acetylation sites indicated that metabolic enzymes are a key target for the action of acetylases and deacetylases (primarily CobB) in these Gram-negative species (Yu, *et al.* 2008; Zhang, *et al.* 2009; Wang, *et al.* 2010).

As streptococci are known to regulate virulence based on the availability of extracellular carbon sources via the carbon catabolite repressor CcpA, it is conceivable that Spy1215 also acts in this manner (Shelburne III, *et al.* 2008). Indeed, examination of the transcriptional responses of streptococci to glucose has indicated that *spy1215* is repressed in the presence of this sugar. Furthermore, CcpA appears to play a direct role in this repression (Kietzman & Caparon 2010). Thus far, CcpA is the only regulator found to affect expression of

spy1215, despite extensive studies of isogenic mutants for the stand-alone regulators PerR, Mga, MtsR, RopB, and RivRX, and the two-component systems CovRS, Ihk/Irr, and TrxRS (Voyich, *et al.* 2004; Dalton, *et al.* 2006; Roberts & Scott 2007; Dmitriev, *et al.* 2008; Leday, *et al.* 2008; Olsen, *et al.* 2010; Grifantini, *et al.* 2011).

Induction of Spy1215 in glucose-limited environments (such as would be found in the oropharynx) provided the first clue that the streptococcal sirtuin may play an important role in virulence regulation. Examining our transcriptome data for the isogenic mutant SF370Δ1215, however, revealed that the story of Spy1215 regulation is far more complex than we originally envisioned. Our initial screen of transcriptional changes induced by exposure to pharyngeal supernatants and pharyngeal monolayers indicated that *spy1215* is upregulated in both host environments. Although carbon source availability (i.e. glucose limitation) may play a role in this induction, our data regarding SF370Δ1215 in this section indicate that the effects of Spy1215 deacetylation on streptococcal virulence expression also appear to be dependent upon close spatial association to and/or transient interactions with host cells.

We limited our analyses of Spy1215 regulation to the transcriptional responses of non-adherent (associated) streptococci in the pharyngeal cell environment. If Spy1215 activity is solely dependent on information concerning extracellular carbon sources, we would have expected to see similar effects on streptococcal transcription following exposure to pharyngeal supernatants and

monolayers. Although possible, it is unlikely that the nutrient availability and host cell milieu would differ significantly between the two cultures due to our experimental setup (detailed below and in the methods section).

For our experiments, we collected pharyngeal supernatants following 18 hours of pharyngeal growth. These supernatants were pooled, filtered, and distributed for use in both transcriptional studies. Thus, the streptococci exposed to pharyngeal monolayers were bathed in the same filtered supernatant used for streptococci incubated with the supernatants alone. Consequently, any carbon source signals affecting *spy1215* induction and subsequent Spy1215 activity would presumably be present in both experimental conditions. Considering the transcriptome results obtained for the SF370Δ1215 mutant in the two host environments, additional signals are required to further fine-tune the activity and/or targets of Spy1215 deacetylase in the infection setting.

As the only significant difference between the two experimental conditions is the presence of epithelial cells, we propose that transient interactions between streptococci and host cell receptors provide the additional signals necessary to modulate Spy1215-dependent regulation. Furthermore, the signals imparted by host cell contact serve to activate a Spy1215-mediated program of virulence repression (as was generally observed in our time course microarray analysis detailed in section 4). Sirtuins are known to repress expression of virulence genes in the eukaryotic parasite *Plasmodium falciparum*. In addition, virulence proteins were identified as possible targets for direct acetylation in *S. enterica,*

although researchers have not explored if acetylation of virulence factors occurs *in vitro* or *in vivo.* Overall, available evidence does suggest that sirtuins may play an important role in controlling virulence in diverse human pathogens.

Although it may initially appear counterintuitive that streptococci would repress known virulence factors when in close contact with host cells, it is possible that the bacteria reduce virulence expression to prevent induction of host defenses and pro-inflammatory signals. In addition, surface adhesins, like the streptococcal pilus, can be a double-edged sword for the pathogen. Although the pilus and other adhesins allow for streptococcal binding to host epithelial cells, they are also targets for the body's innate defenses. The salivary component gp340, for example, binds streptococcal pili causing the bacteria to aggregate, which may increase the rate of bacterial clearance from the oropharynx (Edwards, *et al.* 2008). Downregulation of the pilus has been observed during growth in human saliva and was also seen in our transcriptional analysis of SF370 exposed to pharyngeal supernatants (Shelburne III, *et al.* 2005; section 4 of this thesis).

Streptococci must therefore strike a delicate balance between virulence elaboration and immune evasion. We hypothesized that virulence derepression in SF370Δ1215 would affect the mutant's virulence potential. Surprisingly, our *in vivo* experiments using a murine IP model of infection indicated no difference in lethality between the *spy1215* deletion mutant and wild-type SF370. There could be several reasons for this unexpected result. First, it has been demonstrated

that the parental SF370 strain is generally not virulent in mice when injected IP (Lukomski, *et al.* 2000; personal observations in our laboratory). Thus, any effect on virulence potential may not be readily detectable using this model.

Second, *speB* expression in the hypervirulent M1T1 clone appears to be negatively correlated with virulence (Kansal, *et al.* 2000). It is possible that high SpeB production could cleave bacterial surface adhesins and hydrolyze secreted virulence factors negating any effect of their transcriptional upregulation (Aziz & Kotb 2008). We observed a 64-fold increase in *speB* expression during SF370 co-culture with pharyngeal monolayers. Unfortunately, oversaturation of the microarray signal at the *speB* locus prevented our assessment of its expression in SF370Δ1215. We are currently performing real-time quantitative RT-PCR to ascertain if SF370Δ1215 does indeed express *speB* at high levels in the host environment. Examination of the virulence potential of SF370Δ1215 with other *in vivo* models will be necessary to elucidate the significance of Spy1215-dependent virulence repression within the host.

The mechanism of Spy1215-dependent virulence repression in *S. pyogenes* will certainly require further investigation, but we can propose one potential model that the data support. In *E. coli*, deacetylation of a key lysine residue in the DNA-binding site of the two-component transcriptional repressor RcsB increases its promoter-binding affinity leading to reduced downstream gene expression (Thao, *et al.* 2010). As many of the virulence determinants upregulated in the SF370Δ1215 mutant are known to be repressed by CovR, it is

possible that Spy1215 deacetylation of CovR allows for CovR binding and acts to repress these virulence genes when in the presence of host cells. One of the key residues critical for CovR binding to DNA is a lysine and may serve as an acetylation target site allowing for modulation of CovR activity *in vivo*.

It is also conceivable that Spy1215 can act further upstream by deacetylating other transcription factors which then affect *covR* expression, but our transcriptome analyses do not support this alternative. In fact, we did not see regulation of any of the characterized stand-alone regulators or two-component systems when Spy1215 deacetylase activity was eliminated, suggesting that Spy1215 acts immediately upstream of virulence factor expression and not through a long string of interconnected regulators.

In addition, it is possible that Spy1215 deacetylation of the RNA polymerase complex or its associated cofactors may be responsible for virulence gene repression at particular loci. CobB in *E. coli* was shown to deacetylate the RNAP when in complex with the regulator CpxR, reducing expression from a specific promoter (Lima, *et al.* 2011). Certainly, we cannot rule out the possibility that Spy1215-dependent virulence repression is the result of more distal events. Spy1215 may simply act on metabolic pathways that are then linked to virulence expression by other regulatory proteins. The requirement of host cell contact for Spy1215 virulence repression, however, seems to suggest a more complex scenario for Spy1215-dependent virulence regulation than basic metabolism.

As with CobB and other bacterial sirtuins, a role for Spy1215 in *S. pyogenes* metabolic processes is supported by our data. In both host environments, the absence of *spy1215* affected expression of genes involved in carbohydrate, amino acid, nucleotide, and coenzyme metabolism. We had anticipated a larger overall effect on carbohydrate metabolism than was observed. Although CobB in *S. enterica* and *E. coli* works to activate metabolic enzymes at the protein level, we anticipated that deregulation of protein function would trigger feedback mechanisms affecting transcriptional expression of these genes or their downstream metabolic partners. Only two genes central to carbohydrate metabolism that are acetylated in *S. enterica* (phosphoenolpyruvate carboxylase and fructose bisphosphate aldolase) were downregulated in SF370Δ1215 during incubation with host cells (Wang, *et al.* 2010).

Overall, the lack of Spy1215 deacetylase activity appeared to largely deregulate protein synthesis and posttranslational modifications. This effect was most pronounced in SF370Δ1215 incubated with pharyngeal supernatants in which over 20 proteins involved in translation were downregulated. Many of the proteins involved in translation and posttranslational modifications that were downregulated in response to one or both host environments have also been identified as targets of acetylation in *S. enterica*, including trigger factor, various ribosomal subunits, tRNA synthetases, and xanthine phosphoribosyltransferase. In addition, the inosine biosynthesis pathway was negatively affected in the absence of *spy1215* in both host environments. Thus, it appears that the role of

Spy1215 in supporting protein synthesis and modification is independent of host cell contact.

Interestingly, genes involved in septum formation and cell division were downregulated in the *spy1215* deletion mutant during incubation in cell-free pharyngeal supernatants. Perhaps this could explain the compacted appearance of SF370Δ1215 chains during growth in THY. Any minor defects in septal formation or cell segregation that may result from *spy1215* deletion did not affect the mutant's overall growth rate, however.

We must acknowledge that Spy1215 may also possess ADP-ribosyltransferase activity as has been observed for other Sir2 proteins. Furthermore, it is possible that the effects of Spy1215 on virulence expression could stem from this activity versus the deacetylase action of sirtuins. We are currently in the process of confirming that Spy1215 can indeed remove acetyl moieties from peptide lysine side-chains. Additional work will be necessary to identify deacetylation targets in GrAS and determine the mechanism by which Spy1215 affects virulence expression.

It should also be noted that we currently cannot distinguish between effects of Spy1215 deacetylase activity and indirect effects due to an altered NAD^+/NADH balance resulting from *spy1215* deletion. Sirtuins are Mn^{2+}-binding, NAD^+-dependent enzymes, so their function within a bacterial cell has an effect on the cytoplasmic redox state and metal ion homeostasis. To this point, we observed the upregulation of numerous genes involved in oxidative stress

responses in SF370Δ1215, including NADH oxidase, glutathione reductase, and the molecular chaperones *groEL* and *groES*. These expression inductions were largely confined to streptococci exposed to pharyngeal supernatants, however, indicating that changes in the redox state may not account for Spy1215-dependent virulence repression. Identification of deacetylation targets in transcription factors will be necessary, however, to definitively demonstrate the action of Spy1215 on virulence repression.

Finally, we should focus some attention on putative acetyltransferases in *S. pyogenes* that may act as antagonists to Spy1215 regulation. As previously discussed, protein acetylation is a reversible modification important for modulating protein-protein interactions, protein-DNA binding, and protein stability. While *spy1215* encodes a sirtuin deacetylase homolog, we have not yet discussed the GNAT acetyltransferases of group A streptococci. SF370 carries genes for two acetyltransferases: *spy1356* and *spy1570* (Ferretti, *et al.* 2001). During wild-type SF370 incubation with both pharyngeal supernatants and intact monolayers, these acetyltransferases were upregulated: *spy1356* at all time points examined and *spy1570* at 2.5 hours.

Examining the transcriptional data from SF370Δ1215, we found that only *spy1570* was differentially regulated in the absence of *spy1215.* The *spy1570* gene was downregulated in the mutant, but only during interactions with pharyngeal cells. Thus, the expression of this acetyltransferase mirrors that of *spy1215* in the presence of host cells. It is possible that Spy1570 works to

balance the actions of Spy1215 in group A streptococci to achieve complex regulatory patterns. This would explain why *spy1570* is downregulated in the absence of Spy1215 activity, but this hypothesis remains to be tested.

In conclusion, our transcriptome analyses of SF370Δ1215 have cemented a role for bacterial sirtuins in virulence regulation. One previous study had determined that deletion of a sirtuin homolog in the phytopathogen *Xanthomonas campestris* attenuated virulence, but the researchers did not find significant differences in expression for key virulence factors assessed (Zhou, *et al.* 2011). Hence, this is the first study to our knowledge that describes a general role for the sirtuin deacetylases in virulence for animal bacterial pathogens and a specific function in regulation of virulence factor transcription for any bacterial pathogen. Future work on this regulator will no doubt enhance our understanding of the complex, interweaving network of streptococcal virulence regulators.

6. REGULATION BY SPY1755

6.1 Introduction

Protein homology indicates that the *spy1755* stand-alone regulator is a member of the MarR family of transcription factors (Ferretti, *et al.* 2001). Over 12,000 MarR-like homologs have been identified in 45 species of bacteria and a dozen species of Archea (reviewed in Ellison & Miller 2006; Perera & Grove 2011). Although members of this family share structural similarities, such as a DNA-binding helix-turn-helix domain, there is significant diversity at the amino acid level. This could indicate that MarR regulators have evolved to respond to diverse signaling molecules and affect transcription of divergent DNA targets (Ellison & Miller 2006).

First characterized in *E. coli* as a regulator important for multiple antibiotic resistance, members of this family regulate a wide variety of biological functions, including metabolic pathways, stress responses, and the export of harmful chemicals (Ellison & Miller 2006; Perera & Grove 2010). In addition, MarR-family regulators are involved in virulence regulation in a number of bacterial pathogens, including *Salmonella typhimurium* (SlyA), *Vibrio cholerae* (AphA), and *Staphylococcus aureus* (MgrA) (Libby, *et al.* 1994; Kovacikova, *et al.* 2004; Ingavale, *et al.* 2005). In *S. aureus*, the global regulator MgrA affects expression of capsule, protein A, alpha-toxin, and secreted proteases (Luong, *et al.* 2003; Luong, *et al.* 2006).

MarR regulators affect gene transcription by binding to palindromic sequences within target promoters as dimers. MarR homologs are often encoded between divergently expressed loci and affect the expression of these proximal genes. MarR regulators can also act on distant genes, however, either repressing or activating those loci (Perera & Grove 2011). MarR family regulators have been found to respond to specific ligands, such as aromatic compounds. In the absence of the ligand, MarR binds more readily to target promoters repressing expression of genes involved in catabolism of the ligand. On the other hand, binding of the ligand attenuates DNA binding of the MarR repressor and activates transcription of genes in the catabolic pathway (Wilkinson & Grove 2006).

Transcriptome analyses of SF370 incubated with pharyngeal supernatants and intact pharyngeal monolayers revealed that *spy1755* was upregulated in comparison to control streptococci at 1.5 hours and 2.5 hours in both host environments. This observation combined with the role of MarR regulators in virulence modulation in other bacterial pathogens led us to hypothesize that Spy1755 may affect transcriptional changes important for initiating the streptococcal adaptive response to the host environment. The following chapter details our work to characterize the Spy1755 regulator using an isogenic *spy1755* knockout in the SF370 background.

6.2 Results

6.2.1 Confirmation of *spy1755* deletion

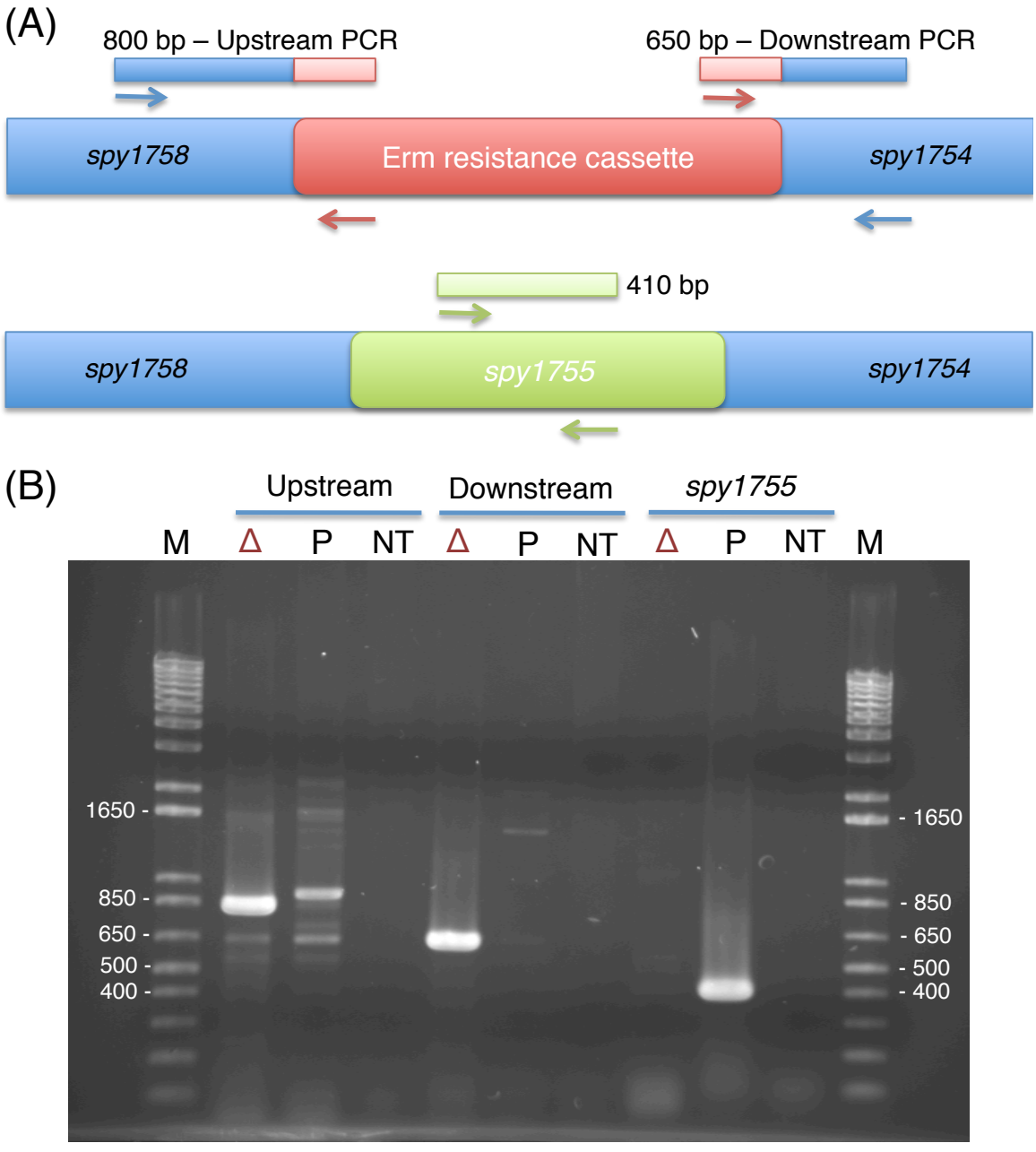
An allelic replacement strategy was employed to delete the *spy1755* ORF using a cassette encoding for erythromycin resistance. Following electroporation of SF370, Erm^R colonies were screened for double recombination events and resulting loss of *spy1755*. PCR amplification of an internal *spy1755* region, as well as the upstream and downstream junctions of the plasmid with the bacterial chromosome confirmed that *spy1755* had been eliminated from the genome of SF370Δ1755 (Figure 6.1).

DNA sequencing provided an additional line of supporting evidence for the generation of the *spy1755* isogenic mutant (data not shown). Furthermore, sequencing analysis determined that no point mutations were introduced into the upstream and downstream genes surrounding the *spy1755* locus as a result of allelic recombination (data not shown).

Figure 6.1 PCR analysis to confirm *spy1755* deletion from SF370

Panel (A) depicts the PCR scheme. Primers internal to the Erm cassette (red) were used in combination with primers internal to genes upstream and downstream of *spy1755* (blue) to confirm plasmid integration. Expected product sizes resulting from plasmid integration are 800 bp for the upstream region and 650 bp for the downstream region. Primers internal to *spy1755* (green) were used to specifically probe for the presence of the ORF (expected product size is 410 bp).

Panel (B) shows the results of PCR analyses using genomic DNA from WT SF370 (P) and the mutant SF370Δ1755 (Δ). Control PCR reactions with primers but no template (NT) were also included. Lane (M) contains 1-kb Plus DNA ladder (1 μg, Invitrogen). PCR products were separated on 1% agarose gels in TAE and visualized by ethidium bromide staining. Non-specific amplification was observed with the upstream primers in the wild-type. Sequencing of the upstream PCR band from SF370Δ1755, however, confirmed integration of the plasmid at this site.


(A)
800 bp – Upstream PCR
650 bp – Downstream PCR
spy1758
Erm resistance cassette
spy1754
410 bp
spy1758
spy1755
spy1754
(B)
Upstream
Downstream
spy1755
M
Δ
P
NT
Δ
P
NT
Δ
P
NT
M
1650 -
850 -
650 -
500 -
400 -
- 1650
- 850
- 650
- 500
- 400

6.2.2 Growth curve comparison and light microscopy of SF370 and SF370Δ1755

Examination of the growth curves of wild-type SF370 and SF370Δ1755 in BHI broth revealed that SF370Δ1755 exhibited significant growth deficiencies (Figure 6.2). Not only did the mutant exhibit a long lag phase, but SF370Δ1755 also did not achieve $O.D._{600}$ readings greater than 0.4, even after 24 hours of growth (data not shown). On blood agar plates, long growth incubations at 37°C (with and without 5% CO_2) resulted only in tiny, beta-hemolytic colonies (data not shown).

Observations of SF370Δ1755 using light microscopy revealed that the mutant suffered severe cell morphology changes when compared to wild-type SF370 (Figure 6.3). In particular, the mutant bacterial cells were not arranged in the typical chain orientation, and individual cells appeared engorged and elongated.

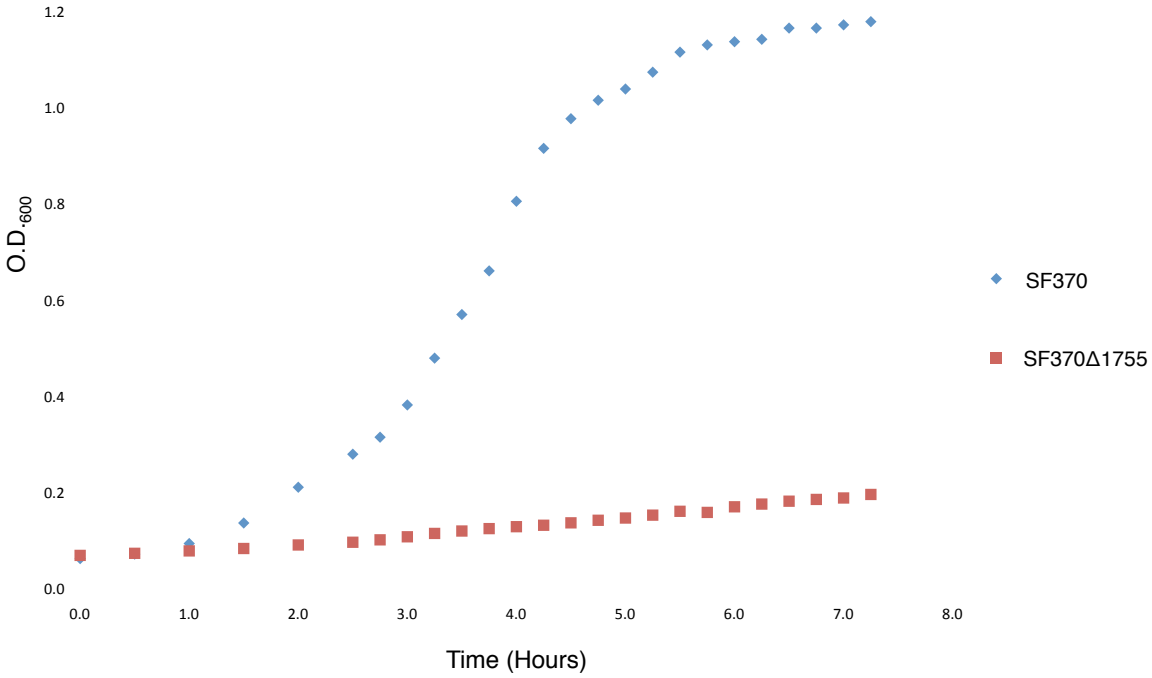


Figure 6.2 Growth comparison of wild-type SF370 and the deletion mutant SF370Δ1755

Overnight cultures were diluted 1:50 in 10ml of pre-warmed BHI and incubated at 37°C. Absorbance of cultures ($O.D._{600}$) was measured at different time points using a spectronic 20D spectrophotometer (Thermo Electron Corporation) and the results were plotted. Each curve represents an average of three experiments performed on different days.

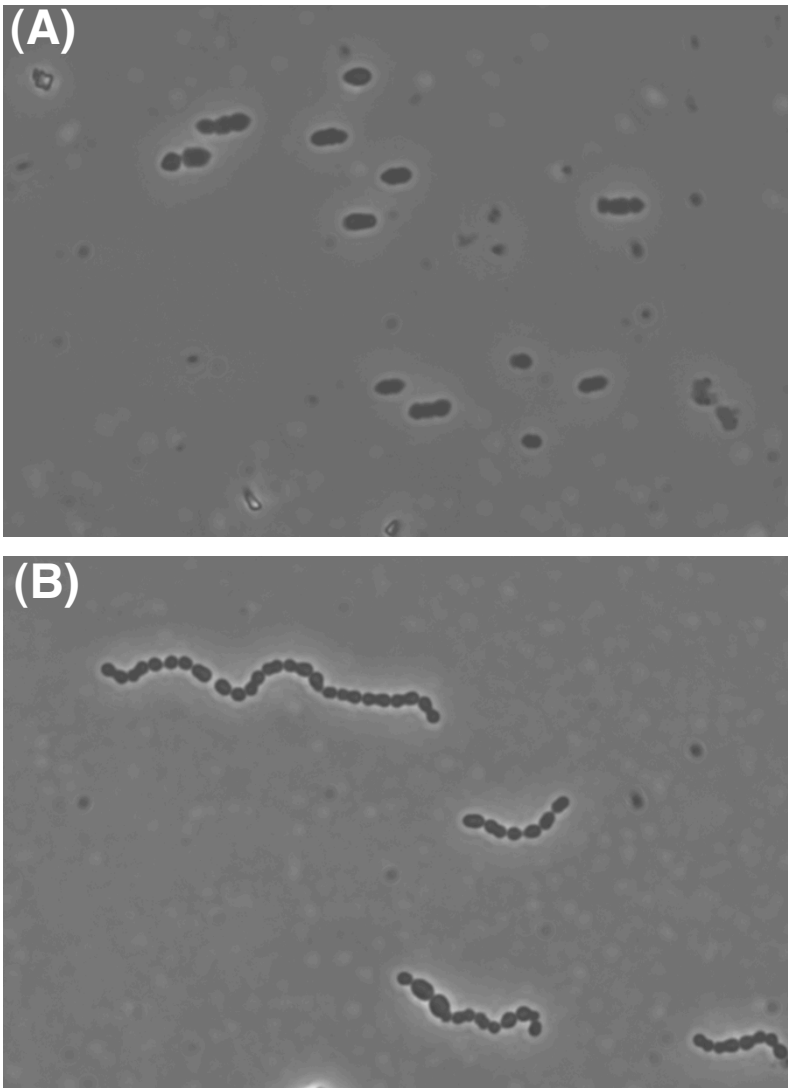


Figure 6.3 Visualization of SF370 and SF370Δ1755 by light microscopy

Panel (A) shows the altered SF370Δ1755 cell morphology and absence of streptococcal chains. Panel (B) demonstrates wild-type SF370 cell shape and chain length. Panels are representative samples taken from cultures grown to stationary phase overnight in THY broth. Images captured under 100X magnification.

6.2.3 Examination of polar effects on downstream gene expression

Reverse-transcription PCR was utilized to examine if deletion of *spy1755* had polar effects on the expression of downstream genes. Researchers had not previously investigated if *spy1755* was part of an operon in *S. pyogenes*. Examination of the genomic region surrounding the *spy1755* locus revealed that the gene resides between ORFs transcribed in the same direction (Figure 6.4). Thus, it is possible that deletion of *spy1755* could eliminate expression of *spy1754* and other downstream genes resulting in the growth deficient phenotype observed *in vitro.*

RT-PCR using primers internal to *spy1754* and *spy1753* revealed that the deletion of *spy1755* did not affect expression of these genes at late exponential or stationary phase growth in THY (Figure 6.5). Furthermore, RT-PCR of *spy1755* in the isogenic mutant again confirmed the loss of *spy1755* at the level of transcription. These data indicate that the growth deficient phenotype of SF370Δ1755 was solely due to the loss of *spy1755* function.

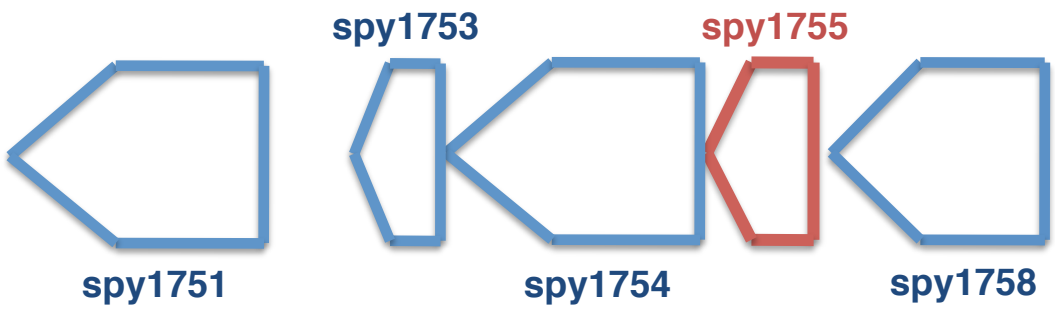


Figure 6.4 Schematic of the *spy1755* genomic region

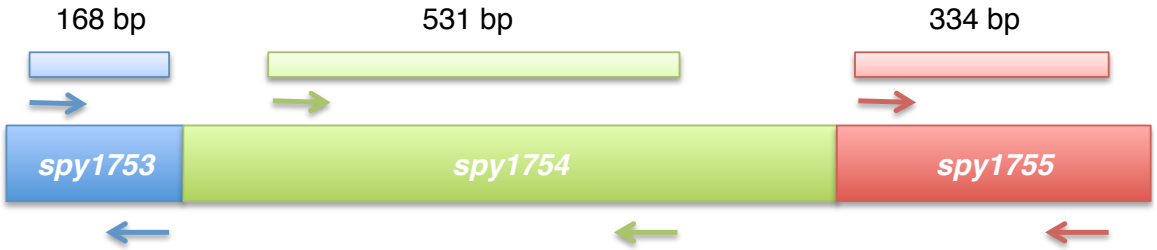
The figure depicts the open reading frames (blue) directly adjacent to *spy1755* (red). All genes are transcribed from the reverse strand, such that *spy1755* lies upstream of *spy1754* for transcription (indicated by direction of arrowheads). ORFs have been drawn in proportion to their nucleotide length and intergenic spaces.

Figure 6.5 RT-PCR analysis of expression from *spy1755* and its downstream genes in SF370Δ1755 and SF370

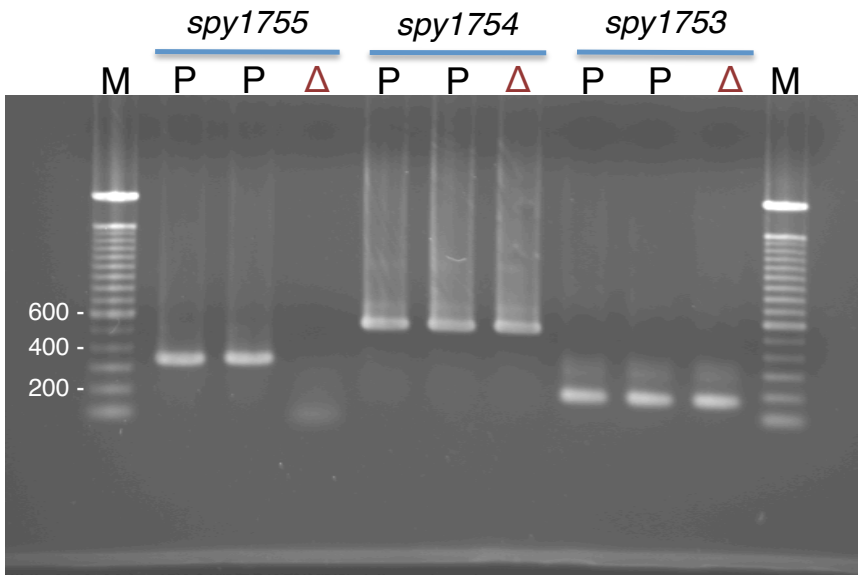
Panel (A) depicts the RT-PCR strategy. Primers were designed to amplify segments of mRNA generated from *spy1753* (blue), *spy1754* (green), and *spy1755* (red). Expected product sizes are 168 bp, 531 bp, and 334 bp respectively.

Panel (B) shows the results of the RT-PCR analysis on total RNA from two WT SF370 (P) samples and the mutant SF370Δ1755 (Δ) grown to late exponential phase in THY broth. Lane (M) contains 100 bp DNA ladder (1 μg, Invitrogen). PCR products were separated on a 1% agarose gel and visualized by ethidium bromide staining. Control reactions performed with Taq polymerase (but no RT enzyme) indicated that the RNA samples were free of contaminating DNA (data not shown). Identical results were obtained from total RNA isolated from SF370 and SF370Δ1755 at stationary phase (data not shown).

(A)


168 bp
531 bp
334 bp
spy1753
spy1754
spy1755

(B)


spy1755
spy1754
spy1753
M P P Δ P P Δ P P Δ M
600 -
400 -
200 -

6.2.4 *In silico* analysis of the *spy1755* genome region

Recognizing that MarR regulators often regulate genes immediately adjacent to them in the genome, we examined the genetic locus of *spy1755* to ascertain if deregulation of nearby genes could account for the SF370Δ1755 growth deficiency. Inspection of gene annotations and protein similarities of adjacent genes highlighted that this region of the SF370 genome is responsible for fatty acid biosynthesis (Figure 6.6). Furthermore, protein homology searches indicated that Spy1755 shares 62% identity at the amino acid level with a regulator of fatty acid biosynthesis in *S. pneumoniae.*

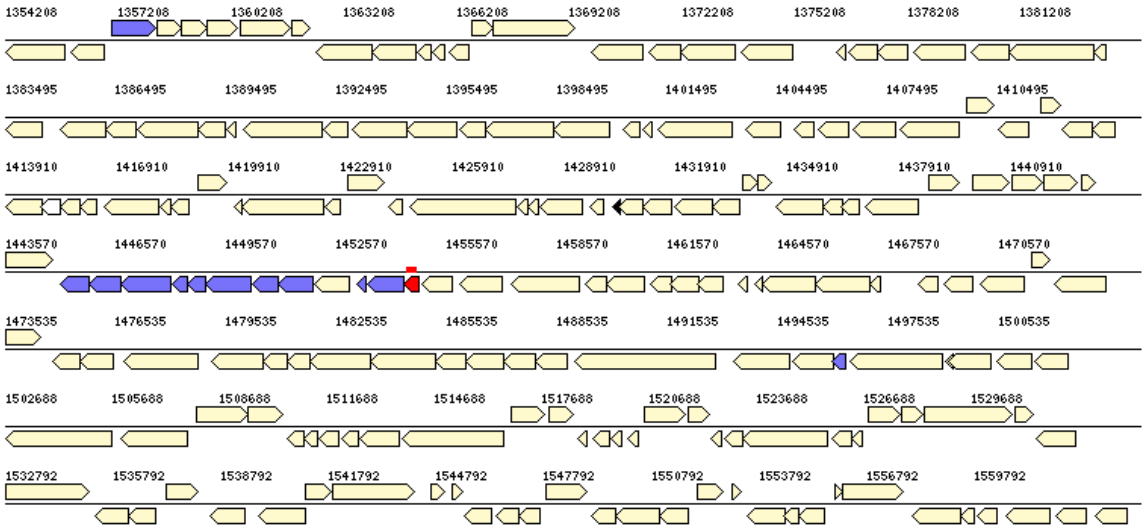


Figure 6.6 Genes involved in fatty acid biosynthesis in the vicinity of *spy1755*

A region of the genome representing *spy1633* to *spy1884* was scanned for genes involved in fatty acid biosynthesis on the basis of TIGRFAM classification. The TIGRFAM model optimized for mining prokaryotic genomes categorizes genes into functional categories based on sequence similarity to genes of known function (Selengut, *et al.* 2007). The ORF for *spy1755* (red) resides directly upstream of a cluster of genes important for fatty acid biosynthesis (blue). Figure generated using the Department of Energy Joint Genome Institute website (http://www.jgi.doe.gov).

6.2.5 Partial rescue of the growth deficiency phenotype with Tween-80

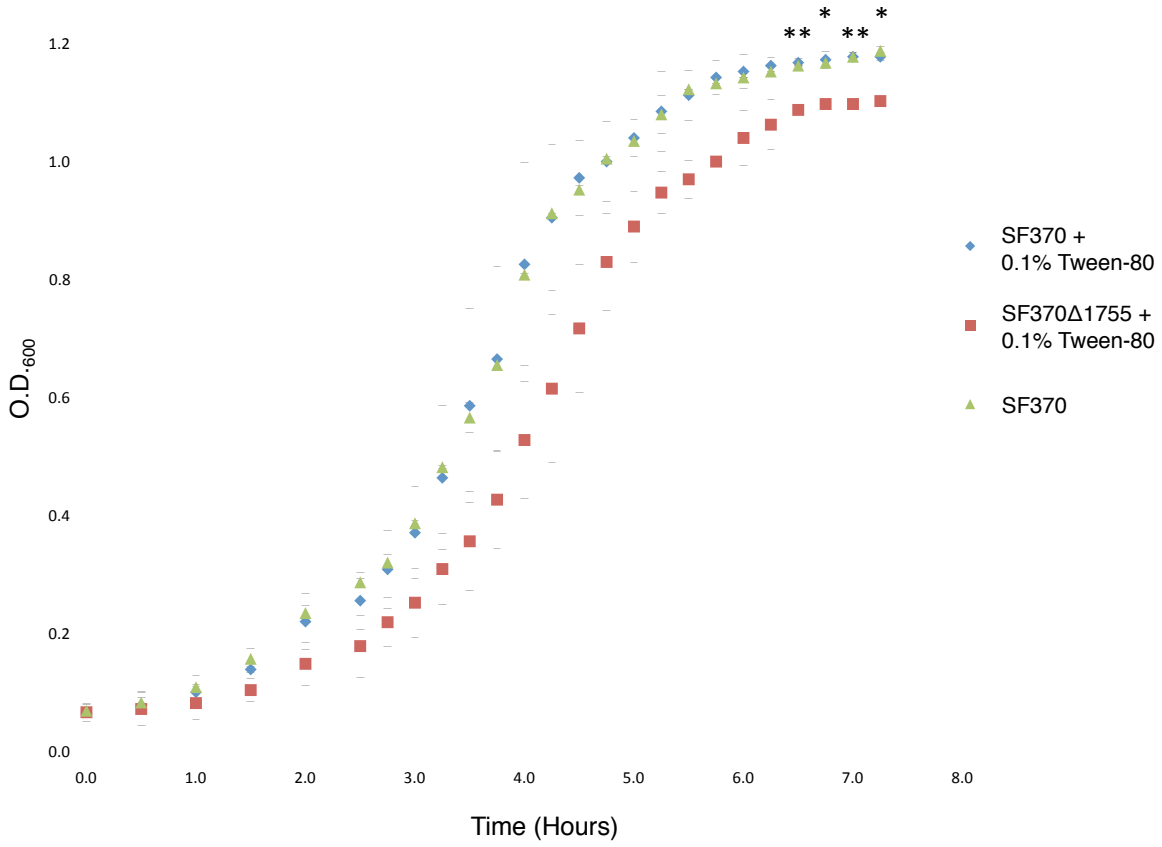
As our *in silico* analysis revealed that *spy1755* lies upstream of a cluster of genes responsible for fatty acid biosynthesis, numerous fatty acid supplements were tested for their ability to rescue the growth deficiency phenotype of SF370Δ1755. Addition of 0.1% Tween-80 (oleic acid) to BHI broth was found to restore growth in SF370Δ1755 to near wild-type levels by growth curve analysis (Figure 6.7). The mutant no longer exhibited an extended lag phase and achieved a rate of growth comparable to wild-type SF370. During stationary phase, however, the maximal cell density of SF370Δ1755 was statistically lower than that of wild-type SF370, as determined by an unpaired Student's *t*-test.

Visually, overnight growth of SF370Δ1755 in THY broth supplemented with 0.1% Tween-80 yielded a bacterial pellet comparable to those observed with wild-type SF370 (Figure 6.8). Under the light microscope, SF370Δ1755 grown in the presence of 0.1% Tween-80 appeared to exhibit cell morphologies and chain lengths indistinguishable from wild-type (Figure 6.8). Furthermore, addition of Tween-80 to SF370 culture broth did not affect the appearance of wild-type streptococci.

Figure 6.7 Growth comparison of wild-type SF370 and the deletion mutant SF370Δ1755 grown with 0.1% Tween-80

Overnight cultures were diluted 1:50 in 10ml of pre-warmed BHI and incubated at 37°C. Broth was supplemented with 0.1% Tween-80 for one wild-type SF370 culture (blue) and for the SF370Δ1755 culture (red). Absorbance of cultures ($O.D._{600}$) was measured at different time points using a spectronic 20D spectrophotometer (Thermo Electron Corporation) and the results were plotted. Each curve represents an average of two experiments performed on different days.

Student's *t*-test was employed to determine if $O.D._{600}$ readings for SF370 and SF370Δ1755 grown in the presence of Tween-80 differed significantly at any points along the curve. Statistically significant differences in growth were only observed for the last four measurements during stationary phase (** $P < 0.01$, * $P < 0.05$). There was no statistical difference between the growth curves of wild-type SF370 grown with (blue) or without (green) Tween-80.


**
*
**
*
1.2
1.0
0.8
0.6
0.4
0.2
0.0
O.D.600
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
Time (Hours)
SF370 +
0.1% Tween-80
SF370Δ1755 +
0.1% Tween-80
SF370

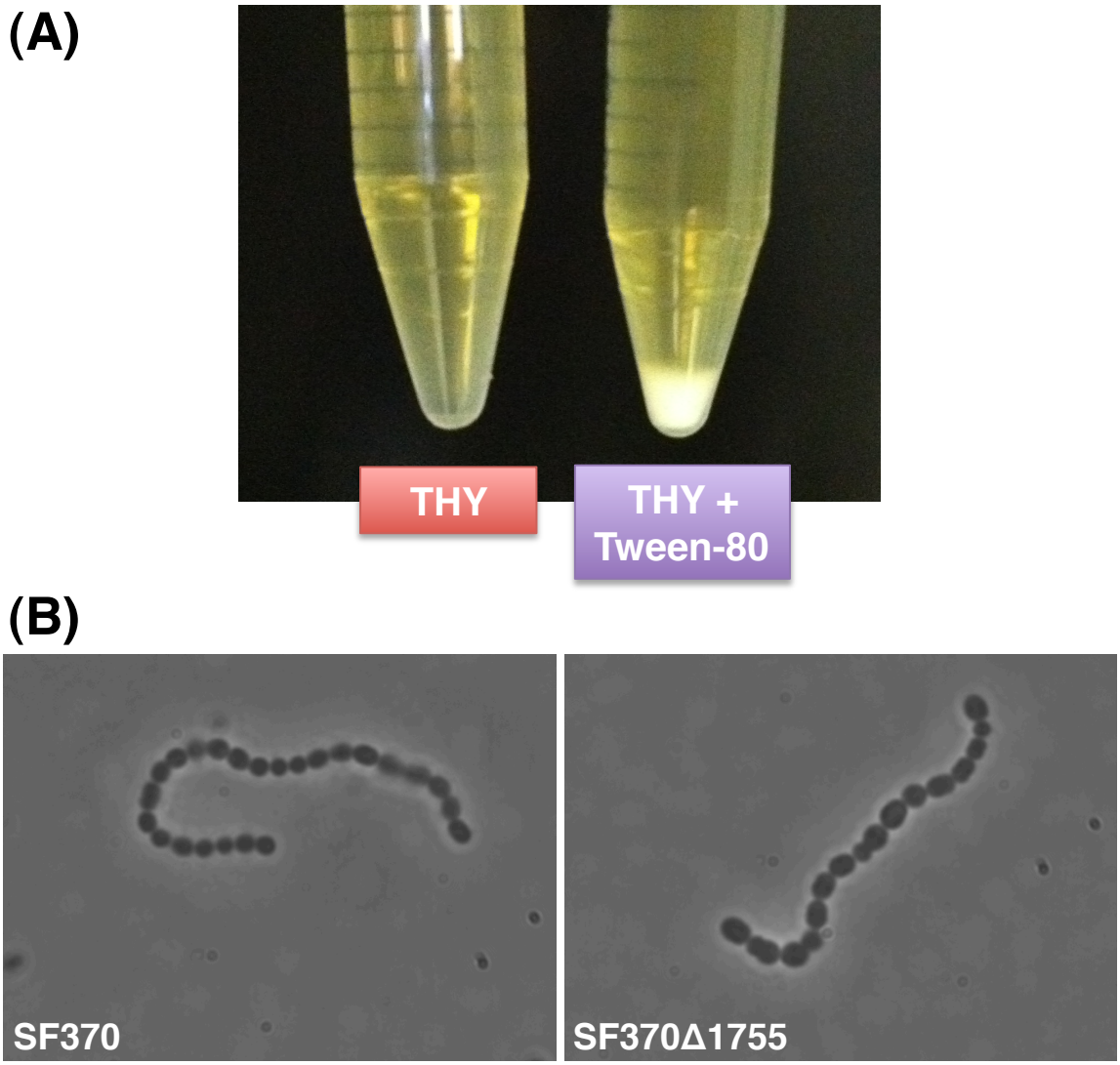


Figure 6.8 Visual growth characteristics of SF370Δ1755 restored to wild-type with Tween-80 supplementation

Panel (A) is an image of overnight SF370Δ1755 growth in THY without (red) and with (purple) 0.1% Tween-80. The bacterial pellet observed with Tween-80 supplementation is characteristic of wild-type SF370 (not shown).

Panel (B) includes images captured at 100X magnification of SF370 and SF370Δ1755 grown overnight in the presence of 0.1% Tween-80.

6.3 Discussion

Fatty acid biosynthesis is vital to bacterial physiology and is accomplished by a series of enzymatic steps, each encoded by a separate gene, known as the type II fatty acid synthetase (Rock & Cronan 1996). Our results strongly support that the MarR regulator *spy1755* is responsible for directly modulating type II fatty acid biosynthesis (FAB) in *S. pyogenes*. Protein database searches revealed that *S. pneumoniae* encodes a MarR regulator FabT with 62% amino acid identity to Spy1755. Deletion of *fabT* leads to overexpression of the FAB pathway indicating that FabT acts as a repressor in *S. pneumoniae* (Lu & Rock 2006).

Elimination of *spy1755* had the opposite effect in our study. Growth of SF370Δ1755 was greatly impaired in THY broth and could only be rescued by addition of exogenous fatty acids (accomplished in our work by supplementation of oleic acids using Tween-80). This growth-deficient phenotype and its subsequent rescue by introduced fatty acids mirrors the phenotype observed when genes encoding enzymes in the FAB pathway are deleted in *S. agalactiae* (Brinster, *et al.* 2009). Thus, we propose that Spy1755 is an activator of FAB in *S. pyogenes*. Notably, the ability of exogenous fatty acids to complement the deletion of *spy1755* indicates that transport systems for the uptake of fatty acids (FA) from the environment are not regulated by Spy1755.

In silico analysis of the *spy1755* genomic region demonstrated that the regulator lies directly upstream of a gene cluster encoding key enzymes in the FAB pathway. The gene *spy1754* encodes FabH, a 3-oxoacyl-ACP synthase

important for initiating cycles of fatty acid elongation. AcpP encoded by *spy1753* is an acyl carrier protein used to carry fatty acid chains during biosynthesis. Thus, any polar effects from the interruption of an operon by *spy1755* deletion would have a significant impact on the biosynthetic pathway and indirectly lead to the observed growth deficiencies.

RT-PCR analysis revealed that deletion of *spy1755* did not completely abrogate expression from these genes during late-exponential growth or stationary phase in THY. The growth deficient phenotype does suggest, however, that the expression of these genes in the absence of the activator Spy1755 may not be sufficient to support normal levels of streptococcal growth. Real-time quantitative PCR assessment of the expression of these enzymes in SF370Δ1755 as compared to SF370 is required to address this open question.

During the course of our study, a proteomic analysis was published indicating that the FAB pathway in *S. pyogenes* is highly expressed during growth in THY broth, but dramatically repressed in human plasma (Malmstrom, *et al.* 2012). Incubating SF370 in THY with increasing concentrations of human plasma (5-20%) led to a dramatic reduction in the intracellular amounts of key FAB enzymes. Levels of Spy1755, however, were too low to be accurately assessed in the study (Malmstrom, *et al.* 2012). These data indicate that THY broth is not a sufficient source of FA, and are in agreement with our observations that streptococci deficient for the putative FAB activator Spy1755 require an exogenous source of fatty acids during growth in THY.

Interestingly, Malmstrom, *et al.* demonstrated that streptococci can scavenge fatty acids from the host environment. Although it has long been recognized that streptococcal species can incorporate free fatty acids into their cellular membranes *in vitro* (Umesaki, *et al.* 1977), *S. pyogenes* may actively recruit fatty acids from the host environment to reduce its metabolic burden. Human plasma is rich in fatty acids, which are mainly transported by the human protein albumin (HSA). The researchers determined that M protein binds FA-carrying albumin leading to active transport of fatty acids into the streptococci for use in membrane biogenesis (Malmstrom, *et al.* 2012).

When exogenous fatty acids were added to THY in the absence of HSA, the researchers reported that repression of FAB enzymes was less pronounced indicating that streptococcal FA scavenging is less efficient when the bacteria must import fatty acids in the absence of carrier molecules (Malmstrom, *et al.* 2012). Perhaps this explains why maximal cell densities were not achieved in our work when growth of SF370Δ1755 was supplemented with free oleic acids without access to carrier molecules.

Although the work of Malmstrom and his colleagues indicated a role for sensing fatty acid levels in repression of the FAB pathway, the mechanism underlying this regulation was not explored. Our studies indicate that the MarR regulator Spy1755 may play a central role in this regulatory network. As MarR regulators are known to bind ligands associated with the pathways under their

control, it is possible that Spy1755 may directly monitor the level of fatty acids in the streptococcal cytoplasm and adjust its activity accordingly.

When exogenous fatty acids are bound and internalized by the bacterial cell through the action of M protein or other as-yet uncharacterized surface proteins, intracellular FA levels rise. If Spy1755 is truly a ligand-binding activator of FA biosynthesis, binding of FA to Spy1755 could reduce the regulator's affinity for promoter regions in FAB genes, thereby repressing the biosynthetic pathway. When exogenous FA is not available, intracellular FA levels fall leaving unbound Spy1755 that can interact with FAB promoters and activate the synthesis of fatty acids essential for normal growth processes.

If this model of FAB regulation via Spy1755 in *S. pyogenes* is correct, then the deletion of the *spy1755* gene prevented Spy1755-dependent upregulation of the FAB pathway in the fatty acid-deficient THY environment. Further support for the direct role of Spy1755 in responses to exogenous fatty acids can be derived from available microarray studies. During incubation in human blood and amniotic fluid (both environments with freely available fatty acids), expression of *spy1755* was greatly reduced (Graham, *et al.* 2005; Sitkiewicz, *et al.* 2010). As MarR regulators have been found to autoregulate, Spy1755 may directly repress its own promoter in FA-rich host environments.

On the other hand, it is also possible that other streptococcal regulators modulate Spy1755 in the presence of exogenous FA. A recent study of the GrAS eukaryotic-type serine/threonine kinase (*stk*) in an M1 strain indicated that this

kinase activates the fatty acid biosynthesis pathway (Bugrysheva, *et al.* 2011). The researchers did not explore the mechanism of regulation, which according to our work could involve activation of Spy1755 by Stk phosphorylation, or could presumably involve Stk phosphorylation of the downstream FAB enzymes. A role for CovR repression in Spy1755 regulation was also demonstrated in this study using the M1 strain (Bugrysheva, *et al.* 2011).

Despite the mounting evidence that Spy1755 could potentially play an important role in modulating the FAB pathway in response to nutrient availability in the host, this regulator's direct effect on streptococcal virulence has not been examined. Malmstrom and colleagues suggested that streptococci connect FA levels to the elaboration of secreted virulence toxins. As streptococci divide and consume local resources, fatty acids become scarce. A transcriptional switch then activates FAB (for which we postulate Spy1755 is the key mediator) and potentially the production of secreted toxins inducing host inflammation. Although seemingly counterintuitive, local inflammation would benefit the pathogen by yielding an influx of plasma containing HSA-bound FA for streptococcal use (Malmstrom, *et al.* 2012).

Little evidence currently exists linking FAB regulation to modulation of virulence factors in *S. pyogenes*, but MarR family regulators are known to control expression of virulence genes in other bacterial pathogens, including *S. aureus*. Just as the carbon catabolite repressor CcpA uses limited glucose availability in the oropharynx as a signal to initiate a program of virulence regulation

(Shelburne III, *et al.* 2008), Spy1755 could conceivably use changes in exogenous FA concentrations to signal a larger program of virulence remodeling.

One enticing clue connecting FAB regulation to the oropharyngeal environment comes from a recent study of *S. gordonii* that reported the induction of FAB enzymes upon *in vitro* interaction of this bacterium with one of the dominant components of saliva (amylase) (Nikitkova, *et al.* 2012). In the future, transcriptome analyses of the SF370Δ1755 mutant certainly promise to yield a great deal of insight concerning the potential role (if any) for Spy1755 in direct virulence regulation.

7. EFFECT OF PROPHAGE ON STREPTOCOCCAL GENE EXPRESSION

7.1 Introduction

The first observations of lysogenic prophage in *S. pyogenes* were made in the 1930s, but the ability of bacteriophage to contribute to streptococcal virulence was not appreciated until the discovery in 1964 that phage transduction underlies acquisition of erythrogenic toxin production (Evans 1934; Evans 1940; Zabriskie 1964). Decades later, researchers found that group A streptococci exhibit a high frequency of lysogeny (> 90%), and these prophage sequences provide the vast majority of genetic diversity among streptococcal serotypes and strains (Yu & Ferretti 1989; Yu & Ferretti 1991; Ferretti, *et al.* 2001).

With the widespread adoption of genomic sequencing in streptococcal research, it has become apparent that lysogenic phages contribute to the virulence potential of their host strain (Smoot, *et al.* 2002; Beres & Musser 2007; McShan, *et al.* 2008). The rise of the hypervirulent M1T1 clone in the late 1980s has been attributed, at least in part, to the acquisition of bacteriophage encoding two virulence determinants: the superantigen *speA* and the highly-active secreted DNase *sdaD2* (Sumby, *et al.* 2005; Sumby, *et al.* 2005b; Aziz & Kotb 2008).

Strain SF370 contains four integrated prophage elements in its genome: Φ3701.1, Φ370.2, Φ370.3, and Φ370.4 (Ferretti, *et al.* 2001). Only one prophage (Φ370.1) has been demonstrated to be inducible by mitomycin C treatment, whereas the other three phage elements appear to be defective (Ferretti, *et al.* 2001; Canchaya, *et al.* 2002). Phage Φ370.1 has a 41 kb genome, and encodes

the *speC* superantigen gene, the *spd1* DNase gene, and the *hylP1* hyaluronidase gene (Ferretti, *et al.* 2001; Canchaya, *et al.* 2002).

Phage Φ370.2 has a 43 kb genome and carries the genes for two superantigens (*speH* and *speI*) and a hyaluronidase (*hlyP2*). This phage was initially thought to have a complete functional genome, but two inactivating mutations were later discovered: one in the replisome organizer (important for initiating induced phage DNA replication) and the other in the portal protein (involved in packaging phage genetic material into the prohead) (Ferretti, *et al.* 2001; Canchaya, *et al.* 2002).

Phage Φ370.3 has a 33 kb genome and encodes another DNase gene (*spd3*) and an additional hyaluronidase gene (*hylP3*). This phage also contains a stop mutation in the replisome organizer. Finally, Φ370.4 represents a phage remnant with a genome of only 13 kb. This prophage has undergone extensive deletions that have eliminated any recognizable structural, lysis, or virulence genes (Ferretti, *et al.* 2001; Canchaya, *et al.* 2002).

Interestingly, Φ370.4 appears to be responsible for growth phase-dependent regulation of the mismatch repair (MMR) system in SF370 (Scott, *et al.* 2008). During exponential growth, Φ370.4 exhibits dynamic excision and reintegration into the 5' end of the *mutL* gene—a critical component of MMR in streptococci. Although most prophage integrate into the 3' end of chromosomal genes leaving their expression intact (Campbell, *et al.* 1992; Campbell 1992), insertion of Φ370.4 into the 5' end of *mutL* interrupts the MMR operon yielding a

mutator phenotype, while excision restores MMR expression and relieves mutation rates. When resources are limited and streptococci enter stationary phase, however, Φ370.4 remains integrated in the bacterial chromosome allowing the pathogen to accumulate mutations at a higher rate, which could potentially be advantageous for survival and dissemination (Scott, *et al.* 2008).

The work described above on the role of Φ370.4 in MMR regulation provided the first evidence that prophages may have more intimate interactions with their streptococcal hosts beyond the introduction of virulence factors. One aspect of the phage-streptococcal relationship that has not been explored is the role of phage encoded regulators on chromosomal gene expression. Although researchers have determined that chromosomally encoded regulators can modulate expression of phage genes, it has not been determined if the reverse could play a role in shaping the streptococcal transcriptome (Becket, *et al.* 2001; Riani, *et al.* 2007; Gryllos, *et al.* 2008; Anbalagan, *et al.* 2011; Shelburne III, *et al.* 2011; Wen, *et al.* 2011).

To enhance our general understanding of the relationship of ubiquitous lysogenic phage to their host *S. pyogenes*, our laboratory created the first group A streptococcal strain completely devoid of phage (Euler 2010). Strain SF370 was systematically cured of Φ370.1, Φ370.2, Φ370.3, and Φ370.4, yielding a deletion mutant referred to as CEM1ΔΦ with a genome 10% smaller than parental SF370. Initial characterization of CEM1ΔΦ indicated that the phage-free mutant was indistinguishable from wild-type SF370 on the basis of growth rate,

blood hemolytic pattern, colony morphology, and microscopic analysis of cell shape and chain length (Euler 2010).

Surprisingly, streptococcal adherence to and internalization by pharyngeal cells *in vitro* and measurements of *in vivo* virulence in murine infection models were similarly unaffected by curing of all lysogenic phage, despite the loss of numerous phage encoded virulence factors (Euler 2010; unpublished observations). Phenotypic differences were observed between SF370 and CEM1ΔΦ in terms of extracellular DNase activity. As phage encode two DNases in SF370 (*spd1* on Φ370.1 and *spd3* on Φ370.3), curing of bacteriophage resulted in a significant reduction of extracellular nuclease activity during growth on agar that could not be compensated for by the chromosomally encoded DNase *spd* (Euler 2010).

The highly restricted phenotype associated with loss of all integrated prophage sequences (representing a substantial portion of the streptococcal genome and numerous virulence factors) was unexpected. Recognizing that curing of phage also eliminated phage encoded regulatory genes that could have an effect on chromosomal gene expression, we sought to compare the transcriptional profiles of SF370 and CEM1ΔΦ during early and late exponential growth in THY. This chapter details the results of that transcriptome analysis.

7.2 Results

7.2.1 Effect of phage deletion on gene expression during early exponential growth *in vitro*

SF370 and the full-phage deletion mutant CEM1ΔΦ were grown in THY until the cultures reached early exponential phase, defined as an $O.D._{600}$ reading of ~0.35 (Figure 7.1). Microarray analysis revealed that 35 genes were differential expressed between the mutant and wild-type at this point in the growth curve, though most differences in expression levels were modest (1.2 to 3.5 fold above or below wild-type levels) (Table 7.1).

Twenty-one genes were found to be downregulated in the mutant as compared to SF370, indicating that these genes are expressed at higher rates when phage are integrated in the streptococcal genome. Using the COG classification system to assign differentially expressed genes into functional groups, we found that these genes were involved in a wide range of cellular activities, including membrane biogenesis and numerous metabolic pathways (Figure 7.2).

The dipeptidase gene *pepD* and a gene encoding an ATP synthase subunit *ntpI* exhibited the greatest decline in transcription, with both downregulated over 2-fold in the mutant compared to SF370. Expression of two transcription factors, one of which is an arginine pathway repressor, was also negatively affected by curing of lysogenic phage. Notably, two virulence factors

were found to be downregulated in the absence of integrated phage: the host macroglobulin-binding protein GRAB (*spy1357*) and a hemolysin (*spy1497*).

A total of eleven genes were upregulated in the mutant, indicating that their expression is normally lower in the wild-type during early exponential growth in THY (Table 7.1). The majority of these genes were classified as encoding either hypothetical proteins or proteins involved in DNA replication, recombination, and repair. The latter group included the operon responsible for DNA mismatch repair (MMR), which was transcribed at rates more than 2-fold higher in CEM1$\Delta\Phi$.

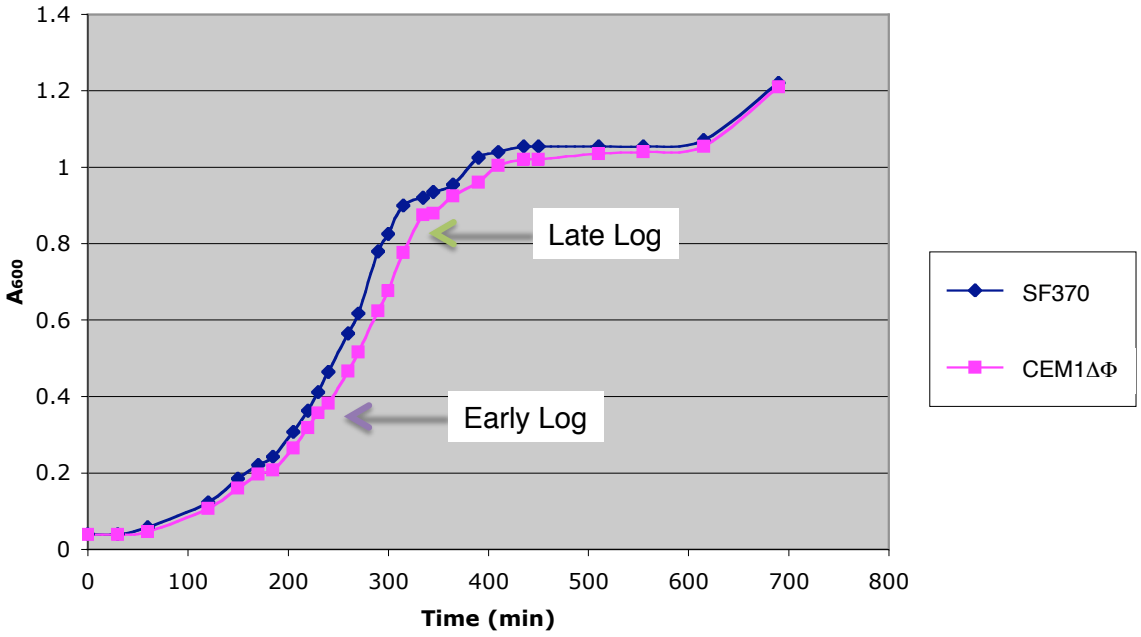


Figure 7.1 Growth curve sampling points for transcriptome studies of CEM1ΔΦ

To generate this graph, overnight cultures were diluted 1:50 in 10 ml of pre-warmed BHI and incubated at 37°C. Absorbance of cultures (A_{600}) was measured at different points using a spectronic 20D spectrophotometer (Thermo Electron Corporation) and the results were plotted.

For the array data, 1000 ml of THY were inoculated with 5 ml flash-frozen aliquots of SF370 and CEM1ΔΦ. Growth was followed using absorbance readings as described above. When cultures reached $O.D._{600}$ ~0.35 (early log) and $O.D._{600}$ ~0.85 (late log), streptococci were harvested for RNA isolation and transcriptome analysis.

Table 7.1 Genes differentially regulated in CEM1ΔΦ as compared to SF370 during early exponential growth in THY (Pages 288-290)

Chromosomal Genes

21 Genes Downregulated in CEM1ΔΦ (Higher expression in wild-type)

Locus Tag	Gene Name	*P* value	Log_2 Fold Change	COG (Functional Category)	Proposed or Known Function
spy0116		0.041	-0.22	Function unknown	Hypothetical
spy0148	*ntpI*	0.002	-1.32	Energy production & conversion	ATP synthase subunit I
spy0160	*purA*	0.004	-0.28	Nucleotide transport & metabolism	Adenylsuccinate synthetase
spy0338	*murE*	0.016	-0.28	Cell wall, membrane, & envelope biogenesis	UDP-N-acetylmuramoylalanyl-D-glutamate--2,6--diaminopimelate ligase
spy0605	*bsaA*	0.004	-0.26	Posttranslational modifications, protein turnover, & chaperones	Glutathione peroxidase (Redox reactions)
spy0713	*pepD*	< 0.001	-1.00	Amino acid transport & metabolism	Dipeptidase A
spy0714	*adcA*	0.004	-0.60	Inorganic ion transport & metabolism	Zinc-binding protein; Putative adhesin
spy0715		0.001	-0.61	Transcription	GntR-family transcriptional regulator
spy0716	*agaS*	< 0.001	-0.71	Cell wall, membrane, & envelope biogenesis	Tagatose-6-phosphate aldose/ketose isomerase
spy1010	*mutX*	0.001	-0.40	Nucleotide transport & metabolism	ADP-ribose pyrophosphatase
spy1011		0.015	-0.34	Function unknown	Predicted permease
spy1012		< 0.001	-0.50	Function unknown	Hypothetical

Table 7.1 Chromomsal genes downregulated in CEM1ΔΦ (Higher expression in wild-type)

spy1357	*grab*	0.005	-0.42	Virulence	GRAB (G-related alpha 2M-binding protein)
spy1420	*murF*	0.033	-0.26	Cell wall, membrane, & envelope biogenesis	UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase
spy1422	*recR*	0.019	-0.29	Replication, recombination, & repair	DNA replication and repair protein
spy1424		0.001	-0.54	Inorganic ion transport & metabolism	Formate/nitrite transporter
spy1432	*pyrD*	< 0.001	-0.93	Nucleotide transport & metabolism	Dihydroorotate dehydrogenase
spy1496		0.024	-0.27	Transcription	Arginine repressor
spy1497	*hlyA1*	0.028	-0.26	Virulence	Hemolysin
spy1499	*xseB*	0.027	-0.28	Replication, recombination, & repair	Exodeoxyribonuclease VII small subunit
spy2189	*sdhB*	0.039	-0.29	Amino acid transport & metabolism	L-serine deaminase (glycine betaine degradation)

Table 7.1 Genes differentially regulated in CEM1ΔΦ as compared to SF370 during early exponential growth in THY

Chromosomal Genes

15 Genes Upregulated in CEM1ΔΦ (Lower expression in wild-type)

Locus Tag	Gene Name	*P* value	Log_2 Fold Change	COG (Functional Category)	Proposed or Known Function
spy0334		0.049	0.19	Function unknown	Hypothetical
spy0412	*exoA*	0.043	0.18	Replication, recombination, & repair	3'-exo-deoxyribonuclease
spy0428		0.002	0.63	Signal transduction	Unknown; Family of ADP-ribosyltransferases
spy0515		0.017	0.24	Cell wall, membrane, & envelope biogenesis	Glycosyltransferase
spy0603		0.011	0.46	Function unknown	Hypothetical (contains a signal peptide)
spy0604		0.001	0.40	Function unknown	Hypothetical (contains a signal peptide)
spy0639	*kgdA*	0.020	0.46	Carbohydrate transport & metabolism	2-keto-3-deoxy-phsophogluconate aldolase
spy1391		0.016	0.30	Function unknown	Putative methyltransferase
spy1542		0.028	0.68	Amino acid transport & metabolism	Dipeptidase (glutathione-mediated detox)
spy2118	*tag*	< 0.001	1.09	Replication, recombination, & repair	DNA-3-methyladenine glycosylase I
spy2119	*ruvA*	< 0.001	1.05	Replication, recombination, & repair	Holliday junction DNA helicase subunit
spy2120	*lmrP*	< 0.001	1.18	Defense mechanisms	Proton motive force-dependent drug transporter (Lipophilic antimicrobials)
spy2121	*mutL*	< 0.001	1.04	Replication, recombination, & repair	DNA mismatch repair protein
spy2205		0.050	0.25	Carbohydrate transport & metabolism	Glucose uptake protein

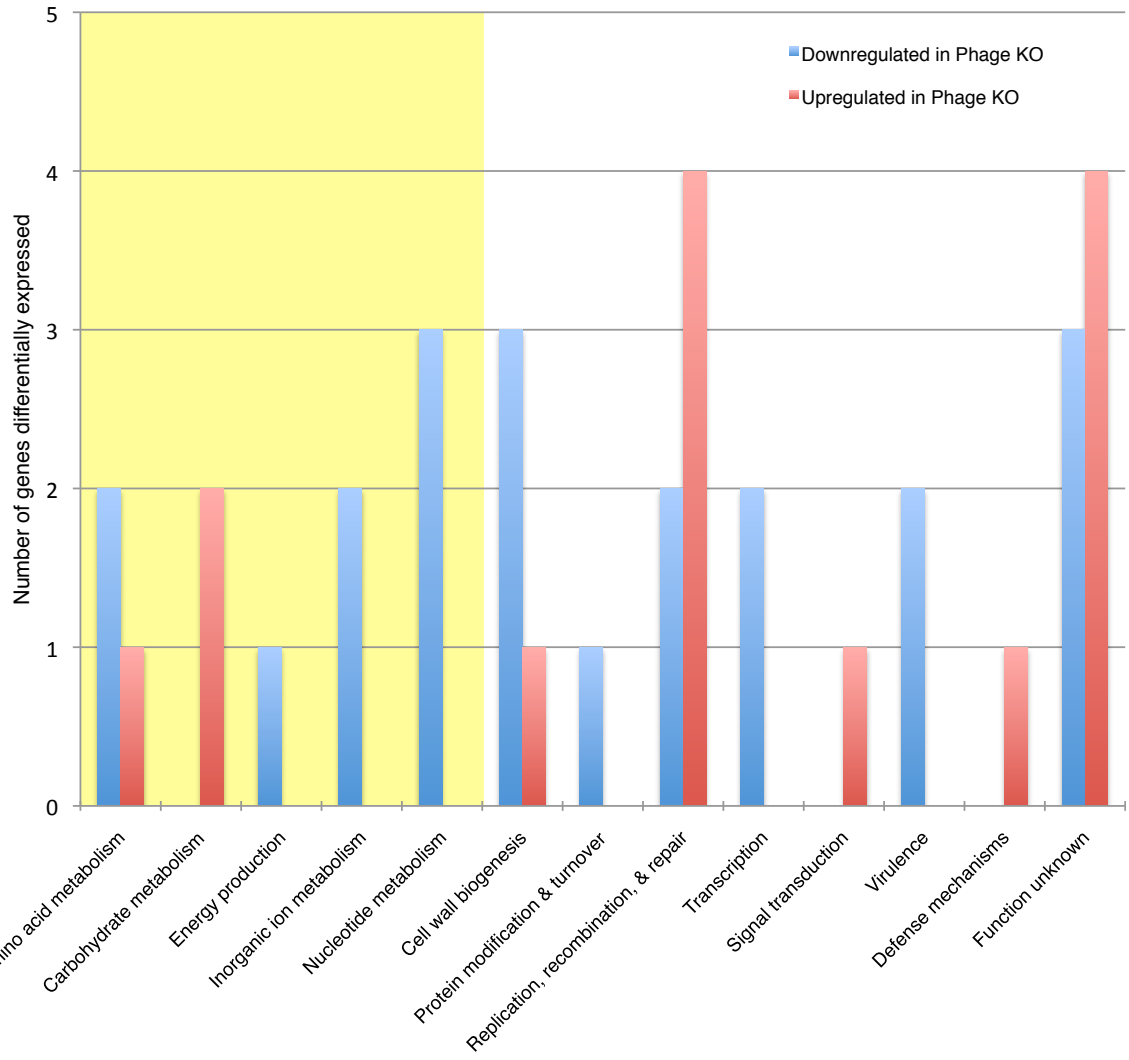


Figure 7.2 COG classification of genes differentially expressed in CEM1ΔΦ during early exponential growth in THY

Genes are classified into functional groups based on protein homology to characterized genes. Each bar represents the total number of genes differentially upregulated (red) or downregulated (blue) in CEM1ΔΦ as compared to wild-type SF370. The area of the graph shaded yellow highlights all functional categories involved in cellular metabolic processes.

7.2.2 Effect of phage deletion on gene expression during late exponential growth *in vitro*

SF370 and the full-phage deletion mutant CEM1ΔΦ were grown in THY until the cultures reached late exponential phase, defined as an $O.D._{600}$ reading of ~0.85 (Figure 7.1). Transcriptome analysis determined that only 24 genes were differentially expressed between CEM1ΔΦ and SF370 at this stage in growth, with most expression ratios again exhibiting modest differences (1.2 to 3 fold above or below wild-type levels) (Table 7.2).

Thirteen genes were downregulated in the CEM1ΔΦ mutant late in growth, meaning that these genes are transcribed at higher levels in wild-type SF370. The greatest effect on transcription rates was again observed for the dipeptidase gene *pepD*, which exhibited 3-fold lower expression in CEM1ΔΦ. Categorizing the affected genes by functional categories indicated that metabolic pathways were once again negatively impacted by phage deletion (Figure 7.3). In addition, two transcription factors identified as downregulated during early exponential growth were similarly affected late in growth. Although expression of the hemolysin identified in our early log analysis was unaffected late in growth, the virulence factor *grab* was once again downregulated in CEM1ΔΦ.

Eleven genes were upregulated in the mutant during late exponential growth in THY. Most of the genes encoded proteins with virulence functions or proteins involved in DNA replication, recombination, and repair. Affected virulence factors included *nga* (NAD-glycohydrolase), *sclA* (collagen-like

adhesin), and *hasA* (capsule). The DNA MMR system was once again upregulated in CEM1$\Delta\Phi$, but the difference in expression level was lower than observed for early exponential growth at 1.5-fold above wild-type SF370 levels.

Table 7.2 Genes differentially regulated in CEM1ΔΦ as compared to SF370 during late exponential growth in THY (Pages 294-295)

Chromosomal Genes

13 Genes Downregulated in CEM1ΔΦ (Higher expression in wild-type)

Locus Tag	Gene Name	*P* value	Log_2 Fold Change	COG (Functional Category)	Proposed or Known Function
spy0172	*metB*	0.003	-0.74	Amino acid transport & metabolism	Cystathionine beta-lyase (Methionine biosynthesis)
spy0713	*pepD*	< 0.001	-1.58	Amino acid transport & metabolism	Dipeptidase A
spy0714	*adcA*	0.022	-0.66	Inorganic ion transport & metabolism	Zinc-binding protein; Putative adhesin
spy0715		0.033	-0.65	Transcription	GntR-family transcriptional regulator
spy0716	*agaS*	0.002	-0.66	Cell wall, membrane, & envelope biogenesis	Tagatose-6-phosphate aldose/ketose isomerase
spy1010	*mutX*	0.035	-0.27	Nucleotide transport & metabolism	ADP-ribose pyrophosphatase
spy1357	*grab*	0.008	-0.59	Virulence	GRAB (G-related alpha 2M-binding protein)
spy1422	*recR*	0.007	-0.34	Replication, recombination, & repair	DNA replication and repair protein
spy1424		0.048	-0.57	Inorganic ion transport & metabolism	Formate/nitrite transporter
spy1425		0.007	-0.36	Function unknown	Hypothetical protein (signal peptide)
spy1427		0.001	-0.52	Function unknown	Hypothetical (Helix-turn-helix domain)
spy1432	*pyrD*	0.002	-0.79	Nucleotide transport & metabolism	Dihydroorotate dehydrogenase
spy1496		0.018	-0.30	Transcription	Arginine repressor

Table 7.2 Genes differentially regulated in CEM1ΔΦ as compared to SF370 during late exponential growth in THY

Chromosomal Genes

11 Genes Upregulated in CEM1ΔΦ (Lower expression in wild-type)

Locus Tag	Gene Name	*P* value	Log_2 Fold Change	COG (Functional Category)	Proposed or Known Function
spy0025		0.016	0.37	Nucleotide transport & metabolism	Phosphoribosylformylglycinamidine synthase
spy0036	*purB*	0.050	0.23	Nucleotide transport & metabolism	Adenylosuccinate lyase
spy0165	*nga*	0.003	1.08	Virulence	NAD-glycohydrolase
spy1543		0.046	0.44	Function unknown	Hypothetical protein (signal peptide)
spy1983	*sclA/scl1*	0.040	0.63	Virulence	Collagen-like protein (adhesin)
spy2003	*dppD*	0.011	0.36	Amino acid transport & metabolism	ABC-type dipeptide/oligopeptide/nickel transport system, ATPase component
spy2118	*tag*	0.001	0.60	Replication, recombination, & repair	DNA-3-methyladenine glycosylase I.
spy2119	*ruvA*	0.002	0.61	Replication, recombination, & repair	Holliday junction DNA helicase subunit RuvA
spy2120	*lmrP*	0.002	0.64	Defense mechanisms	Proton motive force-dependent drug transporter (Lipophilic antimicrobials)
spy2121	*mutL*	0.002	0.49	Replication, recombination, & repair	DNA mismatch repair protein MutL
spy2200	*hasA*	0.037	0.59	Virulence	Capsule synthesis

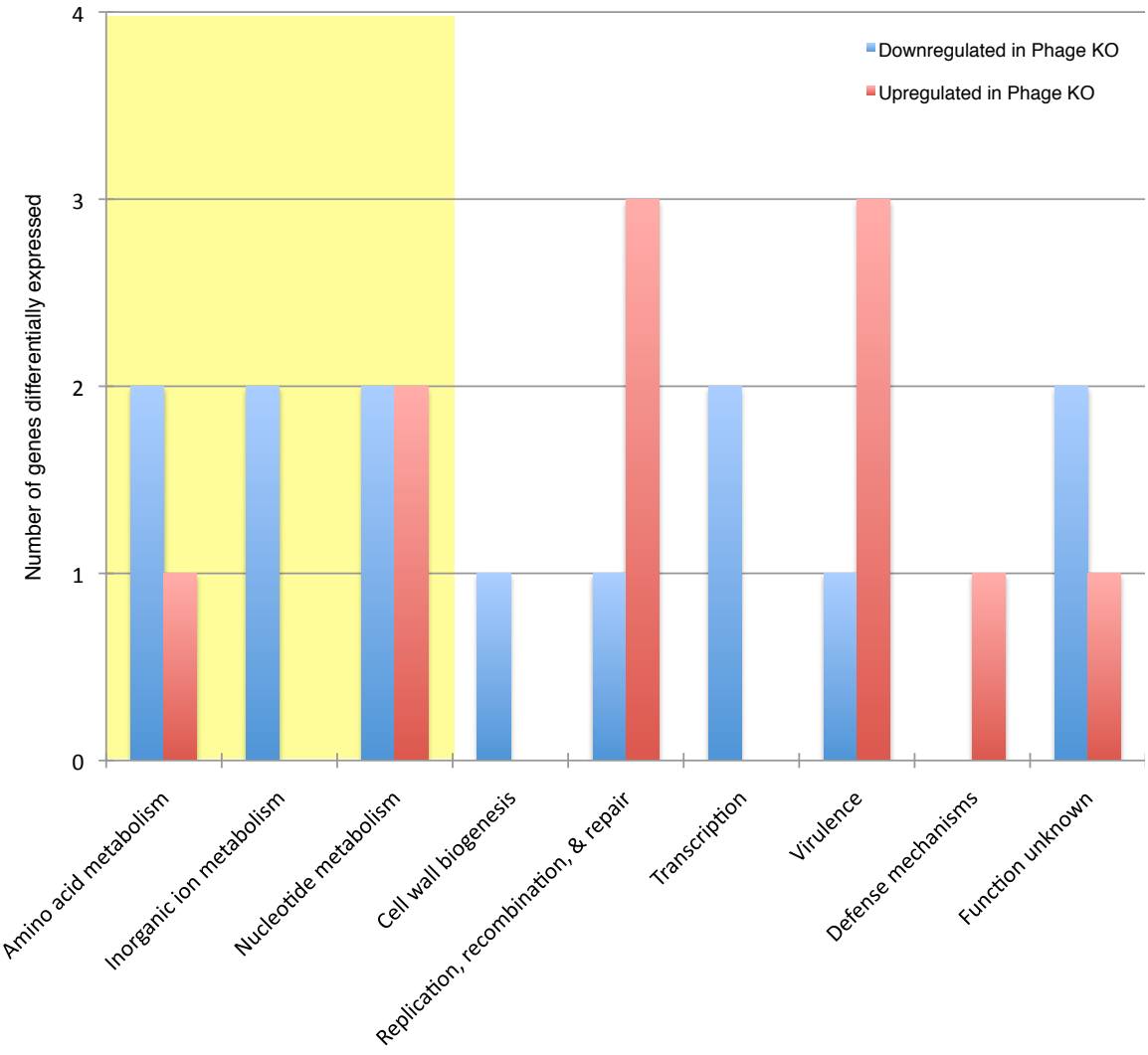


Figure 7.3 COG classification of genes differentially expressed in CEM1ΔΦ during late exponential growth in THY

Genes are classified into functional groups based on protein homology to characterized genes. Each bar represents the total number of genes differentially upregulated (red) or downregulated (blue) in CEM1ΔΦ as compared to wild-type SF370. The area of the graph shaded yellow highlights all functional categories involved in cellular metabolic processes.

7.2.3 Comparison of CEM1ΔΦ transcriptional profiles during early and late exponential growth

In general, genes regulated in CEM1ΔΦ as compared to wild-type SF370 during early exponential growth represented a broader range of cellular functions than those genes identified late in growth. Direct comparison of genes regulated at the two time points was performed to determine the level of overlap between downregulated genes (Figure 7.4) and upregulated genes (Figure 7.5) in the absence of integrated phage.

Overall, there was much greater overlap between genes downregulated at early and late exponential growth. As the Venn diagram demonstrates, genes downregulated late in growth comprised a subset of those observed to be regulated in early log phase. For genes upregulated in the absence of phage, there appeared to be growth phase-dependent regulation of two distinct groups of genes. The only 4 genes upregulated at both time points were the ORFs of the MMR operon.

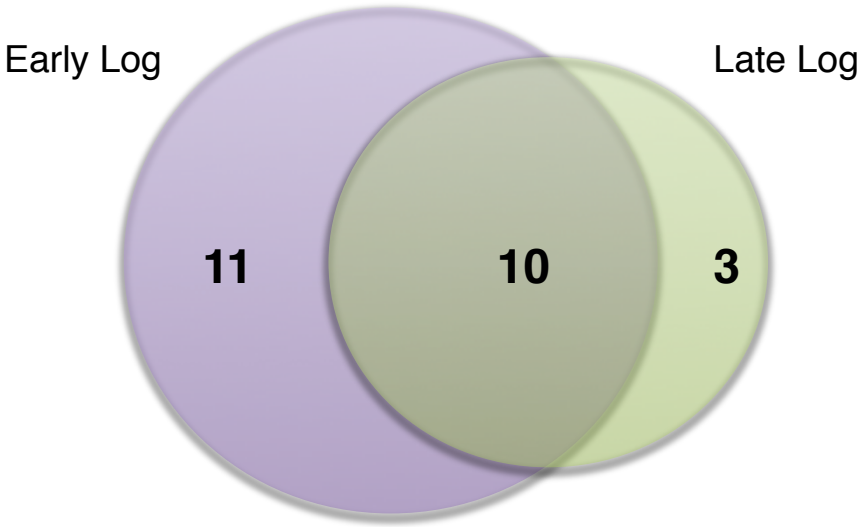


Figure 7.4 Comparison of genes downregulated in CEM1ΔΦ during early and late exponential growth in THY

The Venn diagram depicts the high level of overlap between genes downregulated in CEM1ΔΦ during the two growth phases sampled. During early log, 21 genes were downregulated in the mutant with 10 of these genes similarly affected late in growth. Only 3 genes were found to be regulated in late log phase that were not affected at the earlier time point.

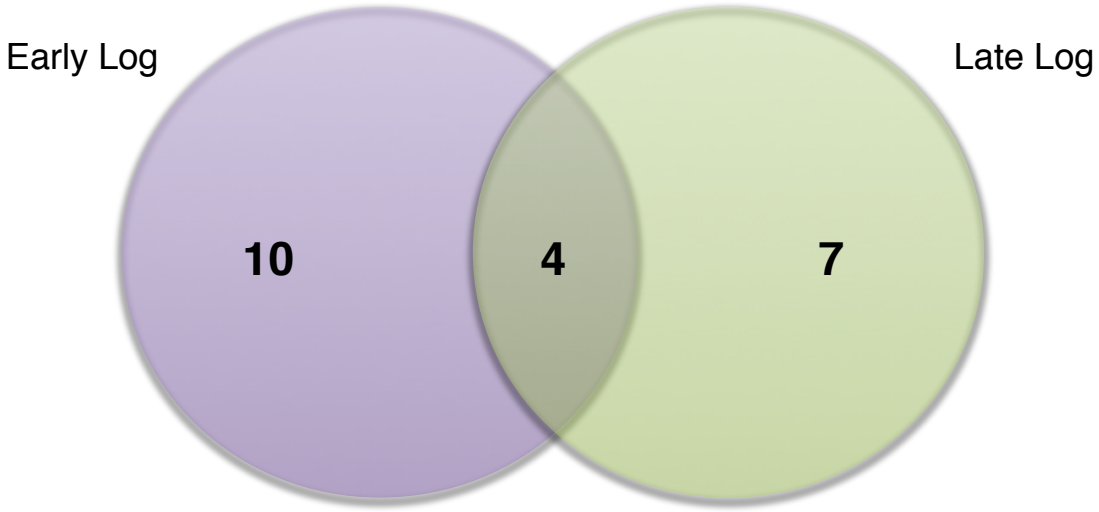


Figure 7.5 Comparison of genes upregulated in CEM1ΔΦ during early and late exponential growth in THY

The Venn diagram depicts the limited overlap of genes upregulated in CEM1ΔΦ during the two growth phases sampled. During early log, 14 genes were upregulated in the mutant with only 4 of these genes (MMR operon) similarly affected late in growth. An additional 7 genes were found to be upregulated in late exponential phase that were not affected at the earlier time point.

7.2.4 Effects of phage deletion on expression of genes downstream of phage insertion sites

Examination of genes with the most significant differential expression in CEM1ΔΦ (as determined by *P* value) revealed that the majority of these ORFs had a phage insertion site upstream of their 5' end (Figure 7.6). In fact, all genes in which a phage inserts in their 5' upstream regions exhibited altered transcriptional profiles in the mutant as compared to SF370.

For some genes, phage deletion reduced expression, as was the case for *spy0713-0716* downstream of Φ370.1 and *spy1010-1012* downstream of Φ370.2. For the MMR operon (*spy2118-2121*) that lies downstream of Φ370.4, the exact opposite was true with increased expression observed following phage deletion. These effects were consistent for both growth phases.

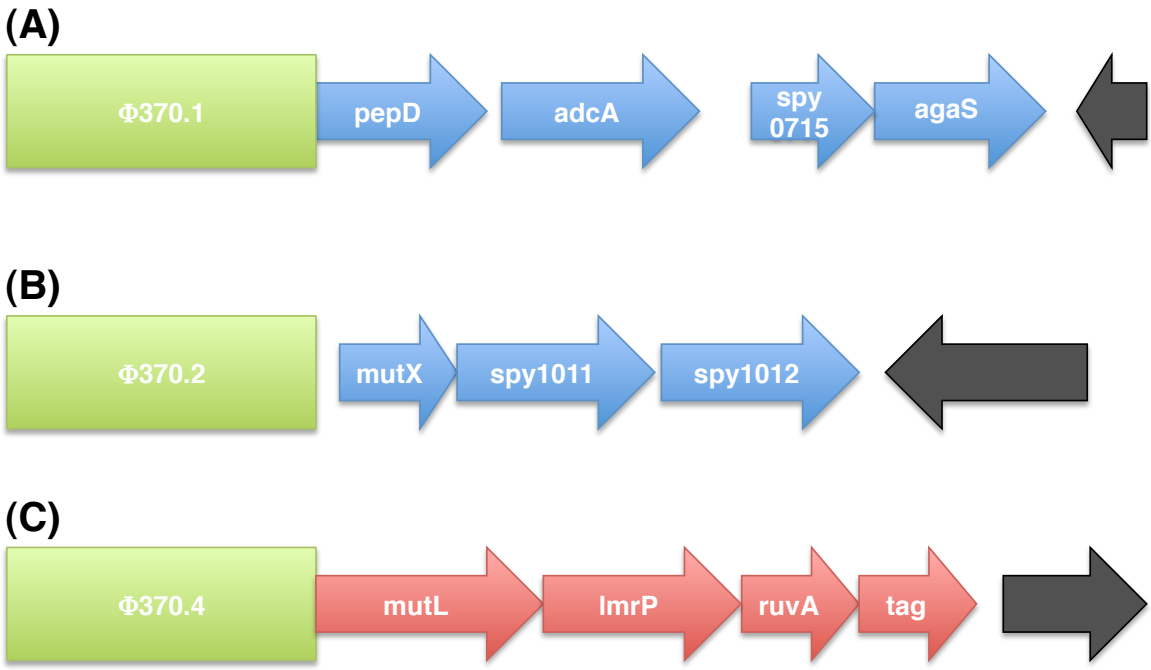


Figure 7.6 Effect of phage deletion on downstream streptococcal genes during growth in THY

Phage boundaries were examined to find junctions where the phage genome (green) had inserted 5' to a streptococcal open reading frame (red and blue arrows). Three occurrences were found in SF370: Φ370.1 insertion upstream of *pepD* (A), Φ370.2 integration upstream of *mutX* (B), and Φ370.4 insertion in the 5' sequence of *mutL* (C). Deletion of phage had differing effects on these downstream genes with some experiencing downregulation in the mutant (blue) and others exhibiting upregulation in the absence of phage (red). ORFs in black indicate neighboring streptococcal genes that were not differentially expressed in CEM1ΔΦ in response to growth in THY. Direction of arrowheads denote 5'-3' transcription.

7.2.5 Expression of phage genes in SF370 during growth in THY

The deletion of integrated phage in CEM1ΔΦ presented a unique opportunity to simultaneously sample the expression of all phage encoded genes in wild-type SF370 during growth in THY. As expression of these genes is absent in the mutant, statistical analysis of our array data examined via *t*-test if the expression of any phage genes in SF370 was significantly different from the baseline background signal.

Our results indicate that numerous genes in Φ370.1, Φ370.2, and Φ370.3 are expressed under normal growth conditions in THY (Table 7.3). Furthermore, comparison of phage genes expressed at early and late log phase growth indicated that the number of transcriptionally active phage genes is highest during late log. Notably, phage genes expressed during early log comprised a subset of genes transcribed late in growth (Figure 7.7). The gene with the highest expression was *speC* (superantigen). Two additional superantigens (*speH* and *speI*) and a phage encoded DNase (*spd3*) were also expressed. No genes from the phage remnant Φ370.4 were identified in our analysis.

Table 7.3 Phage genes expressed in wild-type SF370 during growth in THY (Pages 303-304)

		Early Log		Late Log		
Locus Tag	Phage	*P* value	Log_2 Fold Change[a]	*P* value	Log_2 Fold Change[a]	Phage Gene Function
spy0656	370.1	< 0.001	3.63	0.001	3.37	Lysogeny (Superinfection exclusion?)
spy0661	370.1			0.010	3.53	*Not annotated*
spy0666	370.1			< 0.001	2.34	Replication
spy0680	370.1	< 0.001	3.61	< 0.001	2.95	Head morphogenesis (Terminase large subunit)
spy0681	370.1	< 0.001	4.43	0.001	3.77	Head morphogenesis (Portal protein)
spy0686	370.1	< 0.001	5.36	0.001	4.45	Head morphogenesis (Major head protein)
spy0690	370.1	< 0.001	2.86	< 0.001	2.61	Head-tail joining
spy0696	370.1	< 0.001	3.71	0.002	3.70	Tail gene
spy0702	370.1	< 0.001	3.04	< 0.001	3.45	Tail fiber gene
spy0711	370.1	< 0.001	7.96	< 0.001	7.49	*speC* (Superantigen)
spy0943	370.2	< 0.001	3.76	< 0.001	3.11	Lysogeny
spy0953	370.2	0.001	1.95	0.003	1.93	Replication
spy0968	370.2			< 0.001	1.91	Head morphogenesis (Portal protein)
spy1007	370.2	0.002	3.33	0.001	3.10	*speI* (Superantigen)
spy1008	370.2	< 0.001	3.21	0.001	3.31	*speH* (Superantigen)

Table 7.3 Phage genes identified as expressed in wild-type SF370

		Early Log		Late Log		
spy1436	370.3	< 0.001	2.54	< 0.001	3.72	*spd3* (DNase)
spy1441	370.3	0.001	3.25	< 0.001	2.73	Tail fiber gene
spy1461	370.3			0.045	0.55	*Not annotated*
spy1463	370.3	< 0.001	2.65	0.001	2.56	*Not annotated*
spy1464	370.3	< 0.001	1.67	< 0.001	1.79	Head morphogenesis
spy1466	370.3	0.004	1.06	0.002	0.84	*Not annotated*
spy1469	370.3			0.001	3.39	Regulation
spy1470	370.3			< 0.001	1.56	Replication
spy1471	370.3			0.002	1.96	Replication
spy1487	370.3	< 0.001	4.32	0.001	4.04	Lysogeny

[a] Log_2 fold change values represent the ratio of phage gene expression in wild-type SF370 as compared to the baseline background signal associated with CEM1$\Delta\Phi$ total cDNA hybridization.

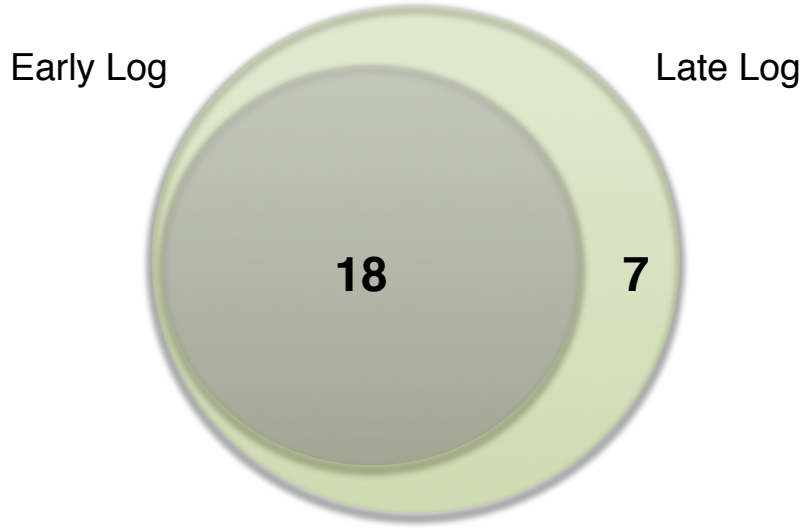


Figure 7.7 Comparison of phage genes expressed in SF370 during early and late exponential growth in THY

The Venn diagram depicts the complete overlap of phage genes expressed in wild-type SF370 during early and late exponential growth in THY. The phage encoded genes transcribed during early log were a subset of the larger group of phage genes expressed late in growth.

7.3 Discussion

Examination of genes differentially expressed in the mutant CEM1ΔΦ as compared to wild-type SF370 revealed a limited effect of phage deletion on the streptococcal transcriptome during exponential growth in THY. These results support the restricted phenotype previously observed for CEM1ΔΦ using a variety of *in vitro* growth measures and *in vivo* virulence tests (Euler 2010). Most differences in expression were relatively modest, but preliminary quantitative real-time RT-PCR validation has supported our findings (data not shown). The greatest influence of phage insertion on chromosomal gene expression was observed for streptococcal genes that had phage insertion sites immediately upstream of their 5' end (Figure 7.6).

Scott and colleagues previously determined that Φ370.4 integration upstream of *mutL* in SF370 interrupts transcription of the MMR operon (Scott, *et al.* 2008). Our data are in full agreement and indicate that expression of the DNA mismatch repair system is 1.5 to 2-fold higher in the phage deletion mutant CEM1ΔΦ during exponential growth. As Φ370.4 undergoes dynamic excision and reinsertion during log phase in the wild-type setting, it is possible that the difference in transcription rates between SF370 and CEM1ΦΔ may be more pronounced in stationary phase when the phage no longer excises.

Integration of both Φ370.1 and Φ370.2 yielded an effect on streptococcal gene expression opposite to that observed for Φ370.4. Insertion of these phage elements into the 5' upstream regions of *pepD* and *mutX*, respectively, actually

increased transcription from these chromosomal loci. Integration of Φ370.1 into the bacterial chromosome replaces the first nine N-terminal amino acids and the upstream promoter sequence of *pepD* with bacteriophage-specific sequence (Canchaya, *et al.* 2002). Our laboratory had previously demonstrated that expression from *pepD* occurred at all phases of growth in wild-type SF370 and an isogenic mutant lacking only Φ370.1 (Euler 2010).

Furthermore, we determined that Φ370.1 was also dynamically excising and reinserting throughout growth in laboratory media. Thus, wild-type *pepD* expression comprises a mixture of transcripts produced from both chromosomal and phage promoters, depending on the status of Φ370.1 integration (Euler 2010). What had not been previously explored, however, was the overall contribution of phage-derived transcript to this pool of *pepD* mRNA. Comparison of transcription rates in the presence and absence of Φ370.1 in the current study indicated that *pepD* expression is higher when the phage is integrated in the genome. Thus, it appears that the phage encoded promoters for *pepD* (and potentially *mutX*) may have evolved to enhance expression of these chromosomal genes.

PepD is a dipeptidase involved in the breakdown of peptides into their constituent amino acids. MutX is a member of the Nudix hydrolase family important for degradation of potentially hazardous materials, such as oxidated nucleotides (Bessman, *et al.* 1996). As both proteins appear to function in important metabolic and stress response processes, enhanced expression of

these genes from phage promoters may provide a selective growth and/or survival advantage to lysogenized strains. This type of phage-dependent gene regulation would be mutually beneficial to the bacterium and the integrated prophage, which is replicated each time its host divides. Comparison of SF370 and CEM1ΔΦ growth in response to a broad range of stressors and energy sources may yield additional insights regarding this hypothesis.

Upregulation of gene transcription in the presence of integrated phage was also observed for genes directly downstream of *pepD* and *mutX* (Figure 7.6). It remains unclear if these gene clusters are encoded in an operon driven by the phage encoded promoter, or if augmented expression of genes downstream of *pepD* and *mutX* represent an alternate mechanism of phage insertion-dependent regulation. Notably, one putative transcription factor (*spy0715*) was upregulated in the presence of prophage, but what this might mean for streptococcal gene expression remains to be determined.

One potential virulence gene downstream of Φ370.1 caught our attention. The zinc-binding protein *adcA* is encoded adjacent to *pepD* and is proposed to be a surface adhesin. Thus, it is possible that enhanced expression of this gene product in lysogenized strains could augment the host bacterium's ability to bind to target epithelial cells in humans. No evidence currently exists to support this hypothesis, however, as the rates of adherence to pharyngeal cells were indistinguishable between CEM1ΔΦ and SF370 in our *in vitro* assay (Euler 2010). On the other hand, it is certainly possible that the conditions necessary for

regulation of this and perhaps other genes have not been or cannot be achieved *in vitro.*

Taking a broader view of the transcriptome data, it becomes immediately apparent that many of the genes expressed more highly in the presence of integrated phage are involved in cellular metabolism. Considering that 25 phage genes were transcribed in SF370 during growth in THY, it is possible that the increased expression of metabolic genes in SF370 could simply reflect the increased metabolic burden on wild-type streptococci due to replication of phage DNA (10% of bacterial genome), transcription of phage genes, and translation of phage proteins.

The picture for genes downregulated when prophage are integrated into the genome is more complex. As previously discussed, phage integration reduced expression of the MMR system at both phases of growth due to interruption of the MMR operon. All other genes downregulated in the presence of lysogenic phage were encoded at loci distant from phage integration sites. In addition, each of these genes was only regulated during one growth phase. In early log, genes negatively affected by phage integration included a few ORFs with no assigned function.

Genes whose expression was negatively impacted by the presence of phage in late log were largely involved in nucleotide metabolism and virulence. One might expect that genes important for nucleotide metabolism would be downregulated in the presence of integrated phage as the DNA mismatch repair

system is also repressed. The virulence genes negatively affected by phage integration late in growth included genes for capsule synthesis (*hasA*) and the collagen-like adhesin (*sclA*). Additional studies will be necessary to determine what effect (if any) the modest reduction in expression (~1.5 fold) of these virulence factors has on phage and/or streptococcal fitness.

Finally, our transcriptome analysis strategy allowed us to ascertain the level of phage gene expression in wild-type SF370 during growth in THY. As the CEM1ΔΦ lacks all phage genes, any signal arising from total CEM1ΔΦ cDNA hydridization on our arrays would represent background noise. Using this background signal as a baseline, we were able to ascertain which phage genes in wild-type SF370 were expressed at levels that were statistically significant from baseline (as determined by a modified *t*-test). Although not as sensitive as RT-PCR, this strategy allowed us to obtain a preliminary snapshot of phage gene expression during growth in THY across all prophage simultaneously.

We determined that at least 25 phage genes are actively transcribed during growth in THY. There was 100% overlap between the phage genes expressed at early and late exponential growth, with genes transcribed in early log comprising a subset of the larger group of genes expressed late in growth (Figure 7.7). Surprisingly, the phage gene exhibiting greatest expression at both growth phases was the superantigen *speC.* The other phage encoded superantigens in SF370 *speH* and *speI* were also expressed. In addition, the phage encoded DNase *spd3* was found to be transcribed in THY. This analysis

suggests that Spd3 may be the phage DNase responsible for phenotypic differences in extracellular nuclease activity previously observed between SF370 and the deletion mutant CEM1ΔΦ (Euler 2010).

In conclusion, it appears that phage integration has limited effects on streptococcal gene expression, mostly confined to genes affected by insertion of phage into their 5' upstream regions. Unanswered questions remain, however, regarding phage insertion-dependent effects on the expression of distant genes in the streptococcal chromosome and what regulators may mediate these changes.

On the chromosomal side, two transcription factors (*spy0715* and *spy1496*) were identified as upregulated in the presence of integrated phage, with at least one appearing to function in the repression of arginine metabolic pathways. Regarding the phage genes identified by our analysis, one ORF (*spy1469*) putatively annotated in the regulatory region of Φ370.3 was expressed in SF370 during growth in THY. Further analysis will be required, however, to determine if this phage gene is indeed a regulator and if it has any role in the modulation of chromosomal gene expression.

8. CONCLUSIONS

The work described in this dissertation reveals that *S. pyogenes* undergoes large-scale transcriptome remodeling in response to *in vitro* host environments. Furthermore, we examined the role of two transcriptional regulators that are induced in response to pharyngeal supernatants and monolayers. The first Spy1215 is a putative deacetylase that acts to repress virulence expression specifically in the presence of host cells. It is the first bacterial sirtuin to be linked directly to virulence regulation. The second Spy1755 appears to be an activator of fatty acid biosynthesis and is required for normal growth in GrAS. Finally, we contemplated the role of integrated phage in the broader streptococcal transcriptome using the first *S. pyogenes* strain to be cured of all lysogenic phage. Interestingly, there was a limited effect on streptococcal gene expression, mostly exerted on genes downstream of phage insertion sites. Overall, this body of work provides new insights into gene regulation in the medically important human pathogen *S. pyogenes.*

Table A.1 Oligonucleotides represented on the *S.pyogenes* SF370 Microarray

Locus Tag	Microarray Oligo Sequence
M1_Spy_0002	CATGCCTATAATAAAATCAAAAACATGATCAGCCAGGACGAAAGCCTTAGGATCGA
M1_Spy_0003	CTGAGGTTGGTAAGGTAAACGAGGATTTAGATATTGTTAGTCAGTCTGGTAGTGATTT
M1_Spy_0004	CAGTGTACTAACTGTCAGCATGTGATTATGATGAGTCGTTATGATTTTGAGCGAA
M1_Spy_0006	TAAATTAACCAGAGCAGCTTATCATCTCTTAGGCCTTGGAACCTATTTTACAGCAG
M1_Spy_0007	CCACATTGGAACTCAGGAATTTAACCGAATCAAAGTTGGTATTGGACGACCTTTAA
M1_Spy_0008	TGAGCTTATGGATCGTTTTGGAGAGTATCCTGATCAAGTTGCCTATTTGTTAGAGA
M1_Spy_0009	CAGTAGCAAAAGAAGTTGCGGATAAGGGACGAATTAAAGTTAATGGGATACTTGC
M1_Spy_0010	GCTAGGGCAAAATACTATTTATCGCGTGAAGGAGAAATGATTTATCCTATTCCAGG
M1_Spy_0012	GTGTTGAAGACCTACTGAAAGCTGTAGCACAACAATCGGATAATGTAGCAACTAATA
M1_Spy_0013	GCTTTAGTAACAGCTCACCATTCAGATGATCAAGCAGAAACTATTTTGATGAGGC
M1_Spy_0015	CTCATAAATTGATTGCTGAAGCTCTTCTCAAATATGAAACTCTAGACGCTGCACA
M1_Spy_0016	TCAAAATGCTACTCTTGTCACTGGACTACTTGCTATGATTTGTGCGGGAATATTTC
M1_Spy_0019	CCGCTAATATGGCAATGGCTGAAGAAAACCAAAATACATTACGTACTCAACAAGCT
M1_Spy_0020	AATTGCAGACTATTTTGATCGTCATGGTTTAGTGGGTGAAGATGTTGTCGTTGTTAGT
M1_Spy_0021	GCACTGATTTACCGCTTGATTTTTCCCATCGTTTTTCAGCTGTACTTTGTTCTGAA
M1_Spy_0022	CGTGGTCGGACAATTAGTAGAAGAATTTGCTAAGGAGACTCAAGTAAATGACTAG
M1_Spy_0023	GACTAGACAGGAGATTTTTGAAAGGCTGATTAACTTAATTCAGAAGCAAAGAAGC
M1_Spy_0024	ATGAACTCTTGAAAGGTTGGTTTGCTCAGATTGGGCTTAACTTGATTGACTTCAAG
M1_Spy_0025	ATCTGGAGCCAATATGTGGACTTTGACGGACAACCATCTATGGATTCTAAATACAAT
M1_Spy_0026	AAGGATTGTCGGATTATTAAGAGAAGCAGGAGCTAGTGAAGTACATGTTGCTATAG
M1_Spy_0027	ATCCATTCAAACGGTTATTCTTTGGTACGTCGTGTCTTTGCTGACTATACTGGTAAA
M1_Spy_0028	TTCCAACTTTCAGGTCATAGCAGAGCAGTTTCCAGTTAGTTTTGTCTTTTCAGATCAT
M1_Spy_0031	CTTATCATCTCAGACAAGTCATTCCTACCCAAGACAACTAAAACACATTTCTCAAGCT
M1_Spy_0032	AAATTACTTGTTGTTGGTTCAGGTGGTCGTGAACATGCGATTGCTAAGAAGTTGTT
M1_Spy_0033	GAAAACTCCAATTATTTCTATTATCATGGGCTCCAAATCTGACTGGGCAACCA
M1_Spy_0034	TTCAGGAAAATATCCACCGCAACAATATCCTGTCAAAAACCATCGTACCAG

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_0035	GGAAGAATTGATAAGAGCGAAGGAAGAGTTATACCCAGAAGCTTATCCAGCTAAT
M1_Spy_0036	CACAACTATCTTGATTGACTACATGCTCAACCGCTTTGGTAATATCGTTAAGAACTTG
M1_Spy_0037	GGACACCAACTTACATGGATGATGGAAAATTACAAGTACGAGAAGAGCAGTTTGA
M1_Spy_0038	AATAACTGATAGAGCTTTGACTATGTTAGATGTTGACCGCGAAGGTCTCGATTACATT
M1_Spy_0039	GGAGCATGGAAACCCTATTCACTCAGGTACACAGTCCATATTAAAAACTTACCAAATT
M1_Spy_0040	CGGGGAGTTTCATAAAGGACAAGCAAATGGCAAAGGTGTGTTAAAAGCAAAGAATAATA
M1_Spy_0041	TTATAGTGAAGATAGTCAAGGAGCAGAACTATATGTCTCCACAATCCAGACTGCT
M1_Spy_0044	TTGTAGCCGTAGGTCTTCCATCAGAATACATGGAACTGTCTATCGTAAAAACTGTT
M1_Spy_0045	TCTTGCCGCTATTTACACAGAATACTGATGCTCAAAGGTCTGCCATGATTGTTTTA
M1_Spy_0047	CGACTCACAAATACAAAGATTCTCGCGAACAATTTGAAATGCGTACACACAAACG
M1_Spy_0049	TTTTGATGACAAACGTGAAGTCTTGAGTAACAAACCTGCCAAAGGCCATGTT
M1_Spy_0050	ATACAAACTTCCACAAAAAGTTCGTCGCCTTGCGTTGAAATCAGTTTACTCAGCAA
M1_Spy_0051	TGAAGCAGGCAAATACACTTTCGAAGTTGATACTCGTGCACACAAACTTTTGATCA
M1_Spy_0052	CCAGCGCTTGGTCTTAAAACTCGTAACAAGAAAGCTAAATCAGACAAACTTATCGT
M1_Spy_0053	TCAACGATTTTCCCAAGTTTCATCGGATATACAATCGCAGTTTATGACGGACGTAA
M1_Spy_0055	GCAAACTTGGTAGTATCTGAAACATTCGCTAACGAAGGACCAACAATGAAACGTT
M1_Spy_0056	TGACACAACTTATGGTAAACTTGGCGTTAAAGTTTGGATTTACCGTGGAGAAGTTC
M1_Spy_0057	ATTAAAATCTTCCCACACAAATCATACACTGCAAAAGCTATCGGTGTACGTATGGG
M1_Spy_0059	AATTGTTTGATCTTCGTTTCCAAGCTGCAGCAGGTCAACTTGAAAAGACTG
M1_Spy_0060	CACTTTCAGCTACAAAACGTTTCCGTCTTGTGGAAGTAGTGGAAAAAGCTGTTAT
M1_Spy_0061	TTGAAAGTTGCTGATAATAGCGGTGCTCGTGAGATCTTGACTATCAAAGTACT
M1_Spy_0062	AAATCCTCAAGGAGCTATCGTCGAAAAAGAAGCACCTATCCATGTCTCTAATGTT
M1_Spy_0063	CAGCTTTGATGATGTTGATAAAGTACGTGGTCTTGATATTGTAATCGTCACTACTGCA
M1_Spy_0064	AAGAACAAACGTCCTGCAAAACACTCTACACAAGCTTATACTCGCTGTGAAAAATG
M1_Spy_0065	GGTTATGACTGACCCAATTGCAGACTTTTTAACACGTATCCGTAATGCTAATCAAG
M1_Spy_0066	AAGGTACTAAACTTGTCCTTTCAGTAGGTAAATCTCACCAAGACGAAGTTGAAGCT
M1_Spy_0067	AAGACGTTTCTAAAGGAACAAAAACAGAACAAGCCGTTGTAGTCGGCAAACTTGTT
M1_Spy_0069	GCAGGTGTTGCTGATATTACTTCAAAATCTCTTGGTTCAAACACTCCAATCAACATTG

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_0071	TTACTTTGACTAAGTCTCCTATCGGACGTAAGCCAGAACAACGTAAAACTGTTGT
M1_Spy_0072	TTAAAAGATGCTGGTATTGTCCGAGCTGAAAAATCAGGCGTTAAAGTGCTTGGTAA
M1_Spy_0073	GCTCATTTTGATTTCAACTGGTATCGAAGGTATGAAACAGCTTGAGGGATATCTTC
M1_Spy_0074	TGTCTTGTAGAGCGTTTGAGTGGTCGTATTATCAATCGTAAAACTGGTGAAACGTT
M1_Spy_0075	GCAAAAGAAGACGTGATTGAAATTGAAGGCAAAGTAGTAGAAACGATGCCAAATGCAA
M1_Spy_0076	GAATACTGTAAAGTTATTCGCCGTAATGGTCGTGTTATGGTAATTTGTCCGACAA
M1_Spy_0077	ACAAAATACTAAAAACAATGCTCGCACTCGTAAAGGGAAAGCTGTTGCGATTGCA
M1_Spy_0078	AAATCTGCACAAGAACACGGACTAAAAACTGTTGAAGTGACTGTAAAAGGTCCTG
M1_Spy_0080	TTTAACAGAAAAATCTGAGCCTGAAATGATGAAAGTCCGTAATCTTGGACGTAAGAGC
M1_Spy_0080a	GATGAAGCTACCGATAAATACACATCAACGACAGCTCTTCAAAAACTTTTCTCAG
M1_Spy_0084	TATCGTTTGGTGGTTCATATTTGCTTGTTAAATGTTAGGGATGAGATGCTGAGTC
M1_Spy_0092	GAACTAAGGATACGGTTGATGCTAGGGTGACTTATTTTGAATTAACCGAGTTAGCT
M1_Spy_0093	ATGCTGATCGGAACATTCATTTAGTCAGAAACCAAAAACTTCCTTGGCGTTGTTTCAA
M1_Spy_0094	GCGGTATGTTATCTGGTATTTTCTTATCTTATTTCTTTGAAACGCCAGCTAGTGCC
M1_Spy_0095	CCTTCATTTGATTCCTCGTTTAACTAATGATAGCTTTTGGGAGGGCATCACCATTA
M1_Spy_0096	AAATGGTGATAATAAAGCAGAGCTTGTCAACAACTACGACTGGTTCTCGCAAATCA
M1_Spy_0097	GGCAGGACATGATGACAATACCTCATTAGCGCCATTAACAGGATATAACAATAATTCT
M1_Spy_0098	GTTTGAAAGCCTATGAAGCGATTACTAAAGGTAAACCAATTCCAAAACCAGGTGTAC
M1_Spy_0099	TGATTGATCATACAGAAGTCTCAGCAGAAGCCGTTTTTACAGCAGAAGCAGAATAA
M1_Spy_0100	GCGAGGACTGTGACGAAGATTTGCAGCAGTTTCATTCTCTTTTACTCCTTAAAAAT
M1_Spy_0101	GCATTAATTGCTTACCAAAGGTTACTTAATGGAGGAGCATTGATTGACTCTACTC
M1_Spy_0102	ATGATTGAATACGGTGAAATCAAATCGAAACTGGGGGCTGAGTTGGAAATCTATG
M1_Spy_0103	CCGCTGTTGTTAAATTAGTGGAGAATCAAGCAGAACTATATGAATTATCTCAAGGCTC
M1_Spy_0104	CAGCAAAAGTTAGCCATATTACAGCAAAAACAGCGCGTCTTGGATATTTCTTCAAC
M1_Spy_0105	GTACGGATAGATACATTAGTATTCACGATGACGAAGGAGAAGTGATGAAGATTGAGTA
M1_Spy_0106	CTCATCAATTGCGAGAGGAGTTAAGTGGAGCAAGGTTTTACAAAGTAGCTGATAAT
M1_Spy_0107	CGAGAGCGAAAAGAGAGATGGATGTTAATTGTTAAGTTAGATCAGCAACGACAGT
M1_Spy_0108	TCTTACAAAAACAAACAGACCACCCAATGGAAACCTTTGTCTATCCAATTCGGGATTT

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_0109	AGATGTTATTGGTGGCTTAACATGGTTCGGAATGGACATTGATCCTGAGAAAAATG
M1_Spy_0110	AGAAGTCTGGTGGCTAAGAAAATGGAGGCACTAGGGAAAATAACAACTAATAG
M1_Spy_0112	TTATGCTATGTCCCACCAAGACCTTATTGACCAGGTTGATCTTGTTATTTTAGGCAT
M1_Spy_0115	TAAAAGGACAAGACCTTAACAACACCCTAATTGCAGGTGCTAACGTTCAAGAAGAA
M1_Spy_0116	CTTTTCGCATCCTAAGCGCCCTACTATTCTTATTAACCAGCTTGTGCTTTATCAT
M1_Spy_0117	AGATTATATTTCAGGTGGGGTTGTTATGTCAAACGGCAAACAGTACCAATTCACC
M1_Spy_0121	TTGACTTCGTTCAAAACCCACAAGATTTGGCGACTGTCTTAAAAATGATTGACACCAA
M1_Spy_0122	TAAGATTTACCACATTGTCAAGGAAGTCACGTCTGTTTTAGATATTCCACTGACTGTC
M1_Spy_0123	TTAATGTGCTTTTAGATGAAAATGATAAGGTTAAGGTTGCTGGTGGCTTTATGGTGCAG
M1_Spy_0124	TAGCCAGTAATTTTATCAATGCTCATCTCCTAACGGATTCTTTTCCAAGGTATTTCTCG
M1_Spy_0127	GATTATTGCTCAAGCAGGTGATGAAGTGGACCTGACAGAACAAGGTGAATTAAAAA
M1_Spy_0128	GGCACCATATATTGCTTTAGGAATTGTAGCAGTTGGTGGAGCTCTTTACTTTGTT
M1_Spy_0129	CCTTATCAACCTGTGAGGATATGACAACAGATGGTAGGATTATCGTTATTGGACAAA
M1_Spy_0130	GGTTATTTACTTATGAGTTTTTGTTTGTTAGACTCCGTAGAGGCAGAGAACCTCAC
M1_Spy_0131	TTCCTAATGAAGAAACTCTCGCTAAAAGAGAAGTGCTAGAAGCTTATTTGCCATGG
M1_Spy_0133	TCAAATCCCAACATGAGTTAGATCTCTTTTCAGGTGCTGTTTATCTCTTCTGTGGT
M1_Spy_0135	AATGAAACTCGTGGATAAATCAAGAATGTGGGCAGAAGTAGTGTGTGCTGCTTT
M1_Spy_0136	GCAGGTATTAAACAAAACAACCCATTAGCAGCATCATTCCCAAGTTATAAAGGGG
M1_Spy_0137	CTTATACTATTCCTTGTGTCTTGGTGGTCACAGGTATTGCCATCATTTACCTATTTGT
M1_Spy_0140	AAAAGCAGTTGAGTTAGGTGCTCAAAAGATTCAAACGGGCAACGCTGATATTGTTTTA
M1_Spy_0141	TTAGTGGCAGAAGGCAAGGAAACAAAAACCATCAAGGGAAAAACCTATCTCTTAGAAT
M1_Spy_0142	GCCAGATGGTATCCAAGTAATTGAAATCAGTGAAGGGTTTACTTTTGATGAGGTTC
M1_Spy_0144	TCTCAAAAGTCGCTGATATTCTATGTATTTCCGAACCAAGTGTTTACCGCTACCT
M1_Spy_0145	AACTTATACGATTGAAGGACATTTTCTATACACCGCAGGCCAATTACCCCTTAATC
M1_Spy_0146	CAGCCCTTGGATGTATTATTATCAGCTTAATTGTGGGTTATATTGGAGGTTCTGTCTTT
M1_Spy_0147	GAAGAAGAGTTAAAGCAGCTGTTCCTTGAGTACGAACAAGAAACTCAGCTACTATT
M1_Spy_0148	GGGGCTTATTTACGGTTCTTTCTTTGGTTTTGACTTACCTGTGGCATTATTATCAACTA
M1_Spy_0149	GGAATCTACGGCTTTGCTATTGGTATTTTAATTTGGATGAAACTGACACCAGAACTTTC

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_0150	CTCAGGAAGCAGGATTTTTAGTCAGTTTAGACCAAGTCGATGACAATTATTTATACCG
M1_Spy_0151	AGCTAATGATTTACCCAAAGAACGTGTCATAGAAAGGATGAGACCAATAGCATGA
M1_Spy_0154	TACTCAGAAGGTTACCAAAGAGATTCACCACGTTTTAGCAAAGGGAGGAATTTAG
M1_Spy_0155	GGTTGGGAATTGTTGTCGATTTTGCCACGTACCGAATTAAAACGCATTAAAGATGA
M1_Spy_0157	ATTCGTGAAAACAATGAGCTTCGGCAAACTATCGAAAAAGAACTTGCTGCCAATATGAA
M1_Spy_0158	GCGGCTTTACAAAAAGACCTAAAAACCAAAGACAGTGCTACACCAGACTATCAAATAAA
M1_Spy_0159	AACTCTTAACCCTCAGCCTTTGAAAACCCCATCTCCTAATCTTATTAACAAAGCAC
M1_Spy_0160	AATTGATGTTCTTTCAGGGCTTGATACGGTTAAGATTTGTGTGGCTTATGACCTTGAT
M1_Spy_0163	TTAGTAAAAGTAGCCGTAAAAACCTCAGAAGACCAATTCCCAGGTGGTCAAATAAC
M1_Spy_0164	TTTATGGGACAAGAAGGCCGTGTGGTAGAAATCGAAAACAACAAAGTGAAACTGAT
M1_Spy_0165	GTTCCTGGTTGCCAAACGTGGTCAGGAAAACACATTGAAAATTCAGAAAGTGAAT
M1_Spy_0166	AAATAGCGGTCAAGCATTAGACATTTACCAAGCAGTTCAAACCCTAAACGCTGAAAA
M1_Spy_0167	GTGGCGAAAAGTGATCGACGAAAGAGATGTGAAACTGTCTAAAGAAATCAATGTCAAT
M1_Spy_0168	AAAGATGGGTACAAAGAGGGTTATAATGAAGGCTGGAAATCTCAGTATCCCGTTTT
M1_Spy_0169	GCCCTCTAACCTGCTTAAAGTATAATGTAGAAGACTGGTACAAAGCAGTAACAAG
M1_Spy_0170	AAATGATGCTAGCTATAGTAACGGTTTCTACGGACACACTGGTCCTGACA
M1_Spy_0171	CCATTTGTGTGGGTTACGAAAATAGAGACATAAAAGAGAGTGAAACTTCTAGCACCAA
M1_Spy_0172	TTTGCTGCGCTTGTCTTGTGGTGTTGAAAATATCGAAGACTTATTAGCCGATTTTGA
M1_Spy_0173	AAATCGCAGGCAAAGACATCATCAAAGTCATCGCAGTACCAAACAAACTCGTTAATAT
M1_Spy_0174	CAGTTTATCTCTTTATCTTGATGCAGGGTGTTCGCATGTTTGTTTCTGAATTGACCAA
M1_Spy_0175	CCAAAGGCCACTTGGTTGGTCTTGATAACCTGATGGATGATAATGAAATTAAAACC
M1_Spy_0176	TGCCAAAGTCCTAAAGAGGTTCTAGAGATGGTAGAAGAATCTAAAGACAGTCCTT
M1_Spy_0177	TTGATGTAGACACTTTGAGACTTTTTGAAGGGGTTGATGTCTTTACCTTTATCGCAG
M1_Spy_0178	GAAGCCCAAGTGATGCTTTCTATTGAGATTATGGATGACCCTTTTATCAACTCTATTG
M1_Spy_0179	AAATTTACGTGGGGCAATGTCTCTGAAGTTTGTCGTGAATTAGGACGTATTGTCAT
M1_Spy_0180	TATGTTGTGGAGTTCTTAGAGACCAGATATAAGGTGTTGGTGTTGGATCAGTTGA
M1_Spy_0181	GAGCAAGAAAAGACCAAACTCGTTATTGTTTCAGATGATGGGATAGGCATTCAAAAGTT
M1_Spy_0182	CATGGTGCGGATTCTCATTTTTCGAACTACTTTGCTAAACATGGACGTGATTATGA

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_0183	ATGATAATAATCGTCTGTTAGGTGTCATTATTCGAGGACGAGTGATTGAAGCCTTG
M1_Spy_0184	ACACAGTTGTTCAATAGCAAACCTCAAGAAAAAGCCAAAGCTGGCAAAACCAATAAATG
M1_Spy_0185	AAAGACACCATGGAAGCTGCTGTTGATTTAGCTGTTCCTTTATGTGTCGATGAAA
M1_Spy_0186	TAGATGTCTTTCGCAGAAGTGAGTTTTTACCAGAAGTTGCCAGAGACTTTTTAGCA
M1_Spy_0187	CAATGTGGTTTGTGAAATATGTGGGAAGATTGCTGACTTTATGGATGTTGATGTTATGG
M1_Spy_0188	TCTTCAGAGTATGACGTAATTGTGACAGATATGATTCTTGAGCAAACAACGGACTC
M1_Spy_0189	TTCGTGGAAGTCGTATACTCAGTTTCTTTTATCAACGCTACCAGTGAGGTTAAGAA
M1_Spy_0190	TGATTTGATGAGTCAGATTGTAAAGGATCTTCATGAGTGTGGGCGAAGTGATAACT
M1_Spy_0191	TTGTACTTAATGCCCTTGATGAGTCTGTTTGTGGCTTCAAGTTTGTCGTTGAATGA
M1_Spy_0195	GCCAAGAAAAACCTTTGATAAAGCCTTCAAACTCTCTGCTGTAAAACTCATCCTTG
M1_Spy_0196	GGCAATCATATCTTTATCGTCAGCATTATCTACCCACTCATCGTCTTGCTTTTATTATC
M1_Spy_0197	ATTGATTACGTCTCCAGTGGTAGTTTAACCCATAGTGCTAAGAGTCTCGATTTTTC
M1_Spy_0198	CTTACCTGAAGGAAAACTCTACTTATCACCTGTTCTTGATGGCTATAACAGTG
M1_Spy_0199	CGGCCAATCTCAGAAACAAACATCCTTAGATTATGCTTTACCAACTTCTAGTATGC
M1_Spy_0201	CCTACAACTTAGCTTCACCGGTTGGTTTCTACTGAATATTGTTACTTTTGGGTTAC
M1_Spy_0203	CTGAAGATTTTACCCCACTTGATCATGACTGTGACTGCTACACTTGTCAAAACTAT
M1_Spy_0205	CTCAAATGTGCTTCTTGTCAGACTTATTATGCTTGTTACCATTGCCATGACCAGTT
M1_Spy_0207	TGATGACCACTTTGATTATTATTTTAGGCTTTATTCCTGCTATCCCGCTAGGCTTTATC
M1_Spy_0208	TAAGTTAAGCATTCCTGATGTTTCAGAAGGTAGAGCCTATGATGTTACTGGAGTTGAA
M1_Spy_0210	TTATTCTAAACGTCATCAAAGCTATATCTCAACGTTTGACAGTGACGCCATCCTTG
M1_Spy_0212	GAAAAATACAATATCTATGATTCGGAATCGCGTTATACATCGGGGAGCCTTTTCC
M1_Spy_0215	CGAATGGTGGAAACAATTAGCTGGTGAATCAGAAGGAAAAGATCAAAAAGGGATTTAC
M1_Spy_0216	GACAAGTCATCAGCGACAACAACTTTACCATTTAATCACCAAAGATCCCGATTATCAT
M1_Spy_0219	AGAGAAAGTTTTCATGCGTACGGCGTGGCTAGTTTCTTGTCAGCCTTGAA
M1_Spy_0223	GTTGGCTTTAGTGAGCTATGGTATTATTTTGGTTGGTGTGTTAGTGCTTGGGTTTTT
M1_Spy_0224	AAAGCCTACATCATCCAACTGGGCAAAGCACTAGAGAAGACAAAACCATA
M1_Spy_0226	AATTAGAAGATATTGAACGCAACATGGGTATGGTTATCGAAGGTATCGCAACAACTAAG
M1_Spy_0227	CACAAGTGATAGGTGATTTACGTGAATTGATACATCAAATCGAACAAATTAGTGATGAG

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_0228	TATAAGCAAGTAGTGTTAGCGCAGGTTGGTAGAGATAAATTGCCTTTGTTGCGA
M1_Spy_0229	GAACACACGCAGAGTTGGTGGCTAATAATGCGATTTATCGTGAAATTTATGAGAC
M1_Spy_0230	CGACCCATATTCATGACTTTATCATGTCCTTACCAAAAGGGTACAATACCTATGTC
M1_Spy_0233	TTGAAATGTGTGAGGAGTATGATATTTACCGCCAATGCTATTGTGGCTGTGTCTAT
M1_Spy_0235	TTACTAATCCTGATTTACTACCAAAACGTGAGACGACTCATGCAGCTGGTTATGA
M1_Spy_0236	GACAATGAATTTTACCTCTATGCCGAAACCAATATGCAAGCTATTCGCACAGAAA
M1_Spy_0237	TTAAAACCCAAGACTAAAGTAGCGATTGTGACTTGTATGGATTCACGGCTACATGT
M1_Spy_0238	AATGGCTTTATGCAGTCAATTGGTACAGGTGAAGGAGTTGTTAATACCTTTAGAGG
M1_Spy_0239	AAAGGGAAAAACCTCTTTATGCCTATTCGTATTGCAGTATCTGGTGAAATGCATGG
M1_Spy_0242	CAGCTCTTGAAGCACAACAACCTCATATGTCAATTGCTTACTTTTTGTTAGGTGAC
M1_Spy_0244	TCAGCCCACTTTTTGACTGTTTTAGATAGTTACCCCCAAATAACGCTTTCGACTAAA
M1_Spy_0245	TACGATATGATGTCTAGTCGTGAGTCAAAGGACATGTTTTTATTTGAGACTCCAC
M1_Spy_0246	TAGGAAATGCAGTCACCAGAAATGCAGTCAAACGAAAGATACGTCATGTTATCAT
M1_Spy_0247	TTTATGTGATGCCGCTAATGATCTTTTTCATGGGCTTTAACTTGGCTAGTGGAGTA
M1_Spy_0248	GTCCATGATTATGTGGAACATCGAACAGAAACCTTGATTGACTTTACGCAAAAAG
M1_Spy_0250	TAAAATCCGTCGTCAACGTAAACACGGATTCCGTCACCGTATGTCAACTAAAAA
M1_Spy_0251	TATTCGACAAGTTAAGGAAAAGTACCCCAATCAGCTTTTGATGGCAGATATTAGTACC
M1_Spy_0252	TTGCTGAATGGACAAAATTCTACTCACCATACTATAACACGATTGATGGGTTTGCTG
M1_Spy_0254	TTTCTTAGCTCCGGTCTTGGTTTTCTTTGTGATTTTTGTCTTGATACCGATGATTATGG
M1_Spy_0255	ATTGTTAATTTTATGGGAATCCATGACACTTTGGCGGCTGTTATTTTGCCTCTTGTG
M1_Spy_0256	TGCTGTTTTATTTTTGGTGGTTATACTCTTTTTGTACGCTTATCCTCAGGCCGTCAAA
M1_Spy_0257	TAATGAGATTATCGGTGTCCTTGTTTCTGCTCACGGCAATATGTATGGGGTTATTAAA
M1_Spy_0258	ATCGGTGGCTGTTTATTGTTTAATTCCCAAGTTTTTCATGGTAGTAGTCATTCAGCTTG
M1_Spy_0259	TTTGACGACTCCTTAGATATTATTCCCGTATCATCAACTCACCAACTACATTATGGGA
M1_Spy_0260	ATGCCCCTTATCTTACACCAGTTCCAAAACGTGGCAAACAAAATCATACAGCTTA
M1_Spy_0261	ATCGTTTTAACAGACCCAGATTATAATGGTGAGCGCATTCGTAAGCTCATTATGG
M1_Spy_0262	TTGAGGTTAAGGACGAAGATTTCTTTTTCAGAGTGTCTCGTCTCAGTTTTGTCCAT
M1_Spy_0263	ATCATTAAATCTCTGGCTGGTTTTTATTACGTGGAGTCAGAAGGACAGGTATATCAGA

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_0264	CAGATGTTGTGGCAAGTATGAGAAAGCACAGTAAATTGGTCTTTGATTGCCATCTA
M1_Spy_0265	AGAAAATGGGCTTCCTTTGAATATGGCTGTTGGTGATTTTGATTCGGTTTCCCAAAAA
M1_Spy_0266	CTTATCAAGACCAAGCAACAAAATCTCTCTTGAACATGCCCTTATTAGAAGAGGA
M1_Spy_0267	ACCAGATAATACAGAATACACCGTTCGTGGTAACTTGATTGGGCATATTTCTCTCATT
M1_Spy_0268	AGTCTCTTGACAGAATTTGACAGCACTTTAGTTGGTGTTGCTGTGTTTGCAGAAAA
M1_Spy_0269	TGCCATTGTGTCTAAAATCACATCTTCGATTACTCAGCCCTCATCTAAGACATCTT
M1_Spy_0271	TAAATTCGCTCGTGTACGTTTGAGCAACCTTATCGAAGTAACTGCCTACAT
M1_Spy_0272	AAAGAAATCATGGATGCTGCAAACAACACAGGTGCATCAGTTAAGAAACGTGAAGA
M1_Spy_0273	TAACAGCCAAATCGTTCGTGCTTATGTTCCACTTGCTGAAATGTTCGGTTAT
M1_Spy_0274	TCCTATGGCTAAAGCTCTTCACGATGCATTCGGTATTCAAAAAGGTCTTATGACTA
M1_Spy_0276	AAAACCTAAATGTATTGGAAAATGCTATCGTCGCTCAAACCACCGTCCTCAAAAGA
M1_Spy_0277	ATTGTTCAATCTATGGTTATCTACTACGGAACAGCGCAAGCCTTTGGTATTTCGAT
M1_Spy_0278	AACCTTTGTGTCTAGCTTGTCTTCAGCTACAACTTCCTCAAATTTCTCAGTCTCTT
M1_Spy_0280	AGCTTGACCGCTTTTGTGGTTAGTTTATTGGCGATTAGGTTCTTGACAGATTATGTTA
M1_Spy_0281	GCTTACAATTGATGCTTGATGGTGCTGTTGAGCAATTACAAAAGATTGAGTTGGGA
M1_Spy_0282	GGCGTCCTCTTGATGCTTGGACTTTTATTTGGTTTAGAAGTTTTTATTGAAGGATTGG
M1_Spy_0285	TTACATTACCCCAGACCTAGTTCATGTTATGATGGATGGTCGTATTGTCTTATCTG
M1_Spy_0287	CGCTGAAATTCCTATTCCATCAGTCCGCCAAGAGATTATTAAGGTTTTAGATGAGAAA
M1_Spy_0288	GCCCAACCTCTTCTTAGTTATTTAGGTGTACCAGCAACTGTTAGAGCAAGTTTTTATA
M1_Spy_0289	TAAAATTGAAGATATTGCTTTTGCAGGCAACGGCTGTACCATTTCCACAGCTTCAT
M1_Spy_0290	TCATAATTCACAGGTGGCGTTAGAACATGAAGCCAAAGTGTCTAAGATTTCTGAAGA
M1_Spy_0292	AAAGGGACAAGTCTTAGGTAGAGCAACCCTTCAAGATAAACATCTTATTGGACAAG
M1_Spy_0294	GGTGTTATGTTTTCCTATATCGTTGGTTTGCCTCTTGGTTTATTTATGGCTCGGTTTAA
M1_Spy_0295	AAATGGGCAATCTCTGTTTGATGGTGTTTGGTATGGGGCACGTAATTCTATTTTAATCT
M1_Spy_0296	CGAGAATATCATTTCACCATTATCTTTATTACGCACGATTTAGGTGTTGTGGCAAGCA
M1_Spy_0297	GTATTGCGGTTATCCATAAAGGGGTTATTGTAGAAGTTGCAGAAACAGAAGAACTG
M1_Spy_0300	CAGTTACTAGAAAGGTACCCAGAGTTAATTGAGGAAGAAGAGCGTTTATATCGCTA
M1_Spy_0303	TTGGAAAATTCGCTCCCCCTTATCAACCATTCTATTTCTTGTCTTCGTTTGTCAGTTA

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_0305	TTTAGTCAAACCACTTGTTGCTTCTGATGCTTGGAATACTAAAATTAACCGCTGGC
M1_Spy_0306	TAGGATGGATTCGTGTTAATGGTCAAAAAGACTCAAAAGCGATAGTTGCTGCATG
M1_Spy_0307	GGTTTCTGCTAAGAAAGAAAACCGCAAGTTGTCCCCTAAAGTTAAAGCAATCTAG
M1_Spy_0308	GCCCAATTATCTCTTACCTAAGCGGGTTTTAGACTACATTACTCAAGAAGGTTTG
M1_Spy_0309	GCCTATTTTCCCACAAACTCTGGATACCTATAATGCGTTTTGCAGCTATTTAAAGG
M1_Spy_0310	TTGTGATTGCGAGTGCAACAAACAGTCGTCAATTAGAGGCTATTGCGGATAATATT
M1_Spy_0312	ATTTTATTCAAGGTAACATGCTGGATTTGTCACAGGTGGGGCAGTTTGATTTTGTAAC
M1_Spy_0314	TTACTAGAATTGGAGCAGAAACTTGGGATGAGATGACACAAAAAGCAGACAGCAT
M1_Spy_0315	CCAAAGCTTTTCAAAGTGCTATAATAAGGGAGTTAGAGTCAATATTAGTAACGTT
M1_Spy_0316	AATTACTTGAAGCGGATGTAGACGTAGATGATGTTGAAGCAGAAGAGGGAACAATAA
M1_Spy_0317	AATGGTCTTCTATCTTTACAGGTTTGGACTCAGGAAAATACCAAATGGGTGGTAACAA
M1_Spy_0319	TCTGCTGATGCAGCTGTTGTCAATAATAGTTACGCTGTTCCTGCAAAAATTGACTA
M1_Spy_0320	TATTCCAGTTGGTGACATGATTGTTGTCTTGGAAGGGCAAGCTGAAAATATTCTTG
M1_Spy_0321	GATCTTATTCGTGTTTCTACAGTCACCTTAATTTCTTTAGTTGGGGAAACTGCCATG
M1_Spy_0323	CAGTCATTATCAACTGGTCAGCTCCTGTTTTATTCCTTTTATACCCATTAACAGTCGA
M1_Spy_0324	AGGATTCTTGTGAGACAGCCTTAAATTCGTCAACCGATGTTCTTTTTACAGCTATTG
M1_Spy_0326	AAACCTTTTGAACCTTTAGAACCGAATACTATCATTGTGGCCATCGCTAATGACCA
M1_Spy_0327	TCTTATTTGAATCTATTTCTGCCATCGCGACAGTTGGGGTATCTATGGATTTGACA
M1_Spy_0329	GTTGAGAAGAAAAAGGAAACACCTAACAAGTATCCAAGAAAAGCTGGCTTGCCTAA
M1_Spy_0330	AAGTGACCAGTTTGTTTGCCGTTGCTGAAAATTACCCAGACTTAAAAGCTAACGAAAA
M1_Spy_0331	TCTTGTAATGAGTATGAACAATGCTAGAGAAGTTACAGAAAAAGAGGCTCCAGGCTTT
M1_Spy_0334	AGTATGTTACTTGAGCGTGACCAACAGATTATTGACATAAAGGCAGTTAAAGCTGTC
M1_Spy_0337	TGAAGGTTCAGTGTTTATCTTACATATTCCTTTGGCCCAGTCTAAAGAGAGTTAG
M1_Spy_0338	AAATGAAGTGTCAAGTACTGCTATTGGTAATTTAGTGATGGATGAGTTGGCCGAAC
M1_Spy_0339	AAAGTAATGTGCCTAAGTGGAGCAATCCTGATTACCAAGAGACAACAAGTCAAGAA
M1_Spy_0340	ATTTTGTGGAACAATACCCATCAGCAGAGCAAAAAGGCTTGTATTTGTACGGAGATAT
M1_Spy_0341	AGCCTTTACCTTTGAAGGGACACCGATTCATCTAATTGCTCGCAAACGAAAATAA
M1_Spy_0342	CTACAGTTTTGGATGGCAATGAAAGCCGAGCAAGTATGTCCATTGAAGAAATAAAAGA

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_0343	AAAGAAATGGCCTATGTTCCTAAACAGGTTTCTAAAAAGCAACAGCCTGCGGATT
M1_Spy_0345	CGCACTATTGCCTTATTAGAGGATTTTGCTTGTGCTTTAAATGAAGCTGACAGTGT
M1_Spy_0346	CGGGGTGACTAAAAATCCTAAAACTGGAGGCTATATTGCTATTACTAGTTTGTCCA
M1_Spy_0348	AACCTATAACTATTACAAAGAAACTACCATGAGAGAACTTGTAGAGGACATGCTGGCA
M1_Spy_0349	CAAAAGATGAAGTCGCTATCGGAAAAACAGTTATCGTTCAAGAAGTTGGAACAACAGA
M1_Spy_0351	GAGTATCAACAAGCACCTTTATGGGTATTGATCTTGGTAGCCGTAGTTTAGTGTTAA
M1_Spy_0352	GATGGGACCGTTGAAATTCTGGCCCAATCAAAAGATTCAGATAAAATAGCCACATTTA
M1_Spy_0356	AAAAATCAGGACAAGTTTTTCGCCACATCTTTGTTTTAGAGGAAATGGTGGAGCGT
M1_Spy_0357	AGAGGGGGCTTACTTCATGATTTCTTTTATTATGATTGGCGCGTGACCAAGTTTAA
M1_Spy_0358	TCTTAGAAAGGCTATGTTTGCTGCTTTGATTGGTGTTGTTGTGGCTAGTCTGATTAA
M1_Spy_0359	CCAGATGGGGCAAAAACCAAGTGAAGCAAAAATCCAACAAACTTATCGTAATATCATC
M1_Spy_0361	ATCCCTTGTTACGACCAATTATCCAAAATGTTATGGGGCCATCTGTTAAGCTGATT
M1_Spy_0362	TATATTGCTACGCAGCCTAAGGGAGAAAATGGTTTTGGGTATGATCCTGTCTTTAT
M1_Spy_0363	TATTTGGGCTGGTATTTATGTCGTTGGCGGTAATTGTGACTATGATACAGGTTAC
M1_Spy_0364	TTATGCACAAGTTAATTGATTTCCCATTCTTACCGGTTGTAGATAGAGCGAATCG
M1_Spy_0365	GAACTTAGCGATATTCTTGGGCTGAAAAGCCCTATGACCTTAGAAAAATACTACA
M1_Spy_0366	CAGCGACTGGAAAAAGAAAACGTTATTAGATTATCAGCGATTTTTGAGGAATGCC
M1_Spy_0367	ATGTGGTTGGGCGTCCTCATTTGTATGCGACAACAGATTATTTTCTGGACTATAT
M1_Spy_0369	CCTTAACTCGAGGAATTGTTATTGATGGTAAAAAGACCAAACCAGCTCGCTACAAT
M1_Spy_0370	TATTAATGGGGATAGCAAGAATATTAAGATGTCACCCTTTTGTTGCTGGTGGTGTC
M1_Spy_0371	GACAACAGGGCTTTCAAGGGTTAGAGTTAAAGCATACTTACGAACACGATAAACTA
M1_Spy_0373	GGTCCTTATTGAAACTCTTTTTGAACAATCGTGGGGTTAATGATTTTATTGGTCTCCC
M1_Spy_0374	GGAGTACACTACCCAAGTGATATTTTAGCAGGCTTTGTATTAGGATTTGGTATTTTGC
M1_Spy_0376	CATTCTTATGATTTGTATAAAGAAACGGTGAGGCGACTAAGGCATTACCCCAACAT
M1_Spy_0377	AACGTTATTAATGGTAGGTGGGCGTATTGCTATTATGGTCTATTATGGGCATGATG
M1_Spy_0378	TCCCAAGGACCGATGCTTTCATGATTGACATTAACGATGATCCGCTTGAAAATATT
M1_Spy_0379	TCCGGATACTTGGGAGATGGAAACCAACAATTCAAAAATCAGAACAGTCAATGATG
M1_Spy_0380	TAACTCAAACTCTGAAATTCTAGCTATTGGAGCAAACATGGACAAGGTTGAAGCAG

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_0382	GCAAAAACGTCCTGATATATTTGTGGAAGCTCTGGTTACACTTGGTTACCTAAAAC
M1_Spy_0383	ATATCCAGACAGGGATTTTAGTGTCGCTTATCGGAGCCCCTTATTTTCTTTACTTAAT
M1_Spy_0384	TGAAACATTTTACACCTCATCATTACCGATACCTTTTGCCGCTTTGTGCAGTTTCT
M1_Spy_0385	TCAAGGCCGTTACAGGTGACAAAGCAACCTTTACTATTATGGGGCTTTATGAAAAA
M1_Spy_0386	GATGTTATGACTTCACCTATCATTCAAGACATCTTCCAAATCAAACCGGTACTGG
M1_Spy_0388	ATATTTCAAAAGAAATTGCCAGCCATATCGCACGCCCAGTAGAGATTATTAGTGAC
M1_Spy_0390	AGTTTTACAGATACCGCTTATTTATTTGCTACATGCTTATGGTCCTTTACTAGCGACGA
M1_Spy_0392	CCGTAATGAAGAAACCCTTGAACCTGTTGAATACCTCGTTAAACTACCAGAAGATA
M1_Spy_0393	CAACCATCAAATAGTAGCCTTTTTAGGCCAATCGTTCTTGATTTTGTTTA
M1_Spy_0395	GCGCAAAATGCTGGAAAAACGATTAAACAAATCCATAAAGATGCTGAGCGTGATT
M1_Spy_0397	CGTATTGGACTCGTCGTTTACCTGTATTATAACCGAGATGCCAGAAAGTTATCAAA
M1_Spy_0399	TCGTTTAGATTTGGAAACAATAGAGATACATCAACGAGTACGAAAAGGATACCTAGCC
M1_Spy_0400	ATAACTCAAGCAGAGCGTTTTGTATCTATTTGGCTGAAAGATCAGTTGCAGGCATA
M1_Spy_0401	GCATGTTGTCCTAATTCCCTTTTCAGCAGAAGAGCACATGGATTTTTACAACTGT
M1_Spy_0405	TTGAGTCACCTAGAACATGAAACGGTAGCTAAAAATCCTTCCAAACAAAGGAAGG
M1_Spy_0406	ATTCAAGCAGGAGCCAAGCCAAATCAGGCTATTAAAACAATTGCTAAAGCTTACCA
M1_Spy_0407	CAGTATTTGGCTTGTTTTTCCCAGGCATTGCTTCTTTGATAGTGGCTTTTGGAAAA
M1_Spy_0408	AAGCTACTCCTATTACAGACAATGTGGAACAGCTTAAAAGTCTTGTGACCTATGC
M1_Spy_0410	TAACAGCTACCGCGAATTGGGACTTAAAGATAAAATAGACCAGCTTAGCCTTGATAAA
M1_Spy_0412	ACTATTGGCTAGCTTCAAACCGACTTGTGGATAAGGTTAAGCGTTCTGAAATGATT
M1_Spy_0414	AGTTAAAAATGGTTATGCAACTGGCTGGGACACAAACTATCCAAGATGTTAAAGCCTT
M1_Spy_0416	TATGACACCAATGCAGCCAAACTAGAGTCAGATAAAATCGTTTCCTTTACCTTGTCA
M1_Spy_0421	GCTTTGATGCCTATTTTAATGCCTTTACTCTATGTCAATTACCTGTTGGCTCAGGAA
M1_Spy_0422	TCAAGGAAGTGTCAAAAGTGGAAGGTTCAGAAAAATTGCTGCGCTTTAGAGTAGAT
M1_Spy_0425	TAACGCTGGAAAATTCCTTCAAAATTTAGGCTATGAGTCGCCCTTCACAGATGAA
M1_Spy_0426	TTAAAGCATCACACACACGACAAGCAAAAACAAACCAACAACCTGATTACAGAAAGGA
M1_Spy_0427	CGCTTTTGCAGGATTTTCGTTATCAACCTATTCAGATGGCACTTATTTTGCCAAGT
M1_Spy_0428	TTGTAGTATACCGCTATGTATACGAGACGTTTTTGCGTGATATCGGTGTTTCACAT

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_0430	AAAGGATATTTAGAGGGATATGAAAAAGGCTTAAAAGGAGATGATATACCCGAACGGC
M1_Spy_0431	CAGTGGGACTGCTCAACACTAATAAAGAATTTTATCAGCCAAGAATTCACCAACC
M1_Spy_0432	TTATAGTTATCTCTACTTACCGCTTGTAGCAAGTAGTCTAGCTCTTATCGGTCTG
M1_Spy_0433	ATCCCTCAAGGATGGTGGGTATATCTTTACAGACAATGTGATCATAACAGTAAAG
M1_Spy_0435	TATTATTAAGTACTGCTGTTTTGACGCTAGCCGGTACTGTCGCCCTATTAA
M1_Spy_0436	GGTGGGGTTACACCATCAGTAAACAGTAATTCGGAAAATAGTAAAATTGTAGGTA
M1_Spy_0437	GGTACCCAACATACTATTAAATTATCATCCCAACTTACAAAAGGTGAGAGAGTCAC
M1_Spy_0439	AAAATTATGTGCAGTTGTTTGATACCCTTGAAGTGGATGGGAAAGTACTACAGGC
M1_Spy_0440	CATTATCTTAGACGTATGGTGGAAAAGCAGTCAGGTGTTATCATCAATATGTGCTCAA
M1_Spy_0441	ATGACAGCAGGAGAATTAGATGAACTGGTGGTAATCGCTAGAAAACGAAAACTCATTT
M1_Spy_0442	TCACAAGTAGCCAATATGTCAGAGGCTCATATCCATTTTGCGATTTCAATGTTAGAGT
M1_Spy_0443	AATATGAAACAGTCATTGGAGATCACGCTTTTATTGGGAGCAACTCGACTCTCATT
M1_Spy_0444	GTGAGCTAGAAGAAGAAACAGCCTATACGGGGACATTGACGTTTTTGTATGAATTTTA
M1_Spy_0446	ATCGAAAAAGCGAGACGAATGGAAACATTTGAGCCAGATGATGCTGTAAACTTTGA
M1_Spy_0447	TTTGTGGCTATCTTCAAACAAGTCCTAAAACACGAAAAGACAAATGGCCAAGTTG
M1_Spy_0448	CAGCATTTGACTGATGAAAAATCACACCTGCAATATAGTAAAGACAACGCACAACTTCA
M1_Spy_0450	AATCCAGTATCTAGAAGCACATCACCTCAATATCAATACCGAGCTAACCTTAACT
M1_Spy_0453	CCATCTGCTTATATCTGGGAAATTAACACCGAAGAAGAAGGAACACCAGATCAAAT
M1_Spy_0454	AAGAATCAGACTATATTTTCTTAGATGAACCGTTTGTTGGCATTGACTCGGTCAGC
M1_Spy_0456	GCTGTGCAAGATAGCGATAAGTGGATTACTATTGGTGTTTCGATTTTTGTTTTAGTGGT
M1_Spy_0457	AAATCAAAGCAAGGGATTATCAAGTACCAACTACCCAAAACCTATCATCTCTGCCTAT
M1_Spy_0458	AATTGGCTGACTTGATGATGGTGGCTAGTAAAGAAGTTGAAGATGCTATTATTCGTTTG
M1_Spy_0459	GCTATTTTGGCGAGTTGGCTTTTTAGTCACCGTTATTTTCTACCTGATTCTAGTTATC
M1_Spy_0460	GCACAAGTACAAGAAATTGCTGAAACTAAGATGCCAGATTTGAACGCTGCAAACAT
M1_Spy_0461	TAAAGCTAAACCTGCAACAGCTAAAGGAACTTACATGGCAAACGTCTCAATCACAT
M1_Spy_0462	GGTGGTTTTCAATATGAATGAAGCTGGCAATATTCAACGTGTTGTCTTTGGAGAAC
M1_Spy_0463	CCGCGATGCTATGGATGATGCTAAAAAGCAAGAAAAAGCAAAAGAAATCACTGAAGA
M1_Spy_0464	GGTGGATTGATGAAAGCTAAACGTATCAAACAAGACGCCACAGGAACTGAATTAA

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_0466	ACTATTGAACCAGCAGAGCCATTTTATCTCGCAGAGGATTATCACCAAGGATTTTATA
M1_Spy_0467	ATGACACAACGTAACCCTAAATCAAATGAACCTGTGGCGATTTTAGCAGATTTGGT
M1_Spy_0469	ATTGACCTAATTTTTCCAGACACGATCCTAACAGCAAACTACAATCAACACGGTCA
M1_Spy_0470	TACAGTTCCTGTTTACATGCCTTATATTACGAGTTACTTTATGCCACGCGTCAAAG
M1_Spy_0471	CCCTCGAAATGTCAAGTCTGGTTTGATTGATGCTACTCAGAAATTACAAGGCATTA
M1_Spy_0472	TTGAGGATTACTTGCCAGACTATTTTCCACTTGTTCATCCTTCTCCAAGAAACCAATT
M1_Spy_0473	GGCACTCTTTTGAGCGTGAAATGGGATTTTTGGCTGTTCATGGATTCTTACATAT
M1_Spy_0475	GCAGGTGCTGTTTTGATGATTTCTGGCTATGCTGTCTTGACAGGACTTATTATTT
M1_Spy_0476	GGTCAAAGTCAAGAAAAACTGGCGGGATAAAAAGCTTGATTTAGCTGATTTTGGC
M1_Spy_0477	GAATGTTTACACTCATTTTGAGGAAGTTTATCCCAACGGTGATGCTGTTCAGACTA
M1_Spy_0478	CTAAGCAAGTATATGACCTTCTAAACGAAATACGTATGCGTTATCCTCATGCTCA
M1_Spy_0479	GGTGCTATTGCGGGTGGATTAACTTGCGTTGGTGGTATGTTATTTAATGGAAAAT
M1_Spy_0480	AAATATTTCTGGTGGAAAAGGAAATATTGGATCTGCTATCGGAGGGTGCTTAGGT
M1_Spy_0484	TATTGACAGGTGGATGTGTTGTAGGATTTACAGTTGGTGGAGCATATTTGGGATATA
M1_Spy_0486	TATCATAAATGGAGGCGGAAAAGACGGAGCAAATATCTTTTTGACAGGGATG
M1_Spy_0488	TAGATGAAAAATCAACAGGGCGGTTTGAGCCTTTTAGACCTAATGAAGATGAGAAG
M1_Spy_0489	AAGATGATGCTGCTATCCTTAATCATTTGGAGGAAGACCGTGATGTCAATAAGTTGTT
M1_Spy_0492	TTTATTCTTGCCGTCAGCCCTAAACCACCAATTCTTAAACACACTATTGAAAGGAAAAG
M1_Spy_0496	TGTGCATGGGTATTATAAATTAAAGCAGGAAGAAATGTCTGGAGAAGAGGACATGC
M1_Spy_0497	TAAGTTAAAAACCTTTGAGGCTGCTCTGCTATCCTCCCAAAAACCCATAAAACCA
M1_Spy_0498	CCCTATGCCAGTCTAGTGATTGATAATAGTGGGGATATTGCAGCCTTAATAAA
M1_Spy_0500	ATACTTTCCGACAAAATCCTGACTTAGATGTGGCAAGGGTGATTACAGAACTAGAA
M1_Spy_0501	TAGCTTTATCATGTATTTTAGCAGTGCCTTAGCGCAGACCAGTTTTCAATTAGGAGTA
M1_Spy_0502	GTACAACTTACTACTTACGATATTGCTAGTACTGTCGGGATTGCTTGAGATTGCT
M1_Spy_0503	GTAGAATTGCCAAATACGATTGAAGGTTTGGTTCATATCACTAGCTTACCGGAATACT
M1_Spy_0504	ATGGCTTTGCTAAAGTTCTGATTGGTCTAGCTAAAGGGAAACACGAGTATGACAAA
M1_Spy_0505	CAAAAGCGAGACCCCAAAGATTTTGCTTTATTGGATACCGTACTAGTTGCTATAGA
M1_Spy_0506	ACCTCGATGATATTACAAGAGGAATTGAGGCTGCTATTTTTGCCATTGTCGATTTCAA

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_0507	CCAGTTATGTTAATGGCTATGATTTCTCTACTTCTTGCCTTGATCCTTCCTGATTTCA
M1_Spy_0508	TTTGCTAACCATTTTCTTCATCACTTTACCGCTTATCGGCTTGTCAGAAACTTATGG
M1_Spy_0510	CTGTCTCTATTATTTTATTCATGGGAGGCATTCAGCTCTTTTGCATGGGTATTATTGGA
M1_Spy_0511	TAACCTACAATTATGGTCATGGCGACTACGACTTAGGAAATGGCTATGGTCATATT
M1_Spy_0512	TATCAAAGAAGGTGATGGTCTCAACAGCCGCCTAAAACTCTATGAATCTTTCCAAAAA
M1_Spy_0513	GCCATGTCACTAAAAATGGCTTTGAAGTCTTTACCCATACTCCAAAAGAGTTGCTT
M1_Spy_0514	AATTTGAGTTCTATCAGTCAACCTGTTTATGACTTAGGTGCTGTTAGCATGCGGAT
M1_Spy_0515	AAATCTTATTTTCCCAGGATACATTAAAGGGGATGTTTATGAAGGTGCCATGACTGGT
M1_Spy_0516	GCTCATGGCAATCCTTATTTAGATGATGTGGTGACTGATAAAATGTTTGGCACTCT
M1_Spy_0517	ATTAATTGTTGGGGACAAAGAAATGGAAGACAAATCAGTCAATGTTCGCCGCTATG
M1_Spy_0518	CAGGGATGTGAAGATGACTGATGTAGATATTGAGGATATTGTTCGTCGTTTCTA
M1_Spy_0519	AGTGCTTAGTTTTCTACCGTTTTCCTCTTTGGTTTACACGCCAGTTATGATTGTCA
M1_Spy_0521	GTTCAATGACTTTTCTAAGTACCCTATGTCCATTTACCATTCCTTTTTGCGGTGGTTA
M1_Spy_0524	AACGAAGCCTTTGCCGTTATTGATGGTCTATTTGAAACCCATTATCCAGATTTATTGG
M1_Spy_0526	TATAGTGTAGAAGGAATGTCACAACCATACAGTGTTTCAGACTTAGGAAAAATGAGCC
M1_Spy_0527	TTTAACTATGGCACAGATGGTCCTAGACATTGGGATTTAGATGTTGATCCGATTAG
M1_Spy_0528	ATGACACGAGAACACTTATTGGAAATTGTTTGGGGATATGATTATTTTGGCGATGTGC
M1_Spy_0529	AACAGATTTGCCTCTTATTTTTGATCGGTTCTATCGTGTAGACAAGGCAAGAAGTC
M1_Spy_0530	CTTTACGGTCCATGATACCTCTCCAGATACTGCTTGTCCATTAACTGATATTTGA
M1_Spy_0531	CGCCGTCGTGATACTATTTTAGGAGACTTATTTGAAGCCTTTTTAGGTGCTCTTTTAT
M1_Spy_0532	TTCCGTTTGTGATTTTAGATGAGGTAGAAGCGGCTCTTGATGAAGCAAATGTTAAG
M1_Spy_0533	CAAAACTTGGTGCCACACTTAGAAAAGTTCGAAAAGGAAAGCAAATTAGTCTGTGC
M1_Spy_0534	GTATACAGGGAGCCAATTAGTGAGAATACAAGAAGTTTGATAAAGAGTTTCCCTG
M1_Spy_0535	GTAAGAATATTTGGTGGGATTGGGTGCTCAGTAGATGACTTCTTGAATAATGAAC
M1_Spy_0536	AAAATTCCAAGAAGAGACTTTGTGTGATATGATTGCAGCTTGTGCTGGCTTAGTAATG
M1_Spy_0537	GCAAGATTGAAGGAGGTGATGCTAAAACAGTAAGAAGAGTTACCATTGATGACAAAAC
M1_Spy_0538	GCTTTTATGACCCAAGTCATTTATCATACGAAGTAGTAGAACGCAAAGGCTTAGGT
M1_Spy_0539	ATGAGCTATTGGGAAGGCATATTTCTGGACAGGTTGCATGTTTTCAATCAGATAATG

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_0540	AAAACAGTATCTACATGGGCTGTCAGAGGTAGAATGGGACGGAATTTTGTCTAATA
M1_Spy_0542	ATAGGAAATTCGCACAATAATCCTTCTTTCGGTTTTGGTGGGTACTGTCTTCCTAA
M1_Spy_0543	TGCCTATTGGGACTGGCTTTTTCTCGGTGGCATTAAATCCTAACAATAGCTTTAA
M1_Spy_0544	GGATTAGTTCTTCCACGATCGCAAAATTGGGAAAAGGGGAAAATATCACTACTGA
M1_Spy_0545	TCTATCCAAAGAAAAATCAGAAACGGACGCTGACTTGAAACGCAGTACAGAAGATAT
M1_Spy_0546	GGATTGCAGGAAAAAGTAATCAAAATACCGATTTCCCCTTACAAGTTTATTCGAGCCA
M1_Spy_0547	GCACCCTTGCTTATTTGCCTAGAGTGCTAAATGAAATCAAAAGTAGAAACTCAAACG
M1_Spy_0549	GCCAACAAGCGATTTCTGATATGAAGTTAGACAGTGGAATTGTTTCAGTTTTGACTAG
M1_Spy_0550	GAGTTGATGATTCAGTAAAAAGTGCGGCAGATGATATTTTGAAACGCTTAGGTATTCC
M1_Spy_0552	TGTTCTGGAAGATGAAGTTATAATTGTTGCGGTCGTCTATGGATCACGTCATATG
M1_Spy_0553	GAGATTGCGACTTCAGTATTATCACGACATCTTGACGCATTTAAGGAATTGACAGA
M1_Spy_0555	GGGACTACTTGATTTAATTGGAAGAAAAAGGGCTAGAGATAAGCCCCAAAACAGTTAT
M1_Spy_0556	ATAAAAGTAGACAAGCACATCAGCCTAACCTTCAAAAATGGCGTACGGATTGATTG
M1_Spy_0558	AACAGTAGAACAAATAACAGCCATTATTGATGGGAAAAGAGTGTTAGGGAACCCC
M1_Spy_0559	AGATTTGTTGTTAGGAATAGGCGAAACTGTAGGTTGTGGAGAAAGATGCGAAACATAT
M1_Spy_0560	GGAAGAGTTTATTGATGGAGAAGAATATAGCTTAGAGAGCGTGGTAAGAAATGGAATCT
M1_Spy_0565	CAACCAGAAATTTAATCCTGACAAACCTAATCAAGTATGGTCTACTGACTTCACC
M1_Spy_0567	TTATCTTAAAAGAGGTGAATAAGGGCTTTGGTATGGAGCATCTTTGTCAAGCCTT
M1_Spy_0568	GATGAGTCTGGAGTTGCAGAAGCTGTGAAGACATTTATTTTAGAAGAGGAGAGTTA
M1_Spy_0569	CCGTCAAGAATTAGACATTCCAGTCAAGTTTATCGGATTTGGTGAAAAAGTGGATGAT
M1_Spy_0570	CAAGCGGTGTCCAGTCTGTCTACCTTCTCTTTACTATTTTTGCTCTACTTGATTT
M1_Spy_0571	GCGGTACAACTACCACCTTAATTCAAGCGAACTTTTGTATTTGACGGTTCATGTT
M1_Spy_0572	ATATTGATTTCATTACTTCAAAAGGCTATTCACTCATCAGTCCTGTGGTCGTGACC
M1_Spy_0574	TTGAAGACGGAGACAAGGAATTATTAGCGGAACATACGGTTGATTTTCTATCCTTTAG
M1_Spy_0575	GATGGAATTGATCTATCTCTACCCAGATGAGTTTCTTGATCGTGACATCCAATACA
M1_Spy_0576	TTGTCCAATTTATCGGCGTATCCGTTCTTATGATTGCCGTTTATTGTTTACTTGTGG
M1_Spy_0577	TATGGCAATTACCCATAAAAAAAATGATGAACTTGAAAAAATGCTAGCAG
M1_Spy_0578	TTTTTTTATGGGTAATTGCCATATCTATGACCTTCTTTCATTCAAGATAA

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_0580	GATGTGAGTCAGAAAAAGTTGAGTGAGAATCTTGGTTTTGTTGTGGATACTGTGGTTA
M1_Spy_0581	TCAAGTAAACGGGCTTCGTTATGTCCCAACTAGCTCTAAATCAAAGACTAATCATCAT
M1_Spy_0583	CGGCTCTTCTTATTTACGGAACTGGTTTTGGAGTTTTGCTTTATATTTTGTTGGCC
M1_Spy_0584	TTCTGGAATTGGTAAAAGTGAGACTGGTTTAGAATTAGTTAAGCGAGGGCATCGTT
M1_Spy_0585	CGCATTCCGACCTTTCTCTATGAATCACTTTGGAATCTTTTGGGCTTTGTCATTATTA
M1_Spy_0587	GCTAATATCCTTGTTGAAGATGTTAATGGTAAAGTAGCAACCATCGATCCACTGT
M1_Spy_0588	CAAAAGAAACCCTTGCCGAAGTTGAAGCGAAAGAAAAGCAAGATGATGTTATCA
M1_Spy_0589	TAGTCTTCTTTGCTCGTATCGCTAGTTTCAGCCTTATTACCGTTTTATTTGCATTTGTC
M1_Spy_0590	GATAGAGATTGCTGTTGATTATCTTGTGGTTGGTGATGCAGGTGTGTTTTATGTCAAT
M1_Spy_0591	ATGTTATGGACCAGGTTTCCGTCATTTTGAAACGGTTGTTAAGGACTTACATGATG
M1_Spy_0593	TATCAGAAAATGATAAAGGTTGGACGAAAGAATTGAACCGCGTCAGCTTTAATGGC
M1_Spy_0595	ACGCCAATGCTTTCACAGAGTTGAATGATCCCATTGACCAATTATCTCGTTTTGAA
M1_Spy_0596	CAATTTTACAGATGGGAATCAATAATGGCTTGATGATGGCAGCAGAGCGTTTACTT
M1_Spy_0598	ACGTTCTTATTGTAGGACACGGCGCTAACTTAACAGCAACTATTCGCTCATTATTA
M1_Spy_0599	GCTATATTCACGGTGCCAATAATCCTGTTGGGATTCGTCAAAAACATTCATATCCT
M1_Spy_0600	ACCCTTTCATTTATTCCAGATTTTGCTGGCTGGTTTAGTCCTTCTTATGGTGCTATT
M1_Spy_0601	AAAAGGTTTCGATTCTGTATGGATTCCGACTTATGGTAGTGATTCTGGCTATTATGAG
M1_Spy_0603	CTTATGTGGCCTCCTTTATGGGGTGTTGATAGATACTTGTTTTGCGTATTTGTATAGT
M1_Spy_0604	AAATACTGGCTTTTTAAGGTGAATGGAAAGATGGCAGATAAGGGTGCCGATCAAATTA
M1_Spy_0605	CATCTCACTAGTTGACTACAAAGAAAAAGTGGTTTTGGTCGTGAATACAGCTACC
M1_Spy_0606	TTGCTCACCGTTTAACAGATACTTACTTTCATAACTTCATCACTCACCTATTGGAAGC
M1_Spy_0608	TTATTTCTTTGAGGCAAGTCCGATTAAGGAAGTTTCCAGTCTCAACATTGGGTCAA
M1_Spy_0609	GTCTCAAGGTGGAAATAGCTTACTTGTCTTATCTGTTGCAGTGGGTTTTGTACTAA
M1_Spy_0611	GGTCAAGTTATTGCTAAACCAAGTTCAATCAACCCACACACTAAATTCAAAGGTGAAG
M1_Spy_0613	GCTGATAAAGTTCGCGTTCAATACGGTGGTTCTGTAAAACCTGAAAACGTTAAAG
M1_Spy_0615	GATGGTTCAGACGGTGTCTTGCGCTTTAAACAGAATTTCAATGGCTTTATTTTGCA
M1_Spy_0616	AACCTTGATTTATGGCGAGACTTCTGAAAATCTTTATGCGGGAATGGACGATGATTAT
M1_Spy_0617	CATTTCTTTGATAAGTACACCATCGTCAAGTCAACCCCTTTTTATTTAGAGTTCATGCC

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_0619	TCACGTCACACGCAGAAATAAGAAATCAGTTGTCAAATCAGCAGAGCAGTTCAT
M1_Spy_0621	ATCCAAGAACTCAGATTGAAATGATGCAAAAAGACGGGAGCCTTGCTGAATTATCT
M1_Spy_0622	GCTATCAGTACCTCATCTATCAAAACACTGACAAGGAAAAGGTTGTCATCAAGTAC
M1_Spy_0623	TAGTGGTTATTATCGGAAGTCTCTTGATGGTTGTTCTTGTTGAATTGGTGAAAGCTGTT
M1_Spy_0627	GAAGAAGGCGAACAGGCTACTAAGATTTTAATCGATGATATTGAAGGTCACAGTCA
M1_Spy_0628	CTGGCATTTAAAAGGAAAGCAGTTTGACTACAGTATCTGTGTTCTCCAAGAGGATT
M1_Spy_0629	ATGTTAGAAAATAATCGCCCTGAAGAAGAAACCCGAAACATCAAAATGGCACTTATGG
M1_Spy_0630	TCCTTTTGTACTAATTGGTTATGTTTGTGCAGCCTACCTCCAAATTCCAACCATTG
M1_Spy_0631	CGCTAACGATGCGGTTTCTGAAGATAAGATTCAACAATCCCTCATGAAAACAGTTA
M1_Spy_0632	TGGTATTCCTTTGACCTATCGTTTCCTAAAAGAACCTGAACTCATTCAACTCTTCAAG
M1_Spy_0634	ATTATCGTTATGGGACATGGACATTTTGCGAGTGGTATTGTTTCAGCCTTAGAATTGAT
M1_Spy_0636	CGGCCATATTCTTTATGTCGACGGTGGCATTTTAGCTTATATTGGAAAACAACCTT
M1_Spy_0637	TTGAATTTGACCTTAATATTTGAACGCCTCTTTGCAGAACCAATGGGTGGTGGTTA
M1_Spy_0638	TACTTTATCAGTTGTTAGAAGGTAACGAAAAGCAGCGCAACCTTGATTTTGCTGTG
M1_Spy_0639	GAGTTTAATCAGTTAGCGAGCCAAAAACAATATAACCTAATCACTAAGAAAGCCGCTC
M1_Spy_0640	GTCAAGCTGATCACAAGATAGATCATTTAGGACAACTGTGTGTAAAAATCGGTTG
M1_Spy_0642	TGTCCAACTTCTTGTTTAATTGGTGATGGCTCAATGAATGCCAAGCGTTGTCTAT
M1_Spy_0643	AAATCACTTGGGGAAGTCAGATTCGTTCTTATGTTTTCACCCCTTATACGATGGT
M1_Spy_0644	ATCGTGTTGTTGCTATTGAAGATGGTCGCATAGTACGTGATGAAGAAAAAGGAGATT
M1_Spy_0645	GATGTATCCGCTTGATCCTTATGTATATTACCTGATTGGTGCTTTGTTTGTCATAGGT
M1_Spy_0646	TTTTCCACTCAGATGAATTAGTCGTTACTGGCGATGCTCTATTTCGTGAAACTATC
M1_Spy_0649	GTCCTATGGACAAGTTATTTTGGAAGGTCTCGGTCAGGAATTTTTGATTTCTCAAC
M1_Spy_0650	TATTTACGGGTCCTTAGTCTATAACGGCAATCAGTTTGTTCCTATTTCAACACTCTCA
M1_Spy_0651	CAACTACGATGCTCTTGTGGCTAAAATGGATGAACTAGGTATGGATAAATCAGAATATG
M1_Spy_0652	TTAATGAAAGCCATCGTGATCAAAATCGTCGTAAGGAAACGGTGAATCGTTCATGA
M1_Spy_0653	AAATCAGCACTTAGGAAGAGATTTGATTGATACGGTTTTGGTAAATGTGGCTAAGGTG
M1_Spy_0654	AAGAGCGGGGTCAACCATCGCTTGCGTAAAATCAATAAAATTGCAGATGACTTATA
M1_Spy_0655	TTTAAAAGTATGCACAGGAAGAGCTAAGAACGATGGTACGAGAAGGCAAACTTAC

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_0656	TAATAGGTGGAACAGTTATGGGATTTTTAGGAGAAAAAGGGAAGAAGCAATGGCACT
M1_Spy_0657	AAGAGGCGATAGCATGTTACCAAAGTACGAGCAAGGAGATATGCTTTATATCGTT
M1_Spy_0658	ACACGAACACCATATGCAAAAAGAGAGAACGGTATTGTTTCAATTGGAGCTGATGA
M1_Spy_0659	CACAATGTGAGCCAGAAAATATAACAGTGCCAATTTCTTTTGCAGGTTACTATCA
M1_Spy_0660	ACTAAGCGATTGTCATCTGAAAAGCAGTATGATGCATTCCACGCTTATATGA
M1_Spy_0661	GACAACGAATTTTTGCGAGACGAAAATCGCAGGTTAAATAATGAATTGGCGGAACA
M1_Spy_0663	CTCAGTGGAAAACAAAGCTCATAACTTTAAAACAGCAGAGGGTGGTAATTTTGCTG
M1_Spy_0664	CCAATGACACCAATTGAAAACTTCCCGTCAGATTATTGGAATATGATTGTGGCCAA
M1_Spy_0665	CCATCGGAGAAGGTGGCTTACAAGAAAAAGTAGACAAGGAATTGGAAAAAGTCTT
M1_Spy_0666	ATGGCTTTGTTTGAAGCAGATGGTGGAAAGTGGCGCTTAGATGCTATCAATAATAT
M1_Spy_0667	TTAAATAAAGATGTCGCACAACCACAGCAAATGACACATCAACAATCTGCTCAACCTA
M1_Spy_0669	AGAGAAACTGGCCTAACAACACCTAAACAAATCAGAATGCTAGAACGATACGGATT
M1_Spy_0670	AGGTTGGAGTGTAGAAAGAATAGTAGAAGAAGCGAGAATGAAAAATGACAGATGA
M1_Spy_0671	ATGGAACGCTACGTGAGTTTGCGAAGTTTCAGCCAGTTAATATGTGGTTTAGTTAT
M1_Spy_0672	CTTGCCCAATTGAACTTTTACAAACGCAATGGCATATTATGGGAGCGTATTTGAA
M1_Spy_0673	TACGAGCCTGAGAGTTTGAAAAATGCTAGGGATAAGTTTACAAGTCTTTTGGCTCA
M1_Spy_0674	TGGGCAAATCAATCTATTGTTGCAATCTGCGAAAAGTACGGAAATGACAGTCTGAT
M1_Spy_0676	TTGAGCGAGAATTTAATGTCAAAGAGTGGATTGGTGATGGGATAGAACACATTGA
M1_Spy_0677	TCAGTATGATACTAGAGCCAGAGAGACGCAGGATTTTGTATGACAAATACTTAGC
M1_Spy_0679	GTTAATTGCTTGTGAGTCAAATGGTCGTCACGCTAGATTAATGGAATATGACCCAA
M1_Spy_0680	TTACAACTACCGAGGTCAAAGATAGCCTGGATAGAAAAGAACAAAGACAGCGAATTAA
M1_Spy_0681	ACAATTCTAAAGATGTATCTAGCAAAGCAAAAGGTATTGAGGTCGGTATCGAACGC
M1_Spy_0682	TTTTACAGATCGAGACGCTGAGTTAGACTACTGGATAAAAGTTGTTAATGCTGGCTTT
M1_Spy_0683	GTCTCGACAGTAATAAACCCGATAGACGGAAATATTGTTACAGTTCATGCTACTAG
M1_Spy_0684	CGATTTTGTCAAAAATGAACTTCCGGGATTACCTGTGGATATAGATGTTAATTCCG
M1_Spy_0685	AAAAACGTAAACTAGAAACAGCAGTTGTGATGCTTGTTGCAGAAAATGCC
M1_Spy_0686	AAGCGAGTCAGAAAAACAAACAGAAATCTTGAATGCTAAAGACAAAGAGCTTGAGGAAG
M1_Spy_0688	GTATCATGCTTAAACGTAACACAATGGTTGAAACAGACCGAGATATCACAAAAGCG

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_0689	AGAATGAGTTACTAGCTGTTGGGTTGGGATATACGGGTATTAGCTATGATCGATA
M1_Spy_0690	CGAACTGCAGGTAAAACTTGTCAAAGATAAAGGTGATTATGGAGGGTTTGTCTATG
M1_Spy_0691	AATTTAAAAAGTACACAACACCAGGTACAGGCAAACGTTGGGATAAACGTGCGTTA
M1_Spy_0693	ACTATCTAACAAGACAAGAAGATTTAGCCATTTATCCAATGCCAGGTGGGAAGGTAA
M1_Spy_0694	TAAGTATAAGCTAGGAGATGAGCGAAAAGTTTGGCATTTGATTGTATCGGCTGATG
M1_Spy_0695	AACTTAGACAACGAAATTGAAGATAAAGGGGTTACGAAAGATGTTGCCCAAAGTGCTT
M1_Spy_0696	ATAACATGCGTAAGCTAAAGGCTAAGTACAGTTTAGATGAAAGAGAGGAGGAGGA
M1_Spy_0697	TCAAATAACAGTTTTACCCTTAATGTCAAGGTTGATGAATCCGACGGTAATAGCCG
M1_Spy_0698	TTGGACAAAATAAAATCTCGTGGACTGGTAGCTTTACAATTACCGCAGTGCCAAA
M1_Spy_0700	TTGGATTTGGGATGGCAATCAATGGGTCAAGGTACTAGATACAGAGGATTTAAAC
M1_Spy_0701	ATTAGCGACAAAAGTCGAGACCGCTCAGAAACTACAACAAAAAGCAGATAAAGAGA
M1_Spy_0702	AATTGAGCAGCGGGTTACTAGAGATGGTGTCATGAGTATTATTAGTGGCGCTGGA
M1_Spy_0703	CTACTCGCAAAAGTAGTCGATAAAATCAACGAGTATGCAAAAGATGAGACCGACT
M1_Spy_0705	GAGAGGCTGGTGAAGGTAACCTAGTCTTCGTACACGTTAACGAAGCATTT
M1_Spy_0706	ATGACAGAGCAAATTAAAAATTTAACAGATGATGTTAAAGATTTAAAAGATATGA
M1_Spy_0707	AAAGGCCTGTCAGATAGTGAGCAAGCATTGACTTACCATGAGCCCAAAAAATA
M1_Spy_0710	GGGATAGAGAAGCTCTTAAAAATAATAAGGAGGAAACGATGACAACCGCAAACGAAAT
M1_Spy_0711	TAAGCGGCAGAATCGAAATTGGCACAAAAGATGGGAAACATGAGCAAATAGACTTATT
M1_Spy_0712	CTACCTTTTCACATATCCAACTGCAAGATAGACACGAAACTAAAGATGTACGCACTAA
M1_Spy_0713	TCATGAAACTGATTTGGACAATTACTTGGAAGAAACCATGGCAAAAGGTCATGCCA
M1_Spy_0714	ATTTCCACCTGTACTGGGGTGGTGACAGCCAAGAAAAATTACATAAAGAGTTAGAA
M1_Spy_0715	GCTTGATCCCGATACAGAAGTTTTTGAATTAGAACGCCTAAGGATTGCAGATGATA
M1_Spy_0716	TAAAATCTTGGCTCTATTGCAAGGAGGGGATATCAACTTTTCAGAGGACCAATTTC
M1_Spy_0717	AAATTTCATCAGATTCACACCCATTCTACACAGGACGTCAAAAGTTCACTCAAGCA
M1_Spy_0720	ATAAAGAGGCTTTGGTCATCATTACAGATACAGCGAATCGACCAAGGATTGATGAT
M1_Spy_0721	TAGAAGGTAAAATCTATGGCGTTGTCGGCTCTGGTGACACTTTTTATGACTATTTTTG
M1_Spy_0722	TTATGTCTCTTTCACGGCAGTATCAAACGCAACACTTAACAGGTGGTGACATTAAT
M1_Spy_0723	GCTACCAAATCCTTATTTCCGGATTGCTTTTATCGGTGCTTTGTTATCTATCTGTC

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_0724	GGTGTTGTTATTTCACGTAAAGGTCAAGGGATCTCAGAAATGTACACTGTTCGTAA
M1_Spy_0726	AGTAATTGGAGATCGGCTAATTGATAAACAAGCAGGACAAGCAGCGGGTTTTAATA
M1_Spy_0727	GTGTTGAACCAAGACGTGATTTCATTGAGGAAAATGCGGTTTATAGTACACTGGA
M1_Spy_0728	GACGCTTACTAATGTCGAGTCAGGTCAAATGGATGTTTTTGCAGCAGTTAAAGATATT
M1_Spy_0729	CTATCAACCTTTACACTTAAAAATCTCTTCCAAGCTGGCACCTCAACTTCAAATACTC
M1_Spy_0731	GCCAAATCAAAACAGGTTCATTGTCACGTACAGACCGTATTGCTAAATACAACCAA
M1_Spy_0732	TCTCATTAGCCAAGCAGTCTTATTTTACTGTTACGAAAACCTCTCCGATGATAGAAG
M1_Spy_0733	CGTGACTTGAGTCGCTTCTATCAAAACTTGACTGAGGATAATATTGTTCGAGAGTT
M1_Spy_0737	GGTGATGCAAACTCAATTGTTCTTGTTGGCTTAGGAGTTATGTCTCTTCTTTTAGG
M1_Spy_0738	TACTGGAAGTGGTAATTCTCAAGGTGGTAGCGGAAGTTATACGCCAGGTAAATAA
M1_Spy_0739	GAATCAGGCGACTGCCTATGCTTTTATTGAATCAGGAGAAATAGCCCAGAATATT
M1_Spy_0740	GGGACGTTTATTAAGCATTTACTTACCGACGCTTGAGATTCAGGTCCAAGATATTT
M1_Spy_0741	TATACCAGCATTTCCTTTTGCTAATCATCCACGAATGAGACAGTTTGGAGGTGTAA
M1_Spy_0742	TTGCGTCTTGATAGTACTGATTGAACTCGTTTTCTTACATGGTTTACGTTGTTGG
M1_Spy_0743	GGCTAGTAGGTGCGATGATATTAACGGTGACTTATTTGATGATTAGTTGCAAAGAG
M1_Spy_0744	GTTGTTCATCAGCTCACACAGGCCAATATTACTTTTAGCGAGATTAGACATAACCA
M1_Spy_0745	TATTCGCCTTTTTAGGAGGCAGTTACGTTCCATTGACAACATTACACAGCTCTATTA
M1_Spy_0746	GGCCATACTTGTTAGCTCTAATGGGAACAGCACTAGCTTTGATTAGTTTTTCAAGT
M1_Spy_0747	GAGATAGTCTTGTTTATGTGATAACGCTACTAGGAACGGCTAGTTTATTAGTGCC
M1_Spy_0749	TTTATAGTTTCGGACTGATTGATGTCTTTGTAGGCGGCGGTTCAACTCTTATTTTTGT
M1_Spy_0751	CTGGAAGTGTATCTAAGAAAACAGATTTAGTTATTGCAGGTAGTGACGCTGGTTCTAA
M1_Spy_0752	ATTACAGATCGCCGTGTCGAATACATTAAGACCAGTAAAATAGTCATTGAACCCCAAT
M1_Spy_0754	TATTTTCGCATTGGCTTTGGCCTGTTTTGGTGTTTCTCTTGCTGAAGGTTTCTT
M1_Spy_0755	CACAAATTTCTTATCACTTGCCATTAGGTTATTCGGTAATATCTTTGCCGGGGAAG
M1_Spy_0756	GCGTTGACAGATATTGAACAAAGCAAATCAGACGCTATTTCAGCAGTCAAAACAGA
M1_Spy_0757	CTGTAACAGCGGGTCGTTTAATTGAAAAAGTAGATCCTTCGCTTATTGGAGGATT
M1_Spy_0758	GCCCTTACTCATGGTTTCTTAGATGATGTTCCTGTAGATGATATTTTGGCTTTTGAAG
M1_Spy_0759	TATATGTCTGTTACAATCACCATGTGAATAGCTTAACCAGCCAAGTCCGTGTTCAA

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_0760	TATTACGTCAACTCAAAAAGGATCTGTTACTTCTATCCAAGCGATTTATGTGCCAGC
M1_Spy_0761	ATGAAATGAAAATTCGCCGAGGTGGTGAAGATGAAAAAGTGGACTGGATTGCAA
M1_Spy_0762	TCTTTTTATTAGCTACTGCCCTAGGGTATTTGGTGAGCCATTGTATGCTGGAATT
M1_Spy_0763	GACTTCAGGCAATGTTTTGATTAGAGATGCTGTTTGGGAACATAATCGTCCATTGA
M1_Spy_0764	GGACTTATGATTGGTTATAGTTTTATGGGAGATGGCCAGAGTCCATGGCATATTT
M1_Spy_0766	GCTAAATCCTCTGATGACAGCTTGACTTTTAACGTGTTTGTTCCAAATGTCCAA
M1_Spy_0768	AAATGTCAGGTGTTGATGCAAAAGAGTATTCAGGCTTTGCCTTTGGTTTAGGTCAA
M1_Spy_0769	AATATGGTTTCAGGGATGTTAGATACGGTTGCCTATAATGTGGCTCGTAAACAAAGTAA
M1_Spy_0770	TATTGGCTTACATTGGAACTTATTGTCGTGTGAAACAACTCCTCAAACCTGAAGTTAC
M1_Spy_0771	TTGGATGCTCAGTTGGAAACTAAATCTGAGGAGATGCTATGCTTTTTACAAGAGATGA
M1_Spy_0772	TGGTTTAGGGTTGCTAGGTACTTGGCTGACATCATTATTATTACCAACAGTAGTAC
M1_Spy_0773	TTTTATCTTACAAGCTTCCAATCTGATACCTTTTTCAACGGTCCAGCAGCAGTTG
M1_Spy_0775	CTCTACCGAGGACTATTGGACTAAGACATTTCGTGAGAAAGGGTTAACTTATCATA
M1_Spy_0776	AAGGAGATCAATTAAAAGCCATTACCCGTTTTGAAGCAGATCTTGACATGGGACA
M1_Spy_0777	CTAAGCGCTATCTTGTTTTAATGGGAGGTGGAAAGCCAGAAATTGTCGAAGTTTA
M1_Spy_0778	GATGAGCAAGAACGGTACAAGTCATTTATTGCAGAGACAATAGCACTAACGAAAA
M1_Spy_0779	TCGCTTCAAACGTTCTGTTACTAAAGCTGGTACTCTTCAAGAATCACGTAAACGT
M1_Spy_0780	AGTTATCATCGGGGGAGCTTTTGGTGCTATTGTCACTTCTTTTGTGAACGATATTAT
M1_Spy_0781	CCTCTACCTTAGAATTACTTTATCAACGGCTGAAGCAACAAGGACACATTACATCTTA
M1_Spy_0782	GAACAAATCGCAGAACGTATGGAAATGACTCCAGACAAGGTTCGTGAAATTCTAAAAA
M1_Spy_0783	ATATGACACTCACAACAATGGGTTGTCCTTTGGCAGACTTGTTGACAGATTACAT
M1_Spy_0784	ATACTTATTAGATGAACGAGGTGTTGATTATGTAGCCGTTGATGTCGCAGAAATGGA
M1_Spy_0786	GCAGGTTTTGATAGTGATCATCTGGGCAAAGAAGCCAAAGCTATTATTCAAGAACA
M1_Spy_0787	CGGCCTATGTTTCTTTATTGGATATGCCTTTTACCAAACGCTTAGCTACCTTGAAA
M1_Spy_0789	TATGTATGCCACTCCAATCATTTATCCGATCACTTTTGTTTTAGATAGCCACCCTTTG
M1_Spy_0790	GCTACTCTTTTCAACAGCAGAACAATCACCCCAGATAGTGATTCAAAGAAACGATA
M1_Spy_0791	CGTAGATAAAACAAAGCAGCAAGAAACACGTGAAGTGGAGTTGCAAAATGTCATAG
M1_Spy_0792	GAGCTCATACCTACGTTAACTTTAATCAAATGGGCGGTATCAAAGGAGCATTGAAA

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_0793	GAGGTACTGAATAAAGCTAGTGTCGATAAATCACCAGATAGTCCTGAAGTTAAAGC
M1_Spy_0794	AAAGTGAAAGAAATTCCTGTTGTAATGAATGAGCGACAAGGCGGTGTGTCATCTAT
M1_Spy_0796	GTCTCACTTCCTGGGTATTCATTCACCAACCAATATGTTATTCTTTTTAGGTTTTTG
M1_Spy_0797	TAGATTTTATGTTGATTATGCTAGGAGGCTCGATTGGTAGTTTTGCGACAGTTATCGA
M1_Spy_0798	TTTGGCGCATTAAGCTATGGTATAACGAACCTTTTCCCGCTTTATGTGTGTTTCTT
M1_Spy_0799	AAGCCTGTAATCGAACCCATACGTGGAGGAACAGATGGCTCTAAAATTTCATTTAT
M1_Spy_0800	GTCGGGTTTTTATTCTATTTGCTATTTTTGCTGTCCTAGTTGGCATTGGATTACATCGT
M1_Spy_0801	AAGTATCTATCATTCCAGAAAAATGCATCGCTTGTGGTCTATGCCAAACCTATTC
M1_Spy_0802	TATTTCTGTGGAGCAGTTACAAGCACTTAATCCAGAGCACATGACTCAAGGATATT
M1_Spy_0803	TTTTGAGACGCTAAAAGAAGAGATTGCAGCTCGTGATTACAAGGATAGTCATCGTA
M1_Spy_0804	TTGCTATCATTGAGCAACGAGCAAAAATGGATGGACGCCAAATGTTTATGCAACTT
M1_Spy_0805	TCGTCATCTTCGTAAAGCTGGTTTGGTTAGCTCAGGAGATTTCAAACGTATCAA
M1_Spy_0806	GATGCACGGTTTGAAACTTGCTGAAATCGAAGTAAACCGTAAAATGCTTGCTGAT
M1_Spy_0807	AGAAACTTTTTAAAGCACCATCTTACTTAGAACTAAATCCCGTAGAAGCTGACGCG
M1_Spy_0808	AGCCAGTTCAAAAAGCAAATTATCAAAGGATTTGGAAGTCGTCGTCCTGAGTCTAT
M1_Spy_0809	AACTATATCTATGGGGAAATTAGGAAGATTGTCTCGTTTTGCGGGAGATGTCATTG
M1_Spy_0810	GGTGCTTGCCAAAGAGATTTTAGAGACTTTTTCTTCTACTACCATGTCTGAATTGC
M1_Spy_0811	GGCCTTATCTGTTTACATGAGTGATATTGAGCGTATTGTATTTGCTCCGTTGAAAGA
M1_Spy_0813	TTGTCAGAAGAAGCAGCAGTCAAACAGTATGGGCAAGAAGCAGTTAAAACATATCAAT
M1_Spy_0814	TTCCTAAAATATCTCCAAAAACGATTCAAAAAGGAATTGAGGCAACAACTTGGCCTGG
M1_Spy_0815	AAAACCATACAGGTACTTCAGAAACCACAGATGATAATAAGACAACTCTTCCGTCATC
M1_Spy_0816	TGAAAAACAAGTGAGCGTCTTACCTAAACTAACAGCGATGCGAGATGTGATTTACAAA
M1_Spy_0817	CAGACCGTCCTAAAACCAACCCTAAACTAGGTAATGCTGAAAAATATGAAGAATGCT
M1_Spy_0818	GCCATCCGCATGTTATTGAACCTTCAGAGACAGTTATAAAAGGTAGGCAAAAGAAA
M1_Spy_0819	CAGTTGTTGAAGGAGCTACTGTCGTTGGAACTGTTGAAAAACAAGGAAAACAAAAG
M1_Spy_0821	GTGGTAAACATGGCTTTGATATTGTTTGTGCCTCTGTTAGTACTCTAGCCATTAAC
M1_Spy_0822	TGGTGGAGATGACACACTTTTTGCTAAAGTTGAAGGTGTTGTACGTTTCGAA
M1_Spy_0824	AGCCAACATTATGACTTTTTGCCACCTCTTATTACAGCTTTTTCGCAACAATACGATG

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_0826	TCTGGTGGAATCGGGAATTTTATTGATCGTTTGCGTTTAGCTTACGTGATTGATATGAT
M1_Spy_0827	TATCATATCAAGGATTTGTCCTCAATCAATGGTGTGGTTCGTCCAGGAATTGTTCAT
M1_Spy_0830	TAGATATTACAGGCAAGGATGTGATTTTGATTGATGATGTGCTTTATACTGGTCGTACC
M1_Spy_0831	TTATTGGAGGTATATCAGTTGCCTTGTTTGGTGTGATAGCTTCTAGCGGTTTGAAAAT
M1_Spy_0832	CCATCAAGCGTTTGGATTGACACAGGAACGTTACCAACAATTAAAAGATTCTGCTA
M1_Spy_0833	GGGGTACAAGGTAAGATTCCTATTTTTGGTATTTGTATGGGACATCAACTCTTTAGTC
M1_Spy_0835	TAAACGCACAGCTGATAAAGACGGACAGATGATTAGAAGTTCAGCTATTGAACAAG
M1_Spy_0836	GACACTTGGAAACGCAGATGCACAACAACAAGAAATTCATAAAGGAGTAGCTGTT
M1_Spy_0837	AACCAACAGGTGCACTAGATACTAAAACAGGTCAGCAAATTATGGAACTCTTGACA
M1_Spy_0838	GGAATGTTGCCAGCTAATAAAGCTAGTAAGTTAGATCCAATTGAGGCATTACGTT
M1_Spy_0839	CTATGTCATTGAAGACTTTTCTTATCGGTCTTATTTGTCGTCCCAAGCTTTTTGG
M1_Spy_0840	CATCTTGTCTAAAGCTGGTGTTATGGCTAAGTTCCACGACCAAAAATTCTCTAAAT
M1_Spy_0841	TGACTTTTTAGAGTATCATCTTGACTTGGATGCTCAAGACATCGGTCGTGTTATC
M1_Spy_0843	GGCGAGGGACAAAAAGACGCTTATGGTAATCCTATATTAAATGTTGACGAGGATAA
M1_Spy_0844	AATTGAAATTGCCTTTGTTGATCGTGAGCGCCTTTTAGGTTACAGGTTTGTAGGAA
M1_Spy_0845	TATTGCACTTTGCACCATGAACTCAGCCAACTTCCTCATATAGTGTCTGTTAATCA
M1_Spy_0846	GAAACCTTAAAATGGTGGCTCCATCAAAGGCAAAGGATGTCTGCAAATGAACTAT
M1_Spy_0847	CTAATGATGTTTGGATAGTGAAACGCCAAGGAAAACGAGACTTGCTCTTACCATATA
M1_Spy_0849	GGAAGAGCGCAAGCTATTGGATAAGATTAAAGAAGCATTGGATCAAGGAGAAGAT
M1_Spy_0850	AGTGTGGAATTATTGAAAGCATCAACGGTCAACCTCTTAACACCTTATGTGCCAAAA
M1_Spy_0851	AAACTTCTTGAAACATCCTATCATGGCTATTCCGATGGTGTTCACTGCAGCAATTA
M1_Spy_0852	ATCGTTGGTCAAGCAGAAGGTGTCGAACTAAACGAAGAAGAAATCACACAATATGTAA
M1_Spy_0853	ATTTCTTGGAATGAATGGTGTTGATGATTCTTACCTGACGACTCCTGATATGGAAGAA
M1_Spy_0854	TAAATGGTCAAGGACCTATCATTAGTCAGGAACAACTGGAGGATTTGAAAACTAAGCT
M1_Spy_0855	TATATCTTGTCTTTGTTGTGATTGGTGCCCTTGTATCAGGTATCTTATTTGGCGCT
M1_Spy_0856	GCAACCTTAACAGGCACTTACGCCACTGATACAACTTACCATACCAAATTAAATG
M1_Spy_0857	CTCAAGAAACTGGTCTAGCAACTTCGGGTTATGTTTGGAATGAATATCGTCGTAATT
M1_Spy_0861	AAACCTGTGGGGAGCTGACTTTGATTCTAACGGGAACCTTAAAGCTATTTATGTAA

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_0864	GTTGAATCCTTAGCTGCTCCTTTGCTAGATATGGTTAAGCGATTGACTTATGAAGT
M1_Spy_0865	ATATGACATTGAAGAGGTTCATTTTGGAGCGGTGATTGCTACTCATTTAGGAGAAG
M1_Spy_0866	TTGAAAAATTGGATATTTACCAGTACGGCAAGAAAATGGCGAGTCTAGTGGAAGAC
M1_Spy_0867	CGCATGTCCTACCTTGAAAAACAAGAATGGGCTCAGATAGAAGATAAAATAGCAACTA
M1_Spy_0870	TGTCGTTATCTGGCTATCGCAAAACACAGCGGTATGATAAGATTACTGTTGAGTAT
M1_Spy_0872	GGAAAAGCTTATGCAGATGTCCGTGGTACGCTAGATACTGATACCAATGATTTTAT
M1_Spy_0873	TAATGCAGAAATGATTACAAGAGAGTTAAAGCGTTGTGCAGACGAGTTGGCATCTT
M1_Spy_0874	ATATTATTATGCTGACGGCTATGGCAGAAATTAATGACCGTGTTACCGGACTAGAT
M1_Spy_0875	CAGCTATTGCTCAAGATGCAGCTGAAGATTTTAAGAGTCTGGTTTTGAAAGATGGT
M1_Spy_0876	GACCATGCCATTAAGTTTATCCGAAACATTGGCTTTGAAACTATCGAAATCGCCTTAA
M1_Spy_0877	TATGGAGGCTGTGAAAGAACTGCGTCAAGAAGGTTTTGCTTGTTATTTTACCATG
M1_Spy_0878	TTGCAAGAAGGTCATAAGGAAGAACTCAAGAAAAGTTTAGCAGGAGCAAGTCATCT
M1_Spy_0879	CCAACCGAGCGTGTGATTGCTATCGTTAATGGCTGGAAAGAAGAATTAAAAATCAT
M1_Spy_0880	CTGTTTCTATCGCACATGATCTTCTTAATCAACCTGATGCCAAAACATTAGCCCAATT
M1_Spy_0881	AATTTGCTCTAACAGCCATCAAGGAGCATCGAAGGATCTATCATACCAATGACAAAAA
M1_Spy_0882	GCCAGTTGCTAGTCAGCCTAAGCTTGTTTTAAATGTTCCAGATAGGACTAATTTCT
M1_Spy_0883	ATTTCACGCAAGAAAGCCAAACGTTCTATGCTCGAGACGCTAAAAATCCTTATGAT
M1_Spy_0884	GTGCGTGATTTGTGTTCTTTGTGCCATTCCAGCTTTTAAAAAGAATCGTTATGGC
M1_Spy_0885	AATATCAGGCTCTTCTTTCTTACGATGGCGTCGAATTGGCATTTGATAAGGAA
M1_Spy_0886	GAGTTTGGTGGATTTACGTCATGCCCCTTCAAAAGAAGATATTCAGATGTATGACT
M1_Spy_0887	ATTGATCAGAAGTTAGGCGATGATTTTGCTAAACGTCTTACTGGTTTGCATCAGAG
M1_Spy_0888	CAAGATACGGAAACTTCTGAGTCTAACATTATGGATCGCATTGCACCTTATTTCAG
M1_Spy_0889	ATCTTGATTTGGGTTGTATTAAAGATCCTGTTGCTTTTGGTCACCTGCTAGATGGAA
M1_Spy_0890	ACATTCCATTGCTTGCCTACTCTGTATCCTTTACTGGAAATGGTCTTATTCCACAA
M1_Spy_0891	CTTATATTAAAGAGGATGGCGAGAGTGTGAACCCTATTGAGGTAAAAGGAGAAAAAG
M1_Spy_0892	ACCATGAAGAAGTTGTGGAAGTGACCCAACATATCAAAGAAGATTTCAAAGGTCTTGT
M1_Spy_0894	TGTCTTTTACACTAACATGCCTGAGCGTAATATGGCTCTTGGTAAATTGGGTGTTA
M1_Spy_0895	GACTTTTTCTGGTGTAATCTACCGACGTGAGCCTTTTTACTTTGATGACATGA

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_0898	CAACTCCTTTTATTTTTCAGAAGAGATGATGTCCCAGATGCCCCACAACAAATCAATT
M1_Spy_0899	TTTAAAAGTGAGTGCTTTGTCACTTAAGGAAGCTCCTATTCCGGATGTGCTCTAT
M1_Spy_0900	TTACTTGCTTAACTCCTGGCATTCGTCCTAAAGGCAGTAATATTGGCGATCAAAAA
M1_Spy_0901	ATTTAATTGAGAATGGGTTTGTGGAAACTATCAAGGCTCATTTCCCAGAAGTTGAGGT
M1_Spy_0902	TTACCTACTTATGAAACTAAGGAAGGTCTCTCAATGGGCATTCAACTAATTGCAGC
M1_Spy_0903	TCTCAAAATCCAAATCTAAATGTCAAAATGAGCGATGTTCAGTACAACCCAACCGAAC
M1_Spy_0904	GATATGGTTAAAAGTTCGTCGCTAGCTGCCATGATTACCGTACCAGATATTTTTCA
M1_Spy_0905	CAAGAACCAATGCTATTTTAGAAAAAGAGGGCATGACTGGCGTAGATTGGCTAAA
M1_Spy_0907	TATGGTTTTGATGCGGGTTATTTGGCAGAAAATGGTCCAGCAGATTTGGTTATTTTTG
M1_Spy_0908	GTGTGGTTTCAGCTATCGTTGGTGTGTTATCTGTTTTAACATTTCCTGCCATTCAT
M1_Spy_0909	TCAACGCTATAAAGGACTGGGTGAAATGAATGCTAACCAATTATGGGAAACCACTAT
M1_Spy_0910	GAAAGAATCAGCAACGGCTCCTTTATTTCAGACACTATTTCGGATCAAGAAGTACT
M1_Spy_0911	ATTTGTTACGCCACTTAGTCCATCCATCTTGCCATCTATTACGAAGTATTCACTCTTA
M1_Spy_0912	GATGGAGTAGATTGCTTCTTCAAAACGCTCGAACAAGCGGTTGAAAATATCATTG
M1_Spy_0913	CCCAGAAACACAAACTGGATTCTCAATGGCTGATCTTTTCGGTGATATTGAATTGTAA
M1_Spy_0914	ATGAACTTTGTTTAGAATATGGGACTTATCCGTTGTCACGTGTTGATGCTTACTGG
M1_Spy_0915	AATGGTTTATCACGTCAAGAATGGGCAGAACGTTTAGAAGCTATTGGAGAATCCTT
M1_Spy_0916	GTAAAACTGGATTCTGAAGACTGCCTTACCATCAATCCTTTGGGAATGGTTTAA
M1_Spy_0917	TGTTTTTAGGCCAATGATCAACCCTCATCGCTACACTATTTCTCAAGACAATCAAG
M1_Spy_0918	GGCGACAGCTTTAGTCACTTATGTTTATGGGGCTTACCTGAGATTTCTATTTCAA
M1_Spy_0919	AAGAAATATCAATTTGTTAAAGGTCTTGCTACTGGCGTCCTAGCAACTGCTGCTA
M1_Spy_0921	AGTGATCAATTAAAACAAAACACGAGACGTTTTGCCAAGCGACAGCTAACTTGGTT
M1_Spy_0922	GATATTTCGGGCTACATTTCCCCAAAACAGCAATGGAGACTTGACGATTTTTATGA
M1_Spy_0923	TAATCTTTCTAAGGCTTTGGGGCTTTTTACTGACCAACCATCTTTTGAGGAAGAAAAG
M1_Spy_0924	GCGTGATTGTCGCCAGATGGAAAAAGATGCCGCCACTATATTTGAAAATGTTAAA
M1_Spy_0925	GAATTTGCCCTTTAGTTTAGCGGTGACCCATCAATTTTACACCCTTTTCCCTAAATT
M1_Spy_0926	CCAAGCGCAAAAACGAGAGATTTCAGAGAGCCACATTTACCAAGACTTAAAAGAATT
M1_Spy_0927	CTGCCAAGAGATGTTGTCTCTGCCGATTACGAAAAAGAATATGGTCTTGATACTTT

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_0928	CTCAAGTGACGATATCAGAGGATTTTCTATCTGCGATGAATTTATGGAGTGATTC
M1_Spy_0929	ATTCGTGATGATTTTAAGGGTCAAGTGCCAAAAACCCACAAAGAGCTTGAAAGTTTAC
M1_Spy_0930	TGATATTTTAGAGGCAGGTAAGGAAAAATTGCAGGGCATTGAACGTCTGGTTTTACAA
M1_Spy_0931	TTGGGATGTCAGCATTATTTCAAGCAAAGCCTCGACCAATCCATTTAGCCATTTATA
M1_Spy_0932	GAGCTTATACCAGTGATTATTCTCCTTTTTATGGTCAGGTTTCAGGATTGAAGAACCT
M1_Spy_0933	CCTATCGCATGGGCTATATCAGTAAAGAAGATGTCCATAAATTGGCGCAATCTTTA
M1_Spy_0935	TTCAAAAGGAATGTTTGTTCCTAGAGGGGTTGCTAATGGCTTTCAAGTTCTTTCAGA
M1_Spy_0936	GTGGAAAGCAGAAAAAGATGCTGTAGAAGCCAAGTATGCTAAAACTCAAGAAGTGATTA
M1_Spy_0937	CTACTCAACAAATCTATACCCACGTCACGAAAAGCATGAAAAATGAAGTCGTCGA
M1_Spy_0938	CCCCGTAGCAAACATTACCGTCCGAAAATGGATACAATCAGACTTAGACAAAATTA
M1_Spy_0939	AGAAGTTGGAAAAACAAAGTCGACAATATCAAAATGGGAAAAAGGGACGCGTTCTC
M1_Spy_0940	CTCTGTTAGTGTTGGGCGGTATTGCTTGGATGATCGCTTGGTTTATTACAAAATA
M1_Spy_0942	GCTAGAACGATAGCTTCAACATTTAATCAGGACAAAGAAAAGGATCTTCACTCAGCTT
M1_Spy_0943	AGAAAATGAAAGCACCTAGCCTAGAAGTTAATCTACGGCTAGGCGAAAAGGAAA
M1_Spy_0944	CTGAATTGCCACAATTAGACAGTTTTAGTAATCTGCAAGAACTAACAGAAGAGCTTTCG
M1_Spy_0945	AGAAAGTTATCGCAACTAAAATGGGTTTTACGCGCTCTGGATTTTATCAGAAACG
M1_Spy_0946	CAGGGCAATCAAACAGAATATACAGACCACGTTCTAAAACTAGACATGGCAAAAGA
M1_Spy_0947	CTACAATAGTCCGATTGATGATGAAGCAATTAGATTGGCTGAGGTAACAGCAGAA
M1_Spy_0948	CGCTCACCTTTATCGCAACTCTAAAGCTCACAAACTCAAATACTACAATCCAGAT
M1_Spy_0949	GGAGTATGTCTCTAAGTCAACCTCTAAATCTACTATCGCAGCTATGACAAATGCAT
M1_Spy_0952	ATAAACGCAGAATTTTTAGAGAAGTGGCTTTAGAAAACAGCAAAGGGTGGAGCGA
M1_Spy_0953	GGAGATACGACAGTAGAAAAGGTCAGAGACAGGTTGCAACGGTTTGACGATAGTA
M1_Spy_0954	TGGATCAGGAGATTTTTAATTTTTTTAACAAACAAATCAAAAAAGATTTT
M1_Spy_0956	AGTTGATGAACTCGGTGGAGTTTTAACTTCGCGAGAAGTCTTTTTGGCAG
M1_Spy_0957	TAAAAGTATGGTAAACGGAATGTTTGAGCAGGTTTTTGGAGGTGAATAGA
M1_Spy_0958	AAAAACAGCACTTGTTCAGACACTTAGAGAGGCTTTTCCTGATGAACTCGGTAATAT
M1_Spy_0959	AATATCAAAAGGAGCAAGAGGTCGAAAAAGCAAAAGCTACCATCTCAGGACAATGT
M1_Spy_0960	AACTAAAGAACAAGAACAGGCTCAGAAATCAGCAGAAATACCACAAATCGACAAGGAAA

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_0961	TCACTTGATGAAAACGGAGATTTGTTTGAGCCTGTATACGAGTTGATGCTTGAAG
M1_Spy_0962	GCGATGAAAGCACAATCGAAAGAAAAAGCTCTCGAATATTGCGGAAAAACAGTA
M1_Spy_0963	AACGATTTTATCGACAAAAACAAGTACGCAAAAGTGACAGGCGACTTATGGCAAG
M1_Spy_0965	GTCAAAATCTAGCAACCGAAAATGTAAAACTCACCGAATCCGAAATCCGCAAAGA
M1_Spy_0967	GATAATGCTTTGTTGTCGTTCTCAGAGCTATATAAAGAGGGAATGTTGCTTGTCG
M1_Spy_0968	GAAAAAGGGAAATTGAACATGGGGATAATGAGGGCCTTGTGGTTGATACAAGATTATT
M1_Spy_0970	CAGCAATTGTAACCGCAATATTCTTTGCTAGTTTTTCATTGATACCAACTTCCGG
M1_Spy_0971	ATAAAGTTAATGGAGCTTACTCAGAAAAACGTGAATTAGAGCATTCTGGAACGGTGGT
M1_Spy_0972	GAAATAAAAAGTAAAGGGGTGTCTGGAATTGTCCCGAGTATTAAAGGTAAAGGGTCAA
M1_Spy_0975	GTAGCATCATATCTCAAAGCGTCCAAAGTGCTATCCTAAAAGGTAAAAATGGCTTAAC
M1_Spy_0976	TGGAAATGATGTCAAGACCGACTCAAGAAGTGCTAACGTTTTCTAAAATCATCCG
M1_Spy_0977	AACGCCCAAGCAATAAATAGAGAGTTTGCAGCAGTTAAAGGTGTGTGTCTTGATAAT
M1_Spy_0978	AAATGGGGCGACCTACTAGCGACCCTAAGACTGTTAAATTGACTGTTAGAATTAA
M1_Spy_0979	ACATTGATTGGGATAGTCAGTGTGATTGGTACGATTTTATTTCTCAACACAGTTCCCA
M1_Spy_0980	GAGAACATGGTTATGAAGCCAAAGGCTGATTATTTTGATGATTTGGTTGACCGCAATT
M1_Spy_0981	AAGATGAATGAAAAAGAGAAAGCAGACTACGAAAAGCAGAAGCTGTTAGACGAATTGCA
M1_Spy_0982	TCCATTTTTGAAGACCGCAAACAGAAAGTAGAAACCGTGACAAATGAAGAAGTCTCTA
M1_Spy_0984	TCACAACAACCAAGACAACTGATCCAGTAAACGTCCAAACCAAGGTATCTATGATT
M1_Spy_0985	ACTAATGCTTATTATGTAGGCGATGATTACAAAGGAGATCGTATCGAGGAATTGACAG
M1_Spy_0986	AAACAACCTACTACGACCATTTGAATGAGTTTGAACCCTACGATGCCATGATTATG
M1_Spy_0987	TACATTTGTTAAAACGACGGATGAGCAATACAATCCCGATTTAGGTGAGTATACGC
M1_Spy_0988	GTCAGAGACAATGGGACGCAGCTTCAAAGGAAAATGATTAATAAAGCGGTATTTAC
M1_Spy_0989	GAGTTCTGACGTTTTCCGCTGGTCATTAAATACTCGTCAATCATCTATTCAAATG
M1_Spy_0991	GTTTGGGGTATTGATAAAAATGGAACAAACAGAGGAAACGGTAAATACCTAGCGAC
M1_Spy_0992	CGAACCACCACGTCCTAGTTTGAATGATATTTACGACTATATTGACGAAGTTGAAG
M1_Spy_0993	ATTAATGACAGGCAAAGCACTAGCGGCTGTTGATGAATCACATAAAGCTCACAAA
M1_Spy_0994	TGTTGTCAAATCAGAGAGTTCGCAATCGTTATCGCAGCAACAAGAGTTTGTACATA
M1_Spy_0995	GATTACCCAGAACGGACGCAATATCCTTGGCGATTTAGATATGATTAATAGTAGGT

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_0996	TCTTTAGAGACTATCACAGACTTAGGGATGACATTTCAGGAATTTTTGCAAGGCA
M1_Spy_0997	GATGGACCATTGATGATTTTACGCTCTAACAAAGATACGTTTGATCAGTCAGTTC
M1_Spy_0998	GTAACCGTGTAGCAATCTATGGAAATAAAAATCGTAGTCCATGGCTCTTTGACTCA
M1_Spy_0999	AGAACTAAACGCACAATTGGATGAAGCAACAGCACCGAAGGAAGAAGGTAAATAA
M1_Spy_1001	ACGTTGAACCCGATATCTACAAAGGTATGTTAGAGCTTATTGAGCCTGCTAAACA
M1_Spy_1002	GAGCGTTTAGGTCGCGTAGAAGACGAAGTTATTATCAACAAGGAGCGTATTATTA
M1_Spy_1006	ATTAGCAGATGATAGTAGCAAAGTGGATATACCTAAGATTGACAAACCACAAAGCCAG
M1_Spy_1007	AGTACAGGGAGTACTGATAAGAAAGAGGTTACGATTCAGGAACTTGATGTGAAATC
M1_Spy_1008	GGTTTGTGAATGTCCAGGGAAAAGGTATGAAGCGTTTGGTGGAATTACATTAACT
M1_Spy_1010	GTACCTTACGATCAAGTGTTGGAAAAGCCAACTTGGGAAGGTGATTATGACATTT
M1_Spy_1011	CGGTTTGTATGACGAAGAAGTATTGGTGATTGAGGAGGTTAAGGACCATGTTAAA
M1_Spy_1012	CCACATGCCAAGCGTATTTATTTGCAGTTAGCTGATGATTACCCAGAACTCAATAT
M1_Spy_1013	AAGTCATGTCATCATCAAAGACAATCTTGACCCAAGTGACGAGGTTAAAACTGATG
M1_Spy_1016	TTAAGGTTATGAATGCTGAGGGGGATCAGAGTAAAATCCAAACAATGAGTAGGCAATTA
M1_Spy_1017	ACCCTCACTGAGATGAATTTGGATGCCAAGTTAGACAATCGTTTTGAGGATGTAA
M1_Spy_1018	TCTCAAAAGGGATTGGAGTTATTGTTATTGGTTTGGCGAGCATAATTGTTGGTGAAGTA
M1_Spy_1019	TTTGGTTAAACGAACAGGAAATGGCCTAGAAAAGCATTTAGAAACACCAGCAGGT
M1_Spy_1020	CCATGCTAATGGGCGTGATTTGCAACTATTGATGAATTTGTTAAAACCCCAGTATC
M1_Spy_1022	GGCAGAAAGTGATGGAAAAACCAAATTTTATGCTTGGTGTGGCTATGAGGATTTTC
M1_Spy_1024	GTGATTCCGTTTTAGCCAATAAGATCTCGATAGTTACTGAGCAAGTGGTGCAAATT
M1_Spy_1025	TGACTATGATTATTACCTTGAGAAAAAGGCAGAGCTAGAAGAATTAGCCCGCCTA
M1_Spy_1026	TGCTTATGAATTTGCACAAAACAGTCCAGATCCAGAACTTTCAGTAGCCTTCGAA
M1_Spy_1028	ATGCTCGTGTACTTGAACAAGCAATCTTACCAGATGTTGAGAAAATCAAAGCTGCT
M1_Spy_1029	TGAAAGCAGCTGAAATGTCTGGATCAACCTTCTCTATCACAAACTTGGGAATGTTT
M1_Spy_1031	TTTCTATCGGACGTGTATCACAAATGAACGGTCTGGAAAATCTTAACCTTGAAATGGAT
M1_Spy_1032	GATGGCGATCGTTATGCCTATATGATGCTTCCAAATATGACTCGTCAAGAATTTGA
M1_Spy_1033	AAGATTGAGTCTAAAGGAGTGAAGTCTGTTCGTGCTCGCGTGACCAATATTTTAAA
M1_Spy_1034	CTACCTAGCTATGATGATATACTGAAACAAGAAATCACTGAAGAATATGCCGACC

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_1035	GACTTAGAGTCACAGGATTTGACGATACTAAGATAAAACAAGCTGAAAAGCTCGAG
M1_Spy_1036	CAGTAACTCGCAAAGGCCAATTTTTACACGATTTAAGCACAGATGAATTTGAAACGGT
M1_Spy_1037	AACGGGGGTTAATAAGGTTGAAGTGACCAGTGATGAATCTACCATTGCTTTAATTGAT
M1_Spy_1038	GAAATGTTGGAGTCAGCCTTGATTGCTGGTCTACTATCTGTTGGTATTGAAGTTTATA
M1_Spy_1039	AGATATGCAAGAGAAACTCCCTTCTTTAAGCATTATGGTCAGTCAAGAATCAAGCAAG
M1_Spy_1040	TTTTGAGGAGCGTTATGGAAATATTGTTAGGGAGCTTCAAAACCAAGGACTGCTT
M1_Spy_1042	TCCAGAAGATTTAGTAGCGCCATTTGAGACTGATTTTGTTAAAAAGCTACACCGTG
M1_Spy_1043	ATTTAATTGATTTAGACGGAACGATTTACCAAGGGAAAAACCGTATTCCAGCAGGG
M1_Spy_1044	GATTCTGTGATTTGGATCCTTCCAGAAGTCTTTTTCTTGCACTGTTTCCTTTTCT
M1_Spy_1046	CGTCTACAAAAGAAGTTTTAGATGCCACTCTTATCCATCAATCCATCACTGGTCTTTA
M1_Spy_1047	TTTATTATTGAGTATGTTTGCGCGTGAAGTGGTTGTGTCTGGATGTATGACTCAGTTT
M1_Spy_1048	CAATGCCATGATTGGTCGGCTGAGGGAGCATAATCCTAATAAAGGAAATATTACAT
M1_Spy_1049	GTCAATAGCGGAGCTTTTCTAACAAAGGATGAAGTGGCTAGTTTACAAGAGTATA
M1_Spy_1053	TTTTAGCGAAGTGTTATGGTGGTGACGTGTCTCGTAAACGTAAATTGCTCGAAAAA
M1_Spy_1054	AATAGAGGTCTAAACAAACCACAAACACAAGGTGGTAATCAGCTCGCAAAAACAC
M1_Spy_1055	CAAAACCTATTGAAAATCGTATGGTCACAAACCACGATGACTCTTCTTATGGGATGA
M1_Spy_1056	GACCAGTTTGGGAGTTTCTGCCGATAGTTTCCATTACCTAAAAACCTTATCAAAATTCT
M1_Spy_1057	CAGTGTGAATCAGAAAATGGTAACCGCTGTAATGAACAGTGGCAAAGACAATATCT
M1_Spy_1058	CGCAAAGGAATTAGTCAGTTTACCAGAACTTGATGTCTTTAACCAAGTCTTACCATCA
M1_Spy_1059	TTCTTTCATTGGTATCTTAGTAGGTAGTGGACCTGTTAATGCCTTTGTGGAACACATT
M1_Spy_1060	GGTAACACTAGCAGCTTTTTGGCTTCTACGAAAGAAAGTGAATATCATCTGGATT
M1_Spy_1061	CCCCAGACCTTGATAGTCTTGAAGTGCCTAAATTGTTCTTGTTACCTTTGATAGAAAA
M1_Spy_1062	GCCCATCATGACAGCTTTTGACATGTTTGAAGTAACAAAAGATCAAACTTACGCC
M1_Spy_1063	ATTGTTTATCCAAAAGAAGGAACGGTGTTTGTGCCCTCCTCTGTTGCTATTATCA
M1_Spy_1064	ATTCATCAGGAGGTTTTTCAAGAGATTGATTGCACGTCCTGTGCTAATTGTTGCAA
M1_Spy_1065	TCTTTGTAGGTCCTTCGACAGGTTTTTACACGGCAAATCACCCTCTAGATTATAAA
M1_Spy_1066	TTGATGATTGGTGATAGTTTATCGGCTGATGTTCAAGGTGGAATTAATGCTGGCAT
M1_Spy_1067	GGTCGTGAATTGAGTGAGCTTGGCTTTACATCTTTTGTGAATGAACACCTTATCTAT

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_1068	CAGTAGTTGAAGCAATACAGCAACAGATAACAGACAATAAAAATGATAGATCGTCCCC
M1_Spy_1069	TTGGTTATTATATGGAGACTTTGCCACCACGTTTTGCTGCTTTTAATGAGGTTCAAAC
M1_Spy_1070	ATGAAGGCTACTGATGGTAAAATTGAAGTAACTATTATTGGTAAGTCTGCTCACGGGT
M1_Spy_1071	GAATTAGAGCAATTACCAGATGACTACATTCCCATTTCCGTTTTAACCAACCAGAACA
M1_Spy_1072	GGTCTTGATGAGTTGAAAGATGTATTCGTAGGACCATCTGCAGTAGCATTTTCTAA
M1_Spy_1073	TGAGCTTAACGATCTTGTAAAAGCTATCGAAGAAGAATTTGGTGTAACTGCAGCTG
M1_Spy_1075	TATATTTGGAGTTTGGTTCATTCCCAATTACGTGAAGGTGGTTTATCACAAGCTGCTA
M1_Spy_1077	TTAATCAGTGCCATGAGTGTATTGGCTTTTGTGAGATTGATAAGTTTGCGAGGCAAT
M1_Spy_1080	TATTGCTCATCTCAATAAGGTTGCGGAGGTCTTGCTTAATCTGAACAACAACGATATA
M1_Spy_1081	AAGATAAAGAATGAGGCTGAGGAGAGAGTGAAGCGTAGTTTTCCACCTATAGAAAT
M1_Spy_1082	ACAAACATTATGGAATCGGTTTGGCATTTGTTAAAGGCGTTGCTATCAAACACGGT
M1_Spy_1083	TAGCCGTTACCTATGTACCCCTGGTTCTTGTTGGAAATTAGTTTGCTTTACAA
M1_Spy_1084	ATTAAAGCAGGAGAGAGGTTAGCAATTGTTGGTGGAAATGGTTCTGGAAAAAGTAC
M1_Spy_1085	TTAGTAAGACAAGTGGTTCTATTATTTTTGAAGGTAGAGAATGGTCACGTCGGGATCT
M1_Spy_1086	ACCTTTTGGAGCGGAGTTTGTTCCAGACTACTCACAGATTTTTATATCATTGTTCCTA
M1_Spy_1087	ATATTTAGTACAGGTGTGGCATTAGGTATTTCAATGGTAGGTGTTATTGCTGCAG
M1_Spy_1088	GAGAGGAGAGGTTGCCTTAATGGCAGATGTTCTTGTGCAGTTTTATAAACTCTAT
M1_Spy_1093	CCAAGAATAACAGATCAGTCAGTGAGGTATTTAAAGGAACCTAAGCGTATTCCTCT
M1_Spy_1094	GATTCGTATCCTCCCAAACATGAGTCCTGAAAATCTTTTACAAACAATGGAACCA
M1_Spy_1096	TTTACTAGTCCTTTAGGAGTTATTTCAGATATTCACATCGCTATGCCAGGACACCA
M1_Spy_1097	TTCTATATGTGAACACCACTTAGTTCCTTTTTATGGCAAGGCTCATATCGCTTACTTG
M1_Spy_1098	TGAAAATTGGAAAGTTTGTGATTGAGGGCAATGCGGCTATCATGGGGATTTTAAA
M1_Spy_1099	ATGGTGTTTGATAGTGTCCGTCAGTTAGTGGAAGGGGAAAAGTTTATTTTGATTGAAC
M1_Spy_1100	CTTATATGACTGAGCGTGCTTTTGTTTTAATCCCCTTATTGGAATTGCAGCCAGAT
M1_Spy_1101	ATACGATCAGCCAAGAAATGAATCGGTTAAATCACCTTCGCCAACTAAAACAACCTTTA
M1_Spy_1102	TTTGATGCCCGTATTGAAGAATATGTGGAAATGGATGAGCCTGAAGATGGATTGATTAA
M1_Spy_1103	TGTCTTTATCCCAAGTTTGAGTCTCTTTATGTTAACCCGTTTGATTGGTGGAAACC
M1_Spy_1104	GGGATTATTGCAGGTTATTTTATGGCCTTTACCTATTCCTTAGATGATTTTGCAGTGAC

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_1105	CTGCTATTGGAATTACCTTTTCTGGTGAAGCCAGTGAGATGTTAGATAGTAACGAA
M1_Spy_1106	AATTAGCTTCCGCTTGTCACTTATCCCATGTCTCTGTCCGTAAATACATTGCTTATTT
M1_Spy_1107	ATTTACCGCTTTATCCATACTAATCTTCTAACCACACTGAGACCCAAATCACTCGTTTT
M1_Spy_1109	CTACCCGTTTAGGTGGGGCTATTACCGTTATCACTATGACAGCTATTTTAAGAAT
M1_Spy_1110	ATATCTTCATTGGCGTATCAGCACCAGGAGTCTTAAAAGCAGAGTGGATTAGTAAAAT
M1_Spy_1111	CCTGATACGATTGATGATGAGGCACTTGTCATGCTTTCTGATATTTTACCAACTTC
M1_Spy_1113	ATGAAGATATAAATGCTGCCAAAGAGGCAGGTGCAAGACCTATTCGCATTTTAAGA
M1_Spy_1114	TCTTTTTGGTCGTTAATGAAGCTGATACAGGTTTTGTCCGTGAGGTATTGATGTAG
M1_Spy_1115	GTCAAGACTATTGTAGAGCTTATTAGCCAGATTCGTAGTCATTGTGTCTATAGTC
M1_Spy_1117	TGAAGGCAATTTTATTTATCCGAAAGTAGTTGGTGGATCGATTGGATTACCGGCT
M1_Spy_1118	GACTGTCCGACGTTCACTTGCAGAACCAAGAGAAATTTTATACTATGCCTGTAAAA
M1_Spy_1119	CTTAGGGATTTGTCGTGGTACACAGTTAATGAATGTTGCCTTAGGAGGAAATCTTA
M1_Spy_1120	CAATCTGTCGATTTGACCAGTGAGCTCCAGACACTACTCTATTTTATGAATCAAA
M1_Spy_1121	AGAACTCACAGGGTTTAAATTAGCTATCACCGATCAGTCTGGTCTTAGATTAGTT
M1_Spy_1122	TAATCCAAGTCATGTTTTAGCTGCGTACTATGGGGATGACTCTTCTCGTTTAAAAG
M1_Spy_1123	CCCGATTGCTATTATCGACTATGCACAAGATGATTCTGAACGTGAACAAGGTTATA
M1_Spy_1124	AACATTTGTAAATAGCGCCGAATTAACCCTAAAAGTGCCAGAAAAAGTGGGGAATC
M1_Spy_1125	CTTTAGAATTAGACGAAGAAATGCGTAAGATTCGAGAAGATATTCGGGAGGCTCAA
M1_Spy_1126	ATATTGTGATTTCTATTGGCGGAGATGGGATGCTCTTATCTGCCTTTCACATGTAT
M1_Spy_1127	TTATGTGGACAATCATTACCTAGACCAACAAGTGCATATCGTGACTCGTTTAGACA
M1_Spy_1128	TATGTTGGTTAAAATGGGATTAGCAGATGGAATGGTATCAGGGGCTATTCATTCAACA
M1_Spy_1131	TCAGATAACCCTTGTAAAACCTTCCTACTTCCTTCGGGTTCATTGGATCATATTTAC
M1_Spy_1133	TGATTAAGACCCACCCTGCTAATGCTTTTGTGGTCAATTTATTTGGAGGTGATGA
M1_Spy_1134	ATCAATTAGCGGGAAAAATTACTGAAAAAGAGATGCAGGACATGAACTACGAGGTATCT
M1_Spy_1135	CGCGGGTGGTAAAGATTTAGACAGTTTGCGTCATGTTGATTATGTTATCGTCAAAA
M1_Spy_1136	GTTTGCTCAAAAATATGCTGAGGCTGGCATTACAAAAGTGGTTACAATCGAAGCTT
M1_Spy_1137	ATGCTTGTTCTCTTTGGTATGGTTGCTCTTCAAGGAATGCAAATGCTTAATCGTGTT
M1_Spy_1138	GTTGTGACTTCTGGAATTTATGAAAGGCAGTTTACATCCAAAGGGAAACAGTATCATC

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_1139	GGTTACCGAAGTAGTCTCACGCATTGCAAAAGCACCTAAAGAAAATATCCATGTT
M1_Spy_1140	GACAGAAAACGGAAAACCTGTCTATGAAGGTGATCAGATACAAATTGGTGGTAATG
M1_Spy_1141	GTTGAAGATCGTTATGAAGAGTTAGGTGAGTTACTTAGTGATCCAGATGTTGTTTCAG
M1_Spy_1142	ATGCACCGTTGAATGTTTTAGATATTGGTACAGGTAGTGGAGCGATTGCTATTTCT
M1_Spy_1143	TAACAGGTAAGGATTCAACCATCTTAGACTTATCAGGTGAGCGTGCTGTGATTTTA
M1_Spy_1144	GCGCATGCTTTTTACCGAAACCTCGACTATGCAGATGATAAAACACAATTACGAT
M1_Spy_1145	GATGTCACTAAGGTTATTGCTAATGGTAAACTAGCGCAAAATCTTCTGGATGAGGT
M1_Spy_1146	CAGCCCGTTTAACCTTTGTGGATCAAGTCAAAGATGTCATTCCAGAACCTATAAAAAT
M1_Spy_1147	TATTTTTATTACCACCCCCTCGCCTTGATTTCTGGAGGTAAGCTTTATAAGAATGG
M1_Spy_1148	GAATTTGCAGTTTGCTGATAAGATTTTAGTGTTGGAACAGGGCCATCAATTGGAT
M1_Spy_1149	TGTCCAATATACCAAACCTTTCAACGATATTTCATCTGTCTTGGCAGAGATACAG
M1_Spy_1150	TTTCTTCTTACCACATTTCAACAAACCTTACAACTACATCACAATGGCAGCACTTGGT
M1_Spy_1151	TTCTGTTTTCCAAGAAGGCCAATACGAAGGTGTTGAAGATTGCTATATTGGTCAAC
M1_Spy_1152	GCAAACAGGTCGTGCTACTTTGGGTGTTAAAATTATGAAACTAGATGCTGATGCTA
M1_Spy_1154	TTGTCAAAGGAGAACTAAAAACAGAATACGATTTTGATAAAGCGCCCGCCGATGTA
M1_Spy_1155	AAGTGACCCCAACTATCAAGCTCCTCCTAAACGTGTAAGTGAACCAGAATTTAAAA
M1_Spy_1156	AACTTACAGAAATGTTACAATTGGTTTTATTAGTGGTTCCTGCTGTAAAACTTGC
M1_Spy_1157	TTCTGGATGCAGTATCGGAAAAAGTCCAAGAATCCAAAGATGCTGTTGAAGTTTAC
M1_Spy_1158	GGATAAAAGTGACTATCTGCCCATTCAATTAGATATCATTCCAAAAGCCGTTTCG
M1_Spy_1159	GATTTGGCATCTCTTTATTTTATTGGCCTCTGCCCTCCAATTTATCGCTATCACT
M1_Spy_1160	AAACGGTATTTCCTATTCAAGATATTAGTCGCGTATTGGAGGAACTTCAGGCACA
M1_Spy_1161	AAATCTACCTTGATGAATCGCTTGGCTGGTAAGAAAATTGCTGTAGTTGGCAATAAAC
M1_Spy_1162	ATATTGCTATTTCGCAGCAGTCTATTCTTAAAGGCGATGCCAATTCCTTGTCTATTG
M1_Spy_1163	TTCAGGTTCCTTGATTACTTGTCAAATTGGGATAGAAGAAGGCCGAGACATTTTTG
M1_Spy_1164	TTGTGATCAGTATCCAGATTGTGAGTTTATCTCGTGGGATTTACCTATTGGACGT
M1_Spy_1169	TGTCTCTCCTTATTGCCATGATTTTCTGCTTCCTTATTGTTTCTCCAATTACAACGGTA
M1_Spy_1170	GCTTATTATACTGATGAAGCCGTTAAAAACCTAGTTGAAGGTGCCCTTAATGCTAC
M1_Spy_1171	CGAGCTAGTCAATGGGAAATTTTTGACACGCTTTTAGATTTGGGCATTTATGAGGA

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_1172	TTACCACTTGATTGGAGTTAGCATTCCCCTAATTTGTTTAACCCTCATTATCCCACAA
M1_Spy_1173	TTCACCAAATCTTTTAACCGAAACCTTCCAATCTCGGAGCAATCCAAACCTTTTCTTT
M1_Spy_1174	TTACCAGATGGTGTCTAAGGAAATTAAACAATACTTGGCAGGCGACTATGGCAAAA
M1_Spy_1175	TAGCCAAAGACGAATTGGCTAGAATTGCTGTTATGACTAGTCTTGCTTATGCTAG
M1_Spy_1176	ATTCAGGTAAAGGCTCCAATGTCTGGAACAGTCTTGTCTATCTTTGCTACAGAA
M1_Spy_1177	CTTGGTTGGCTGTTTGATGCTGGGAAATCTGGTCAGAGAAATTAAAATTGTTCCTA
M1_Spy_1178	GATTTATCAACAACTGACCTAAGATTGGCTTTGTCCTCCTACAATCAATGCCTCATTA
M1_Spy_1179	CTGTGAAACCTTTATCGCAGAGGATGACACCAATCGTATTTTGGATAATTTTAATGCC
M1_Spy_1180	ATGGGGGAATGGCAAAAAGAGGCTGCTAAATATGCCCTTATCATCTTTGTTATCTTT
M1_Spy_1181	GGGCAAAGATTCCCGCTTATATTCCTGCAGACAAAAGTGATTACAAAAAGGTTAC
M1_Spy_1183	ATTGATTTTAGAAGCCATGAAGATGGAAAATGAGATTGTGGCCTCATCAGCAGGAA
M1_Spy_1184	AATCATCGTCTTTGGTTTACTTGCTTTTATCATGGATTCTATCGGTGGGGTCATGTTT
M1_Spy_1186	TTTGGCAATCACATTCGTCAACTCATTCATCAAACCCTAGTGAATTTGAAGGTGAC
M1_Spy_1188	GCTGCCATTGATACTGTTTATTCTGATGTCAATAATACCGAAGGGTTCCAAAACGA
M1_Spy_1189	TGACAGATAATTTGGTTCCTTATCCTAATACCCCAATCAGCATTCCTCAAACAGATGT
M1_Spy_1190	GTCGTCTCATGGATTTAGATGTCTTGGTCTTGCAAAATGATCTGCCTCATTCAATT
M1_Spy_1191	TTACAAGGTAGTTCCAAATGAAATCAAAGACTATGTTCGAGGGCTATACGGTCAATCA
M1_Spy_1192	AAAAAGATACGTTGGAGAAGAGCCAACCAGCCGAGTAACTGCTATTTACAATGAAA
M1_Spy_1193	GAATAAGAAAAGCTCTAGGAGTAGTTGGGAAACTGATGTCAATTGTTCCTGACACT
M1_Spy_1196	TAGTTAGTCATCAATTAGGCCATTCCTCTACTCAAGTCACGGATCTTTATACCCATATT
M1_Spy_1198	GGTACTGGTTACTCCTATTTTGGTGATGGTAACTTTGATGTTGTCTTTTACGATGAAC
M1_Spy_1200	GGCCTTGGTGGTGATATGGATATGAGTCAACTATTTGGTAAAGGGTTTAAAGGAAA
M1_Spy_1201	ATGTTGTTCGAAGTGAAATCTTTGACGACATGATTGCTCACTACCCTCATGATGA
M1_Spy_1202	ATTTTGGCTCTAACTCAGGTGTCTTACTTTACAGACGGCAAACCTTTTGAGTATG
M1_Spy_1203	GGTTTATGGAACCTATTCATTTTCCACCAAATTCACCGGTTATTGTCGCAGGTAAT
M1_Spy_1204	AAGAAATTGCTAAGGCTGGACTTGATCGTGACGTGTGGCAATACTTTACAGTTAA
M1_Spy_1205	TGTTTTTCAGGATGCTTATCAAGATGGTATTATTTGGGCTAACATGGGAGGAGTAGAA
M1_Spy_1206	ACCTGATTTCTACAATGGGTGGGTAACCGATACTTGGGATTTTAGTAAGTTAACCT

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_1208	GGTAGTTTCGGAACATCTTTTGTGACTTTAGTTGCTGTTCCTCTTTTGTATCCTGT
M1_Spy_1209	GAGGGCAGCATTGGTTTTGCTTACTATCAGTCAGATTCAGATCAATTAACATTCCA
M1_Spy_1210	CCTTTTCCTAATACGGGCAAAACAATTCTCCTTGAAAAGCCTACTTATCATCGGAT
M1_Spy_1211	ACATTACATTTCTTATCGTCTTCAGCGAGATTTAGAGCGCAATGGCTATGGTGATAA
M1_Spy_1212	GACTATCGTAGCCTTTATCATCATTTTGAATGCGCGACTTATTTATATCGTGTGTCAG
M1_Spy_1213	CCAACCGATTTTGACATTACCATTCGTGAATTTGTTCCTAAAACAGGAGCAGGTTT
M1_Spy_1214	AACTTGAAGGTCACTTGATAGGAACACGAATGGAAAAAGAGGATGTCTTAGCTAC
M1_Spy_1215	CTAGAACTGACAGAACACATTGCACAATTGATTAGCGCCACAAAAACCATTTACCA
M1_Spy_1216	ATGTTTTAGTCCTAACCATGGCTGTATTGATAATGCGATTCATACGTTTGCCGGAA
M1_Spy_1217	TTGAGAAAACAGATGACCGCTATACCATTAGCATGACACCAGAATTACAAGACGATATT
M1_Spy_1218	TTTAAGACCTACCCTGATGTTGAGGAGGCTAGAAACTATCACTTAACAGAGAAACAAA
M1_Spy_1219	GGGGAAATGATATTGGTTATAGTATTGACGAGTTTTTGCAGTTGATGGATTGGGTACT
M1_Spy_1220	TGTCGCTGATGATGGGATTTTGAATTTTTCGCTTGTTATTCCAGACCATTTTTCGGAA
M1_Spy_1221	GCTGATTATATTCTCGCCAATGATTTAGTTGATATCCAAACAGGTATGCACAAGGC
M1_Spy_1222	GGATGAGCATGACCCAAAAGTTATCAACCATATCGAATTAGCAAAACGCACAGATTTAT
M1_Spy_1223	CGCGACAGTTTTTGATGGAAATATTAAAGCACTACTAACAGCCATCATCTCATCA
M1_Spy_1224	AAACCATTTGTCCGTGATAAAGACGCTATTCAAGCAGTTCTTTTGGTTGCTGAGAT
M1_Spy_1225	CTAGCCTTATTGGTTACTTCGCCATTGCTTTTTCGTTCTTTGCTTGGTTTTTGCTTTAT
M1_Spy_1226	CTACATCATTTTATATGTCGGAAATGCTATTGTTCAACGTGGTTATCCTGAGAGTGTC
M1_Spy_1227	AAAAATCTTGAAGTAGATGAAAATAGAGGGGTTCCAGCAGTTAAGGGGCTATCTCTA
M1_Spy_1228	CAACTGTCTATGGTCTAAAAGATGGCGGTGTTGAAATCGCAACTACAAATGTTTC
M1_Spy_1230	CATTTTCCAGTAGGAGCAGCTTTAAAAACAAAAGATGGAACGATCTATACTGGCTG
M1_Spy_1232	TTATTAAAGACACTAAATTTCAAGGAGAACGAACGTGTACTGGACTTAGGTTGCGG
M1_Spy_1233	CTAGATTCCATCAAAAATGGTCAAACTGCTTTTGCACCCGTCTATTCTCATGATA
M1_Spy_1234	TCAAAAGGTTTGATTCACAAAAACAAAGCAAGCCGCGATAAAGCACGCCTT
M1_Spy_1236	AACGAAACGATATTAGAAAATAGTGAGCATCTCGCTTCTAGTTTAGACGAGGTTCG
M1_Spy_1237	AATTTAGTGGTTGACTTAGCTCGAAAAGAGGTGAAGGTTGAAGGGAAAGTAGTTGAATT
M1_Spy_1239	GTTTATTGGCAGATAGTGGCTTGATTTCCTATGCTGAATTGGTTGACTTAATTGCTCA

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_1240	AGAACAAATCAACCTTATGGGAAAAGCTGTGAAACAGATGGTAGAAGAAGCCCTTAAT
M1_Spy_1241	AGGTATCAGAGATAAATCAATCTTAGACCATGCTGTTGAAAGCTCTCTTAAAGGGG
M1_Spy_1242	CAGCAACAACGCTTGTGTATTGCTAGAGCCATTTCTGTCAAACCTGATATTCTATT
M1_Spy_1243	TTCATATTTGGAAAGTCAATAGCGAAGGGACAATTCCAGATGCTACTCTTGTATCC
M1_Spy_1244	GGAATCCTATCTGGGGTCTGTGTCTTGTTTGTTATGATTTTACCAACAGTGACTTT
M1_Spy_1245	TCCTACGAACACATGTATACCAAAGATAAACCAACAGGGTTGACCAAGGAATTTCT
M1_Spy_1246	GCTAAAAGATAAGCGTCAAGAAGGACAAAGCACTAAACTAAAAGCACCTAAATCCAACT
M1_Spy_1247	GTACTAGGCGATAAACTGGGTTATGTTTTACTAACTATTGACGGATATGAGCCTGA
M1_Spy_1248	GCTCAGGAAAAGCAAATCGATCGTGATTTTCAAAAAGTTAGTGGTTATTCGACCT
M1_Spy_1249	ATGAAATTAGAGCGTTTTTATCGAGAGATTATCGCAAGCAAGAACTACGTCAAGCAAC
M1_Spy_1250	GAGATTGTTTGGTTAGATAAGATTCGAGACATGGAAAAATTCGAGGGAATCGAAG
M1_Spy_1251	GAGTATGGCGTTGCTGATCTTCCTAAAATGGTTATTGATGATACTGAATTGACTG
M1_Spy_1252	TGCTAAGAACTATCAGTCTGCCAGCAACAACTTTAAAAAGGCTATTGACGAGATTG
M1_Spy_1253	CACAAGATCAGCAAATTCGGTACTATCACCAAAAAGTTGAATCGGATCTGAAAGGT
M1_Spy_1254	AAACTGTTAAGGGGCTACTATATCAAGTTCTTTTTGGACTCACCTATTGGTCAAGC
M1_Spy_1255	TGATTAAGGTAAAAGACAAAGATAGCAAGCACATAAAGGAGACAGCCAGTGACTTGTT
M1_Spy_1257	TTGACTACCAAACAGGAAAGCAAATTCTAACCCTCTTACAGGATATGGCACAAACTAA
M1_Spy_1258	CTATGCTTTAGAAGTTTACCTTGCCAGTATTGAGAGCATTATCAGCTATTGGATTGC
M1_Spy_1259	GCTATTGAACATGCTGGTCAGTTTATCGTTTCACCAGGTCCTTATTCTGTTATTGA
M1_Spy_1260	GATGGTGTTTCAGTTGAAGTTGGTAGCAAGGAAGTAGCAGTTGATTTAGCAATCAT
M1_Spy_1261	CTGGAAAAGTTAAAGAGGTTATTGCAGACGCCAAAGATACTGTAAAAGGATTAGC
M1_Spy_1262	CAGTGGATAACGGTGTTGAAAAACTTCAAGATCAAAAAGCTGAGCCACGTGTAAA
M1_Spy_1263	GTGGGGCTAATTATTGCCATATTGCTCATGGCTTTTGGTTTATTCAAAACGCTAC
M1_Spy_1264	TAGCAGTGGCAGATCAAATTAGTGGCGTGAAAGAAGGGATCGAAAAAGGTTTAA
M1_Spy_1265	TTTAGCAGGAATGTCTTTGATCCCGTCAGTTATTGGTGCCGTTATCGTTGTTAT
M1_Spy_1267	GAAGTTCTCTTTATGACCAATGCCAACACCAGAACCCTCTTTGGTAAAAGTAGTTA
M1_Spy_1270	TAACCTTTTCCCTAGTTCTCTTCGCCTTTACAACGATTTTAGGATGGAGTTATTATGGA
M1_Spy_1272	CTTCCACCAAATTACCCCTATCGCTAAGAAACAATTACATCACACACACTATCGT

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_1273	CATTTGACGTTTACAATAGACTTAACAAAGCTATCACACATGCGGTTGGTGTCCAA
M1_Spy_1274	TATGGGCTATAAGGATGAAAGCGGCAGATGCAAAGGTTTTGATATTGATTTGGCTAA
M1_Spy_1275	GACGGTAGAAGCCTTGATTGTTCAAAACCGTGAAATGGGAATAACTCAAATTGTAG
M1_Spy_1276	ATGCAACCTTAGCCCCTATGTTTATCGCTGGTCTTATCTATTTGCTTTTAATTGGACT
M1_Spy_1277	ATTACTGTGGTTAAAGATTTGAAAGTAAAAGGCGCTCCAAAAGACCTGAAACAAGGGA
M1_Spy_1280	TCCAACTCAACTGATTGCTTACTACGCTTCATTACAACGTGGACTTGATGTTGATA
M1_Spy_1281	AAGGTTTAGTGACCTTTAGAGTTTTGCCTCTCAGCGATTTTGGATTTGTAGAAGTAGA
M1_Spy_1282	TTTGATGATTAACTGGGGTGTTATTCCTGTTCTTGCTGAAAAACCTGCATCTACTGAT
M1_Spy_1283	TTAAAGATGGCAAAGGTGGTTTGGCTGTTGGTATTCACAATGAAGAACTTGTTGAAAG
M1_Spy_1284	AATATTCTTTGGAACAGGAGATCGTTGGAGTTGGCATGAGCAAGCATCCTTTAATT
M1_Spy_1285	ATTAGCCAAGAAATCAAGAGTGGTGATCAACTTCCAACTGTTCGTGAATATGCAGA
M1_Spy_1286	AAATGCTTCTGTTATTATCTCAACCCACTTGATTTCGGACATTGAACCTATCCTTGAC
M1_Spy_1287	CGGCGAGGCTTGATGGCCTTTATTGCATATTTTATATTAGTTATTCTGATTAGCG
M1_Spy_1288	TTATGTAGGCATCATTATTGGCAGTATAGCCCTCTTCTTGCTCGTTAAGACTT
M1_Spy_1289	TTTTATTGTCGTGCAGATCATACAGATTGTTTTTCCTGTGATTCCTGGAGGCCTTA
M1_Spy_1290	GCAAGAGAGTTAGGCTTATCGCAAAAGGAATACCAACAATTACAAAAAGGAATGTC
M1_Spy_1291	TTCTCATCTGACCGTACCATTGAGCAGTATAACGAAGATATTTGGCATTCACGAT
M1_Spy_1292	GCAATGGCGTATGCTTGACGGTGAATTGAACCAAGATCACAAAGATTACCTTATTTAT
M1_Spy_1293	GCCTTGGTTGTTGTTGGTGATGTTCTGGCTATTCGTATGGTACAGTTATTATCTTTTT
M1_Spy_1294	AATCAAACGAACTTGCTAAAACCGTTATCACTATGGGTTCTTCTTCAGACTACACTGT
M1_Spy_1295	TTGCGACTGGTATTATCATGAACCTTCCAAGTGAACAAATTGAGGCTGCTGAAATT
M1_Spy_1296	AATCATTGTCTATACTGCCCTTCTTGCTTTTATGGGGCCTTGGATTGACTTTATCTTT
M1_Spy_1297	TAATCGTGAGCGATATGAGCATGTTTCTGCCTTCTTTCCAAGTTTGCACATTGTA
M1_Spy_1298	TATGATTCGAAACTTCTCTGAAGGACTTGGATTGATGGTTAATTGTTTGGCTTGGC
M1_Spy_1299	CTTTTAAGGGCGCAAGAAAATTATACGGTAGCAGTTGGTCTTCAATCGTTTATTACGA
M1_Spy_1301	TTCTGATTTCTTGGATTTACAAGTTGACCACACAAACCTCGCCTCAGTTTTCGAT
M1_Spy_1302	AGACCATTGCCAAGTACCCAAACTGGTTCTTTGATACTCATCTACCAATCAATAAAGA
M1_Spy_1304	ATTGCTCTTTATCTTTATGGGCATTCCTTGCTTGTATTATGGGACAGAAGTGGCAAT

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_1306	GGTAAGGAAGAAGCATCTAAAGCTGCAAAAGAAGCAGCAAAAACTATTAAGGAAGC
M1_Spy_1308	TCCTTTGTATGGTGATTATGAGGATTTGCCTGAAATAACGACCTTTGTTGGGACAA
M1_Spy_1309	GGCGGATTATACCGGCTTAAATCAAGATAAGTATCAAGCGGCAGTTCGTAAAATAA
M1_Spy_1310	GATGATTGGAACACAGTAACTAAGATTGTTCAAGGTGTAGAGGAATTGCAACATGC
M1_Spy_1311	GCACATGAGTTTGTCCTTCTGGTTTAGAGATTATGTGTTTATGCGATTGGTTCATCTA
M1_Spy_1312	TATATCCCAGTAGATGTGCATTCAGCACCAGAACGTATTTTAGCGATTATTGAGATTG
M1_Spy_1314	AGAAGAGATGGATTATGAAAGTATGTCAAGAGGAGAACGAAAAGAAGCGATCAATGCT
M1_Spy_1315	AATAACTACAAACAACTATTGGCAGGACTTGGAACCACGCTCAGTTTAACCCTTATTT
M1_Spy_1316	GGTACTCCTGAAGAGATTTTTGATCATCCTAAGCACCCTCGTCTCATTGAATTTT
M1_Spy_1322	AACATTATGGTAAATACAAAGAACGTACAGTGAGGGTGCTTATTACGGCCCTAGAA
M1_Spy_1323	GCCTTAGCCTTTATTGATTTAGCCAAGGAATTGGTTACCGTTTACGACAAAATGTC
M1_Spy_1324	GCTAATAGCATTGACGTTCTCTTGTTAGGTCCACAAGTCAAATTCTTACTAAAGG
M1_Spy_1325	TATTGGAGAACAAGAAGGTATCAAGAAAGAGGTCAGTTTACCGTCACAGATGAGTTTA
M1_Spy_1326	ACTATCTTAGTTTGTTAGATGATTTGCGACCTTGGCAGGAATTTTGTTTGGAGATTGC
M1_Spy_1328	GCAAAGGCTACCTCTTGTGGACTTTTATTGATTGCTGGTCTTGGTTAAATGCTTAT
M1_Spy_1329	TATTTACTTGTCTCCACTTTATCGATTGCCACTTCTTTCTCAGCGGTTTATCTCAG
M1_Spy_1332	AATCCCAATAGACGTTTGCGCTTAGAACGTGAAAAATTGAAAAAGGATGAGGCTT
M1_Spy_1333	AACGTGGAGCAAAAGATGGGGATCCTGTTCGTATTGGCAAGTTTGAATTTGAATTT
M1_Spy_1335	GCAAACAGGTCGTGCTACTTTGGGTGTTAAAATTATGAAACTAGATGCTGATGCTA
M1_Spy_1336	ATAACTGTGAGATTATTGATTTCACTCTGTCTCGGTCTCCTAACTTGAAACAGGC
M1_Spy_1337	TGACGTATCAAGGTACGCCCTTGGTCTATGAATCCTTTGTTTATTACCTGTTAAAC
M1_Spy_1339	GTCATACTAGAGTTAGGGTGAACAGTAAACATTTTATGGCCAAAGTAGCGGAGTAA
M1_Spy_1340	TTCTCTGTATGTGATTAGTTTTGGAGCCATGTGGTCTATTGGTCAGTTAAAAGCAGAA
M1_Spy_1343	CAAATCAATGGGGTTAAGTCTGGCTATGCTAAATTTGAAACCTTCCCAGTTTGGAA
M1_Spy_1344	GGTTGATGTGACGATTCATAACCAAACAGGAGCTCTTCTAACAAAAACAAGCTT
M1_Spy_1345	CATTTATGCTTGGAAGTGCTCAATACGCTAGAAAAGGATGGAAAGGCTACTGATTTTT
M1_Spy_1346	TTGGAAACAACTATGCCATTTCTGCTCAGTCTTTCTACCAAGTGAATACGGTTATG
M1_Spy_1350	AAAAATGGTGTTTCTGTTTTAGGAAGTGGGAAAAATGGAGTTGAGCACGTGACCAT

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_1351	TATCATCGTAACAGGTGGCGGTGTAGTGGTTTTACAAGAAAATCGACAATTACTGA
M1_Spy_1352	CACAAGGAACAACCTGTATTAAAGATGCTCAAGAATTACGAGTCAAAGAAACAGATCG
M1_Spy_1353	CATTTTTGAGTAATCTGGTTGGCAATTACGTTGTGTATAATGTTGAGCGGATGGTAGA
M1_Spy_1354	AGCAGCTTTAGATTTTGATAACGTACCTGAGATGAAGAAATGGACAGGATCTTATACC
M1_Spy_1355	TATAAAAGAAAAGTGATTGGTGTAGTTAGTATGCGTGATGTGGTGGATCAGTTGCCAA
M1_Spy_1356	AAAGTTTCCTTTAGGTCAGTGGGCAATAGAAGATAAAGCAACCCACCAAGTAATAG
M1_Spy_1357	TTCAGATGCCTTAGAAGCATTAGCGGATCAAACAGACGCTTTACAATCAGAAGAA
M1_Spy_1358	GTGGAACGATTATTGACACTATATATGAAAAGCGCATTAACCATGTGCCAGAGTTG
M1_Spy_1359	GGGAGAACAGATATTGATTTGCCATGGGAGCGTTTAAATAAGGTTGATGCTTTAGT
M1_Spy_1361	GAAAGAAAGTGCTCAAGAAGCATCGGAATCACATGACTACAACCATAATCATACCTAT
M1_Spy_1362	CGCGATTAACAGGCATTGATACAGAAATCAAATGGGTAAACGACATCTATCTAGGT
M1_Spy_1363	GGCTTGTTGCTCTTTAGGGATTTAAGAAGAGTGCATACCATTAACCAACTGACTA
M1_Spy_1364	CAGCAAATGAAATCGCAAAAAGACAGTGTTCAAGAAGAACAAGAAGTAGCGCTTGA
M1_Spy_1365	CATGACACTACCCAATTCTGTTTTTACGGGATTTTACCTTTTTGATGGGCAGGAATTA
M1_Spy_1366	GTTGTTGCTTTACTTTCTGGCATGGGCTTTCAACTAACAGAAGATATCCGTTATT
M1_Spy_1367	GATAACAGTTTTGTTTGGAGGTTTTGGAGCATTACTTGCAGCTAAAAAGTACCACC
M1_Spy_1368	TATTGAGCCAAGTAAACGCTATGCTGATATTGTTATTCCAGAGGGAGTTAGTAATGTC
M1_Spy_1369	GCTCGGGGCATTGATATTGATAATCTTGACTATGTCATTCATTTTGATGTTGCTCG
M1_Spy_1370	TATGGAATATCTAAAAGCAGAAGGATACGAATGTGTGACTGTATCAGAACTCTATGCG
M1_Spy_1371	CAGGTGCAGGGGTACAAGGAGTTAAATATTCTATCGAAGCTATGACAACTGTTAA
M1_Spy_1372	GTGTAAAACTTCCAAATCCAGCTACAATCAATGAAGAATCAATCGTTATCGCACACGA
M1_Spy_1373	AAAATCAATCATGGGTGTTATGAGTCTTGGTGTTGGTCAAGGTGCAGATGTTACTAT
M1_Spy_1374	AAAAGTGGATTATGTGAAAAGTCTTGGCTTCACATCTGCTCCTGTTATCGAAGCA
M1_Spy_1375	CAATCACACAACGCATCGAAGAGCGTCAAGAAAAGAAAATTGGTAAAATCTACTATCC
M1_Spy_1378	CCCAGATACTGCAAACGATGTTAACCCAATTGTTATGAATGGTATTTCAACAGGA
M1_Spy_1379	AGCTATCCTTAGTCAGTACCTTATTCCCTTGTCTCTATCAAATACGTTAGCCATTATG
M1_Spy_1384	CGCTTTATACTGCCCTATTTGTACTTGTGATAGCTTTTATCGCTCCTATTATGGAAGA
M1_Spy_1385	ACCATGTTATTGATTGCCGTAGCTTCTATTTTATCCATGTGCAATGTGCTACTTG

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_1386	ATGAAAATTGCAAAGGTATTTCAGGAACCAGTTGAAGAGGTGTTTCGTCTAGTGGA
M1_Spy_1389	ATTTGCTGATAACTGGAAACAAAAAGACTACTCTGATGTGCTTGTTCTCGTAGCAG
M1_Spy_1390	ATGAGGGTGGCATATCAGACGTTATCTCGGTTTTAGATCCAACTTCTTATCAAAAG
M1_Spy_1391	AAGATATTCGACGAGGTCAGCGTACTATTTATCGTGGCTTACAGTCTTTGTTTGAT
M1_Spy_1392	TGATCTTTTTGGGGCAAAGGAACTGGCTACATTACATGGGTATATATTGACAGCTT
M1_Spy_1393	TTGCTAAAGCAGGCGTTGATATGGAAAAAGGTGATTATTTGGAAGCCGCCTTTAAA
M1_Spy_1395	AACAACTGATGCTTTTTATCCTCAATGGAGACCAGTTCAATCTTCCAGCGGTTTTT
M1_Spy_1398	TTAAAGCCGATACAGACCAGTCACAGGCTAGTTTGACCATTTCAGAAGGAAAATT
M1_Spy_1399	AGTCGTTTCTTTACAAGTATTGAGGATGTGCCAAAACAAGCTATCTCTATGGGAATTG
M1_Spy_1400	CCCGATACTCATGGTCATGTGGAATTGCTTTTGCTTAAAAATACACAAGGAGATCA
M1_Spy_1401	GCTTCACCATTATCAAGAAACAAGAGGTGACAATAAGACCTCAAAAACACAGCAT
M1_Spy_1402	TAGGTGCCATTGTTTGCGAAGAGTCTCCTTATTTGATTGCTCTTTATTCATCTGGTTT
M1_Spy_1404	TTGAGTTTGGTCAATACGGTCAAGGCGTATTTAAAGGAATTTGGCATTGGTGAATC
M1_Spy_1405	TAGGGATTATTTTAGGAATGATGATAGTGACCTTACTTGTCGGCTGTTTGACTGGTA
M1_Spy_1406	TAAAGAAGGACAACTCGAAATCACATCAACCGCTAACCAAGATACTCCAATTTCAGAA
M1_Spy_1407	TAACAGGAGCGGTGAAAACCTTGATTGAGACAGATTACCAGATAAAAACAGGACTTTA
M1_Spy_1408	CTTGGAAAAAGAGGGGGAAAACGAGATCATAAAACGTTATCCTCAACTAAGAGTAG
M1_Spy_1409	ATGTACCTAAACAAGGAGAAGAGATTTCTGTGCTACCAAGATCATTAGTTTCTGG
M1_Spy_1410	TTTTCCTATTGATCGTGACAAACCGAGTCCAGATGCTATTCGTTACCCTGTTAATAT
M1_Spy_1411	AAGACGGTTCTATTGAAGGGATGACTATTTTACCTCCTTTAGTTGTCCACAAGGAA
M1_Spy_1412	TTCTTTACTTTATTACGAAGTGTTTGATAGCAAAGAGGCTGCGATGAGCGCTGAA
M1_Spy_1414	TATACTCCATTTTAGACAAGCGGCCAAAACGCGCTCAAGTTTATTGGTTTGTAAATGT
M1_Spy_1415	TAACTACGATATTACCCAAGATCCAGAAAGTTATGTTCACCGTATTGGTCGTACAG
M1_Spy_1416	TATTGCTTTTGTGCGTATTGTTTCTGGTGAATTTGAGCGCGGTATGGGAGTTAATTT
M1_Spy_1419	CTTTGTTAGTGAATGGTTTGATTTACCTCTTGCGTTTTTACGATGCCAGTCAGTTAAG
M1_Spy_1420	TATGGTAGCAGCCTATGTAGGAAAACTATTGGCAGTAACAGACGAGGATATTATAG
M1_Spy_1421	TTTCCAAAGCCCAAACAAAAGTAGAATTAAGAAAAGCGATTCAGTTAGCCCTGACCTAT
M1_Spy_1422	TAAAAGAGAACTGACCTATTGTTCGATTTGTGGAAACCTTACCGATGACGATCCTT

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_1424	TGTGATTGGAGCTGTTTTTGTAGCTTTTGTGTTTGGTCATTTTCTAGGCTTAACATCAG
M1_Spy_1425	TTCTATGTCTTATTTTTGGTCTATGCTAGGCTTGATTGGCTTAGGTTCGCTTATTCATG
M1_Spy_1427	TTTTAGCTCATCAAGGGATTATTACGACGGTAGAGGTAGAACATCCTATTTACGGTAT
M1_Spy_1429	AGTATTGACACGTGCTATCAAAACAACTAACCTTGCCCTTGAAAATGCAGGTCAAT
M1_Spy_1432	AGCTATCTTTGAACGGGTCACTAAGGAACTTAAAACGATTATGGTAGAAAAAGGCTAC
M1_Spy_1434	GACTCGTATTCCATTTTCGATTCAACTTTCCCGCACAATGAAGACCATTATCAAACAA
M1_Spy_1436	CAACTCAAAACGAAGAAGATAGTAATTTAGAAACCGAAGAGTTTGAAGAAGCGGC
M1_Spy_1437	GTGCAATATTTCTTACAGGTTGTTCTATTTTAGCACTACTTCCATTCCTCCTTCATAGG
M1_Spy_1438	CTTCAGGAATGGGATGAAGAGGCTATTGTCAGAAAGGAGAAGCAAATGATTAGTT
M1_Spy_1440	AAAAGGCCTGTCAGACAGTGAGCAAGCATTGACTTACCACGAACCAAAACAAT
M1_Spy_1441	GGCTTGAAGAGCACGCTGAGCAAAATAAAACGTTGATTACAATGACAGAGCAAATT
M1_Spy_1442	AAGACGGTGAAGCAGGCGAAGGGAACCTAGTCTTTGTACACGTTAATGAA
M1_Spy_1443	GCAAAAGTAGTAGATAAATTCAAAGAGTACGCCAAGGATGAGGGTGACCTTATTG
M1_Spy_1444	ACATCAGTCCAATCTGTAACACTATCAGCTAGCAAAATTACTGGAGGTGTATTAGC
M1_Spy_1445	CAGATGGTGACTTTTGGTCATGTGCAAGTTCAATTGTGGATGGTAATCTAACAGTT
M1_Spy_1446	CCCTATGGCTAATCGGTTAAAATCCATCATCTTTGGAGAGATTAAGACCAACTTAG
M1_Spy_1447	CAACAATGCGTCTATGATGCTGAAGGTAATGTGAAAAACTCAATCCGAATAAGAGGAA
M1_Spy_1448	TCAGTAGTTTTAGTAGCTTAGTGTCTAGTGTTGGTTCAGCAGTAAATGGAGTTATCGA
M1_Spy_1449	GCATTATCTGGAGAGACTGAGGCTTTGGATACTGTTTTGTTAGCTGGTATTTATGA
M1_Spy_1450	CCAACAGCGGTAGTTAAAATCGTTAATTTGTTACTTGGTGATGCTGCTAAGTCTCTA
M1_Spy_1451	ACGAATTGTCTTACCAAATGCAAAAGTCGATGAGGTAGGTGAAATTAAGTATGTTGACG
M1_Spy_1452	GTGTACATCTTAACGCTGACTACAATTTTACAGATACAGCAACTAAGCGCTATCGT
M1_Spy_1453	CCAAGATATCCATGTTGGGAAAAATAGGGCTAATGCTATGGTCAGTACTAAAACCA
M1_Spy_1454	CTATACCAAAAGGAGACGAACACGATTGGGAAAATAAGGAAGTTAGATTTTTCGG
M1_Spy_1455	GAATGGAAGCTGACAAAGTTGGCAAAGACTTAGATAAAACGATGGTTGATAAGCCT
M1_Spy_1456	AACAGCAGACAGGCGAAAAAGCAAAAACAGTAGCAGAAATTAAACGTCAGTTAGATG
M1_Spy_1457	GTCAATACTACTGTTGGTGCTGGAGCTGATGAAGCAGAATCCAAAGATTTAGTAAT
M1_Spy_1459	GATAGTAATGCAGACGCAAATTACAGAGCGTTAGTGCAAGGATTAAGTACAGAAGATT

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_1460	CTCAGGAGATGAAAGATCAAGGTCTAAAGAAACCAGAATTGCCTAAAGTTGCTGAAAT
M1_Spy_1461	GATGAATAAGCGAATCAAAAAGAAACGTAAACTCGAACGGGCTATTGTGA
M1_Spy_1462	CATGAATAAAGTCAATGAAACAAAAGAAGCTTACGAAAAAGCCTTAGAAGCTGCGG
M1_Spy_1463	CAGACGTTTACTTTGATGAGTGGAAAAAGGTTCTACCAGATGCAACACTGGAATT
M1_Spy_1464	GATTTAGTAAGATTAGAGAAAGCCTGGGTAGATAAAAGAACTGCTAATGAAGCTGAGG
M1_Spy_1465	ATGGAGCTATCAAACTTAATCAAGCTATCCCTGGTTTCATGGATGCAGATGTTATC
M1_Spy_1466	GCAATCGTGAGAAACAACGTAAATTGATGAAACAAAAACGGGCTGAACAGAGGAA
M1_Spy_1468	GACAAGTCTCTTAAGTATCCACATCCATTAAGCGCAGCAATAGATCATATAGTTCC
M1_Spy_1469	AAGATGACTAGATGGCAAGTGATTGATGAGCTAAATTGCAGCGAAAGCACCTATTT
M1_Spy_1470	TTGAGCGAGAATTTAATGTCAAAGAGTGGATTGGTGATGGGATAGAACACATTGA
M1_Spy_1471	TAAAAGCTATTATGAACCGCAAATATACGGACTACGTACACAGCTAAGCAGGACA
M1_Spy_1473	GCAAGAAGTCCATGAAATTACAGTCAAAAATAAAAGTGGAGCAAGAATTTAATAAATCGG
M1_Spy_1474	CCGAATCTTTGCCATCTCTGGCAGTTTGACTATGCAATGGGATTTTATTAGTGAT
M1_Spy_1475	TTTGCAGATGGCTGCTTACAAAACCATGCTAGAAGCTAAATACAATAAGCCGTTTGAA
M1_Spy_1476	GTTGGAGATAGGGAAAGAGAAGAGTTTGTCTTTTGCGATACACCTATTGATGAAG
M1_Spy_1477	CAGGGGAAATTAAAGCTAGCGAAGGCAACTTATTTGATAACCTCGGAGACTTAAT
M1_Spy_1478	AAAAGTATGGTAAACGGAATGTTTGACGAAATTTTAAGAGGTAGTATCTA
M1_Spy_1479	ATCAGGCAATTGATGATCTCGGTGGAGTTCTAACTTCACGAGAAGTCTTTTTAG
M1_Spy_1481	GATGGATCAGGAGATTTTTAATTTTTTTAACAAACAAATCAAAAAAGATT
M1_Spy_1482	AAACGTCCCAAGCTGGTCGAATCCAGACTACAAAGAACCAGATTTAGAAGAATTT
M1_Spy_1483	TATCTTAGCGGTCATTGCTAGGGTGCAACACGAAGTCATTAAAAAACACAATTCG
M1_Spy_1484	GCGACAGAAATGCGTGATCATCCAGACTTTAAACAGTTTGTATTAAATCCAACGC
M1_Spy_1485	ACTCAAACATCTATCAGCAACTGGGAAAAAGACCAGACAAAGATTTCAGGTGAGTAT
M1_Spy_1486	AATGGAACCACTTATTCAAGACAACGATTTGCTATTCATCAAGGTATCTAGCCAAGTC
M1_Spy_1487	GGTCGTGGTGGACAATACAAAACACTCAAAAACGATTTAAAGACCGTGGTTACTA
M1_Spy_1488	AAAATCAATCATGGAACGTGTAGGACATGCAGACGCTAAAACTACTGCCCAAATT
M1_Spy_1489	GAAGCTTTCCTTGCTGAAGGTGAAAAAGTACAATTGATCGGTTTTGGTAACTTCGA
M1_Spy_1491	GCTACAAATTGCCAAGCTTTGTTAAAGTGATGCCAAATCAATCAGCTATCGTTGT

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_1492	CCAGCTCGTCAGTATCAAAAACGATTAAGACAGATTATCGAGTTAGCCAGAAAAGATA
M1_Spy_1493	TCGCAGCTTATTACAACCACTCTATTTCGGTTTTAGAAACAGGCTCTATCATCCAA
M1_Spy_1494	ATGTAATAATGCCTAGTGACGATGAGCTATTTGATTTAACTGATGCTGTTGAGACACG
M1_Spy_1495	ATGGGCAAGTTCTCGCTATTTCACACTTACCTCAAGTTATTGCTATTGCTGATTATC
M1_Spy_1496	CATGAATGAAATTGGTATAGTAAAGATTCCTTCTGGCAGTGGACGTTACATCTATGGT
M1_Spy_1497	TTTACAGTTAAGGGACTTGATTTTTCTCCTATTCAGGGTGGTCATGGCAATATCGAAT
M1_Spy_1498	TAACGGTGTATCTGAAGAGTTGATTGATGCTATCTTGTATTCTGTTGACAGTGGTG
M1_Spy_1499	CAGAATTTCAAAAAGGAATGCTTCTCTCAAAAGAACTTCAAAAGACCTTACAAGCGGC
M1_Spy_1500	TGATGAATACGATGAAAGATCGTCTGAGTTCCGCCAAAGAAAACAAGGTTCAGTT
M1_Spy_1502	TATAAAAGATGGTGCGATAGTGATTGATGTAGGAATGAACCGTGATGATAATGGCAAG
M1_Spy_1503	AAGAAAGTATTGGTTTTTGCTATGGCACTTTTGTTCGTGACAAAGACGCTGTTAGC
M1_Spy_1505	CGATCATGCTAAACAACGTGAACAAGCTATTGCAAAGTATGAGTGGGCAAAAGAA
M1_Spy_1506	ATATCACTTTATCGCCAATCAAAACTAAGGGAATGGCTAAAGCAGAGGCTGACAAAA
M1_Spy_1507	TACTTATTCTCCAATTTCCCCTTTACTGGTGGCTGCTTTTTACTACTTAATGGTCACA
M1_Spy_1508	ATTTTGAACGCGATGGTGTTGTTGTTGCAGAAGTTGCCTTTTCAAATGATCCTGAA
M1_Spy_1509	TTAAAAGAAAAGGAACAGTCTCAACTCGTAAACTTAGCCAATGATCTGAAAGCACACGT
M1_Spy_1510	TATTCTTGTTCAAGCTCCTAATGGTTCCTGGTTTTTACCTGGTGGTGAAATTGAAG
M1_Spy_1511	GTCGAAGTATACTCAGCAGGAAATACAGATGTCATTTTTACCCAAGCACCAAAAC
M1_Spy_1513	TACCGTAAAATCCGTAACACCCTTCGCTTCTTGATTGCTAATACATCTGATTTCAAC
M1_Spy_1514	GTGTTGATGAAAGCAATAAGGAGTTAGAGGCTTACCAACTTGACTCTCAATCTGAT
M1_Spy_1515	CCAGACCCTGGATATTCTAGTTTCTAGTTTTCGATTAGATGGTGTAGTTGCTACT
M1_Spy_1516	CTGGGGCATATGATTCAAAAATTGGGCGTTTGATTAGTGGTATCGTTGAACCAATT
M1_Spy_1518	TTATGCTAGATGCTCAGGCTAGACGGTGTTTAGATTTTATTGATGGTGCTAGTAAAGT
M1_Spy_1519	CGATTATCCAATTGCTATTCAAGAAGGCTCAACTTTTATTCGGATTGGTAGAGCT
M1_Spy_1520	GGTAACAAACGTGGTAATTTTGCTATTGAAGGTATCGAAGAACTACGTGAACAAGTTG
M1_Spy_1521	TTATTTCAGCGCGTATTCGTCATATTTTAGATCGTGTGAAGCAAGATTTGGAAAGAGGT
M1_Spy_1523	TTGAAAATCAACCTGAAGTTCCTCTTACGCCTGAACAAAACGCAGCTGATAAAGAA
M1_Spy_1524	TCAGTTAGAAAATGCCACTTATTTTGAGAAGAGGGGCTACGCTAAACAATTACAGGAA

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_1525	TTTTGAGGTTCGTGGGGATGAATTTTTGGCAACCTTTGATTGTTTAAGAGGAGATG
M1_Spy_1526	ATTGGTTTAGTTGGCTTGGTTGCTCTGCTTATTGTTTTAGCGGGCTTGAGTTTTAT
M1_Spy_1527	GAATCTATCCGCTTGCGTAAACAAATCTTGAATAAAGCTGCGCGTGATAAAGCTAATA
M1_Spy_1528	CAATTTGATGCTGCTATTAAGGGACTACGCAAGGACAAACCTGTTCTTATTTACGA
M1_Spy_1529	TTAATTGGAAAGAAACCCAAGAAGTCGGTTCCGTTGTTGAAAAAGAATTGGGCATTC
M1_Spy_1530	AACTTCAGCGTCTCTATGATAGCGGCTTATTGGATAAGAGGGATTACTTAAATGC
M1_Spy_1531	TATATGCGTGGTCCAGGTTTCTTGTACCTTCACCCCAAAATGGATGAATTACTT
M1_Spy_1532	GGGCTAGTATGGTACATTGGGTTAGTGCTAGTTACTGTTACTTGTTACTCTTTTC
M1_Spy_1533	TCATTCTGTTTGTGTGACGACTCAAGTTGGCTGTAACATTGGCTGTACTTTTTGT
M1_Spy_1534	AAGAAATCACCTAAATCAAAATCAAATCGTCGCAAGCCAAACTATTCAAATCAGCAGCC
M1_Spy_1535	CTATTCATCAATCCTCTTATTTGATGGGCCGTTATGCCGCCGAATTAGTTTATACT
M1_Spy_1536	ATGAAGATTGTTCCTGTTACGACTGTTCAAGAGGCACTGGTTTATCTTCGCAAAT
M1_Spy_1537	ATTCATTTTCAGTCTTCCTTAGAAGGTTTGGTTCCTCAGTCGGTTATTGCTCAAGT
M1_Spy_1538	TTAAAAATGGAAGCTGAGCGAGCTATTGATTGCTTGACTGGTCGATTTGACTTAGT
M1_Spy_1539	ACTGAAAATGGCTATCATGGGCTTAATGGTGATATTTTGGTTTGGAATGACCAACTTG
M1_Spy_1541	TTCAGAATTGGTGGACGCAGACCTCTTTATCGTTTTGACAGGAGTTGATAATGTTTA
M1_Spy_1542	GGCCTAACCTATGAAGAATTTGATTATTTGGCCCCATTATATGTGCCATTAGACAG
M1_Spy_1543	ATGGCCTTTTACCCACTTTTAATTCCTGTTATGATTGCGGTTGGTTTTGACAGTATTG
M1_Spy_1544	TTATGGTAAAGACGTTGCTGAAAAATTCGGCGTGAAAGAAATGGAAGTCACTGATGAA
M1_Spy_1546	TAGCTGAAACGAAAATAGAAGCACGATTGACACGCATTGTAACCTTAGCAGATTATTGT
M1_Spy_1547	GAAACCCGTAACCGTGAAACCCTTTATGGTAAATACATTTTCACTCATCACCCAAT
M1_Spy_1548	TAACAACAACAGATATTGCTCAAATAAGTGGGACGACAAGAGAAACAGTTAGCCATGT
M1_Spy_1549	AATTCCAGAAATTGTAGCAACGGTCTGTGGTGATGATACTTGCTTGATTGTCTGT
M1_Spy_1551	TTTTGGGAACTTTTTCCAGGTGCCAAACTAGGTGTCGTCTTATTAAAGGATGTTCA
M1_Spy_1552	AAAATCCTAACATTGACAAACTCATGCGTCTCCGAAAATGCCAACAGTACAAGGAT
M1_Spy_1556	ATTTTTGACGGGCTACGATGATTTTAACTATGCCTTATCTGCTTTGAAATTAGGGGCA
M1_Spy_1557	TTTCTAATCGCTACTGGGATTCTTTTGATGCAGGAATTTACGTAGACGTTGTGACA
M1_Spy_1558	TAAAATGTCAGATAAAGACTATGTGGTCCTAACAGTTGTCTCGCCAGGTCATCAA

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_1559	ATTATTTATACTCTTGGATTGGGCATTCCTTTCCTACTCATCTCTTTTGCGTCAGG
M1_Spy_1561	TATTCGCTTTTATTTATTGGGGAAAAATTGGCAGAGGCGTGTGGAAACACTTGGT
M1_Spy_1562	GAGCTATTTTTATTGATTTGTGGCAGAAGCGTAAGCATACTGAGGTAGAACATCCT
M1_Spy_1563	AAGAAACGCTAGACTGCGACATTAACGAAGGTTGTCTTTATTACCATAGTGTCAATCA
M1_Spy_1564	AGATGCTGAGGCTATTAAAGAAGTTTTGGTTAGCTTGTTTGAAAATGATGCGTCGTCT
M1_Spy_1565	CAATGCTGAACGTTATTGGACAGCCTATACTGGACAACCAACAAAATTGATGATGT
M1_Spy_1566	CGAATTACAGGGTGTCAGGGCTCTGTTACATGATTATAGTGCAGATTTAAGTTATG
M1_Spy_1567	TTCTGGTTTTGACGTCGGAGTATTATGACACGGAAAGAGGAGTTAAATACGATTCA
M1_Spy_1568	GCTAAGTACGACGCAACCCAAGAACGGATTGCAGAGATGAAGAAAATTAAATCAT
M1_Spy_1569	TTAAACGCAATCGCACAGAAAATCGTCTCAAACATAAACCTCTTAAACGCAACATCGA
M1_Spy_1570	TTTGCATAAAATTGAGCTGGGTTGCTACGACTACAACAAGCAAAGTCAGG
M1_Spy_1571	TTTCTGCTGGTAAAAATCTTGTGGAAAAAGGCAGCCAAACAGTTGAAAACCTGACA
M1_Spy_1572	GATGAAAGGGCTAAAGATGCCTTTATTCGTTATGAAAGTAAGTTAGACGCCTACGA
M1_Spy_1574	TGTTGAAAAATCTAGGGGACAGATTGAAGTTAGAGAATACTGGGTGTCTTCCGATAT
M1_Spy_1575	GGTATCATTTCACAGGCTAAGGGTGCTTCATGGATAACAACGAGATTTTGTATTC
M1_Spy_1576	ATTCAACAAAAGACAGATTTACCGATTATTGTGGACGTATCTCATTCGACAGGCAGA
M1_Spy_1577	TTCAAAAAGAAACCGATAAGCAGGGACTTGAACGTCTGATTTACCAATCCATTTCCAA
M1_Spy_1580	TAACTCAAGAAAGTCTCATAGCCATACCTGCCCAAAAAGTCCATCATTTAGAAGCTAA
M1_Spy_1581	GAGCAAGATCAAATGTTAAGTAGAACACTAGTCCAAAGGCAAGACCTAGGAATAAC
M1_Spy_1582	CATTATGAGACGAGGCCTTAAGAAAGAGAACAGACAACAGTTTTTAAAGATGCGTC
M1_Spy_1584	GTGCCTTAGGAATTCGTGGCTCCAATGTGTCTATGCCAAATAAAGAAGCTATTTT
M1_Spy_1586	GCTGATAGCCACTTGAGCCAGTTAGAAGAGGTTATTTTCTTCGATGATATTTTGAC
M1_Spy_1587	AAAATTGAACTACCAGAGTCCCGTTTGCAGTTCTTTGCTTTTAACAAGGCGTTGAT
M1_Spy_1588	GATAGCTTCGCTTATCATGTAAAAATTGATGAGAGTGTTGCTGACTTAGCCATTCC
M1_Spy_1589	GTCTTTATCTCTTTTTAACCTTCGGAGCTCTAGTCTTTTGCTTGGACTTTGTCACT
M1_Spy_1591	GGGACAAGGTGCAAAAAGAAGTTCAAAAACAATTGGACGACTTTGTAGCTAAAGAC
M1_Spy_1592	AAGTCCTTTCTCTTCTTAATAGCGATCCCGAATTGTTAAATGGGCTTGTTTATGGTGT
M1_Spy_1593	CTTATTGGAATGATTGGTTTAACGCCTTGCTTTATGTGCAAAGTGAGAATCTTTACCC

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_1595	ATACTTATTATGAAGCTGCTATGGTGGATGGTGCAAGCAAATGGCAACAAATTAGGAA
M1_Spy_1596	TTTTAACATTCAACACGCCTTTGATCCTCAACTGATTTTAATCGGGGGTGGTGTTT
M1_Spy_1599	CATCCAATTTTTGAAAGAACATTTAACCTATCTCCACAAAGGAATAGAAGCAGGCTCG
M1_Spy_1600	TACTATCAACAGCAAGATAAACTAACAGCTAATCAAGGTCAAGTTACCCAAGCAAGAC
M1_Spy_1602	AAATTAAGCACAGCAGCTCAAGATTTTTACCAAATGGGGCAAGAAGCAGCTAAAGT
M1_Spy_1603	CATGATGTTCTGTGAACTACTTCTTGACTATCTGGGCTTTAGTATTGCTCCTTAG
M1_Spy_1604	AACTACAATGCCAAGGTCACTAATTTGTTAGAAAAAGACAGCAAGCAAAGCACACC
M1_Spy_1605	CGTTATGACTATCTCACAATTAGCTCGCAGATTCATGATGGCATTTTAACCCAGT
M1_Spy_1606	AAAGGTTTACGATGTTCACTATATCCAAAGTGTAGACATGTTCCCCCATACAGCTA
M1_Spy_1607	AAAAATTGCCACCAAAAGCCTTGAAAGATAAAATCACCCAAGCATTACTGACCAAAGG
M1_Spy_1608	GGACCATTTTCCAAGGCCTATATCACTATTTGGTACAAACGTTATCTTGAACTGA
M1_Spy_1610	CAATCATCTTCTTGGAAACTTGAAAACCGTCCATAACCCTGAGATTATCCTTAAAACG
M1_Spy_1613	TGATTTCTTTATCTACACTGATTCTGAAGATGGCGCAACAAACATTTTATACCGTCGC
M1_Spy_1615	TCGAGAAAGACAATTTAATCAAGTGAGCGCTATTCTTGAGGCAGCTAATGTTAGC
M1_Spy_1616	TTTAGAAAGGGGTGTGACATTTCCCCAAATTGATGTTTTTGTTCTTGGGTCTCATCAT
M1_Spy_1617	GGTCAGATAAGCTGTGAGAAAATCGGATCCAAAGTTATTGAATTCCCCTGTGATTA
M1_Spy_1618	TACGCCGCTATTGAAGTCGCTAAACAGTTAGGAAAAGGCAAACATGTGTTAACTAT
M1_Spy_1619	GAGCCTTTGTTGCCCTTGAAAATGGGACAACAGGTCTCATTCATATTTCAGAAAT
M1_Spy_1621	AAGCGGTTGTCTATGCTTTTCGACATCATTTAGTCCCCCAAGACGATAATT
M1_Spy_1622	TGATGGTGTAGGATTTGATATGGATCAGGTAAGGGATTTGAGTTATGGTCTGAAGAATA
M1_Spy_1623	GATTCGTTTCAAGGAAGAAAAGATTGAAGTGAACAATACCAAGCATCAATGGATTGG
M1_Spy_1625	CTAATGAATATCCTGAAAATACAGTCATCAGTCAATCGCCAAGTGCGGGTGATAAATT
M1_Spy_1626	AAGCTGCTAGCCATCCACAAAGAAATATTATCACTCAATCTATTGGTCAAGCTTCC
M1_Spy_1627	TCTTATTATCACACCAGAACAATACCAGACTGATGGCTTCTTTATTGGACAAGTCAGA
M1_Spy_1628	CTTTGTCCTTGATCGTTGTGCAACCGGCTGGTAAACCTAAAATGTCAATAATAGA
M1_Spy_1629	ACCGTTTTGAAGATAACCTAGAAGAGACGCTCAATCGTATTCTTGATTGGAGTCAA
M1_Spy_1630	ACGCTTGGCCAAAGAGGAAGAGGAACGTAAAATCAAAGAACAAATTGCCAAAGAA
M1_Spy_1632	CGTGGTACAGATAGTCAAGAAGTCATAGCTCAACGTATTGAAAGAGCAAAAGAAGA

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_1633	TCTGTTGAGGTTGGTAAGTTAGCTGGTATTTTAGCTGGTGAGTTAGGTGAAAATGTT
M1_Spy_1634	CCAACAGGCATCGCTGATAATTTGGTTGAGACTTATGAAATCCAGAGATCAGAATT
M1_Spy_1637	ACTTTGCATTGGTGGAGGACAAGGAACTGCTGTTATCGTGAAAAACAATACTTAG
M1_Spy_1638	TAATTGATGCACTTGTGGAAAAAGGTGTCAAAGACCTCACTTTAATCTGTAACGATGC
M1_Spy_1639	TATCAAGATGAAGGTCTATGCGCCCTTGAAATTAACCCAGATTACACCTTTGAAGA
M1_Spy_1640	TTCCTCAAAAACGCCTCATTGACGTTCAAGAAATTGCAGACTATGTGTCTTTCCTT
M1_Spy_1641	TAATCCCGAAATGATTCACCGTGTTGCCACTATTGCTTCTAATATCTTTACCATTGTG
M1_Spy_1642	ATTTTGATGTTCGCCTAGTGCAGCCTAACCAAAATTCTATTGAAACAGCCGGTTT
M1_Spy_1643	GCCTGATGGCACTTATCTGTTCTCTATTAATTGTAAGACGGGTTATTTTGTAGGA
M1_Spy_1644	TAAGCAAATGCGTCTGCAAGACTTTCGAACAGACAAGGTTAATGGTGTAGTGATAT
M1_Spy_1646	CCGACTCGTGTTGCTCAATCAGCTACTAATTTTGACATCTTGAAACGTATTAGTA
M1_Spy_1647	AAAATGGTGTGGATTGGTTTATCTTTACAGGGAACTTGGGTTTTGAACAATGGGCTTT
M1_Spy_1648	CTTGCGTTTTACATCAAAAAGGAATCTGCTTTGTCCTTCTTCACTTTTCCACACTG
M1_Spy_1649	TTTACAACAATCTTTACAGCAATAACACGACAACAGCTTCTAGCCAAACGACTTCAGAT
M1_Spy_1651	AAGGCTGGACGTTTGGATTATAGTGAAAGTTTGATGACACATGCTATGGTTTTAACAG
M1_Spy_1652	GTATGAAAAAGTTCCAACTGCTGATTTAGAAGATCAAAAACCAGGCTTGGCTGATG
M1_Spy_1653	GTGTGGCAAAACAAGATGGCCTTAATTGATAACATTCGCAAAGATGCTTATGGA
M1_Spy_1654	CAATAACGAACTAGAAAATGGAATGGTAAGGGGCTTAGAAAATCGTCATGTGCAGTTA
M1_Spy_1656	GGACTTTATGGCGAGCAAAAACCAAAAGAGATAAAACATTGATTGAAAGACAAGC
M1_Spy_1657	TTTAGCAGATCGAAAACATCATTACCCACGTCATTTATCAGGGGGACAAAAACAAC
M1_Spy_1658	CTATTCCAACCTTGATTAATGGGTTAATTGGCTTAACTAAAGGAACATCGCTTGCC
M1_Spy_1659	CGTGATCGCCGTCAAAACCGTGAAAAAGCCTATCAAAAATTAGACACAGAAATGA
M1_Spy_1662	ATGGACTCTCCTGATTATTGGGATCGTTTATGTTCTTGAAACAAGTTCTGTGATGTTG
M1_Spy_1664	GCTGGACAAAATCCAATGTTAAAACCTTTGCTAAATGGACTGGAATAGACATCAGC
M1_Spy_1665	CAAAAACTAGAGCTTAATAATGCCAAGCAAGAAGTAAATGAGTTATCTCGCCGTG
M1_Spy_1666	AATCTATTCAGGACGCTATGGAATTATTAGCCCTTGATGGTCGTATCTCAGTTATTAC
M1_Spy_1670	AACACGATTTACCGATGGTTTTGTTTTTGGGCTTGGTGCTGAAATTGGGATTTCTA
M1_Spy_1672	AAAGGACAGATGGTCTTGATGAATGGAGCTAATCCCAGAGATATTTTAAGAGTGCTT

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_1673	GGACTTATCTTTGGTAATTTAATAGTTGCAGGTGTGCTAGGTTTTGGAGCTGTTATCA
M1_Spy_1674	TTTGTTGGAACGATTGATGAGTTGAAAGAACACCATCCTGACAAAGACTTGGAGA
M1_Spy_1675	GAAAAGAAACGACAGCGCTTATACGATGTGATTAGACAGGCTTATGATTATCCAGA
M1_Spy_1676	AAAATATCTTTGACGAACAGTCAGCAGAGTATAAAGAAAGCATCCTACCAGCAGCT
M1_Spy_1678	CTTACATTAGCCCATTTATCGGTCGTTTGGAAGATATTGGCACAGATGCTTATCAATT
M1_Spy_1680	CAACCAACCAATAGCCAATACCTTTATCAATTAGTAGGCGAAGATAAAAGAGCGCT
M1_Spy_1681	TTTGGCTGAAATTAATAGCTAGAAAAACGGATGGGATTTTAATAGGCGCTCAGCTC
M1_Spy_1682	GTGAAATCTTTGGGACCTTTGTCTTGATGCTTGGTATTTTGGCATTTGGCTTATATGAT
M1_Spy_1683	ACAAGTTACTCAAGAAGATGTGGATTATTTGTTAGGCGTTGTCAATAACCGCTTTCCA
M1_Spy_1684	CTGTTAAAGCAACTCAAGTTTTCACCCAAGAAGAAGATGCAGATGACGATGCAAAA
M1_Spy_1686	AAGAATACAGCTATACCATTGACGCCAACTCAGGGGATATTGTGGAAAAATCTTCT
M1_Spy_1687	TTGACTGGACCTGAAAAAGTGGAACAGGCAAAACTCCGTGAAGAATACATTGAA
M1_Spy_1688	CAGCAGTTAGAACAGGTCTTTGCCCTTAGCCCAGTGATTAATGATTTCTTTGATAA
M1_Spy_1689	AAGGTTTGGTTCACCCAGCTTATGATTACGTGCTCAAATGTTCTCATACGTTTAAC
M1_Spy_1691	AAAACGATTAAACATCCTAACTACTTTTTAAATATCGCCCCTGAGCTGGTGGGCAT
M1_Spy_1693	CAACAGTAAAAATGACAAGTGGCTATGAGATTCCCGTTTTAGGATTTGGAACTTACCA
M1_Spy_1694	TCAAAGCTGGGCATGCGGCTGACTTTATTGTTCTTGATAAGGATTTAACACTTGTT
M1_Spy_1695	TTATTTCCCACTATACCCGCATCACAGATCATGCCTTGAATTTAGCAGAGAAAGTA
M1_Spy_1697	GCTTTACAATCTCGGTTAGACGCTATTTTACCAGCGAAACCCATTATTGATGAAT
M1_Spy_1698	AGGTTTGTGTAGCTTTTATGCTGAAGACGGAGGACTTTTAATGGGCTACGAAATT
M1_Spy_1699	CGATTTCAGAATTGGTGACGAAAGCAGGAGTATCTCGTAACGCTTTTTATCGTAATT
M1_Spy_1700	TTATTAAACCAGGACAAACAGATTCCAAAAGAGAATATCACAGCCATCCAAGAAGCAG
M1_Spy_1701	AATTTGATATTTTAGGGATTTTTATTTTAGGTTTCGTGACAGCCTTTGGAGGCGGAGC
M1_Spy_1704	TAAGCGTTTTGGCGTTGATGTGCTAAAAGTAGAGGTTCCTGTTAATATGGCTTATGTA
M1_Spy_1707	GAGAATAAGAAGCTAATTGCTAAAATAGCCCACTTGGAAAGTCATCATGCCAACCA
M1_Spy_1708	AGAGATTGTAGGTCCAGAATTAGCGAAGAATATCGTCAAAGGTTTTGTGACTGGT
M1_Spy_1709	GGTATGCTAACCAAATGAAGGCACGTAACGCTGAATATGCGAAAACAATGAAAT
M1_Spy_1710	CGTTATGATATTTTTACGCCGATTGCAAAAACTGACTTGGGATTTGAGATGCCGAT

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_1711	AATATTCTATTTACTGAAGCAAGGACGCAGCCAGAGCTTTTTGATCTTGTTGCTAG
M1_Spy_1712	CGAACGTTATAGTCAGTTCACGCAATTATTAGTAGCCCACGAAAACAAGGAGTAA
M1_Spy_1714	GGACGTCCTTTAACGTTCCTAATCAAGCGTGCTTTGAAGGATACCAAATTTGAAT
M1_Spy_1715	CAGGTTTTGCCATGAGTTTTAGTTCAGTATCAGTTGTTTTGAATGCCCTGCGATTAAA
M1_Spy_1717	TATCAAGCTCTTTTGGATGAGACTTTGCTACAAAAAGAAGCACTAGCGACTGTTT
M1_Spy_1718	ATTACCTTTATTACCCAAGAAGGCATGAACCACGTTTACCCAATTTATCCTATCGAAGA
M1_Spy_1719	TAATCATCGTATTGACCGCGTTGGGATGGAAATCAAGCGCGAAGTAAATGATATT
M1_Spy_1721	GATGACGTTAAAGAAGTCGGTAATGCTCAAGAAGGTGGGTTGATGATTGAAAACTT
M1_Spy_1722	AGCTCAACGGGCTGGAAAAGTGATTTCAGGTGAAGAACTAGTAGTAAAAGCTATT
M1_Spy_1723	CAACTGGTAAACAAAATGGTCGTGGTGCTTATATCAAATTAGACAATCAAGAGGCC
M1_Spy_1724	ATGCTCTTGCTATCAGTTCTGCCTATGAATTGGGTGATAAGATTCGCTTTGAAGAAT
M1_Spy_1725	CGCTGTTAGATACGATTGATCCAGATCCTTTTCCAAACCAATATATGCTAGAAGTC
M1_Spy_1726	TACAAGGATTTCTTAGACACTTACAAGCGTATCTTGCCAGAACACGGTGAAATTC
M1_Spy_1727	CTTACCAGAGTTCAAATCAGAAGTAGCAACGATTGTGCATGGAGATATTAAACATAGC
M1_Spy_1728	GGCTGTCCCTTTATGCTAGAGCCTTTTTACGTAGTTCAGATTATTTGGGCTTATTT
M1_Spy_1729	ATGTTTTGGACTCTGCTGAAAGAATGTGTGATCGTTTTGTGATTTTGCATCATGGACA
M1_Spy_1730	GCTACTGCCATGAATATCATTAACAATAACGAAGCCTTAGCGGGACAAACTGTTT
M1_Spy_1731	AGAGCTGTTATCCATCAGTAGACAAGCAAAAGATGCTGCCCAACTTGATTTAGATAA
M1_Spy_1733	TTCAGGCCAGGCTCCATCTTATTCTGATAGTCATAGCTCTTACGCTAATTATTCAA
M1_Spy_1734	GAAGTATTATCCCAAGGCCAAGCTGAAGCAGATGTATTTATGTTAGTTGCTAAGACT
M1_Spy_1735	GTTACCGTTATTGAGTGGGGAGAATTATTAGGAGAGGGATTATTACAAGACTATTTGC
M1_Spy_1736	GCTGTGTTACTCTATTTCCTATGGTATTGCCGCTGCCTTTATTTTCTATTGTCTAG
M1_Spy_1737	CAATGACACTGAGATGCTAGCTTTTGCTGGAACTGGTTATGCTATGAAAAATGCTA
M1_Spy_1738	AAGCTTCTAAAGATCCTCGTTTTGGTAACACACACGCACTTATTCTTTTCCAAACC
M1_Spy_1739	ATTTCGATTATTTCTGCAATTTTGGTCGTTATCATTGCCTTCTTCGCTGGTCTTGAAG
M1_Spy_1740	CTTATCCCAGGTTTAGCTGGATTACTCCTTACATTCCTTTGCATGTGGTTATTGAA
M1_Spy_1741	CACTTTGAAATTTTTACGGACCAAGGCAAGTTTTTATTTGCTTCTGGAGACTCTGG
M1_Spy_1742	ATTCTTCAAAAACTTGGTTTGCCATATCGCGTCATTTCCCTTTGTACTGGAGACAT

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_1743	CAAGTGAAATCGTTGACATCATCAAAGCTAACCTCATCGAACAAATAACCAGTTTGCA
M1_Spy_1744	GTCATTGAGACCACAGTTCGTGAAAACTTGCCAGATGATTTCCAAAAAGCAGAATTT
M1_Spy_1745	ATTTCAGATAAGCGAGTGATTGCAGGAGATTATGATACCTCTTTCTTGATGGAAACGT
M1_Spy_1746	ATGTGACTATTAATGAGCCTTTCTTTAACGGGCATTTTCCTCACTATCCTGTGATG
M1_Spy_1747	TTTTGTAGCAGAAGGTGATATTGTTGAAAGTCCTTTAGTCGGTGTAGCCTATCTTG
M1_Spy_1748	ACAACTGAAGATCCAAGCAGAAGCTCTATTCCATTTGATAAAGACCGCAATGGTTTTA
M1_Spy_1749	TAAGTCAAATCCCAATGAAACGTATTGGAAAAGCTCAAGAAGTTGCTCACTTAGC
M1_Spy_1750	GGCTAAAGAGTTGGAAAGATACAACTTCAGTGACTTCAAGATTCCTTTGGTTGGTA
M1_Spy_1751	TTATTATGGTGCAGCTCGTGTTATTCAAAATGAAGCTAAGCGCTGGCAATCTGTTT
M1_Spy_1753	CAGAAGAAGGATTAAATACTGTTGGTGACCTTGTTGCTTACGTTGAAGAAAAGTC
M1_Spy_1754	CGTCGTATACTTGATAAAATAGCACGCAAGATTGATGTTCCTCGGGAAAAATTCCT
M1_Spy_1755	CCACTAGCTTAAATAAATTAGAAGCAAAAGGTTATATCGCAAGAACGCGATCACGC
M1_Spy_1758	TTAAAGAAGGTGTCATAGCTTTTGGAGAAAGGCGCCGACCAAATTTTCAAGGTAA
M1_Spy_1759	GGAGAAGCTGGCTTTAATGGCGGACCTTATGGTGATTTATTTGTTATTTTGAATG
M1_Spy_1760	GCGAAACGTAAAGAAGAAGTTGACCTTAAAAACGAAGTTGACCAAGCTATCTTTGC
M1_Spy_1761	ATGAACATCCAGCCGATAGCATTGCAGAAGTCTTTCAAAAGGGATATAAATTGCAC
M1_Spy_1763	CTCCTGAGTTTGATTGGTCCTATTGATATGGATTATCGTAGGTCTGTTAGTTTGGT
M1_Spy_1764	GCCATTACCCTAAAGTCCCCTAAACAAACCAACCAAAACGTGAGATATGCTATTTATA
M1_Spy_1765	CTTGCTATTGACATGAGCACTGTAGATACTTTAAATGCCAGTGATCCATCAGTAG
M1_Spy_1766	TTTGATGATGGCCAATTTACGATAAAGACTATTGGAGATATGAGTTACCGCTACCG
M1_Spy_1768	CCCGTCCATTTTGGGAGCAGCAATTGTAATTGTACTTGTTTCAGCTATTTTTGGA
M1_Spy_1769	GTTAGTGACCGCCTATAAAAAGACCATCATCTATGCTAAAATAAGACTGACAGGT
M1_Spy_1770	TTCTTGATGAAGGCAACCAAAGGTCAAGCTAATCCACAAGTCGCTCAACAATTATT
M1_Spy_1771	TTGAAGCGGTGACAGATTATCACAAGCAACAACCGATTATTTTTGGAGGTGACAA
M1_Spy_1772	TTACAACAACCATGGCAGATAAGAAAAATGTGATGCGTCAAGATGTCGCTGAAGAA
M1_Spy_1776	CCATCTTTATGGTCCACTAGCTGAAGTTTACAGTTATATGAAGCAACATCCCAGA
M1_Spy_1777	TAAACTAGAAAGTGCAGGGATTATTGAAAGTCGTTCTCTTGGTATGAAAGGGACATAC
M1_Spy_1779	CCAGATCACTTCCGTATTGTTTACCTACCAAGTGTAGAAGAATTAGAAGATGTCCA

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_1780	AGTACATTATGCGACACGCTAAGATTGACTTACTTGTAGTCCGAGATAGCACTAAA
M1_Spy_1781	GCCTTAGCGGATTTGCTTTATTTTACTTATGGTTCTTTTGTACTGATGGGAGTAGATC
M1_Spy_1782	TTGCTCTCAACGCCGGACTTACTGGTCAAGAGCTAAAAGATTATATTGAAGGATAA
M1_Spy_1783	TTCTTGTATGCTACTTGTTGTCATTGCTGTTGGTGTAGCCTATAGTTTTCGCAAGA
M1_Spy_1784	TTTAGAATTGGGGCTTAGAGTTGAGACTGATGGTCAGATGCTACTAATAAAACCAACT
M1_Spy_1785	AGCACGCAAAGTTTCTGCAGCGATTGTTTCTGATCCTAACTGGATATATGAAAAAC
M1_Spy_1787	TAAAGCCTATCTTACCCAATTAAAAGCAGCAGGAAAAACCATTATTGTAGCCGAGCAT
M1_Spy_1788	GTAATCCCGCTATCGATTTTCCCTTCTTATCTTGACCACTTATTGAGTTTTGTCTC
M1_Spy_1789	AGTGGCCCTATTTTGCTGATGTGGTTGTTGAAAGATGCCTATGTTGAAAGTTTGTTA
M1_Spy_1790	TTAAAACACATTGTCAAGGATTGGTCTTCTTAATTTCTCACAGGCCATCAACCCTAG
M1_Spy_1791	GCAGGTTCTTTATGTATCGGACCAATCTACCTTATTGAACCGTAGCATTTACGATA
M1_Spy_1793	CAGTTTATCAGAGATGTCTTTGGTGTTGAATTGCCCTTTGTTGAGAGAAATGGTCA
M1_Spy_1794	TTTTTCAGCTTTGCTTGGAGCCTTTGTCTTTTTGCTAGCCGATACTTTAGGAAGAA
M1_Spy_1795	AACATTTTACGGCAGTCAAGGAAGGGAAAGTCTATGATTTGGACAATACCCTGTTT
M1_Spy_1796	CAGGTGCTATGATAACTCAGAATAAGCCTAAAGCCAACTCATCAAATAACAAGAGTCT
M1_Spy_1798	TTGCAGGTCTATCTAGCTTTATGATCGTAGCTCTGGGATTCATTATTGGTCGAAA
M1_Spy_1801	TGGCACTTATGGCCACGTATCAATTGTAGAAGATGTTAGAAAAGATGGGTCTATTC
M1_Spy_1802	AAGATGATTTCAGCTGGTGATACAGTCGGTTATGGAGCTACTTATACTGCCAAAAA
M1_Spy_1804	CTGAGCAGGAATTGGCTATTTTTGAGAGTTTTCCTTACAAACGTCGACTCAACTAT
M1_Spy_1805	GAGTTTGATGTAACACGTACCATGATGAAAGCACAAATTCACGAACAAGAACGTGA
M1_Spy_1806	TTTCAAAATTGATGTCCGGGCACAAAAGGATGAAGAGAAAGTTTTCATGCGTACG
M1_Spy_1807	TTGGAAAATTCGCTACCCCTTATCAACCATTCTATTTCTTGTCTTCGTTTGTCAGTTAG
M1_Spy_1810	ACTTATCGGGTTTATGATTTTGACCGTAAGGACGTTAATGGTAATCTTCGTGACCTT
M1_Spy_1811	ATGTGGATTTGTTAGGACAACTTTCGGCTGCTTTTAAGATTCCTTTTGATGTGACAAC
M1_Spy_1813	GCCCGAAATCCTGAGACAACCAATAAACCTATTCAGGAAGCAAGTCTACAAATTTT
M1_Spy_1815	AAATTGTCTTTGAAACAGGTCATGCCTATGCTATAACGTCAAGTCAAGGAGCAGAA
M1_Spy_1816	ACCAAAAAGGGAATGGCCTAAGATTGAAAGTGGATACAACAAAAGGACAGTTAAGCAT
M1_Spy_1817	GAAGTGCAGAAATCATCAAATTACCAAATAACCTATCTCCTGTTAGACGCGAAATGGAA

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_1818	TTTATTTAACATAGAGATGGGAGCTGAGTTATTGGCAGCTTCTCAGTTTGCCTATG
M1_Spy_1820	CTGCAGTTAATATCCATGTTGAAGGTATTGTTGCGGAAAAGACACCAAAACCAGA
M1_Spy_1821	TCATTGAAACTGTCCCAGCACAATACCTATACAAAATGGATGACACTGCTTACTTCAT
M1_Spy_1823	GGGTCATTATATGGAAGACGGACATTGTATTCGCACTGTTCATGCTGAAATGAAT
M1_Spy_1824	ATGGTGTCCGTATTGAAGATGACTTGGTTATCACAAAAACTGGTTGTCAAGTCTT
M1_Spy_1825	TAGACGATGGTAATACGGTTCTTGTGATTGAGCATAATTTGGACGTTATCAAATCTGC
M1_Spy_1827	GCCTAGAACATGCTTTTTGGTATATCACCTTGATTGCTATGCTCCTTAGTTCCTT
M1_Spy_1828	GCTTTACGCTATTACCTACAACGCAAATACAACATTCAAAACACTATGTCACCTGT
M1_Spy_1829	GATTACAAAGATACTGAGCTTCTTAGCCGTTTCGTTTCAGAACGTGGAAAAATTC
M1_Spy_1830	TAATGGTGGTTTTAACAATAACACTTCATCATCAAACAGTTACTCAGCGCCTGCACAA
M1_Spy_1831	CGAGTTTGACCGTCTTTCAAAAATCAATGGTGACATTCTTCGTCACATGATCGTT
M1_Spy_1832	CATGACGCAAGTTTTTCCTGGATTAGGTATCGTATGTGCCATGGTTGCTATTATT
M1_Spy_1833	AAGTGGACAATAGAACTCATAGAGGGGGTGGTCAAAGCAACAGATTTACCTAAT
M1_Spy_1834	CCCCTTATTGTCACTGTATTTTTCCTCATTACTGTTGCTTTTGCTTTTGCAAGTCG
M1_Spy_1835	AAAGAAGGTTTAGTACTCATTGATTTTTGGGCAACTTGGTGTGGTCCATGTCGTAT
M1_Spy_1836	GGCAAGGGATGACATTTCAATTATGGTTAAGGTTATTAATTATTCCTGCTGTTGGC
M1_Spy_1837	TATCATTCATGGTATTGGTACAGGCGTTATTCGTGAGGGAGTGACAAAATATCTTC
M1_Spy_1839	CTTCAATCGTTTTATGTCTTAGGAGCCCTACTTTCTCTTTTGGTAGCTAATCGAT
M1_Spy_1840	CAATCTCTTTATGGAAGAAGTTGAGCGGGTAGCTAAAGAAAAGTATCAGGCTCTTA
M1_Spy_1841	GCTAAGACAGTTTTTGAAGCAAGCAACAATAGCCAAGATATACCTATAATCGGCAG
M1_Spy_1842	CCTTATCGGTGAAGTGAAAGCTCGTTTTTGGCCACTCAATAAAATGACCGTTTTT
M1_Spy_1844	GACTCTTGCAACGAAATGTGATTTACACGGCCATTACTCGGTCTAAAAGTAAGTTA
M1_Spy_1845	TTACAAGGCTAAAACCCTTAACGAAAAAGGTGATGATACAGACCAAGCTATGCTGT
M1_Spy_1846	CAAGACCATAAAAAGTTAGGCAAGACCATTGTGCTCAAAGTGCGTTATGCTGATTT
M1_Spy_1849	CTAACAAAGCAAAAGGTGGCTGGTTACAAAACCTTAACTCACTTGCTAAACTGGAATT
M1_Spy_1850	GACCAACCGTACTTACGACGAGGATGATAAAAGTAAATGGTTAAAAGAACGTCAG
M1_Spy_1851	ATTGCTATCTTTTCATCTTGGGGAGAAGGGCAGGAATGTTTTGTAATTGAAGAGTCA
M1_Spy_1852	TGCTGGAATTGTTTCTGGTGGTATTATTGGCGTTTTACTAGCTGTGTTTTTCTTAACGA

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_1854	ATTGGCGGTTGCTCACATTGGTATTGGTTTCTTAGTTATGGTCTTGGTTACTTCATTA
M1_Spy_1856	CACTTATTCTGGTTAGCTGCTGTGATTCCACTGATTTGTACCTTGCTTTACTTGTTAA
M1_Spy_1857	CGTATCGGTGCCATTTCTACTCAAATATATGACTTAATGACCCTATTTGGCGAAG
M1_Spy_1858	CATCTTATACACAACCGATTTTGAACATACTGTCCGTGACAACAGCAACTATGCTT
M1_Spy_1861	GGTCTATTAAGTGATATTGCTGCTATCTTTGATGTTAGTTTAACCGCCCTAATGGAAG
M1_Spy_1862	GCCCTTACGCAAATTGATGAAACAACTAAGAAAACATTAGGCGCTTTTGCTGGTAA
M1_Spy_1863	GTTGCTATGGGACTATTTCTACCAGGTTTCAGTTGGTTGGTAATTTATTTTAGGAAGC
M1_Spy_1864	ATACAAACAAAGCATATCTCAGCCAGCAAGAAGAGGTAACCACAGATAACCCTTT
M1_Spy_1865	CTAAAATTAGAATTGGAAGAAGCTAAAGCAGAAGCCAACCAAGCTCGCTTACAAGT
M1_Spy_1866	ATCAGAAGATTACCCCGAAAAGGTCAAACAACTCTCTCCAAAACAAGAAAAGGAATTG
M1_Spy_1867	AAGAAGAACTTATCGAACTTTGTGGAGTTGTCACACGTTCAGGTGCAGACTTTATTAA
M1_Spy_1868	TGAGATAATGGTTGTGTTGGTATCAGGTATGGGAAGTATGTCAGTTTCTATTCTTGGT
M1_Spy_1869	ATTAAATAAATGGGAAGCATGGAAACGTCTGGGAACAAAAGCGTCTGAAATGGAATCT
M1_Spy_1870	AGAAAATCTATGAATCTGGTAAAGCTTCTCATTCCTGCAACCAAATCGGCTATACC
M1_Spy_1871	TTACATGCGTAAGTTTGGTGTTAGCCGTATCAATTTCCGTGACTTAGCTCACAAA
M1_Spy_1872	ACATATAGAAGTTGTGATTCCTAAACTAAGACTCTGTGGAGATAACGCGGGTATGATT
M1_Spy_1873	GTCTCCTTGGACTTTAGAACAAGTATTAATCGATATACGGCGGGATCAAACAGATT
M1_Spy_1874	AGAGAGTGGAAGCTGAAGAAAACTGGCTCAAAGATAATGAGATAAAAGATGATAGTCAC
M1_Spy_1875	TATCAAGGCTCGTCGTCTCGTTGAAGATAACACCTATTATAACGTGGAATTTATCG
M1_Spy_1876	ATCAGTTAACGGTGCTATCGTCAATGCTCTTCGTCCATTCTTGTATGAAAAGACT
M1_Spy_1877	CAAAATTGAAGCTCCAGAACCCGTTGAAGCTAACATTTATACCATGACAATGGAAG
M1_Spy_1878	GCTGACACAAAGCGGTTTTTCTACTCCCTCACAGCATATTGGTAATTTTCGTATTT
M1_Spy_1879	TAAATCAGGTATTATTGGGGCAGTGGCTGCTCTTTTGATTATTACACTGTCTATTCCA
M1_Spy_1881	TCTTATCGGTGGTGGTATGACTTACACATTCTACAAAGCTCAAGGTATCGAAATC
M1_Spy_1882	AGAAGAGTTTGGAAGACGCTTTATCATTTTCCCTAATCCTATGTATGGTTCATGGG
M1_Spy_1884	GAAATTGCCTTGGAAGTACAACATCAAGTGGCAGAAGAAATGTCAACCTATGGATATA
M1_Spy_1885	TCTTTGTTGGTGAAGAAGGTGATCAAGACTTAGCAGAAGAACTTGCTGGGTATTTA
M1_Spy_1886	ACCTTGAAAGTCAGCTTGGTCTCACTGCTGATATGGTTAATGTTTACGTGCAAAATAT

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_1888	CACACGCGATGAACCAAACAAAACGTACAGTTAAACCAAACCTTCAAAAAGTTAC
M1_Spy_1889	AGAAGCTAAACAAGCTCCAGTTCTTATCCAAACATCAATGGGTGCAGCAAAATACAT
M1_Spy_1892	ATTGTTAAAGGGGCAAAACACGCCAAATCCTTTGAAACAAACCCAGAACAATACCA
M1_Spy_1894	AAATTGATATTGAAGATATGGGAGGTACGCTTCGTCTGGGATTGTACCCATGTAAATT
M1_Spy_1895	GCTGAGGTTGAATATGATGAAGAAGATCCAGATGATGAAAAATCAGAAGTGGAGTCAT
M1_Spy_1896	AGTGAATGAATTTATGGGCAACATGCAACGTCAAGGAATCTCACCTGAAATGTACT
M1_Spy_1897	GACTTTATCAAGAAGCTTTACTCAAAGAAGGCATTCAATTACCGACTGCAATCAGC
M1_Spy_1898	ATACTGCTATAGGAATGCATATTAAGCGTGTTCAGATCCCTCTGATTCCTTGTCAA
M1_Spy_1899	AACAGGGATATTGACAGCATTGGTTGTTGTTTTAGGAAGATTTGTCATGCTACCTACT
M1_Spy_1900	ATAATATTGGCGCAGGCTGTACTTTTGCATCTAGTATCGCTAGTCAGTTAGTAAA
M1_Spy_1901	TCTATAAGCAGGTGAGGAATATGGTGGGAACTTTGCTGAAAATTGGTAATGGTCAAAT
M1_Spy_1902	CAGTTACTAGAAAGGTACCCAGAGTTAATTGAGGAAGAAGAGCGTTTATATCGCTA
M1_Spy_1903	ACAACAGCAGCCAATTTCCTTAGAAGCAGCGATAGATAAAACAACAACACTTTCT
M1_Spy_1904	GCATGTCAACCTTAAAAGAATCCCTAGACTACCAAGCCTATAAAGAAAAACAAGGTGA
M1_Spy_1905	AATGGTACTCAATGATAAGACAGCGAATGGGGCAATAATCGGAAGATGTCTACTTAT
M1_Spy_1906	ATAACTTAAATATCCCACGCTATGTTGATACCTTTGAGGAAGTCCCAGTTAAACCACT
M1_Spy_1908	GAGATAGCAAAAGAACTCATACAGTCTACTCCTCATTTGAAAGTTGTTATGTTGACAGG
M1_Spy_1911	GAGTTAATGAAAGCTCAAGGCAGAACAAATTCTATTGAAGGTAGTAAGTCACTCC
M1_Spy_1912	ATTATTTTTGCTGACGAACCAACTGGAGCACTTGACTTGAAATCTTCTGAGGAAACAA
M1_Spy_1914	GGATACCAGAGGAAACGTCTATAATTCTGTAGGGACATTAATTGGAAATCTAGGAG
M1_Spy_1915	TGACTAATGCTATCGAAGAAGTTTCTGAAAAAGAACTTATGGAAGTAGCTGGTGG
M1_Spy_1916	CCTTGGTATTAACTACTATATGAGTGATTGGATGCAAGCTTTTGATGGTGAGACTG
M1_Spy_1917	TTGGAATCACTATTATCTTCGGTGCATTTGCTTTCTTCTGGTTTGTTGGTATTCATGGA
M1_Spy_1918	CTACTCTTTTAGGTTTTGAAATCGTAGCCTATGCTGGTGATGCCCGTTCAAAATT
M1_Spy_1919	TTATTTGAGTGCTGGTGTTTCTGCCAAATTGTTCCAAGATACTTTGGTGTTTGCAG
M1_Spy_1921	ACGTTGACATTCCTAAAATCAAAATTGTAAGCGCTGTAGGTTCAGGGGATTCTACA
M1_Spy_1922	TAGCTTGCAACATGCTAGCCAAGATCAAAACAACCCACACTTCTTTGATGAATTC
M1_Spy_1923	CCTTAAAGAAGGTAAATACGAAGTCGTTGATGTTAGTGAAAACGAAGTACGTGAC

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_1924	TATTGCGACGTACAGTGATCTCGAAGGAGAAGTTCAAAGGGTTGCATTGAATAAT
M1_Spy_1926	GTCACAAACGAGGTTGACTTATTGACGGAAGAGGAGTTAGAGAAAGAAAAACTTT
M1_Spy_1927	TGTCCTATACCATATCGAGGGGGAAGAAAATAGGATCGTTATTGATTACTTGCTTC
M1_Spy_1931	CAACCAACCTTTTGCAGTTACATCAACTGAAGGTTCATACGACGTTTTCGTTAAC
M1_Spy_1932	TGATTGAAAAATCAGTTAAAGGCATGCTTCCACATAACACTCTTGGACGTGCACAA
M1_Spy_1934	AATGAAGAACCTCAAATTAAAAGCAGCGCGTGCAGGCAAAGATTTGTCTCAACAA
M1_Spy_1935	ATACACTATTGGGTTTTGGTTTATGATTGTGGTTATCTGGCTGTCACTCTTTATTAGCC
M1_Spy_1936	AAATGGTGGCTCAAGTGGAAAAAGATATTGCGGATTCGACGGTTATTGTCTCTTATA
M1_Spy_1937	CGTGTCACTGCAAGAGAATTAGAGCAAAGAGTTCATACTGTCAAAGCTGATTTAGAT
M1_Spy_1938	TGAATGGAACACCGTCTGATTGCTGGAACACTAATGGTAAACTTGCTTTAGTCATT
M1_Spy_1939	CCCTTGGGTCTTATTAGGTCTAGTAGTCAGTTTAGCTGCAGGTTTATTTATAGCT
M1_Spy_1940	GAAAAAGAAGAAGACATCTATAAGCGTGGTCGCAATACCAATAGCCATACTAAAGCT
M1_Spy_1941	GTTTTTGGCATTATCTTTGAAGAAGAGGTGCTTGAGGTTGACATTGAAGCCTTGAT
M1_Spy_1942	TACACTGGTGGCTAGTCTAATCTTTTTACTGAGACAAGACATGGTTAAAGCGATT
M1_Spy_1944	TGCCAGCTAAGATAGTACGAGTGCATGGGCAAAAAGATAATCGTCAAATTCAAAGTTT
M1_Spy_1945	TTAAATACGCTTATGAGTGATGACAATGTGGCGGCAGATACGATGGAAAAGGTTTA
M1_Spy_1946	ACTAAAGAAATTATTGCAGGTCTTGTTCGTGAAGCTAAGGTAGGCGAAGTTTACCAT
M1_Spy_1947	CTTGTACCACTGATTTGATTGCCAAGTTGCCTCTTCAAGGAAAAGATTTAGAAGACTA
M1_Spy_1949	AGCAGAAGAATTAGCAGCAGGTCAAAACCCATTTATTTTTGCCATTAAGAGTGGTCTT
M1_Spy_1950	ATTGTAACGGTTTGTGGAAACGGTATTGGTAGTAGCTTGTTACTTCGCATGAAAGT
M1_Spy_1952	GAAGGCTTTGCAGGAACTGTCGTTGATTTTAGATGATGATGAACATATTGAGCAA
M1_Spy_1955	AAGTAGCAGTTCTTACTTGGGAAATCAACCACTTGAACAGCCACATCAAACAACA
M1_Spy_1956	CCATCTTACCTCTTTTAGCTGGTTATCTTATTAGGCAAGCAATCGCACAGGTTTA
M1_Spy_1957	TACATTATTAGCAAACCGTTTTGGAACAAGCGATCGCCATCTTAAGCACGTACTT
M1_Spy_1958	TTGATGTGTCAAAACGTATCATCGCTGTGCTCGTTCCTAATCTTCCTGATAAAGAA
M1_Spy_1959	TATTGCCGTTTATCCGAACTTCAGTAGCAGGAGAGTTTACAAAAGCAACTGTGAAT
M1_Spy_1960	GATTAGCAAGTGCAAAGACAAAACGGATAAAAGAGCCTATGTGGTTACCTTAACAG
M1_Spy_1961	GATGATGTAACGGCTTCTTGGCAGACAACTCACTTTAACTTCCATGATATTGATGAA

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_1962	GCTGAAGGTATCGTGGAAATTAAAATCAAGGCAACAGGTGACAGCATTGAAGTTA
M1_Spy_1963	CTACTATTGTTGAAGAAGACGGTACTGAGATAAGAATTGCTCCGCTTGATGTTCAATA
M1_Spy_1964	GGGACATGGTGGTATTTTAGACCGCTTTGATTCCATGATTTTTGTTTTTCCCATCA
M1_Spy_1965	ATCCAGGAGATATTACGGAGGATTTGATTGCTAATTACTTGATGACAGACCATCTG
M1_Spy_1968	GCGTATTGTAACAAAAGCTACTACAGAAACGACACTGGTTGAAACAGAAGAAACC
M1_Spy_1971	ACATTTGAAGAAATCGTTGCGAATTTTATACCGTCGAGTGTTGCAGAAGTCACATCA
M1_Spy_1972	GACAGACAATCATCAAACCAAAACACCAGATGGCTCAAAAGACCTAGACAAATCATTA
M1_Spy_1973	ATAAAGTGTTTGCTTATCAGCGTCAGCTTGGAGAAGAGACGTATGTGATTGTTGT
M1_Spy_1976	AAATTGCTAAAATTCACCGTCGTATCGGCTCAACAACTATTTACGTTACCCATGAC
M1_Spy_1978	ATCCGTTAGGAGATAAAGATTGGATGAGTATCAAAGAAGGGGACCAATATGTGCATTT
M1_Spy_1979	ACGATCCTCGTGATAAGGCTAAACTACTCTACAACAATCTCGATGCTTTTGATATC
M1_Spy_1980	AGTCTATCCAAGACATCAAGGGCAGTATCTTATCTGTGTCGCAATTTACCCTTTAT
M1_Spy_1981	CGTAGTGGTCTGCTTAATGATGTGCTCCAAATTTTATCAAACTCAACCAAGAGCAT
M1_Spy_1983	GCTGAGGTTTCTTCTACGACTATGACGTCGAGTCAAAGAGAGTCAAAAATAAAAG
M1_Spy_1984	AGCGGCTATCCCTTTATCTAGCAGACAATTACGATTACCATGTCAAACAGATTAAC
M1_Spy_1985	GAGAAATTAGAAAAAGAACTGCTCACACTAAACTGTCCCTTGCTACTTATGGGAGATT
M1_Spy_1986	AAGTCAATATGTCACAGCTCACTAGCAAACCGACTCCCGCAAAAGATTTTAAACA
M1_Spy_1987	TGGATTTCCTAAAGGCGATAAGTTAGACACGATTGCCCAGAAAGTGACTGAATT
M1_Spy_1988	CAATATCTTGGCGGATATCTTAGTCTTATTGACTGATGATGCTTACCGTTTGGTCA
M1_Spy_1989	CAAAATAAAGAGGCTATAAAAACCATTGACGATAGTGGCGAAGTGACCCAAGATGATA
M1_Spy_1990	CTACTTTAAGACCAGCTACCGGCCTCATATTGAGCAAAAATCTTATGAACAGCTAT
M1_Spy_1991	AAAAGGTTTTAGCGTAACCGCTAGAGACTGTGACGATCAAGAAATCATGGCATTT
M1_Spy_1992	ACACGCCTACCCTGAAAAATGGGTCAAACAACAATACCTACCAGATAAATTAGTAG
M1_Spy_1994	TTATTCTTCTATTCCCAAAACAAATGAGGTCTTCTTGCACCGTCTCTTCATCAAACCAA
M1_Spy_1995	GGAATTATGCCAATTGTCTCTATGAAGAGCGCATGAGTGAAGCTGATTACATTATC
M1_Spy_1998	CCTATGGTGGAATGACGCCTTATCAAGAAGAACCAATGTCTAAAAATATCCCTGTTAAT
M1_Spy_1999	TACTTCGTTCGCAGAAACAGGAAATCGGGTATCACATTAGCAGAGTAGTTCAT
M1_Spy_2000	ATATTGGTGATAAACGTATCAATGTAGGTAAACAAGGCGTCCACAGTCATGGTCAT

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_2001	GTCACCCTTCATACGAGAACTAAAATGATGTCGGTGCTTTCTAGTGAATATGTCTTAT
M1_Spy_2002	TTTTATGATTCTCATTTCTTTTGTTGTTGGGAAAGGGGCTCAAGGGGTTATCATTGCA
M1_Spy_2003	AAACGCATCAAACAGTTGCGAGGAAAAGAAATGACGTTGATTCCACAATCCGTTAATT
M1_Spy_2004	GCTTTTCGATTGTGCGTTCATTACATCCAGAGACGAAATACCTTATTGCAGATGAA
M1_Spy_2005	AGAAGGTGCTGGTAAGTTGACAGGTGATAAAGAGCTAGAAGCAAAAGGATTTGTT
M1_Spy_2006	AAGAGCAATGAAAACCAACAGCCAAGTGAAGCCAGTAAAGAAGAAGAAAAAGAATCAGA
M1_Spy_2007	ATTTTCTTATCTGGCTAAACGATTCGGCTTGAAACAACTTGGTATCTCGGGCATTT
M1_Spy_2009	GCACAGCCACTCTTTATAGCTACTATGACTTTGATGTCTCTATTTGGCAGTCTTT
M1_Spy_2010	GCTACAAAAGCATCAACAAAAGATCAGTTACCAACGACTAATGACAAGGATACAAATCG
M1_Spy_2013	GTACGACCAGTAAGAACTACCAAAAAGTTGTCACACGGCATATTTGGAAAGACTATTT
M1_Spy_2016	ATATTCCTAAACCTGAGAACGAACAATCACCAAACCCACTTCATATTCCTGAACCT
M1_Spy_2018	AAGCAGGTACAAAACCTAACCAAAACAAAGCACCAATGAAGGAAACTAAGAGACAGTTA
M1_Spy_2019	ACAGTATGATGTGATCGTGACAGATGTTATGGTAGGAAAAAGCGAAGAGCTAGAAATT
M1_Spy_2023	CGAGTGTTGCCATGTTAGGAAGTGTCTGTTTCTTTATTATCTTTGCTCTAAACCGTA
M1_Spy_2025	CTATTCGTCACATGGTAGAAGACACCATCATTGCCAACAAAAACCAAACCTTTGAA
M1_Spy_2026	CAGAACTAAAACACCTCAAAGTGACTATCAATCTCGAAGAACAATTCGTCAAAGCCAA
M1_Spy_2027	ATCAAACGTATCGAAAATGTATTACGCGTCTCTACTCCAGATGAAAAGCGCCAAAT
M1_Spy_2029	CGAATATCCCTATATCCTATCCTTGTTCTCAGTTGTCTTGAGTTTAGCGTTTTGTTGT
M1_Spy_2031	AAGTAGCTGCCCTATGCAAAAAGACGGTGATCCTAAGAGATGGTAATATAGAACATT
M1_Spy_2032	CCAAGGTGGCAATGACCAACAAGATAATCCAAACCAAGCAAAATTCCCTTATGTTAT
M1_Spy_2033	GTTGATGGCGAAGAAGTTACAGAATACACCACAAAAGATGGAAACGTGATTCAGA
M1_Spy_2034	AACCAACTACTAGCTGATATCTTAGACTTGGCCCCTATCATGTACAAGCATATGT
M1_Spy_2037	GACCCTGACTTCCAAAACAAAGTCATCGCTAAAGCTCTTGATAAAGCAAATGTCAA
M1_Spy_2039	CGTGGCGACTTTAGCAAACAAGATTGGGAAGCACAAATTGACAAAGAATTATCTCA
M1_Spy_2040	CCCTATCACGATCCTTATCACAGAATCAGTTATTAACAAAATGAGAACGGTCCCA
M1_Spy_2041	GTTTGTCAATGCTTTTATTGACTTCTCATTTTTATCTACTACCATTTTGCAAAAGGAAC
M1_Spy_2042	TATTAGCAACTCTTACTGAGGAAGACTGTCTGACAGAGCGGACTTATTTGTCAAA
M1_Spy_2043	GTAAAAATCAAAACCCCGCAGGCTGGACTGGAAACCCTAATCATGTCAAATATAAAAT

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_2045	AATTATGGGTATCTGGACAAGACAAAACCGGGAATAGTTGACAAACTCAACCACAAA
M1_Spy_2047	AACTCTTCTTGGAAAATCGTTCGCGTGAAGAAATTGACCGCTACATCGACTTTTA
M1_Spy_2048	ATGTGTTTGAAGCAGGATTTGCCATGCCATCTATTCAAAAAGCGGTTGACGATTTT
M1_Spy_2049	TGTGCTCTCAAAAGCAACCCAAGATGACATTATTGAACGTACAGAACACACACTTT
M1_Spy_2050	TATCAGTAAGGTTTGGTTTGGTGGTCAAGGGATTATTGGTGCTATTGTGATTGGTTTAA
M1_Spy_2051	AATGATATCCCTATTGCAGTTATCCCAATGGCTGATTATGGAATGTTAGACGGCAA
M1_Spy_2052	AGTGAAAAAGAAATGGCCTTAGCAGATGATGCCTTATTAGAAGCCCACAATTTACAGA
M1_Spy_2053	CTAGTGACGGATAAAGAGACCATGCTAGCTGTTTTAGCGTTAGATGAAGATATTGA
M1_Spy_2054	TCAATCGCGTCATTACAGACCAAGCACTAGACAAGCAAACACAAGAGTTATTAAGT
M1_Spy_2055	GAGCAATTTGTCACCCTTGTTGACTACGTGGACTTTATCTATACCGATTTGAAACATT
M1_Spy_2058	CTTGAGTACACAGCTTTCTTTACGGTGATTATCTACATCTTTGATCAGCTCTTAGC
M1_Spy_2059	GATCAGTGGGTTATTGGTTATACGCCAGATGTTGTTATTAGTCAATGGGTAGGATTTAA
M1_Spy_2060	AAGGACTACTCTTTCAAGTGGATGGTGTGGCTTATTCAAAGCATATTTCGATGAC
M1_Spy_2063	TCGAGTCCATTTAGCCCATCAAGGTCATGTTTTATTTGGTGATCCTTTGTATTCCAAT
M1_Spy_2065	GCAGCATTAACACATGAATTTGATTATACCAAGCCACTTTCGGGAGTAACTTTGC
M1_Spy_2066	TAAATTTCATATTGCCAAATCTTACAATCCACCACTAAACGATGCCAACCGCTCTC
M1_Spy_2070	TTAATGCTGCAACAGGTGAGTGGGTTGATATGATTAAAACAGGAATCATTGACCCT
M1_Spy_2072	AAAACCATTAGGTGACCGTGTGGTCGTAAGATTTGATGATGAAAAAGAGCAGACA
M1_Spy_2073	GACCATCAAGCTATGGAGAAACGTATTTTAGAAGAATTAAGAAAAACTTACCGCCCAGA
M1_Spy_2074	TTGCTTTTTGACGAACACTTATTGACAGAGCGTGAGGGAAATATCATTTTGGCTGT
M1_Spy_2077	TAAAACGTTAGAAGAAGGACAAAAAGTGGCATTTGACGTCGAAGAAGGTCAACGT
M1_Spy_2079	AATTAATGCTGATGGTATTGGACGTGACGCTAGCACCTTGATTGATAAAATTCACG
M1_Spy_2080	CGCGCAGCTAAGACTAACAATATGACCATCATCAAAAATGTTGCTACTAAGGACAT
M1_Spy_2081	TAACAGATGATGGTATCGCTAAGTTGATTGGTGCTGGTGTTATTGGTAATTTGCTTC
M1_Spy_2082	AATGGTGGTTTCGGACTTGTTTTAGATGGCAGCGAACGCATTGATGAGATTATTAAAT
M1_Spy_2083	CGATGTGGCTGAATACTACTTACAAGTTGAGGACTTTGACTACCATAAACAGATTC
M1_Spy_2084	TCGGTAAGTCTAATACAAATGCTGCTAGCGACTTAGGAGTTGCTGCTTTAAACT
M1_Spy_2085	TGAGGAAGACGATAGCATTGAAACCAAACTGACTAAAATCGTTACCAAAGTTTACGGT

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_2087	AACTACTTTATGCCCTAAGCAAACTGACTTATTCTGTGACCAATCAGGCGAATCAA
M1_Spy_2088	CGAGTACCATTTGGCCCTATTATCCCAATTCTTGCAGTTATCGTTAGTTTAGTTATGAT
M1_Spy_2089	TTGATCTCAAACCTGAAAATCATGAACTAGGTAAAGGGACTAAGGTTGCCTACGAT
M1_Spy_2090	ATAACCACACCGCCAATTTAGCAGCCACCTTTATCTTTTACCTCGTACAGATTAT
M1_Spy_2091	CGAGATTGTCATTTGGTGAGTGAGTTGAACGATTTGCGTCAACAATTATCTGCTAA
M1_Spy_2092	TTCTTAACAAACAACGCGCTCGTCTTGAAAAATTCTTGGGCGGTATCGAAGATAT
M1_Spy_2093	TACACTCTTCTTGCACAAGTTTACATCATGGACGACAGCAAAACTGTTGAAGCTTA
M1_Spy_2095	CTGCGAACAAATGTCACCTTAACCAACTTTGATGCTTTCCATGAGACCTTTGATAT
M1_Spy_2096	TATTTTTCCTTTCTACCAACGCTTGATTGCTTTGCGGAAGGAACTCCCTATTATTG
M1_Spy_2097	TTAACTTAGATGGTCAAGGATTTGAAGCTTTGGTGAAGCAAGGTGATCAGGTTAAG
M1_Spy_2099	AAGTCTACCTTTCCAATAATAACCAATTCCAATTCACCGAGAGTCGCCATAAACTG
M1_Spy_2103	ATGTCCATCTGAGTGTCAAAAATGCTCTGGCTTCTTCACTTCTCTATCAAAAGGTTT
M1_Spy_2104	AGCATCCCCAAGTGATTTCCCTTTTATCCAGTGAATTTGACGATGTCTTTTCCAAA
M1_Spy_2105	CCAGACAAACTGGAAATGCTGTCCTTAATTGATATTCTGGTAGATGGTCGTTTTGATA
M1_Spy_2106	TTTACGGTTAAATGAGAGATTACTTGAAAAAGGTGGGCATATTGGTTACTCTGTCCGT
M1_Spy_2107	TTCTTGGGACACTACCTTGCACAAAAGAGGTTTTGAAACAATGATCGATGCCTTTT
M1_Spy_2110	GTTTATTCGACTCCATCAGAAAGTTTGACAGATCGCTTTTGTCGTTTAGATACGGA
M1_Spy_2111	GTCATCGCAGACGTCATGGTAAAAATGGTGTTGGTCGAGTAATGATGATTTATCTT
M1_Spy_2112	AGCGGGATCTGAAGAAGATGAGTCTGGTGAAATTGAAATCCAAGCCTATTCATTTA
M1_Spy_2113	AATGAGAATAATGGGACTTGATGTTGGCTCGAAAACGGTTGGTGTAGCTATTAGT
M1_Spy_2114	ATTTACAGATGAAACAGTCCGCTTTAAGTTGGATGATGGCGACAAACGACAAATTAG
M1_Spy_2115	AACCGAAAATGGAGTGGAGAGCATTGTTTCATCTAAAAATCGTTATGCCAAAGCTC
M1_Spy_2116	GCCGATCACCCAGAATTGTTTGATGAAATCGACCTTAAAGTACGTGTTAAATTTGG
M1_Spy_2117	CTCAAGTATTCAATGGAGGCTTTGTGACTTATAGCATGGAAGAAAAAGCGAAAATGCT
M1_Spy_2118	CAGCAACTGTCAACAACGCTATCGCAGTACAGAAAATTCAAAAAGAGTTTGGTAGT
M1_Spy_2119	TCTGAGACGGCAGAGCAATACATCAAATCAGCATTGAAACTGTTAATGAAAGGGT
M1_Spy_2120	ATTTACTCTAGGGATGCTCTTGTCATTTACCTTTACGCAGTTCTATGCTATCTGGTTAT
M1_Spy_2121	AGACAAAAGTTATCTCAAATGCTAACCCGTTTGGAAAATGAAGGGCAATCAGTGTTTC

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_2122	TTTCACGCTTTTAGACACACTCACGCTAGTTTATTGCTGAACGCTGGTATTAGTTATA
M1_Spy_2125	CGGACTTCTTCGGAGTTCTAAAATCAGACATTGACCCCCGTTTTGATTCTAATAA
M1_Spy_2126	AATTACACTAAAAGCTGCCCGAATCAACGCTGGTTACACTTTAAAACAAGTAGCTG
M1_Spy_2127	TTTCATGGACAACCTTTAGACATTTACGGTGATATTCAGGAGCCTTTATTTTTGGCTAG
M1_Spy_2128	TATGAATAGGCTTGTCGATGGCTTACAAAATGCAACTAAGAAAGACCTTATCAAAGCC
M1_Spy_2129	CTCATGCTTGATGACAAAGTGCTAATCTATAATCGCAAAGCAGAGCAAAAGCGTTT
M1_Spy_2130	TTTGAAGAAGCATGGGACGCTTTAGACTATCAACGAACCACAGAGATTGTCTTTA
M1_Spy_2131	GTGCAATTATAAACACGAGTTGTAGCGCTTTGAAGTGCCTTATTTCTGACCATGAT
M1_Spy_2132	AGACGCTTATTCTATTCTTTGTTTTAGGTCTCTTAGGGCTTCTTCTTAGCCGTTCTAA
M1_Spy_2133	AATCAAGTACAAAAGGAACAGGAACGCCTAAGTGTGTTATTGAATGCCGATTTAACCA
M1_Spy_2134	TACCAACCACCACTAGAAGACACCAGGAAACATTATTACAAGGTCAGTGATATTCTTA
M1_Spy_2135	TAAATGGGTGGATTGTGATTTAGAGACGGCTTATGAGCTGGTACAAATAGCTAACA
M1_Spy_2136	CGGTGATAAGTCCGCCATTGATAGACGCTTTAGGATTTTACCTTTTACCAAAGTATTTA
M1_Spy_2140	GCGCGTGGTTTCTTTATCTAAAAGTACTCAAGGGAAAGTTAATAATCGAGACACAC
M1_Spy_2142	ATATCATTAAACCAATGAAGACACAGGGGAAGACAAGAGTTACAGGATCAATAGGTGA
M1_Spy_2144	TATTAAGGCTAGATACGTGGATGACTTATCATGGGACGAGATACTCGATAAACTAG
M1_Spy_2145	TTTACCAGTTTGATATAGAGTACCATCCCACGCGCAGAAAACACCTTGAAATAAG
M1_Spy_2147	CAGTTACTACAAGTATAGATGGATGGACAATAGGTTATGGAGAATGGACTGTGTG
M1_Spy_2148	ATGATGGAAGCAAACCAAGCAATCAAACGCGCAAGTGACAACTCTCTTATTCTATTT
M1_Spy_2149	GATGCAAGATGCAAGTGATGTGATTCAGTATATCACCAAACGTATAGAAGATCAG
M1_Spy_2150	CTTGCTTGTGATTTGCAGAGACGAAGAGATTGCTAAACGCTTTGAAAAAGATTTGG
M1_Spy_2151	CCCACGCATTATCAAGCGAACATCAGATAACTTTGAGCCTTCTATTATGGCTAAAT
M1_Spy_2152	GATCTTAGTCGCTTGGTAAAAACAAAAGCTAGCCATTACAAGGGAACTGCTATATC
M1_Spy_2153	AACTTTGCTTTTGGTGGATGCTCTTGTATTGACAGCTTCGTTGACTTATGTGGATTTAA
M1_Spy_2154	ATGATTGTCTCTTTGACTTATATTTCCCTGCCGCAAATGATGTATGCTCTGATTGCTA
M1_Spy_2155	CCTTTGTTTCAGTGGCTGAGAATGTTAGAATTATCGGTCGTTTTGTGGAAGATGAT
M1_Spy_2156	AAAGTGATGAAAGAGACAAAAGAAATAGATGTAACCCTACCATTCCCACGTATGAGCTA
M1_Spy_2157	TAAATTGTTTGCGCCAGAGGTCCAAAAACCCGTCAAACTTTATTATATCGGTTCCAT

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_2159	AATTCGACGAAACTACTGGAGATTACTCACGTTCTCACCGTGTATCCCTTAAA
M1_Spy_2160	GCGCGTAAATATTACACTTGAACATAAAGAATCTGGTGAACGCTTGTACCTTACT
M1_Spy_2162	CAAAAATTGGCACAAGTCCCTTCTGTTGGAGAAACTTTGGAAAAATATAGCAGATGG
M1_Spy_2163	CATCTTCGTAAACTAGCCAATCAAAACATCTTGGACACTAGAAGAGAGGGGAAAATTA
M1_Spy_2164	TTACACAAAGCGAACTAGGCAATGCAGGTCAAGTTAGTCAAGTTGAAAGTGGTAA
M1_Spy_2165	TACTATCACAAATTGCCTTGTTGGGACATGCTACTAAAATCCATCTATTCTTGGGAAG
M1_Spy_2166	GAGATTGTCATCCAACACACAGACTATTATTTCTCTTCTCCTGTTCGAGTTCGTA
M1_Spy_2169	GGAATTGGTGGTATTTTAGGTATTGCTCTCTTTTCTTTCCCCGTTGAGTTTTTGCTTAA
M1_Spy_2170	TACCTTTACGTCAGCTACTTTTCACTGATCCTGTCTATTATCGTTTAGAAGTCACTC
M1_Spy_2172	TAAATTTGCCTTAGACGGGATTATTGAAGGGAGCTTAAGACATGACAAGGACTGA
M1_Spy_2173	TTGTGTTTTGGTGGTGGTCTTCTTATTTGGATTATTGCCACAGCATTCCTTCGTTTTT
M1_Spy_2174	TAAAAGCAAAGAAGAAGAGGAGAACGACTACTATGACAATGATGCTATCTTCACCG
M1_Spy_2176	GGAGTAGAAGTGAAAGTCTTAACTGGTTTCTTACTGGCATTTATGGTATTAGCACTAC
M1_Spy_2177	CATCACTACTGATTTACAGGACAAGTTCAGTACCGAAGAGTTATCTCAAACGGAA
M1_Spy_2178	CTTCCAGAACGCGATGAAATTAACCCAGAAATCAATGAAGCACTTGTCGTTGAAT
M1_Spy_2180	TTGGAAAATTCGCTACCCCTTATCAACCATTCTATTTCTTGTCTTCGTTTGTCAGTTAG
M1_Spy_2181	TTAAAGAGGACATAAGAGCCCATGAAGGACAGTTAGTAGAATTAACCCTTGAGAATG
M1_Spy_2182	ACAGTTGATTACAGGAACTAAACCCGAAAATCGTCAGCAAGAGGTTTCAGATATTTCA
M1_Spy_2183	GTTTTAGATCATCCTATCCGCGCCATTGGTCTAATTGAAGTTCCTGTGAAATTAC
M1_Spy_2184	GCTCACGTTGAGGCTAGCTTTGTATTAGTTGAAACAGCTTCTCATAAGATTGCTAT
M1_Spy_2185	TCGTTACTGCCCATCTATTGAAGATAAGATTGTTCGCTTTGCTGATAAAGAACGTCAT
M1_Spy_2186	AGAAGACCCTAAACTAGAAATGATAGAGAATGCAAGCGACAGGTTTGTGAATGGATT
M1_Spy_2188	GCTGATAATGGCAAAGACCAAACCTACTTTTTAAGTCAACTATCACAGGAACAACTAC
M1_Spy_2189	CGATTCTAGAGAGTGCCAAGAAGCAGCCAACCAAATTGCAAAAATTCCTCATATTT
M1_Spy_2190	CGCAGCTGATATGGCTTTAGCTGATATTGATTCTCAAATTCCTGTTGACGAAGTTA
M1_Spy_2191	AACTGTTCAGCAGGTATCTTCAGTAGCCTATAATCCAAACAATGTGGTACTTTCCAAT
M1_Spy_2193	GACTAATGCTTTCTTTAAGTTTACGTTTTGTGCCTACTTTAATGGATGATACCACGCG
M1_Spy_2194	GAGTTCCTAAGGTGACTAAACTTGCTCAAAGGCTTGTTGATAGAGGAATACCTAT

Table A.1 Oligonucleotides on the SF370 Microarray (continued)

M1_Spy_2195	CTGTTGTACAGATGCTTCAAGAAGAAGGTTATCCTGTTGACTATGGATATCTCAC
M1_Spy_2196	ATAAGATGCTTGTCATGAGTGCCTTTATCATGTTAGTTGGACTTGGCTTAGTTCCT
M1_Spy_2197	ATACAGCGACTTTACCTGCAGAAATAACAGAATCATTGTTGACACTTGGTGTGACA
M1_Spy_2198	CTTGATACACCAGAGCCTATCGCAATGTCTAACTATATCCGTCAAAAATTAGCTAC
M1_Spy_2199	GCTTTAGTTCTTTTAAATGGGTTGTTAGGCTCTTTTGCACATTCCCGTCTCTTTA
M1_Spy_2200	CGTTGCCTTATGGACTATTTTCGAAGTCGTTATGTTTATGATGTTGATTGTCGCA
M1_Spy_2201	GTTGCTGGATCAGGATATGTTGGATTATCACTAGGAGTTCTTTTATCACTTCAAAACG
M1_Spy_2202	CGACTATGCCTTAGAACACCCACAGGTCAAAGAGGACTTGAAAAATTACATTATC
M1_Spy_2203	GGGGATAAGATTTCTCTACCTGACCAAGACTTAATTATCACTATTGTTGAGCCTAG
M1_Spy_2204	CTTTTACTAGACGATGTCATGAGCGAGCTAGACAATACACGACAAACAAAGTTACT
M1_Spy_2205	CGTTGGGTTGTCACAGGAATTATTTGTTTTATCGTAGGTGCTATTTTATTAGGTGTGG
M1_Spy_2206	AAAACGATTATTGCTGATGGTGGTATCAAGTATTCTGGCGATATTGTTAAGGCTCTTG
M1_Spy_2207	ATATGGGAGAGGTGTTCCGTATGTTACAAGAAGGTAGTCAAAAAGCAAGAACTGT
M1_Spy_2209	GATCGTGGAGTGACTAAACTTCCTATTCGCGGTGGTTATACTACTTCTGATAAAATTA
M1_Spy_2210	TATAGATGAAACTTATGACGAATTCCTTGACAATCCAGAAGTCCAAGCCCGAGTT
M1_Spy_2211	AGGTTCTCTTATTTTTACACTTCCTTACGATAAAGGTTGGTCAGCACAAAAAGATGGG
M1_Spy_2215	GTCTAGTCCTTACTGAGCAAATTTATCGTGCTTTTATGATTACACAAGGCAGTCC
M1_Spy_2216	GGTAATAGTGGTGCTGTTGAAGGAATTGGTTTTGCTATCCCATCTACTGATGTTATTA
M1_Spy_2217	CTTTAGAGAAACAATTGGCTAAATCATTGGGACTCTCTGTCAATATGAAGCTGAC

Table A.2 Primers used for PCR detection of contaminating genomic DNA in total RNA samples

Locus Tag	Primer Name	Primer Sequence
M1_Spy_0930	Spy0930For	TTTTGCTATAATAGAACCATTACG
M1_Spy_0930	Spy0930Rev	GCTTCATAGGCATCAATTAGG

Table A.3 Primers and probes used for real-time quantitative RT-PCR

Reference Genes

Locus Tag	Primer / Probe Name	Primer / Probe Sequence
M1_Spy_0764	Spy0764 QPCR F	GCCATTGGACTTATGATTG
M1_Spy_0764	Spy0764 QPCR R	CTGCCCACTTATCCATAG
M1_Spy_0764	Spy0764 Probe	[6FAM]CCATGGACTCTGGCCATCTCC[BHQ1]
M1_Spy_1309	Spy1309 QPCR F	GTGGTGAGTCCTACTTTA
M1_Spy_1309	Spy1309 QPCR R	GGTTGCACTTTCTTATCA
M1_Spy_1309	Spy1309 Probe	[6FAM]ACCAGCCATTCCAACCGAGA[BHQ1]
M1_Spy_1390	Spy1390 QPCR F	CCAGCGAATCATCAAAAG
M1_Spy_1390	Spy1390 QPCR R	GTCTCAGTTGGTTCTTCA
M1_Spy_1390	Spy1390 Probe	[6FAM]CCATCAGAATCAGAGCAAACACAGAC[BHQ1]
M1_Spy_1877	Spy1877 QPCR F	GAGGCACCTGTTTATGTC
M1_Spy_1877	Spy1877 QPCR R	CCATCTAATCCAGCTTCC
M1_Spy_1877	Spy1877 Probe	[6FAM]AGTAATCGTTCACCGCTTATCCGT[BHQ1]
M1_Spy_1912	Spy1912 QPCR F	TTCCTCAGAAGATTTCAAGT
M1_Spy_1912	Spy1912 QPCR R	TCAGAGCAATCATTAAAGAAC
M1_Spy_1912	Spy1912 Probe	[6FAM]TGACGAACCAACTGGAGCACT[BHQ1]
M1_Spy_2189	Spy2189 QPCR F	GTCAGAATTGGTAAGGTAG
M1_Spy_2189	Spy2189 QPCR R	TCAGGATTATCAGTACCC
M1_Spy_2189	Spy2189 Probe	[6FAM]CAAGAGCCTTATCAGTACCATGTCCT[BHQ1]

Validation Genes

Locus Tag	Primer/Probe Name	Primer/Probe Sequence
M1_Spy_0028	Spy0028 QPCR F	GTATTATCAATATTCACCCAGC
M1_Spy_0028	Spy0028 QPCR R	GCTTTCTAGGCTATCGTC
M1_Spy_0028	Spy0028 Probe	[6FAM]CGCACTTGTTGGATGACCTGACC[BHQ1]
M1_Spy_0946	Spy0946 QPCR F	GCAGAATGGGGTAAATATTG
M1_Spy_0946	Spy0946 QPCR R	GGTGTCTTGCTTACTGTA
M1_Spy_0946	Spy0946 Probe	[6FAM[TCGTCTCTTCACTTGGCTTCGT[BHQ1]
M1_Spy_1215	Spy1215 QPCR F	GTGAGTAATTGGACAACC
M1_Spy_1215	Spy1215 QPCR R	GCGACCCGATATAAGTAA
M1_Spy_1215	Spy1215 Probe	[6FAM]AGACATGCCAGCTCCAATACCA[BHQ1]
M1_Spy_1357	Spy1357 QPCR F	GGCTGTTCGATAGGTGAG
M1_Spy_1357	Spy1357 QPCR R	TGCGTAGATCAGCTTTTG
M1_Spy_1357	Spy1357 Probe	[6FAM]ACCGACTAATACTGATGCTGACACC[BHQ1]
M1_Spy_1432	Spy1432 QPCR F	ATGGTTTCTACTGCTACAC
M1_Spy_1432	Spy1432 QPCR R	AAGACGAGTATCTGCGTA
M1_Spy_1432	Spy1432 Probe	[6FAM]TCTGGATTACCTGGACGAACTTCAA[BHQ1]

Table A.4 Primers used for cloning genes to create plasmid standards for QRT-PCR

Reference Genes

Locus Tag	Primer / Probe Name	Primer / Probe Sequence
M1_Spy_0764	Spy0764_For	AATTTGGTTCATTTAGATCGTGGC
M1_Spy_0764	Spy0764_Rev	TGCTCTTGCACCCTTTTTCG
M1_Spy_1309	Spy1309_For	GAATTTGGTCGTGATGATTGGAAC
M1_Spy_1309	Spy1309_Rev	AAAAATCACCACAGCAATAATGACC
M1_Spy_1390	Spy1390_For	TTCGTAAAAATCAAGCAGATATTG
M1_Spy_1390	Spy1390_Rev	CCGTCACTTTTCTGTATCTAATCC
M1_Spy_1877	Spy1877_For	GCGGTTTTTCTACTCCCTCACAG
M1_Spy_1877	Spy1877_Rev	AATAGCTCATCCAAAAGACCCACTC
M1_Spy_1912	Spy1912_For	TTACTCTCCTCTATCGGATA
M1_Spy_1912	Spy1912_Rev	ATGAAACAATTAGTATTAAA
M1_Spy_2189	Spy2189_For	GAAATAAAAAACGGAGTTGCC
M1_Spy_2189	Spy2189_Rev	AAATTGTTGGTCAGCTTGTTTG

Validation Genes

Locus Tag	Primer/Probe Name	Primer/Probe Sequence
M1_Spy_0028	Spy0028_For	ATGAAAATCGCTGTTTGCTTCTGGTA
M1_Spy_0028	Spy0028_Rev	CTAGATTACTTTCCTCTCCACTCCC
M1_Spy_0946	Spy0946_KPN_For	CGGGGTACCCCGAATAACACAACACTGGAAAGGAC
M1_Spy_0946	Spy0946_KPN_Rev	CGGGGTACCCCGTTTTCTCCCTTCGTTAGTTTTG
M1_Spy_1215	Spy1215_New_For	CCTTTACATCAGAAGACAAGGC
M1_Spy_1215	Spy1215_New_Rev	TACGTTTACCGTTACGAATCG
M1_Spy_1357	Spy1357_For	CTAATTTTCTTTGCACTTTG
M1_Spy_1357	Spy1357_Rev	ATGGGAAAAGAAATAAAAGT
M1_Spy_1432	Spy1432_For	ATGGTTTCTACTGCTACACAAA
M1_Spy_1432	Spy1432_Rev	TTAATCTTTGTAACGTAAGTTCC

Table A.5 Primers used for allelic replacement of *spy1215* and *spy1755*

spy1215:

Primer Name[1]	Primer Sequence
1215upNheI	AATTTGTTGCTAGCCTTTTTCCGTTAAA
1215upXhoI	ATTTACCAAAACTCGAGACCAAAGCC
1215doNdeI	GGTTGTCCATATGCTCACAGTTATTCTCC
1215doXmaI	TGCTTTGCCCGGGTCAAAAGATTAC

spy1755:

Primer Name[1]	Primer Sequence
1755upEagI	ATCTGATTCGGCCGTTAGTGTGAG
1755upBamHI	TTTGGAGGATCCGGTTTAAATGATT
1755doNdeI	GTAAATTTTGTCATATGCCAAGTAAACACC
1755doXmaI	TGTAGCTGGCCCGGGTTTTAATATAG

[1] Up =MCSI; Do = MCSII

Table A.6 Gene-specific primers for *spy1215, spy1755,* and downstream genes used for PCR and RT-PCR

Primers internal to one ORF:

Locus Tag	Primer Name	Primer Sequence
M1_Spy_1212	Spy1212_RT_For	CCAAATGTGGGGAGAAATCCTTAG
M1_Spy_1212	Spy1212_RT_Rev	TTTATCAGTATCAAGCGGGGAATC
M1_Spy_1213	Spy1213_RT_For	CCAGCAGGAGAAGGAAAATCAAC
M1_Spy_1213	Spy1213_RT_Rev	TCAAGTCACGCACATAGACAGGC
M1_Spy_1214	Spy1214_RT_For	GAAATGTCTGGACGGAATGACTTG
M1_Spy_1214	Spy1214_RT_Rev	CTGCCTTGCCAAAAAAATCTCC
M1_Spy_1215	Spy1215_RT_For	TTGTGGCGCTAATCAATTGTGC
M1_Spy_1215	Spy1215_RT_Rev	ACCTATCCTCAGAAAAACCTCACCC
M1_Spy_1215	1215chkF	TTGTGGCGCTAATCAATTGTGC
M1_Spy_1215	1215chkR	ACCTATCCTCAGAAAAACCTCACCC
M1_Spy_1753	Spy1753_RT_For	GAAGAACTTGGTAAGGAGAC
M1_Spy_1753	Spy1753_RT_Rev	GTAAGCAACAAGGTCACC
M1_Spy_1754	Spy1754_RT_For	CAGTCAGGTAGCCCACTATGTTCC
M1_Spy_1754	Spy1754_RT_Rev	CCATCTGTGTGTAATGTTTCCGC
M1_Spy_1755	Spy1755_RT_For	CGAGTCAGTTTAGTGATGTCTC
M1_Spy_1755	Spy1755_RT_Rev	TGATGTAGATTCCCTAGTCC
M1_Spy_1755	1755chkF	AATCCTCCAAAAATTGATGTAGATTCC
M1_Spy_1755	1755chkR	TACCCATACTTAGTTGATATTTTCAACCG

Primers spanning multiple ORFs (*spy1215* to *spy1214*):

Primer Name	Primer Sequence
Spy1215-1214_RT_F	AGGTCAACTGATTCCTCATTGCC
Spy1215-1214_RT_R	ATAACCAAGTCATTCCGTCCAGAC

Table A.7 Microarray results for SF370 exposed to pharyngeal supernatants or pharyngeal monolayers for 0.5h, 1.5h, and 2.5h

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
Locus Tag	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change	*P* value	Log_2 Fold Change
M1_Spy_0002	0.096	0.67	0.021	0.72	0.1269	1.07	0.8455	0.29	0.1386	0.72	0.0353	0.89
M1_Spy_0003	0.0274	0.73	0.0467	0.76	0.2355	0.76	0.3497	0.64	0.124	0.79	0.2135	0.74
M1_Spy_0004	0.9715	0.21	0.9942	0.24	1	0.06	1	-0.02	0.991	-0.29	0.6554	-0.48
M1_Spy_0006	0.0445	1.13	0.0016	1.13	0.1605	1.16	0.3067	1.01	0.0337	1.50	0.2475	1.12
M1_Spy_0007	0.1516	0.55	0.7352	0.38	0.9899	0.32	0.9755	0.36	1	-0.12	1	-0.10
M1_Spy_0008	1.00E-04	2.30							0.0207	2.63		
M1_Spy_0009	0.6351	0.38	0.7239	0.45	0.6417	0.50	0.9543	0.32	0.9948	0.25	0.9972	0.26
M1_Spy_0010	0.0514	1.55	0.0348	1.44	0.1599	1.81	0.0061	1.31	0.0189	1.89	0.2242	1.28
M1_Spy_0012	0.0037	1.25	0.0068	1.12	0.1556	1.25	0.001	1.33	0.0207	1.41	0.0293	1.14
M1_Spy_0013	0.0124	2.05	0.0387	2.59			0.0035	2.36	0.0622	3.18	0.0031	1.82
M1_Spy_0015	0.6108	0.50	0	1.74	0	1.55	0	1.23	0	2.18	0	2.03
M1_Spy_0016	0.1954	0.90					0.0196	1.79	0	3.22		
M1_Spy_0019	0.0088	-0.74	0.9996	0.11	1	-0.10	0.1889	-0.71	1	-0.01	1	0.03
M1_Spy_0020	1	0.07	1	-0.04	0.9917	-0.28	1	0.16	1	0.09	0.9991	-0.17
M1_Spy_0021	0.031	0.70	0.9661	0.26	1	0.03	0.9811	0.35	1	0.05	0.9733	-0.40
M1_Spy_0022	0.2722	0.32	0.4379	-0.40	0.9483	-0.44	0.9717	0.18	0.0868	-0.59	0.4753	-0.55
M1_Spy_0023	0.0327	0.48	0.8833	-0.26	0.9899	-0.33	0.6085	0.35	0.9217	-0.33	0.2142	-0.53
M1_Spy_0024	0.0374	0.78	1	0.08	0.9974	0.30	0.0427	0.84	1	0.31	1	0.00
M1_Spy_0025	0.3232	-0.44	0.9524	-0.44	0.9998	-0.14	0.8617	0.36	1	0.16	1	0.02
M1_Spy_0026	0.8028	-0.32	0.9972	-0.29	1	-0.06	0.0659	0.56	1	0.18	1	-0.21
M1_Spy_0027	0.2449	-0.37	0.9976	-0.23	0.9999	-0.16	0.4936	0.58	1	0.20	0.9887	-0.33
M1_Spy_0028	0.3493	-0.41	1	0.02	1	0.12	0.5483	0.49	0.961	0.49	1	0.02

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_0031												
M1_Spy_0032	0.5875	0.23	0.3067	0.65	0.0199	0.65	0.1157	0.69	0.5491	0.47	1	-0.07
M1_Spy_0033	0.1246	0.37	0.0224	0.96	7.00E-04	1.02	0.0853	0.76	0.0494	0.83	0.3889	0.47
M1_Spy_0034	0.0809	0.63	0.032	0.99	0.0165	1.07	0.0403	1.03	0.0093	0.90	0.7168	0.69
M1_Spy_0035	1.00E-04	0.84	8.00E-04	1.01	0.0045	0.88	3.00E-04	1.37	0.0566	0.85	0.7738	0.44
M1_Spy_0036	0	1.00	4.00E-04	1.23	6.00E-04	1.13	0	1.47	4.00E-04	1.22	0.0051	0.94
M1_Spy_0037	0	1.49	0.0678	0.73	0.2648	1.29	0	1.58	0.9292	0.51	0.9869	0.60
M1_Spy_0038	0.073	0.42	1	-0.02	0.9983	0.16	0.7173	0.30	1	-0.04	1	0.12
M1_Spy_0039	1.00E-04	-0.75	0.0141	-0.58	0.2399	-0.59	2.00E-04	-1.07	0.433	-0.67	0.0415	-0.65
M1_Spy_0040	0.0638	-0.80	0.7593	-0.45	0.6492	-0.39	0.0018	-0.85	0.3501	-0.55	0.1694	-0.51
M1_Spy_0041	1	0.04	0.6577	0.25	0.9716	0.22	0.8652	0.22	0.9854	0.26	0.9454	0.20
M1_Spy_0044	0.5907	-1.05	0.3035	-0.54	0.9928	0.22	0.5983	-0.40	0.6119	0.89	0.1213	1.46
M1_Spy_0045	1	0.09	2.00E-04	2.39			0.729	0.47				
M1_Spy_0047	0	2.31	0	2.46	0	2.27	0	2.97	0	3.24	0	3.14
M1_Spy_0049	0	2.22	0	2.29	0	2.21	0	2.91	0	3.00	0	3.04
M1_Spy_0050	0	2.74	0	2.84	0	2.79	0	3.38	0	3.76	0	3.73
M1_Spy_0051	0	2.40	0	2.55	0	2.57	0	3.31	0	3.41	0	3.02
M1_Spy_0052	0	2.48	1.00E-04	2.38	1.00E-04	2.44	0	3.43	0	3.04	0	2.83
M1_Spy_0053	1.00E-04	1.53	1.00E-04	1.66	0.0137	1.42	0.0526	2.40	4.00E-04	1.74	0.014	2.19
M1_Spy_0055	0	2.47	0	2.69	0	2.71	0	3.45	0	3.55	0	3.31
M1_Spy_0056	0	1.76	0	1.86	0.0028	1.78	0	2.76	0	2.02	5.00E-04	2.24
M1_Spy_0057	0	2.59	0	2.70	0	2.59	0	3.60	0	3.59	0	3.55
M1_Spy_0059	0.0069	1.48	4.00E-04	1.82	0.0467	1.68	0.0116	2.56	7.00E-04	1.78	0.0636	1.99
M1_Spy_0060	0	2.64	0	2.60	1.00E-04	2.49	0	3.57	0	3.15	0	3.01
M1_Spy_0061	8.00E-04	1.53	0	1.73	0.0036	1.36	0.173	2.50	4.00E-04	1.85	0.0535	2.34

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_0062	0	2.68	0	2.98	0	3.01	0	3.82	0	4.08	0	3.59
M1_Spy_0063	0	2.09	0	2.39	6.00E-04	2.26	0	3.15	0	2.53	0	2.68
M1_Spy_0064	0	3.02	0	2.97	0	2.87	0	4.06	0	4.16	0	3.88
M1_Spy_0065	0.006	1.93	0	2.11	3.00E-04	2.11	0.0012	3.02	0	2.62	0	2.71
M1_Spy_0066	0	2.70	0	2.89	0	2.70	0	3.84	0	3.38	0	3.22
M1_Spy_0067	0	2.05	0	2.11	6.00E-04	2.04	1.00E-04	3.18	0	2.66	0	3.00
M1_Spy_0069	0	2.92	0	3.19	0	3.07	0	3.83	0	4.03	0	3.89
M1_Spy_0071	0.0011	1.69	1.00E-04	1.74	2.00E-04	1.71	0	2.82	0	2.14	0	2.38
M1_Spy_0072	0	2.80	0	3.04	0	3.07	0	4.00	4.00E-04	4.40	0	4.06
M1_Spy_0073	0.0027	1.45	0	1.97	0	1.98	6.00E-04	2.67	0	2.55	0	2.72
M1_Spy_0074	0	2.70	0	3.19	1.00E-04	2.50	0	3.37	0	4.23	0	3.08
M1_Spy_0075	0	1.20	2.00E-04	1.39	0.0493	0.93	0.0087	1.53	0.0095	1.33	0.0088	1.23
M1_Spy_0076	1.00E-04	1.54	0	1.55	0.0081	1.33	0	1.94	4.00E-04	1.66	0.0052	1.22
M1_Spy_0077	0.0122	1.07	0	1.25	0.0405	1.01	0.0013	1.39	0.0372	1.29	0.0858	1.10
M1_Spy_0078	1.00E-04	1.22	0	1.35	4.00E-04	1.23	0	1.62	0	1.63	1.00E-04	1.44
M1_Spy_0080	1.00E-04	1.28	0	1.73	0	1.57	0	1.97	0	2.30	0	2.15
M1_Spy_0080a	0	1.48	0	1.68	0	1.51	0	1.91	3.00E-04	2.25	0	2.03
M1_Spy_0084	1	-0.08	1	0.05	1	0.21	0.9129	-0.34	1	-0.01	1	0.05
M1_Spy_0092	1	-0.05	0.3451	-0.37	0.9978	-0.21	0.8613	-0.31	1	-0.04	1	0.02
M1_Spy_0093	0.8725	0.20	0.976	-0.29	0.9967	-0.22	1	0.11	1	0.02	1	0.09
M1_Spy_0094	0.2003	0.86	0.9954	0.62	0.8108	0.60	0.9188	0.59	0.5458	0.87	0.7	0.66
M1_Spy_0095	0.6025	0.53	0.0018	1.62			0.1132	1.00	0.0189	2.10	0.0296	1.50
M1_Spy_0096	0.8858	0.46	2.00E-04	2.17			0.1568	0.71	0.2337	2.18	0.0071	1.88
M1_Spy_0097	0.0145	0.85	0.0202	0.90	0.5704	0.80	0.0444	0.91	0.0195	1.19	0.0528	0.80
M1_Spy_0098	0.7518	0.27	1	-0.09	0.2105	-0.69	0.0903	0.86	0.5197	0.38	0.9953	-0.15

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_0099	0.9897	0.30	0.5362	0.50	1	-0.04	0.0083	1.12	0.0065	0.93	0.7954	0.40
M1_Spy_0100	0.0016	-0.91	0.9781	-0.20	0.9956	-0.20	0.0018	-1.05	0.1685	-0.63	0.7866	-0.34
M1_Spy_0101	1	-0.01										
M1_Spy_0102	0.1678	-0.92	0	-1.81	0.0035	-2.03	0.904	-0.88	2.00E-04	-1.68	0.1412	-1.68
M1_Spy_0103												
M1_Spy_0104												
M1_Spy_0105												
M1_Spy_0106	1	0.05										
M1_Spy_0107	0.9999	-0.12							1	-0.25	1	0.31
M1_Spy_0108	0.7023	-0.26	0.4642	0.47	0.9976	0.16	0.4247	-0.37	1	0.09	0.5044	-0.50
M1_Spy_0109	0.2242	0.53	0.0084	0.68	0.9007	0.26	0.0104	0.74	0.2147	0.50	0.92	0.22
M1_Spy_0110												
M1_Spy_0112	1.00E-04	1.14	0.8873	0.33	0.824	0.83	0.8595	0.52	0.9774	0.42	1	0.18
M1_Spy_0115	0.0115	1.05	0.9961	0.30	0.2788	0.95	1	0.26	1	0.29	0.9222	0.38
M1_Spy_0116	1	0.10	0.2306	-0.45	0.6271	0.42	0.0232	-1.07	0.0934	-1.16	0.9948	-0.23
M1_Spy_0117	0.9994	-0.20	1	-0.05	1	-0.01	1	-0.01	0.696	0.58	1	0.17
M1_Spy_0121	1	0.04	0.4762	0.46	0.9929	0.23	1	0.07	0.366	0.47	0.2525	0.58
M1_Spy_0122	0.0387	0.86	0.002	2.12	0.0253	1.32	0.0619	1.23	3.00E-04	2.15	0.0015	1.67
M1_Spy_0123	0.0092	-0.66	0.3444	-0.64	0.1033	-0.71	0.0542	-0.86	0.0055	-1.01	0.0503	-0.88
M1_Spy_0124	8.00E-04	-0.77	0.005	-0.84	0.0023	-1.18	0.0021	-0.85	0.0225	-1.16	1.00E-04	-1.49
M1_Spy_0127	0.5971	-0.26	0.9416	0.54	0.5301	0.97	1	0.01	0.4473	0.60	0.0848	1.10
M1_Spy_0128												
M1_Spy_0129	0.0162	-0.76	0.2022	-0.66	0.8287	-0.44	0.228	-0.87	0.9288	-0.49	1	0.13
M1_Spy_0130												
M1_Spy_0131												

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_0133												
M1_Spy_0135	0.031	-0.66	0.0054	-1.31	0.008	-1.41	0.0254	-1.03	2.00E-04	-1.68	0.0034	-1.57
M1_Spy_0136	0.8172	-1.06	0.0037	-1.59	6.00E-04	-1.56	0.8572	-0.76	0.1338	-1.10	0.1377	-0.97
M1_Spy_0137	0.6551	-0.42										
M1_Spy_0140												
M1_Spy_0141	0.6583	-0.35										
M1_Spy_0142												
M1_Spy_0144	0.966	-0.22	1	0.00	1	0.09	0.9443	-0.28	1	-0.08	1	0.06
M1_Spy_0145	1	0.04					0.9476	0.44				
M1_Spy_0146	0.3778	-0.37	1	0.04	1	0.07	1	-0.18	0.8289	0.65	0.8751	0.63
M1_Spy_0147												
M1_Spy_0148	0.5406	-0.64					1	0.33	1	-0.01	0.9947	0.43
M1_Spy_0149	0.7956	-0.30										
M1_Spy_0150												
M1_Spy_0151												
M1_Spy_0154	0.8649	-0.40										
M1_Spy_0155												
M1_Spy_0157												
M1_Spy_0158	0.4835	0.37	0.9999	-0.21	1	0.00	0.9842	0.53	1	0.29	0.9701	0.43
M1_Spy_0159	0.9994	0.15	0.9997	-0.17	0.9995	-0.20	1	0.08	1	-0.02	1	0.12
M1_Spy_0160	0	1.70	0.6765	-0.39	0.0757	-1.03	0	1.26	0.2363	-0.79	0.2642	-1.44
M1_Spy_0163	0	1.56	0.0258	0.73	0.9953	0.23	0	1.57	0.8967	0.48	1	-0.03
M1_Spy_0164	0	1.14	0.0342	1.40	0.1069	0.97	0.0035	1.71	0.0087	1.65	0.1538	1.03
M1_Spy_0165	0.9048	-0.26										
M1_Spy_0166												

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_0167	0.0743	-0.64	0.2303	-1.30			0.2612	-0.69	0.8563	-0.64	1	0.16
M1_Spy_0168												
M1_Spy_0169												
M1_Spy_0170	0.0278	-0.91	5.00E-04	-1.53	6.00E-04	-1.61	9.00E-04	-1.13	0.2183	-1.32	0.1448	-1.25
M1_Spy_0171												
M1_Spy_0172	0.7308	-0.45	0.0959	1.05			1	0.20	0.281	1.85	0.5735	0.78
M1_Spy_0173	0.0216	1.16	0.0043	1.39	0.0047	1.06	0	1.79	0.0016	1.87	0.0045	1.51
M1_Spy_0174												
M1_Spy_0175												
M1_Spy_0176												
M1_Spy_0177												
M1_Spy_0178												
M1_Spy_0179	0.0077	-0.81	0.0013	-1.40	0.0012	-1.62	0.5125	-0.91	3.00E-04	-1.71	0.0126	-1.72
M1_Spy_0180												
M1_Spy_0181	0.3027	-0.54	0.0032	-1.34	0.0869	-1.34	0.4154	-0.68	0.0092	-1.36	0.0055	-1.27
M1_Spy_0182	1	-0.07	0.5469	0.64	0.5268	1.31	1	0.00	0.8918	0.90	0.3406	2.20
M1_Spy_0183	0.8781	0.19	0.0505	0.87	0.9858	0.22	0.1923	0.53	1	0.23	0.8667	-0.34
M1_Spy_0184	0.0459	0.67	3.00E-04	1.75	0.001	1.14	0	1.38	0.0035	1.47	0.0051	0.97
M1_Spy_0185	0.7767	-0.21	0.0157	-0.88	0.0772	-0.83	0.9966	-0.21	9.00E-04	-1.20	0.0855	-1.06
M1_Spy_0186	0.9954	-0.13	0.0668	-0.61	0.2064	-0.60	0.6683	-0.26	0.9073	-0.58	0.0136	-0.76
M1_Spy_0187	0.4322	-0.34	0.0057	-0.81	0.0544	-0.77	0.4352	-0.48	0.0889	-0.72	0.0638	-0.65
M1_Spy_0188	0.1459	-0.41	0.8671	-0.46	0.7954	-0.43	0.9686	-0.40	1	-0.06	0.6804	-0.41
M1_Spy_0189	0.1583	-0.56	5.00E-04	-1.02	0.0213	-0.85	0.0014	-0.84	0.0133	-0.85	0.0208	-0.87
M1_Spy_0190	8.00E-04	-0.74	8.00E-04	-0.78	0.9938	-0.25	0.0068	-0.90	0.2682	-0.79	0.3692	-0.48
M1_Spy_0191	0.6693	-0.36					0.3849	-0.70	0.0381	-1.13	0.046	-1.25

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_0195	1	-0.01	0.8041	-0.38	0.9411	-0.56	0.9912	-0.21	0.9273	-0.57	0.0192	-1.30
M1_Spy_0196												
M1_Spy_0197												
M1_Spy_0198	0.9013	-0.28							0.0453	-1.34		
M1_Spy_0199												
M1_Spy_0201	0.9852	-0.18	0.9997	0.12	0.9959	0.31	1	-0.10	0.9905	0.54	0.9772	0.36
M1_Spy_0203	1.00E-04	1.05	1	0.16	1	-0.17	0.0302	0.98	0.1634	0.93	1	0.21
M1_Spy_0205	0.0163	0.62	9.00E-04	2.54	1.00E-04	2.65	0.0192	0.97	2.00E-04	2.57	0.006	3.23
M1_Spy_0207	0.006	1.38					0.0366	2.10				
M1_Spy_0208	0.999	-0.09	0.0031	-0.79	0.0163	-0.78	0.881	-0.35	0.0015	-1.08	6.00E-04	-1.05
M1_Spy_0210	0.8978	0.23	0.1849	0.77			1	0.19	0.5779	1.22	0.3573	0.82
M1_Spy_0212	1	-0.02	0.8817	-0.93	0.6251	-1.08	1	-0.04	0.9598	-0.61	0.844	-0.92
M1_Spy_0215	0	1.36	4.00E-04	2.02	9.00E-04	1.82	0	2.06	0	2.50	0	2.19
M1_Spy_0216	0.6031	-0.93	0.115	-1.01			0.0303	-1.03	0.9944	-0.50	0.1861	-1.21
M1_Spy_0219	0.9999	-0.14									1	-0.02
M1_Spy_0223	1	0.07	0.0034	1.82			0.4585	0.58	0.0689	1.92	4.00E-04	2.13
M1_Spy_0224	0	1.04	0.5925	-0.28	0.015	-0.90	0.004	0.75	0.6785	-0.53	0.0085	-0.92
M1_Spy_0226	0	1.53	0.9997	-0.15	0.1492	-0.65	2.00E-04	1.06	0.9104	-0.43	0.0716	-0.73
M1_Spy_0227	0.2681	-1.37	0.3871	-1.04			0.0036	-2.29	0.0061	-1.30	1	-0.11
M1_Spy_0228	0.5317	-0.89	0.9576	-0.45			0.2925	-1.32	0.3784	-0.81	0.8635	0.59
M1_Spy_0229	0.7187	-0.30	1	0.08	1	0.07	1	0.08	0.5992	0.51	0.2815	0.67
M1_Spy_0230							0.995	0.60			0.099	2.09
M1_Spy_0233	0.5308	0.47	0.039	1.86			0.0911	0.77	0.0425	2.18		
M1_Spy_0235	0.9909	0.16	0.0033	2.00	0.0156	1.58	0.9932	0.34	0.0065	1.85	0.2437	1.29
M1_Spy_0236	0.8561	0.33	4.00E-04	2.14	9.00E-04	1.73	0.0478	0.74	0.0537	2.47	0.2216	1.80

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_0237	1	-0.04	5.00E-04	1.18	0.0269	0.73	0.9979	-0.17	0.0766	0.77	0.8818	0.32
M1_Spy_0238	0.4993	0.45	0.1679	0.71	0.3695	0.92	1	0.05	0.972	0.48	0.9633	0.33
M1_Spy_0239	0.059	0.56	2.00E-04	1.29	0.0157	0.88	0.0081	0.98	4.00E-04	1.38	0.008	1.09
M1_Spy_0242												
M1_Spy_0244												
M1_Spy_0245	0.9994	-0.14					1	0.18	1	0.09	0.3941	0.83
M1_Spy_0246	0.0029	-0.78	0.9812	0.20	1	-0.16	0.0206	-0.66	1	0.13	0.9968	-0.17
M1_Spy_0247	0.1568	-0.51	0.9997	0.08	0.9946	0.19	0.1582	-0.53	1	0.15	0.992	0.20
M1_Spy_0248	0.2501	-0.33	0.623	0.29	0.7794	0.30	0.938	-0.23	1	0.10	0.9971	0.15
M1_Spy_0250	0.2502	0.44	5.00E-04	1.36	3.00E-04	1.06	0.053	0.82	0.0062	1.60	5.00E-04	1.43
M1_Spy_0251	0.0232	-0.59	0.331	-0.78	0.266	-0.71	0.0245	-0.87	0.0636	-0.94	0.2233	-0.68
M1_Spy_0252												
M1_Spy_0254												
M1_Spy_0255												
M1_Spy_0256	0.0671	-0.53					0.6537	-0.54	0.0819	-1.20	0.1746	-0.92
M1_Spy_0257												
M1_Spy_0258	0.3211	-0.46	0.3797	-1.06			0.2416	-0.64	0.0481	-1.29	0.1477	-1.12
M1_Spy_0259	1	0.04	0.4531	0.82			1	0.06	0.1026	1.09	0.6659	0.67
M1_Spy_0260	0.9791	0.18	0.999	0.15	0.9971	0.29	1	0.17	0.9578	0.29	0.9954	0.20
M1_Spy_0261	0.0016	0.82	0.135	1.09	0.2022	0.91	0.503	0.95	0.031	1.36	0.0432	1.13
M1_Spy_0262	0.136	0.70	0.2507	0.56	0.9201	0.37	0.0078	0.80	0.1521	0.65	0.6604	0.46
M1_Spy_0263	0.2802	-0.45	0.0298	-0.86	0.0045	-0.92	0.0215	-0.68	0.0093	-1.20	0.0019	-1.08
M1_Spy_0264	0.7295	-0.23	0.002	-0.94	0.0172	-1.01	0.1374	-0.51	6.00E-04	-1.16	3.00E-04	-1.16
M1_Spy_0265	1	-0.01	0.1932	-0.45	0.1415	-0.69	1	-0.11	0.834	-0.56	0.0886	-0.75
M1_Spy_0266	0.3835	0.45	0.9957	-0.21	0.1251	-0.63	0.6323	0.39	0.9484	-0.33	0.3373	-0.51

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_0267	0.0054	0.67	0.998	-0.17	0.9924	-0.37	0.6131	0.62	1	-0.14	0.6244	-0.54
M1_Spy_0268	1	-0.05	1	0.01	1	0.03	1	-0.09	1	0.07	1	0.08
M1_Spy_0269	0.9706	0.28	0.9891	0.19	1	0.11	0.0899	0.57	0.978	0.24	1	0.04
M1_Spy_0271	0.0028	0.76	0.0029	0.76	0.2675	0.75	0	1.00	3.00E-04	1.26	0.0015	1.23
M1_Spy_0272	1.00E-04	1.28	0.0117	1.28	0.0322	1.19	0	1.68	0.0015	1.89	1.00E-04	1.74
M1_Spy_0273	0	1.35	0.0542	0.68	0.8419	0.33	0.0043	1.37	0.3898	0.53	0.9955	0.17
M1_Spy_0274												
M1_Spy_0276	0.6701	0.41	0.3287	0.86	0.1348	1.36	0.0027	1.11	2.00E-04	1.84	0.0053	1.69
M1_Spy_0277	0.0752	0.63	0.0042	1.36			0.0222	1.29	3.00E-04	2.79	5.00E-04	2.57
M1_Spy_0278	0.8382	0.19	0.3137	-0.76	0.472	-0.76	1	0.09	0.7839	-0.56	0.6654	-0.58
M1_Spy_0280	0.3796	0.32	0.2354	-0.63	0.3589	-0.84	0.9867	0.28	0.9968	-0.40	0.7636	-0.57
M1_Spy_0281	0.3551	0.56	0.0698	0.71	0.5036	0.61	0.7966	0.47	0.4192	1.03	0.9087	0.59
M1_Spy_0282	0.0093	0.76	0.0363	1.30	0.0272	1.18	0.0958	0.95	0.0046	1.80	0.0269	1.35
M1_Spy_0285	8.00E-04	0.94	0.1294	0.75	0.9796	0.37	8.00E-04	1.32	0.0589	0.90	0.5706	0.55
M1_Spy_0287	0	1.31	0.0024	1.14	0.3418	0.94	0	2.07	2.00E-04	1.80	0	1.59
M1_Spy_0288	0	1.30	4.00E-04	1.51	0.0808	0.95	0	1.98	0.0052	2.04	0.0062	1.62
M1_Spy_0289	0	1.18	6.00E-04	1.11	0.1392	0.70	0	1.83	0.0102	1.70	0.0082	1.44
M1_Spy_0290	1.00E-04	1.52	5.00E-04	1.67	0.001	1.43	0	2.30	0.0032	2.49	0.004	2.21
M1_Spy_0292	1	-0.06	0.4975	0.60	0.6724	0.63	1	0.15	0.2307	1.07	0.6844	0.55
M1_Spy_0294	0.7584	0.44	0	1.38	0.0097	1.41	0.0072	0.84	0.0285	1.90	0.0114	1.99
M1_Spy_0295	0.9994	0.10	0.0646	0.97	0.0722	1.12	0.0089	0.79	0	1.71	0.0014	1.80
M1_Spy_0296	1	0.03	0.0084	1.29	0.0043	1.15	0.0838	0.67	3.00E-04	2.03	0.0086	2.07
M1_Spy_0297	1	-0.04	0.0096	1.03	0.0012	1.10	0.0471	0.64	7.00E-04	1.91	0.0011	1.94
M1_Spy_0300												
M1_Spy_0303							1	-0.05				

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_0305	1	-0.07	0.544	0.46	0.9995	0.19	0.9945	-0.30	0.9389	0.30	1	-0.03
M1_Spy_0306	0.0216	0.67	0.1718	0.48	0.9975	0.16	0.0423	0.62	0.3612	0.47	1	0.14
M1_Spy_0307	0.1318	0.60	0.1286	0.52	0.9228	0.24	0.0713	0.56	0.2818	0.48	0.9946	0.19
M1_Spy_0308	0.0228	0.77	0.4105	0.44	1	0.07	0.2103	0.67	0.885	0.48	1	-0.09
M1_Spy_0309	0.0254	0.98	0.0327	0.67	1	0.11	0.032	0.90	0.407	0.72	1	-0.07
M1_Spy_0310	0.0082	1.04	0.0102	0.73	0.9916	0.17	0.0078	1.04	0.2222	0.75	1	0.15
M1_Spy_0312	0.4631	0.51	0.535	-0.35	0.1181	-0.79	0.1825	0.64	0.6382	-0.42	0.1003	-0.74
M1_Spy_0314	8.00E-04	1.33	0.7394	0.47	1	0.08	3.00E-04	1.63	0.9665	0.46	0.9515	-0.34
M1_Spy_0315	0.0572	0.68					0.0943	1.06				
M1_Spy_0316	0	0.89	0.5311	0.39	0.9929	0.29	2.00E-04	1.11	0.0817	0.87	0.7613	0.46
M1_Spy_0317	0	-1.43	0.0158	-0.85	0.0137	-1.05	0.0517	-1.63	0	-1.90	0	-2.01
M1_Spy_0319	0.008	-0.72	0.0015	-0.85	0.4791	-0.58	0.003	-1.06	1.00E-04	-2.07	0.0022	-2.24
M1_Spy_0320	1.00E-04	-0.84	0.0385	-0.72	0.3982	-0.43	0.0038	-0.83	2.00E-04	-1.68	0	-1.64
M1_Spy_0321	0.0065	-0.84	0.9064	-0.24	0.9458	0.29	0.0171	-0.71	0.7389	-0.50	0.2696	-0.51
M1_Spy_0323	0.0787	0.78	0.5048	1.04			0.2556	0.82	0.009	2.00	0.0026	2.02
M1_Spy_0324	1	0.09	6.00E-04	1.83	0.0045	1.94	1	0.16	0.0013	2.57	0.018	2.50
M1_Spy_0326	1	-0.01	0.0051	-0.74	0.0026	-1.01	1	-0.11	0.6171	-0.45	0.0499	-0.78
M1_Spy_0327	0.9788	0.16	0.8985	-0.53	0.0901	-0.85	0.9895	-0.23	1	0.19	0.8914	-0.63
M1_Spy_0329	1	-0.03	0.0089	0.75	0.1017	0.92	1	-0.11	0.8257	0.58	0.0711	0.72
M1_Spy_0330	0.9994	-0.10	0.2559	-0.41	0.9878	-0.20	1	-0.16	0.397	-0.60	0.9377	-0.24
M1_Spy_0331	0.9994	0.17	0.9997	-0.08	1	-0.01	1	0.12	0.3607	-0.67	0.5495	-0.52
M1_Spy_0334	0.0995	0.61	0.6299	0.49	0.9571	0.48	0.6414	0.35	0.5603	0.70	0.9656	0.51
M1_Spy_0337	0	1.55	0.2042	0.64	0.6364	0.44	0	1.45	0.543	0.52	0.6466	0.42
M1_Spy_0338	0.0011	-0.73	0.9072	0.23	1	0.10	0.0179	-0.79	1	0.06	0.8061	-0.43
M1_Spy_0339	0.7641	-0.29	0.9954	0.23	0.7189	0.31	0.9454	-0.37	1	0.13	0.9955	-0.15

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_0340	1	0.09	0.1118	0.66	0.3256	0.63	0.9416	0.29	0.0551	1.20	0.041	0.80
M1_Spy_0341	0.3861	0.38	0.0016	1.12	0.0185	1.04	0.0387	0.88	4.00E-04	1.46	0.0183	1.20
M1_Spy_0342	0.0052	-0.71	0.4588	0.73	0.7134	0.40	1	-0.09	0.0879	1.18	0.6494	0.60
M1_Spy_0343	1	-0.04	0.3434	-0.37	0.0479	-0.60	0.9872	-0.19	0.3224	-0.50	0.1983	-0.60
M1_Spy_0345	0.1205	0.42	0.6996	0.55	0.9681	0.38	0.1948	0.58	0.7077	1.19	0.2455	0.53
M1_Spy_0346	0.7742	0.21	0.9561	0.21	1	0.12	0.6603	0.28	0.9997	0.18	1	0.08
M1_Spy_0348	8.00E-04	0.81	9.00E-04	1.41	6.00E-04	1.36	0.018	1.04	0	1.75	0.0357	1.13
M1_Spy_0349	1.00E-04	1.10	5.00E-04	1.38	0.0038	1.21	3.00E-04	1.11	6.00E-04	1.74	0.1115	1.30
M1_Spy_0351	0.006	-0.81	0.0099	-0.97	0.0344	-0.98	5.00E-04	-1.05	3.00E-04	-1.26	0.0014	-1.11
M1_Spy_0352	0.0045	-0.71	0	-1.51	1.00E-04	-1.63	0.0028	-0.82	0	-1.78	0	-1.65
M1_Spy_0356	0.048	-0.70	1	0.04	0.9805	0.29	0.122	-0.94	1	-0.21	1	0.02
M1_Spy_0357	0.2299	-0.57	0.0693	-0.65	0.0552	-0.70	0.0177	-0.70	0.0291	-0.78	0.5344	-0.70
M1_Spy_0358	0.1806	0.40	0.0309	0.83	0.5134	0.69	0.3575	0.60	0.033	0.94	0.1315	0.69
M1_Spy_0359	0.9852	0.14	0.9781	0.20	0.9681	0.28	1	0.06	0.9943	0.32	0.9704	0.33
M1_Spy_0361	1	0.04	1	0.08	0.9897	-0.25	1	0.02	1	0.18	1	-0.11
M1_Spy_0362	0.0527	0.64	0.174	0.60	0.9863	0.30	0.042	0.74	0.1221	0.75	1	0.20
M1_Spy_0363	0.0036	0.75	0.3049	0.57	0.9884	0.29	0.0517	0.77	0.2816	1.03	0.9942	0.36
M1_Spy_0364	0.0022	0.83	0.1142	0.77	1	0.11	0.0117	1.00	0.1392	0.84	1	0.18
M1_Spy_0365	0.0027	1.17	0.1341	1.51	0.4602	0.68	0.0632	1.59	0.0063	1.58	0.9603	0.57
M1_Spy_0366	1.00E-04	0.97	0.1048	0.66	1	0.16	0.0035	1.06	0.0347	1.01	0.994	0.25
M1_Spy_0367	1.00E-04	1.02	0.8642	0.26	0.9995	-0.21	0.0038	1.26	0.1549	0.91	1	-0.03
M1_Spy_0369	0	1.16	0.9644	0.44	1	-0.01	0.0296	1.50	0.5736	0.73	1	-0.11
M1_Spy_0370	0.0029	0.94	0.9171	0.34	1	-0.02	0.0833	1.21	0.4389	0.76	1	-0.12
M1_Spy_0371	0.9698	0.20	0.0034	2.61	0.001	2.09	0.892	0.35	0.0188	2.78	0.1788	1.73
M1_Spy_0373	0.004	-0.77	1	0.05	1	-0.13	0.1366	-0.89	1	0.11	0.8192	-0.42

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_0374	0.9221	-0.34	0.0018	0.95	0.131	0.89	1	-0.20	0.1045	1.17	0.0379	0.96
M1_Spy_0376	0.6752	-0.24	0.0149	0.82	0.0439	0.82	1	-0.10	0.0275	0.91	0.2787	0.83
M1_Spy_0377	1	0.01	0.5379	0.37	0.3011	0.71	1	-0.16	0.8368	0.64	0.7209	0.62
M1_Spy_0378	0.9874	-0.15	0.0498	0.68	0.1514	0.70	0.6036	-0.35	0.0492	0.80	0.0434	0.75
M1_Spy_0379	6.00E-04	-0.72	4.00E-04	-1.76	0.0185	-1.53	0	-1.24	0	-2.03	0.0063	-1.36
M1_Spy_0380	0.7151	-0.20	0.002	-0.92	0.0018	-0.92	0.9408	-0.26	0.0372	-0.71	0.2638	-0.52
M1_Spy_0382	0.9145	-0.24	0.0562	-0.53	0.4156	-0.54	0.3819	-0.38	0.2932	-0.54	0.4039	-0.46
M1_Spy_0383												
M1_Spy_0384												
M1_Spy_0385												
M1_Spy_0386												
M1_Spy_0388	1	-0.06	1	0.02	1	0.18	1	0.11	0.5684	0.52	0.3472	0.60
M1_Spy_0390	0.02	0.86	0.0011	1.72	0.013	1.44	0.407	0.72	0.0716	2.09	3.00E-04	2.08
M1_Spy_0392	0	1.42	0.004	1.96	0.0171	1.82	0.0878	1.63	0.0305	2.76	0.0171	1.85
M1_Spy_0393	0.9203	0.30	0.2937	0.72	0.6619	0.65	0.9936	0.26	0.6585	0.55	0.2065	0.86
M1_Spy_0395	0.642	-0.51	0.0013	-1.06	0	-1.22	0.0175	-0.81	4.00E-04	-1.60	1.00E-04	-1.55
M1_Spy_0397	0.1707	-0.55	0	-1.60	0.0224	-1.67	0.0089	-1.11	0	-2.13	0	-2.30
M1_Spy_0399	0.9609	0.26	0.9995	-0.13	1	-0.14	1	-0.01	0.8546	-0.45	0.1052	-0.64
M1_Spy_0400	0.9998	-0.10	0.0057	-0.67	0.024	-0.73	0.2962	-0.37	0.0052	-0.89	0.0019	-1.08
M1_Spy_0401	1	0.08	0.9534	-0.29	0.9999	-0.25	1	-0.14	0.3166	-0.47	0.3641	-0.68
M1_Spy_0405	0.6704	0.25	0.1501	-0.41	0.3073	-0.59	1	-0.06	0.1432	-0.62	0.0071	-0.84
M1_Spy_0406	0.838	0.22	0.0338	-0.64	0.1438	-0.71	1	-0.06	0.0987	-0.63	0.2766	-0.77
M1_Spy_0407	0.6186	0.26	0.3105	-0.52	0.2933	-0.52	1	-0.01	0.1375	-0.55	0.5239	-0.55
M1_Spy_0408	1	0.04					1	0.12	0.6087	0.85	0.9566	0.40
M1_Spy_0410	0	0.93	6.00E-04	1.36	0	2.04	0.471	0.52	4.00E-04	1.45	0.0045	2.07

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_0412	0.3707	-0.33	0.4031	0.40	0.6863	0.55	0.9822	-0.18	1	0.13	0.6376	0.44
M1_Spy_0414	0	-2.74	1	-0.06	0.9971	0.53	0	-2.40	1	0.10	0.9015	0.56
M1_Spy_0416												
M1_Spy_0421	0.174	-0.44	0.9995	-0.35	0.939	-0.59	0.9621	-0.33	1	-0.37	1	-0.24
M1_Spy_0422	1	0.06	5.00E-04	1.73	0.0036	1.79	0.1524	0.68	0	2.25	0	1.99
M1_Spy_0425					0.1804	1.48						
M1_Spy_0426												
M1_Spy_0427												
M1_Spy_0428	0	-1.15	0.0239	-1.26	0.3934	-0.77	0.0046	-1.39	0.9953	-0.41	0.8752	0.42
M1_Spy_0430												
M1_Spy_0431												
M1_Spy_0432	0.0381	-0.77	0.5196	-0.40	0.7848	-0.48	0.0191	-0.94	1	-0.23	0.9932	-0.27
M1_Spy_0433												
M1_Spy_0435												
M1_Spy_0436	0.6015	-0.44	0.0098	-1.55	0.3131	-1.51	1	0.28	1	-0.28	1	-0.28
M1_Spy_0437												
M1_Spy_0439	0.7547	-0.26	0.3408	0.86	0.0515	0.95	1	-0.13	0.0786	1.03	0.156	1.14
M1_Spy_0440	0.9985	-0.15	0.0104	1.24	0.1432	0.96	1	0.00	0.0032	1.46	0.0299	1.41
M1_Spy_0441	0.988	-0.15	0.0106	1.14	0.0018	1.12	1	0.15	4.00E-04	1.43	0.051	1.57
M1_Spy_0442	1	-0.03	0.0124	1.62			0.0791	1.01	2.00E-04	3.22	0.0025	2.93
M1_Spy_0443	0.2554	0.30	0.5228	0.36	0.9967	0.15	0.5065	0.35	0.8553	0.61	0.6465	0.38
M1_Spy_0444	0.348	0.34	0.9999	0.08	1	-0.08	0.5739	0.31	0.9978	0.26	1	0.07
M1_Spy_0446	1	-0.05	1	0.02	0.9833	-0.18	1	0.07	0.9999	0.19	1	-0.05
M1_Spy_0447	1	-0.06	0.9729	-0.27	0.1797	-0.50	1	-0.03	1	-0.18	0.4976	-0.38
M1_Spy_0448	0.9998	-0.07	0.9905	-0.19	0.6998	-0.34	1	-0.07	1	-0.02	0.9348	-0.22

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_0450	0.0257	-0.87	0.2867	-0.67	0.9956	-0.22	1.00E-04	-1.05	0.5692	-0.69	1	-0.10
M1_Spy_0453	0.3744	0.38	0.4729	0.36	0.5361	0.45	0.7009	0.28	0.9729	0.35	0.4995	0.73
M1_Spy_0454	0.3503	0.63	0.0977	0.93	0.0026	1.09	1	-0.09	0.3449	1.14	0.0119	1.40
M1_Spy_0456	0.0693	0.66	0.0602	1.48	0.0083	1.69	1	0.27	0.0627	1.93	0.0211	1.91
M1_Spy_0457	0.8793	0.34	0.6501	0.76	0.9113	0.76	0.999	0.27	0.2907	0.84	0.8781	0.87
M1_Spy_0458	0.0028	1.01	0.0011	1.52	0.0157	1.26	0.0055	1.40	0.0041	1.80	1.00E-04	1.66
M1_Spy_0459	0.5003	-0.71	1	0.11	0.9955	0.64	0.0126	-0.95	1	0.23	0.9982	0.24
M1_Spy_0460	0.058	0.64	0.0026	1.26	0.0187	1.10	3.00E-04	1.10	0.0486	2.42	0.0312	2.16
M1_Spy_0461	0.0303	0.87	4.00E-04	1.48	0.0336	1.78	3.00E-04	1.58	0	2.69	0	2.74
M1_Spy_0462	0.0665	0.43	0.2775	0.49	0.455	0.60	0.5777	0.34	0.0995	0.72	0.1327	0.59
M1_Spy_0463	0.7413	0.43	0.0909	0.64	0.0541	0.71	0.842	0.47	1	0.23	0.9414	0.35
M1_Spy_0464	8.00E-04	1.20	5.00E-04	1.77	0	1.74	0.0055	1.34	2.00E-04	2.34	1.00E-04	1.92
M1_Spy_0466	0.9994	0.13	0.9141	0.23	0.9946	0.24	1	-0.11	0.9999	0.26	1	0.13
M1_Spy_0467	0.9979	0.18	0.6409	0.35	0.972	0.24	1	-0.04	0.9816	0.56	1	0.14
M1_Spy_0469	0.9572	-0.21	0.984	0.17	0.9971	0.25	0.5866	-0.35	1	-0.08	1	-0.18
M1_Spy_0470	1	-0.04	0.851	-0.27	0.5499	-0.47	0.628	-0.31	0.9993	-0.35	0.0645	-0.75
M1_Spy_0471	0.9616	0.20	0.7613	-0.47	0.5668	-0.53	0.9843	0.17	0.2502	-0.63	0.1535	-0.70
M1_Spy_0472	0	1.16	0.1886	0.65	0.9956	0.50	0.0055	1.06	0.0075	1.09	0.9895	0.34
M1_Spy_0473	0.0265	0.65	0.5914	0.32	0.99	0.37	0.0981	0.60	0.4091	0.58	0.9763	0.26
M1_Spy_0475	0.0037	0.99	0.8158	0.58	0.9973	0.34	0.0079	0.76	0.6421	1.16	0.9809	0.74
M1_Spy_0476	0.0256	0.68	0.9091	-0.23	0.7872	-0.43	0.0034	0.84	1	-0.04	0.9574	-0.24
M1_Spy_0477	0	1.86	0.0429	0.93	0.6939	0.51	1.00E-04	1.91	0.1081	1.65	0.7226	0.76
M1_Spy_0478												
M1_Spy_0479	8.00E-04	1.24	0.6851	0.81			0.2746	0.76	0.2741	1.04	0.0016	1.77
M1_Spy_0480	1	0.05	0.0674	-0.71	0.7315	-0.43	0.935	-0.31	0.0601	-0.82	0.6985	-0.43

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_0484	0.9973	0.20			1	-0.22	0.9686	-0.33			1	-0.18
M1_Spy_0486	0.3108	-0.47	6.00E-04	-1.18	0.0038	-1.13	0.0265	-0.79	6.00E-04	-1.53	0.0022	-1.35
M1_Spy_0488												
M1_Spy_0489												
M1_Spy_0492												
M1_Spy_0496	0.0098	-0.73	0.0086	-1.18	0.0245	-1.25	0.109	-0.90	0.0071	-1.51	0.0062	-1.38
M1_Spy_0497	1	0.03	0.9999	0.21			1	-0.03	0.9915	0.53	1	0.08
M1_Spy_0498	1	-0.04	1	-0.19			1	0.02	1	0.10	1	-0.06
M1_Spy_0500	0.1425	0.36	0.4912	-0.54	0.8119	-0.50	0.9984	0.15	0.6751	-0.54	0.1929	-0.57
M1_Spy_0501	0.3175	0.72	0.8494	0.49	0.9209	0.52	0.4515	0.66	0.1595	0.90	0.3731	0.83
M1_Spy_0502	0.5555	-0.28	0.0038	-1.07	0.0232	-1.16	0.1806	-0.84	0.0071	-1.37	6.00E-04	-1.31
M1_Spy_0503	0.0442	0.68	0.9997	-0.08	0.7421	-0.42	0.1063	0.43	0.3712	-0.48	0.0046	-0.81
M1_Spy_0504	0.0529	0.46	0.9855	-0.16	0.4012	-0.47	0.629	0.26	0.3187	-0.59	0.0072	-0.78
M1_Spy_0505	8.00E-04	1.04	0.9876	-0.23	0.8963	-0.51	0.2007	0.67	0.5239	-0.56	0.002	-0.96
M1_Spy_0506	0.1507	-0.49	0.2547	0.65	0.1135	0.65	0.8692	-0.26	0.0387	1.08	0.1295	0.85
M1_Spy_0507	0.991	0.14	6.00E-04	1.73	0.0163	1.78	1	0.14	0.0251	1.68	0.0125	1.40
M1_Spy_0508	0.9893	0.15	0.0377	0.76	0.2328	0.92	1	0.10	0.1221	0.93	0.4366	0.80
M1_Spy_0510	0.102	1.29					0.7238	1.12				
M1_Spy_0511	0.2671	0.41	5.00E-04	-1.43	0.0025	-1.85	1	0.04	3.00E-04	-2.01	0	-2.31
M1_Spy_0512	6.00E-04	0.83	0.2239	-0.66	0.0425	-0.98	0.1111	0.50	0.0061	-1.31	0.0052	-1.44
M1_Spy_0513	8.00E-04	0.93	0.4483	-0.52	0.2331	-0.81	0.0624	0.62	0.0821	-0.92	0.0035	-1.17
M1_Spy_0514	0.9766	0.16	0.9954	-0.20	1	-0.05	0.6138	0.30	0.9055	0.42	0.753	0.41
M1_Spy_0515	0.0131	-1.10	0	-1.61	5.00E-04	-1.65	0.0011	-1.06	0.0042	-1.59	0.1271	-1.16
M1_Spy_0516	0.9727	-0.26	8.00E-04	-0.99	0.038	-1.01	1	-0.01	0.2119	-0.68	0.5912	-0.51
M1_Spy_0517	2.00E-04	0.88	7.00E-04	1.02	0.0197	1.11	0	1.27	2.00E-04	1.45	0.0054	1.32

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_0518												
M1_Spy_0519												
M1_Spy_0521												
M1_Spy_0524												
M1_Spy_0526												
M1_Spy_0527	0.6927	-0.36										
M1_Spy_0528	0.5876	-0.26	0.5176	-0.33	0.9753	-0.36	0.708	-0.42	0.9017	-0.38	0.7303	-0.39
M1_Spy_0529	0.9998	-0.07	1	-0.07	0.9808	-0.23	1	-0.10	0.9994	-0.18	0.9992	-0.14
M1_Spy_0530	0.0955	0.72	0.736	0.52	0.9957	0.39	0.919	0.43	0.973	0.68	0.968	0.53
M1_Spy_0531												
M1_Spy_0532	0.1063	0.62					0.1097	1.75	0.9876	0.70	1	0.20
M1_Spy_0533	0.0807	-0.58					0.2528	-0.77	0.9415	-0.97		
M1_Spy_0534	0.0454	-0.51	0.4154	-1.01	0.7216	-0.80	0.6199	-0.66	0.0321	-1.25	0.1779	1.64
M1_Spy_0535	0.7504	-0.34									0.0944	3.15
M1_Spy_0536												
M1_Spy_0537	0.9834	0.36					0.7266	0.69	0.5045	1.67	0	5.35
M1_Spy_0538											0.0032	7.49
M1_Spy_0539	1	-0.02	0.6058	0.80	0.0259	1.70	1	0.18	0.8636	0.72	1.00E-04	4.78
M1_Spy_0540												
M1_Spy_0542	0.6424	0.39									0	5.95
M1_Spy_0543												
M1_Spy_0544	0.4174	-0.41										
M1_Spy_0545												
M1_Spy_0546												
M1_Spy_0547	0.4808	-0.40					0.894	-0.38	0.8869	-0.71	0.9968	-0.32

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_0549	0.0363	-0.79	0.0024	-1.45	0.0053	-1.43	0.0354	-1.17	2.00E-04	-1.85	0.0062	-1.60
M1_Spy_0550	0.0583	-0.61	0.0145	-1.00	0.0276	-1.17	0.0437	-1.01	0.1037	-1.26	0.0347	-1.74
M1_Spy_0552	0.4966	-0.47	0.182	-0.69	0.1065	-0.93	0.3142	-0.63	0.1527	-1.03	0.0456	-1.61
M1_Spy_0553	0.8676	-0.40	0.4003	-0.60	0.5974	-0.97	0.4743	-0.67	0.5208	-0.96		
M1_Spy_0555												
M1_Spy_0556												
M1_Spy_0558	1	-0.02	0.0839	-0.56	0.9929	0.23	0.2649	-0.45	0.8693	-0.53	0.9824	-0.36
M1_Spy_0559	0.9967	0.12	1	-0.05	0.9728	0.31	0.8236	-0.25	0.9978	-0.22	0.9883	-0.23
M1_Spy_0560	0.6383	0.27	1	0.04	0.6022	0.42	1	-0.13	0.9972	-0.18	1	0.02
M1_Spy_0565												
M1_Spy_0567	1	0.00	0.0919	0.99	0.8553	0.54	0.9879	0.19	0.2209	1.16	0.5945	0.70
M1_Spy_0568	0.9179	-0.22	0.9995	0.21	1	0.04	0.9996	-0.15	0.3129	0.46	0.9966	0.25
M1_Spy_0569	0.9989	0.13	0.0972	0.63	0.0873	0.63	0.7139	0.32	0.1307	0.95	0.5906	0.69
M1_Spy_0570												
M1_Spy_0571												
M1_Spy_0572												
M1_Spy_0574												
M1_Spy_0575	6.00E-04	-0.80	0.0612	-0.68	0.0644	-0.74	0.0179	-0.92	0.5039	-0.61	0.32	-0.53
M1_Spy_0576	0.1988	-0.55	0.0377	-0.64	0.3145	-0.65	0.1922	-0.66	0.6735	-0.50	0.635	-0.56
M1_Spy_0577	0.0011	-0.78	6.00E-04	-1.38	0.0017	-1.57	0.0027	-1.25	0	-1.89	1.00E-04	-1.66
M1_Spy_0578												
M1_Spy_0580	0.5983	0.43					0.0389	1.07				
M1_Spy_0581	0.9861	-0.14	0.2764	0.45	0.0357	1.22	1	-0.07	0.2494	1.07	0.0786	1.65
M1_Spy_0583	0.0085	-0.86	0	-2.19	5.00E-04	-2.38	0	-1.07	0	-2.47	0.0136	-1.71
M1_Spy_0584	0.0016	-0.78	0	-1.32	1.00E-04	-1.31	0.0016	-0.89	1.00E-04	-1.47	0.0032	-1.28

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_0585	0.5486	-0.29	0.1614	-0.50	0.2175	-0.52	0.9962	-0.16	1	-0.16	0.545	-0.59
M1_Spy_0587	0.9968	-0.13	0.0011	-0.86	0.0424	-0.91	0.7476	-0.26	6.00E-04	-1.17	1.00E-04	-1.33
M1_Spy_0588	0.8702	-0.22	0.1046	-0.48	0.8894	-0.45	1	0.01	0.1901	-0.69	0.1277	-0.69
M1_Spy_0589	0.8894	0.36	0.0013	2.33			0.018	1.12	0.1469	2.51		
M1_Spy_0590	0.4928	0.41	0.036	1.26			0.8331	0.44	0.0226	1.68	0.0935	1.53
M1_Spy_0591	0.9323	-0.24	0.9894	-0.28	0.2822	-0.70	1	-0.16	0.9888	-0.28	0.9152	-0.48
M1_Spy_0593	0.4398	-0.43	4.00E-04	-1.19	0.001	-1.08	0.0272	-1.03	7.00E-04	-1.50	0	-1.58
M1_Spy_0595	0.0407	0.61	0.001	1.16	0.0169	0.96	0.0047	0.91	0.0052	1.11	0.0296	0.85
M1_Spy_0596	0.7057	0.32	0.7701	0.56	0.9796	0.74	0.6544	0.51	0.2347	0.96	0.536	0.97
M1_Spy_0598	0.8011	0.30	0.2079	0.64	0.916	0.48	1	0.06	0.5043	0.92	0.994	0.65
M1_Spy_0599	1	0.08	0.6596	0.59	0.9053	0.64	1	0.07	0.5836	1.02	1	0.26
M1_Spy_0600	0.9861	0.24	0	1.86	0.0106	2.24	0.9995	0.20	0.0052	2.11	0.0907	1.67
M1_Spy_0601	0.2553	-0.40	0.0099	-0.96	0.0054	-1.06	0.0167	-0.84	0.2597	-0.73	0.0402	-1.11
M1_Spy_0603	0.0146	-0.78	0.9969	0.25	1	-0.01	0.0115	-0.78	0.9998	0.50	1	0.20
M1_Spy_0604	0.0319	-0.93	0.8441	-0.41	0.2772	-0.46	0.141	-0.88	0.9451	-0.50	0.2413	-0.72
M1_Spy_0605	0	1.21	0.971	0.35	1	-0.03	0	1.36	1	0.21	0.8437	-0.36
M1_Spy_0606	0	2.23	0.026	1.49	0.712	1.03	0.0172	2.23	0.036	1.69	0.865	0.73
M1_Spy_0608	0.2008	-0.41	2.00E-04	-1.24	0.0925	-1.24	1	-0.03	0.0033	-1.48	0.0809	-1.87
M1_Spy_0609	0.0028	1.15	0.0161	1.83	0.0165	1.78	0.0051	1.43			0.1058	2.09
M1_Spy_0611												
M1_Spy_0613	0.3623	-0.48	0.4438	-0.33	0.7406	-0.47	0.5675	-0.44	0.7345	-0.56	0.2445	-0.73
M1_Spy_0615	0.0972	0.41	0.0412	0.60	0.6053	0.48	0.2699	0.50	0.3905	1.02	0.5443	0.63
M1_Spy_0616	0.1308	0.56	0.4099	0.86	0.489	0.97	0.0136	0.68	0.0409	1.40	0.6144	1.08
M1_Spy_0617	0.0285	0.69	0.0034	1.27	0.3548	1.33	0.2146	0.79	0.0967	1.53	0.6191	0.91
M1_Spy_0619	0.006	0.70	0.2329	0.54	0.9479	0.37	0.091	0.84	0.4777	0.51	0.726	0.43

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_0621	0.0585	0.48	0.413	0.49	0.9868	0.32	0.0097	0.81	0.7141	0.46	0.9616	0.26
M1_Spy_0622	0.003	1.96					0.0306	1.73	0.2477	2.19	0.0011	2.32
M1_Spy_0623	0.0029	0.86	0.0295	1.80	0.0357	1.63	0.001	1.37	0.0186	2.30	7.00E-04	1.87
M1_Spy_0627	0.0065	-0.84	0.0054	-0.75	0.0045	-0.99	0	-1.20	3.00E-04	-1.46	5.00E-04	-1.52
M1_Spy_0628												
M1_Spy_0629												
M1_Spy_0630												
M1_Spy_0631												
M1_Spy_0632												
M1_Spy_0634												
M1_Spy_0636	0.9998	0.14					0.5408	0.59				
M1_Spy_0637							0.5003	0.65				
M1_Spy_0638							0.9967	0.25	1	0.22	1	0.17
M1_Spy_0639												
M1_Spy_0640	0.9968	0.12	1	-0.06	1	0.15	1	-0.11	1	-0.01	0.9992	0.21
M1_Spy_0642	0.9079	-0.18	5.00E-04	-1.03	0.006	-1.13	0.0483	-0.62	3.00E-04	-1.23	1.00E-04	-1.41
M1_Spy_0643	0.3966	0.30	0.5516	-0.32	0.9995	-0.15	1	0.14	1	-0.17	0.9342	-0.32
M1_Spy_0644	0.9857	0.15	0.2404	-0.35	0.9091	-0.30	1	0.14	1	-0.13	0.9999	-0.14
M1_Spy_0645	0.4002	0.46	0.999	-0.18	1	-0.11	0.843	0.46	1	0.18	1	0.01
M1_Spy_0646	0.6565	-0.36	0.0364	0.69	0.3374	1.22	0.0138	-0.83	0.9625	0.42	0.5375	0.63
M1_Spy_0649	0.0697	1.33	0.1194	0.77	0.6276	0.86	0.0073	1.26	0.4754	1.33	0.432	0.83
M1_Spy_0650	1.00E-04	1.06	5.00E-04	1.76	0.0271	1.85	0	1.25	2.00E-04	2.46	0.0093	1.66
M1_Spy_0651	6.00E-04	1.17	3.00E-04	2.34	0.0163	2.61	1.00E-04	1.97	0.0082	3.20	0.008	2.51
M1_Spy_0652	0.8147	0.21	0.1759	-0.51	0.3725	-0.60	0.9957	0.15	0.2103	-0.55	0.224	-0.55
M1_Spy_0653	0.0419	0.61	1	0.02	0.9995	-0.16	0.0195	0.70	1	0.11	1	-0.01

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_0654	0.0026	0.92	1	-0.01	1	-0.15	0.042	1.15	0.8949	0.39	1	0.10
M1_Spy_0655	0.1035	-0.47	0.0854	-1.05			0.2139	-0.66			0.273	-1.13
M1_Spy_0656	0.0102	-0.69	0.0018	-1.23	0.006	-1.10	0.0051	-1.06	0.0026	-1.27	0.0026	-1.45
M1_Spy_0657	0.0368	-0.48	0.0107	-1.17	0.0596	-0.96	0.0616	-0.84	0.0071	-1.17	0.041	-1.26
M1_Spy_0658	0.0148	-0.73	5.00E-04	-1.24	0.1204	-0.91	0.0085	-1.45	0.1173	-1.36	0.0315	-1.29
M1_Spy_0659	0.2807	-0.89	0.027	-1.29	0.0499	-1.17	0.0068	-1.50	0.0162	-1.46	0.0068	-1.66
M1_Spy_0660	0.2996	-0.82	6.00E-04	-1.33	0.3927	-1.12	0.0101	-1.67	0.3027	-1.36	0.0045	-1.70
M1_Spy_0661	0.1549	-0.69	0.0012	-1.25	0.3879	-0.93	0.0133	-1.36	0.1964	-1.30	0.0174	-1.41
M1_Spy_0663	0.2929	-0.56	0.0868	-1.03	0.0886	-0.95	0.1713	-0.96	0.1029	-0.96	0.2542	-1.24
M1_Spy_0664	0.4423	-0.57	0.0161	-1.15	0.1908	-0.97	0.1822	-1.20	0.4762	-1.00	0.0451	-1.31
M1_Spy_0665	0.4821	-0.51	0.2958	-0.79	0.4535	-0.69	0.0354	-1.03	0.5262	-0.81	0.0857	-1.03
M1_Spy_0666	0.088	-0.73	0.0064	-1.22	0.0159	-1.07	0.0055	-1.23	0.0738	-1.19	0.002	-1.46
M1_Spy_0667	0.6802	-0.64	6.00E-04	-1.22	0.0449	-0.84	0.0642	-1.32	0.5951	-0.97	0.0113	-1.40
M1_Spy_0669					0.858	-0.64			0.6368	-0.73	0.9502	-0.47
M1_Spy_0670							0.7099	-0.73	0.9993	-0.50		
M1_Spy_0671	0.7712	-0.47			0.7345	-0.72	0.3283	-0.79	0.9244	-0.74	0.5781	-0.72
M1_Spy_0672												
M1_Spy_0673	0.162	-0.65	0.0044	-1.12	0.0434	-1.04	0.1206	-0.76	0.1219	-1.06	0.0508	-1.11
M1_Spy_0674	0.4182	-0.69	0.0169	-1.22	0.3833	-1.02	0.5996	-1.06	0.9948	-0.86	0.2392	-0.99
M1_Spy_0676	0.2615	-0.49					0.0863	-0.79				
M1_Spy_0677	0.5125	-0.51					0.2641	-0.74				
M1_Spy_0679	0.8306	0.21	0.9987	0.20			1	0.04	0.7875	0.57		
M1_Spy_0680	0.9289	-0.19	0.0127	-0.83	0.9944	-0.31	0.5787	-0.71	0.017	-1.15	0.5983	-0.63
M1_Spy_0681	0.9947	-0.14	0.0629	-0.74	0.8804	-0.57	0.3422	-0.54	0.0395	-0.98	0.2094	-0.76
M1_Spy_0682	1	-0.04	0.6267	-0.72			0.6842	-0.41	0.9843	-0.46	1	-0.13

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_0683	0.367	-0.38	0.7477	-0.35	1	-0.02	0.1347	-0.59	0.9999	-0.22	1	0.06
M1_Spy_0684	1	-0.04	1	0.00	1	0.10	1	-0.17	0.9916	0.36	0.9952	0.33
M1_Spy_0685	0.9999	-0.08	0.7076	-0.51			0.1629	-0.67	0.9991	-0.41		
M1_Spy_0686	0.9954	-0.10	0.1122	-0.51	0.9116	0.37	0.0259	-0.62	0.3183	-0.67	0.6123	0.42
M1_Spy_0688	0.968	0.17	0.9911	-0.25	0.9908	0.42	0.9566	-0.29	1	0.11	0.4567	0.75
M1_Spy_0689	0.961	0.20	0.9995	0.20			0.9466	-0.31	0.9785	0.79		
M1_Spy_0690	0.1876	-0.41	0.039	-1.10	0.8266	-0.73	0.0116	-0.94	0.0185	-1.29	0.1738	-0.91
M1_Spy_0691	1	-0.02	0.8909	-0.36			0.2867	-0.57	1	-0.22	1	0.17
M1_Spy_0693	1	-0.03	0.9868	-0.25			0.3362	-0.58	1	-0.14	1	0.22
M1_Spy_0694	0.103	0.49	0.9974	0.34	0.3189	1.15	1	0.01			0.9947	0.40
M1_Spy_0695	0.2414	0.54	1	0.01			1	0.16	0.9999	0.39		
M1_Spy_0696	0.9999	0.14					1	-0.05				
M1_Spy_0697	0.1402	0.72										
M1_Spy_0698												
M1_Spy_0700												
M1_Spy_0701	0.73	0.53	1	-0.05			0.9996	0.28				
M1_Spy_0702	0.8085	0.30	0.7142	-0.59	1	-0.15	0.9962	-0.34	0.0725	-0.99	1	0.02
M1_Spy_0703	0.4705	0.62	0.999	0.26			0.9951	0.27			0.6014	0.92
M1_Spy_0705	0.2327	0.42	0.9997	-0.19			1	-0.07	1	0.07		
M1_Spy_0706	1	0.04										
M1_Spy_0707	0.1355	0.55	1	-0.08			1	0.06	1	0.18	0.9749	0.46
M1_Spy_0710	0.0941	0.64	0.9879	0.29	0.5336	1.08	0.9971	0.21	1	0.45	0.0584	1.11
M1_Spy_0711	1	-0.02	0.9988	-0.17	0.86	0.53	1	0.04	0.857	0.38	0.1598	0.79
M1_Spy_0712	0.8917	0.35	0.9961	0.22	0.901	1.14	0.7071	0.39	0.2545	0.96	0.0442	1.25
M1_Spy_0713	0.9921	-0.25	0.3864	-0.38	1	0.07	0.9411	-0.23	1	-0.15	1	0.09

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_0714	0.0011	1.08	0.0918	-0.57	0.013	-1.24	0	1.37	0.8519	0.53	0.5337	-0.54
M1_Spy_0715	0.8824	0.25	0.0044	-1.13	0.7605	-0.71	1	-0.05	0.6492	-0.78	0.7767	-0.59
M1_Spy_0716	0.7481	-0.30	0	-1.87	0	-1.69	0.1386	-0.44	0.0015	-1.37	0.0091	-1.43
M1_Spy_0717	1	-0.03	1	-0.09	0.9838	-0.26	1	-0.01	0.9999	-0.25	0.9671	-0.22
M1_Spy_0720	0.988	0.16	0.8857	-0.34	0.9171	-0.45	0.9441	0.21	1	-0.01	0.9994	-0.22
M1_Spy_0721	0.542	-0.40	0.6642	-0.36	0.1888	-0.49	0.5732	-0.41	0.9307	-0.34	0.0509	-0.73
M1_Spy_0722	0.3822	0.57	0	1.62	0.0021	2.32	0.97	0.66	4.00E-04	2.45	0.0529	1.86
M1_Spy_0723	8.00E-04	1.04	0	2.14	0.0026	2.43	0.2134	1.20	7.00E-04	2.62	0.0014	1.79
M1_Spy_0724	2.00E-04	0.82	0	1.48	0	2.10	2.00E-04	1.37	0.0077	2.66	1.00E-04	2.86
M1_Spy_0726	0.4506	-0.41	1	-0.07	0.8938	-0.34	0.0205	-0.56	0.9999	-0.15	0.7218	-0.49
M1_Spy_0727	1	0.06	0.3833	0.68	0.7572	0.46	0.082	0.59	0.0012	1.28	0.158	0.82
M1_Spy_0728	0.9977	-0.14	0.2298	0.53	0.8525	0.36	0.5741	0.43	0.0764	0.93	0.053	0.60
M1_Spy_0729	0.9314	0.28	0.0076	-1.12	1	-0.29	0.8493	-0.61	0.0551	-1.35	0.3359	-1.07
M1_Spy_0731	0.1297	1.16										
M1_Spy_0732	0.6637	-0.36	0.4057	-0.65	1	-0.18	0.3619	-0.78	0.9999	-0.38	0.9977	-0.43
M1_Spy_0733	0.0167	-0.70	0.0025	-1.11	0.9748	-0.57	0.0117	-1.06	0.1041	-0.92	0.4218	-0.99
M1_Spy_0737												
M1_Spy_0738			0.0372	0.57	0.8764	0.39			0.7897	-0.63	0.5959	0.99
M1_Spy_0739	0.0029	-1.21	0.3092	0.60	0.0093	1.31	0.0296	-0.71	0.3917	1.73	0.0619	1.99
M1_Spy_0740	0.4653	-0.68	0.0489	0.82	0.1173	1.13	0.9411	-0.26	0.076	1.59	0.0263	2.10
M1_Spy_0741	0.3253	-0.49	0.0435	1.11	0.1361	1.39	1	-0.02	0.0199	1.65	0.1855	2.04
M1_Spy_0742	0.058	-0.51	1	-0.05	1	-0.01	0.5083	-0.42	1	-0.26	0.995	0.29
M1_Spy_0743	1	-0.09	0.163	2.34			0.9387	0.30	0.0066	2.42	0.1107	2.78
M1_Spy_0744	1	0.07	3.00E-04	2.23			0.8727	0.45	0.0488	2.72	0.0031	2.94
M1_Spy_0745	0.9629	0.34	0.2882	2.53			0.2205	0.71			0.0046	3.44

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_0746	1	0.04	0.0854	0.88	0.0294	1.65	1	0.11	0.0419	1.19	0.0079	2.07
M1_Spy_0747	1	-0.07	0.0274	1.07	0.0997	1.09	0.3237	0.46	0.2562	1.34	0.1789	1.53
M1_Spy_0749	1	-0.02	1	-0.11	0.9908	0.35	0	2.42	0.0649	2.12	0.2593	2.13
M1_Spy_0751	0.0281	0.68	0.999	-0.17	0.9036	-0.44	0.0945	0.90	1	-0.16	0.9397	-0.46
M1_Spy_0752	0.0832	0.65	0.1957	-0.67	0.5751	-0.89	0.0839	0.71	1	-0.30	0.0734	-0.96
M1_Spy_0754	0.9774	-0.19	0.9996	0.11	0.8258	0.62	0.0635	-0.50	1	0.22	0.9008	0.56
M1_Spy_0755	0.9557	0.17	0.4054	0.42	0.1075	0.66	0.9997	0.12	0.416	0.46	0.1411	0.62
M1_Spy_0756	0.9994	0.12	0.0308	0.67	0.001	1.21	0.9218	0.28	0.0162	1.09	0.0127	1.14
M1_Spy_0757	0.178	0.40	0.0012	0.99	0.0999	1.71	0.4352	0.48	0.0763	1.37	0.008	1.27
M1_Spy_0758	0.0354	1.17	5.00E-04	2.32	0.0309	3.08	0.0439	1.41	0.1159	2.80	3.00E-04	2.00
M1_Spy_0759	8.00E-04	1.00	0	1.72	0.0155	2.31	0.0147	1.29	0.0049	2.31	0.0057	2.13
M1_Spy_0760	0.0029	1.01	0	1.69	0	2.00	1.00E-04	1.41	6.00E-04	2.09	0.0014	1.80
M1_Spy_0761	1.00E-04	1.09	0	1.82	0	2.17	0	1.52	0	2.27	0	2.10
M1_Spy_0762	0.0058	-0.63	0.997	0.28	0.9176	0.46	0.0369	-0.67	0.928	0.46	0.9832	0.32
M1_Spy_0763	0.534	-0.29	0.937	-0.22	0.9679	-0.25	0.1797	-0.39	0.9999	-0.18	0.5639	-0.42
M1_Spy_0764	0.6155	-0.35	1	-0.03	1	0.11	0.9415	-0.24	0.9993	0.21	1	0.00
M1_Spy_0766	0.79	0.45	0.0122	1.47			0.9421	0.40	0.0207	1.52	0.2715	1.27
M1_Spy_0768	1.00E-04	2.07					2.00E-04	2.69	0.0053	2.90	0.0086	1.72
M1_Spy_0769	0.0784	1.42	0.0512	2.46			0	2.36	0	3.75	0.0092	2.25
M1_Spy_0770	0.6373	0.32	0.1175	1.14	0.6278	1.00	0.6351	0.52	0.0666	1.17		
M1_Spy_0771	1	-0.07	0.4069	0.58	0.8654	0.60	1	-0.01	0.4246	0.66	0.9442	0.71
M1_Spy_0772	0	-1.05	0.9999	-0.10	1	-0.02	6.00E-04	-0.84	1	-0.19	0.8474	-0.28
M1_Spy_0773	8.00E-04	-1.00	1	0.03	1	0.06	0.1251	-0.70	1	-0.17	0.9999	-0.15
M1_Spy_0775	0.9288	-0.24	0.0475	-0.91	0.8401	-0.66	0.0486	-0.86	0.0119	-1.03	0.0206	-1.07
M1_Spy_0776	1	-0.03	1	0.10	1	0.25	0.9771	0.31	0.9435	0.47	1	0.16

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_0777	0.7775	0.25	0.7462	0.40	0.9908	0.40	0.4348	0.78	0.1268	1.15	0.9121	0.52
M1_Spy_0778	1	0.00	0.9468	0.54	0.8863	0.67	0.8658	0.43	0.329	1.08	0.9023	0.49
M1_Spy_0779	0.9996	0.12	0.068	0.54	0.039	0.77	0.1643	0.40	6.00E-04	1.00	1.00E-04	1.13
M1_Spy_0780	0.0644	-0.49	1	0.04	1	0.06	0.0987	-0.59	0.9989	-0.22	0.8748	-0.30
M1_Spy_0781	0.9999	0.08	1	0.08	1	0.11	1	-0.24	1	0.18	0.9797	-0.29
M1_Spy_0782	0.1517	0.39	0.81	0.33	0.9976	0.17	0.6255	0.43	0.8536	0.33	1	0.09
M1_Spy_0783	0.0717	0.51	0.9637	0.33	0.9966	0.24	0.3151	0.54	0.8569	0.61	0.9969	0.21
M1_Spy_0784	0.0943	-0.51	0.0018	-0.99	0.0115	-0.96	0.009	-0.88	3.00E-04	-1.26	0.003	-1.25
M1_Spy_0786	0.4577	0.34	0.9865	-0.21	0.8476	-0.35	1	0.08	1	-0.02	0.6808	-0.41
M1_Spy_0787	0.069	0.71	0.6488	0.65	1	0.08	0.6441	0.75	0.7632	0.87	0.9909	0.39
M1_Spy_0789	1.00E-04	1.35	0.3271	1.18	0.5469	0.75	0.0169	1.27	0.3437	1.80	0.7263	0.69
M1_Spy_0790	0.0116	0.77	0.1022	0.77	0.6295	0.46	0.0241	1.21	0.0032	1.28	0.5362	0.50
M1_Spy_0791	0.0456	1.00	0.0965	1.05	0.7778	0.63	0.0169	1.39	0.0556	1.71	0.4663	0.83
M1_Spy_0792	0.0086	0.65	0.0869	1.10	0.2057	0.94	0.0043	1.26	0.0099	1.52	0.1853	0.84
M1_Spy_0793	0.0942	1.30	0.0464	1.56	0.096	1.40	0.0118	2.10	7.00E-04	2.27	0.0309	1.36
M1_Spy_0794	0.0799	0.56	0.0027	0.93	0.3215	0.72	0.0022	1.39	4.00E-04	1.33	0.0833	0.87
M1_Spy_0796	0	1.90					0.162	3.35			0.0065	2.13
M1_Spy_0797	0.0278	0.75	0.02	1.23	0.9073	0.82	0.0109	1.57	0.0754	1.84	0.1482	1.12
M1_Spy_0798	0.0029	1.22	0.0022	1.86	0.3916	0.91	0.0494	2.36	0.0046	2.87	0.099	1.39
M1_Spy_0799	1	0.03	0.067	-0.65	0.0077	-0.81	0.9574	-0.22	0.0556	-0.76	0.0196	-0.78
M1_Spy_0800	0.9992	0.11	0.0951	-0.69	0.626	-0.73	1	-0.06	0.1484	-0.66	0.0253	-0.78
M1_Spy_0801	0.0104	-0.80	0.0378	-1.07	0.0327	-1.57	0.013	-1.14	0.2381	-0.87		
M1_Spy_0802	0.9972	0.11	0.0546	-0.74	0.0255	-0.90	1	-0.11	0.0117	-0.87	0.0135	-0.84
M1_Spy_0803	0.888	0.20	0.0012	-0.80	0.0011	-0.88	1	0.11	0.0608	-0.88	0.0056	-0.91
M1_Spy_0804	0.3771	0.34	0.1341	-0.52	0.378	-0.52	0.6952	0.29	0.9999	-0.23	1	-0.07

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_0805	0.2548	0.37	0.1455	-0.47	0.8819	-0.41	1	0.11	1	-0.02	0.9948	0.17
M1_Spy_0806	0.3021	0.30	0.2294	-0.45	0.9361	-0.28	0.7362	0.28	1	-0.06	0.9849	0.27
M1_Spy_0807	1	0.02	0.039	0.78	0.3261	0.55	0.1472	0.62	0.024	0.91	0.0758	0.86
M1_Spy_0808	0.9979	0.10	0.7028	0.53	0.7745	0.52	1	0.13	0.2753	0.73	0.5916	0.59
M1_Spy_0809	0.0265	0.48	0.2178	0.53	0.6916	0.45	0.2158	0.48	0.123	0.72	0.6199	0.53
M1_Spy_0810	0.0029	0.84	0.0672	0.72	0.9601	0.39	0.1315	0.75	0.3404	0.86	0.8756	0.35
M1_Spy_0811	0	0.99	0.998	0.19	1	0.01	0.0215	0.70	0.9743	0.31	1	0.08
M1_Spy_0813	6.00E-04	-0.89	4.00E-04	-1.08	0.0756	-0.76	0.0074	-0.86	0.1291	-0.81	0.7734	-0.51
M1_Spy_0814	8.00E-04	1.25	1	-0.04	0.9949	-0.37	0.0179	1.01	0.9926	0.71	1	0.16
M1_Spy_0815	0.0039	1.50					0.0679	1.32			0.9364	0.51
M1_Spy_0816	0.0374	0.60	5.00E-04	1.84			0.0393	1.24	8.00E-04	2.69	0.0055	1.70
M1_Spy_0817	3.00E-04	0.94	4.00E-04	1.77	0.0035	1.41	3.00E-04	1.60	0.0296	3.22	5.00E-04	1.98
M1_Spy_0818	0.7288	-0.31	0.2523	-0.71	0.9804	-0.38	0.9614	-0.38	0.9963	-0.37	0.9631	-0.36
M1_Spy_0819	0.0394	0.54	0.1302	0.43	0.4389	0.43	0.0022	0.81	0.0046	1.23	0	1.42
M1_Spy_0821	0.0019	0.65	0.7663	0.36	0.891	0.39	0.0046	0.79	0.0504	1.21	0.0047	1.37
M1_Spy_0822	6.00E-04	0.75	0.9987	0.15	0.9681	0.28	0.003	0.77	0.0792	0.98	0.0024	1.28
M1_Spy_0824	0.5526	-0.50	0.935	-0.27	1	-0.13	0.5674	-0.65	1	-0.11	0.9971	-0.20
M1_Spy_0826	0.0168	-0.52	0.0089	-0.68	0.5943	-0.46	0.0038	-0.77	0.428	-0.51	0.1298	-0.59
M1_Spy_0827	0.3793	-0.31	0.1101	-0.67	0.9988	-0.26	0.0889	-0.61	1	-0.25	0.9633	-0.41
M1_Spy_0830	0.0011	1.40	0.0072	-1.24	0.001	-2.35	0.0082	0.94	6.00E-04	-1.36	0.9879	-0.33
M1_Spy_0831	6.00E-04	1.72	0.1705	-0.60	0.3286	-1.58	0.0013	1.43	0.8492	-0.58	0.9334	0.40
M1_Spy_0832	1.00E-04	2.03	0.9148	-0.49	0.1978	-1.36	0.106	1.66	0.868	-0.46	0.8225	0.55
M1_Spy_0833	0	1.74	1	0.04	0.719	-0.86	0.0034	1.70	1	0.15	0.0401	1.33
M1_Spy_0835	0.0152	1.50	0.8524	-0.45	0.0083	-2.00	0.0038	1.61	0.9775	-0.50	0.9532	0.52
M1_Spy_0836	0	1.70	0.0254	0.96	0.6557	0.90	0	1.55	0.0396	1.01	0.0251	1.32

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_0837	1.00E-04	2.52	0.2716	1.85			0.0489	2.16	0.599	1.59	0.0656	1.79
M1_Spy_0838	8.00E-04	1.49	0.0534	1.03	0.7709	0.52	0.2357	1.42	0.5182	0.67	0.0947	1.12
M1_Spy_0839	0	2.89	0.0507	2.66			0.0706	2.74	0.0061	2.60	1.00E-04	2.60
M1_Spy_0840	0.0358	0.92	0.001	1.44	1.00E-04	1.74	0.001	1.06	7.00E-04	2.09	0	2.27
M1_Spy_0841	0.0337	0.85	2.00E-04	1.69	0	1.80	0.0057	0.93	3.00E-04	2.42	0	2.34
M1_Spy_0843	0.9129	-0.34										
M1_Spy_0844												
M1_Spy_0845	0.7388	-1.31					0.4109	-1.20				
M1_Spy_0846	1	-0.03	1	-0.06			1	-0.21	0.9615	0.55		
M1_Spy_0847	0.996	-0.16	0.825	0.27	0.9985	0.19	1	-0.08	1	0.06	1	-0.04
M1_Spy_0849	0.4014	0.41	0.0018	1.35	0.0671	1.09	0.0089	1.00	0.0218	1.95	0.0889	1.55
M1_Spy_0850	0.2887	0.37	0.0144	1.05	0.2184	1.22	0.0188	1.15	0.2022	1.75	0.0086	1.58
M1_Spy_0851	0.7354	0.47	0.0171	1.60			0.0871	1.33			0.0523	2.11
M1_Spy_0852	0.255	0.40	0.0243	1.17	0.1907	1.01	0.005	1.29	0.0668	2.18	0.2283	1.78
M1_Spy_0853											0.0213	2.72
M1_Spy_0854	0.9847	0.29					0.2128	1.02			0.0032	1.84
M1_Spy_0855	1	-0.09					1	0.00	0.9999	-0.30	0.9555	0.60
M1_Spy_0856	0.9949	0.39	0.5847	-0.87			1	-0.15	0.7412	-0.83	0.4741	-0.79
M1_Spy_0857	0.0256	-0.87	0.0018	-0.81	0.5555	-0.52	0.0013	-1.03	0.0195	-0.98	0.1054	-0.89
M1_Spy_0861												
M1_Spy_0864	0.9969	0.11	1	-0.02	1	-0.10	0.6479	-0.27	0.9961	-0.19	0.0901	-0.55
M1_Spy_0865	0.5112	0.31	0.7088	0.28	1	0.09	0.9757	0.17	1	0.17	1	0.01
M1_Spy_0866	0.9422	0.19	0.0048	1.51	0.1698	1.60	0.4381	0.47	0.0099	1.62	0.0071	1.65
M1_Spy_0867	0.3313	0.84	0.0393	2.44			0.0031	1.53	6.00E-04	3.01	0	2.37
M1_Spy_0870	0.006	-0.73	0.0372	0.93	0.115	1.09	0.1557	-0.85	0.0528	1.06	0.0628	0.91

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_0872	0.4117	0.41	0.0567	1.70			0.2295	0.63	0.0799	1.99	0.0055	1.67
M1_Spy_0873	1	-0.05	0.0032	-0.75	0.6406	-0.55	0.1404	-0.45	0.0255	-0.95	0.0071	-0.89
M1_Spy_0874	0.9972	0.10	0.0135	-0.67	0.9374	-0.37	0.6939	-0.29	0.458	-0.57	0.0768	-0.62
M1_Spy_0875	1	0.07	0.0015	-0.86	0.8814	-0.56	0.9777	-0.33	0.7862	-0.42	0.4534	-0.68
M1_Spy_0876	0.7734	-0.36	0.5411	0.39	0.8917	0.43	0.1833	-0.55	0.9948	0.65	1	0.04
M1_Spy_0877	1	0.08	0.4252	1.05	0.1096	1.10	1	0.21	0.5759	1.10	0.9512	0.56
M1_Spy_0878	0.9808	0.17	0.3643	0.61	0.445	0.70	1	0.14	0.3219	0.89	0.9296	0.44
M1_Spy_0879	0.9893	0.17	0.9974	0.19	0.9922	0.25	0.9284	0.26	0.9119	0.65	1	0.20
M1_Spy_0880	6.00E-04	0.95	9.00E-04	2.33	0.0247	2.73	0.0013	1.76	0.0045	2.77	0.1895	1.87
M1_Spy_0881	0.0708	0.69	0.0573	1.70	0.4236	1.92	0.0212	0.91	0.2755	2.14	0.1115	1.43
M1_Spy_0882	0.0028	1.38					9.00E-04	1.86			0.0378	2.01
M1_Spy_0883	0.3828	0.38	0.0099	-0.85	0.6187	-0.60	0.9842	0.20	0.9091	-0.50	0.6727	-0.46
M1_Spy_0884	0.0714	0.74	0.6133	-0.59	0.9724	-0.48	0.5147	0.47	1	-0.06	0.9932	-0.35
M1_Spy_0885	0.0092	0.66	0.0582	-0.74	0.0086	-1.01	0.0577	0.88	1	-0.24	0.4876	-0.65
M1_Spy_0886	8.00E-04	0.98	0.9971	-0.32	0.7496	-0.52	0.0021	1.24	0.9999	0.25	0.9992	-0.21
M1_Spy_0887	1	0.02	0.0278	0.78	0.9935	0.28	0.4525	0.39	0.0231	1.01	0.2687	0.73
M1_Spy_0888	0.0239	-0.92	5.00E-04	-1.75	0.0101	-1.60	0.009	-1.51	0.0071	-1.98	8.00E-04	-2.15
M1_Spy_0889	0.9976	-0.10	0.0113	-0.70	0.1876	-0.65	1	-0.11	0.6331	-0.49	0.3381	-0.48
M1_Spy_0890	0.2495	0.31	0.9936	-0.19	1	-0.05	0.8818	0.43	0.9984	0.28	0.6885	0.36
M1_Spy_0891	0.3487	0.37	0.999	-0.14	1	-0.03	0.0018	0.75	0.926	0.46	0.343	0.41
M1_Spy_0892	0.3084	0.38	0.9995	-0.11	0.9919	-0.19	0.0011	0.77	0.9978	0.20	0.9893	0.15
M1_Spy_0894	0.0685	0.83	0.5603	0.48	0.2466	0.54	3.00E-04	1.21	0.3571	1.28	0.0603	1.10
M1_Spy_0895	0	1.70	0.0241	1.83	0.1634	1.77	0.0047	2.11	0.0052	2.34	7.00E-04	1.82
M1_Spy_0898	0.2165	0.46	0.9645	0.45			0.987	0.45	0.4264	1.30	0.2055	0.88
M1_Spy_0899	0.0445	-0.46	1	0.03	1	0.14	0.1916	-0.53	1	-0.04	1	0.00

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_0900	0.0288	1.31	0.2026	-0.81	0.2979	-1.63	0.1114	0.90	1	-0.15	1	-0.10
M1_Spy_0901	0.0517	0.78	0.0056	-1.11	0.011	-1.66	0.123	0.60	0.0399	-0.95	0.574	-0.61
M1_Spy_0902	8.00E-04	2.40					0.0157	2.39				
M1_Spy_0903	0.0039	0.79	1	0.02	1	0.14	0.2376	0.64	1	0.06	0.7055	0.57
M1_Spy_0904	0.0167	1.28	0.9941	0.19	0.9969	0.45	0.1741	1.15	0.9869	0.39	0.2343	0.76
M1_Spy_0905	0.9639	-0.14	0.0021	-0.98	0.0157	-1.08	0.2468	-0.41	0.0047	-1.21	0.0324	-1.28
M1_Spy_0907	1	0.02	0.0056	-0.95	0.0483	-1.03	1	0.06	0.1212	-0.95	0.0208	-1.21
M1_Spy_0908	0.0898	-0.93	0.8321	0.62	0.3042	1.42	0.5715	-0.82	0.293	1.62	0.1593	1.22
M1_Spy_0909	0	1.52	0.0044	1.77	0.0364	1.63	3.00E-04	1.64	0.0228	1.90	0.0083	1.80
M1_Spy_0910	0.0019	1.28	0.0174	1.75	0.0351	1.64	6.00E-04	1.70	4.00E-04	1.79	0.0014	1.62
M1_Spy_0911	0.6562	0.49	0.0121	2.26	0.0061	2.22	0.2434	0.84	6.00E-04	2.53	0.017	2.52
M1_Spy_0912	0.9698	0.25	4.00E-04	2.16	1.00E-04	2.14	0.5278	0.94	0.001	2.24	0.0954	1.99
M1_Spy_0913	0.0092	1.36	0	3.13	0	3.31	0	2.20	0	3.40	0	3.54
M1_Spy_0914	0.02	-0.56	0.0635	-0.66	1	-0.11	6.00E-04	-0.97	0.0096	-0.94	0.3039	-0.42
M1_Spy_0915	0.9817	0.16	0.4294	0.39	0.5704	0.38	1	0.12	0.969	0.33	0.4789	0.38
M1_Spy_0916	0.001	2.02	0.002	1.91	9.00E-04	2.04	0.0047	2.01	0.028	1.21	0.1452	0.99
M1_Spy_0917	0	2.38	0.0091	1.90	0.0157	2.41	3.00E-04	2.24	0.0537	1.56	0.0598	1.62
M1_Spy_0918	0	2.41	0.0012	2.21	0.001	2.05	0.0017	2.38	0.0012	2.06	0.2947	1.61
M1_Spy_0919	0.9869	-0.18	0.9462	0.22	0.9939	0.29	0.9952	-0.24	0.8348	0.40	0.4128	0.64
M1_Spy_0921	0.5476	0.80										
M1_Spy_0922	0.0146	0.78	0.4373	0.66	1	0.16	0.0172	0.92	0.1194	0.97	0.9992	0.21
M1_Spy_0923	0.0082	1.04	0.1854	0.65	1	0.15	3.00E-04	1.28	0.0711	1.14	1	0.20
M1_Spy_0924	1.00E-04	1.14	0.2801	0.71	0.9986	0.35	0.0136	1.84	0.0131	1.71	0.4631	0.63
M1_Spy_0925	0	1.38	0.0604	1.12	0.9916	0.26	0.0038	1.87	0.047	1.83	0.5173	0.67
M1_Spy_0926	0.0243	2.20					6.00E-04	3.32			0.107	2.02

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_0927	0.4436	-0.34	0.7924	0.35	0.9941	0.20	0.9626	-0.21	0.5084	0.61	1	0.09
M1_Spy_0928	0.9963	0.14	0	1.77	5.00E-04	1.31	0.9123	0.30	0.0054	1.95	0.0732	1.05
M1_Spy_0929	0.9989	0.14	0.0089	1.40	0.0011	1.21	0.4856	0.45	0.0046	1.72	0.1331	1.21
M1_Spy_0930	0.4138	0.36	0.0017	1.55	0.3017	1.07	0.7066	0.57	0.3085	1.70	0.4682	0.80
M1_Spy_0931	0.9184	0.23	0.0305	1.19	0.0274	0.95	0.9374	0.37	0.1262	1.95	0.7549	1.09
M1_Spy_0932	0.0894	0.76					0.3953	1.02			0.3621	1.30
M1_Spy_0933	6.00E-04	0.77	0.0781	0.55	0.4702	0.46	0.0516	0.75	0.6449	0.50	0.8696	0.40
M1_Spy_0935	0.0032	0.85	0.6415	0.27	0.9617	0.22	0.0455	0.72	0.9731	0.34	0.998	0.20
M1_Spy_0936	0	1.27	0.422	0.52	0.6706	0.38	5.00E-04	1.13	0.2591	0.55	0.9789	0.27
M1_Spy_0937	6.00E-04	-1.18	0.736	-0.33	0.9981	0.42	0.3303	-0.82	1	0.19	1	0.26
M1_Spy_0938	1.00E-04	-0.88	6.00E-04	-1.02	0.9775	-0.38	0.0963	-0.88	0.0087	-0.88	0.994	-0.27
M1_Spy_0939	0	-1.28	4.00E-04	-1.28	0.6448	-0.78	0.0057	-1.37	0.0182	-1.21	0.5537	-0.74
M1_Spy_0940	6.00E-04	-1.17	0	-2.40	0	-2.44	0	-1.61	0	-2.21	1.00E-04	-2.79
M1_Spy_0942	1	-0.02	1	-0.06	0.9082	0.53	1	-0.21	1	0.19	0.9817	0.41
M1_Spy_0943	0	-1.46	0	-2.48	0	-2.16	0	-1.78	7.00E-04	-2.20	0	-2.43
M1_Spy_0944	1.00E-04	-0.83	0.0017	-0.85	0.7531	0.62	0	-1.25	1	-0.27	0.042	0.82
M1_Spy_0945	0	-1.21	0	-2.03	1.00E-04	-1.93	1.00E-04	-1.52	0.0102	-1.88	1.00E-04	-2.15
M1_Spy_0946	0	-1.33	0	-2.31	1.00E-04	-2.02	0.0047	-1.62	0.0049	-2.11	0	-2.40
M1_Spy_0947	0	-1.14	0	-2.07	0	-1.92	0.0011	-1.46	0.0033	-1.92	0	-2.40
M1_Spy_0948	1	-0.09	0.982	-0.30	1	-0.17	0.9181	-0.31	1	-0.19	0.9645	-0.51
M1_Spy_0949												
M1_Spy_0952	0	-0.97	0	-2.01	3.00E-04	-1.83	2.00E-04	-1.40	0.0061	-1.84	1.00E-04	-2.34
M1_Spy_0953	0	-0.96	2.00E-04	-1.86	1.00E-04	-1.64	1.00E-04	-1.36	2.00E-04	-1.80	0	-2.04
M1_Spy_0954												
M1_Spy_0956	8.00E-04	-0.78	0	-1.92	4.00E-04	-1.73	0.0027	-1.28	0.0307	-1.75	6.00E-04	-2.21

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_0957	0.0799	-0.68	2.00E-04	-1.53	2.00E-04	-1.65	0.0035	-1.10	0.0478	-1.69	0.0037	-1.85
M1_Spy_0958	0.0155	-0.72	0	-1.71	0.0235	-1.69	0.0547	-1.08	0.0149	-1.45	8.00E-04	-1.84
M1_Spy_0959	0.0026	-0.78	0	-1.86	0.0313	-1.79	0.0189	-1.13	0.0231	-1.73	0.003	-2.04
M1_Spy_0960	0	-1.11	6.00E-04	-2.06	1.00E-04	-1.95	0.0865	-1.38	0.1756	-1.76	1.00E-04	-2.07
M1_Spy_0961	0.0739	-0.56	4.00E-04	-1.49	0.1306	-1.45	0.0785	-0.98	0.3871	-1.24	5.00E-04	-1.68
M1_Spy_0962	0.0052	-0.86	0	-1.82	1.00E-04	-1.75	0.0039	-1.23	0.0392	-1.65	0	-2.22
M1_Spy_0963	0.0162	-0.99	1.00E-04	-1.78	9.00E-04	-1.86	0.0011	-1.25	0.0744	-1.63	7.00E-04	-1.94
M1_Spy_0965	0.0109	-0.88	2.00E-04	-1.92	0.0058	-1.87	0.0179	-1.13	0.134	-1.52	0	-1.94
M1_Spy_0967	0.0491	-0.88	0	-1.97	0.0083	-2.20	0.0442	-1.34	0.0681	-2.37	5.00E-04	-2.78
M1_Spy_0968	0.9997	-0.10	1	0.01	0.9999	0.24	1	0.02	1	0.27		
M1_Spy_0970	0.9979	0.19	0.7743	0.69			1	0.20	0.4268	1.23	0.0772	1.23
M1_Spy_0971	0.4741	-0.30					0.3905	-0.66	0.1051	-1.00	0.3634	-0.81
M1_Spy_0972												
M1_Spy_0975												
M1_Spy_0976	0.02	-0.65	0.1315	-0.72	0.9949	-0.33	0.0027	-0.88	0.0396	-0.84	0.8609	-0.41
M1_Spy_0977												
M1_Spy_0978	0.0848	0.59	0.0313	1.01	0.0144	1.42	0.9845	0.43	0.0482	1.61	0.0479	1.49
M1_Spy_0979												
M1_Spy_0980												
M1_Spy_0981												
M1_Spy_0982												
M1_Spy_0984												
M1_Spy_0985												
M1_Spy_0986												
M1_Spy_0987												

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_0988												
M1_Spy_0989												
M1_Spy_0991												
M1_Spy_0992												
M1_Spy_0993												
M1_Spy_0994												
M1_Spy_0995												
M1_Spy_0996												
M1_Spy_0997												
M1_Spy_0998												
M1_Spy_0999												
M1_Spy_1001	0.1893	-0.45	0.333	-1.01	0.6217	-1.22	0.4458	-0.59	0.0173	-1.32	0.0278	-1.12
M1_Spy_1002	0.1405	-0.69	0.0792	-1.20	0.0018	-1.44	0.6129	-0.99	0.0033	-1.64	0.0045	-1.36
M1_Spy_1006	0.0203	-0.68	0.0056	-1.39	0.0252	-1.47	0.0307	-1.12	6.00E-04	-1.88	0.0026	-1.70
M1_Spy_1007	0.0772	-0.55	0.1289	-0.78	0.3322	-0.73	0.0192	-0.89	0.1501	-0.83	0.1185	-0.77
M1_Spy_1008	8.00E-04	-0.85	0.0052	-1.12	0.0568	-0.89	0	-1.26	0.0049	-1.30	0.0089	-1.02
M1_Spy_1010	0.7843	-0.23	0.7356	0.40	0.7325	0.51	1	-0.01	0.223	0.61	0.2374	0.65
M1_Spy_1011	0.1844	-0.45	1	-0.10	1	-0.12	0.9995	-0.31	1	-0.11	1	-0.07
M1_Spy_1012	1	-0.01	0.0016	1.25	0.4019	1.24	0.5072	0.60	3.00E-04	1.66	0.0049	1.46
M1_Spy_1013	0.9779	-0.18	0.9997	0.21	1	0.09	0.9988	-0.24	1	0.11	1	0.09
M1_Spy_1016	0.531	-0.50	0.857	-0.66	0.6219	-0.65	1	-0.15	1	-0.08	1	-0.27
M1_Spy_1017	0.1331	-0.48	0.9901	0.39	0.9957	0.38	0.8223	-0.41	1	0.21	0.9982	0.27
M1_Spy_1018	0.1688	-0.60	1	0.13	0.9999	0.24	0.3912	-0.61	1	0.09	1	0.15
M1_Spy_1019	0.5235	-0.35	0.7785	0.49	0.8102	0.61	1	-0.17	0.4976	0.64	0.5298	0.66
M1_Spy_1020	0.8082	0.19	1	-0.04	1	-0.09	1	0.06	0.9882	-0.24	0.3676	-0.48

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_1022	1	-0.04	0.1199	0.41	0.9986	0.18	1	-0.04	1	0.05	0.6484	-0.39
M1_Spy_1024	0.9994	0.11	0.0653	0.59	0.4417	0.49	1	0.03	0.6458	0.33	1	-0.05
M1_Spy_1025	0.3162	0.49	2.00E-04	1.33	0	1.52	0.0623	0.60	0.0223	1.16	0.0016	1.14
M1_Spy_1026	1	0.05	0.953	-0.30	0.9912	-0.20	1	0.14	0.449	-0.50	0.3394	-0.42
M1_Spy_1028	0.0126	0.80	5.00E-04	0.89	5.00E-04	0.85	0.0086	1.07	0.132	0.75	0.0669	0.66
M1_Spy_1029	0	1.15	1.00E-04	1.07	0.001	1.03	0	1.54	0.0417	1.08	0.0016	0.92
M1_Spy_1031	0.0115	0.68	0.1848	0.48	0.2119	0.54	0	1.08	0.1076	0.66	0.4196	0.46
M1_Spy_1032	0.0273	-0.63	1	-0.05	0.9454	0.39	0.4065	-0.54	1	0.14	0.9639	0.47
M1_Spy_1033	0.067	-0.48	1	-0.02	1	-0.13	0.8805	-0.44	0.9342	-0.35	0.5452	-0.44
M1_Spy_1034	1.00E-04	-1.04	9.00E-04	-0.86	0.5164	-0.55	1.00E-04	-1.10	0.0043	-1.02	0.2293	-0.65
M1_Spy_1035	0.0088	-1.34	0	-1.33	0.0017	-1.25	0	-1.24	0.001	-1.37	5.00E-04	-1.41
M1_Spy_1036	1	-0.06	1	-0.09	0.999	-0.23	1	0.03	0.5982	-0.54	0.9532	-0.57
M1_Spy_1037	0.9989	0.15	0.9995	-0.23	0.9778	-0.34	0.8757	0.41	0.9983	0.18	1	-0.08
M1_Spy_1038	0.8136	0.21	0.3133	0.46	0.9803	0.35	0.9572	0.32	0.9999	0.25	1	0.09
M1_Spy_1039	0.1453	-0.49	0.9978	-0.31	0.4936	-0.72	0.98	-0.47	0.2784	-0.80	0.2386	-0.97
M1_Spy_1040	0.0145	-0.52	0.0131	-0.65	0.2163	-0.61	0.006	-0.67	0.0107	-0.82	0.0325	-0.77
M1_Spy_1042	0.0542	-0.57	0.1942	-0.49	0.3123	-0.47	0.0848	-0.58	0.3992	-0.49	0.1964	-0.54
M1_Spy_1043	0.0381	-0.70	0.0034	-1.02	0.0474	-0.98	0.0024	-0.80	7.00E-04	-1.17	0.013	-1.05
M1_Spy_1044	0.9998	0.10	0.8379	0.38	0.7475	0.52	0.9385	0.34	0.7899	0.91	0.8254	0.48
M1_Spy_1046												
M1_Spy_1047	0.9381	-0.22					1	-0.18	0.2635	-0.89		
M1_Spy_1048	0.0559	-0.66	0.0127	-1.07	0.0038	-1.33	0.7114	-0.77	7.00E-04	-1.41	0.0192	-1.35
M1_Spy_1049	0.029	-0.56	0.3624	-0.83	0.0908	-0.99	0.3627	-0.66	0.7112	-0.63	0.3131	-0.66
M1_Spy_1053	0.9341	-0.18	0.9995	-0.10	1	-0.11	1	-0.04	0.9299	0.25	0.9989	0.15
M1_Spy_1054	0.9985	-0.16	1	0.08	1	0.07	0.9911	0.18	0.5961	0.63	0.9666	0.33

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_1055	2.00E-04	-0.96	0.0031	-0.90	0.4357	-0.59	0	-1.41	0.002	-1.28	0.0362	-1.01
M1_Spy_1056	0.0029	-0.99	0.0416	-0.81	0.9805	-0.31	0.0017	-1.37	0.3926	-0.82	0.9716	-0.38
M1_Spy_1057	8.00E-04	-1.29	0.2422	-1.41	0.9933	-0.60	0.2238	-0.97	0.1806	-0.83	1	-0.27
M1_Spy_1058	0.1147	-1.28	0.2696	-0.88	1	-0.14	0.6802	-0.74	1	-0.18	0.9965	0.33
M1_Spy_1059	0.1322	-1.24			0.9935	-0.32	0.6784	-0.72	1	-0.03	1	0.30
M1_Spy_1060	0.0114	-0.89	0.0362	-0.97	0.8386	-0.52	0.2616	-0.65	0.8057	-0.53	1	0.28
M1_Spy_1061	0.1474	-0.48			0.9486	-0.65	0.8869	-0.41	0.8695	-0.59	0.9787	-0.42
M1_Spy_1062	0.0746	-0.50	0.7838	-0.47	0.778	-0.72	0.9966	-0.30	0.2243	-0.71	0.8781	-0.72
M1_Spy_1063	1	-0.07					0.986	0.41	0.9993	0.73		
M1_Spy_1064	0.0383	-0.68	1	-0.03	1	-0.02	0.2372	-0.65	0.8229	-0.51	0.8452	-0.43
M1_Spy_1065	0.5883	-0.36	0.0239	0.97	0.1733	1.14	0.96	-0.25	0.0199	1.16	0.8779	1.06
M1_Spy_1066	0.0288	-0.93	0.0092	-1.35	0.1912	-1.45	0.0283	-1.12	0	-1.87	0.0612	-1.55
M1_Spy_1067	1.00E-04	-1.35	4.00E-04	-1.48	0.0015	-1.06	1.00E-04	-1.24	9.00E-04	-1.14	0.2398	-0.97
M1_Spy_1068	0.732	-0.36	0.0335	-0.90	0.1024	-0.63	1	-0.11	1	-0.19	0.9467	-0.40
M1_Spy_1069	1	-0.05	0.3719	-0.45	0.0806	-0.65	0.9965	0.16	0.8412	-0.34	0.1912	-0.53
M1_Spy_1070	0.1098	-0.48	6.00E-04	-1.12	1.00E-04	-1.27	0.9948	-0.26	2.00E-04	-1.35	0	-1.63
M1_Spy_1071	0.0126	0.90					0.0662	1.54				
M1_Spy_1072	1.00E-04	1.82	0.1083	1.96	0.0357	2.49	0.0032	3.10	2.00E-04	2.49	0.0055	3.36
M1_Spy_1073	0.0638	0.92	0.5859	0.91	0.1147	1.69	0.0655	2.26	0.1928	1.65	0.0025	2.29
M1_Spy_1075	0	-1.01	2.00E-04	-1.73	0	-1.86	0.1498	-1.42	0	-2.35	0	-2.23
M1_Spy_1077	0.1068	-0.76	6.00E-04	-1.68	0	-1.84	0.1093	-1.23	0	-2.05	1.00E-04	-2.11
M1_Spy_1080	0.3753	-0.43	0.0656	-1.06			0.2443	-0.76	0.0882	-1.17	0.074	-1.36
M1_Spy_1081	0.6286	-0.30	0.0026	-1.06	0.0291	-0.88	0.2726	-0.50	0.0344	-1.03	0.0613	-1.10
M1_Spy_1082	0.9974	-0.18	0.0087	-0.79	0.1912	-0.67	0.8835	-0.28	0.0399	-0.86	0.0699	-0.91
M1_Spy_1083	0.9957	-0.16			0.95	-0.53			0.9501	-0.56		

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_1084	0.8792	-0.22	0.4315	-0.63			0.664	-0.44	0.5727	-0.73	0.8055	-0.66
M1_Spy_1085	0.9953	-0.19	0.948	-0.50	0.9202	-0.60	1	-0.12	0.7568	-0.56	1	-0.26
M1_Spy_1086												
M1_Spy_1087	1	-0.09							1	-0.14		
M1_Spy_1088	1	-0.02							1	-0.24		
M1_Spy_1093	0.9136	-0.30	0.9947	-0.27	1	-0.07	0.3284	-0.50	1	-0.16	0.8901	-0.39
M1_Spy_1094	0.0241	-0.76	0.0083	-0.85	1	-0.23	0.1233	-0.72	0.9985	-0.26	0.9386	-0.32
M1_Spy_1096	0.5481	0.38	3.00E-04	-1.09	0.0015	-0.87	1	-0.06	0.3878	-0.96	0.0305	-0.95
M1_Spy_1097	0.598	0.33	6.00E-04	-0.90	0.0092	-0.92	1	-0.09	0.0028	-1.02	0.0011	-1.00
M1_Spy_1098	0.8236	0.33	4.00E-04	-0.95	0.0092	-1.06	1	-0.09	0.0071	-1.16	0	-1.30
M1_Spy_1099	0.9979	0.10	1.00E-04	-1.11	0	-1.36	0.9879	-0.21	0.0028	-1.28	0	-1.31
M1_Spy_1100	0.8493	-0.19	1.00E-04	-1.27	1.00E-04	-1.34	0.3885	-0.39	2.00E-04	-1.39	0	-1.31
M1_Spy_1101	0.0791	0.96	0.0404	1.03			0.0199	1.24	0.8783	1.38	0.2022	1.00
M1_Spy_1102	0.9864	-0.14	1	0.06	1	-0.10	1	0.06	1	-0.10	1	0.15
M1_Spy_1103	0.9996	-0.09	1	-0.16	0.9996	-0.29	1	0.08	0.8732	-0.45	1	-0.08
M1_Spy_1104	0.9999	0.09	1	-0.06	1	-0.22	0.9499	0.37	1	-0.16	1	0.11
M1_Spy_1105	0.1251	-0.63	0	-1.47	0.0163	-1.46	0.1764	-0.77	4.00E-04	-1.55	0.0108	-1.43
M1_Spy_1106	0.993	-0.18					0.9986	-0.23				
M1_Spy_1107												
M1_Spy_1109	0.9421	-0.55	0.4382	-1.42	0.7668	-1.41	0.9935	-0.48	0.1336	-1.01	0.8627	-0.86
M1_Spy_1110	0.0275	-0.56	0.1183	-1.17	0.1177	-1.18	0.2063	-0.80	6.00E-04	-1.68	0.0359	-1.36
M1_Spy_1111	1	-0.04										
M1_Spy_1113	0.0014	-0.86	0.0506	-0.63	0.4311	-0.47	0.0025	-0.94	0.1014	-0.65	0.4838	-0.44
M1_Spy_1114	0.9922	0.24	0.4397	1.33	0.9985	0.87	0.0907	1.07	0.3131	1.47	0.0875	1.64
M1_Spy_1115	8.00E-04	-0.95	0.9932	-0.24	0.9992	-0.20	0.0154	-0.98	1	-0.25	0.9915	-0.31

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_1117	0.0864	-0.44	0.9763	-0.28	0.8509	-0.33	0.5954	-0.42	1	-0.06	0.9759	-0.26
M1_Spy_1118	0.9999	-0.12					0.2245	-0.68	0.0035	-1.81	5.00E-04	-2.14
M1_Spy_1119	0.4322	-0.33	0.3609	0.40	0.9727	0.30	0.7927	-0.26	1	0.17	0.9905	-0.16
M1_Spy_1120	0.6499	-0.26	0.0255	-0.60	0.3485	-0.62	0.9652	-0.19	0.3512	-0.54	0.0169	-0.78
M1_Spy_1121	0.9966	0.13	0.1362	-0.48	0.9896	-0.27	1	0.10	0.9995	-0.19	0.4672	-0.50
M1_Spy_1122	0.9996	0.10	0.7492	-0.32	0.8613	-0.37	0.9929	0.17	1	-0.13	0.35	-0.48
M1_Spy_1123	1	0.03	0.9768	-0.23	0.7215	-0.44	1	0.03	0.9977	-0.19	0.2849	-0.47
M1_Spy_1124	0.4428	-0.30	0.9995	-0.16	1	-0.07	0.252	-0.56	0.1743	-0.62	0.718	-0.49
M1_Spy_1125	0.1824	-0.38	0.0759	-0.82	0.0789	-0.89	0.2574	-0.46	0.0029	-1.22	0.0031	-1.22
M1_Spy_1126	0.7962	-0.24	0.1146	-0.54	0.0547	-0.69	0.1285	-0.42	0.0188	-0.84	0.0328	-0.85
M1_Spy_1127	0.8611	0.25	0.3933	-0.44	0.5163	-0.50	1	-0.07	0.2801	-0.60	0.2271	-0.70
M1_Spy_1128	0.7462	-0.28	0.8742	-0.25	1	-0.04	0.458	-0.39	0.8082	-0.38	0.8877	-0.25
M1_Spy_1131	0.0269	-0.74	0.1972	0.77	0.004	1.15	0.025	-0.82	0.9062	0.33	0.131	0.69
M1_Spy_1133	0.0018	-0.75	1	-0.01	1	0.09	0.1352	-0.78	0.6425	-0.59	0.9759	-0.32
M1_Spy_1134	0.0651	-0.44	0.006	1.04	0.0018	1.29	0.9991	-0.17	0.5111	0.73	0.0221	1.02
M1_Spy_1135	0.0061	-0.76	0	-2.51	1.00E-04	-3.08	0.0038	-1.07	0	-3.12	0.0055	-3.92
M1_Spy_1136	0.157	-0.67	0	-1.65	0	-2.29	0.1735	-0.62	0	-2.07	5.00E-04	-3.03
M1_Spy_1137	0.047	-0.53	0.0089	-1.31	5.00E-04	-1.68	0.6312	-0.44	4.00E-04	-1.48	0.0018	-2.01
M1_Spy_1138	0.9968	0.19	0.0205	0.83	0.4797	0.65	1	-0.13	0.9999	0.23	0.9982	0.27
M1_Spy_1139	0.9996	-0.14	0.9971	0.20	1	0.23	0.9389	-0.44	0.9999	-0.34	0.9907	-0.30
M1_Spy_1140	0.9998	-0.07	0.9556	0.19	0.9975	0.15	1	-0.04	1	-0.09	0.9536	-0.22
M1_Spy_1141	1	-0.04	0.9808	0.18	0.9999	0.13	0.9996	-0.12	1	-0.07	0.996	-0.15
M1_Spy_1142	0.0374	0.44	0.038	0.67	0.1013	0.72	0.0733	0.52	0.0489	0.66	0.2224	0.49
M1_Spy_1143	8.00E-04	0.71	0.0012	0.91	0.0014	1.01	0.0066	1.03	4.00E-04	1.05	0.035	0.82
M1_Spy_1144	1.00E-04	0.81	4.00E-04	0.99	0.0348	0.89	1.00E-04	1.18	0.01	1.14	0.0079	0.87

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_1145	0.0018	0.74	0.0465	0.77	0.2312	0.71	1.00E-04	1.07	0.0282	0.85	0.1129	0.62
M1_Spy_1146	0	1.12	0.0018	1.22	0.0522	1.10	0	1.62	0	1.55	0.02	1.06
M1_Spy_1147	0.6177	0.45	0.1147	0.55	0.9899	0.25	0.0165	0.88	0.9661	0.37	0.9771	0.33
M1_Spy_1148	0	1.14	0.0905	1.04	0.3844	0.83	0	1.79	0.0054	1.22	0.029	0.88
M1_Spy_1149	0.4914	0.45	0.9976	0.26	1	-0.01	0.2654	0.75	0.8476	0.40	1	0.02
M1_Spy_1150	0.1801	-0.48	0.9997	0.11	0.9838	0.28	0.838	-0.25	1	0.20	1	0.07
M1_Spy_1151	0.0115	0.94	0.999	-0.16	0.6979	-0.50	0.1591	0.74	0.9811	-0.22	1	-0.14
M1_Spy_1152	0.6913	0.34	0.0055	0.68	0.0132	0.71	0.8736	0.27	1	0.14	0.9945	0.26
M1_Spy_1154	0.7629	0.29	0.0224	0.77	0.0282	0.83	0.2186	0.47	0.9053	0.38	0.3219	0.46
M1_Spy_1155	0.0011	0.95	0.0041	0.84	0.1793	0.68	0	1.02	0.1953	0.62	0.9775	0.37
M1_Spy_1156	1	-0.04							0.9342	-0.39	0.9341	-0.28
M1_Spy_1157												
M1_Spy_1158	0.9147	-0.17	0.377	-1.08	6.00E-04	-1.83	1	0.06	0.0279	-1.44	0.0062	-1.79
M1_Spy_1159	0.8669	0.31	0.2453	-0.96	0	-1.61	0.8415	0.43	7.00E-04	-1.35	0	-1.58
M1_Spy_1160	1	0.03	0.0018	-1.29	0.0201	-1.44	0.959	-0.17	0.002	-1.86	0.0011	-1.63
M1_Spy_1161	0.9384	-0.21	0.8314	-0.23	1	0.00	0.3304	-0.47	0.3475	-0.59	0.5833	-0.60
M1_Spy_1162	1	-0.06	1	-0.01	0.9999	0.19	0.9957	-0.20	0.9985	-0.31	1	-0.09
M1_Spy_1163	0.09	-0.56	4.00E-04	-1.54	5.00E-04	-1.55	0.1667	-0.92	2.00E-04	-1.96	1.00E-04	-1.99
M1_Spy_1164	0.1691	-0.57	0.047	-0.89	0.0422	-1.02	0.6078	-0.64	2.00E-04	-1.43	0.1509	-0.99
M1_Spy_1169												
M1_Spy_1170	0.0373	-0.67	0.007	-2.12			0.067	-0.98	0.0028	-1.88	3.00E-04	-2.37
M1_Spy_1171	0.9994	-0.17	0.5079	-0.70	1	-0.04	0.8632	0.37	1	0.17	0.963	0.37
M1_Spy_1172	0.9996	-0.14	0.9932	-0.36			0.9777	0.35	0.9995	0.95	0.5593	0.83
M1_Spy_1173	0.3286	0.45	0.194	1.17	0.3825	1.39	0.028	0.71	0.0753	1.80	0.2749	1.16
M1_Spy_1174	0.0651	0.88	0.5096	1.01	0.1296	1.44	0.0454	0.97	1	0.39	0.9385	0.44

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_1175	0.3779	0.83	0.1091	0.84	0.929	0.52	0.0269	0.96	1	-0.17		
M1_Spy_1176	1.00E-04	1.76										
M1_Spy_1177	0.7734	0.40	0.1333	0.90	0.1213	1.30	0.9394	0.30	1	0.19	1	0.16
M1_Spy_1178	0.2771	0.46	0.4562	0.96			0.1437	0.84				
M1_Spy_1179	0.1131	-0.44	1	0.03	1	0.02	0.9997	-0.19	1	0.18	1	0.25
M1_Spy_1180	0.0574	-0.48	0.4564	-1.09	0.0132	-1.31	1	-0.13	0.4546	-0.62	0.8867	-0.97
M1_Spy_1181	1	0.06	0.9471	-0.48			1	0.12	0.2225	-0.91		
M1_Spy_1183	0.7276	-0.44	0.0621	-1.19	0.037	-1.33	0.437	-0.55	0.0054	-1.63	8.00E-04	-1.51
M1_Spy_1184	0.9239	-0.57	0.4672	-1.13	0.7359	-1.28	1	-0.12	0.6202	-0.63	0.9107	-0.74
M1_Spy_1186	0.7659	-0.30	0.0244	-0.82	0.0373	-0.97	0.9551	-0.30	0.013	-1.14	0.0155	-1.17
M1_Spy_1188	1	-0.05					1	0.23	0.9025	-0.52	1	-0.20
M1_Spy_1189	0.8326	-0.23	0.5256	-0.54	0.3368	-0.79	0.9988	-0.21	0.0526	-0.89	0.0697	-0.91
M1_Spy_1190	0.931	-0.28	0.0614	-0.76	0.0463	-0.89	0.9532	-0.29	0.0059	-1.21	0.1025	-1.06
M1_Spy_1191	0.9936	0.16	1	0.13			0.9998	0.32	1	-0.29	1	0.12
M1_Spy_1192	0.4899	0.31	5.00E-04	-0.93	0.006	-1.01	1	-0.13	6.00E-04	-1.17	0	-1.34
M1_Spy_1193	0.6064	-0.38	0	-1.76	0.0011	-1.60	0.0123	-0.98	0	-2.37	0.0141	-2.10
M1_Spy_1196	0.9953	-0.13	0.9505	-0.30	0.9999	0.28	0.7274	-0.42	0.9872	0.39	1	-0.04
M1_Spy_1198	0.1559	-0.42	0.0338	-0.96	0.0054	-1.24	0.1842	-0.62	0.0071	-1.19	0.0062	-1.34
M1_Spy_1200	0.1817	0.36	0.0639	0.62	0.0691	0.75	0.0771	0.67	0.0179	0.88	0.0232	0.78
M1_Spy_1201	0.9796	-0.22	0.9954	-0.14	0.9957	0.21	0.4299	-0.38	1	-0.10	1	0.05
M1_Spy_1202	0.9996	-0.11	0.9998	-0.10	1	0.04	1	-0.18	1	-0.08	0.994	-0.20
M1_Spy_1203	0.8412	-0.25	0.9997	-0.14	0.8584	-0.40	0.9217	-0.27	0.9086	-0.52	0.9685	-0.46
M1_Spy_1204	0.964	0.43	0.0131	0.93	0.9049	0.39	0.2311	0.90	0.1801	0.77	0.4668	0.41
M1_Spy_1205	0.7137	-0.28							0.9148	-0.66		
M1_Spy_1206	1	-0.07					0.9905	0.30	1	0.02	0.9962	0.40

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_1208	1.00E-04	-1.84	0.999	-0.30	0.9924	0.58	0.013	-2.14	1	0.27	1	-0.02
M1_Spy_1209	1.00E-04	-2.31	0.9947	-0.28	0.9927	0.44	0	-2.45	1	0.08	1	-0.21
M1_Spy_1210	0.9989	-0.22					0.999	-0.22				
M1_Spy_1211	0.1455	-0.71	0.0024	-0.85	0.0447	-0.62	0.1324	-0.97	0.028	-1.39	0.0025	-1.20
M1_Spy_1212	0.0791	0.54	6.00E-04	1.17	1.00E-04	1.26	0	1.23	2.00E-04	1.28	0.0015	1.06
M1_Spy_1213	0.0239	0.60	8.00E-04	1.28	6.00E-04	1.16	0.0018	1.36	2.00E-04	1.32	0.0107	0.94
M1_Spy_1214	1	0.06	0.0068	0.96	0.0223	1.01	0.0139	0.86	0.0019	1.15	0.0032	1.17
M1_Spy_1215	0.5363	0.28	6.00E-04	1.23	0	1.58	0.0013	0.96	0	1.75	0.0051	1.61
M1_Spy_1216	0.9979	-0.11	0.0467	0.51	0.3301	0.57	0.5902	0.34	0.7775	0.29	0.3922	0.54
M1_Spy_1217	1	-0.01	0.0075	0.66	0.0173	0.88	0.027	0.55	0.017	1.10	0.0062	1.06
M1_Spy_1218	0.8726	-0.18	0.2528	0.49	0.0206	0.76	0.9943	0.16	0.009	0.86	0.0019	0.99
M1_Spy_1219	0.0292	-0.52	0.5075	-0.29	1	0.03	0.2757	-0.40	1	-0.11	0.9958	0.17
M1_Spy_1220	0.0095	-0.64	0.8485	-0.22	1	-0.04	0.0279	-0.59	1	-0.13	1	0.07
M1_Spy_1221	0.8199	-0.21	0.9995	-0.12	1	0.12	0.3918	-0.42	0.897	-0.43	1	-0.12
M1_Spy_1222	1	-0.07	1	-0.01	0.5655	0.53	1	-0.09	0.9568	0.34	0.8072	0.39
M1_Spy_1223	0.9998	0.11	1	-0.06	0.9978	0.32	1	-0.04	0.7859	0.43	1	0.18
M1_Spy_1224	0.5495	0.29	0.5122	0.33	0.6832	0.49	0.0587	0.50	0.713	0.49	0.1679	0.78
M1_Spy_1225	0.0354	0.89	0.0203	1.00	0.0192	1.31	0	1.40	0.0426	1.40	0.0044	1.02
M1_Spy_1226	0.0092	0.64	0.1307	0.70	0.0458	0.79	0	1.07	0.0185	0.83	0.0217	0.67
M1_Spy_1227	0.7995	0.23	0.9976	-0.18	1	0.06	0.9862	0.20	1	-0.07	1	-0.15
M1_Spy_1228	0.1316	0.56	0.9935	-0.17	0.4248	-0.39	0.578	0.37	0.4318	-0.58	0.0174	-0.83
M1_Spy_1230	0.493	-0.28	0.3207	-0.41	0.9777	-0.28	0.465	-0.36	0.6958	-0.43	0.8747	-0.40
M1_Spy_1232	1.00E-04	-0.92	0.0175	-0.73	0.4652	-0.44	0.0076	-1.19	0.0377	-0.83	0.0042	-0.93
M1_Spy_1233	0.3678	0.52	0.0453	-1.00	0.9725	-0.35	1	0.03	0.0831	-1.00	0.4215	-0.61
M1_Spy_1234	0.4799	0.41	0.0424	1.62	7.00E-04	1.35	0.1312	0.88	0.0049	1.97	0.0249	1.74

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_1236	0.017	0.85	0.6515	0.44	0.9877	0.40	2.00E-04	1.38	0.975	0.33	0.9995	0.29
M1_Spy_1237	0.0016	0.89	0.4929	0.39	0.9762	0.32	0.0014	1.44	0.4993	0.53	0.874	0.34
M1_Spy_1239	0.0011	0.81	7.00E-04	0.87	0.076	0.72	0	1.35	2.00E-04	1.33	0.0014	1.06
M1_Spy_1240	0.9619	-0.19	0.2113	0.47	0.9468	0.58	0.9169	0.40	0.4405	0.76	0.5738	0.83
M1_Spy_1241	0.0082	-0.68	0.4392	-0.71	0.3518	-0.65	0.9993	-0.21	0.9982	-0.22	1	-0.01
M1_Spy_1242	1	0.09	0.071	0.99	0.2319	0.95	0.0369	0.76	0	1.62	4.00E-04	1.60
M1_Spy_1243	1	-0.08	0.4919	0.45	0.9213	0.48	1	0.18	0.2055	0.69	0.3638	0.79
M1_Spy_1244	0.1538	-0.47	0.202	-0.60	0.182	-0.65	0.8528	-0.48	0.031	-0.70	0.9877	-0.38
M1_Spy_1245	0.4754	0.33	0.0788	0.65	0.1814	0.58	0.1817	0.44	0.0181	0.84	0.011	0.87
M1_Spy_1246	0.0027	0.82	0.1879	0.58	0.1843	0.75	0.0955	0.82	0.0205	0.89	0.1964	0.86
M1_Spy_1247	0.3236	-0.41	4.00E-04	-1.13	0.0092	-1.15	0.3729	-0.66	2.00E-04	-1.34	5.00E-04	-1.23
M1_Spy_1248	0.419	-0.34	0.0118	-0.83	0.0291	-0.97	0.0856	-0.55	2.00E-04	-1.16	0.0095	-1.03
M1_Spy_1249	0.9904	-0.13	0.8759	0.22	0.8044	0.29	1	-0.06	0.8271	0.42	0.6078	0.38
M1_Spy_1250	0.0431	-0.61	0.0122	-0.68	0.3053	-0.49	0.0661	-0.72	0.0291	-0.90	0.0782	-0.69
M1_Spy_1251	0.047	-0.63	0.988	-0.24	1	-0.11	0.0342	-0.78	0.7761	-0.39	0.7749	-0.34
M1_Spy_1252	0.328	-0.32	0.4326	-0.59	0.3828	-0.62	0.4127	-0.60	0.0087	-1.06	0.062	-0.91
M1_Spy_1253	0.5326	0.38	0.9907	-0.29	0.998	-0.29	0.671	0.42	1	-0.02	1	-0.05
M1_Spy_1254	0.9935	-0.17	0.0498	-0.99	0.0381	-1.02	1	-0.17	0.0362	-0.98	0.2771	-1.00
M1_Spy_1255	1	0.07	0.0694	-0.95	0.0286	-1.06	1	-0.04	0.0172	-1.07	0.0335	-1.07
M1_Spy_1257	0.0039	0.75					0.7627	0.70				
M1_Spy_1258	0.2353	-0.53							0.5847	-0.75		
M1_Spy_1259	0.1105	-0.70	0.0849	0.51	5.00E-04	1.18	0.9098	-0.30	2.00E-04	1.38	0	1.56
M1_Spy_1260												
M1_Spy_1261												
M1_Spy_1262												

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_1263	0.2085	-0.49	0.4522	-0.36	0.9718	-0.24						
M1_Spy_1264												
M1_Spy_1265												
M1_Spy_1267	0.1667	-0.46	0.1375	-0.84	0.0307	-1.04	0.9956	-0.32	0.0537	-0.97	0.1357	-1.04
M1_Spy_1270	0.9602	0.17	0.9394	0.67	1	-0.24	0.1094	1.18	1	0.21	1	-0.11
M1_Spy_1272	2.00E-04	1.00	0.0476	1.49	0.4667	0.69	5.00E-04	1.78			0.0763	1.62
M1_Spy_1273	0.0216	-0.88	0.6068	-0.39	0.1736	0.70	0.0036	-1.38	0.013	-0.79	1	-0.03
M1_Spy_1274	0.0011	-0.65	0.9385	0.50	0.9664	0.45	0.8647	-0.38	0.9948	0.32	0.9925	0.41
M1_Spy_1275	0.4042	-0.54	0.3324	0.81	0.0311	0.97	0.998	-0.17	0.4799	0.91	0.7161	0.98
M1_Spy_1276	0.0887	-0.58	0.0279	0.94	0.2613	0.99	0.9118	-0.28	0.0019	1.20	0.096	0.96
M1_Spy_1277	0.0055	-0.74	0.198	-0.52	0.3946	-0.67	0.0016	-0.95	0.9288	-0.29	0.9917	-0.25
M1_Spy_1280	0.1042	-0.54	0.0056	-1.75	5.00E-04	-1.84	0.0395	-1.16	0.1571	-1.00	0.0247	-1.00
M1_Spy_1281	0.0604	-0.51	0.5913	-0.35	0.9511	-0.23	0.0149	-0.76	0.4077	-0.50	0.4331	-0.39
M1_Spy_1282	0.295	0.31	4.00E-04	1.10	0.0028	1.05	0.0046	0.81	0	1.42	0	1.38
M1_Spy_1283	0.9998	0.07	0.0419	0.51	0.1924	0.50	0.4617	0.38	0.0979	0.63	0.0877	0.60
M1_Spy_1284	0.1373	-0.65	0.4168	-0.45	0.5002	-0.40	0.2599	-0.46	0.1905	-0.56	0.1195	-0.68
M1_Spy_1285	1	-0.07	0.1817	-0.47	1	-0.10	1	-0.13	1	-0.12	1	0.11
M1_Spy_1286	0.1861	0.45	0.6398	0.38	0.2906	0.88	0.9034	0.42	0.2967	1.13	0.3249	1.18
M1_Spy_1287	0.9968	0.19	0.984	0.29	0.6199	0.88	0.9995	0.31	0.0614	1.02	0.0371	1.07
M1_Spy_1288	0.6821	0.24	0.1221	-0.49	0.9178	-0.30	0.9833	-0.22	0.0834	-0.66	0.0466	-0.65
M1_Spy_1289	1	0.00	0.0161	-0.79	0.1053	-0.61	0.607	-0.38	0.1975	-0.80	0.0505	-0.65
M1_Spy_1290	0.9829	-0.27	6.00E-04	1.66	0.0189	2.10	1	-0.03	0.0206	1.94	1.00E-04	2.24
M1_Spy_1291												
M1_Spy_1292	0.9976	-0.16					1	-0.13				
M1_Spy_1293												

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_1294							0.4523	0.60				
M1_Spy_1295	0.1997	-0.62	0.2842	-0.68	0.564	-0.71	0.2818	-0.51	0.0766	-1.10	0.3251	-0.81
M1_Spy_1296	0.7045	-0.36					1	0.00	0.9999	0.47	0.3935	0.91
M1_Spy_1297	0.3787	-0.43	0.352	-0.67	0.8652	-0.70	0.306	-0.60	0.1718	-0.91	0.908	-0.55
M1_Spy_1298	0.8196	-0.24	0.9989	-0.18	0.9453	-0.38	1	-0.17	0.5689	-0.53	0.9979	0.31
M1_Spy_1299	0.2655	-0.44	0.2192	-0.73	0.1829	-0.79	0.4044	-0.51	0.0073	-1.08	0.7203	-0.50
M1_Spy_1301	0.9968	0.14	0.4095	0.70	0.8393	0.70	0.2276	0.71	0.0441	1.57	0.0118	2.02
M1_Spy_1302	0.8605	-0.27	0.9995	0.13	0.9971	0.25	0.7208	0.39	0.1647	0.85	0.2531	1.12
M1_Spy_1304	0.064	-0.79	0.0135	-0.79	0.9814	-0.36	0.9978	-0.26	1	-0.16	0.993	0.31
M1_Spy_1306	0.0093	-1.08	6.00E-04	-1.06	0.5462	-0.57	0.7857	-0.55	1	-0.12	1	0.01
M1_Spy_1308	0.9503	0.17	0.984	0.26	0.5802	0.42	0.4469	0.46	0.6899	0.41	0.4619	0.48
M1_Spy_1309	1	-0.01	0.9808	-0.19	1	-0.02	1	0.13	1	0.00	0.9888	0.19
M1_Spy_1310	0.2857	-0.32	0.3504	-0.47	0.807	-0.53	1	-0.20	0.0509	-0.70	0.9875	-0.38
M1_Spy_1311	0.6138	0.39	0.0893	0.65	0.484	0.57	0.2686	0.77	0.0745	1.01	0.1132	0.72
M1_Spy_1312	0.8803	0.25	1	-0.07	0.9993	-0.21	0.9682	0.30	1	0.18	0.8728	-0.28
M1_Spy_1314	0.8592	0.31	0.2384	-0.77	0.0839	-0.90	0.9992	0.13	0.0985	-0.69	0.0123	-0.91
M1_Spy_1315	0.5472	0.41	0.2418	0.80	0.8082	0.97	0.5103	0.49	0.9001	1.65	0.5109	1.38
M1_Spy_1316	1	0.04	0.2983	0.75	0.1986	1.43	1	0.24	0.0668	1.45	0.0016	1.58
M1_Spy_1322	0.9537	0.21	0.0196	-2.48								
M1_Spy_1323	1	0.14	4.00E-04	-1.85			0.4079	-0.59				
M1_Spy_1324	0.9858	0.22					0.6004	-0.56	0.0071	-2.01		
M1_Spy_1325	1	-0.06	5.00E-04	-2.18	0.0453	-2.50	0.1069	-0.66	0.007	-1.94	0.0522	-2.07
M1_Spy_1326	0.7263	0.39	0.0017	-1.82	0.0407	-2.41	0.9313	-0.29	0.0098	-2.24	0.0315	-2.76
M1_Spy_1328	0.4642	0.37	0.0011	-1.31	0.3568	-1.54	0.9998	-0.16	0.0071	-1.30	0.2014	-1.81
M1_Spy_1329	1	0.01	0.161	2.19			1	0.22				

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_1332	0.4328	-0.46	1.00E-04	-1.51	0	-1.55	0.1087	-0.78	0	-1.86	0.0014	-1.67
M1_Spy_1333	0.9998	-0.14	4.00E-04	-1.07	0.007	-1.19	0.8446	-0.28	0.0022	-1.41	0.0485	-1.20
M1_Spy_1335	0.0279	0.55	0.0272	0.78	0.0575	0.64	0.2828	0.54	0.4892	0.43	0.8146	0.30
M1_Spy_1336	0.024	-0.75	0.0248	-1.39	9.00E-04	-1.58	0.0546	-1.09	2.00E-04	-1.94	0.0014	-1.75
M1_Spy_1337	0.1763	-0.50	1	0.06	1	-0.13	0.3829	-0.71	0.9332	-0.60	0.9963	-0.47
M1_Spy_1339	0.9541	0.30	2.00E-04	-1.42	0.0165	-1.18	1	-0.09	2.00E-04	-1.84	0.0037	-1.38
M1_Spy_1340	1	-0.02	0.9995	-0.36	0.5747	-0.77	1	0.09	1	0.28	0.3031	0.85
M1_Spy_1343	0.225	0.56	0.1106	0.66	0.6486	0.65	0.2212	0.58	0.8897	0.48	0.7911	0.72
M1_Spy_1344	0.0661	0.60	5.00E-04	1.29	8.00E-04	1.23	0.0231	0.77	0.0014	1.29	0.0439	1.08
M1_Spy_1345	0.9563	0.20	0.3578	0.50	0.9605	0.31	0.6595	0.33	1	0.14	1	0.06
M1_Spy_1346	0.9199	0.19	0.1189	0.58	0.952	0.35	0.867	0.24	0.9923	0.26	0.9974	0.19
M1_Spy_1350	0.6357	0.27	1	-0.05	1	-0.13	0.9513	0.19	0.8171	-0.33	0.9954	-0.19
M1_Spy_1351	0.9988	0.12	0.1499	-0.84	0.4515	-0.60	0.851	-0.29	0.5053	-0.68	0.1598	-0.69
M1_Spy_1352	0.0315	0.56	0.9995	0.18	0.8956	0.66	0.7292	0.47	0.9999	0.44	1	0.17
M1_Spy_1353	0.9604	-0.17	0.9992	0.10	1	-0.10	1	-0.12	0.9614	0.30	1	-0.11
M1_Spy_1354	0.0488	-0.46	1	0.00	0.9986	-0.18	0.0635	-0.53	1	0.04	0.9944	-0.23
M1_Spy_1355	0.0658	0.47	0.9958	0.25	0.4479	0.58	0.3036	0.41	0.0763	0.70	0.114	0.71
M1_Spy_1356	0.0011	0.83	8.00E-04	1.14	0.001	1.51	0.0035	0.88	0.001	1.62	0.0068	1.44
M1_Spy_1357									0.5843	0.74		
M1_Spy_1358	0.5583	0.32	0.2602	1.07	0.252	1.25	0.6824	0.59	0.3564	0.96	0.1628	0.78
M1_Spy_1359	0.3167	0.37	0.1689	0.50	0.3386	0.54	0.0608	0.72	0.1264	0.73	0.1797	0.64
M1_Spy_1361												
M1_Spy_1362	0.5008	-0.38	1	0.08	1	0.12	0.6436	-0.60	0.9994	-0.26	0.9988	-0.28
M1_Spy_1363	1	-0.10	0.9819	-0.24	1	-0.07	0.9967	-0.22	1	-0.11	0.7306	-0.39
M1_Spy_1364	0.0442	-0.74	0.0214	-1.00	0.0223	-1.03	0.043	-0.85	2.00E-04	-1.22	0.0092	-1.23

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_1365	0.0113	-0.55	0.0741	-0.53	0.4815	-0.54	0.0022	-0.88	0.0736	-0.74	0.0316	-0.85
M1_Spy_1366	1	-0.14					1	-0.15	1	0.33	0.9622	0.69
M1_Spy_1367	0.9807	0.48					0.9845	0.38	1	-0.36		
M1_Spy_1368	0.0422	1.03	0.0025	2.21	1.00E-04	2.34	0.0074	1.48	0	2.51	0.0025	2.25
M1_Spy_1369	0.9706	-0.17	0.8651	-0.29	1	-0.13	0.338	-0.37	0.9048	-0.31	1	-0.11
M1_Spy_1370	0.0021	0.71	0.0807	0.55	0.7614	0.42	0.0661	0.58	1	-0.06	1	-0.10
M1_Spy_1371	1.00E-04	1.25	0.0235	0.63	0.6794	0.34	3.00E-04	1.12	0.1038	0.70	0.9725	0.21
M1_Spy_1372	0	1.63	0	1.46	6.00E-04	0.93	0	2.11	1.00E-04	1.57	0.0304	0.95
M1_Spy_1373	0.0155	0.82	0.8226	-0.27	0.0019	-0.91	0.0176	0.87	0.9974	-0.23	0.0331	-0.88
M1_Spy_1374	1	0.03	0.002	1.23	0.8746	0.48	1	-0.07	0.316	0.65	0.9956	-0.25
M1_Spy_1375	0.1036	0.47	0.0015	1.20	0.864	0.46	0.0035	0.82	0.1814	0.73	1	0.05
M1_Spy_1378	0.0736	0.48	0	1.29	0.0137	0.77	0.0036	0.78	0.0019	0.90	0.1311	0.61
M1_Spy_1379	0.9978	0.16	0.9978	0.20	1	-0.11	0.6758	-0.39	0.7755	-0.72	0.0161	-1.30
M1_Spy_1384							9.00E-04	1.60				
M1_Spy_1385	0.9252	0.40	0.0013	1.85	0.131	1.62	0.0069	1.22	0.0061	1.66	0.6631	2.18
M1_Spy_1386	0.5874	-0.41	0.9529	0.37	0.4262	0.68	1	-0.04	1	0.14	0.8318	0.39
M1_Spy_1389	0.2483	0.40	0.1996	0.74	0.1368	0.71	0.0324	0.90	0.0395	0.84	0.0054	1.26
M1_Spy_1390	0.6139	0.34	1	-0.06	1	0.01	1	0.06	1	0.01	1	0.03
M1_Spy_1391	1	-0.09	0.0015	1.25	8.00E-04	1.46	1	0.10	0.0188	1.19	0.0216	1.50
M1_Spy_1392	1	0.00	0.9996	0.15	0.9896	0.27	1	0.15	0.9786	0.40	0.9957	0.34
M1_Spy_1393	0.7917	-0.22	0.1077	0.58	0.1008	0.84	1	0.07	0.9923	0.37	0.6503	0.69
M1_Spy_1395												
M1_Spy_1398	0.0405	-0.72	0.0027	-0.96	0.2019	-0.81	0.0194	-0.86	0.0713	-0.93	0.046	-0.89
M1_Spy_1399	0.008	-0.91	0	-1.73	0	-2.04	3.00E-04	-1.23	0	-2.16	0	-2.15
M1_Spy_1400	0.0622	-0.77	0.1805	-0.89	0.0289	-1.13	0.1319	-0.98	7.00E-04	-1.52	0.0223	-1.40

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_1401	0.3005	0.43	0	1.83	1.00E-04	1.85	0.1354	0.85	4.00E-04	1.95	0.0118	1.74
M1_Spy_1402	0.7397	-0.35	0	-1.83	0.0231	-1.87	0.0103	-1.35	2.00E-04	-2.88	0	-2.71
M1_Spy_1404	0.4764	-0.41	1.00E-04	-1.68	0	-2.02	0	-1.52	0	-2.92	0	-2.77
M1_Spy_1405	0.0092	-0.56	0.0011	-1.69	0.0022	-1.99	2.00E-04	-1.52	0	-3.03	0	-2.91
M1_Spy_1406												
M1_Spy_1407	0.9845	-0.14	0.9986	-0.19	1	0.14	1	-0.10	1	0.10	1	-0.04
M1_Spy_1408	0.1397	-0.71	0.0338	-1.65	0.0118	-1.89	0.9977	-0.55	0.0134	-1.25	0.0795	-1.62
M1_Spy_1409	0.5542	-0.43										
M1_Spy_1410	0.6833	0.27	0.0048	1.31	0.1501	1.02	0.8808	0.32	0.0921	1.54	0.1945	0.93
M1_Spy_1411	0.0573	-0.59	0.1169	-0.76	0.2403	-1.17	0.1117	-0.80	0.3697	-0.92	0.0219	-1.31
M1_Spy_1412	0.0602	-0.80	0.0148	-0.84	0.0665	-1.00	0.0187	-0.95	0.0028	-1.35	0.038	-1.28
M1_Spy_1414	1	-0.02	0.0089	1.53	0.0399	1.44	0.945	0.33	0.0011	1.90	0.0337	1.39
M1_Spy_1415	0.0022	0.73	0	1.79	1.00E-04	1.77	1.00E-04	1.55	0	2.25	0	2.17
M1_Spy_1416	0.9968	0.13	6.00E-04	1.32	0.005	1.44	0.0231	0.74	1.00E-04	2.02	1.00E-04	1.87
M1_Spy_1419	0.4653	0.28	0.1464	0.73	0.0486	0.81	0.8971	0.29	0.0772	0.97	0.0754	1.12
M1_Spy_1420	0.2562	0.43	0.7848	0.32	0.3642	0.55	0.8673	0.30	0.091	0.62	0.5025	0.58
M1_Spy_1421	0.0173	0.67	0.001	0.96	0.0161	0.85	0.0051	0.75	0.0149	1.26	0.0889	0.96
M1_Spy_1422	0.053	0.46	0.6991	0.35	1	-0.07	0.3425	0.44	0.7723	0.43	1	-0.15
M1_Spy_1424	8.00E-04	1.27	0.0071	2.02			0.001	1.45	2.00E-04	2.87	0.1987	0.93
M1_Spy_1425	6.00E-04	0.85	1	-0.14	0.8081	-0.78	0.837	0.30	0.7903	-0.35	0.0076	-1.38
M1_Spy_1427	0	1.28	0.9909	-0.30	0.9954	-0.54	0.5391	0.53	0.0682	-1.11	0.0045	-1.29
M1_Spy_1429	1.00E-04	1.06	1	-0.05	1	-0.11	0.0047	0.84	1	-0.13	1	-0.13
M1_Spy_1432	0.8775	0.31	0.0104	1.58	0.0285	1.50	0.9583	0.55	0.0967	1.15	0.4566	1.40
M1_Spy_1434	0.9891	-0.21	0.6045	-0.58	0.9971	-0.35	1	0.17	0.3772	0.79	0.5374	0.70
M1_Spy_1436	0.0095	-0.83	0	-2.00	6.00E-04	-1.90	0.1019	-1.17	0	-2.29	0	-1.84

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_1437	0.0207	0.76					0.8465	0.54				
M1_Spy_1438	0.1467	-0.69	2.00E-04	-1.39	0.0017	-1.56	0.0588	-1.07	0	-1.91	1.00E-04	-1.87
M1_Spy_1440	0.8882	0.33					1	-0.11				
M1_Spy_1441	1	-0.07	0.0596	-0.78	0.6424	-0.47	0.3289	-0.50	0.0121	-1.11	0.3869	-0.53
M1_Spy_1442	0.0577	-0.84	6.00E-04	-1.46	5.00E-04	-1.58	0.0711	-1.16	0	-1.97	2.00E-04	-1.86
M1_Spy_1443	0.5276	-0.38					0.4321	-0.67	0.1311	-1.07	0.4092	-1.08
M1_Spy_1444												
M1_Spy_1445	0.3643	-0.43	0.0148	-1.15	0.0509	-1.24	0.2808	-0.77	0.0032	-1.48	0.006	-1.43
M1_Spy_1446												
M1_Spy_1447	0.1587	-0.40	0.0283	-0.95	0.0339	-1.12	0.099	-0.64	0.007	-1.16	0.0495	-1.36
M1_Spy_1448	0.8175	-0.26							0.0118	-1.87		
M1_Spy_1449												
M1_Spy_1450	0.5633	-0.38										
M1_Spy_1451	0.8821	-0.36	0.0042	-1.09	0.045	-1.23	0.1485	-0.77	0.0022	-1.46	0.0189	-1.43
M1_Spy_1452												
M1_Spy_1453												
M1_Spy_1454	0.4896	-0.37	0.0046	-1.13	0.0275	-1.26	0.207	-0.78	0.0016	-1.39	0.0152	-1.41
M1_Spy_1455	0.2241	-0.45	0.0152	-1.22	0.1202	-1.22	0.1168	-0.77	0.1035	-1.17	0.0238	-1.24
M1_Spy_1456												
M1_Spy_1457	0.0907	-0.44	0.0028	-1.06	0.0385	-1.17	0.054	-0.73	6.00E-04	-1.43	0.0128	-1.36
M1_Spy_1459	0.2773	-0.35	0.0261	-1.17	0.2585	-1.03	0.0893	-0.69	0.0466	-1.10	0.0734	-1.04
M1_Spy_1460	0.0035	-0.84	1.00E-04	-1.70	4.00E-04	-1.77	0.0055	-1.33	0	-2.27	0.0048	-2.10
M1_Spy_1461	0.3543	-0.49	0.0068	-1.49	0.0206	-1.81	0.0482	-1.05	2.00E-04	-2.07	0.0201	-2.03
M1_Spy_1462	0.0213	-0.75	0	-1.78	6.00E-04	-1.77	0.0079	-1.31	0	-2.27	0	-2.12
M1_Spy_1463	0.0927	-0.55	1.00E-04	-1.33	0.0069	-1.15	0.0317	-0.96	3.00E-04	-1.63	0.0407	-1.29

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_1464	1.00E-04	-0.80	3.00E-04	-1.44	0.0087	-1.43	0.006	-1.16	4.00E-04	-1.89	1.00E-04	-1.68
M1_Spy_1465	0.0404	-0.77	0	-1.74	6.00E-04	-1.77	0.0026	-1.24	3.00E-04	-2.11	0	-2.05
M1_Spy_1466	0.0162	-0.67	0.0533	-1.10	0.1256	-0.92	0.1103	-0.95	0.0016	-1.50	0.0167	-1.27
M1_Spy_1468												
M1_Spy_1469	0.0752	-0.52	0.3646	-0.52	0.9675	-0.37	0.1172	-0.63	0.1119	-0.82	0.9184	-0.47
M1_Spy_1470	0.0638	-0.54	0.0372	-1.10	0.0939	-0.96	0.1233	-0.89	0.0133	-1.24	0.0791	-1.15
M1_Spy_1471	0.1397	-0.52	0.0694	-1.04	0.1401	-1.02	0.1611	-0.96	0.0019	-1.66	0.0144	-1.48
M1_Spy_1473	1	-0.08										
M1_Spy_1474												
M1_Spy_1475	0.4161	-0.45					0.795	-0.97			0.3489	-0.73
M1_Spy_1476	0.6771	-0.34	0.9634	-0.44			0.6301	-0.46	0.3689	-0.88	0.5406	-0.64
M1_Spy_1477												
M1_Spy_1478												
M1_Spy_1479	0.2022	-0.55	0.0945	-0.87	0.1154	-1.11	0.1398	-0.76	0.0216	-1.35	0.0052	-1.31
M1_Spy_1481												
M1_Spy_1482	0.0165	-0.93	0	-2.03	1.00E-04	-1.70	0.0116	-1.47	2.00E-04	-2.14	0	-2.35
M1_Spy_1483												
M1_Spy_1484	0.0056	-0.93	0	-2.02	0.0026	-1.79	1.00E-04	-1.63	2.00E-04	-1.87	0	-2.29
M1_Spy_1485	0.9787	-0.34	0.0029	-1.30	0.2337	-1.14	0.0859	-0.90	0.0049	-1.57	0.0086	-1.84
M1_Spy_1486	0.007	-0.62	0.433	-0.68	0.4905	-0.69	0.015	-0.87	0.7193	-0.70	0.0805	-1.16
M1_Spy_1487	0.0418	-0.58	0.1621	-0.57	0.8962	-0.55	0.0475	-0.82	0.365	-0.60	0.7338	-0.71
M1_Spy_1488												
M1_Spy_1489	0.69	0.38	0.0076	-1.18	0.044	-0.84	1	-0.28	7.00E-04	-1.37	0.2249	-0.87
M1_Spy_1491	0.9917	0.14	0.9995	0.12	0.9995	0.14	0.9998	0.14	0.7553	0.35	0.6519	0.37
M1_Spy_1492	0.7737	0.20	0.9413	0.21	0.9532	0.25	0.8927	0.27	0.2596	0.49	0.4719	0.47

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_1493	0.3453	0.34	0.7302	0.35	0.81	0.41	0.9577	0.30	0.2993	0.67	0.361	0.61
M1_Spy_1494	0.0878	0.58	0.2565	-0.49	0.0716	-0.67	0.1957	0.51	0.4906	-0.44	0.4159	-0.52
M1_Spy_1495	0.0022	1.13	0.9989	-0.16	0.5435	-0.50	0.0074	1.01	1	0.11	0.9968	-0.26
M1_Spy_1496	0.123	0.75	0.1035	-0.58	0.0777	-0.78	0.1653	0.57	0.2798	-0.52	0.084	-0.74
M1_Spy_1497	0.0594	0.57	0.0875	-0.62	0.1726	-0.69	0.3052	0.45	0.9268	-0.34	0.9504	-0.39
M1_Spy_1498	0.8125	0.32	0.0122	-0.80	0.0186	-0.95	1	0.04	0.0182	-0.82	0.0344	-0.94
M1_Spy_1499	0.1447	0.45	0.0025	-0.78	0.0277	-0.90	0.5302	0.40	0.2053	-0.57	0.2501	-0.59
M1_Spy_1500	0.9987	0.11	0.0184	-0.95	0.0123	-1.00	1	-0.09	0.0464	-0.85	0.0425	-0.81
M1_Spy_1502	0.2201	-0.68	0.1213	-0.72	0.3318	-0.71	0.0549	-0.84	0.0062	-1.04	0.2215	-0.80
M1_Spy_1503	0.0092	-0.60	0.0451	-0.75	0.5329	-0.61	0.009	-0.75	0.5824	-0.64	1	-0.19
M1_Spy_1505	0.0655	-0.68	0.143	-0.68	0.5914	-0.54	0.0244	-0.88	0.0096	-0.98	0.0684	-0.80
M1_Spy_1506	0.798	-0.33	0.0759	1.24	0.0809	1.79	0.9839	0.31	3.00E-04	2.15	0.01	1.68
M1_Spy_1507			0.2402	2.47			0.6737	0.79				
M1_Spy_1508	0.299	-0.29	0.0859	-0.51	0.9718	-0.33	0.6443	-0.55	0.2874	-0.74	0.5459	-0.46
M1_Spy_1509	0	-1.76	0	-1.75	0	-1.65	0	-1.44	6.00E-04	-1.74	0	-1.70
M1_Spy_1510	0	-1.16	0	-2.03	6.00E-04	-1.91	0	-1.70	0	-2.48	0	-2.31
M1_Spy_1511	6.00E-04	-0.82	2.00E-04	-1.16	0.002	-1.21	0.0011	-1.11	4.00E-04	-1.62	0	-1.73
M1_Spy_1513	0	1.92	0	3.00	0	2.56	0	2.43	0	3.46	0	2.60
M1_Spy_1514	0	1.16	4.00E-04	1.06	0.1233	0.60	0	1.44	9.00E-04	1.07	0.154	0.49
M1_Spy_1515	0.0092	0.89	0.0112	0.68	0.7336	0.35	5.00E-04	1.01	0.167	0.53	0.9511	0.20
M1_Spy_1516	8.00E-04	0.75	0.0264	0.76	0.49	0.46	3.00E-04	0.92	0.0352	0.68	0.9247	0.37
M1_Spy_1518	0.0053	0.75	0.0037	0.73	0.2652	0.48	3.00E-04	0.95	0.0399	0.74	0.153	0.47
M1_Spy_1519	0.0027	0.90	0.0016	0.86	0.451	0.47	1.00E-04	1.10	0.0191	0.75	0.6006	0.37
M1_Spy_1520	0.0503	0.51	0.0344	0.59	0.8785	0.32	0.1216	0.45	0.2519	0.51	0.6099	0.35
M1_Spy_1521	0.0216	0.50	0.0391	0.61	0.3099	0.42	0.0413	0.56	0.1191	0.59	0.9838	0.17

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_1523	0.9003	-0.18	0.2574	-0.43	0.6704	-0.39	1	-0.15	0.9999	-0.18	1	-0.08
M1_Spy_1524	0.6744	-0.24	0.0277	-0.65	0.0586	-0.77	0.8011	-0.29	0.3306	-0.52	0.2941	-0.53
M1_Spy_1525	0.693	-0.24	4.00E-04	-1.04	0.0026	-1.02	0.0751	-0.48	0.0045	-1.02	0.016	-0.93
M1_Spy_1526	0.0034	1.46	4.00E-04	2.83	0.012	2.71	0.001	1.97				
M1_Spy_1527	0.0151	0.63	0.0826	0.68	0.5112	0.54	0.0049	0.92	0.0069	0.95	0.0338	0.88
M1_Spy_1528	0.8931	-0.18	0.8732	-0.33	1	-0.10	0.8639	-0.25	1	-0.02	1	0.15
M1_Spy_1529	0.7102	-0.22	0.8187	-0.23	0.9117	-0.28	0.5401	-0.29	0.8871	-0.27	1	-0.12
M1_Spy_1530	0.7694	-0.21	0.3305	-0.50	0.241	-0.53	0.2627	-0.43	0.0675	-0.68	0.3831	-0.62
M1_Spy_1531									1	-0.20		
M1_Spy_1532												
M1_Spy_1533	0.0012	0.73	0.0399	0.94	0.1488	0.76	0.0218	0.86	0.0165	1.11	0.1876	0.89
M1_Spy_1534	0.251	0.41	0.0619	0.65	0.3083	0.70	0.6382	0.34	0.7312	0.76	0.364	0.70
M1_Spy_1535	0.5576	0.27	0.0366	0.78	0.0554	0.87	1	0.07	0.8438	0.53	0.7262	0.59
M1_Spy_1536	0.2221	0.38	0.7093	0.27	0.8072	0.36	0.8862	0.26	0.9595	0.24	0.4463	0.43
M1_Spy_1537	0.9997	0.08	0.994	0.16	0.9693	0.33	1	0.01	0.8827	0.31	0.8866	0.38
M1_Spy_1538	0.6564	-0.26	0.9852	0.18	0.9839	0.22	0.4731	-0.37	1	0.09	0.9969	0.16
M1_Spy_1539	0.0057	-0.58	0.1076	-0.78	0.0804	-0.82	0.1204	-0.73	0.0192	-0.91	0.2846	-0.69
M1_Spy_1541	0.0253	-1.03	1.00E-04	-1.98	0	-2.36	0.0693	-1.23	0	-1.99	0.008	-1.67
M1_Spy_1542	0.0011	-0.80	0.0013	-1.40	6.00E-04	-1.71	0.049	-0.87	0.1608	-0.97	0.0486	-0.99
M1_Spy_1543	0.0027	-2.01	2.00E-04	-1.98	0.4887	-1.18	0.0295	-0.82	0.1354	-1.04	0.0243	-1.29
M1_Spy_1544	0	-3.20	0	-2.74	0	-1.59	0.0016	-2.22	0.036	-2.22	0	-2.37
M1_Spy_1546	3.00E-04	-2.64	0	-2.09	1.00E-04	-1.20	0	-2.56	7.00E-04	-2.87	0.0019	-2.74
M1_Spy_1547	0.0088	-1.75	0	-2.03	0	-1.39	2.00E-04	-2.23	2.00E-04	-3.43	0.0208	-3.10
M1_Spy_1548	0.955	0.21	0.6434	0.51			0.7206	0.50	0.9883	0.72	0.068	1.16
M1_Spy_1549	1	0.02	0.9943	0.37	0.9638	0.42	0.9995	0.19	1	0.18	0.9927	0.36

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_1551	0.3176	-0.58	0.0285	-0.75	0.1085	-0.70	0.4578	-0.83	0.2272	-0.93	0.0313	-1.09
M1_Spy_1552	0.0045	1.01	0.5266	0.52	0.9838	0.51	0.114	1.11	0.0706	0.96	0.9746	0.58
M1_Spy_1556	1	0.01	0.9687	0.29	1	0.07	1	0.06	1	0.22	1	0.22
M1_Spy_1557	0.1468	-0.59					0.0483	-0.95	0.0263	-1.25	0.0491	-1.17
M1_Spy_1558	0.4491	-0.58										
M1_Spy_1559												
M1_Spy_1561	0.1744	0.52	0.9974	0.23	1	0.15	0.3485	0.57	0.9976	-0.32	0.8411	-0.43
M1_Spy_1562	0.556	0.58	0.9995	0.15	1	0.10	0.1888	0.64	0.9948	-0.31	0.8549	-0.40
M1_Spy_1563	0.9979	0.14	1	0.09	1	-0.12	0.9921	0.30	0.3734	-0.73	0.7213	-0.62
M1_Spy_1564	0.0577	0.89	0.0239	1.27	0.0163	1.15	0.0027	1.21	0.9936	0.50	0.7278	0.63
M1_Spy_1565	0.9888	0.22	0.9997	-0.11	1	-0.20	0.9985	0.23	0.201	-0.71	0.4184	-0.65
M1_Spy_1566	0.8826	0.30	0.8983	0.41	1	0.14	0.5338	0.46	0.9462	-0.39	1	-0.23
M1_Spy_1567	0.1318	0.74	0.7418	1.04	0.4433	1.19	0.0019	1.47	0.9795	0.65	0.6873	0.87
M1_Spy_1568	0.1826	0.57	0.0092	1.00	0.0803	1.00	0.0055	1.13	0.374	1.03	0.0111	1.24
M1_Spy_1569	0.956	0.68	0.5951	2.10	0.0226	2.73	0.7396	1.16	0.168	2.36	0.0125	2.59
M1_Spy_1570	0.9255	0.54	0.1799	1.61	5.00E-04	1.61	0.9573	0.92	0.0798	1.46	0.0014	1.49
M1_Spy_1571	0.1032	0.48	0.0017	1.33	0.0053	1.10	0.5509	0.66	0.0304	0.95	0.2309	0.66
M1_Spy_1572	0.9339	-0.26	1	-0.11	0.8371	-0.54	0.73	-0.38	0.4436	-0.72	0.8151	-0.80
M1_Spy_1574	0.0124	-0.72	0.0153	-1.34	0.0035	-1.54	0.0302	-0.96	2.00E-04	-1.69	0.0026	-1.56
M1_Spy_1575	0.9999	-0.13	0.9746	-0.41	0.9368	-0.76	1	-0.07	0.8908	-0.50	1	-0.17
M1_Spy_1576	0.0037	0.83					0.1909	0.90	0.6599	0.99	0.5556	0.68
M1_Spy_1577	0.0559	0.69					0.3108	0.91	1	0.21	1	-0.03
M1_Spy_1580	0.6425	0.24	0.2407	-0.45	0.8396	-0.30	0.9932	-0.16	0.6287	-0.49	0.2179	-0.52
M1_Spy_1581	1	0.05	0.0104	-0.63	0.0636	-0.67	0.5566	-0.38	0.0359	-0.73	0.0557	-0.62
M1_Spy_1582	0.8348	-0.19	7.00E-04	-1.13	0.0039	-1.10	0.0175	-0.73	0.0149	-1.20	0.006	-1.06

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_1584	1	0.07	0.9995	0.22	1	0.08	1	0.09	1	-0.09	1	0.10
M1_Spy_1586	0.5731	-0.35					0.9852	-0.28	0.9999	-0.27	1	-0.03
M1_Spy_1587	0.9873	-0.17	0.9989	-0.39	0.912	-0.71	1	0.00	0.986	-0.57	1	-0.24
M1_Spy_1588	0.6119	-0.33	0.9997	0.24	1	0.14	1	0.04	1	0.29	0.5146	0.81
M1_Spy_1589	1	0.13					0.9943	0.23			0.0853	1.59
M1_Spy_1591	0.0806	-0.56	0.0329	-1.25	0.0186	-1.25	0.3258	-0.52	0.0079	-1.26	0.9615	-0.57
M1_Spy_1592	0.5173	-0.37							1	-0.11	0.7596	1.03
M1_Spy_1593	1	-0.08					1	0.17	0.93	0.52	0.1706	1.12
M1_Spy_1595	0.7217	-0.24	0.995	-0.22	0.9919	-0.25	0.9525	-0.26	1	-0.15	0.7481	0.56
M1_Spy_1596	0.1102	-0.85	5.00E-04	-1.72	0.0018	-1.91	0.3272	-0.98	0.0024	-1.66	0.0313	-1.76
M1_Spy_1599	0.0031	-0.72	0.8005	-0.33	1	-0.11	0.5631	-0.42	1	-0.02	1	0.14
M1_Spy_1600	0.0039	-1.18	0.998	-0.28	1	0.30	0.4287	-0.50	0.823	0.45	0.9036	0.54
M1_Spy_1602	8.00E-04	-0.79	0.697	-0.43	1	-0.08	0.0224	-0.74	1	0.02	0.9998	0.19
M1_Spy_1603							1	-0.12			0.3791	0.89
M1_Spy_1604	0.2337	-0.46					0.9253	-0.31	1	-0.06	0.9696	0.42
M1_Spy_1605	0.2015	-0.51	0.9907	-0.23	1	-0.11	0.65	-0.39	0.9999	-0.22	1	-0.10
M1_Spy_1606	0.9998	-0.12					1	-0.14				
M1_Spy_1607	0.1078	0.78	0.5953	-0.60			1	0.17	1	-0.41		
M1_Spy_1608	0.6588	-0.40	0.9379	-0.21	0.9856	-0.27	0.0167	-0.77	0.4359	-0.42	0.385	-0.42
M1_Spy_1610												
M1_Spy_1613	0.8187	0.30	0.0015	-0.98	0.0208	-0.77	1	-0.11	0.0275	-1.14	0.0035	-0.86
M1_Spy_1615	0.9909	-0.20										
M1_Spy_1616	0.7684	-0.29	0.3893	-1.06	0.5098	-1.15	0.3609	-0.71	0.0391	-1.17	0.1298	-1.06
M1_Spy_1617	0.1152	-0.47	0.4807	0.44	0.9416	0.35	0.168	-0.61	1	0.19	1	0.19
M1_Spy_1618	1.00E-04	-2.31	9.00E-04	-1.80	0.8077	0.63	0	-2.47	0	-1.93	1.00E-04	-1.88

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_1619	0.2679	-0.34	0.9682	-0.22	0.4229	-0.40	0.3032	-0.34	0.9997	-0.19	0.7191	-0.34
M1_Spy_1621	0.9999	-0.07	0.302	-0.48	0.8655	-0.44	0.2899	-0.50	0.036	-0.74	0.3364	-0.66
M1_Spy_1622	0.9953	-0.11	0.2885	-0.38	0.9365	-0.36	0.0662	-0.53	0.0426	-0.69	0.2868	-0.71
M1_Spy_1623	0.9979	-0.14	0.9945	-0.28	1	-0.20	0.3572	-0.57	0.2947	-0.50	0.2233	-0.64
M1_Spy_1625	0.6041	0.28	0.1585	0.42	0.263	0.45	0.4819	0.32	0.3886	0.44	0.3316	0.41
M1_Spy_1626	0.9998	0.11	0.9997	-0.08	1	-0.02	1	-0.02	0.9968	-0.18	0.9312	-0.24
M1_Spy_1627	0.9998	-0.10	0.3145	-0.35	0.971	-0.26	0.6135	-0.29	0.6878	-0.41	0.4709	-0.39
M1_Spy_1628	0.1602	-0.40	1	0.02	1	0.05	0.2676	-0.46	1	-0.14	1	-0.11
M1_Spy_1629	0.7575	-0.27	1	-0.10	0.9778	-0.27	0.2101	-0.54	0.9759	-0.30	0.8748	-0.42
M1_Spy_1630	1	-0.03	0.0036	0.89	0.0021	1.07	1	-0.07	0.0071	0.90	0.0559	1.37
M1_Spy_1632	0.9735	-0.16	0.0449	0.89	0.2352	0.90	0.9823	-0.27	0.3079	0.86	0.0213	1.04
M1_Spy_1633	0.9998	-0.07	0.735	0.29	0.9106	0.30	1	0.03	0.2414	0.45	0.0577	0.65
M1_Spy_1634	0.9896	-0.13	0.7362	-0.40	0.9953	-0.29	0.494	-0.43	0.274	-0.69	0.8886	-0.49
M1_Spy_1637	0.9998	-0.09	0.9025	0.40	0.9891	0.35	1	-0.16	1	0.22	0.9741	0.39
M1_Spy_1638	1	0.01	0.0924	0.91	0.3523	0.83	1	0.17	0.6168	0.63	0.3327	0.89
M1_Spy_1639	1	0.09	0.0358	0.98	0.0718	0.91	0.8033	0.33	0.0163	1.31	0.0992	0.97
M1_Spy_1640	1	0.02	0.1005	0.92	0.4177	0.69	0.763	0.39	0.1914	1.01	0.4488	0.80
M1_Spy_1641							0.1278	1.16				
M1_Spy_1642	0.9952	0.11	0.8246	0.37	0.9688	0.31	1	0.10	0.8284	0.36	0.4829	0.42
M1_Spy_1643	0.1698	0.47	0.9651	0.21	0.9974	0.19	0.9561	0.20	1	-0.01	1	-0.04
M1_Spy_1644	0.4176	0.34	0.0615	0.58	0.7003	0.35	1	0.03	0.9569	0.26	1	-0.03
M1_Spy_1646	0.663	-0.29	0.0414	-0.65	0.0317	-0.72	0.0065	-0.86	0.0011	-1.09	0.0014	-0.92
M1_Spy_1647	0.9979	0.18	0.0627	-0.84	0.0437	-1.17	1	0.07	0.0494	-1.06	0.329	-1.32
M1_Spy_1648	0.0513	-0.60	0.0018	-0.85	0.0361	-0.80	0.0014	-0.84	0.0804	-0.93	0.0049	-0.96
M1_Spy_1649	0.9931	-0.12	0.195	-0.55	0.0097	-0.76	1	-0.10	0.1633	-0.79	0.006	-0.95

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_1651	0.9992	-0.15	0.9949	-0.17	1	-0.12	0.0221	-0.68	0.3628	-0.70	1	-0.18
M1_Spy_1652	0.1922	-0.47	0.6257	0.38	0.9917	0.18	1	-0.04	1	0.11	1	0.12
M1_Spy_1653	0.1073	-0.57	0.681	0.28	1	0.01	0.9921	-0.21	1	-0.04	1	-0.10
M1_Spy_1654	0.3013	-0.48	0.1313	0.63	0.5152	0.50	0.9971	-0.15	0.0134	0.82	0.061	0.71
M1_Spy_1656	0.0029	-0.72	0.9515	-0.23	1	-0.08	0.0199	-0.69	0.8286	-0.33	0.9898	-0.22
M1_Spy_1657	0.013	-1.15	0.15	-0.63	0.5026	0.38	0.0055	-0.89	0.1717	-0.58	0.8924	-0.27
M1_Spy_1658	0	-1.27	0.0102	-0.65	0.4335	0.48	1.00E-04	-1.33	0.3721	-0.63	0.5265	-0.41
M1_Spy_1659	0.6363	0.53	0.0013	1.10	0.0026	1.12	0.0038	0.97	2.00E-04	1.49	0.0194	1.36
M1_Spy_1662	0.7799	0.35	0.9961	0.27	1	-0.06	0.8524	0.50	1	0.16	1	0.05
M1_Spy_1664	1.00E-04	1.02	0.0939	0.58	0.8073	0.35	0.0027	0.93	0.2557	0.58	0.4163	0.50
M1_Spy_1665	0.9764	0.19	0.6607	-0.29	0.9464	-0.31	1	0.04	0.3438	-0.60	0.5897	-0.56
M1_Spy_1666	0.2419	0.34	0.858	0.29	1	0.11	0.7537	0.30	0.9817	0.24	1	0.05
M1_Spy_1670	0.9989	0.10	0.5529	0.47	0.9996	0.20	0.3343	0.50	0.0866	0.86	0.5904	0.53
M1_Spy_1672	0.8773	-0.22	0.6888	0.38	0.9996	0.19	1	-0.08	1	0.21	0.9715	0.28
M1_Spy_1673	2.00E-04	1.27	0.0033	1.36	0.1114	0.99	0.001	1.57	0.0106	1.71	0.1268	1.10
M1_Spy_1674	0	1.23	8.00E-04	1.26	0.0498	1.03	0	1.67	0.0049	1.23	0.1804	0.86
M1_Spy_1675	0	1.34	0.0169	1.26	0.0679	0.82	0	1.69	0.0045	1.20	0.7363	0.66
M1_Spy_1676	0	1.84	0.0225	1.01	0.8482	0.31	0	2.08	0.9782	0.62	1	-0.10
M1_Spy_1678	1	-0.01	1	0.15	1	0.13	1	0.01	1	-0.08	0.965	0.41
M1_Spy_1680	0.4843	0.47	0.0651	1.46			0.4025	0.80	0.9019	1.70	0.3322	2.38
M1_Spy_1681	0.2437	-0.35	0.6771	0.36	0.7863	0.52	0.9044	-0.32	1	-0.18	0.9976	0.23
M1_Spy_1682	0.2708	0.42	0.0092	1.14	0.3278	1.04	0.7916	0.63	0.2801	1.13	0.0533	1.23
M1_Spy_1683	1	-0.02	0.5246	0.49	0.9817	0.33	1	0.08	0.98	0.30	0.9773	0.28
M1_Spy_1684	0.0063	-0.72	0.5309	-0.36	0.752	-0.50	0.0074	-0.86	0.3046	-0.63	0.0939	-0.83
M1_Spy_1686												

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_1687	1.00E-04	1.01	9.00E-04	1.31	0.0406	1.12	0	1.18	0.0802	1.04	0.0035	1.19
M1_Spy_1688	8.00E-04	0.97	1.00E-04	1.28	0.0052	1.09	2.00E-04	1.11	9.00E-04	1.16	0.0032	1.14
M1_Spy_1689	0.0749	0.42	0.701	0.39	0.9501	0.34	0.3252	0.39	0.9997	0.18	0.9544	0.26
M1_Spy_1691	9.00E-04	-1.30	0.104	-0.82	1	-0.25	0.0035	-1.08	0.9974	-0.34	1	0.00
M1_Spy_1693	0.0164	-1.02	0.332	-0.44	0.9696	-0.37	0.0095	-1.18	0.1817	-1.27	0.0259	-1.25
M1_Spy_1694	0	-1.47	4.00E-04	-1.36	0.3636	-0.99	0	-1.63	0.0469	-1.12	0.0079	-0.94
M1_Spy_1695	1.00E-04	1.69	0	2.50	0.001	2.41	0.0191	2.33	0	3.37	4.00E-04	2.60
M1_Spy_1697	1	0.03	1	0.04	0.9953	0.30	1	-0.27	1	0.03	1	0.01
M1_Spy_1698	0.8008	0.37	0.9991	-0.13	0.9995	-0.16	0.1414	0.58	1	0.01	1	0.13
M1_Spy_1699	0.005	-1.03	1	0.01	1	0.13	0	-1.24	0.9945	-0.33	1	-0.16
M1_Spy_1700	1	-0.04	0.3349	-0.62	0.9593	-0.37	0.9408	-0.25	1	0.04	1	0.01
M1_Spy_1701	0.8605	-0.21	0.4117	-0.43	1	0.00	0.3673	-0.46	1	-0.17	1	0.15
M1_Spy_1704	0.9942	-0.22										
M1_Spy_1707												
M1_Spy_1708	0.6494	-0.49	0.0015	-1.57	0.0962	-1.42	0.2904	-0.86	2.00E-04	-1.86	0.5135	-1.35
M1_Spy_1709	0.3559	-0.40	0.7178	-0.62			0.9534	-0.42	0.2715	-0.99	0.3669	-0.68
M1_Spy_1710	0.5852	-0.40	0.79	-0.89	0.3143	-0.94	0.7044	-0.63	0.0355	-1.30	0.6136	-0.92
M1_Spy_1711	0.011	-0.92	4.00E-04	-1.69	0.0028	-1.73	0.0275	-1.33	0	-2.12	0.0305	-1.78
M1_Spy_1712	1	0.06	0.8421	0.49	0.6981	1.00	0.9991	0.21	0.8316	0.56	0.506	0.94
M1_Spy_1714	0	-1.62	0	-2.34	1.00E-04	-1.99	0	-2.01	2.00E-04	-2.20	1.00E-04	-2.20
M1_Spy_1715	0	-1.72	1.00E-04	-2.34	0	-2.08	1.00E-04	-1.90	3.00E-04	-2.06	0	-2.13
M1_Spy_1717	1.00E-04	1.76	0.9988	0.38	1	0.17	1	0.63	1	-0.62	1	-0.19
M1_Spy_1718												
M1_Spy_1719	0.9998	-0.07	5.00E-04	1.16	0.0983	0.95	0.3643	0.44	0.0012	1.23	0.0373	1.04
M1_Spy_1721	0.993	0.12	0.0064	0.90	0.8698	0.56	0.169	0.50	0.0063	1.01	0.0355	0.98

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_1722	1	-0.03	0.0247	0.63	0.2262	0.47	1	0.09	0.523	0.54	0.8612	0.34
M1_Spy_1723	0.7506	0.20	0.0065	0.78	0.5569	0.60	0.8773	0.24	0.0979	0.75	0.9006	0.28
M1_Spy_1724	0.7145	-0.21	0.1819	0.42	0.9959	0.15	0.5203	-0.29	0.9997	0.18	1	-0.09
M1_Spy_1725	0.069	-0.44	0.8076	0.26	1	0.12	0.015	-0.68	1	-0.01	0.8901	-0.25
M1_Spy_1726	1	-0.05	1	0.08	1	0.14	1	-0.10	1	0.06	1	0.05
M1_Spy_1727	0.9894	-0.15	0.9997	-0.12	0.9996	-0.15	0.9902	-0.15	1	-0.02	1	-0.13
M1_Spy_1728	1	0.05	0.3783	0.46	0.563	0.50	1	0.04	0.9846	0.32	0.7949	0.40
M1_Spy_1729	1	0.06	1	0.03	1	0.09	1	-0.01	1	0.11	0.9891	0.24
M1_Spy_1730	0.9972	-0.14	0	-1.31	0.0458	-1.45	0.1561	-0.48	2.00E-04	-1.46	0	-1.55
M1_Spy_1731	0.106	-0.43	0	-1.59	0.0118	-1.56	0.0035	-0.74	0	-1.74	0	-1.64
M1_Spy_1733	0.9749	0.22	8.00E-04	1.12	2.00E-04	1.52	0.4551	0.41	0.005	1.93	0.0028	1.89
M1_Spy_1734	1	-0.04	2.00E-04	1.41	0.0054	1.71	0.7595	0.31	6.00E-04	1.83	0	1.87
M1_Spy_1735	0.9936	0.14	0.0016	1.81	8.00E-04	1.84	0.5869	0.59	5.00E-04	2.50	0	2.61
M1_Spy_1736	0.6171	0.51					0.331	1.40	2.00E-04	4.84		
M1_Spy_1737	0.0138	0.68	0.9954	-0.18	1	-0.09	0.9991	0.19	0.6322	-0.43	0.9902	-0.25
M1_Spy_1738	0	2.21	0.0125	2.45	0	2.53	0	2.40	0	2.85	0	3.26
M1_Spy_1739	0	1.91	4.00E-04	2.07	0	2.27	0	2.30	0	2.65	0	3.19
M1_Spy_1740	0	2.03	5.00E-04	2.57	0	2.58	0	2.70	0	3.09	0	3.47
M1_Spy_1741	1	-0.03	0.9989	0.18	1	-0.02	1	-0.08	1	0.13	1	-0.03
M1_Spy_1742	0.1502	0.85	0.121	1.64	0.1396	0.98	0.0536	1.03	0.0036	1.69	0.0414	1.43
M1_Spy_1743	1.00E-04	1.73	0	3.02	4.00E-04	1.88	0	2.70	0	3.07	0	1.84
M1_Spy_1744	0.0131	1.55	0	2.98	5.00E-04	1.97	0	2.71	0	3.01	0	2.17
M1_Spy_1745	0.0011	1.66	0	3.26	0	2.35	0	2.77	0	3.47	0	2.44
M1_Spy_1746	0	1.43	0	3.14	0	2.14	0	2.34	0	3.19	0	2.28
M1_Spy_1747	0.0039	1.11	0	2.72	0.0056	1.85	0.0028	2.10	0	2.82	0	2.02

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_1748	0.0075	1.38	0	2.98	6.00E-04	2.17	0	2.40	0	3.08	0	2.40
M1_Spy_1749	0	1.26	0	2.48	1.00E-04	1.83	0	2.13	0	2.72	2.00E-04	1.92
M1_Spy_1750	0.0011	0.83	0	2.31	6.00E-04	1.72	0	1.58	0	2.33	3.00E-04	1.70
M1_Spy_1751	0	1.05	0	2.59	6.00E-04	2.04	0	1.74	2.00E-04	2.60	1.00E-04	2.04
M1_Spy_1753	0.0164	0.56	0.0373	0.69	0.0644	0.55	0.0517	0.55	0.0324	0.80	0.1611	0.53
M1_Spy_1754	0.14	0.51	5.00E-04	1.35	0.0604	0.86	0.0026	0.81	0.0149	1.36	0.0035	1.06
M1_Spy_1755	0.2626	0.50	5.00E-04	1.24	0.0128	0.78	0.1239	0.67	0.0112	1.27	0.0335	1.01
M1_Spy_1758	0.9669	0.16	0.5742	0.48	0.9971	0.19	0.8939	0.31	0.6801	0.45	0.7188	0.35
M1_Spy_1759	0.2342	-0.55	0.0043	-1.02	0.0217	-1.06	0.0034	-0.77	0.0704	-1.07	6.00E-04	-1.19
M1_Spy_1760	0.3527	-0.36	0	-1.38	8.00E-04	-1.51	0.1628	-0.44	2.00E-04	-1.43	0	-1.79
M1_Spy_1761	0.0557	-0.65	0	-1.97	6.00E-04	-2.06	0.011	-0.87	0	-2.19	0	-2.47
M1_Spy_1763	0.0184	-0.67	0	-1.99	0.0025	-2.00	0.003	-0.96	3.00E-04	-2.17	0	-2.40
M1_Spy_1764	0.0109	-0.67	0.1853	-0.63	0.6815	-0.59	0.0803	-0.57	0.945	-0.31	0.8334	-0.39
M1_Spy_1765	0.0157	-0.56	0.001	-1.05	0.0132	-0.98	0.0019	-0.79	0.0013	-1.00	0.0127	-0.90
M1_Spy_1766	0.0235	-0.49	0.8375	-0.30	0.9763	-0.38	0.0892	-0.55	0.9149	-0.31	0.9188	-0.34
M1_Spy_1768	1.00E-04	-0.90	4.00E-04	-1.28	0.0099	-1.02	2.00E-04	-1.25	0.028	-1.08	0.2702	-0.66
M1_Spy_1769												
M1_Spy_1770	0	1.01	0	1.93	5.00E-04	1.57	1.00E-04	1.93	2.00E-04	2.43	0	2.16
M1_Spy_1771	0.042	0.47	0.0013	1.05	0.6629	0.73	0.0108	1.09	0.0675	1.12	0.0117	1.13
M1_Spy_1772	0.9204	0.18	0.1651	0.58	1	0.21	0.7575	0.44	0.9303	0.44	0.9986	0.19
M1_Spy_1776	0.2767	0.82	0.0016	2.07	0.7065	0.45	0.2386	1.25	0.0014	1.96	1	0.20
M1_Spy_1777	1	-0.05	0.5568	-0.29	0.2251	-0.45	1	-0.04	1	-0.12	1	0.11
M1_Spy_1779	1	0.01	1	0.06	0.9933	-0.22	1	-0.05	1	0.12	0.9932	-0.17
M1_Spy_1780	6.00E-04	-0.78	0.003	-0.86	0.0143	-0.95	0.0041	-0.80	0.0017	-1.16	0.1419	-0.94
M1_Spy_1781	1	-0.08	0.0015	1.83	2.00E-04	1.58	1	0.13	0.2458	1.45	0.0127	1.67

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_1782	1	0.08	0.9995	-0.12	0.9971	-0.26	1	0.05	1	-0.25	0.9927	-0.29
M1_Spy_1783												
M1_Spy_1784												
M1_Spy_1785	0.992	-0.14	0.8764	-0.30	0.9039	-0.33	1	0.06	1	-0.07	1	-0.01
M1_Spy_1787	0.0788	-0.57	2.00E-04	-1.36	0.0017	-1.59	0.3174	-0.54	0.7674	-0.78	0.0952	-1.05
M1_Spy_1788	1	-0.02	0.177	-0.79	0.0152	-1.27	1	-0.17	1	-0.09	0.8619	-0.50
M1_Spy_1789	0.852	-0.29	0.0161	-0.99	0.0113	-1.07	0.6176	-0.42	0.0993	-0.83	0.1122	-0.79
M1_Spy_1790	0.997	-0.17	0.0028	-1.09	0.2734	-1.02	0.528	-0.50	0.808	-0.69	0.0451	-1.03
M1_Spy_1791	0.3074	-0.54					0.3289	-0.84	0.0083	-1.84		
M1_Spy_1793	0.365	-0.54										
M1_Spy_1794	0.2073	-0.44					0.4554	-0.77	0.0808	-1.01	0.8498	-0.71
M1_Spy_1795	0.3419	-0.45	0.0278	-1.05	0.1379	-0.97	0.4067	-0.67	0.0076	-1.19	0.1235	-0.94
M1_Spy_1796												
M1_Spy_1798	0.4926	-0.48							0.808	-0.70		
M1_Spy_1801	0.6061	-0.26	0.0042	-0.94	0.0589	-0.77	0.072	-0.52	4.00E-04	-1.29	0.0071	-1.24
M1_Spy_1802	1	0.04	0	1.75	0.0204	1.70	0.3736	0.40	0	1.83	0.0045	1.65
M1_Spy_1804	0.4927	-0.28	0	1.38	0	1.51	1	-0.04	0.0011	1.45	0.0071	1.32
M1_Spy_1805	0.9866	-0.15	0.0319	0.80	0.0066	0.84	0.1836	0.43	0.2196	0.74	0.0654	0.68
M1_Spy_1806	0.4304	-0.35	0.1904	-0.71	0.3747	-0.63	0.5504	-0.44	0.618	-0.52	0.57	-0.55
M1_Spy_1807	0.9949	0.21			0.9887	0.48	0.9926	0.31	0.6161	0.87		
M1_Spy_1810	0.0622	0.48	0.0696	-0.78	0.0316	-1.24	0.5086	0.43	0.7224	-0.67	0.2608	-1.07
M1_Spy_1811	1	-0.01										
M1_Spy_1813	0.9998	-0.16					1	0.07				
M1_Spy_1815	0.999	-0.13	1	0.02	0.9759	0.34	1	0.05	1	-0.11	1	0.04
M1_Spy_1816	0.9457	0.20	0.9964	-0.38	1	-0.36	0.997	0.19	0.994	-0.62	1	0.03

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_1817	0.1361	0.54	0.4273	0.65	0.8601	0.59	0.2414	0.66	0.3264	0.72	0.9634	0.52
M1_Spy_1818	0.9267	0.16	1	0.01	0.9028	-0.29	0.9792	0.15	0.954	-0.50	0.0378	-0.84
M1_Spy_1820	0.5548	0.24	0.8149	0.23	1	-0.01	0.9225	0.22	1	0.01	0.9619	-0.25
M1_Spy_1821	0.9999	0.09	0.9954	0.20	1	-0.05	1	-0.03	0.9796	-0.29	0.0165	-0.69
M1_Spy_1823	1.00E-04	-0.82	0.0094	-1.26	0.0436	-1.18	2.00E-04	-0.93	6.00E-04	-1.21	0.0062	-1.35
M1_Spy_1824	0.0809	-0.43	0.1744	-0.76	0.1328	-0.90	0.1416	-0.56	0.0506	-0.77	0.0087	-1.00
M1_Spy_1825	0.0052	-1.20	0	-1.35	1.00E-04	-1.26	5.00E-04	-1.12	2.00E-04	-1.39	0	-1.77
M1_Spy_1827	0.1786	0.77					0.0524	1.61	0.0727	2.59	0.0011	2.39
M1_Spy_1828											0.0098	3.85
M1_Spy_1829	0.143	0.85	6.00E-04	0.80	0.0338	0.80	0	1.26	2.00E-04	1.41	0	1.60
M1_Spy_1830	1.00E-04	0.97	4.00E-04	1.03	0.0028	0.93	0	1.38	4.00E-04	1.60	0	1.62
M1_Spy_1831	6.00E-04	1.00	4.00E-04	1.03	0.006	0.80	0	1.38	0	1.51	0	1.64
M1_Spy_1832	0.6515	-0.34	0.0538	-0.97	0.0827	-0.88	0.037	-0.80	0.001	-1.37	0.0837	-1.17
M1_Spy_1833	0.1358	-0.78	0.0822	-1.19	0.2897	-1.19	0.8485	-0.61	0.2622	-1.12	0.1491	-0.95
M1_Spy_1834												
M1_Spy_1835	0	-1.52	0	-1.19	5.00E-04	-1.12	8.00E-04	-1.75	2.00E-04	-1.69	0.0022	-1.50
M1_Spy_1836	1	0.08	1	-0.03	1	-0.14	1	0.10	0.6264	-0.50	0.9962	-0.30
M1_Spy_1837	0.0318	0.69	0.2686	0.58	0.3574	0.56	0.1721	0.67	0.5356	0.50	0.4949	0.55
M1_Spy_1839	0.913	-0.24	0.9891	-0.38	1	-0.18	0.2128	-0.56	0.861	-0.45	0.3029	-0.58
M1_Spy_1840	0.0204	-0.69	2.00E-04	-1.18	6.00E-04	-1.27	0.009	-0.97	0.0033	-1.36	0.0021	-1.36
M1_Spy_1841	0.9998	-0.09	0.2951	-0.41	0.9794	-0.34	0.4172	-0.39	0.7435	-0.47	0.4993	-0.54
M1_Spy_1842	0.3829	0.34	0.8677	-0.24	0.9971	-0.19	0.9997	0.18	0.8027	-0.34	0.9991	-0.15
M1_Spy_1844	0.0016	0.79	0.9988	-0.15	1	-0.09	0.1073	0.63	0.9514	-0.37	0.7079	-0.45
M1_Spy_1845	1	0.04	0.599	-0.40	1	0.04	0.4765	-0.32	0.9615	-0.27	0.9998	0.15
M1_Spy_1846	1	-0.04	0.0542	-0.74	0.1332	-0.76	0.7683	-0.41	0.147	-0.86	0.1812	-0.77

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_1849	8.00E-04	-1.56	1	0.10	0.322	1.08	0.9376	0.65	0	2.23	0	2.37
M1_Spy_1850	0.2358	0.53	0.9976	0.30	1	-0.03	0.1052	0.76	1	0.18	1	0.20
M1_Spy_1851	0.9826	0.26	0.9998	-0.10	0.9995	-0.29	1	0.19	1	0.02	0.9979	-0.19
M1_Spy_1852							0.9398	0.46	0.8335	1.64	0.0857	2.33
M1_Spy_1854	0.1576	0.76	0.0015	3.84	0	3.73	0.0065	1.55	0.0022	4.02	0	3.30
M1_Spy_1856												
M1_Spy_1857	0.9994	0.09	0.2857	-0.46	0.6056	-0.52	0.9857	-0.19	0.1631	-0.63	0.2831	-0.64
M1_Spy_1858	0.9976	-0.16	0.5173	-0.74	0.1024	-0.85	1	-0.13	0.375	-0.42	0.0575	-0.75
M1_Spy_1861	0.0943	-0.45	0.9988	-0.16	0.9999	-0.16	0.2229	-0.66	0.8962	-0.35	1	-0.07
M1_Spy_1862	8.00E-04	-0.76	1	-0.03	1	-0.09	0.005	-0.98	0.7003	-0.33	0.5595	-0.37
M1_Spy_1863	0.2943	0.57	0.4819	0.61			0.8231	0.36	1	-0.01	0.9932	-0.33
M1_Spy_1864	0.1095	1.34	0.0308	1.52	0.5166	1.15	0.1788	0.99				
M1_Spy_1865	0.0831	-0.59	0.3143	0.36	0.9169	0.52	0.097	-0.62	1	0.00	0.8753	0.43
M1_Spy_1866	0.4472	-0.27	0.9954	0.13	0.9924	0.24	0.8377	-0.26	1	0.13	0.854	0.35
M1_Spy_1867	0.9967	0.12	0.9995	-0.10	1	-0.04	0.7905	0.24	1	-0.10	1	0.02
M1_Spy_1868	0.2054	0.38	0.2603	0.48	0.251	0.54	0.2078	0.42	0.3556	0.49	0.1314	0.64
M1_Spy_1869	0.0842	-0.56	0.9846	0.25	0.9774	0.49	1	0.02	0.5722	0.51	0.0682	0.87
M1_Spy_1870	0.9882	0.19	0.9999	-0.15	1	0.28	1	-0.08	1	0.13	0.9932	0.33
M1_Spy_1871	0.0073	1.57	0.0072	-1.31	1.00E-04	-3.07	0.003	1.77	1	0.64	0.026	-1.50
M1_Spy_1872	0.0084	-0.76	0.056	0.55	0.5904	0.38	0.5778	-0.34	0.2786	0.50	0.3429	0.41
M1_Spy_1873	1.00E-04	-1.05	1	0.02	1	-0.11	0.003	-0.98	0.948	-0.23	0.8164	-0.27
M1_Spy_1874	0.0012	-0.97	0.3494	0.39	0.9242	0.24	0.0327	-0.71	0.946	0.26	1	0.05
M1_Spy_1875	0.077	-0.41	0.0266	-0.63	0.0052	-0.98	0.0338	-0.79	0.0188	-0.85	0	-1.35
M1_Spy_1876	0.9989	0.13	1	0.10	0.9775	-0.28	1	0.06	0.9661	0.32	0.9849	-0.19
M1_Spy_1877	0.6253	-0.28	1	0.00	1	-0.09	0.8905	-0.24	1	0.00	1	-0.09

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_1878	1	-0.02	0.9453	-0.26	1	-0.17	0.9979	-0.23	1	-0.02	0.5495	-0.54
M1_Spy_1879	0.9921	-0.21	1	-0.05	0.7041	-0.47	1	-0.24	1	-0.03	0.9	-0.39
M1_Spy_1881	0.0074	0.60	0.0112	0.74	0.9809	0.19	0.013	0.72	0.2727	0.64	1	-0.05
M1_Spy_1882	0.9727	-0.17	0.1695	0.49	0.2887	0.48	1	-0.13	0.6783	0.41	0.1223	0.64
M1_Spy_1884	0.9555	-0.22	0.4347	0.56	0.4855	0.42	1	-0.15	0.239	0.60	0.4021	0.42
M1_Spy_1885	1	0.07	0.0732	0.48	0.9917	0.20	0.7385	0.35	0.2003	0.57	0.9441	0.27
M1_Spy_1886	0.1306	-0.43	0.7651	-0.26	0.168	-0.57	0.1794	-0.49	0.3121	-0.46	0.0014	-0.93
M1_Spy_1888	0.2647	0.47	4.00E-04	1.81	5.00E-04	1.62	1.00E-04	1.03	2.00E-04	2.17	0	2.03
M1_Spy_1889	0	1.45	0.005	0.95	0.9867	0.19	1.00E-04	1.33	0.0231	0.81	0.8232	0.36
M1_Spy_1892	0.9411	0.27	4.00E-04	1.38	0.0011	1.51	0.0345	0.68	0.0026	2.10	0.0011	1.92
M1_Spy_1894	0.5227	-0.29	0.0489	1.23	0.1849	1.03	1	0.06	0.5132	0.99	0.5791	0.83
M1_Spy_1895	0.8837	0.19	0.0044	0.93	0.0163	1.00	1	0.09	0.0707	0.70	0.0247	0.79
M1_Spy_1896	8.00E-04	0.75	1.00E-04	1.10	0.0057	1.01	0.0085	0.87	6.00E-04	1.10	0.0025	1.25
M1_Spy_1897	0.9968	-0.12	0.9671	0.28	0.9828	0.32	1	-0.13	1	0.20	1	0.08
M1_Spy_1898	0.6964	-0.34	0.0198	-0.65	0.0566	-0.83	0.5346	-0.36	0.0574	-0.83	0.0299	-0.87
M1_Spy_1899	0.5596	-0.39	0.0092	-0.96	0.015	-0.95	0.1244	-0.57	0.0102	-0.92	0.0055	-1.02
M1_Spy_1900	0.0259	-0.57	0.0132	-0.77	0.0095	-0.83	0.0142	-0.68	0.073	-0.79	0.0043	-0.89
M1_Spy_1901	8.00E-04	-0.77	0.0027	-1.12	2.00E-04	-1.09	1.00E-04	-1.04	0.0023	-1.18	1.00E-04	-1.25
M1_Spy_1902	0.0029	-0.80	5.00E-04	-1.66	1.00E-04	-1.90	0.1123	-1.18	0	-2.14	1.00E-04	-2.05
M1_Spy_1903												
M1_Spy_1904	0.5801	-0.31	0.9985	0.14	1	0.14	1	0.05	1	0.20	0.999	0.17
M1_Spy_1905	0.1076	-0.49	0.1669	-0.64	0.5609	-0.61	0.7692	-0.43	0.0215	-0.87	0.7389	-0.54
M1_Spy_1906	1	-0.04	0.3179	0.46	0.6736	0.50	0.7151	0.39	0.5433	0.59	0.598	0.54
M1_Spy_1908	0.3315	-0.43	0.001	-1.20	0.0028	-1.24	0.0774	-0.64	5.00E-04	-1.48	0.0012	-1.54
M1_Spy_1911	1	0.01	0.4126	-0.42	1	-0.09	0.5999	-0.39	0.7776	-0.47	1	-0.12

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_1912	0.1322	-0.55					0.4294	-0.82	0.0248	-1.70		
M1_Spy_1914	0.0058	-0.79	0.0011	-1.84	2.00E-04	-2.21	0.1224	-1.11	2.00E-04	-2.27	1.00E-04	-2.19
M1_Spy_1915	0.0246	-0.85	4.00E-04	-1.67	0	-1.92	0.1035	-1.24	2.00E-04	-2.11	0	-1.97
M1_Spy_1916	0.0319	-0.88			0.0089	-2.42	0.1586	-1.14	3.00E-04	-2.20	0.0147	-2.66
M1_Spy_1917												
M1_Spy_1918	0.3422	-0.49										
M1_Spy_1919												
M1_Spy_1921	0.2494	-0.42	0.053	-1.02			0.5039	-0.56	0.1344	-1.01	0.0888	-1.09
M1_Spy_1922	0.2838	-0.38					0.9742	-0.43	0.9993	0.37		
M1_Spy_1923	0.2974	-0.86	0.1322	-0.93	0.3665	-0.74	0.0566	-1.26	0.0473	-1.05	0.3419	-0.64
M1_Spy_1924	0.1655	-0.43	0.9837	0.25	1	-0.02	0.3035	-0.53	1	-0.16	0.998	-0.20
M1_Spy_1926	0.0065	-0.87	5.00E-04	-1.05	0.0892	-0.71	0.0024	-1.50	0.0084	-1.30	0.0107	-1.18
M1_Spy_1927	0.1613	-0.56	0.2014	-1.38	0.0313	-1.22	0.6532	-0.53	0.318	-0.83	0.7501	-0.67
M1_Spy_1931	2.00E-04	0.89	0.0076	0.67	0.8791	0.51	0	1.30	0.0034	1.05	0.0177	1.00
M1_Spy_1932	0	1.04	0.0277	0.90	0.1899	0.81	0	1.39	6.00E-04	1.57	0.0035	1.55
M1_Spy_1934	0.1692	-0.48	0.9995	-0.23	1	-0.16	0.2053	-0.69	0.9911	-0.31	0.4997	-0.56
M1_Spy_1935	0.7348	-0.44	1	0.11			0.127	-0.81	1	0.07		
M1_Spy_1936	0.0106	-0.63	0.7256	0.35	0.2742	0.48	0.1768	-0.88	0.9936	-0.28	0.6011	-0.34
M1_Spy_1937	0.824	-0.20	0.0166	-0.66	0.0673	-0.64	0.18	-0.55	4.00E-04	-1.19	5.00E-04	-1.31
M1_Spy_1938	0.1142	-0.37	4.00E-04	-1.09	0.0069	-1.13	0.0369	-0.76	0	-1.77	0	-1.83
M1_Spy_1939	0.5049	-0.45	0.8176	-0.44	0.9995	-0.23	0.4483	-0.57	0.9848	-0.33	1	0.05
M1_Spy_1940	0.0844	0.67	6.00E-04	2.54	0.0028	2.01	6.00E-04	1.90	0.0093	3.26	5.00E-04	2.65
M1_Spy_1941	0.9645	-0.26	1	-0.04	0.9875	-0.29	1	0.03	0.9835	-0.27	1	0.29
M1_Spy_1942	0.996	-0.17	0.9981	0.35	1	0.24	1	0.24	0.9899	0.42	0.3454	0.80
M1_Spy_1944	0.2485	0.45	0.0536	0.92	0.4908	0.77	0.1193	1.18	0.0316	1.28	0.0023	1.26

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_1945	0.1944	1.09	0.0468	1.89	0.0083	1.78	9.00E-04	2.30	0	3.65	0.0083	2.76
M1_Spy_1946	0.0176	0.69	0.0197	1.13	0.0176	1.11	0.0017	1.48	0	2.04	0	1.82
M1_Spy_1947												
M1_Spy_1949												
M1_Spy_1950	0.9254	-0.29							0.0661	-1.34		
M1_Spy_1952	0.9989	-0.17										
M1_Spy_1955	0.0102	1.01	0.0662	0.83	0.0018	1.25	0.2631	0.70	0.214	1.11	1.00E-04	1.48
M1_Spy_1956												
M1_Spy_1957												
M1_Spy_1958	0.0331	0.60	0.0147	1.75			0.1977	0.76	0.0866	1.64	1.00E-04	1.83
M1_Spy_1959	0.0157	-0.59	0.0623	-0.49	0.8111	-0.36	0.0357	-0.65	0.7016	-0.48	0.1465	-0.62
M1_Spy_1960	0.4612	0.48	0.2267	0.80	0.403	0.82	0.7643	0.48	0.5973	0.89	0.9847	0.57
M1_Spy_1961	1	0.03	0.9927	0.20	0.9971	0.21	1	0.05	1	0.11	1	0.16
M1_Spy_1962	0.7744	-0.41	4.00E-04	1.61	0.0091	1.56	0.3433	0.59	0	2.23	0	2.04
M1_Spy_1963	0.9263	-0.21	4.00E-04	1.16	0.0277	1.02	1	0.10	4.00E-04	1.22	0.0051	1.21
M1_Spy_1964	0.8336	-0.26	0.0032	1.29	6.00E-04	1.14	1	0.00	0.0027	1.31	0.0672	1.09
M1_Spy_1965	0.0238	-0.70	0.259	0.41	0.7942	0.30	0.032	-0.63	0.9897	0.21	0.943	0.22
M1_Spy_1968	0.0011	-1.28	0.9869	-0.17	0.9999	-0.13	0.0038	-0.95	0.7062	-0.39	0.9998	-0.18
M1_Spy_1971	0	-1.51	0.9773	-0.25	0.2643	-0.48	0.0056	-1.38	0.3318	-0.59	0.3848	-0.46
M1_Spy_1972												
M1_Spy_1973	0.6027	-0.47	0.4786	-0.83	0.4699	-0.84	0.9996	-0.28	0.0089	-1.23	0.8407	-0.67
M1_Spy_1976	0.4822	0.39	0.2258	0.68	0.0163	0.96	0.1427	0.63	0.0381	1.35	0.0014	1.29
M1_Spy_1978	0.4272	-0.37	0.9827	-0.29	0.9971	-0.24	0.8345	-0.35	0.9946	-0.26	1	-0.09
M1_Spy_1979	0.0265	-0.97	2.00E-04	-1.81	0.121	-1.30	5.00E-04	-1.38	8.00E-04	-1.79	0.765	-0.79
M1_Spy_1980	0.9957	0.16	0.0925	1.02	0.1659	0.97	0.8702	0.27	0.195	1.23	0.5532	1.11

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_1981	0.9038	0.18	0.0016	0.92	0.0497	0.94	0.9842	0.22	0.0126	1.01	0.0701	0.95
M1_Spy_1983												
M1_Spy_1984	0.54	0.49	0.0042	0.99	0.4318	0.49	0.8225	0.36	0.2225	0.59	1	0.11
M1_Spy_1985												
M1_Spy_1986					0.9985	0.54			0.5226	1.36	0.7858	1.69
M1_Spy_1987	0.5669	-0.25	1	0.02	1	0.01	1	-0.05	0.9886	0.26	0.9998	0.15
M1_Spy_1988	0.7619	-0.23	0.9996	-0.13	0.9974	-0.26	0.9999	-0.15	1	0.05	0.998	-0.17
M1_Spy_1989	0.0029	-0.85	0.014	-0.87	0.1279	-0.83	2.00E-04	-0.96	0.0375	-0.88	0.3171	-0.72
M1_Spy_1990	0.7125	-0.21	0	-1.41	0.0119	-1.33	0.1286	-0.57	0.0235	-1.43	1.00E-04	-1.60
M1_Spy_1991	0.6374	-0.27	0	-1.48	0	-1.62	0.5221	-0.35	0	-1.63	0	-1.64
M1_Spy_1992	1	0.06	4.00E-04	-1.29	0.0229	-1.37	1	-0.05	0.1788	-1.17	0.0035	-1.41
M1_Spy_1994												
M1_Spy_1995	1	-0.05							0.993	0.73		
M1_Spy_1998												
M1_Spy_1999	0.9968	0.23	0.075	-1.16			0.7609	-0.40	0.0138	-1.71		
M1_Spy_2000	0.0035	0.94	0.8691	-0.36	0.4441	-0.57	0.0487	0.84	1	0.08	1	0.07
M1_Spy_2001												
M1_Spy_2002	0.8964	0.29					0.5159	0.63				
M1_Spy_2003	0.2846	0.70					0.1062	1.61	0.5926	1.20	0.4264	1.40
M1_Spy_2004												
M1_Spy_2005	0.1491	-0.60	0.5144	-0.53	1	-0.05	0.0972	-0.88	0.3867	-0.80	0.9255	-0.40
M1_Spy_2006							0	3.37			0.8635	0.81
M1_Spy_2007												
M1_Spy_2009												
M1_Spy_2010	0.2454	0.60					0.4	1.31	0.0282	3.07		

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_2013	0.7048	0.45	0.3669	-1.07	0.0353	-1.19	0.9995	0.32	0.3681	-1.09	0.1672	-1.03
M1_Spy_2016	0.0077	-0.58	0.0044	-0.72	0.8681	-0.32	0.0197	-0.67	0.4045	-0.53	0.3615	0.67
M1_Spy_2018	0.9535	-0.20	0.4874	0.54	0.0742	0.94	0.5885	-0.42	0.638	0.54	0.0266	0.69
M1_Spy_2019	0.9639	-0.23	0.9501	0.29	0.9303	0.31	0.9797	-0.20	0.8929	0.37	0.9175	0.52
M1_Spy_2023	0.0679	0.72	0.8298	-0.39	1	-0.19	1	0.26	1	-0.47	0.1618	-0.79
M1_Spy_2025	0.0281	0.73	0.9995	-0.21	1	0.33	0.9942	0.34	1	-0.13	1	-0.18
M1_Spy_2026	0.0019	0.75	0.9738	-0.22	1	-0.01	1	0.11	0.9968	-0.45	0.4212	-0.77
M1_Spy_2027	0.0127	0.68	0.9013	-0.30	1	0.08	0.9909	0.35	1	-0.28	0.5626	-0.63
M1_Spy_2029	8.00E-04	0.98	0.9999	-0.14	1	-0.04	0.9932	0.36	0.9981	-0.41	0.3771	-0.79
M1_Spy_2031	0.0711	0.51	0.339	-0.74	0.2401	-0.60	0.9688	-0.25	0.0061	-1.32	0.0208	-1.39
M1_Spy_2032	0.0931	0.59	0.3212	-0.51	1	-0.16	0.9996	0.21	0.8855	-0.73	0.5553	-0.98
M1_Spy_2033	0.0525	0.40	0.0311	-1.02	0.0038	-1.02	0.9419	-0.36	1.00E-04	-1.76	6.00E-04	-1.94
M1_Spy_2034	0.0207	0.76	0.0042	-0.96	0.384	-1.00	1	-0.16	3.00E-04	-2.13	0.0015	-2.28
M1_Spy_2037	0.9998	-0.12	1	0.06			1	0.10				
M1_Spy_2039											0	6.02
M1_Spy_2040												
M1_Spy_2041	0.5825	-0.61	0.9964	0.21	1	0.01	0.1773	-0.81	1	0.17	0.9873	0.30
M1_Spy_2042	1	-0.07	0.9995	0.13	0.0483	0.64	0.9101	-0.34	0.9582	0.26	0.1605	0.60
M1_Spy_2043	0.9182	-0.37	0	-2.80	0	-3.31	0.1464	-0.67	2.00E-04	-2.11	5.00E-04	-1.25
M1_Spy_2045	0.7163	0.37	0.9997	0.10	0.9793	0.34	1	0.07	1	-0.16	1	0.04
M1_Spy_2047	0.0402	-0.55	0.0084	-1.64	0	-2.01	0.1158	-0.90	5.00E-04	-1.58	0.0034	-2.13
M1_Spy_2048	0.0632	-0.65	0.0043	-1.61	5.00E-04	-2.10	0.0489	-0.79	0.0038	-1.65	0.0691	-2.27
M1_Spy_2049												
M1_Spy_2050												
M1_Spy_2051	0.0919	-0.93	6.00E-04	-1.87	0	-2.15	0.0253	-1.35	0	-2.31	3.00E-04	-2.22

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_2052												
M1_Spy_2053	0.0974	-0.67	5.00E-04	-1.57	0.001	-1.94	0.0522	-0.99	0.0084	-1.40	0.0164	-1.99
M1_Spy_2054	6.00E-04	-1.03	0.0018	-1.36	0.0017	-1.46	0.0028	-1.15	0.7442	-0.77	0.1486	-1.20
M1_Spy_2055	0.1263	-0.51	0.0694	-1.12	0.3823	-1.01	0.3432	-0.70	0.9577	-0.56	0.9486	-0.48
M1_Spy_2058	0.7015	-0.23	2.00E-04	-1.52	0	-1.71	0.0117	-1.06	0	-1.99	0	-2.33
M1_Spy_2059	0.6113	0.42	0.0011	-1.30	0.0903	-1.17	0.9917	-0.24	0.0677	-1.30	5.00E-04	-1.41
M1_Spy_2060	0.9211	0.28	0.001	-1.55	0.0168	-1.27	0.0585	-0.55	3.00E-04	-1.83	0	-1.64
M1_Spy_2063	0.9968	0.10	0	-1.63	0.1579	-1.46	0.8416	-0.33	0.0581	-1.26	0.0183	-1.48
M1_Spy_2065	0.0124	0.80	0.0159	1.15	0.0878	1.14	0.0686	0.89	0.0187	1.56	0.0837	1.45
M1_Spy_2066	0	2.48					0.2749	0.97				
M1_Spy_2070	0.9966	-0.16	0.015	-1.06	0.0077	-1.39	0.9167	-0.22	0.0222	-1.26	0	-1.82
M1_Spy_2072	0.5614	-0.30	6.00E-04	-1.14	0	-1.58	0.6822	-0.42	4.00E-04	-1.62	1.00E-04	-2.41
M1_Spy_2073	0.775	0.23	0.0013	0.91	0.0205	0.78	0.6595	0.26	0.0643	0.80	0.2685	0.49
M1_Spy_2074	0.711	-0.21	0.0012	0.91	0.1829	0.84	0.7042	-0.24	0.0065	0.87	0.0415	0.75
M1_Spy_2077												
M1_Spy_2079	0.9713	0.18	0.1456	0.41	0.0161	0.89	0.9874	-0.29	0.9667	-0.44	1	-0.02
M1_Spy_2080	0.3167	0.33	0.1718	0.52	9.00E-04	1.17	1	-0.03	1	-0.10	0.7873	0.43
M1_Spy_2081	0.9885	-0.29	0.9946	-0.27	0.9971	-0.36	0.9984	-0.26	0.5702	-0.62	1	0.06
M1_Spy_2082	0.9967	0.24							1	-0.37	0.6181	0.80
M1_Spy_2083												
M1_Spy_2084												
M1_Spy_2085	0.7589	-0.30			0.7081	-0.89	0.7058	-0.52	0.0517	-1.12	0.172	-0.86
M1_Spy_2087												
M1_Spy_2088												
M1_Spy_2089												

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_2090	1	-0.22										
M1_Spy_2091	0.6837	-0.35					0.9005	-0.58	0.2446	-1.19		
M1_Spy_2092	0	1.41	4.00E-04	2.18	8.00E-04	2.41	0	2.28	6.00E-04	3.09	0	2.49
M1_Spy_2093	1.00E-04	1.41	0	2.85	0	2.67	0.001	2.82	3.00E-04	3.78	0	3.13
M1_Spy_2095	0	1.63	0.0177	1.92	0.0097	1.54	0	2.15	0.0121	2.22	0.0048	1.69
M1_Spy_2096												
M1_Spy_2097	0.1988	-0.77	0.0103	-1.34	0.0937	-1.43	0.071	-0.96	0.0015	-1.69	0.0859	-1.39
M1_Spy_2099												
M1_Spy_2103												
M1_Spy_2104	0.0423	-0.53	1	0.01	0.997	0.29	0.0312	-0.88	1	-0.12	1	0.00
M1_Spy_2105	0.7233	-0.25	0.7015	0.36	0.9995	0.17	1	-0.10	0.8018	0.43	0.9682	0.31
M1_Spy_2106	0.9904	-0.18	0.5188	0.64	0.9995	0.20	0.9943	0.21	0.1898	0.85	0.5319	0.48
M1_Spy_2107	0.9994	-0.15	0.7052	0.95	0.6331	0.66	0.8479	0.39	0.4553	1.15	0.6817	0.64
M1_Spy_2110	0.0046	-0.81	0.999	-0.26	0.0326	-0.93	0.0081	-0.99	1	0.04	0.5249	-0.63
M1_Spy_2111	0.0505	-0.52	0.0459	-0.77	0.0299	-0.90	0.0066	-0.85	0.0073	-1.05	0.2408	-0.78
M1_Spy_2112	0.891	0.25	1	0.03	1	0.12	0.9991	0.15	1	-0.29	0.4515	-0.47
M1_Spy_2113	0.9735	-0.34	0.0324	-0.78	0.2271	-0.66	0.6401	-0.61	1.00E-04	-1.59	0.0045	-1.29
M1_Spy_2114	0.4383	-0.44	0.5448	-0.37	0.9604	-0.25	0.2367	-0.50	0.5427	-0.79	0.2227	-0.61
M1_Spy_2115	0.4748	-0.35			0.0396	-1.42	0.8908	-0.28	0.434	-1.44	0.3797	-1.75
M1_Spy_2116	0.9989	-0.14	0.8335	0.24	0.9962	0.22	1	-0.05	1	-0.05	0.9968	0.15
M1_Spy_2117	0.4056	-0.35	0.2744	-0.61	0.1242	-0.96	0.4557	-0.49	0.0071	-1.04	0.0231	-1.05
M1_Spy_2118	0.77	0.33	1	0.11	0.9982	-0.21	0.2344	0.64	0.9952	0.34	1	-0.03
M1_Spy_2119	0.7689	0.29	0.999	-0.14	0.9105	-0.41	0.7318	0.39	1	0.13	0.9848	-0.27
M1_Spy_2120												
M1_Spy_2121	0.7014	0.34	0.999	-0.14	0.9977	-0.20	0.9979	0.18	0.95	-0.35	0.902	-0.35

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_2122	0.9941	0.23					1	0.15	0.9975	0.39		
M1_Spy_2125	1	0.06	0.9593	-0.37	1	-0.17	1	-0.05	1	0.00	1	0.04
M1_Spy_2126	0.4012	-0.48	0.0202	-1.71	0.0337	-1.96	0.1393	-0.82	0.2294	-1.36	0.0135	-1.44
M1_Spy_2127												
M1_Spy_2128	0.9895	-0.23										
M1_Spy_2129	0.5671	-0.44					0.4263	-0.54	0.1587	-1.13	0.3545	-0.95
M1_Spy_2130	0.4547	-0.46										
M1_Spy_2131												
M1_Spy_2132												
M1_Spy_2133	0.5603	-0.43	0.2153	-1.19	0.092	-1.30	0.4056	-0.72	0.016	-1.35	0.1744	-1.06
M1_Spy_2134												
M1_Spy_2135	0.2571	-0.51					0.8436	-0.56	0.299	-1.03		
M1_Spy_2136	0.4361	-0.43							0.7751	-1.05		
M1_Spy_2140	0.3661	-0.45	0.2169	-1.12	0.0354	-1.22	0.3175	-0.91	0.0061	-1.32	0.1868	-1.13
M1_Spy_2142												
M1_Spy_2144	0.513	-0.44			0.4143	-1.19	0.8283	-0.66	0.0683	-1.38	0.2513	-1.10
M1_Spy_2145												
M1_Spy_2147	0.36	-0.48					0.9822	-0.66	0.509	-1.05		
M1_Spy_2148												
M1_Spy_2149	0.336	-0.58	0.0165	-1.21	0.0269	-1.16	0.2938	-0.98	0.0191	-1.45	0.0051	-1.36
M1_Spy_2150	0.9638	0.35	0.9995	-0.21	1	0.05	0.9956	0.24	1	0.09	0.8514	0.54
M1_Spy_2151	0.0413	0.87	0.0791	0.56	0.0373	0.74	0.0035	1.22	5.00E-04	1.12	0.0075	0.96
M1_Spy_2152	0.0051	0.74	0.2346	0.49	0.0208	1.08	0.5568	0.62	0.8559	0.46	0.4124	0.62
M1_Spy_2153							0.0055	2.02				
M1_Spy_2154	0.1179	0.48	0.0399	1.49	0.1816	1.25	0.4502	0.77	0.0162	1.97	0.0026	1.91

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_2155	0.0082	-0.68	1	-0.14	0.8778	-0.52	0.9967	-0.47	0.9953	-0.45	1	-0.13
M1_Spy_2156	0.3204	0.44	0.0011	1.41	0.0671	1.01	0.2019	0.82	3.00E-04	1.54	0.0171	1.31
M1_Spy_2157	1	-0.05	1	0.03	1	-0.24	1	0.04	0.9995	0.41	0.9718	0.31
M1_Spy_2159	1.00E-04	1.00	4.00E-04	2.29	1.00E-04	2.32	0.005	1.18	0	3.24	0	3.07
M1_Spy_2160	0.0088	0.63	2.00E-04	1.27	0.0048	1.50	9.00E-04	0.89	0	2.05	0	2.28
M1_Spy_2162	0.4088	-0.45							0.4233	-1.01		
M1_Spy_2163	0.9857	-0.24										
M1_Spy_2164	0.5619	-0.47							0.9808	-0.79		
M1_Spy_2165	0.6298	-0.38							0.7397	-0.73		
M1_Spy_2166												
M1_Spy_2169	0.5806	0.40	0.24	0.82	0.0308	1.27	0.9991	0.25	0.6375	0.65	0.144	0.88
M1_Spy_2170	0.0051	-1.01	0.001	-1.54	0.0116	-1.75	0.0111	-1.31	9.00E-04	-1.80	0.0014	-1.98
M1_Spy_2172	0.009	-0.82	0.0017	-1.33	0.0113	-1.47	0.0439	-0.97	3.00E-04	-1.72	0.0198	-1.59
M1_Spy_2173												
M1_Spy_2174	0.1244	-0.71	0.0012	-1.19	0.0156	-1.38	0.1651	-0.80	0.2902	-0.96	0.0109	-1.22
M1_Spy_2176	0.0139	-0.71	5.00E-04	-1.56	1.00E-04	-1.67	0.1418	-1.09	2.00E-04	-1.95	0.0024	-2.00
M1_Spy_2177												
M1_Spy_2178	0.1767	1.26	4.00E-04	2.46	0	2.68	0.013	1.97	0	2.58	3.00E-04	2.90
M1_Spy_2180	0.9426	0.36	0.9995	0.34			0.9686	0.35	0.9948	0.77	0.9969	0.36
M1_Spy_2181	0.0162	-0.60	0.9269	-0.20	0.9987	-0.17	0.0536	-0.49	0.9993	-0.18	0.9974	-0.19
M1_Spy_2182	0.0414	-0.78	0.6667	-0.27	0.9392	-0.36	0.1832	-0.59	0.9959	-0.20	0.9301	-0.30
M1_Spy_2183	0	-1.16	0.8053	-0.27	0.7761	-0.34	3.00E-04	-1.07	0.9974	-0.28	0.9301	-0.27
M1_Spy_2184	0.002	-1.01	0.1572	-0.44	0.6327	-0.46	0.0016	-0.87	0.6477	-0.46	0.1548	-0.54
M1_Spy_2185	0.461	0.42					0.0025	1.73	0.0337	3.23	5.00E-04	2.94
M1_Spy_2186	0.6085	0.38					0.0095	1.38	3.00E-04	2.80	0.0123	2.80

Table A.7 SF370 time course microarray results (continued)

	Pharyngeal Supernatants						Pharyngeal Monolayers					
	0.5h		1.5h		2.5h		0.5h		1.5h		2.5h	
M1_Spy_2188	1	0.06	0.5788	0.58	0.9803	0.46	0.686	0.43	0.5278	1.02	0.9783	0.30
M1_Spy_2189	1	-0.05	1	0.04	1	-0.10	1	0.00	0.9251	0.72	0.9996	0.23
M1_Spy_2190	1	0.00	0.9995	-0.14	0.9975	-0.20	1	-0.06	1	0.09	1	-0.10
M1_Spy_2191	0.0468	0.61	0.0011	1.25	6.00E-04	1.44	0.5184	0.72	0.0123	1.36	0.0243	1.45
M1_Spy_2193	0.1715	0.46	0.7176	0.60	0.9599	0.34	0.6961	0.58	0.9562	0.37	0.9828	0.43
M1_Spy_2194	0.0537	0.65	0.0049	1.02	0.0502	0.89	0.1959	0.74	0.4325	0.71	0.4937	0.72
M1_Spy_2195	0.9916	0.12	0.9637	0.27	1	-0.02	1	0.18	1	-0.01	1	-0.08
M1_Spy_2196	0.2389	0.44	0.0242	0.74	0.7518	0.46	0.619	0.50	0.1245	0.82	0.6891	0.34
M1_Spy_2197	0.3054	0.46	0.0016	0.91	0.0455	0.98	0.2376	0.44	0.005	1.03	0.0136	0.81
M1_Spy_2198	0	1.58	0.0026	0.98	0.0197	1.32	0.013	1.33	0.2096	1.03	0.3166	0.86
M1_Spy_2199	0.0029	1.63	0.0252	1.68	0.1885	1.70	0.1129	1.33	0.3488	1.25	0.1799	1.08
M1_Spy_2200												
M1_Spy_2201												
M1_Spy_2202	0.9526	-0.38	0.5496	-0.75			0.7052	-0.56	0.1664	-0.89	0.9662	-0.59
M1_Spy_2203	1	0.08	0.9173	-0.61	0.9646	-0.37	1	0.06	1	-0.03	1	0.09
M1_Spy_2204	0.1991	0.40	0.9918	-0.27	0.9956	-0.21	1	0.00	0.6712	-0.46	0.6789	-0.44
M1_Spy_2205	0.0017	-0.83	0.0263	0.84	0.0028	1.05	0.1939	-0.53	0.9682	0.51	0.9741	0.28
M1_Spy_2206	1	0.07	5.00E-04	2.24	0	2.40	0.2641	0.78	2.00E-04	2.31	0	2.40
M1_Spy_2207	1	0.03	0.005	1.60	0.0253	1.95	0.0912	0.55	0	1.98	1.00E-04	2.13
M1_Spy_2209	0.0029	0.89	4.00E-04	2.75	0.0052	2.03	0.0186	1.15	0	2.94	0.0837	2.29
M1_Spy_2210	0	1.19	4.00E-04	1.83	1.00E-04	1.77	3.00E-04	1.53	0	2.13	6.00E-04	1.86
M1_Spy_2211	0.9364	0.43	2.00E-04	2.36	0.0011	2.07	0.0822	1.26	2.00E-04	2.78	0.1603	1.99
M1_Spy_2215	0.9648	-0.29	0.4508	-0.55	1	-0.03	0.0414	-0.75	0.6797	-0.60	1	-0.15
M1_Spy_2216	0.993	-0.13	0.8437	-0.24	1	0.02	0.4374	-0.37	0.5679	-0.48	0.9893	-0.20
M1_Spy_2217	1	-0.01	0.9545	0.23	0.2362	0.53	0.9896	-0.17	0.9769	0.22	0.7479	0.29

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