Pathoadaptation of a Human Pathogen Through Non-Coding Intergenic Mutations

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Pathoadaptation of a Human Pathogen Through Non-Coding Intergenic Mutations

PhD Thesis

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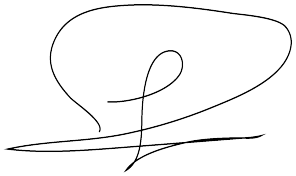
Technical University of Denmark

March 2017

Preface

This thesis is written as a partial fulfillment of the requirements to obtain a PhD degree at the Technical University of Denmark (DTU). The work presented in this thesis was carried out from October 2013 to March 2017 at the Infection Microbiology Group (IMG), Department of Biotechnology and Biomedicine at DTU under the supervision of Professor MSO Lars Jelsbak.



Seyed Mohammad Hossein Khademi

Mashhad, Iran, March 2017

Abstract

Most knowledge gained from evolutionary studies of bacteria in natural and experimental settings center around contribution of intragenic mutations on bacterial evolution. While cases of adaptive intergenic mutations have sometimes been reported or explored, none of these studies consider intergenic mutations in broader context as key players in evolutionary adaptation of bacteria.

The focus of this thesis has been to provide novel insights on contributions of non-coding intergenic mutations in natural evolution of bacteria. The model system used for these investigations is adaptation of opportunistic pathogen *Pseudomonas aeruginosa* in long-term chronic airway infections of Cystic fibrosis (CF) patients. Using sequenced genomes of *P. aeruginosa* isolated from this setting, 88 intergenic regions under positive selection for adaptive mutations within and across isolates of different *P. aeruginosa* lineages were identified. Mutations within core promoter are more frequently found than other elements in these intergenic regions and intergenic mutations made a larger numerical contribution to selection of adaptive genes than intragenic. Several genes present within these regions had established roles in CF adaptation of *P. aeruginosa* and their expressions are altered by the mutation. It was established that mutations upstream *ampR* increased tolerance of *P. aeruginosa* to some β-lactam antibiotics.

Mutations in promoter of *phuR*, encoding receptor of *pseudomonas* heme uptake system, conferred growth advantage in the presence of hemoglobin demonstrating that *P. aeruginosa* has adapted towards utilization of iron from hemoglobin. Further investigation of *phuR* promoter mutation revealed pleiotropic effects on expression of many other genes. The pleiotropic effect by this mutation was contingent on epistatic effects of other mutations in CF adapted genotype of *P. aeruginosa*. It was also established that this mutation leads increased inhibition of *S. aureus* and decreased fitness of *P. aeruginosa* during anoxic growth.

The findings presented in this thesis provide a new dimension for bacterial evolution through intergenic mutations. The knowledge gained here can be applied to future treatment of patients suffering from chronic bacterial infection. Moreover, direct evolution or genetic manipulation of intergenic region offer ample opportunities for better outcomes in biotechnological applications of bacteria.

Resumé

Den meste viden fra evolutionære studier i bakterier i natur- og forsøgsomgivelser er centreret omkring bidraget af intragenetiske mutationer på bakterieevolution. Mens tilfælde af adaptive intergenetiske mutationer nogle gange bliver rapporteret eller undersøgt, så er der ingen af disse studier der betragter intergenetiske mutationer i en bredere kontekst som centrale aktører i den evolutionær tilpasning af bakterier.

Denne afhandlings fokus har været at give nye indsigter i ikke-kodende intergenetiske mutationers bidrag på bakteriers naturlige evolution. Det modelsystem der er blevet brugt i disse undersøgelser har været den opportunistiske bakterie *Pseudomonas aeruginosa* i langvarige kroniske luftvejsinfektioner i cystisk fibrose (CF) patienter. Ved at bruge sekvenserede genomer af *P. aeruginosa* isoleret fra disse omgivelser, identificerede vi 88 intergenetiske regioner under positiv selektion for adaptive mutationer inden for og på tværs af forskellige isolater. Mutationer inde i indre promotorregioner findes hyppigere end andre elementer i disse intergenetiske regioner og intergenetiske mutationer bidrog i større antal med selektering af adaptive gener end intragenetiske mutationer. Flere gener i disse regioner havde etablerede roller i CF tilpasning af *P. aeruginosa* og havde deres ekspression ændret af mutationen. Det blev fastslået at opstrømsmutationer af *ampR* forøgede tolerancen af *P. aeruginosa* mod nogle β -lactam antibiotika.

Mutationer i promotoren for *phuR*, kodningsreceptor for *pseudomonas* hæmaoptagelsessystem, gav vækstfordel ved tilstedeværelsen af hæmoglobin, hvilket viser at *P. aeruginosa* har tilpasset sig til at udnytte jern fra hæmoglobin. Yderligere undersøgelser af *phuR* promotor mutationer afslørede pleiotropiske effekter på mange andre genes ekspression. Den pleiotropiske effekt fra denne mutation var betinget af epistatiske effekter fra andre mutationer i CF tilpassede genotyper af *P. aeruginosa*. Det blev også vist at denne mutation ledte til forøget inhibering af *S. aureus* og nedsatte *P. aeruginosa*'s fitness under anoksisk vækst.

Resultaterne i denne afhandling giver en ny vinkel på bakterieevolution gennem intergenetiske mutationer. Den viden der er opnået kan blive anvendt til fremtidig behandling af patienter der lider af kroniske bakterieinfektioner. Derudover giver direkte evolution eller genetisk manipulation af intergenetiske regioner rigeligt med muligheder for et bedre udbytte i bioteknologiske anvendelser af bakterier.

Acknowledgements

During the past 3.5 years of my PhD, I have enjoyed acquaintance and company of many individuals that made outstanding contributions to accomplishment of this work. First and foremost, I would like to acknowledge Lars Jelsbak for trusting in my abilities and daring me to become better at what I do. He taught me how to become resilient during hard and hopeless times. His unending support and inspirational guidance was very valuable during my time as a PhD student. So, thank you Lars!

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I should give a special acknowledgement to administrative and technical staff at building 301 including Susanne (Søs) Koefoed, Lisse St. Clair-Norton, Brian, Anna Joensen and Lone Hansen for their remarkable support during my times as a PhD student. I also like to thank all students I have supervised during my PhD.

I have to acknowledge Søren Molin and Helle K. Johanson for their great inputs and feedback to improve my thesis. I specially like to appreciate Lea M. Sommer and Sandra B. Andersen for their helpful discussions and support throughout my PhD.

Some of the works included in this thesis were made possible through strong collaborations with other research groups. I like to acknowledge Oana Ciofu, Tina Wassermann, Lasse A. Kvich and Thomas Bjarnsholt for contributions to some of the works presented in this thesis. I also like to thank Morten Sommer, Lone Gram and Mogen Kilstrup labs for being excellent neighbors and providing equipment supply during my time as a PhD student.

Lastly, I should thank my family and friends (including Kosai Al-Nakeeb and Ali Mohebbi) for being understanding during difficult times of my PhD. Thanks to my beloved wife Zahra for being by my side all the time and my little son Hadi who despite his occasional nuisance showed me the sweetness of life.

Contents

List of publications

Research articles included in this thesis

Marvig RL*, Damkiær S*, **Khademi SMH***, Markussen TM, Molin S, Jelsbak L. (2014) Within-Host Evolution of *Pseudomonas aeruginosa* Reveals Adaptation Towards Iron Acquisition from Hemoglobin. *mBio* 5(3):e00966-14. doi:10.1128/mBio.00966-14.

Khademi SMH, Jelsbak L. (2017) Contribution of non-coding intergenic mutations on within-host evolution of a human pathogen. *Manuscript submitted to Nature Microbiology*.

Khademi SMH, Wassermann T, Kvich LA, Bjarnsholt T, Ciofu O, Jelsbak L. (2017) Adaptive mutation in a bacterial intergenic region cause pleiotropic effects on gene expressions. *Manuscript in preparation*.

Published works that are not part of this thesis

Michelsen CF, **Khademi SMH**, Johansen H, Ingmer H, Dorrestein P, Jelsbak L. (2015) Evolution of metabolic divergence in *Pseudomonas aeruginosa* facilitates a mutualistic interspecies interaction. *ISME J*. doi:10.1038/ismej.2015.220.

Wassermann T, Jørgensen KM, Ivanyshyn K, Bjarnsholt T, **Khademi SMH**, Jelsbak L, Høiby N, Ciofu O. (2016) The phenotypic evolution of *P. aeruginosa* populations changes in the presence of sub-inhibitory concentrations of ciprofloxacin. *Microbiology*. doi: 10.1099/mic.0.000273.

* Denotes equal contribution

Abbreviations

HIV	Human immunodeficiency virus
WGS	Whole genome sequencing
RNA-seq	RNA sequencing
CF	Cystic fibrosis
sRNA	small RNA
UPEC	Uropathogenic *E. coli*
UTI	Urinary tract infection
HGT	Horizontal gene transfer
SNP	Single nucleotide polymorphism
NS	Non-synonymous
CRE	*cis*-regulatory
TAF	*trans*-acting factors
TRE	*trans*-regulatory element
RNAP	RNA polymerase
ncRNA	Non-coding RNA
NTP	Nucleoside triphosphate
mRNA	messenger RNA
NGS	Next generation sequencing
PMN	Polymorphonuclear neutrophils
LPS	Lipopolysaccharide
ROS	Reactive oxygen species
TTSS	Type III secretion system
Phu	*Pseudomonas* heme utilization
WT	Wild type
LB	Luria-bertani
MM	Minimal medium
MIC	Minimum inhibitory concentration
ChIP-seq	Chromatin immunoprecipitation sequencing
EMSA	Electrophoretic mobility shift assay

List of figures

Chapter 1

"Nothing is as it seems, but something is everything it is made out to be."

- Carroll Bryant

Introduction

Understanding how organisms evolve is not only essential to comprehend development of life on earth but to tackle modern day challenges of antibiotic resistance, hereditary diseases in human, and emergence of rapid evolving viruses like HIV. The on-going process of evolution has honed the ability of organisms to adapt to new environments. With modern day technologies like WGS, RNA-seq and metagenomics, we can unravel detailed changes that we were unable to detect before. The more we discover the more we realize that a molecular and mechanistic knowledge of evolution is vital for solutions to modern day challenges.

Bacterial species have incredible capacity to evolve and genetically adapt to different environments. This unique feature not only offers ample opportunities to use bacteria in industrial applications but also makes them potentially aggressive infectious agents. Experimental and natural studies of bacterial evolution have endowed a wealth of knowledge to their evolutionary dynamics and genetic basis of adaptation. While the greater emphasis of these studies are on genetic changes in transcriptional regulators or genes, the role of mutations in non-coding intergenic regions is surprisingly neglected.

The focus of this thesis is to uncover the function of non-coding intergenic mutations on natural evolution of bacteria. The model we selected for this investigation is within-host evolution of *Pseudomonas aeruginosa* in long-term chronic airway infection of Cystic Fibrosis (CF) patients.

1.1 Thesis outline

This thesis is organized into eight chapters. While this chapter introduces the thesis, **chapter 2** briefly describes phenotypic acclimation and genetic adaptation, two main routes by which adaptation to novel environments are facilitated. This chapter sets

the stage for the proceeding two chapters where detailed mechanisms of phenotypic acclimation and natural evolution of bacteria are discussed. **Chapter 3** outlines detailed mechanisms of prokaryotic gene regulation where phenotypic acclimation can play a role. The chapter mainly elucidates gene regulation at the transcriptional and post-transcriptional level with a description of sigma factors, promoter recognition, transcription factor regulation, termination of transcription and post-transcriptional regulation by sRNA. The main aim of this chapter is to describe involvement of non-coding *cis*-regulatory intergenic elements in prokaryotic gene regulation. **Chapter 4** introduces *P. aeruginosa* within-host evolution of CF host, a well characterized natural model of genetic adaptation of bacteria. It begins by describing the CF environment and infection, continues with description of *P. aeruginosa* and concludes with genetic adaptation of *P. aeruginosa* in CF host where I describe how routine sampling of this bacterium from CF patients provide opportunities to study molecular mechanisms of evolution and genetic adaptation in natural systems. **Chapter 5** describes cases of evolutionary changes that integrate genetic adaptation and phenotypic acclimation. It mainly highlights examples of genetic changes in transcriptional regulators leading to gene expression changes and phenotypic acclimation in bacteria. **Chapter 6** presents investigations conducted as part of the PhD project. It provides background information and objectives of the studies and summaries of each of three individual research papers. The full length of published articles or prepared manuscripts are provided in **Chapter 8**. Finally **chapter 7** discusses conclusions and futures perspectives of this PhD thesis.

Chapter 2

Bacterial adaptation to new environments

Based on fossil records isolated from submarine-hydrothermal environments, bacteria began evolving on earth from at least 3.7 billion years ago[1]. From extreme conditions of seabed to gut of mammalian species, from depths inside earth crust to ice glaciers of snow in South Pole, bacteria have displayed remarkable survival instincts in hostile and lethal conditions[2,3]. What distinguishes these intriguing micro-organisms from other life-forms is their extraordinary ability to evolve and adapt to new environments. Smaller genome size and faster reproduction pace allow bacteria to adapt at far greater speeds than many other organisms. Bacterial adaptation to new environments is facilitated through two different mechanisms: (*i*) phenotypic acclimation and (ii) genetic adaptation. While the former involves phenotypic changes through altered regulation of genes, the latter is rise of adaptive phenotypes through inheritable genetic changes[4]. The following section (2.1) briefly describes phenotypic acclimation by demonstrating two examples of metabolism and morphological acclimation. The second section (2.2) describes the basic principles of genetic adaptation in bacteria.

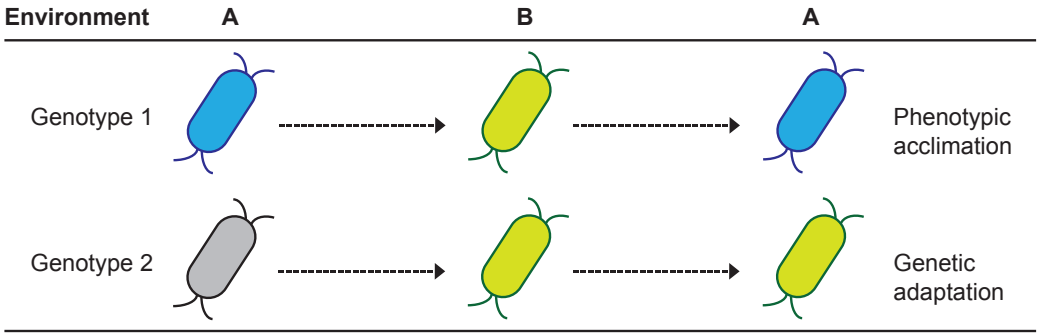

Figure 1 | Differences of phenotypic acclimation and genetic adaptation. Two distinct genotypes of A and B are grown in environment A and exhibit different phenotypes (blue and grey). Both genotypes are then grown in the new environment B with changed properties than A and they now exhibit similar adaptive phenotype in environment B (green). To find out if the presence of new phenotype was from phenotypic acclimation or genetic adaptation, both genotypes are transferred back to ancestral environment A. Genotype 1 reverts to its ancestor phenotype (blue) whereas genotype B exhibits the same phenotype it presented in environment B. Therefore the reversible phenotype genotype 1 exhibited in environment B was due to phenotypic acclimation, while genotype 2 exhibited permanent inherited phenotype due to genetic adaptation. Figure adapted from Rainey 2004[4].

2.1 Phenotypic acclimation

Bacterial species respond to environmental cues by altering their behavior, morphology or metabolism related phenotypes. These reversible responses are not due to any inheritable genetic changes but essentially controlled by built-in complex regulatory networks where signal transduction and the consequent effects on gene expression plays a central role in formation of new phenotypes[4,5].

Historically, the *lac* operon in *Escherichia coli* was the first characterized bacterial regulatory system and it is a typical case of metabolism involved phenotypic acclimation. The discovery of this regulatory system was instrumental for progress of gene regulation theory in bacteria. While glucose is the preferred carbon source in many bacteria, it is absent in some conditions where the *lac* operon product effectively utilizes available lactose. In such conditions, the *lac* operon initiates transcription of genes necessary for breakdown of present lactose as an alternative carbon source. The operon is strongly repressed by the constitutively expressed LacI protein when lactose is absent. This prevents unnecessary fitness costs associated with expression of β-galactosidase enzyme[6].

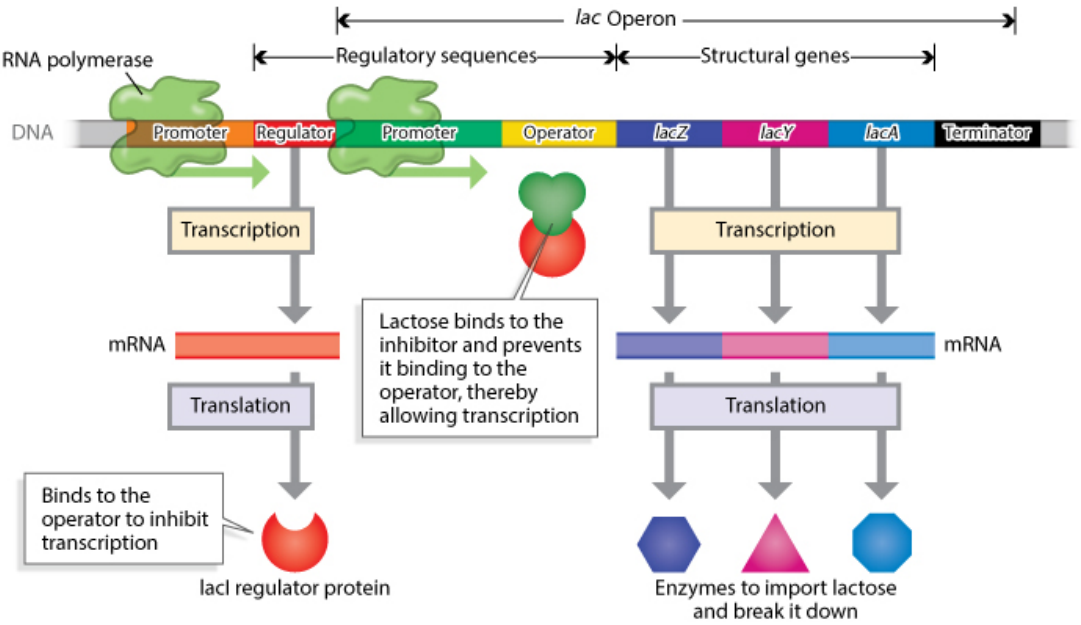

Figure 2 | Overview of the lactose operon in *E. coli*. The *lac* operon contains three genes *lacZ*, *lacY* and *lacA*. *lacZ* expresses β-galactosidase enzyme that cleaves lactose into glucose and galactose. *lacY* expresses β-galactosidase permease that facilitates import of lactose into the cell through cytoplasmic membrane. *lacA* encodes β-galactosidase transacetylase. In the absence of lactose, the *lac* operon is heavily repressed by the constitutively expressed LacI that blocks the binding of RNAP to the promoter of the operon. Repression of the promoter is only lifted when lactose binds to LacI and cAMP-bound CAP protein aids the binding of RNAP to the promoter. Figure adapted from Ralston 2008[6].

One clear example of morphological phenotypic acclimation is filament development of bacteria in stressful conditions such as presence of host effectors, eukaryotic protist predators and antibiotics[7–10]. Filementation occurs when cell growth continues while divisions is arrested and the lengths of filaments are between 10-50 times longer than bacillary cells. Interestingly, size is among the most controlled properties of bacteria and its variation is seldom observed in bacteria grown under similar conditions[11]. In one example, UPEC bacteria in UTI respond to host immune effectors within bladder epithelial cells by forming filaments. Upon epithelial cell death and exposure of filamentous and bacillary UPEC to the surface, neutrophil phagocytosis kills bacillary cells but filamentous UPEC survive the innate immune system[12]. In another example, marine bacterial *Flectobacillus spp*. evade invasion threat of protists by filamentation and this provides a competitive advantage compared to other marine bacteria lacking filamentation tactic and being consumed by protists[7]. Filamentous bacteria are also commonly isolated in samples taken from patients undergoing antibiotic therapy. In one study, exposure to β-lactams induced SOS response in *E. coli* leading to filamentous phenotype through arrest of cell-wall synthesis and cell division[9]. Presence of filamentous phenotype provides another reversible acclimation tactic whereby morphological plasticity offers survival advantage in the presence of environmental stress.

2.2 Genetic adaptation

As discussed in previous section, bacteria have complex built-in regulatory system to adjust against environmental changes through phenotypic acclimation. But while it provides far-reaching effects in response to subtle temporary fluctuations, phenotypic acclimation can function within certain limits and it is unable to provide the peak of phenotypic states essential for effective long-term adaptation in new environments with permanent changes[13]. Inheritable genetic changes through natural selection offer permanent beneficial phenotypes that are necessary for survival in response to permanent condition of new environments. Adaptive mutations chosen by natural selection improve the fitness and reproductive success

of bacteria in new environments. The process of genetic adaptation is especially fruitful in bacteria because of their shorter generation times.

Generally, genetic changes arise from two different mechanisms: (i) horizontal gene transfer (HGT) and (ii) *de novo* mutation in present coding or non-coding intergenic regions. *De novo* mutation can include single nucleotide polymorphisms (SNP), indels (insertion or deletion) and rearrangements like duplication, inversion or translocation[14,15]. These type of mutations occur at stochastically low rates. Based on experimental evolution studies, the incidence rate of mutations in *E. coli* and other related bacteria is around 10^{-10} per base pair per generation[16]. Furthermore, many mutations are neutral in terms of fitness or even detrimental to the reproductive success of an organism in its environment. These mutations can also become fixed in a population through genetic drift or hitchhiking.

Genetic drift occurs when neutral mutations drift to high frequencies in the population. This can either happen randomly or due to bottlenecks where population size is significantly reduced and odds of survival of any individual within the population is purely random and independent of any specific inherent genetic advantage[14]. Hitchhiking is propagation of neutral or detrimental mutations through genetic links to beneficial mutations in another locus. This phenomenon is particularly dominant in asexual populations where the whole genome acts as a single linkage group[14,17].

In addition to hitchhiking and genetic drift, the real phenotypic effect of some mutations may be contingent upon their interactions with other mutations in a process known as epistasis. To dissect the real effect of these mutations they will have to be constructed in ancestor backgrounds and the fitness effect of the resulting strain is measured against its isogenic parent. If the mutation by itself confers no effect on fitness of the strain, it could also be classified as non-adaptive[18]. It can therefore be difficult to tease apart carrier adaptive mutations from passenger non-adaptive mutations. To begin this process, researchers measure the ratio between number of non-synonymous (NS) and synonymous mutations fixed in the population. In this simple approach, NS mutations changing protein function are inferred as those with radical consequences and therefore more likely to have fitness

effects. Therefore a larger ratio depicts signs of adaptive evolution through natural selection whereas a smaller ratio indicates neutral evolution[19,20].

Ultimately, neutral mutations are always present in a population but they seldom become dominant because they lack reproductive advantage. In contrast, beneficial mutations with increased reproductive potential of an individual become more frequent by substitution of neutral alleles and finally get fixed in the population. In this process known as selective sweep, variants with most advantageous mutation or combination of mutations overtake all less fit variants and become the dominant genotype by sweeping all genetic variations in the population[21,22].

Permanent changes in gene expression are common products of adaptation to new environments. These changes are usually established through *cis*- and *trans*-regulatory element mutations. Mutations in non-coding *cis*-regulatory elements (CRE) target binding sites of transacting factor (TAF) and they often induce major adaptive phenotypes in higher eukaryotes[23–25] and bacteria[26–29]. On the other hand, NS mutations in *trans*-regulatory elements (TRE) can alter their function by rewiring their binding to promoters and changing their affinity for the core RNA polymerase[30]. Given their more conservative nature, CRE mutations are suggested to occur more frequently than TRE mutations as they do not pose deleterious effects by altering protein structure and function[23,31,32]. In contrast, TRE mutations putatively provide more radical phenotype advances necessary for fast adaptation in new environments[33]. In agreement with this theory, adaptive mutations in global regulators of gene expression are commonly found in artificial and natural evolution studies of bacteria[31,34,35].

Interestingly, the studies conducted as part of this PhD project demonstrate that non-coding intergenic mutations targeting potential *cis*-regulatory elements make a significant contribution to adaptation of bacteria in complex natural environments. It is of utmost importance to consider these types of adaptive mutations with intragenic mutations to grasp the full evolutionary pathway of bacterial populations.

Chapter 3

Prokaryotic gene regulation

As mentioned in previous chapter, phenotypic acclimation is defined by regulation of gene expression in response to environmental changes. Evolution has shaped a complex and organized regulatory system in bacteria that can perceive signals and translate them into controlled changes in gene expression. All steps of this highly organized process from transcription initiation to RNA processing and translation can be fine-tuned by regulatory elements such as sigma factors, transcription factors, small non-coding RNA, etc. In the following sections, I will briefly describe regulatory mechanisms of gene expression at the transcriptional and post-transcriptional levels.

3.1 Transcription

The process of transcription in bacteria is contingent upon promoter recognition and transcription initiation by RNA polymerase (RNAP). However, RNAP core enzyme composing of $\beta\beta'\alpha_2\omega$ subunits is only competent for DNA-dependent RNA synthesis and unable to initiate transcription without the sigma factors. The formed complex of sigma factor and the core enzyme known as RNA polymerase holoenzyme can facilitate transcription from specific promoters[36]. The sigma subunit facilitates specific recognition of promoters, positions the core RNAP at the promoter and triggers unwinding of DNA duplex near transcription start site[37,38]. Sigma factors are categorized by two different phylogenetic families: σ^{70} and σ^{54}. While most bacteria have more than one sigma factor of the σ^{70} family, they usually contain one from σ^{54} [38–41]. The primary sigma factor in *E. coli* and many other bacteria responsible for transcription of most genes under normal conditions is σ^{70} (RpoD). This sigma factor is commonly referred to as the housekeeping sigma factor. Alternative sigma factors modulating expression of specific genes in response to stress conditions are FecI, σ^{E} (RpoE), σ^{S} (RpoS), σ^{32} (RpoH), σ^{F} (FliA), and σ^{N} (RpoN)[42]. The expression profile and phenotypic picture of bacteria is determined by competition of a pool of different sigma factors for limited number of RNAPs in the cell[39,40]. Different regulatory

mechanisms are triggered by specific physiological factors to facilitate association of alternative sigma factors for the core RNAP.

These regulatory mechanisms include concentration of different sigma factors, anti-sigma factors, small molecule secondary messenger such as ppGpp, small non-coding RNA (ncRNA) and sigma factor affinity for different promoter sites[39,40,43,44].

The process starts by RNAP holoenzyme interacting with the promoter at a specific location and unwinding the DNA duplex at the transcription start site. Positions +1 and +2 within uncoiled template strand enter the active site of RNAP holoenzyme to form the open complex. The subsequent transcription cycles continues with escape of the associated sigma factor, elongation and termination of transcription[45–48].

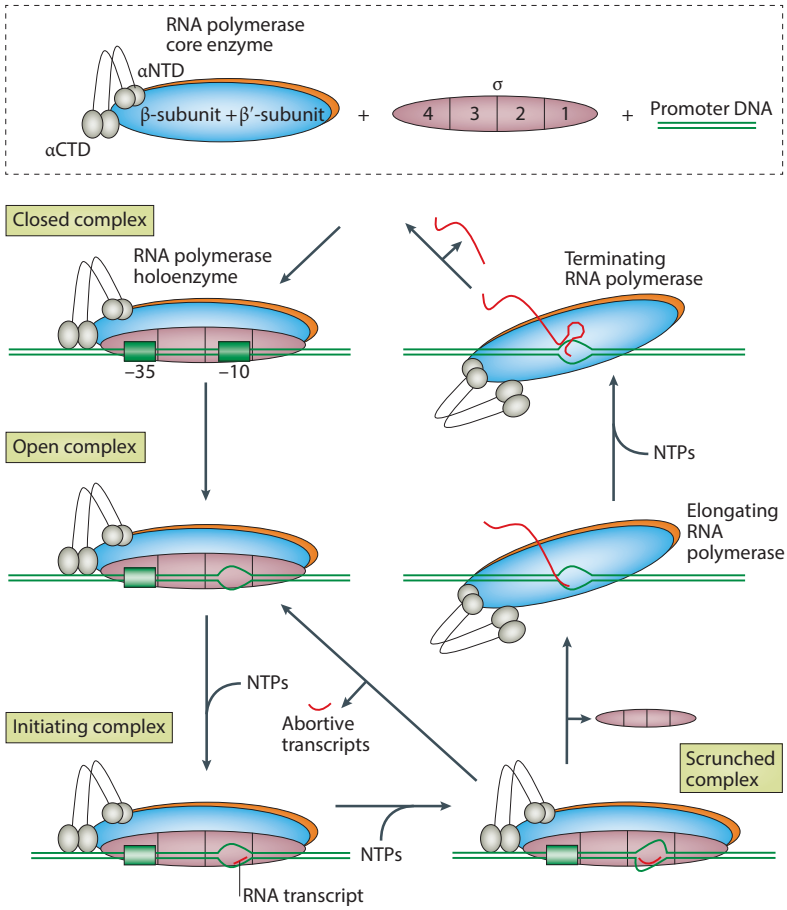

Figure 3 | Overview of transcription cycle in bacteria. RNAP holoenzyme interacts with specific promoter to form the closed complex. Unwinding of DNA duplex in the transcription start site leads to formation of open complex. Transition to the initiating complex is driven by addition of nucleoside triphosphates (NTPs). The template strand is pulled into the initiating complex to abort (scrunch) transcription. The cycle leading to scrunched complex can alternatively be directed to elongation of the RNA transcript by escape of the sigma factor and addition of NTPs. Transcription is stopped when RNAP meets a transcriptional terminator and the polymerase is released to bind another sigma factor. Figure modified from Browning and Busby 2016[49].

Two different methods are currently proposed for termination of transcription in bacteria: Rho-dependent and Rho-independent termination. Rho-dependent termination is destabilization of template and messenger RNA (mRNA) interaction by Rho protein releasing the newly formed mRNA from the elongation complex. Rho-independent termination is when RNA transcription is paused because mRNA forms a G-C- hairpin loop followed by several U's. Upon the formation of this structure, the mechanical stress breaks the mRNA bond with the template and releases the poly-U transcript region out of the elongation complex[50].

3.2 Regulation by transcription factors

In addition to sigma factors, transcription factors (TF) also regulate gene expression by targeting promoters. The expressions of these proteins are regulated by environmental cues and they coordinate environmental signals with specific promoter activities. TFs are generally composed of two units of sensor and regulator domains. The sensor domain receives signals through binding of small ligands or proteins or covalent modification and enables regulator domain to bind specific target sites in the DNA[38]. Two-component systems are another type of TF, where a kinase protein located on inner cell membrane responds to extracellular signal by phosphorylating itself and a cognate response regulator protein. Thereafter, the phosphorylated response regulator binds specific target in DNA[51]. Most TFs regulate more than promoter and most promoters in *E. coli* are regulated by more than one TF. Furthermore, expressions of many genes encoding TFs are regulated by other TFs providing a diverse transcriptional regulatory network capable of robust acclimation to different environments[52–55].

Interaction of TFs with promoter can be mediated through operators containing direct or invert repeats of specific sequence of 4-5 base pairs. Generally, homo-or-multi dimerized structures of TFs containing specific motifs bind to target promoter operators and either repress or activate transcription of specific genes[49]. The repressive or activating function is dependent on where TF binds with regards to transcription start site of the target gene. Additionally, some TFs have dual repressor and activator functions depending on target promoter. While activators increase

transcription by a promoter through improving its association with RNAP, repressors prevent transcription by steric hindrance of RNAP binding or by cooperation with other repressors to decrease promoter affinity for RNAP[30,38].

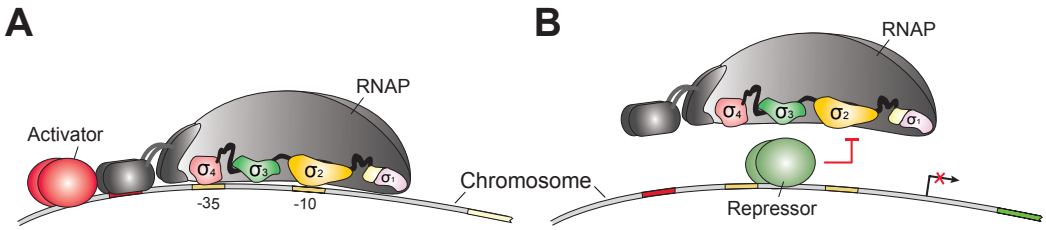

Figure 4 | Activator or repressor function of transcriptional factors. A) Dimerized TF containing special motif structures binds to operator in upstream of the promoter and interacts with αCTD of the RNA polymerase to facilitate its binding to promoter region. **B)** Dimerized repressor containing special motifs blocks binding of the RNAP within the core promoter through steric hindrance. Figure modified from Browning and Busby 2004[38].

3.3 Regulation by small non-coding RNA

Small non-coding RNA (sRNA), ranging between 70-500 bp, are a group of highly structured RNAs containing several stem loops that regulate gene expression in bacteria. Through interaction with mRNA, they either control mRNA stability; affect transcription termination or translation initiation. *cis*-encoded sRNA are positioned in overlap with their target genes whereas *trans*-encoded sRNA are separated by a distance from their target gene. The inherent ability of sRNA to modulate gene expression in response to environmental cues allows them to participate in a diverse set of adaptation processes such as coordination of virulence, carbon metabolism, cell envelope hemostasis, transcriptional reprogramming and iron homostasis[56,57].

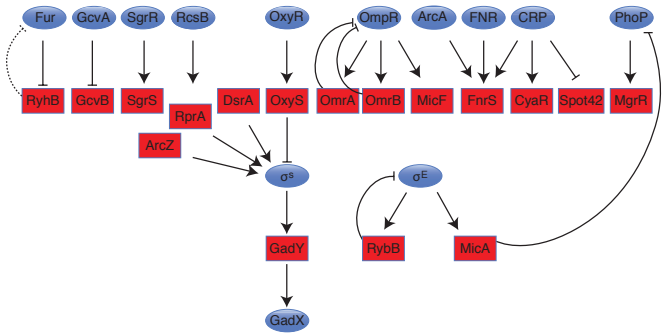

Figure 5 | Regulatory circuit of major known sRNAs in bacteria. Transcription factors (blue ovals) regulate expression of sRNA shown in red boxes. Some sRNA feedback regulate their transcription factors levels. The complex regulatory circuit depicts fundamental involvements of sRNAs in prokaryotic gene regulation. Figure adapted from Gottesman and Storz 2011[58].

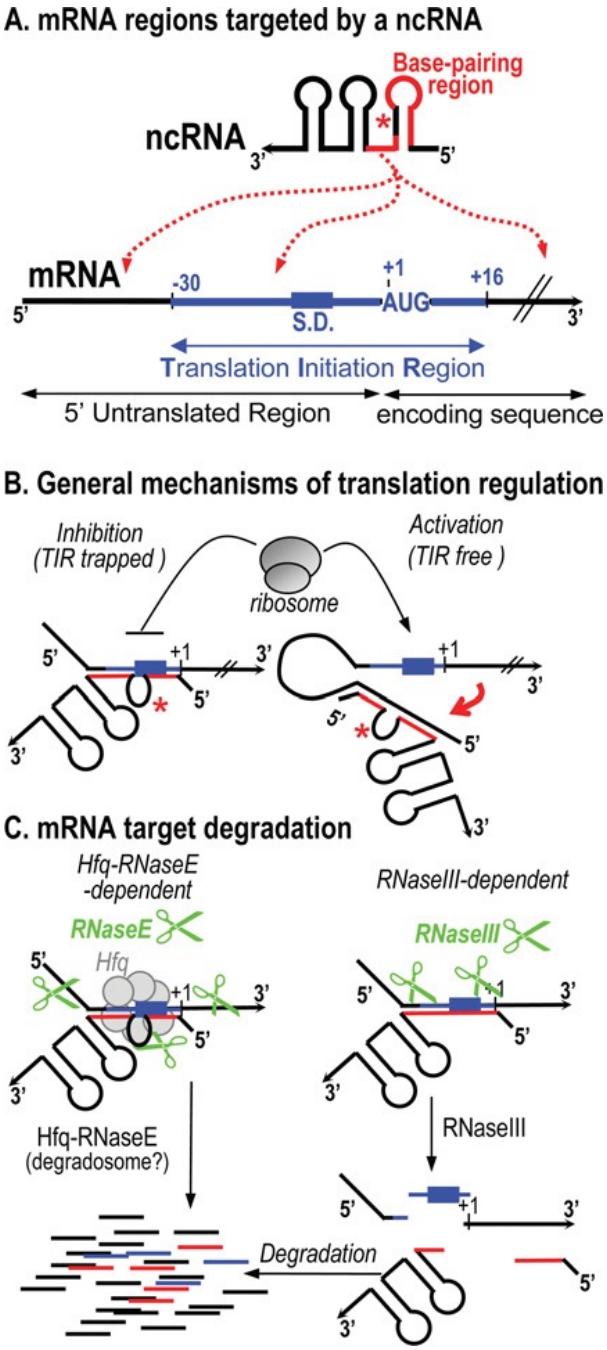

Figure 6 | Properties and regulatory mechanisms of sRNA. A) sRNA can target different sections of mRNA. The interacting region within non-coding sRNA is marked by red and named 'base-pairing' region. Parts of sRNA not interacting with target mRNA are marked by red asterisk. sRNA may interact with Translation Initiation Region (TIR) of mRNA normally bound by 30S subunit of ribosome to initiate transcription. Alternatively, sRNA can also interact with upstream of TIR or within the coding sequence of the gene. **B)** sRNA can both function as repressor or activator of target mRNA. On the right, sRNA binds to the TIR of mRNA thereby blocking recognition by ribosome and intiation of translation. On the left, sRNA binds to another region within mRNA that was base-paired with TIR and blocked access of ribosome, therefore the sRNA is activating translation by ribosome. **C)** mechanisms of mRNA target degradation by RNaseE. On the left, RNaseE interacts with Hfq protein to facilitate degradation of target mRNA. On the right, RNaseE recognition of cleavage sites within mRNA facilitates its degradation. Figure adapted from Repoila and Darfeuille 2009[57].

Chapter 4

Evolution in natural environments

Whole genome sequencing (WGS) is the most applicable tool to study relatedness of organisms. Until recently, the high cost of sequencing entire genomes discouraged sequencing of related organism to study their phylogenomics. With the advent of WGS and next generation sequencing (NGS) techniques, analysis of detailed changes in related isolates of bacteria is no longer a dream. More than hundred thousand bacterial isolates have been sequenced[59] and evolutionary biologist can easily discover genome alternations to understand the evolutionary pathways of bacteria. Evolutionary biologists have performed experimental evolution studies to provide novel insights to comprehension of bacterial adaptive evolution[4,14]. However in real life, natural evolution of bacterial species occurs under much more complex conditions than in laboratory. The more limited number of studies on natural evolution of bacteria reflects difficulties related to systematic sampling within those populations. Sampling habitats are difficult to define and target population is often too small. Despite such limitations, sampling pathogenic bacteria from chronic human infections provide more fruitful results on their within-host evolution because of well-defined boundaries of the host[20,35].

Studying microevolution of organisms is instrumental in grasping the underlying basis of their genetic adaptation. With information deriving from experimental and natural evolution studies, researchers can genetically engineer organisms to improve their fitness in industrial application or identify mechanisms of their pathogenic manners in host infections.

4.1 Cystic fibrosis model

Chronic airway infections in cystic fibrosis patients provide monumental opportunities to study natural evolution of bacteria in clinical settings. CF environment contains a complex repertoire of selection pressures that can shape adaptation of colonizing pathogens. Routine samplings of expectorated sputum and nasal lavage from CF patients in different countries have produced a goldmine of

bacterial isolates that can be used in longitudinal studies of bacterial evolution in chronic infections[20,60–64]. In addition, there are real values in any contribution to potential treatment of patients suffering from this condition.

The following sections will present an overview of cystic fibrosis genetic condition, its clinical manifestation, its environmental habitat, involved selection pressures, colonizing pathogens and their adaptation in CF.

4.1.1 Cystic fibrosis

CF is a human recessive genetic disorder caused by the combination of two mutant alleles in cystic fibrosis transmembrane conductance regulator (CFTR) gene. There are at least 1500 possible mutations targeting CFTR gene but the most dominant mutation affecting 70% of CF patients is ΔF508. The disease is mostly affecting Caucasian population with 1 in 2500 live birth incidence rate and approximately 70 thousand people have been diagnosed with CF worldwide[65]. The mutations lead to loss-of-function or malfunction of CFTR, a cyclic-AMP regulated transporter of chloride ion and water across epithelial membranes. Loss of CFTR function impairs electrolyte transport and results in production of viscous mucus in the airways. The thick and dehydrated layer of mucus in CF airways intrudes with mucuciliar clearance of inhaled microbes and makes CF patients particularly vulnerable to infections by different microbes[66,67]. If left untreated, CF patients succumb to airway infection at a young age. The life-expectancy of CF patients in 1974 was 8 years old but in recent years with intensive antibiotic treatments, a CF diagnosed patient can live to a median age of 40 years[68].

4.1.2 Cystic fibrosis airway environment

There are three compartments in the human airway. The upper part of the airway contains paranasal sinuses extending to nasal cavities. The conductive zone comprising of trachea, bronchi and terminal bronchioles is located in the lower airway. These two compartments are more prone to bacterial colonization because of the thick mucus production providing optimal conditions for bacterial growth. The last sector of the airway is also located in the lower part of the respiratory zone and

it includes respiratory bronchioles and the alveoli[68,69]. This part is usually immune to infections except in cases of severe lung damage[70].

The spatially separated compartments of the CF airway generate environmental heterogeneity and induce diversification of bacterial populations. In two separate studies on within-host evolution of *Pseudomonas aeruginosa* colonizing CF airways, related clones of this bacterium from different locations of the airways demonstrated diverse phenotypes and genotypes. These results demonstrate that clones of the same ancestor evolved to the properties of their environmental niches[71,72].

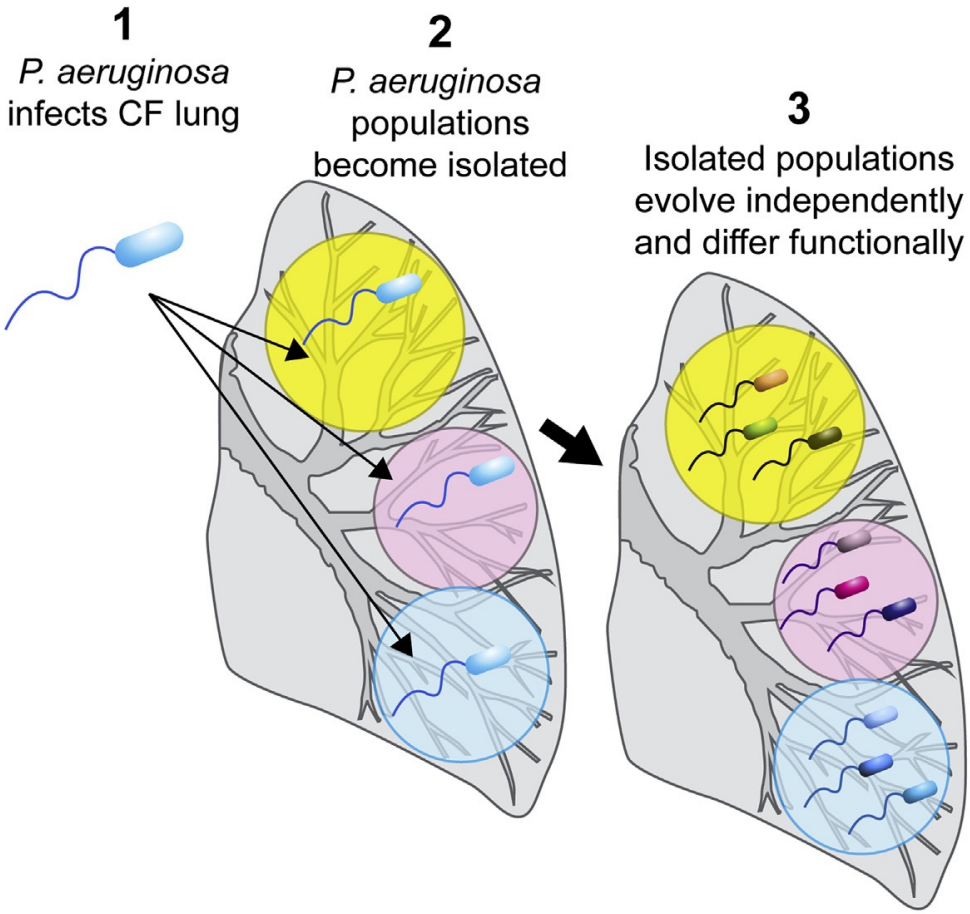

Figure 7 | Diversification of *P. aeruginosa* genotypes in different compartments of CF airway. Bacteria of the same ancestor clone colonizing spatially isolated compartments diversify independently within CF airways. Environmental heterogeneity of different locations offer various selection factors and colonizing *P. aeruginosa* adapts to optimal phenotypes to survive in each compartment. Figure adapted from Jorth *et al.* 2015[71].

In addition to environmental heterogeneity within different locations of the airways, the open system of CF environment is subject to several known selection pressures that vary in both time and space[73]. In the following sections an overview of most obvious selection pressure are provided.

Antibiotics are regularly administered to CF patients to inhibit and eradicate bacterial pathogens, depending on the present condition. Aminoglycosides, β-lactams, antimicrobial peptides, macrolides and fluroquinolones are the different classes of antibiotic often present within compartments of the CF airways. While antibiotics are administered both orally and intravenously, different outcomes are expected on population organization and evolution. For example, intravenous administration of antibiotics results in higher concentration in mucus of respiratory zones, but lower concentration in that of the conductive zones. In contrast, oral inhalation of antibiotics will have the opposite effect[69,73]. Additionally, mucus accumulation blocks access to sinus cavities and pathogens within this region are less susceptible to antibiotic treatments[74]. Antibiotics selection pressure in CF environment leads to adaptive resistance phenotypes in colonizing pathogens[64,75].

The Immune system is another challenging selection pressure on pathogens of the CF environment. Failure of the mucuciliar clearance prompts early recruitment of inflammatory polymorphonuclear neutrophils (PMN)[76]. Additional components of the immune system including defensins, macrophages and secroty IgA are also activated in response to infection but their site of action depends on the compartment of the airway. For example, PMN attachment to colonizing microbes, facilitated through microbial lipolysaccharide (LPS) and flagellin structures, is more predominant in the lower airways whereas secretion of IgA antibody is more common in the sinuses[77]. Activated PMNs or macrophages trigger phagocytosis and liberation of reactive oxygen species (ROS). The release of ROS provides oxidative stress in lower airway conditions for pathogens but also deteriorates lung tissue damage overtime[78]. In response to recognition by the immune system, colonizing pathogens adapt by reducing their immunogenicity[79].

Oxygen availability is another limiting factor for bacterial pathogens of CF airways. While lung is presumed to contain an abundance of oxygen, there are really different levels of oxygen in different CF compartments. Gas exchanges occur in the respiratory zone and oxygen level is sufficient in this compartment. On the other hand, mucus enriched regions vary between aerobic to micro-aerobic and strictly anaerobic[69,74,80]. Oxygen is poorly dissolved deep inside the mucus matrix. Here,

facultative aerobes take advantage of sufficient amounts of alternative electron acceptors like nitrate or phenazines to exploit anaerobic respiration[81–84].

Nutrients such as free amino acids, glucose, lactate and different types of fats are richly found in the CF environment[85]. Nonetheless, the distribution and abundance of different nutrients varies from one compartment to another and pathogens adapt by optimizing differently to varying presence of nutrients[86].

Iron presence is a limiting factor for pathogens colonizing CF airways because the host withholds iron reserves by binding to proteins like ferritin, transferrin and lactoferrin[87]. This makes colonizing pathogens like *P. aeruginosa* to utilize iron through heme and siderophore uptake systems[88].

Salts such as Na^+, K^+ and C^- are abundantly found in CF airways because of the impaired function of CFTR in transport of electrolytes and water across epithelial membrane[89,90]. In response, pathogens need to adapt to high osmotic pressures to survive in CF airways[35].

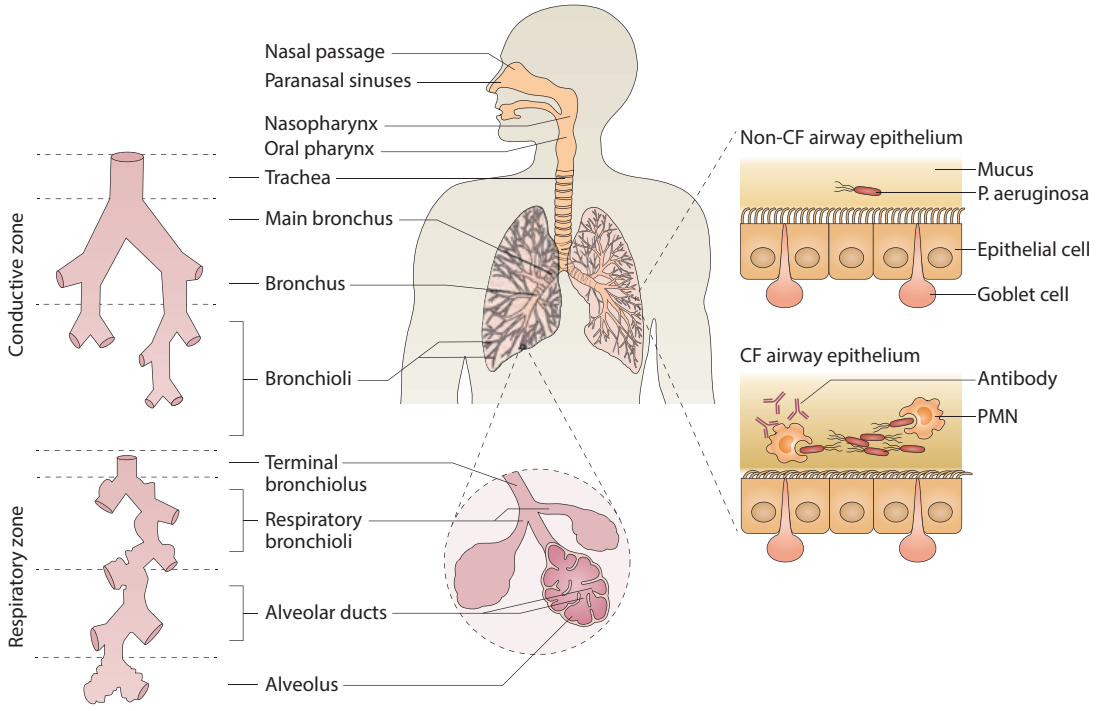

Figure 8 | Compartments of the CF airways. There are three distinct anatomical regions in human respiratory system: the paranasal sinuses, the conductive zone and the respiratory zone. Due to mutations in CFTR, transport of electrolyte and water across epithelium is interrupted leading to impair physical removal of inhaled microbes. The thick dehydrated mucus within sinuses and the conductive zone provides an optimal reservoir for growth of CF pathogens. Increased concentration of microbes such as *P. aeruginosa* initiates an immune response by the host with recruitment of inflammatory PMN agent and antibodies leading to impaired lung function and exacerbated lung tissue. Figure adapted from Folkesson *et al.* 2012[68].

4.1.3 Ecology of the CF airway

The microbial habitat of CF airway is composed of a highly complex and mixed ecosystem where multispecies of microbial communities coexist[91]. It is proposed that from 100 to 1000 different species colonize CF airways and 10^9 CFU per ml of bacteria are present in CF sputum[92,93]. However, a range of organisms including *Pseudomonas aeruginosa*, *Haemophilus influenza*, *Burkholderia cepacia* complex, *Staphylococcus aureus* are found to be more frequently isolated from CF patients than others. The emergence of these bacteria in CF patients is proposed to be dependent on age[68]. While *H. influenza* and *S. aureus* dominate in infections of early childhood, *P. aeruginosa* eventually overtakes others and become the main infectious agent in the CF host. In this context, around 60-70% of adult CF patients have chronic *P. aeruginosa* infection demonstrating that this opportunistic pathogen is main agent causing morbidity and mortality in CF hosts[94].

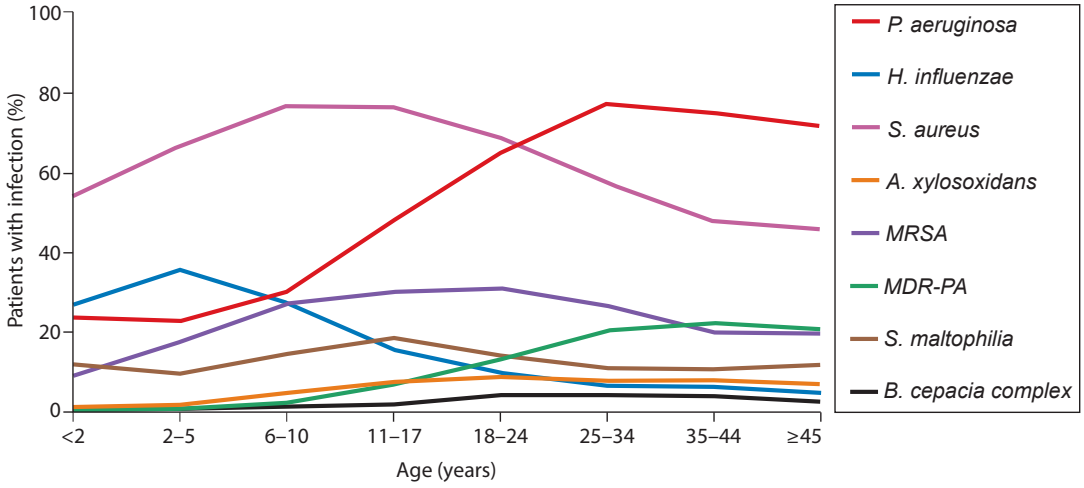

Figure 9 | Development of different species prevalence in CF patients as a function of age. *A. xylosoxidans*, *Achromobacter xylosoxidans*; *B. cepacia*, *Burkholderia cepacia*; *H. influenzae*, *Haemophilus influenzae*; MDR-PA, multidrug-resistant *P. aeruginosa*; MRSA, methicillin resistant *S. aureus; S. aureus*, *Staphylococcus aureus*; *S. maltophilia*, *Stenotrophomonas maltophilia*. *S. aureus* and *H. influenza* are the predominant agents colonizing CF patients at early childhood. As age of patients progress, *P. aeruginosa* dominates against all other pathogens in CF patients and become the main cause of mortality and morbidity. Figure adapted from Folkesson *et al*. 2012[68].

4.1.4 *Pseudomonas aeruginosa*

The gram-negative bacillus *Pseudomonas aeruginosa* is a motile, aerobic bacterium inhabiting a variety of environmental niches like soil, water, plants, animals and humans. This opportunistic pathogen seldom infects healthy humans but it has received particular attention due to its ability to cause bloodstream infections, UTI,

ulcerative keratitis and nosocomial pneumonias while being very infective in immune-compromised patients (e.g. HIV and cancer) and those with CF disorders[95]. The most extensively annotated reference genome of *P. aeruginosa* is laboratory strain of PAO1[96–98]. The chromosome size of *P. aeruginosa* ranges from 6.2 to 6.9 million base pairs and the GC content is around 66%. The relative large genome of *P. aeruginosa* contains a large repertoire of regulatory proteins potentiating its extraordinary ability to thrive in different environment[96]. This built-in versatility is augmented with fast growth rate and inherent resistance to toxic and antimicrobial agents enabling this pathogen to survive in extreme conditions of CF airways[99,100].

4.1.5 *P. aeruginosa* adaptation in CF

The pattern of *P. aeruginosa* settlement in the CF host commences with a period of intermittent colonization. During this period, recurrent cycles of colonization and eradication are observed[101]. Eradication and delay of chronic infection onset can be established by intensive antibiotic treatments[76]. *P. aeruginosa* strains colonizing patients during this period exhibit typical phenotypes of environmental strains such as fast doubling time, non-mucoid morphology and being susceptible to antibiotics. Indeed, genetic analysis has also verified that these unique strains trace back to unidentified environmental niches[102]. The intermittent colonization by *P. aeruginosa* may last from a few months to a few years in early lives of CF patients depending on treatment and adaptive status of the infecting strains[102]. Most patients acquire new genotypes after eradication of earlier ones, however in some cases recolonization with a previously eradicated genotype is also observed demonstrating a persistent environmental source or protected host location, e. g. the sinuses, difficult to reach by common treatments[68,103].

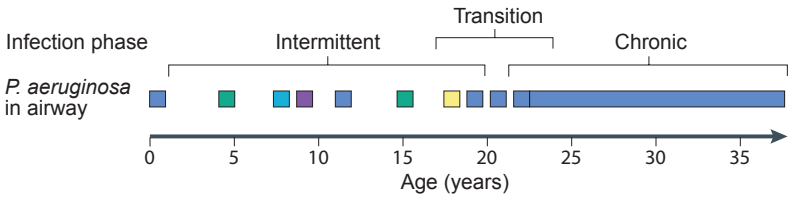

Figure 10 | Development of *P. aeruginosa* infection in CF patients. Phylogenetically distinct clones of *P. aeruginosa* (different colors) colonize CF patients and are eradicated by antibiotic treatments. Periods of *P. aeruginosa* absence are also observed until when chronic infection by a persistent clone is established. Figure adapted from Folkesson *et al.* 2012 [68]

Inevitably sooner or later, due to unknown reasons, a chronic infection with *P. aeruginosa* happens in CF patients[102]. In 60-70% of cases, patients are colonized by chronic infection before reaching 20[94]. Signs of this type of infection include uninterrupted presence of one *P. aeruginosa* genotype for more than six months, elevated inflammatory response and development of antibodies specific to *P. aeruginosa*[76]. The persistence of *P. aeruginosa* in chronically infected CF airways results in immune complex-mediated chronic inflammation that worsens lung tissue destruction on top of the damage already caused by the bacteria[104]. Despite vigorous antibiotic treatment and the inflammatory response of the host, most persistent chronic infections lead to respiratory failure and complete lung tissue destruction requiring lung transplantation or result in death of patients[105].

All causes of transition from intermittent to chronic infection if CF patients have not been discovered yet, but multiple studies point to genetic adaptation of *P. aeruginosa* to the CF environment as a key factor[20,35,60–62,106–109]. Several reasons have been proposed for genetic adaptation of *P. aeruginosa* in CF airways. To begin with, chronic infections are often induced by total dominance of a unique clone type that can colonize for many years until demise of the CF patient. As this clone type is capable of outcompeting all other invading *P. aeruginosa* clones, it must have gained advantageous mutations increasing its fitness in the CF environment[79,103]. Furthermore, phenotypes observed in chronically infecting *P. aeruginosa* clones differ significantly from those presented during intermittent colonization, which exhibited phenotypes of environmental strains[68]. Finally, these typical phenotypes are observed in multiple strains of *P. aeruginosa* isolated from unrelated chronically infected CF patients across the world. Therefore, this parallel evolution of adaptive traits in independent settings indicates natural selection[18]. The following describes some of the adaptive phenotypes of *P. aeruginosa* in chronically infected patients.

Mucoidity is a typical and perhaps most characterized phenotype of chronically infected isolates of *P. aeruginosa* in CF patients. It is presented by exopolysaccharide alginate overproduction leading to slimy colony morphology of *P. aeruginosa*. Alginate production is proposed to shield *P. aeruginosa* from immune system response and antibiotics[110–112] emphasizing its significance in chronic infection scenario where alginate production is associated with poor outcomes for

CF patients[113]. In majority of cases, the mucoid phenotype arises from loss of function mutation in *mucA*, expressing anti-sigma factor that represses AlgT[114]. AlgT is an alternative sigma factor controlling stress response genes in *P. aeruginosa* including those of alginate production expressed by *algD* gene cluster[68].

Antibiotic resistance is another commonly observed phenotype in chronically infected isolates of *P. aeruginosa*. It is predictable to observe antibiotic resistance trait due to regular administration of antibiotics to CF patients providing strong selection pressures on *P. aeruginosa* to genetically adapt to resistance mechanisms. *P. aeruginosa* is inherently resistant against multiple classes of antibiotics through low outer membrane permeability, function of several outer membrane multidrug resistance (MDR) efflux pumps and expression of an AmpC β-lactamase[115]. Commonly observed mechanisms of resistance are: A) mutations affecting regulation of MDR efflux pumps[116,117]. B) Mutations modifying topoisomerase IV and DNA gyrase structures[118,119]. C) Mutations that increase expression and specificity of β-lactamases[120]. D) Deleterious mutations in membrane OprD leading to decreased import of carbapenems[121]. E) Mutations increasing resistance to cationic antimicrobial peptides through changing the composition of LPS[122].

Loss of ***virulence factors*** is commonly observed in late stage chronic infection isolates *P. aeruginosa*. It is logical that manifestation of virulence factors draws attention and lowering expression of immunogenic agents[79] provides evasion of the immune system. Loss of virulence factors is commonly facilitated through structural mutations affecting global regulators and sigma factor such as Vfr, LasR, RpoN, AlgT and PvdS[35,60,72,106,123,124]. Lost virulence factors include flagella, LPS, type IV pili, proteases, phenazines, pyoverdines, pyocins, siderophores and TTSS factors[123,125–129].

Hypermutation has been frequently observed in several adapted strains of *P. aeruginosa* isolated from chronic infections[20,106,130,131]. It remains to be elucidated how the hypermutator phenotype is advantageous for *P. aeruginosa* but higher rate of mutations may increase chances of rapid genetic adaptation and survival in CF airways[130]. Loss-of-function mutations in *mutS* and *muL*, encoding DNA mismatch repair system factors, are the most frequent cause of this phenotype[132].

Chapter 5

The interplay of phenotypic acclimation and genetic adaptation

In chapter 2, I presented a brief introduction on phenotypic acclimation and genetic adaptation, two main pathways by which bacteria and more generally all organisms adapt to new environments. The principle of phenotypic acclimation relies on gene regulation, a topic that was presented in chapter 3. In chapter 4, I presented an example of bacterial genetic adaptation in natural systems, *P. aeruginosa* evolution in airways of CF patients. Here, I will present putative cases where genetic adaptation has modulated phenotypic acclimation response in bacteria.

Remodeling of regulatory systems through genetic adaptation ensures adaptation to highest average performance under different conditions. In essence, the occurrence of these mutations reshapes the pre-existing regulatory networks in place for phenotypic acclimation to environmental cues. The immense pleiotropic effect associated with such changes is because of regulatory effects of targeted proteins controlling expression of many genes.

In a study by Yang *et al.* in 2011, it was discovered that strains of *P. aeruginosa* DK2 isolated from chronic CF patients over a period of 200,000 bacterial generation were more affected by mutations within regulatory genes at the start of their adaptive history. NS mutations in global regulators such as *mucA*, *lasR* and *rpoN* are responsible for half of later expression changes of all genes confirming the extreme pleiotropic effect caused by these type of mutations. Furthermore, early establishment of many phenotypes necessary for initial colonization in the CF airways are established through these types of mutations. As an outcome, isolate DK2-CF30-1979 containing all of these mutations acquired the peak of adaptive phenotypes and all later evolved isolates were mostly similar in phenotypes to this ancestor[106]. In a later study by Damkiær *et al.* in 2013, the detailed contribution of each DK2 global regulatory mutations on adaptive phenotypes were investigated. Through construction of each global regulatory mutation in laboratory strain of PAO1, the authors show that global regulatory mutations change the way *P. aeruginosa* DK2 responds to the CF environment by becoming mucoid or non-

mucoid at different stages of its adaptive history. Furthermore, epistatic interactions of all these mutations significantly increase tolerance to antibiotics[35].

Additionally, studies on experimental evolution of bacteria also report importance of regulatory network alterations in evolution of adaptive phenotypes. In controlled evolution of bacterial populations in laboratory, global regulators of gene expression are commonly targeted by adaptive mutations and establish fundamental phenotypic changes in bacterial species[31,133–135].

In conclusion, it is clear that genetic adaptation targets regulatory systems to accommodate different phenotypic acclimation patterns in response to these environments. The consequent changes are not optimal for one condition but accommodate highest average performance in different conditions. Hence, the adaptive nature of global regulator mutations accommodate increased fitness through altered phenotypic acclimation pattern.

In addition to changes of regulatory systems, intergenic mutations in non-coding regions can also have potential effects on regulatory systems facilitating phenotypic acclimation. This is because the bacterial transcription machinery is composed of regulators of gene expressions controlling expression of genes through binding to *cis*-regulatory intergenic elements. Genetic changes within these elements affect binding of regulatory proteins causing changes in expression of downstream genes. Changes in binding of a global regulator to one region may also cause pleiotropic effects on expression of other related genes. Two separate studies have investigated evolution of *cis*-regulatory elements through horizontal gene transfer[136] and *de novo* mutations[27] where pathogen adaptive phenotypes emerge as a consequence of such changes. Furthermore, experimental evolution studies also emphasize the importance of mutations in *cis*-regulatory elements in functional innovation and adaptation of bacteria[28,137].

Chapter 6

Present investigations

This PhD thesis builds on previous studies of *P. aeruginosa* evolution in natural system of CF airways. Before I begin, I have to acknowledge that the collection of *P. aeruginosa* isolates from CF patients paved the path for conducting all these investigations including those of this thesis. In this context, professor Niels Høiby and his colleagues at the Danish Copenhagen CF center in Rigshospitalet collected and stored clinical isolates of *P. aeruginosa* from Danish CF patients since 1973. Similar comprehensive programs of *P. aeruginosa* collection from CF patients were also carried out elsewhere across the world. The depth of knowledge gained from these valuable resources of clinical isolates may have been limited when the programs started years ago but with recent advances in technology several studies have dissected the phylogeny, evolutionary dynamics and important adaptive stages of *P. aeruginosa* evolution in the CF environment.

6.1 Background

The majority of studies on evolution of bacteria in natural systems focus on the following major themes:

- Evolution of bacteria in natural systems and correlations of findings with those of experimental evolution settings
- Remodeling of global regulatory networks and its effect on emergence of major adaptive phenotypes
- Identification of genes under selection for adaptive mutations
- Adaptive phenotypes caused by gene mutations
- Role of hypermutation in evolution of bacteria

While these studies embark on major discoveries that can be utilized in understanding bacterial evolution in natural setting, they still neglect the extent of knowledge that can be gained from their collected data. One common alarming notion is following the intuition that all adaptive changes occur only through intragenic mutations. Recent studies document that regulatory intergenic mutations

are contributors to bacterial adaptation in natural[26,27] and experimental setting[28,29]. In this thesis, I have made an effort to study the role of intergenic mutations on evolution of *P. aeruginosa* in CF airways.

6.2 Aim of study

The following thesis uses adaptation of *P. aeruginosa* in CF airway environments as a model to reach the following objective:

- *To provide novel insights on evolution of bacteria in natural setting through non-coding intergenic mutations.*

The aims of research articles included in this thesis are the following:

- *To investigate the qualitative and quantitative contributions of non-coding intergenic mutations on within-host evolution of P. aeruginosa in CF airways.*

- *To investigate local and pleiotropic consequences of mutations in one intergenic region (phuS//phuR) mutated across different genotypes of CF adapted P. aeruginosa.*

6.3 Results and discussion

The following sections present summaries of three research articles included in this thesis. Detailed description of methods and figures can be found in chapter 8 where full-length published articles or prepared manuscripts are provided.

Article 1 | Within-host evolution of *Pseudomonas aeruginosa* reveals adaptation toward iron acquisition from hemoglobin

In this paper, we investigated the most densely mutated intergenic region in *P. aerugi*nosa DK2 genotype. A total of 13 mutations were found in a 180 bp region upstream of *phuR* and *phuRSTUVW* encoding the receptor and other components of *Pseudomonas* heme uptake system (*phu*). These mutations occurred in the genome of independently evolved isolates of DK2 in different patients. In addition, we also

found isolates of two distinct CF adapted genotypes of *P. aeruginosa* DK1 and Clone C with mutations within the same region confirming that this observation is not unique to DK2 genotype. In all three genotypes, loss of pyoverdine production through NS mutations preceded the occurrence of *phuR* intergenic mutation

We sought to investigate the effect of these mutations on local transcription of *phuR* gene. For this purpose, we cloned the mutated region from nine genomes upstream of luciferase reporter on a plasmid and integrated the plasmid on the genome of *P. aeruginosa* laboratory PAO1 strain. Measurements of *lux* normalized by the cell density at a specific point demonstrated that almost all of the mutated regions increased promoter activity of *phuR*. Mutation from two DK2 isolates increase *phuR* promoter activity by 93 and 112 folds compared to that of the wild type (WT). We also inspected available transcription data from these isolates and found out that the expressions of *phuR* and *phuRSTUVW* genes were significantly increased compared to isolates without the mutation.

To demonstrate the phenotypic effect of these mutations, we engineered the mutation conferring highest expression change (112 folds) in a CF adapted DK2 background without the mutation. We measured the doubling time of isogenic strains of *P. aeruginosa* with and without the mutation in rich Luria-bertani (LB) and minimal medium (MM) with abundance of iron and demonstrated that there was no significant change. Interestingly the doubling time of the strain with *phuR* mutation was significantly shorter that its isogenic WT in MM with hemoglobin showing that the overexpression of the *phu* system confers a growth advantage in the presence of hemoglobin.

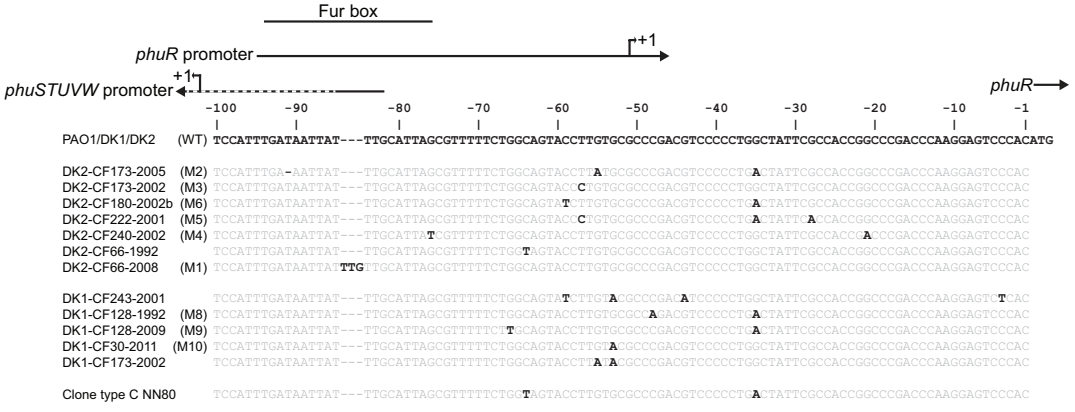

Figure 11: Overview of the intergenic region upstream of *phuR*. The alignment shows sequences from different isolates with genetic variation in the region. Figure adapted from Marvig *et al.* 2013[27]

Articles 2 | Contribution of non-coding intergenic mutations on within-host evolution of a human pathogen

In research article 1, we discovered a novel adaptive mechanism with important implications of *P. aeruginosa* survival in the CF airway. This mechanism was activated through nothing more than intergenic mutations. For this reason, we hypothesized that this specific example can be the tip of the iceberg. *P. aeruginosa* and any other bacteria evolving in a natural or experimental condition may evolve through acquisition of mutations in intergenic regions. This hypothesis inspired us to perform a comprehensive analysis of intergenic mutations in *P. aeruginosa* sequenced genomes isolated from chronic CF patients. We utilized available data from several longitudinal studies investigating evolution of *P. aeruginosa* in CF airways where intergenic and intragenic variants between genome-sequenced isolates of the study have been detected. In total, our study consisted of 534 genome sequenced isolates belonging to 44 genotypes of *P. aeruginosa* isolated from CF patients. To discover intergenic regions under positive selection for adaptive mutations (pathoadaptive regions), we defined our selection criteria based on the occurrence of intergenic mutations within all 534 genomes (see methods). We identified a total of 88 intergenic regions under positive selection for mutations within and across isolates of different genotypes.

We then sought to map the position of mutations in putative intergenic elements within these regions. Interestingly we found that in 33% of regions, the mutations occurred in putative intergenic elements and the most targeted element within this portion was the core promoter. This result confirms a high number of actual pathoadaptive regions in our initial list despite limitation such as low annotation of elements in all regions.

To provide additional evidence for effectiveness of mutations within pathoadaptive regions, we randomly selected 25 regions and tested the activity of 32 genes downstream of mutations through construction of *lux* reporter fusions. Comparing the expression of fusions from these regions to their isogenic WT demonstrated that 15 fusions have significantly altered expression in at least one of LB or MM. Looking at the list of 15 genes, we identified PA4837, *exsC*, *cerN*, *motY*, *pyrC* and *ampR* with associated CF airway adaptive phenotypes in *P. aeruginosa*.

We finally investigated adaptive phenotype of mutation upstream *ampR* in *P. aeruginosa* through replacement of the mutation in *P. aeruginosa* PAO1. We established that this mutation significantly increased minimum inhibitory concentration (MIC) of β-lactams imipenem and ampicillin in *P. aeruginosa*.
In conclusion of this study, we identified several genes associate with fitness in CF airways affected by intergenic mutations in *P. aeruginosa*. Furthermore, we showed that intergenic mutations make a numerically larger contribution to adaptation in *P. aeruginosa* DK2 (2:1). This was in accordance with expectations that CRE intergenic mutations occur more frequently than TRE because of their less deleterious potentials[23,31,32]. A recent study in experimental evolution of bacteria also suggests that 'regulatory' intergenic mutations were more strongly overrepresented than expected[28].

Article 3 | Adaptive mutations in an intergenic region cause pleiotropic effects on gene expressions

In this study, we tried to investigate whether intergenic mutations really confer local and subtle regulatory effects on expression of immediate genes. For this purpose, we chose the *phuR* promoter mutation that we investigated in research article 1. We hypothesized that overexpression of the *phu* system confers additional effects than the *phu* system. To test this hypothesis, we examined transcriptional changes caused by *phuR* promoter mutation using DNA genechip microarray in LB. Interestingly, in *P. aeruginosa* DK2-CF30-1979 isolate the expressions of 118 genes were significantly altered as a result of *phuR* promoter mutation (> 2 or -2 < fold changes). We repeated the microarray for *P. aeruginosa* PAO1, where only transcriptions of *phu* system and two additional genes were affected by the *phuR* promoter mutation. This confirmed that epistatic effects and genetic variations between DK2 and PAO1 genotypes play an important role for induction of the pleiotropic effect. However, one particular gene PA4711 located right after *phuR* was consistently upregulated in both PAO1 and DK2 genotypes with the mutation. In addition, we also performed microarray experiments with DK2-CF30-1979 strains in MM. Interestingly again only expression of *phu* system and two other genes were significantly altered because of

the mutation. Nonetheless, PA4711 was still upregulated and unlike the rest of pleiotropic effects, this upregulation was independent of environmental context. Since PA4711, encoding a Rieske-like iron-sulfur protein of unknown function, was upregulated in all tested conditions and genotypes with the *phuR* promoter mutation, we sought to investigate its expression in original isolates where *phuR* promoter mutation was detected. Interestingly, previous microarray experiments from these isolates showed that PA4711 was also upregulated in these isolates compared to ancestor isolate lacking *phuR* promoter mutation.

As *narK1* and *narK2* were two genes most downregulated in DK2 genotype with *phuR* promoter mutation in LB, we tested the growth of this strain and its isogenic WT during anoxic conditions. We were able to show that the strain with *phuR* promoter mutation grew slightly but significantly slower than its isogenic WT.

To investigate additional phenotypes developing through *phuR* promoter mutation, we spotted the DK2 genotype strains in different solid surface agar plates alone or in combination with *S. aureus* JE2 WT. Interestingly; we observed a change in pigmentation from white to green/yellow along with increased inhibition of *S. aureus* in MM agar plates.

In conclusion, we demonstrate that overexpression of the *phu* system through an intergenic mutation leads to pleiotropic effect on expression of other genes. The effect was most dominant in adapted DK2 genotype and highly contingent on the environmental context. Furthermore, expression of PA4711, a gene located downstream of *phuR* is constantly upregulated along with the *phu* system genes. As this gene encodes an iron-sulfur protein possibly involved in energy metabolism of the cell, we propose that its upregulation leads to imbalance of the normal redox state of *P. aeruginosa*. Possible evidence for this hypothesis is enriched presence of '*energy metabolism*' class of genes among those affected by the pleiotropic effects. Furthermore, we showed that *P. aeruginosa* isolate with the pleiotropic effect is slightly less fit to grow under anoxic conditions and this is possibly related to imbalance of the energy metabolism and redox state. We also propose that the pigmentation and increased inhibition of *S. aureus* are due to increased production of phenazines because phenazines are putative electron carriers involved in respiration under anaerobic conditions.

Chapter 7

Conclusions and perspectives

Investigations of bacterial genetic adaptation require a depth of knowledge on molecular mechanisms of evolution. All apparent pieces of the puzzle have to be considered in order to study bacterial evolution in new environments. With remarkable advances of NGS in recent years, a new chapter in the history of bacterial evolution has started. Evolutionary biologists have been able to reproduce evolution in controlled laboratory conditions and utilize sequencing technology to map patterns of genetic adaptation across genomes of related bacteria. Furthermore, feasible models of natural evolution have also been exploited to study evolution of bacteria in natural environments. The main variable considered in these investigations is evolution of bacteria through acquisition of intragenic mutations. The critical role of global regulators in phenotypic acclimation makes them common target of adaptive mutations facilitating large phenotypic changes in new environments. While intergenic regions are also frequently targeted by mutations in evolving isolates of bacteria, the potential adaptive role of these mutations have been ignored. Many of the assumption about evolutionary dynamics of bacteria and systems under positive selection in an environment are based on intragenic mutations leading to partial consideration of facts to draw important conclusions.

The work presented in this thesis reveals significant contributions by intergenic mutations to natural evolution of bacteria. We have considered natural evolution of bacteria in the CF airways and taken advantage of *P. aeruginosa* sequenced genomes isolated from this environment. The first study demonstrated that mutations in the promoter region of *phuR* encoding receptor for the *phu* system confer a growth advantage in presence of hemoglobin. As access to free iron is limited in CF airways, this intergenic mutation increased fitness in that environment. The observation of such pathoadaptive intergenic mutation acted as an inspiration to perform the second study. Here a comprehensive analysis was performed to

identify intergenic regions under positive selection for evolution in 534 genomes of *P. aeruginosa* isolated from 68 patients with chronic CF airway infection.

By performing this study, we established higher numerical contribution of intergenic mutations on within-host evolution of this *P. aeruginosa* in CF airways. Furthermore, we identified several genes and systems with previous established role in adaptation of *P. aeruginosa* in CF environment. Modulation of these genes through intergenic mutations should be considered for future studies of pathoadaptive systems in *P. aeruginosa*. We also provided a long list of hypothetical genes in regions under positive selection by intergenic mutations and the potential function of these genes on within-host evolution of *P. aeruginosa* remains to be elucidated by future studies. Functional investigation of these genes will unravel new details regarding their role in *P. aeruginosa* adaptation in CF environment. Testing the effect of remaining pathoadaptive mutations within our list through construction of reporter fusions and allelic replacement provides new paths for discovery of genes important for pathoadaptation of *P. aeruginosa* in CF airway.

We demonstrated that the core promoter is the main target by intergenic mutations and mutating this element leads to downregulation or upregulation of genes. Nonetheless, a number of mutations occur in unidentified intergenic elements but they significantly alter transcription of downstream genes. Future studies may identify presence of additional CRE or post-transcriptional regulatory element such as sRNA and define molecular mechanisms by which intergenic mutations target these elements. For this purpose, researchers can use RNA-seq, ChIP-seq, DNase footprinting, primer extension, EMSA and promoter probe experiments.

Intuitively, one can hypothesize that intergenic mutations confer more local and subtle regulatory changes in expression of downstream genes compared to intragenic mutations causing more deleterious effects on their targets. This can explain the larger numerical contribution of intergenic mutations on selection of pathoadaptive genes. In this way, intergenic mutations allow essential genes to become target of evolutionary changes. With a few exceptions, we also observed subtle changes in expression of genes affected by intergenic mutations.

In the third study, we sought to investigate this hypothesis on *phuR* intergenic mutation. We selected this mutation because it conferred more radical expression

changes on local genes. Interestingly, we discovered that the mutation upstream of *phuR* triggers extreme pleiotropic effects on expression of several other genes. This surprise finding goes against the hypothesis that intergenic mutations confer local effects. The *phuR* intergenic mutation conferred additional phenotypes such as increased inhibition of *S. aureus* through possible production of phenazines. Presence of additional microbial organisms such as *S. aureus* have previously been proposed to drive evolution of *P. aeruginosa* in CF airways[138,139]. Nonetheless, there is little evidence for interaction of microbial species in CF airways and how that affects evolutionary dynamics of *P. aeruginosa*. Our study suggests that inclusion of intergenic mutations may provide new paths for investigations of microbial interactions in the CF environment.

The findings of the third study raise interesting perspectives for pleiotropic effects of intergenic mutations where major adaptive phenotypes can be established through acquisition of an intergenic mutation. Previous studies demonstrated roles for hypermutation and global regulatory mutations in rapid and permanent adaptation of *P. aeruginosa* in the CF environment[20,35]. With results of this study, intergenic mutations with pleiotropic effects can be added to the list of important adaptive changes in this pathogen. However, it is unknown how widespread these pleiotropic effects are caused by intergenic mutations and whether they follow similar patterns. This can be investigated by allelic replacement of other mutations in laboratory strains or reversion of natural mutations to WT in adapted strain and further application of high-throughput RNA-seq or microarray to study pleiotropic effects.

While intergenic mutations confer independent roles in expression of genes, we identified multiple cases where presence of additional mutations was necessary for induction of the effect. In this context, the pleiotropic effect of *phuR* mutation was mostly present in adapted isolate of DK2-CF30-1979. This isolate contains all global regulatory mutations essential for rapid adaptation to the CF airway. We therefore propose that epistatic interactions are vital for induction of intergenic mutations effect. While intergenic mutations may confer independent effects on expression of downstream genes, the occurrence and contribution of intergenic mutations are largely intertwined with intragenic mutations. In essence, targets of intergenic mutations are components of regulatory network involved in phenotypic

acclimation and regulation of genes. Therefore in reality adaptation occurs through interaction of changes in both intergenic and intragenic regions.

One related limitation of our study is that we tested the effect of intergenic mutations in neutral laboratory backgrounds because it is easier to genetically manipulate and grow such strains in phenotype experiments. Although, we observe independent localized effects for many intergenic mutations in laboratory background, this compromise has to be considered when extrapolating results to actual conditions in CF airways. The same argument goes for testing mutations under controlled conditions of rich or minimal media. We demonstrated in all three studies that local or global effects of intergenic mutations are highly contingent on environmental context. Therefore it is difficult to extrapolate these results to actual condition of CF airways. To overcome these limitations, intergenic mutations can be tested in their naturally occurring isolates and be screened in *in vitro* models mimicking in *vivo* CF environment[140]. Alternatively feasible animal models like mouse lung can be utilized for *in vivo* analysis of intergenic mutations[141]. Intergenic mutations affecting biofilm developments can be tested in flow-chamber biofilms[142].

Studying evolution of bacteria through intergenic mutations is vital for comprehension of their pathogenic behavior in infections. When considering infection caused bacteria, major issues such as antibiotic resistance are common emerging threats posed by pathogens. With diminishing success in production of new antibiotics, alternative novel strategies have to be designed for control and eradication of bacterial infections. Investigating molecular mechanisms of resistance evolution is critical for design of these strategies. In our study, we demonstrated that genes related to antibiotic resistance and susceptibility are common targets by adaptive intergenic mutations. Considering interactions of intergenic and intragenic mutations is a new dimension in evolution of resistance. For example, we observed frequent co-occurrence of mutations upstream of *ampC* and within its coding regions where expression and activity of this β-lactamase can be controlled by intergenic and intragenic mutations.

Investigations of bacterial adaptation through intergenic mutations should not be limited to *P. aeruginosa* in the CF environment. Adaptive intergenic mutations have been observed in experimental or natural evolution of other

bacteria[28,136], therefor one can anticipate that this type of mutation is a major mediator of adaptation in bacteria. Nonetheless, while general results such as higher numerical contribution of intergenic mutations can be extrapolated to adaptation of other bacteria, considering intergenic mutations is critical for comprehension of evolutionary dynamics and adaptive systems in other bacteria. The methods and objectives of this thesis can serve an inspiration for future investigations of intergenic mutations in other bacteria.

Modulating expression of genes can be a key factor in biotechnology where productions of important life-saving molecules are carried out in bacterial cell factories. Fine-tuning of promoters in prokaryotic systems can increase expression of a desired protein[143]. Directed evolution of genes lead to selection of desired proteins for production of molecules[144]. Alternatively, evolution of *cis*-regulatory elements potentiates greater success for overexpression of products. Studying evolution provides critical knowledge for manipulation of bacteria because natural selection favors beneficial traits important for fitness. By experimental evolution, bacteria are forced to genetically adapt in new environments. Harnessing this knowledge can be applied for genetic manipulation of *cis*-regulatory elements in bacteria to improve yields of desired products or induce production of new novel products.

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Chapter 8

Research papers

The following chapter consists of full-length published articles or manuscripts prepared as part of my PhD project. The articles are enclosed in the following order:

Article 1

Marvig RL*, Damkiær S*, **Khademi SMH***, Markussen TM, Molin S, Jelsbak L. (2014) Within-Host Evolution of *Pseudomonas aeruginosa* Reveals Adaptation Towards Iron Acquisition from Hemoglobin. *mBio* 5(3):e00966-14. doi:10.1128/mBio.00966-14.

Article 2

Khademi SMH, Jelsbak L. (2017) Contribution of non-coding intergenic mutations on within-host evolution of a human pathogen. *Manuscript submitted to Nature Microbiology*.

Article 3

Khademi SMH, Wassermann T, Kvich LA, Bjarnsholt T, Ciofu O, Jelsbak L. (2017) Adaptive mutation in a bacterial intergenic region cause pleiotropic effects on gene expressions. *Manuscript in preparation*.

* Denotes equal contribution

RESEARCH ARTICLE

Within-Host Evolution of *Pseudomonas aeruginosa* Reveals Adaptation toward Iron Acquisition from Hemoglobin

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ABSTRACT ***Pseudomonas aeruginosa*** **airway infections are a major cause of mortality and morbidity of cystic fibrosis (CF) patients. In order to persist, *P. aeruginosa* depends on acquiring iron from its host, and multiple different iron acquisition systems may be active during infection. This includes the pyoverdine siderophore and the *Pseudomonas* heme utilization (*phu*) system. While the regulation and mechanisms of several iron-scavenging systems are well described, it is not clear whether such systems are targets for selection during adaptation of *P. aeruginosa* to the host environment. Here we investigated the within-host evolution of the transmissible *P. aeruginosa* DK2 lineage. We found positive selection for promoter mutations leading to increased expression of the *phu* system. By mimicking conditions of the CF airways *in vitro*, we experimentally demonstrate that increased expression of *phuR* confers a growth advantage in the presence of hemoglobin, thus suggesting that *P. aeruginosa* evolves toward iron acquisition from hemoglobin. To rule out that this adaptive trait is specific to the DK2 lineage, we inspected the genomes of additional *P. aeruginosa* lineages isolated from CF airways and found similar adaptive evolution in two distinct lineages (DK1 and PA clone C). Furthermore, in all three lineages, *phuR* promoter mutations coincided with the loss of pyoverdine production, suggesting that within-host adaptation toward heme utilization is triggered by the loss of pyoverdine production. Targeting heme utilization might therefore be a promising strategy for the treatment of *P. aeruginosa* infections in CF patients.**

IMPORTANCE **Most bacterial pathogens depend on scavenging iron within their hosts, which makes the battle for iron between pathogens and hosts a hallmark of infection. Accordingly, the ability of the opportunistic pathogen *Pseudomonas aeruginosa* to cause chronic infections in cystic fibrosis (CF) patients also depends on iron-scavenging systems. While the regulation and mechanisms of several such iron-scavenging systems have been well described, not much is known about how the within-host selection pressures act on the pathogens' ability to acquire iron. Here, we investigated the within-host evolution of *P. aeruginosa*, and we found evidence that *P. aeruginosa* during long-term infections evolves toward iron acquisition from hemoglobin. This adaptive strategy might be due to a selective loss of other iron-scavenging mechanisms and/or an increase in the availability of hemoglobin at the site of infection. This information is relevant to the design of novel CF therapeutics and the development of models of chronic CF infections.**

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Iron is an essential component for virtually all forms of life. This includes bacterial pathogens that depend on acquiring iron from their hosts in order to replicate and cause disease (1). A general defensive mechanism of the host is therefore to withhold iron from invading bacteria to prevent their growth, but this defense is countered by bacterial pathogens since they possess specific systems to scavenge iron from their hosts. While the regulation and mechanisms of several of such iron-scavenging systems are well described (1), not much is known about how the within-host selection pressures act on the pathogens' ability to acquire iron. This is especially relevant in relation to long-term chronic infections in which invading bacteria acquire adaptive mutations in response to the selective pressures encountered in the host.

The opportunistic pathogen *Pseudomonas aeruginosa* is a common environmental inhabitant which is capable of causing long-term chronic infections in the airways of patients with cystic fibrosis (CF), and *P. aeruginosa* infections are directly associated with the morbidity and mortality of CF patients. Chronic infections in CF patients provide an opportunity for long-term monitoring of the battle between the infecting bacteria and the host (2–6) and thus offer an opportunity for observing evolutionary adaptation of *P. aeruginosa* to the human host environment.

Most iron in the human body is bound in hemoglobin, which is an oxygen transport protein in red blood cells (1). If not bound by essential proteins, such as hemoglobin, iron is withheld and stored by binding to proteins like transferrin, lactoferrin, and ferritin. *P. aeruginosa* is known to scavenge iron from the human host by both siderophore-based systems and heme acquisition systems (7).

Siderophores are low-molecular-weight molecules secreted by

bacteria. The strong association of iron to siderophores enables them to remove iron from the human iron storage proteins, whereupon the siderophore-iron complex can be taken up by cognate receptors at the bacterial surface. The major siderophores secreted by *P. aeruginosa* are pyoverdine and pyochelin (7), and iron-loaded pyoverdine and pyochelin are taken up by the outer membrane receptors FpvA and FptA, respectively (8–10).

Alternatively, iron contained in the heme group of hemoglobin can be taken up by either of two heme uptake systems in *P. aeruginosa*. The two systems are the *Pseudomonas* heme utilization (*phu*) system and the heme assimilation system (*has*) (11). The two systems are different in the sense that the *phu* system is dependent on the direct uptake of heme by the outer membrane receptor PhuR, whereas the *has* system encodes a secreted hemophore, HasA, that returns heme to an outer membrane receptor, HasR.

Furthermore, *P. aeruginosa* can take up ferrous iron through the *feo* system (12) or ferric citrate through the *fec* system (13).

It is not clear in which way the different iron uptake systems in *P. aeruginosa* play a role for survival in the lungs of CF patients. Detection of pyoverdine in the sputa of some CF patients has led to the suggestion that pyoverdine plays a key role in the infection process (14, 15). On the other hand, measurements of the transcription levels of iron uptake systems in sputum samples have suggested that multiple systems are active and that siderophore-mediated uptake may not be the dominant iron acquisition mechanism in all patients (16, 17).

In an effort to understand the genetic adaptation of *P. aeruginosa* to the CF airways, we recently mapped all mutational changes in the *P. aeruginosa* DK2 lineage as it spread among 21 Danish CF patients by interpatient transmission (2). The study showed that the selective forces driving the evolution of *P. aeruginosa* in the CF airways could be inferred from convergent evolution of DK2 sublineages evolving in parallel in separate hosts. Here we further analyzed the genomic data, and we provide evidence that within-host evolution of *P. aeruginosa* is characterized by adaptation toward iron acquisition from hemoglobin.

RESULTS AND DISCUSSION

Parallel evolution of mutations in the promoter regions of the *phu* system. It is known that *P. aeruginosa* undergoes genetic adaptation to CF patients during long-term chronic infections, and several studies have sequenced the genomes of *P. aeruginosa* isolates sampled longitudinally from the airways of CF patients to map the mutations that accumulate during infection (2–6). In one such study, we mapped all the mutations that had occurred in the *P. aeruginosa* DK2 lineage during 36 years of infection (2). Whole-genome analysis of 55 DK2 isolates enabled a fine-grained reconstruction of the evolutionary relationship of the DK2 lineage, and the study identified several genes to be targeted by mutation to optimize pathogen fitness within the host environment (pathoadaptation). Nonetheless, only intragenic mutations (i.e., mutations within genes) were examined to identify such pathoadaptive patterns of mutation. Here, we therefore reanalyzed the data with respect to intergenic regions, since selection might also act on such sequences due to their role in regulation and transcription of neighboring genes.

The 6,402,658-bp genome of the *P. aeruginosa* DK2 strain contains 4,883 intergenic regions with an average size of 146 bp, and the intergenic regions constitute a total of 714,368 bp. Marvig et al. (2) found 1,365 intergenic mutations, meaning that one would expect an average-length intergenic region to be hit by 0.3 mutations (or 0.0019 mutation/bp). Searching for recurrent patterns of mutation of the same genetic loci makes it possible to identify positive selection for mutations affecting genes important for host adaptation (2, 18, 19). We therefore focused on the intergenic regions with the highest densities of mutations and interestingly found the 180-bp intergenic region containing the promoters of the *phu* system to be the most frequently mutated, with a total of 13 mutations (0.072 mutation/bp) (Fig. 1). This number of mutations is 38-fold higher than what would be expected by chance and represents a significant increase in mutation density [$P(X \geq 13) \sim \text{pois}(X; 0.342) = 2.22\text{e-}16$, where $P(X \geq 13)$ is the probability of observing ≥13 mutations given a Poisson distribution with a mean of 0.342 mutations (0.0019 mutation/bp ∗ 180 bp)].

All of the 13 mutations are located within a narrow region from position −91 to −21 relative to the start codon of *phuR*, and eight of the mutations are within the annotated promoter regions of the *phu* system (Fig. 2). Furthermore, two positions (positions −35 and −57) were subject to convergent evolution, since they were independently mutated in parallel evolving DK2 sublineages.

Correlation between promoter mutations and *phu* transcription in isolates DK2-CF173-2005 and DK2-CF66-2008. Using Affymetrix GeneChips, we have previously measured the full transcriptomes of six of the 11 DK2 isolates listed in Fig. 1 (4), including four early DK2 isolates without *phu* promoter mutations and two isolates, DK2-CF173-2005 and DK2-CF66-2008, with *phu* promoter mutations. We hypothesized that the mutations, due to their location immediately upstream of *phuR* and *phuSTUVW*, could cause an effect on the transcription of the *phu* system. Accordingly, we found the transcription of the *phuRSTUVW* genes to be upregulated in both of the mutated isolates (DK2-CF173-2005 and DK2-CF66-2008) relative to that for their ancestors and a laboratory reference strain PAO1 (Fig. 3). Most highly upregulated was *phuR*, showing 116- and 25-fold upregulation, respectively, but also, the genes of the *phuSTUVW* operon were on average upregulated 8- and 4-fold, respectively.

The *phu* system is negatively regulated by the ferric uptake regulator (Fur) (11). As an alternative hypothesis, we therefore speculated that the increased transcription of the *phu* system in DK2-CF173-2005 and DK2-CF66-2008 might be due to a decreased level or activity of the Fur protein. Nonetheless, no mutations or changes in transcription of the *fur* gene were found (Table 1) (2).

Furthermore, in order to determine if iron acquisition systems in general were subject to evolutionary changes in transcription, we searched the transcriptomes for other iron acquisition systems to be differentially transcribed. This search revealed that the *feo* operon, encoding a ferrous iron uptake system (12), was upregulated in DK2-CF66-1973 and the four isolates sampled after 1973 (Table 1), indicating that several iron acquisition systems might play a role in adaptation of *P. aeruginosa* to the human host airways.

Effect of intergenic mutations on activities of *phu* system promoters. To further investigate the effect of the *phu* promoter mutations on the activity of the *phuR* promoter, we cloned the *phuR* promoter region from six of the mutated DK2 clones in front of a luciferase reporter (*luxCDABE*) and chromosomally integrated the transcriptional fusion into *P. aeruginosa* PAO1 at the *attB* site by use of the mini-CTX2-derived plasmid pHK-CTX-lux. The transcriptional fusions enabled us to compare *phuR*::*lux*

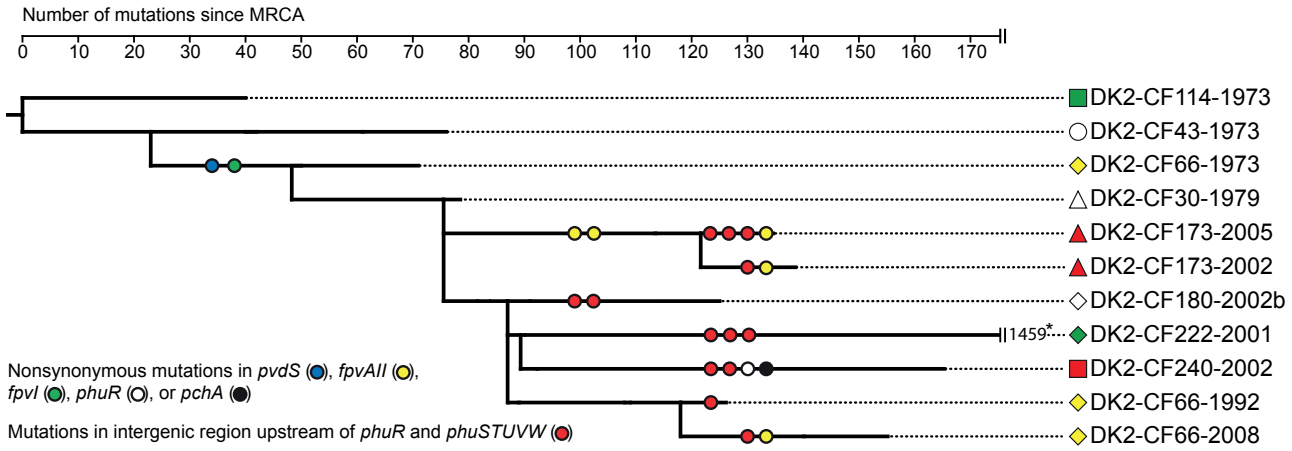

FIG 1 Maximum-parsimony phylogenetic tree showing the genetic relationship of the 11 DK2 clones included in this study. The phylogenetic tree is a subset of a phylogenetic tree from the work of Marvig et al. (2), who recently reported the genome sequences of 55 DK2 isolates. The shown tree depicts the genetic relationship of the 11 DK2 isolates included in this study, and it represents a total of 1,827 mutations (1,486 SNPs and 311 insertion/deletions) identified from whole-genome sequencing. Lengths of branches are proportional to the numbers of mutations except in the case of the truncated branch leading to isolate DK2-CF222-2001. For this hypermutator isolate, the large number of mutations is indicated at the end of the truncated branch. We searched the genomes for nonsynonymous mutations within genes encoding components of the pyoverdine, pyochelin, *phu*, *has*, *feo*, and *fec* iron acquisition systems (7, 11–13), and circles on the evolutionary branches denote that the specified gene is mutated in the branch. Due to the large number of mutations in the branch leading to the hypermutable isolate DK2-CF222-2001, only *phuR* and *phuSTUVW* intergenic mutations are specified. *, in addition to the three *phuR* and *phuSTUVW* intergenic mutations, this branch also contains nonsynonymous mutations in *pvdS*, *pvdL*, *fpvI*, the FpvAII gene, *fpvR*, *phuR*, *fptA*, *pchH*, *pchG*, *pchF*, *pchE*, and *pchD* (2).

expression from the mutated promoter regions (M1 to M6) (Fig. 2) relative to the expression from a construct with a wild type promoter region (WT) (Fig. 2). A construct without an inserted promoter region was used to correct for background expression from *lux* gene cassette integration.

Measurements of *phuR*::*lux* expression at exponential growth (optical density at 600 nm [OD_{600}] = 0.15) in Luria-Bertani (LB) medium revealed that all six mutant alleles (M1 to M6) caused a significant increase in promoter activity, with changes in expression from 5- to 112-fold (Table 2). The largest increases in expressions (93- and 112-fold) were observed for the alleles M1 and M2, originating with clones DK2-CF66-2008 and DK2-CF173-2005, respectively. The M1 and M2 alleles contain a 3-bp insertion and a 1-bp deletion, respectively, in the repressor-binding site (Fur box) of the Fur regulator, known to control the expression of the *phuR* promoter (11). Since Fur mediates strong repression of *phuR* under iron-rich conditions (11), we find it likely that the indels in the M1- and M2-derived *phuR* promoters alleviate Fur repression (if there is any repression from Fur).

Using the same cloning strategy, we tested a *phuS*::*lux* reporter fusion to compare the expression from the mutated promoter region of DK2-CF173-2005 to the expression from a construct

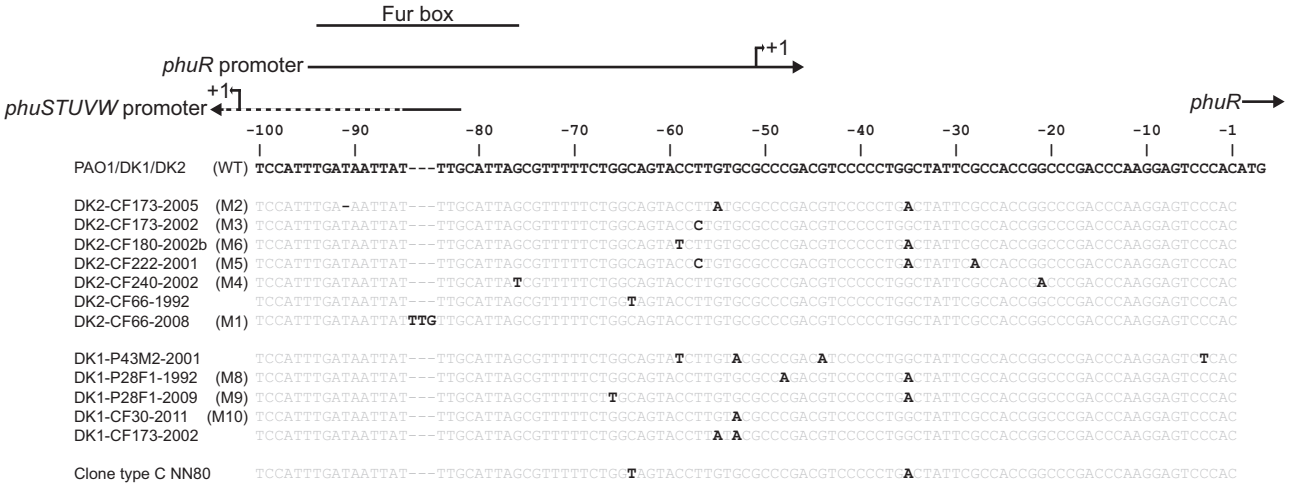

FIG 2 Overview of the intergenic region upstream of *phuR*. The alignment shows homologue sequences from different isolates with genetic variants highlighted in bold. Wild-type sequences of *P. aeruginosa* strains PAO1, DK1, DK2, and C are shown at the top of the alignment. Abbreviations of sequence alleles from different isolates are indicated in parentheses (WT and M1 to M10). Positions of promoters and a Fur box are indicated with black lines above the alignment (the *phuSTUVW* promoter is only partially shown). Positions are relative to the start codon of *phuR*.

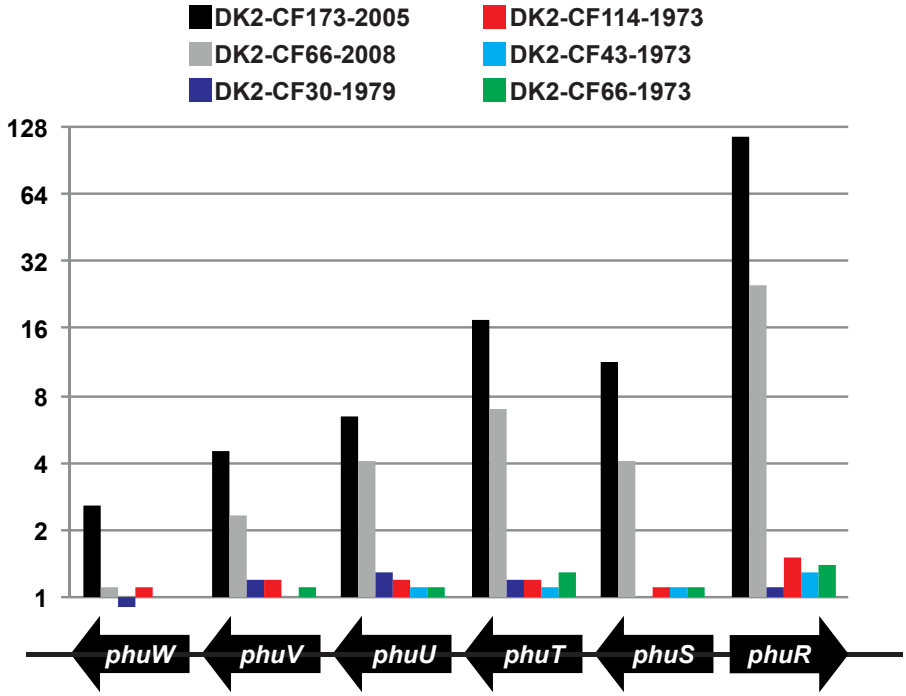

FIG 3 Relative transcriptional levels of genes encoding the *phu* system. The transcriptomes of six of the DK2 isolates included in this study have previously been measured at exponential growth phase in LB medium (4). The expression of the *phu* genes is shown for each of the six clinical isolates relative to that for laboratory reference strain PAO1. Values are averages for three replicates, and the values are normalized relative to the transcription of the respective gene in strain PAO1.

with a wild-type promoter region. Similar to the results for the *phuR* promoter, we observed that the mutations also resulted in a significant ($P = 0.01$) increase in *phuS* promoter activity (Table 2), albeit the mutations had a larger effect on the activity of the *phuR* promoter.

***phuR* promoter mutations confer a growth advantage in the presence of hemoglobin.** The increased expression from the mutated *phu* promoters suggested that there has been positive selection in the CF airways toward iron acquisition from hemoglobin. To test this hypothesis, we replaced the wild-type *phu* promoters of isolate DK2-CF30-1979 with the mutated *phu* promoters of isolate DK2-CF173-2005 by allelic replacement and tested whether the constructed mutant strain, DK2-CF30-1979-M2, had a growth advantage relative to the isogenic wild-type strain, DK2-CF30-1979. We chose to test the consequence of the *phu* promoter mutations in the genetic background of isolate DK2-CF30-1979 because this isolate is an immediate ancestor of isolate DK2-CF173-2005 (4). For the growth experiment, we used $FeCl_3$-free ABTGC minimal medium (which contains glucose and Casamino Acids), supplemented with hemoglobin and apotransferrin.

Confirming our hypothesis, we found that the allelic replacement mutant DK2-CF30-1979-M2 grew significantly faster than its isogenic wild-type counterpart when hemoglobin was present as the sole iron source (Table 3), while no difference was observed for rich medium and medium supplemented with Fe^{3+} as the sole iron source. We suggest that the growth advantage of the mutant is facilitated by an enhanced uptake of iron derived from hemoglobin.

Adaptation toward heme utilization is a general adaptive mechanism. Our results demonstrate parallel adaptation of the DK2 lineage toward hemoglobin utilization in five different CF patients. This indicates that similar selective conditions for heme utilization exist across different patients. Next, we speculated on whether the acquisition of *phu* promoter mutations is an adaptive mechanism specific to the DK2 lineage or if *phuR* promoter mutations constitute a general adaptive genetic mechanism of

TABLE 1 Relative transcriptional levels of *fur* and genes encoding the *feo* iron acquisition pathway[a]

Gene	Relative transcription in strain:						
	PAO1	DK2-CF114-1973	DK2-CF43-1973	DK2-CF66-1973	DK2-CF30-1979	DK2-CF173-2005	DK2-CF66-2008
feoA	1	2.9	1.6	16.7	21.2	21.6	28.1
feoB	1	2	1.6	5.1	6	6.8	13.4
feoC	1	1.3	1.5	2.3	2.8	2.4	4.4
fur	1	1.1	1.5	1.4	0.9	1.1	1

[a] The transcriptomes of six DK2 isolates included in this study have previously been measured at exponential growth phage in LB medium (4). We searched the transcriptomes for genes encoding components of the pyoverdine, pyochelin, *phu*, *has*, *feo*, and *fec* iron acquisition systems (7, 11–13), and the table lists the transcription profiles of those systems in which at least one gene showed differential expression (>3-fold change) in the post-1973 isolates relative to that in the 1973 isolates or strain PAO1. Also, the transcription of the *fur* gene is shown. Values are averages for three replicates, and the values are normalized relative to the transcription of the respective gene in reference strain PAO1.

TABLE 2 Activities of the *phuR* and *phuS* promoters originating with different clinical isolates of *P. aeruginosa*[a]

Strain	Promoter	Origin of promoter	Allele	Mean luminescence (± SD)	Fold change	*P* value
PAO1	*phuR*	PAO1	WT	365 (±1,018)	1	
PAO1	*phuR*	DK2-CF66-2008	M1	34,111 (±3,379)	93	0.00021
PAO1	*phuR*	DK2-CF173-2005	M2	40,726 (±3,422)	112	0.00004
PAO1	*phuR*	DK2-CF173-2002	M3	1,879 (±3,422)	5	0.16
PAO1	*phuR*	DK2-CF240-2002	M4	7,584 (±496)	21	0.00038
PAO1	*phuR*	DK2-CF222-2001	M5	8,968 (±610)	25	0.00023
PAO1	*phuR*	DK2-CF180-2002	M6	6,723 (±701)	18	0.00088
PAO1	*phuR*	DK1-P28F1-1992	M8	13,329 (±1,482)	37	0.00024
PAO1	*phuR*	DK1-P28F1-2009	M9	12,205 (±603)	33	0.00007
PAO1	*phuR*	DK1-CF30-2011	M10	9,563 (±1,586)	26	0.0011
PAO1	*phuS*	PAO1	WT	7,444 (±1,777)	1	
PAO1	*phuS*	DK2-CF173-2005	M2	12,030 (±3,191)	1.6	0.01

[a] Luminescence production from laboratory reference strain PAO1 (37) with *phuR*::*lux* reporter fusions was measured at exponential growth (OD_{600} = 0.15) in Luria-Bertani (LB) medium and normalized for differences in cell density. Mean luminescence production and standard deviations (SD) were calculated for three biological replicates. Statistical analysis concerning the difference between two means was done using a Student *t* test, and the *P* values denote the probability of the mutated alleles having expression equal to that of the wild type (WT).

P. aeruginosa toward heme utilization in the CF airways. To further investigate the generality, we compared our findings to other lineages of *P. aeruginosa* isolated from CF infections.

In addition to the DK2 lineage, our previous investigations have revealed another distinct clone type, known as the DK1 clone type, which has also spread among Danish CF patients (21). We sequenced and analyzed the *phuR* promoter region of five DK1 isolates sampled in the years 1992 to 2011 in addition to an ancestral DK1 isolate from 1973. Whereas the sequence of the *phuR* promoter of the ancestral 1973 isolate (DK1-P33F0-1973) was identical to the wild-type sequence of strains PAO1 and DK2, all five evolved DK1 isolates had accumulated 1 to 4 single nucleotide polymorphisms (SNPs) in the promoter region, and three of the DK1 SNPs were identical to SNPs found in the evolved DK2 isolates (Fig. 2). We tested the activities of three of the mutated promoters from the DK1 isolates (M8 to M10) and found that all three mutated promoters resulted in increased levels of transcription, similar to what has been observed for mutated DK2 alleles (Table 2). Our results provide strong evidence for convergent adaptive evolution of different lineages of *P. aeruginosa* toward iron acquisition from hemoglobin.

To rule out that the adaptive trait was specific for *P. aeruginosa* CF infections at the Copenhagen CF Center, we analyzed the available public data for the genomic evolution of the *P. aeruginosa* C lineage, which was isolated from a patient attending the CF clinic at Hannover Medical School, Germany (6). Interestingly, the C lineage, which has colonized this patient for a period of more than 20 years, also accumulated two SNPs in the *phuR* promoter region (Fig. 2). Remarkably, the two SNPs are identical to SNPs found in the DK1 and DK2 lineages, and this observation suggests that these mutations were also positively selected for in the host environment.

The research team at Hannover Medical School also investigated the microevolution of a PA14 lineage as it infected a patient over 14 years. Nonetheless, the PA14 lineage did not accumulate SNPs in any iron acquisition systems. Likewise, a lineage investigated by Smith et al. (5) over an infection course of 90 months also did not reveal any mutations in iron acquisition systems, except for a nonsynonymous mutation in *pvdS* (which correlated with the loss of pyoverdine production) and an intergenic SNP upstream of *fptA* (5). We therefore conclude that despite an apparent selection for *phu* promoter mutations in three independent *P. aeruginosa* lineages, not all lineages accumulate *phu* promoter mutations during CF infections.

Selection against pyoverdine secretion might lead to a shift in iron source. The siderophore pyoverdine has previously been found in sputum of CF patients, and thus pyoverdine-mediated uptake of iron has been considered important for the survival of *P. aeruginosa* in the CF airways (14). Nonetheless, we observed that all three lineages (DK1, DK2, and C) had accumulated nonsynonymous mutations in the alternative sigma factor PvdS, which is required for pyoverdine synthesis (Fig. 1 and Fig. 4). Accordingly, the evolved C clone NN80 was observed to have lost its ability to produce pyoverdine, in contrast to its predecessors (C clones NN2 and NN11) (6).

This led us to examine the production of pyoverdine in the DK1 and DK2 isolates, and we observed a negative correlation between pyoverdine production and mutations in PvdS (Fig. 5).

TABLE 3 Growth rates of strains DK2-CF30-1979 and DK2-CF30-1979-M2 at exponential growth phase in different media[a]

Growth medium	Doubling time (h)		*P* value
	DK2-CF30-1979	DK2-CF30-1979-M2	
LB	1.27 ± 0.05	1.35 ± 0.07	0.16
ABTGC + 10 μM Fe^{3+}	2.74 ± 0.02	2.69 ± 0.03	0.23
ABTGC + 10 μM Fe^{3+} + 100 μg/ml apo-TF	3.08 ± 0.10	3.07 ± 0.04	0.91
ABTGC + 2.5 μM Hb + 100 μg/ml apo-TF	2.76 ± 0.24	2.13 ± 0.09	0.01

[a] The abbreviations Hb and apo-TF are used for hemoglobin and apotransferrin, respectively. Note that the ABTGC minimal medium standard recipe was modified so that no iron source other than the one stated in the table was added to the growth medium. Mean doubling times were calculated from three biological replicates. Statistical analysis concerning difference between two means was done using a Student *t* test, and the *P* values denote the probability of the two strains having equal means.

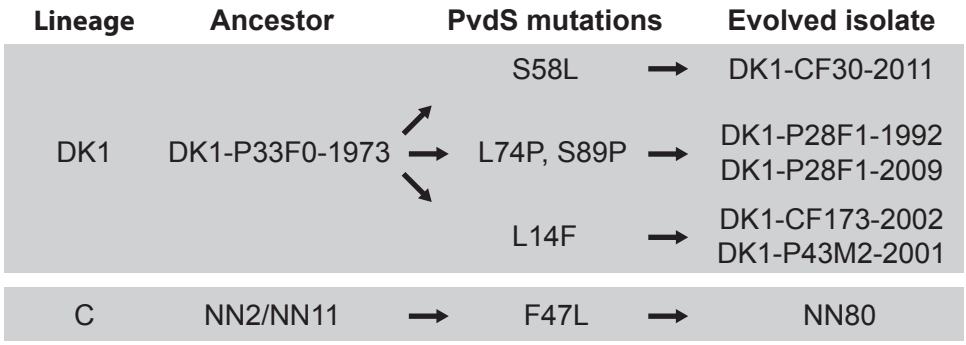

FIG 4 Overview of *pvdS* mutations in the DK1 and C lineages. Mutations that have accumulated in evolved isolates relative to sequences of their ancestor are shown. The *pvdS* mutation found in the DK2 lineage is shown in Fig. 1.

Accordingly, only the ancestral DK1 and DK2 isolates carrying wild-type alleles of *pvdS* were able to produce pyoverdine, whereas all isolates carrying mutated alleles of *pvdS* were unable to produce pyoverdine (DK1-CF173F-2002 was not tested).

Siderophores are generally regarded as highly immunogenic (22), and selection against pyoverdine production might have driven the accumulation of *pvdS* mutations, leading to a loss of pyoverdine production in the evolved isolates. At the same time, we observed a positive selection for *phuR* promoter mutations in the CF airways, leading to a bacterial growth advantage when acquiring iron from hemoglobin. We therefore propose a model in which the CF airways impose selective pressure on the invading bacteria, forcing them to adapt toward a shift to hemoglobin as an alternative iron source. This is of particular interest because inflammation may cause microbleeds, which lead to the presence of hemoglobin at the delicate CF lung epithelia in the presence of both host and bacterial proteases (23). Also, hemoglobin is reported to be expressed by alveolar epithelial cells (24).

Other iron acquisition systems might be affected by mutations. Several iron acquisition systems and mutations other than the ones that we have investigated in detail here might play a role in survival of *P. aeruginosa* in the lungs of CF patients. Accordingly, we also found nonsynonymous mutations in the FpvAII gene and the genes *fpvI*, *fpvR*, *phuR*, *pchA*, *pchDEFGH*, and *fptA* when searching for mutations in genes of the pyoverdine, pyochelin, *phu*, *has*, *feo*, and *fec* iron acquisition systems (Fig. 1). We anticipate that the identification of such mutations can facilitate further investigations of the adaptation of *P. aeruginosa* to human

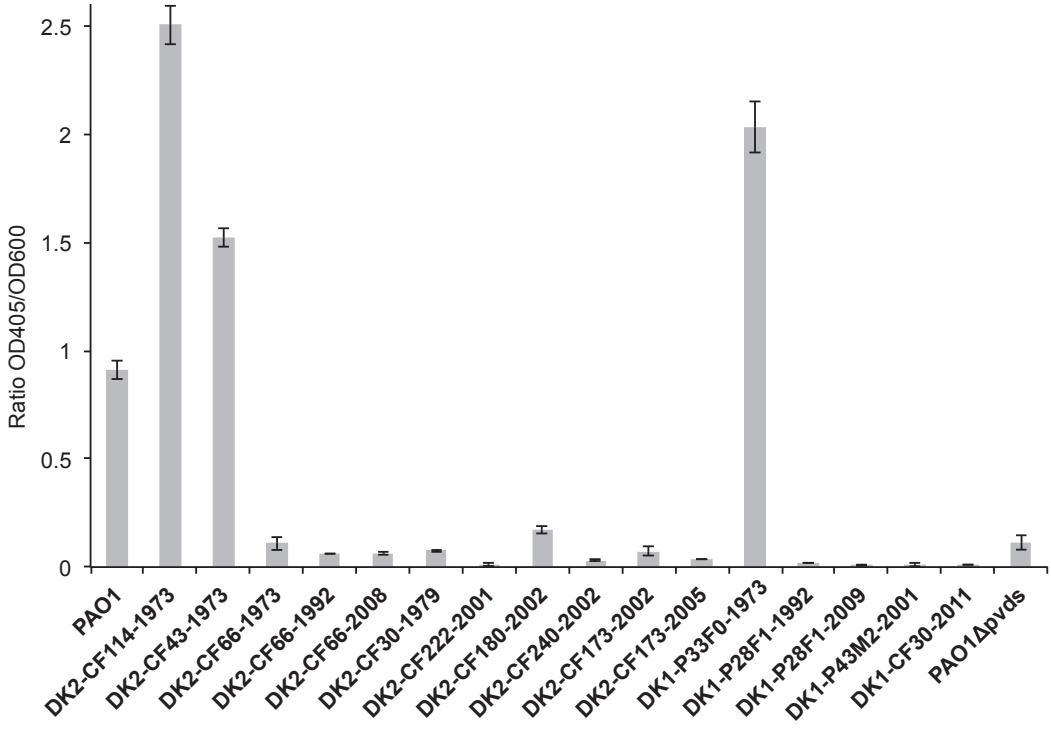

FIG 5 Pyoverdine production in isolates of *P. aeruginosa*. The presence of pyoverdine secreted into the supernatant of bacterial cultures grown in pyoverdine-inducing medium was quantified by measurement of the absorbance at OD_{405} and normalized against the cell density (OD_{600}). The means and standard deviations calculated from three biological replicates are shown in the bar plot.

host airways. For example, it remains to be elucidated whether the mutations in the *pch* and *fptA* genes affect the function the pyochelin iron uptake system in the DK2 lineage and if isolates with mutations in the pyoverdine system are unable to cheat on other pyoverdine producers.

Conclusions and implications. Our results provide evidence that the selective conditions by which evolution is directed in the CF airways can result in acquisition of *phu* promoter mutations in *P. aeruginosa* during chronic CF infections and that such mutations provide a growth advantage in relation to acquisition of iron from hemoglobin. This adaptive trait may be directly selected for due to an abundance of heme-bound iron in the CF lung. Furthermore, we also observed that *phu* promoter mutations coincided with the loss of pyoverdine production, suggesting that selection for increased heme utilization may be secondary to the loss of the pyoverdine iron uptake system. Therefore, targeting heme utilization might be a promising strategy for the treatment of CF infections.

CF patients commonly experience iron deficiency, and *P. aeruginosa* possibly contributes to iron deficiency by depletion of the host iron storage and by causing inflammation (25, 26). In this regard, expanding our knowledge of adaptation of *P. aeruginosa* to the CF lung may help to lessen the impact of *P. aeruginosa* infection and improve the condition of patients.

MATERIALS AND METHODS

Bacterial strains and media. Isolates of the *P. aeruginosa* DK1 and DK2 clone types were sampled from Danish CF patients attending the Copenhagen Cystic Fibrosis Clinic. Isolation and identification of *P. aeruginosa* from sputum were done as previously described (27). The isolates are named according to their clone type, the patient from whom they were isolated, and their isolation year (e.g., isolate DK2-CF30-1979). Luria-Bertani (LB) broth was used for routine preparations of bacterial cultures. ABTGC minimal medium was composed of 2 g/liter $(NH_4)_2SO_4$, 6 g/liter Na_2HPO_4, 3 g/liter KH_2PO_4, 3 g/liter NaCl, 1 mM $MgCl_2$, 0.1 mM $CaCl_2$, 0.01 mM $FeCl_3$, 2.5 mg/liter thiamine supplemented with 1% glucose, and 0.5% Casamino Acids. For the growth rate experiments (Table 3), no $FeCl_3$ was added to ABTGC minimal medium unless otherwise stated. Human hemoglobin (Sigma-Aldrich) and human apotransferrin (Sigma-Aldrich) were added to concentrations of 2.5 μM and 100 μg/ml, respectively. Pyoverdine-inducing medium was composed of ABTGC minimal medium with 50 μM iron chelator 2,2′-dipyridyl (DIPY). *Escherichia coli* strain CC118(λ*pir*) was used for maintenance of recombinant plasmids (28) in medium supplemented with 8 μg/ml of tetracycline. Allelic replacement constructs were transferred to *P. aeruginosa* by triparental mating using the helper strain *E. coli* HB101/pRK600 (29). For marker selection in *P. aeruginosa*, 50 μg/ml of tetracycline was used. Genetic techniques were performed using standard methods, and Sanger sequencing was used for verification of genetic construct and allelic replacement mutants.

Sequencing of *phuR* promoter region and *pvdS* gene in DK1 isolates. Sequencing of DK1 isolates was performed as described earlier (4). Accordingly, genomic DNA was purified from *P. aeruginosa* isolates using a Wizard Genomic DNA purification kit (Promega, Madison, WI) and sequenced on Illumina's GAIIx or Hiseq2000 platform. Reads were mapped against the reference genome sequence using the software program Novoalign (Novocraft Technologies, Selangor, Malaysia) (30), and pileups of read alignments were produced by the software program SAMtools, release 0.1.7 (31).

Construction of reporter fusions and luminescence measurements. The *lux* gene cassette (*luxCDABE*) was subcloned from the plasmid pUC18-mini-Tn7T-Gm-*lux* (32) fragment into mini-CTX2 (33) using the restriction sites XhoI and PstI to produce pHK-CTX2-*lux*, used for the transcriptional fusion experiments. For *phuR*::*lux* reporter fusions, a 220-bp fragment containing the intergenic region upstream of *phuR* was amplified from genomic DNA using Phusion polymerase (Thermo Scientific) with the primers PhuR_F-PstI (5′ GAGACTGCAGAGGCTGGGAG TGCTGCTCAT 3′) and PhuR_R-XhoI (5′ ACATCTCGAGAAGGGCGG GGAGAGCGGCAT 3′) and ligated with T4 DNA ligase into pHK-CTX2-*lux* after double digestion of the PCR fragment and vector with the restriction enzymes XhoI and PstI. For *phuS*::*lux* reporter fusions, a 220-bp fragment containing the intergenic region upstream of *phuS* was amplified with the primers PhuS_F-XhoI (5′ ACATCTCGAGAGGCTG GGAGTGCTGCTCAT 3′) and PhuS_R-PstI (5′ GAGACTGCAGAAGG GCGGGGAGAGCGGCAT 3′) and ligated into pHK-CTX2-*lux* after double digestion of the PCR fragment and vector with the restriction enzymes XhoI and PstI. The resulting plasmids were introduced into *P. aeruginosa* strain PAO1 by transformation as previously described (32).

Allelic replacement of *phuR* promoter region in DK2-CF30-1979. A 1,296-bp fragment containing the intergenic region upstream of *phuR* was amplified from genomic DNA of DK2-CF173-2005 using Phusion polymerase (Thermo Scientific) with the primers PhuSi_F-XbaI (5′-ACATT CTAGACGGACGTCGCTGGCCTCG-‘3) and PhuRi_R-SacI (5′-GAGA GAGCTCTCTCGTGGCCCTGGCGGTAG-3′). The PCR fragment was ligated into the vector pNJ1 (34) after digestion with the restriction enzymes XbaI and SacI. The allelic replacement construct was transferred into strain DK2-CF30-1979 by triparental mating, and merodiploid mutants were selected by plating the conjugation mixture on LB agar plates with tetracycline. Colonies were restreaked on selective plates before being streaked on 8% (wt/vol) sucrose-LB plates without NaCl. Sucrose-resistant and tetracycline-sensitive colonies were restreaked on sucrose-LB plates and screened for the presence of mutated alleles by PCR followed by restriction fragment length polymorphism (RFLP) analysis. Positive mutants were finally sequenced by Sanger sequencing at LGC genomics (Germany).

Measurement of growth and luminescence in reporter fusion strains. Overnight cultures of the reporter fusion strains were diluted 40 times in fresh LB, and aliquots of 100 μl were transferred to a black (clear-bottom) 96-well microtiter plate (Nunc). Three technical replicates were used for each strain, and measurements of growth (OD_{600}) and luminescence were recorded in a Synergy Hybrid H1 reader (Bio-Tek) with 6-min intervals for 10 h and under shaking conditions (200 rpm) at 37°C. Data were analyzed using a custom-made script in the R software environment, version 2.15.2 (35). The experiment was repeated three times to obtain biological replicates.

Growth rate measurements. Growth rate experiments were carried out in 250 ml baffled shake flasks containing 50 ml of growth medium under shaking (200 rpm) at 37°C. Culture flasks were inoculated to a starting OD_{600} of 0.005 in 50-ml minimal medium, and measurements of OD_{600} were started 9 h after the inoculation and recorded every 30 min. In the experiment where the cells were cultivated in LB, the measurements were started after 2 h. The experiment was stopped when the cells reached stationary growth phase, typically after around 23 h of growth in minimal medium. Growth experiments were repeated three times for each strain under each condition to obtain biological replicates.

Pyoverdine quantification assay. Pyoverdine concentrations were quantified as previously described (36). All strains were grown in pyoverdine inducing medium for up to an OD_{600} of >1.5. Cultures were moved into 2-ml microcentrifuge tubes and centrifuged at 16,000 × *g* for 2 min. The supernatants were diluted in 100 mM Tris-HCl buffer (pH 8), and pyoverdine concentrations were quantified by measurement of the absorbance at OD_{405}. Finally, the values of absorbance at OD_{405} were normalized against the cell densities (OD_{600}) for each strain. The procedure was repeated for three independent biological replicates.

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Contribution of non-coding intergenic mutations on within-host evolution of a human pathogen

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Bacterial pathogens evolve during the course of infection as they adapt to the different selective pressures that confront them inside the host. The evolutionary mechanisms that operate *in vivo* are not fully understood and determining the molecular basis of beneficial changes that underlies host adaptation remains a central challenge. Broadly defined, adaptive mutations can be divided into two functionally distinct types: Mutations that change protein structure and function (*i.e.* mutations within coding regions) or mutations that modify protein expression levels (*i.e.* mutations in intergenic *cis*-regulatory elements). Studies of pathogen adaptation have focused predominantly on molecular evolution within coding regions whereas the role of adaptive mutations in intergenic regions has received comparably less attention. As a consequence, the extent to which intergenic mutations contribute to bacterial host adaptation remains unclear.

Here, we analyze recurrence of evolution in intergenic regions in 44 clonal lineages of the opportunistic pathogen *Pseudomonas aeruginosa* as they adapt to their human hosts. We identify 88 intergenic regions in which parallel molecular evolution occur in multiple lineages or isolates. At the genetic level, we find that mutations in these regions under selection are most often located upstream of transcriptional start sites, and within regulatory elements. At the functional level, we show that these mutations may both create or destroy regulatory interactions in connection to transcriptional processes, and that they are directly responsible for the evolution of important pathogenic phenotypes such as reduced sensitivity to antibiotics. Importantly, our results show that intergenic mutations are more likely to be selected than coding region mutations, and thus contribute more to this pathogen´s host adaptation than previously realized.

Results

Parallel evolution in intergenic regions in P. aeruginosa.

To investigate the contribution of intergenic mutations to bacterial adaptation to the selective pressures in the host, we considered data from seven studies[1–7] in which multiple clonal *P. aeruginosa* isolates have been sampled and sequenced during the course of infection in subjects with cystic fibrosis (CF). We focused our analysis exclusively on intergenic regions in which mutations were acquired during infection, and included only intergenic regions also present in the PAO1 reference genome[8]. In total, we identified 3,489 mutations (2,025 SNPS and 1,464 indels) in the intergenic regions of the 44 different *P. aeruginosa* clone types included in our data set (Supplementary Table 1). Since the majority of regulatory elements in the bacterial genome range between 5-30 bp in length[9], we considered an intergenic mutation within a region as potentially beneficial only when at least two additional distinct clone types contained a mutation in the same intergenic region and when these mutations would all be clustered in a narrow region of less than 30 bp. Furthermore, we imposed the criteria that this cluster of mutations should be positioned less than 200 bp from at least one of the neighboring genes. The probability of finding three distinct clone type mutations within a narrow cluster of 30 bp in an intergenic region within our dataset is 23 folds higher than what would be expected by chance and a significant increase in mutation density (Online Methods, Poisson, P = 1.07e-5). Applying these criteria, we identified 62 intergenic regions in which mutations have accumulated in parallel in different clone types (Figure 1).

Since certain *P. aeruginosa* clone types are transmissible and can form clinic-specific outbreaks among patients[4,10,11], we also analyzed if distinct intergenic mutations had

accumulated in parallel among clonal isolates within each of the 44 clone type. We identified 41 intergenic regions in which three or more distinct mutations (less than 30 bp apart) had accumulated in isolates of the same clone type (Figure 1). Interestingly, 15 of these regions are also represented among the 62 regions identified in our analysis of parallel mutations between clone types providing further support for the importance of these mutations in adaptation of *P. aeruginosa* to the CF environment (Figure 1). In total, we identify 88 intergenic regions that evolved under the pressure of natural selection within the hosts. The connection between these 'pathoadaptive' regions and their flanking genes identify genetic systems with importance for pathogen adaptation and provide insight into the selective forces that operate on the pathogen.

Pathoadaptive intergenic mutations target distinct cellular functions.

To investigate cellular functions that were potentially affected by pathoadaptive intergenic mutations, we recorded the PseudoCap functional class[12] of the two genes flanking each of the 62 intergenic regions that had acquired mutations in parallel in different clone types (Supplementary Table 4). This analysis revealed an over-representation of the classes '*antibiotic resistance and susceptibility*' and '*energy metabolism*' (Binomial, $P < 0.05$, n = 124, Supplementary Table 5).

Successful bacterial pathogenesis depends on both metabolic adaptation to exploit the available nutrients for growth[13] as well as mechanisms to tolerate antibiotics and other inhibitors in the host[14]. In the case of *P. aeruginosa*, our data show that these two critical processes are targets of molecular evolution in intergenic regions during CF infection. Similar functional targets have been found in several other studies

84 focusing on pathoadaptive coding regions[15,16,1,4,6], which suggest that little if any
85 qualitative difference exist between adaptive mutations in intergenic and coding
86 region sequences at this level of analysis. We also note that our data revealed a
87 substantial level of interaction between intergenic and coding sequence mutations,
88 suggesting that these mutational processes are not completely disconnected. The
89 average frequency of co-occurrence between intergenic mutations and mutations in
90 the flanking coding sequence was 11% among the 62 pathoadaptive regions selected
91 across clone type (Supplementary Table 6). For example, 36% of the isolates that
92 contain adaptive mutations in the intergenic region of *phuR-phuSTUVW* genes
93 (which result in increased expression of the *phuR* and *phuSTUVW* encoded heme
94 uptake system)[17], also contain mutations in the outer membrane heme receptor
95 *phuR* gene (Supplementary Table 6). Regulatory mutations can potentiate evolution
96 of complex phenotypes by increasing the effect of other (structural) mutations[18], and
97 it is possible that the co-occurrences of intergenic and coding sequence mutations
98 discovered here exemplify related interplays between regulatory and structural
99 mutations.
100
101 *Intergenic mutations frequently target promoter sequences.*
102 We next analyzed the genomic distribution of intergenic mutations. Non-coding
103 intergenic regions are distributed across the genome in three possible orientations:
104 1) upstream of two genes, 2) downstream of two genes and 3) upstream of one gene
105 and downstream of one gene (Figure 2a). We found an over-representation of
106 mutations upstream of two genes among the pathoadaptive regions selected across
107 clone types (Binomial, P = 0.003, n = 62, Figure 2b). This bias towards selection of

intergenic mutations upstream of genes suggest that the majority of intergenic mutations target potential *cis*-regulatory elements such as the core promoter, transcription factor binding sites, ribo-regulators, or translational elements, and consequently influence protein expression levels by affecting transcriptional or posttranscriptional processes.

To further explore this hypothesis, we analyzed the complete set of 88 pathoadaptive regions for the presence of known regulatory elements (Online Methods), and mapped the overlap between these putative regulatory sites and the identified adaptive mutations. While bacterial intergenic regions are home to a wide range of regulatory elements many of which are not well characterized, we nevertheless observed 28 regions (32%), in which the cluster of adaptive mutations was positioned within one or several putative regulatory elements. The majority of mutations within these 28 regions target the putative core promoter alone or in combination with other elements (Figure 2c), suggesting that intergenic mutations frequently target sequences important for transcriptional processes. In support of this, we observed that intergenic mutations were more frequently located upstream of known transcriptional start sites (TSS) (37 cases) than downstream (10 cases) (Supplementary Table 7).

Pathoadaptive intergenic mutations change transcriptional activity of genes involved in host interaction, metabolism, and antibiotic susceptibility.

To further explore this potential relationship between intergenic mutations and transcription, we quantified the effects of a subset of intergenic mutations on transcription of downstream genes. To this end, we constructed transcriptional

fusions of both wild-type and mutant intergenic alleles with the luciferase reporter (*luxCDABE*) genes and integrated single copies of the fusions at the neutral *attB* site[17] in the chromosome of *P. aeruginosa* PAO1. The DK2 clone type contains a large proportion of the 88 pathoadaptive intergenic regions (Figure 1), and we measured the transcriptional activity of DK2-specific alleles of 25 randomly selected regions in which pathoadaptive mutations were located upstream of either one or two genes. This selection resulted in a total of 32 transcriptional fusions, which represent 33% of all possible fusions within the complete set of 88 pathoadaptive regions. In addition, for one of the intergenic regions (*ampR*//*ampC*), we tested two alleles each with different mutations (Supplementary Table 9 and Supplementary Figure 1).

Measurements of *lux* expression during exponential growth in Luria-Bertani (LB) medium and ABTGC minimal medium[19] revealed significantly altered expressions in 16 of 34 tested fusions in at least one of the two conditions (Student t test, $P < 0.05$) (Figure 3). Altered expression was in most cases moderate (<3-fold change) and ranged between -3.1 to 22.1 fold changes for the mutant allele compared to that of wild type (Figure 3). Interestingly, ten of these 16 fusions exhibited altered expressions only in either LB or ABTGC minimal medium[19], but not in both conditions, which suggest that many adaptive intergenic mutations alter transcriptional levels while not interfering with conditional control mechanisms. Overall, our results reveal that a substantial fraction of the intergenic mutations are associated with functional (transcriptional) effects despite the fact that we recorded these effects in the non-native PAO1 genetic background (*i.e.* with removal of potential epistatic effects from the additional mutations found in DK2) and in a

155 narrow range of conditions, which most likely mean that we are not capturing the
156 full spectrum of functional effects connected to the intergenic mutations.
157 Several of the 16 fusions with altered expression relate to genes that encode
158 proteins with known functions in bacteria-host interactions, cellular metabolism, and
159 antibiotic resistance. For example, *cerN* expresses a ceramidase involved in
160 utilization of host produced sphingolipids[20], *exsC* expresses a protein involved in
161 positive regulation of the type III secretion system[21], and PA4837 is the first gene in
162 an operon (PA4837-34) involved in expression of a siderophore system essential for
163 survival in airway mucus secretions[22]. Other genes are known to play a role in
164 pyrimidine and aromatic amino acid metabolism (*pyrC* and *hmgA*, respectively).
165 Finally, two genes are linked to antibiotic resistance *rluC*[23] and *ampR*[24]. Seven genes
166 encode proteins of unknown functions and their role in relation to host adaptation
167 remains unclear.
168 Interestingly, expression changes were observed in both directions (seven mutant
169 alleles resulted in increased expression, and nine mutant alleles resulted in
170 decreased expression) (Figure 3), suggesting that pathoadaptive intergenic
171 mutations may equally well either create or destroy regulatory interactions.
172
173 *Mutations upstream of ampR and ampC enhance resistance to several antibiotics*
174 Finally, we explored the direct effects of intergenic mutations on the physiology of
175 the pathogen. As resistance towards antibiotics is a common phenotype that
176 emerges during CF infections, we selected the mutations found in the two alleles of
177 the *ampR//ampC* intergenic region for further study. Mutations in this intergenic
178 region resulted in enhanced expression of the global antibiotic resistance regulator

AmpR, but had no direct effect on expression of the AmpC β-lactamase (Figure 3). To this end, we introduced these mutations in the genome of *P. aeruginosa* PAO1 through allelic replacement (Online Methods). Since a SNP mutation (G7A) was present at the start of *ampC* gene in one of the alleles, we also made an allelic replacement of this mutation alone in the PAO1 genome to separate the effects caused by the intergenic mutations (supplementary Figure 1). For each strain and their isogenic wild type, we measured the Minimal Inhibitory Concentration (MIC) of various β-lactam antibiotics such as imipenem, ceftazidime and ampicillin from carbapenem, cephalosporin, and penicillin classes of β-lactams respectively. For both intergenic alleles, we observed a small but significant increase in the MIC of to imipenem and ampicillin (Student t test, $P < 0.01$, Figure 5), but not ceftazidime. AmpR regulates β-lactam resistance both through direct activation of AmpC expression as well as via an AmpC-independent manner[24]. Irrespectively of the mechanism, our results show that acquisition of intergenic mutations between *ampR* and *ampC* is directly linked to a relevant phenotypic alteration (*i.e.* reduced β-lactam susceptibility).

Discussion

It is now possible to begin to assess the relative contribution of intergenic and coding region mutations to pathogen adaptation. Focusing on the DK2 lineage, previous work documented parallel molecular evolution in 65 genes in this lineage[4], and here we have identified 15 intergenic regions with convergent evolution within DK2 (Figure 1). Although coding region mutations are numerically dominant over intergenic mutations, normalization to the mutational targets available for intergenic

and coding region mutations (89.8% of the *P. aeruginosa* genome contains coding regions), reveal that the ratio of adaptive intergenic to coding region mutations is close to 2:1. In other words, intergenic mutations are more likely to be selected than coding region mutations, and thus play a quantitatively more prominent role in relation to this pathogen´s host adaptation. The factors that influence the relative contribution of intergenic versus coding region mutation are difficult to disentangle, but may be related to the composition of the adaptive environment. The CF host niche is characterized by a complex combination of multiple stressors that must be mitigated for successful bacterial colonization. As such, our result resonates well with recent results showing that adaptive intergenic mutations underlie the innovation of novel functions in laboratory-evolving *Escherichia coli*[25,26].

At the functional level, our data demonstrate that the transcriptional process is the primary target of adaptive intergenic mutations. Combined with previous reports documenting that mutations in transcription factors leading to systemic remodeling of transcriptional network is frequently observed in *P. aeruginosa* CF isolates[27], our results suggest that mutations that either locally or globally change transcriptional regulatory interactions to change protein expression levels are a major mediator of *P. aeruginosa* host adaptation.

Determination of the quantitative and qualitative contributions of different categories of mutations is crucial for predictions of evolutionary trajectories during host colonization, and may inspire new therapeutical directions.

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Author contributions

S.M.H.K and L.J. conceived study and designed research. S.M.H.K. performed research. S.M.H.K and L.J. analyzed data and wrote the manuscript.

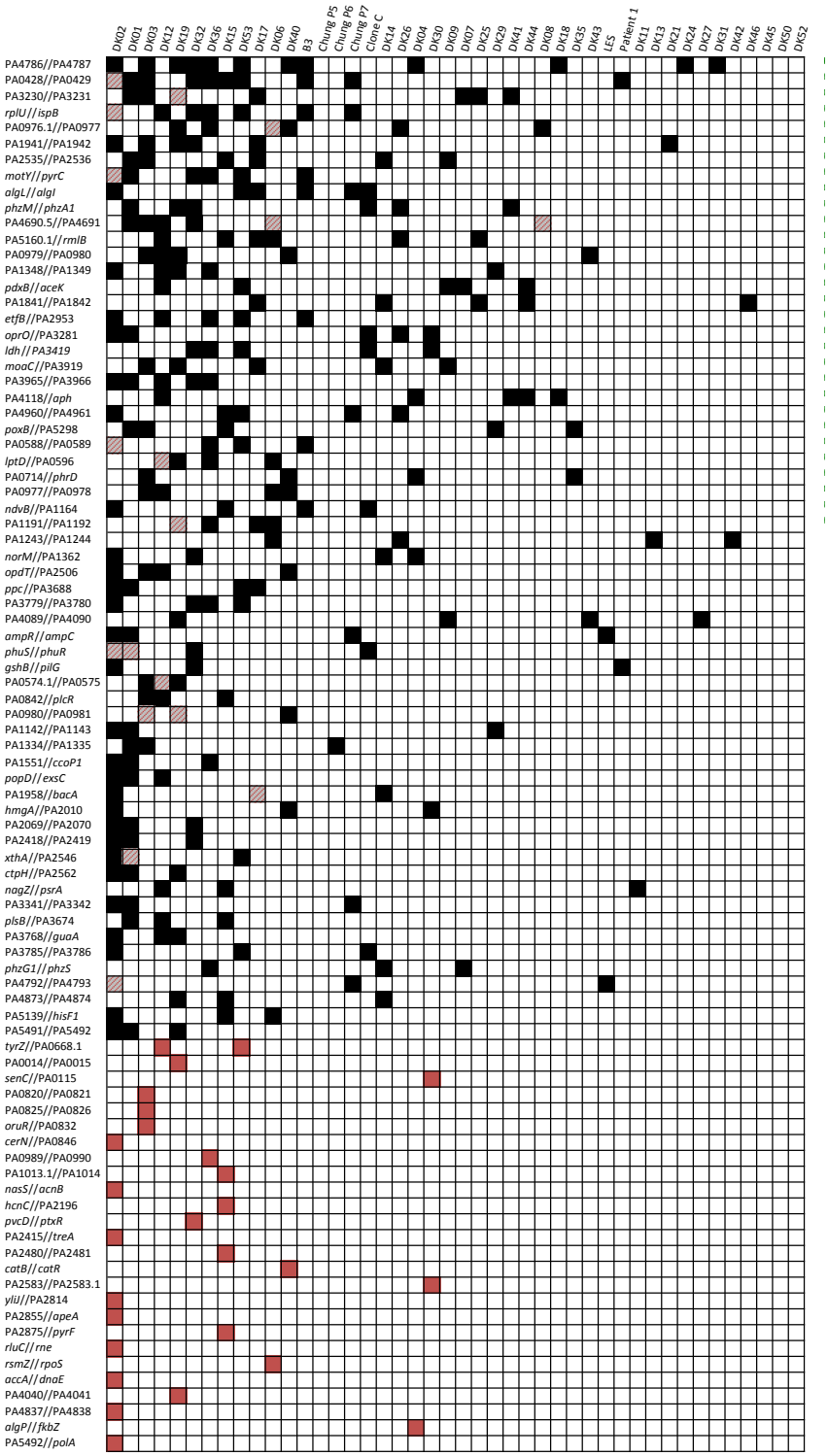
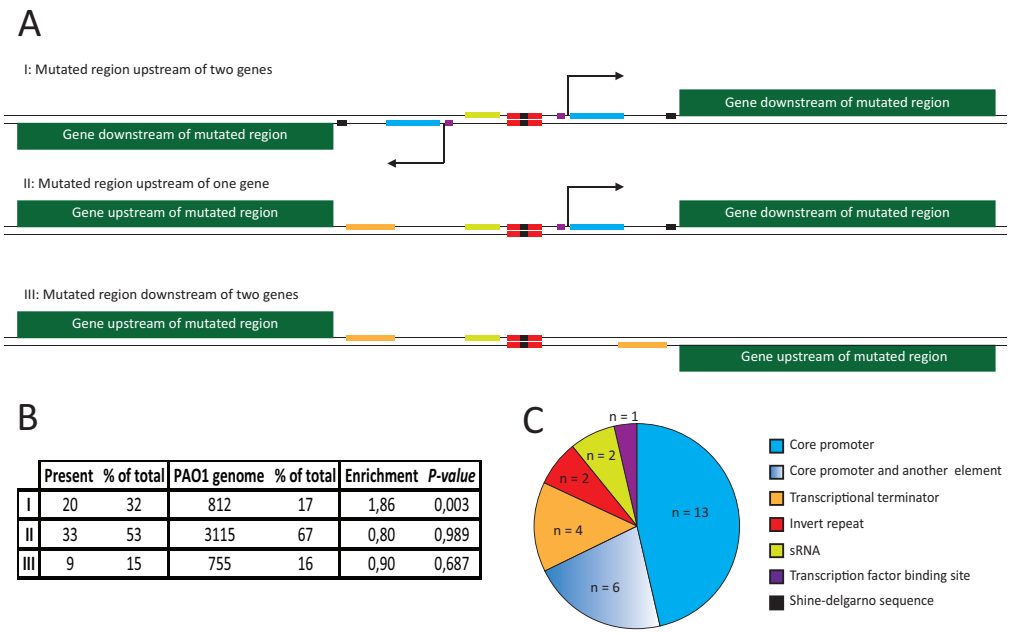


Figure 1 Pathoadaptive intergenic regions. Regions targeted by mutations involved in host adaptation through parallel evolution across or within clone types. The black squares in the matrix demonstrate whether the intergenic region acquired mutations in isolates of the respective clone type. The red squares in the matrix show that the intergenic region has been selected for mutations within isolates of a distinct clone type alone. Squares with striped red color indicate regions that have been selected by mutations within isolates of that distinct clone type in addition to being selected by intergenic mutation across other clone type.

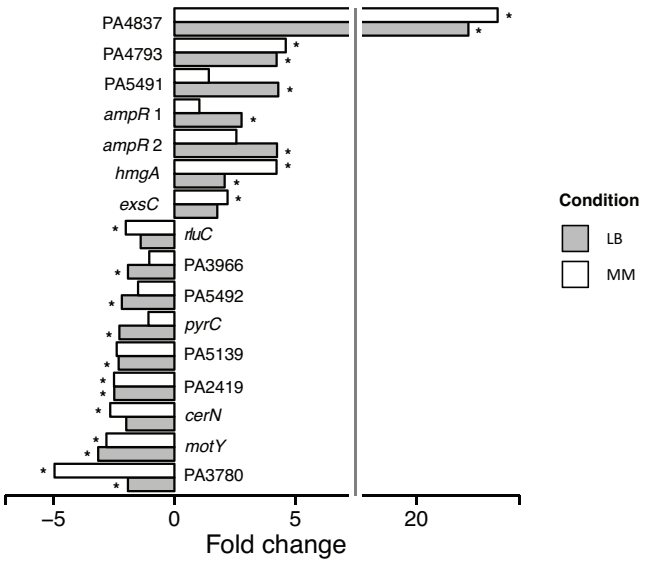


B

	Present	% of total	PAO1 genome	% of total	Enrichment	*P-value*
I	20	32	812	17	1,86	0,003
II	33	53	3115	67	0,80	0,989
III	9	15	755	16	0,90	0,687

241

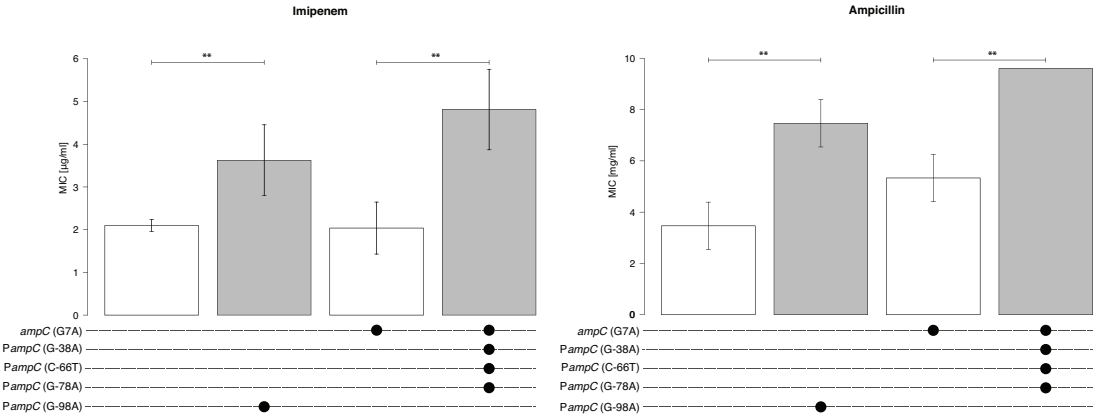
242 **Figure 2** A) Overview of the three different orientations of intergenic regions and the possible location of
243 potential elements within each type. B) Distribution of different orientations of intergenic regions (I-III) within
244 PAO1 genome and the pathoadaptive regions selected across clone types (n = 62). C) pie chart demonstrating the
245 distribution of putative intergenic elements targeted by pathoadaptive intergenic mutations among regions
246 where the mutation cluster was within any known element (n = 28).



247

248 **Figure 3** Overview of transcriptional fusion results. Expression of *lux* from transcriptional fusions with selected
249 mutated regions were measured at OD_{600} = 0.15 and normalized by cell density. Transcriptional fusions are
250 examined under two different condition of Luria-Bertani (LB) and ABTGC minimal media [19]. Mean luminescence
251 was calculated for three biological replicates of fusions with mutated and wild type regions and the relative fold

252 change caused by the mutation was consequently calculated. Statistical analysis of the difference between two
253 means was performed by a two-tailed student t test and the asterisk denotes $P < 0.05$. Detailed description of the
254 results with origin of the mutated regions, mutations not causing a significant change and presence of mutations
255 within putative intergenic elements can be found in Supplementary Table 9.



256

257 **Figure 4** Mutations in the intergenic region between *ampC* and *ampR* cause an increased tolerance towards
258 imipenem and ampicillin. The values for Minimal Inhibitory Concentration (MIC) and the constructed mutations
259 in each strain of PAO1 are shown. Mutation G-98A upstream *ampC* derives from isolate DK2-CF173-1995. Three
260 mutations G-38A, C-66T and G-78A upstream of *ampC* originate from DK1-P43-M2-2002. A SNP mutation at the
261 start of *ampC* (G7A) in DK1-P43-M2-2002 was also constructed in laboratory strain PAO1 to isolate the effect of
262 this mutation and the effect of intergenic mutations from DK1-P43-M2-2002. Error bars indicate standard
263 deviation from three different biological replicates. Double asterisk indicate significant difference between mean
264 MIC of the strains (Student t test, $P < 0.01$).

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Online Methods

Assembly of the dataset used for identification pathoadaptive intergenic regions

We imported called variants in the intergenic regions of CF adapted *P. aeruginosa* isolates from six longitudinal studies[1–6]. To have all variants against one common reference genome, we only considered those with coverage in *P. aeruginosa* PAO1 reference[8] genome and omitted all other variants. In addition, Marvig *et al.* 2013[7] reported the draft genome sequence of four *P. aeruginosa* B3 strains isolated from a chronically infected Danish CF patient that underwent antibiotic chemotherapy, over a period of 4 years. Here, we called for the variants in the genomes of these isolates and identified a total of 315 mutations (237 SNPs and 78 indels) when mapping the reads to the reference PAO1 genome.

In total we identified 3,489 intergenic mutations across 44 different clone types. Detailed description of the dataset can be found in Supplementary Table 1 and 2.

Definition of clone types

To establish existing genetic variation between all 44 recognized clones of *P. aeruginosa* used in this study and avoid parallel observation of identical clones, we performed MLST analysis on genome of each clone. Briefly, available whole genome sequence or assembled contigs of DK1, DK2, B3, PACS2, LES were used as source material for query of MLST profile by the Pseudomonas aeruginosa MLST website[36]. For all remaining clones, sequence reads from one isolate of each clone were retrieved from the sequence read archives database and *de novo* assembled in Geneious 7.1.7[37] using Velvet assembly 7.0.3[38] plugin with Velvet optimizer defined

parameters. Sequence reads from Chung P5, Chung P6 and Chung P7 clones were unavailable and the determined ST are reported by the publication itself[3]. Assembled contigs were analyzed for MLST allele profiles using Pseudomonas aeruginosa MLST website[36]. Overview of MLST results can be found on Supplementary Table 3.

Identification of pathoadaptive regions

We defined a clone type mutation as one mutation within an intergenic region when one or multiple mutations within that region are observed in isolates of that clone type. Using this definition, we observed a total of 2,715 clone type mutations. Pathoadaptive intergenic regions are characterized as regions important for adaptation to the host environment. They are therefore expected to be targeted by multiple mutations acquired in parallel by different isolates. In order to distinguish such mutations from random mutations introduced by genetic drift, we defined an intergenic region as pathoadaptive when it is targeted by 3 or more distinct clone type mutations occurring in a cluster of less than 30 bp apart from each other. Furthermore, the cluster has to be less than 200 bp away from at least one of the flanking genes to have a potential effect on that gene. We also included regions targeted by multiple similar clusters each containing two distinct clone type mutations. To rule out the contribution of any sequencing artifact in intergenic mutations, identical mutations among different isolates from the same study were counted as one clone type mutation. As *P. aeruginosa* PAO1 genome has 4,682 intergenic regions constituting a total of 631,498 bp, we expect 0.0043 clone type mutation/bp rate (2,715 distinct clone type mutations in total) for intergenic regions. However observing three distinct clone type mutations in a 30 bp intergenic region

cluster (0.1 mutation/bp) is 23 folds higher than what would be expected by chance and a significant increase in mutation density [$P(X \geq 3) \sim pois(X; 0.13) = 1.07e\text{-}5$, where $P(X \geq 3)$ is the probability of observing ≥ 3 mutations given a Poisson distribution with a mean of 0.13 mutations (0.0043 mutation/bp * 30 bp)]. We applied these criteria for identification of pathoadaptive regions selected across clone types. Furthermore, for identification of pathoadaptive regions selected within each clone type, we applied the same criteria but only looked for 3 distinct isolate type mutations within a narrow cluster of less than 30 bp.

Identification of putative intergenic elements

The position of putative intergenic elements including the core promoter, transcription factor binding site, transcriptional terminator, invert repeat, small RNA (sRNA) and shine-delgarno sequence were mapped within pathoadaptive regions. We used BPROM[39], CollecTF[40], PRODORIC[41], RegTransBase[42] and the Pseudomonas Genome Database (PGD)[12] to map putative promoters, transcription factor binding sites, shine-delgarno sequences and invert repeats. To increase the number of annotated promoters in *P. aeruginosa*, we utilized the findings of a recent study that validated putative binding sites of sigma factors in *P. aeruginosa* genome with RNA and/or ChIP-seq. A detailed description of present promoters and whether they have been targeted by intergenic mutations are available in Supplementary Table 8. We also used ARnold and PGD[12,43–46] for identification of putative transcriptional terminators. Presence of sRNAs within pathoadaptive intergenic regions were confirmed by a recent study reporting over 500 novel sRNAs within intergenic

regions of *P. aeruginosa* genome[47]. We mapped the position of mutations to the identified putative elements (Supplementary Table 7).

Construction of reporter fusions

Twenty five intergenic regions upstream of 32 genes were randomly selected from isolates of DK2 with mutations represented in the cluster. We also included regions upstream of *ampC* and *ampR* from DK1-P43-M2-2002[48]. Mutated intergenic regions upstream of 32 genes were amplified from genomic DNA of corresponding isolates (Supplementary Table 9) using Phusion polymerase and primers described in Supplementary Table 10. The PCR fragments and the pHK-CTX2-*lux*[17] plasmid were doubled digested with restriction enzymes *Xho*I and *Pst*I and ligated together with T4 DNA ligase (Thermo Scientific). Similarly, wild type region upstream of all 32 genes were also amplified from DK2-CF30-1979 and cloned upstream of *lux* in pHK-CTX2-*lux*. The presence of mutations and the intergenic regions in resulting plasmids were verified using Sanger sequencing at LGC Genomics. The plasmids were introduced into *P. aeruginosa* strain PAO1 by transformation as previously described[49].

Measurements of growth and luminescence in reporter fusion strains

Overnight cultures of reporter fusions strains were diluted 200 times in fresh Luria-Bertani (LB) medium and aliquots of 100 µl were transferred to black clear bottom 96-well microtiter plate (Greiner). Three biological replicates were prepared for each fusion on the same day and measurements of growth (OD_{600}) and luminescence were recorded by Cytation 5 multimode reader (BioTek) every 6 minutes for 8 hours at 200 rpm shaking condition and 37 C temperature. The luminescence values at

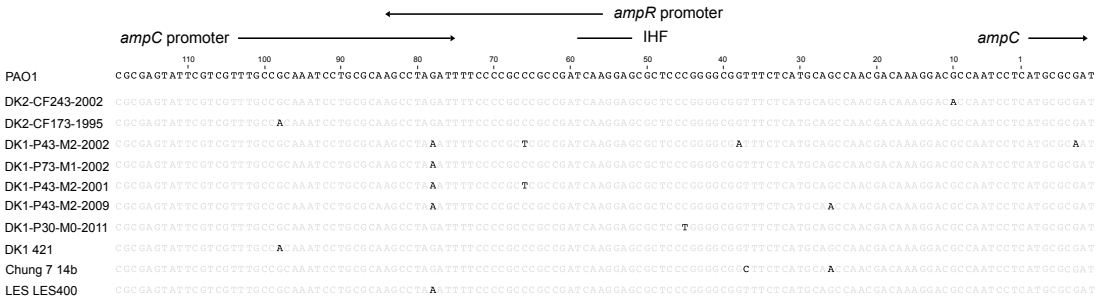
512 OD_{600} = 0.15 were normalized by cell density and recorded for all fusions.
513 Background luminescence from a PAO1 strain containing the promoterless *lux*
514 cassette was measured in the same way and it was corrected for on luminescence
515 expressions of all strains. Data were analyzed using a custom-made script in the R
516 software environment, version 3.1.3[50]. Student t test was performed to examine the
517 statistical difference between the mean of three biological replicates.
518
519 *Allelic replacement of intergenic region upstream ampC and ampR in PAO1*
520 A 1,361 bp fragment containing the intergenic region upstream of *ampC* and *ampR*
521 was amplified from genomic DNA of DK1-P43-M2-2002 and DK2-CF173-1995 using
522 Phusion polymerase and primers *ampR*i-F-*Xba*I and *ampC*i-R-*Sac*I (Supplementary
523 Table 10). The PCR fragments and vector pNJ1[51] were doubled digested with *Xba*I
524 and *Sac*I and ligated together using T4 DNA ligase. As the sequence of *ampC* gene
525 from laboratory strain PAO1 differed from that of DK2 and DK1 isolates, we
526 amplified the 1,361 bp fragment from DK2-CF30-1979 to obtain a pNJ1 plasmid with
527 wild type copy of the *ampR*//*ampC* intergenic region. Moreover, an additional
528 mutation (G7A) was found at the start of *ampC* in DK1-P43-M2-2002. To isolate the
529 effect of *ampR*//*ampC* intergenic mutations from this isolate, we created the *ampC*
530 mutation (G7A) in the pNJ1 plasmid containing wild type region using QuickChange
531 Lightning Multi site directed mutagenesis kit (Agilent Technologies). All ligation
532 mixes were electroporated into *E. coli* CC118λpir[52] and transferred into strain PAO1[53]
533 by triparental mating using helper strain *E. coli* HB101/pRK600[54]. After incubation
534 overnight, merodiploid mutants were selected by plating the conjugation mixture on
535 LB agar plate with 50 μg/ml tetracycline. Colonies were streaked on 6% (wt/vol)

sucrose-LB plates without NaCl for several times until when they became sensitive to tetracycline. Sucrose-resistant/tetracycline sensitive colonies were finally streaked on sucrose-LB plates and allelic replacement mutants were verified by Sanger sequencing at LGC Genomics.

Minimal Inhibitory Concentrations

MICs were determined using two ways. For MICs of imipenem and ampicillin standard broth microdilution. Overnight cultures of PAO1 strains with and without intergenic mutations upstream *ampR* and *ampC* were diluted in Mueller-Hinton (MH) broth to an OD_{600} = 0.02. Serial dilutions were performed in clear 96-well microtiter plates (Greiner) to obtain gradient concentrations of imipenem and ampicillin in MH broth. Aliquots of 100 µl were inoculated in each well containing 100 µl of MH broth with different concentrations of imipenem and ampicillin. We inoculated two technical replicates of each strain on each microtiter plate. Microtiter plates were incubated overnight at 37 C with 200 rpm shaking condition. Minimal Inhibitory Concentration (MIC) was defined as the lowest concentration of antibiotic where visible growth was observed. We repeated the experiment five times to obtain five biological replicates. For ceftazidime, MIC was determined using E-test provided by manufacturer protocols (BioMerieux). Briefly, cultures of strains grown overnight in MH broth were diluted to OD_{600} = 0.5, 100 µl was spread on MH agar plates and a sterile strip of ceftazidime E-test was placed on the plate. The values were measured after 22 hours incubation of the plates at 37 C and the E-test was performed in triplicate.

Supplementary Information



Supplementary Figure 1 Overview of the intergenic mutations upstream of *ampR* and *ampC*. The alignment shows similar sequences of this region from different isolates of four clone types where genetic variants are highlighted in bold. Position of putative elements identified (Online Methods) and the start codon of *ampC* are demonstrated (IHF: Integration Host Factor). Positions are relative to the start codon of *ampC*. Wild type sequence of the region from reference genome PAO1[8] is shown at the top of the alignment.

Supplementary Table 1: Overview of the dataset used in this study to identify pathoadaptive intergenic regions. Intergenic mutations from seven longitudinal studies of *P. aeruginosa* adaptation to the CF environment were imported and mapped against reference strain PAO1 genome. Pathoadaptive intergenic regions selected across clone types (interclonal) or within clone types (intraclonal) were identified using certain criteria (Online Methods). Detailed description of the dataset is available at Supplementary Table 2.

Isolates	534
Patients	68
Clone types	44
Total mutations	22.491
Intergenic SNPs	2.024
Intergenic indels	1.465
Total Intergenic mutations	3.489
Intergenic clone type mutations	2.715
Total mutated intergenic regions	1.610
Intergenic mutations frequency (mut/bp)	0,0043
Pathoadaptive regions selected intraclonally	26
Pathoadaptive regions selected interclonally	47
Pathoadaptive regions selected both intraclonally and interclonally	15
Interclonal pathoadaptive regions shared by different geographical locations	24%

Supplementary Table 2: Overview of the dataset used in this study. The identified MLST type, number of patients, isolates and description of mutations representing 44 clones. ND: not determined

	MLST	Patients	Isolates	Total SNPs	Intergenic SNPs	Total indels	Intergenic indels	Total mutations	Total intergenic mutations	Number of intergenic regions mutated
B3	ST-17	1	4	237	23	78	26	315	49	47
DK01	ST-387	1	10	3271	333	353	132	3624	465	393
DK2	ST-386	21	55	6785	686	1085	301	7870	987	685
DK03	ST-560	2	26	864	124	134	58	998	182	108
DK04	ST-2238	1	18	32	4	61	31	93	35	27
DK06	ST-845	4	35	319	52	158	45	477	97	72
DK07	ND	1	6	9	1	25	11	34	12	11
DK08	ST-1068	2	14	274	42	60	25	334	67	52
DK09	ST-1822	1	20	40	3	104	43	144	46	40
DK11	ST-160	1	2	3	2	3	2	6	4	3
DK12	ST-443	2	23	577	90	180	64	757	154	122
DK13	ST-381	1	15	58	4	56	16	114	20	17
DK14	ND	1	14	17	0	78	32	95	32	29
DK15	ND	2	23	648	68	247	109	895	177	150
DK17	ST-2192	1	28	35	2	125	54	160	56	41
DK18	ST-389	1	7	8	1	12	7	20	8	7
DK19	ST-253	4	36	184	20	142	73	326	93	67
DK21	ST-379	1	5	15	0	13	6	28	6	6
DK24	ND	1	6	8	2	11	6	19	8	7
DK25	ST-207	1	6	15	2	18	9	33	11	11
DK26	ST-27	3	14	218	35	73	27	291	62	58
DK27	ST-709	1	8	21	4	24	12	45	16	12
DK29	ST-676	1	13	9	2	50	19	59	21	18
DK30	ST-235	2	2	161	23	16	6	177	29	23
DK31	ND	1	7	9	2	21	10	30	12	10
DK32	ST-132	1	18	462	53	176	60	638	113	96
DK35	ST-179	1	14	28	3	47	15	75	18	17
DK36	ST-395	3	33	1329	150	321	110	1650	260	209
DK40	ST-252	2	3	400	70	22	10	422	80	45
DK41	ND	1	18	15	2	103	30	118	32	28
DK42	ST-1455	1	2		0	2	2	2	2	2
DK43	ND	1	2	14	0	6	3	20	3	3
DK44	ND	1	7	3	1	19	12	22	13	13
DK45	ND	1	4	3	0	9	7	12	7	6
DK46	ST-926	1	2	1	0	5	1	6	1	1
DK50	ND	1	2	0	0	2	2	2	2	2
DK52	ST-1677	1	2	3	1	0	0	3	1	1
DK53	ST-809	1	12	464	59	131	45	595	104	80
Chung P5	ND	1	2	51	2	38	8	89	10	10
Chung P6	ST-245	1	2	1	0	8	5	9	5	4
Chung P7	ND	1	2	342	22	93	19	435	41	40
Clone C	ND	1	3	916	87			916	87	85
PACS2	ST-1394	1	2	46	5	22	3	68	8	8
LES	ST-146	7	7	416	44	49	9	465	53	49
Total		68	534	18311	2024	4180	1465	22491	3489	2715
Average		2	12	426	46	97	34	511	79	62
Median		1	7	40	4	49	15	105	31	25

Supplementary Table 3: Descritption of the identified MLST pattern in isolates of each clone type. ND: the full MLST pattern is not determined and only recognized partially with some of the alleles recognized. NR: all 7 alleles of MLST pattern are recognized but the pattern has not been reported before. NA: the MLST pattern is not available either due to lack or low quality of isolate sequences

	MLST	acs	aro	gua	mut	nuo	pps	trp
B3	ST-17	11	5	1	7	9	4	7
DK01	ST-387	28	5	11	11	4	12	3
DK2	ST-386	17	5	11	18	4	10	3
DK03	ST-560	5	5	57	13	1	40	3
DK04	ST-2238	6	10	1	3	27	4	7
DK06	ST-845	11	5	1	7	4	4	7
DK07	ND	15		36	11	64	13	1
DK08	ST-1068	23	5	11	7	1	12	137
DK09	ST-1822	142	14	25	6	1	1	8
DK11	ST-160	11	5	6	32	4	6	26
DK12	ST-443	15	5	5	5	50	4	1
DK13	ST-381	11	20	1	65	4	4	10
DK14	NR	5	43	109	6	1	16	131
DK15	ND	140		42		48		32
DK17	ST-2192	35	8	27	3	15	7	3
DK18	ST-389	17	22	5	3	1	14	3
DK19	ST-253	4	4	16	12	1	6	3
DK21	ST-379	39	5	11	28	4	4	63
DK24	NR	11	5	11	5	3	4	3
DK25	ST-207	47	4	5	33	1	6	40
DK26	ST-27	6	5	6	7	4	6	7
DK27	ST-709	40	6	19	11	4	15	9
DK29	ST-676	28	5	11	77	3	4	92
DK30	ST-235	38	11	3	13	1	2	4
DK31	NR	11	5	11	3	1	4	7
DK32	ST-132	6	20	1	3	4	4	2
DK35	ST-179	36	27	28	3	4	13	7
DK36	ST-395	6	5	1	1	1	12	1
DK40	ST-252	6	28	4	3	3	4	7
DK41	NR	40	5	17	2	4	14	7
DK42	ST-1455	15	5	11	3	58	42	9
DK43	ND		8	7	6	8	11	40
DK44	NR	19	5	11	34	4	15	26
DK45	NR	23	5	7	30	1	4	10
DK46	ST-926	29	1	97	99	24	20	87
DK50	ND	11		3	98	1	6	80
DK52	ST-1677	32	8	57	3	1	15	25
DK53	ST-809	36	3	6	13	3	6	26
Chung P5	NA							
Chung P6	ST-245	39	6	12	11	3	15	2
Chung P7	NA							
Clone C	NA							
PACS2	ST-1394	11	5	6	3	74	13	7
LES	ST-146	6	5	11	3	4	23	1

Supplementary Table 4: Description of the 88 pathoadaptive intergenic regions in clinicaly adapted isolates of *P. aeruginosa*. *Pseduomonas aeruginosa* gene number and name of flanking genes, Genome position of intergenic region in PAO1 reference genome, products of flanking genes, function of flanking genes' products, length of the intergenic region, orientation of the flanking genes with regards to the intergenic region and number of clones with mutation in the intergenic region.

Region	Genes	Genome position	Products	PseudoCap Function Class	Length	Orientation	Observed Clone types
PA4786//PA4787		5375479-5375589	probable short-chain dehydrogenase//probable transcriptional regulator	Putative enzymes//Transcriptional regulators	111	→ ←	12
PA0428//PA0429		479806-480055	probable ATP-dependent RNA helicase//hypothetical protein	Transcription, RNA processing and degradation//Hypothetical, unclassified, unknown	250	→ ←	10
PA3230//PA3231		3618468-3618725	conserved hypothetical protein//downstream hypothetical protein	Hypothetical, unclassified, unknown//Membrane proteins	258	← ←	7
PA4568//PA4569	*rplU//ispB*	5116625-5116864	50S ribosomal protein L21//octaprenyl-diphosphate synthase	Translation, post-translational modification, degradation//Energy metabolism; Biosynthesis of cofactors, prosthetic groups and carriers	240	← →	7
PA0976.1//PA0977		1060432-1060509	tRNA-Lys//hypothetical protein	Non-coding RNA gene//Hypothetical, unclassified, unknown	78	→ ←	6
PA1941//PA1942		2125793-2126103	hypothetical protein//hypothetical protein	Hypothetical, unclassified, unknown//Hypothetical, unclassified, unknown	311	← ←	6
PA2535//PA2536		2863940-2864169	probable oxidoreductase//probable phosphatidate cytidylyltransferase	Putative enzymes//Fatty acid and phospholipid metabolism	230	→ ←	6
PA3526//PA3527	*motY//pyrC*	3946963-3947094	probable outer membrane protein precursor//dihydroorotase	Membrane proteins//Nucleotide biosynthesis and metabolism	132	← →	6
PA3547//PA3548	*algL//algI*	3974118-3974358	poly(beta-d-mannuronate) lyase precursor AlgL//alginate o-acetyltransferase AlgI	Cell wall / LPS / capsule; Adaptation, Protection; Secreted Factors (toxins, enzymes, alginate)//Cell wall / LPS / capsule; Adaptation, Protection; Secreted Factors (toxins, enzymes, alginate)	241	→ →	6
PA4209//PA4210	*phzM//phzA1*	4713100-4713795	probable phenazine-specific methyltransferase//probable phenazine biosynthesis protein	Putative enzymes//Secreted Factors (toxins, enzymes, alginate)	696	← →	6
PA4690.5//PA4691		5269260-5269802	16S ribosomal RNA//hypothetical protein	Non-coding RNA gene//Hypothetical, unclassified, unknown	543	← ←	6
PA5160.1//PA5161	*//rmlB*	5810046-5810280	tRNA-Thr//dTDP-D-glucose 4,6-dehydratase	Non-coding RNA gene//Carbon compound catabolism; Cell wall / LPS / capsule	235	→ →	6
PA0979//PA0980		1062370-1062600	conserved hypothetical protein//hypothetical protein	Related to phage, transposon, or plasmid//Hypothetical, unclassified, unknown	231	← →	5
PA1348//PA1349		1463404-1463585	hypothetical protein//conserved hypothetical protein	Hypothetical, unclassified, unknown//Hypothetical, unclassified, unknown	182	→ →	5
PA1375//PA1376	*pdxB//aceK*	1493056-1493089	erythronate-4-phosphate dehydrogenase//isocitrate dehydrogenase kinase/phosphatase	Carbon compound catabolism; Amino acid biosynthesis and metabolism; Biosynthesis of cofactors, prosthetic groups and carriers//Central intermediary metabolism	34	→ ←	5
PA1841//PA1842		1999461-1999511	hypothetical protein//hypothetical protein	Hypothetical, unclassified, unknown//Hypothetical, unclassified, unknown	51	→ ←	5
PA2952//PA2953	*etfB//*	3312471-3312790	electron transfer flavoprotein beta-subunit//electron transfer flavoprotein-ubiquinone oxidoreductase	Energy metabolism//Energy metabolism	320	← →	5
PA3280//PA3281	*oprO//*	3674325-3674569	Pyrophosphate-specific outer membrane porin OprO precursor//hypothetical protein	Transport of small molecules//Membrane proteins	245	← ←	5
PA3418//PA3419	*ldh//*	3825774-3826018	leucine dehydrogenase//hypothetical protein	Amino acid biosynthesis and metabolism//Hypothetical, unclassified, unknown	245	← →	5
PA3918//PA3919	*moaC//*	4387088-4387335	molybdopterin biosynthetic protein C//conserved hypothetical protein	Biosynthesis of cofactors, prosthetic groups and carriers//Hypothetical, unclassified, unknown	248	← ←	5
PA3965//PA3966		4445488-4445688	probable transcriptional regulator//hypothetical protein	Transcriptional regulators//Membrane proteins	201	→ →	5
PA4118//PA4119	*//aph*	4607455-4607577	hypothetical protein//aminoglycoside 3'-phosphotransferase type IIb	Hypothetical, unclassified, unknown//Antibiotic resistance and susceptibility	123	→ ←	5
PA4960//PA4961		5568945-5569089	probable phosphoserine phosphatase//hypothetical protein	Amino acid biosynthesis and metabolism//Membrane proteins	145	← →	5
PA5297//PA5298	*poxB//*	5966578-5966705	pyruvate dehydrogenase (cytochrome)//xanthine phosphoribosyltransferase	Central intermediary metabolism; Energy metabolism//Nucleotide biosynthesis and metabolism	128	→ →	5
PA0588//PA0589		648653-648930	conserved hypothetical protein//conserved hypothetical protein	Hypothetical, unclassified, unknown//Energy metabolism	278	← ←	4
PA0595//PA0596	*lptD//*	656528-656653	LPS-assembly protein LptD//hypothetical protein	Adaptation, Protection//Hypothetical, unclassified, unknown	126	← →	4
PA0714//PA0714.1	*//phrD*	785175-785497	hypothetical protein//PhrD	Hypothetical, unclassified, unknown//Non-coding RNA gene	323	→ →	4
PA0977//PA0978		1060834-1061206	hypothetical protein//conserved hypothetical protein	Hypothetical, unclassified, unknown//Related to phage, transposon, or plasmid	373	← ←	4
PA1163//PA1164	*ndvB//*	1263167-1263377	NdvB//conserved hypothetical protein	Putative enzymes; Antibiotic resistance and susceptibility//Hypothetical, unclassified, unknown	211	← →	4
PA1191//PA1192		1293165-1293266	hypothetical protein//conserved hypothetical protein	Hypothetical, unclassified, unknown//Hypothetical, unclassified, unknown	102	← →	4
PA1243//PA1244		1347825-1348059	probable sensor/response regulator hybrid//hypothetical protein	Two-component regulatory systems//Hypothetical, unclassified, unknown	235	← ←	4
PA1361//PA1362	*norM//*	1473981-1474390	NorM//hypothetical protein	Membrane proteins; Transport of small molecules//Hypothetical, unclassified, unknown	410	← →	4
PA2505//PA2506	*opdT//*	2823921-2824282	tyrosine porin OpdT//hypothetical protein	Transport of small molecules; Membrane proteins//Hypothetical, unclassified, unknown	362	← →	4
PA3687//PA3688	*ppc//*	4130393-4130598	phosphoenolpyruvate carboxylase//hypothetical protein	Energy metabolism; Central intermediary metabolism//Hypothetical, unclassified, unknown	206	← →	4
PA3779//PA3780		4238734-4238807	hypothetical protein//putative TRAP-type C4-dicarboxylate transport	Hypothetical, unclassified, unknown//Membrane proteins	74	→ →	4
PA4089//PA4090		4573073-4573284	probable short-chain dehydrogenase//hypothetical protein	Putative enzymes//Hypothetical, unclassified, unknown	212	→ →	4
PA4109//PA4110	*ampR//ampC*	4593881-4594028	transcriptional regulator AmpR//beta-lactamase precursor	Antibiotic resistance and susceptibility; Transcriptional regulators//Adaptation, Protection	148	← →	4
PA4709//PA4710	*phuS//phuR*	5289037-5289216	PhuS//Heme/Hemoglobin uptake outer membrane receptor PhuR precursor	Putative enzymes//Transport of small molecules	180	← →	4
PA0407//PA0408	*gshB//pilG*	449385-449638	glutathione synthetase//twitching motility protein PilG	Amino acid biosynthesis and metabolism; Biosynthesis of cofactors, prosthetic groups and carriers//Two-component regulatory systems; Chemotaxis; Motility & Attachment	254	← →	3
PA0574.1//PA0575		630511-630526	tRNA-Met//conserved hypothetical protein	Non-coding RNA gene//Membrane proteins	16	← ←	3
PA0842//PA0843	*//plcR*	918530-918616	glycosyl transferase//phospholipase accessory protein PlcR precursor	Putative enzymes//Secreted Factors (toxins, enzymes, alginate)	87	← ←	3
PA0980//PA0981		1062886-1062920	hypothetical protein//hypothetical protein	Hypothetical, unclassified, unknown//Hypothetical, unclassified, unknown	35	→ →	3
PA1142//PA1143		1234014-1234094	probable transcriptional regulator//hypothetical protein	Transcriptional regulators//Hypothetical, unclassified, unknown	81	← ←	3
PA1334//PA1335		1446919-1447225	probable oxidoreductase//probable two-component response regulator	Putative enzymes//Transcriptional regulators; Two-component regulatory systems	307	← ←	3
PA1551//PA1552	*//ccoP1*	1689340-1689556	probable ferredoxin//Cytochrome c oxidase, cbb3-type, CcoQ subunit	Energy metabolism//Central intermediary metabolism; Energy metabolism	217	← ←	3
PA1709//PA1710	*popD//exsC*	1855737-1855861	Translocator outer membrane protein PopD precursor//ExsC, exoenzyme S synthesis protein C precursor	Protein secretion/export apparatus//Translation, post-translational modification, degradation; Protein secretion/export apparatus	125	→ →	3
PA1958//PA1959	*//bacA*	2142890-2143172	probable transporter//bacitracin resistance protein	Membrane proteins; Transport of small molecules//Cell wall / LPS / capsule; Adaptation, Protection; Antibiotic resistance and susceptibility	283	← →	3
PA2009//PA2010	*hmgA//*	2198731-2198890	homogentisate 1,2-dioxygenase//probable transcriptional regulator	Carbon compound catabolism//Transcriptional regulators	160	← →	3
PA2069//PA2070		2269363-2269541	probable carbamoyl transferase//hypothetical protein	Putative enzymes//Membrane proteins	179	← ←	3
PA2418//PA2419		2702067-2702163	hypothetical protein//probable hydrolase	Hypothetical, unclassified, unknown//Putative enzymes	97	→ →	3
PA2545//PA2546	*xthA//*	2877368-2877476	exodeoxyribonuclease III//probable ring-cleaving dioxygenase	DNA replication, recombination, modification and repair//Putative enzymes	109	→ ←	3
PA2561//PA2562	*ctpH//*	2896615-2896740	probable chemotaxis transducer//hypothetical protein	Adaptation, Protection; Chemotaxis//Hypothetical, unclassified, unknown	126	→ ←	3
PA3005//PA3006	*nagZ//psrA*	3366755-3366969	beta-N-acetyl-D-glucosaminidase//transcriptional regulator PsrA	Putative enzymes; Antibiotic resistance and susceptibility; Amino acid biosynthesis and metabolism//Transcriptional regulators	215	← ←	3
PA3341//PA3342		3752479-3752595	probable transcriptional regulator//hypothetical protein	Transcriptional regulators//Hypothetical, unclassified, unknown; Membrane proteins	117	← ←	3
PA3673//PA3674	*plsB//*	4114790-4114932	glycerol-3-phosphate acyltransferase//hypothetical protein	Fatty acid and phospholipid metabolism//Hypothetical, unclassified, unknown	143	→ →	3
PA3768//PA3769	*//guaA*	4225499-4225659	probable metallo-oxidoreductase//GMP synthase	Putative enzymes//Amino acid biosynthesis and metabolism; Nucleotide biosynthesis and metabolism	161	← ←	3
PA3785//PA3786		4244186-4244314	conserved hypothetical protein//hypothetical protein	Hypothetical, unclassified, unknown//Hypothetical, unclassified, unknown	129	← ←	3
PA4216//PA4217	*phzG1//phzS*	4720064-4720300	probable pyridoxamine 5'-phosphate oxidase//flavin-containing monooxygenase	Secreted Factors (toxins, enzymes, alginate)//Putative enzymes	237	→ →	3
PA4792//PA4793		5380449-5380579	conserved hypothetical protein//hypothetical protein	Hypothetical, unclassified, unknown//Hypothetical, unclassified, unknown	131	← →	3
PA4873//PA4874		5471452-5471625	probable heat-shock protein//conserved hypothetical protein	Chaperones & heat shock proteins//Hypothetical, unclassified, unknown	174	→ ←	3
PA5139//PA5140	*//hisF1*	5788443-5788613	hypothetical protein//imidazoleglycerol-phosphate synthase, cyclase subunit	Hypothetical, unclassified, unknown//Amino acid biosynthesis and metabolism	171	← ←	3
PA5491//PA5492		6182690-6182872	probable cytochrome//conserved hypothetical protein	Energy metabolism//Hypothetical, unclassified, unknown	183	← →	3
PA0668//PA0668.1	*tyrZ//*	721557-722095	tyrosyl-tRNA synthetase 2/16S ribosomal RNA	Amino acid biosynthesis and metabolism; Translation, post-translational modification, degradation//Non-coding RNA gene	539	→ →	2
PA0014//PA0015		16608-16899	hypothetical protein//hypothetical protein	Hypothetical, unclassified, unknown//Hypothetical, unclassified, unknown	292	← ←	1
PA0114//PA0115	*senC//*	135895-135933	SenC//conserved hypothetical protein	Hypothetical, unclassified, unknown//Hypothetical, unclassified, unknown	39	→ ←	1
PA0820//PA0821		897229-897334	hypothetical protein//hypothetical protein	Hypothetical, unclassified, unknown//Hypothetical, unclassified, unknown	106	→ ←	1
PA0825//PA0826		900166-900407	hypothetical protein//translated portion of tmRNA gene ssrA	Hypothetical, unclassified, unknown//Hypothetical, unclassified, unknown	242	← ←	1
PA0831//PA0832	*oruR//*	906746-906846	transcriptional regulator OruR//conserved hypothetical protein	Transcriptional regulators//Hypothetical, unclassified, unknown	101	→ →	1
PA0845//PA0846	*cerN//*	923805-924181	CerN//probable sulfate uptake protein	Hypothetical, unclassified, unknown//Transport of small molecules	377	← ←	1
PA0989//PA0990		1070855-1071238	hypothetical protein//conserved hypothetical protein	Hypothetical, unclassified, unknown//Hypothetical, unclassified, unknown	384	← ←	1
PA1013.1//PA1014		1096957-1097295	tRNA-Ser//probable glycosyl transferase	Non-coding RNA gene//Putative enzymes	339	→ →	1
PA1786//PA1787	*nasS//acnB*	1934858-1935034	NasS//aconitate hydratase 2	Hypothetical, unclassified, unknown//Energy metabolism	177	← ←	1
PA2195//PA2196	*hcnC//*	2415508-2415660	hydrogen cyanide synthase HcnC//probable transcriptional regulator	Central intermediary metabolism//Transcriptional regulators	153	→ →	1
PA2257//PA2258	*pvcD//ptxR*	2486119-2486354	paerucumarin biosynthesis protein PvcD//transcriptional regulator PtxR	Secreted Factors (toxins, enzymes, alginate); Amino acid biosynthesis and metabolism//Secreted Factors (toxins, enzymes, alginate); Transcriptional regulators	236	→ ←	1
PA2415//PA2416	*//treA*	2698168-2698525	hypothetical protein//periplasmic trehalase precursor	Membrane proteins//Carbon compound catabolism	358	← →	1
PA2480//PA2481		2799735-2799909	probable two-component sensor//hypothetical protein	Two-component regulatory systems//Hypothetical, unclassified, unknown	175	→ ←	1
PA2509//PA2510	*catB//catR*	2827080-2827240	muconate cycloisomerase I// transcriptional regulator CatR	Carbon compound catabolism//Carbon compound catabolism; Transcriptional regulators	161	← →	1
PA2583//PA2583.1		2922570-2923221	probable sensor/response regulator hybrid//tRNA-Gly	Transcriptional regulators; Two-component regulatory systems//Non-coding RNA gene	652	← ←	1
PA2813//PA2814	*yliJ//*	3167168-3167281	probable glutathione S-transferase//hypothetical protein	Central intermediary metabolism//Hypothetical, unclassified, unknown	114	← ←	1
PA2855//PA2856	*//apeA*	3208618-3208672	hypothetical protein//lysophospholipase A	Hypothetical, unclassified, unknown//Fatty acid and phospholipid metabolism	55	← ←	1
PA2875//PA2876	*//pyrF*	3229255-3229483	conserved hypothetical protein//orotidine 5'-phosphate decarboxylase	Hypothetical, unclassified, unknown//Nucleotide biosynthesis and metabolism	229	← ←	1
PA2975//PA2976	*rluC//rne*	3332305-3332880	ribosomal large subunit pseudouridine synthase C//ribonuclease E	Transcription, RNA processing and degradation//Transcription, RNA processing and degradation	576	← →	1
PA3621.1//PA3622	*rsmZ//rpoS*	4057659-4057908	regulatory RNA RsmZ//sigma factor RpoS	Non-coding RNA gene//Transcriptional regulators	250	← ←	1
PA3639//PA3640	*accA//dnaE*	4075008-4075156	acetyl-coenzyme A carboxylase carboxyl transferase (alpha subunit)//DNA polymerase III, alpha chain	Fatty acid and phospholipid metabolism//DNA replication, recombination, modification and repair	149	← ←	1
PA4040//PA4041		4523754-4523984	hypothetical protein//hypothetical protein	Hypothetical, unclassified, unknown//Putative enzymes	231	→ ←	1
PA4837//PA4838		5429843-5429983	probable outer membrane protein precursor//hypothetical protein	Membrane proteins; Transport of small molecules//Hypothetical, unclassified, unknown	141	← →	1
PA5253//PA5254	*algP//fkbZ*	5916102-5916226	alginate regulatory protein AlgP//probable peptidyl-prolyl cis-trans isomerase, FkbP-type	Transcriptional regulators//Translation, post-translational modification, degradation; Chaperones & heat shock proteins	125	← →	1
PA5492//PA5493	*//polA*	6183521-6183783	conserved hypothetical protein//DNA polymerase I	Hypothetical, unclassified, unknown//DNA replication, recombination, modification and repair	263	→ ←	1

Supplementary Table 5: Distribution of flanking genes PseudoCap function class enrichment among pathoadaptive intergenic selected across clone type (n=62). P(X≥x) ~ binom(X; p), where P(X≥x) is the probability of observing ≥x of the 124 genes to belong to a functional class of genes.

	Total genes	% of total no. of genes (*p*)	Genes present (*x*)	% of genes	Fold enrichment	P(X≥x)~ binom(X; p)
Antibiotic resistance and susceptibility	74	1,3	5	4,0	3,1	0,024
Secreted Factors (toxins, enzymes, alginate)	104	1,8	5	4,0	2,2	0,078
Non-coding RNA gene	111	2,0	5	4,0	2,1	0,096
Energy metabolism	206	3,6	9	7,3	2,0	0,037
Central intermediary metabolism	108	1,9	4	3,2	1,7	0,211
Nucleotide biosynthesis and metabolism	86	1,5	3	2,4	1,6	0,289
Chemotaxis	64	1,1	2	1,6	1,4	0,407
Fatty acid and phospholipid metabolism	64	1,1	2	1,6	1,4	0,407
Related to phage, transposon, or plasmid	65	1,1	2	1,6	1,4	0,415
Putative enzymes	472	8,3	14	11,3	1,4	0,148
Adaptation, Protection	208	3,7	6	4,8	1,3	0,301
Amino acid biosynthesis and metabolism	246	4,3	7	5,6	1,3	0,290
Biosynthesis of cofactors, prosthetic groups and carriers	160	2,8	4	3,2	1,1	0,462
Two-component regulatory systems	123	2,2	3	2,4	1,1	0,504
Hypothetical, unclassified, unknown	1923	33,8	44	35,5	1,0	0,379
Cell wall / LPS / capsule	193	3,4	4	3,2	1,0	0,610
Transcription, RNA processing and degradation	55	1,0	1	0,8	0,8	0,700
Chaperones & heat shock proteins	56	1,0	1	0,8	0,8	0,707
Membrane proteins	675	11,9	12	9,7	0,8	0,812
Transcriptional regulators	487	8,6	8	6,5	0,8	0,842
Carbon compound catabolism	193	3,4	3	2,4	0,7	0,796
Protein secretion/export apparatus	142	2,5	2	1,6	0,6	0,818
DNA replication, recombination, modification and repair	88	1,5	1	0,8	0,5	0,855
Translation, post-translational modification, degradation	198	3,5	2	1,6	0,5	0,932
Transport of small molecules	607	10,7	5	4,0	0,4	0,998
Motility & attachment	140	2,5	1	0,8	0,3	0,955

Supplementary Table 6: Analysis of co-occurrence of intergenic mutations with mutations in the flanking genes.

Region	Genes	Isolates with the intergenic mutation	Isolates with the intergenic mutation and mutation in the upstream gene	% of isolates with intergenic mutation occuring together with mutation in the upstream gene	Isolates with the intergenic mutation and mutation in the downstream gene	% of isolates with intergenic mutation occuring together with mutation in the downstream gene	% of isolates with intergenic mutation occuring together with mutation in at least one of the flanking genes	Frequency of intergenic mutations co-occuring with gene mutations
PA4109//PA4110	*ampR//ampC*	6	3	50	3	50	100	1,00
PA0976.1//PA0977		104			80	77	77	0,77
PA0428//PA0429		4			3	75	75	0,75
PA0977//PA0978		54	35	65			65	0,65
PA0979//PA0980		61			25	41	41	0,41
PA2505//PA2506	*opdT//*	8	3	38			38	0,38
PA4709//PA4710	*phuS//phuR*	14			5	36	36	0,36
PA0842//PA0843	*//plcR*	48	17	35	17	35	35	0,35
PA1163//PA1164	*ndvB //*	6	1	17	1	17	33	0,33
PA1709//PA1710	*popD//exsC*	3			1	33	33	0,33
PA1243//PA1244		57			16	28	28	0,28
PA4786//PA4787		116	29	25	1	1	26	0,26
PA0588//PA0589		20	4	20			20	0,20
PA0574.1//PA0575		54			9	17	17	0,17
PA5160.1//PA5161	*//rmlB*	119	18	15			15	0,15
PA1551//PA1552	*//ccoP1*	18	1	6	1	6	11	0,11
PA2952//PA2953	*etfB//*	10	1	10			10	0,10
PA3687//PA3688	*ppc//*	36	3	8			8	0,08
PA3341//PA3342		13			1	8	8	0,08
PA3526//PA3527	*//pyrC*	40	2	5	1	3	8	0,08
PA0980//PA0981		17	1	6	1	6	6	0,06
PA1361//PA1362	*norM//*	28	1	4	1	4	4	0,04
PA5297//PA5298	*poxB//*	65	2	3			3	0,03
PA4568//PA4569	*rplU//ispB*	34			1	3	3	0,03
PA1958//PA1959	*//bacA*	37			1	3	3	0,03
PA3547//PA3548	*algL//algI*	38	1	3			3	0,03
PA4690.5//PA4691		62			1	2	2	0,02
PA1191//PA1192		100			1	1	1	0,01
							Average	0,25
							Average including regions with no flanking gene mutations	0,11

Supplmentary Table 7: Characterization of putative elements present among 88 pathoadaptive intergenic region.

Region	Genes	Orientation	Shine-delgarno sequence		Transcription factor binding site		Core promoter		Invert repeats		Transcriptional terminators		sRNA		Mutation not in intergenic elements	Mutation in intergenic element	No known element	Core promoter	Core promoter and another element	Transcriptional terminator	Invert repeat	sRNA	Transcription factor binding site	Shine-delgarno sequence
			Present	Targetted	Present	Targetted	Present	Targetted	Present	Targetted	Present	Targetted	Present	Targetted											
PA0595//PA0596	*lptD//*	← →	2	0			2	1								1		1						
PA0714//PA0714.1	*//phrD*	→ →			3		1	1	1		2					1		1						
PA0825//PA0826		← ←					1	0	1	0	2	1				1				1				
PA0842//PA0843	*//plcR*	← ←					1	0	1	1						1					1			
PA0979//PA0980		← →			3	0	2	1								1		1						
PA1163//PA1164	*ndvB//*	← →			3	0	3	1								1		1						
PA1191//PA1192		← →	1	0			1	1								1		1						
PA1361//PA1362	*norM//*	← →	1	0	2	0	2	1					1	0		1		1						
PA2195//PA2196	*hcnC//*	→ →					1	1								1		1						
PA2257//PA2258	*pvcD//ptxR*	→ ←					0	0	6	2						1					1			
PA2505//PA2506	*opdT//*	← →			1	0	2	1								1		1						
PA2535//PA2536		→ ←					0	0	1	0	1	1				1				1				
PA3280//PA3281	*oprO//*	← ←	1	0	6	0	1	0	1	0	2	1				1				1				
PA3526//PA3527	*motY//pyrC*	← →	2	0	1	1	3	1								1			1					
PA3673//PA3674	*plsB//*	→ →	1		4	1	1	1								1			1					
PA3768//PA3769	*//guaA*	← ←			2	0	1	1	2	1	2	0				1			1					
PA3918//PA3919	*moaC//*	← ←					1	1	2	1						1			1					
PA3965//PA3966		→ →	1	0			1	1								1		1						
PA4109//PA4110	*ampR//ampC*	← →	1	0	2	0	2	1								1		1						
PA4209//PA4210	*phzM//phzA1*	← →			9	1	2	0					1	0		1							1	
PA4568//PA4569	*rplU//ispB*	← →	2	0	2	0	3	1								1		1						
PA4709//PA4710	*phuS//phuR*	← →			3	1	3	1								1			1					
PA4837//PA4838		← →			4	1	2	1								1			1					
PA4960//PA4961		← →	1	0	1	0	1	1								1		1						
PA5492//PA5493	*//polA*	→ ←			1	0	0	0			1	1				1				1				
PA0588//PA0589		← ←	1	0	2	0	1	0					1	1		1						1		
PA3005//PA3006	*nagZ//psrA*	← ←					2	0			1	0	1	1		1						1		
PA1709//PA1710	*popD//exsC*	→ →	1	0	2	0	2	1			1	0				1		1						
PA0114//PA0115	*senC//*	→ ←					0	0									1							
PA0574.1//PA0575		← ←					0	0									1							
PA0820//PA0821		→ ←					0	0									1							
PA0831//PA0832	*oruR//*	→ →					0	0									1							
PA0976.1//PA0977		→ ←					0	0									1							
PA0977//PA0978		← ←					0	0									1							
PA0980//PA0981		→ →					0	0									1							
PA0989//PA0990		← ←					0	0									1							
PA1375//PA1376	*pdxB//aceK*	→ ←					0	0									1							
PA1841//PA1842		→ ←					0	0									1							
PA2418//PA2419		→ →					0	0									1							
PA2813//PA2814	*yliJ//*	← ←					0	0									1							
PA2855//PA2856	*//apeA*	← ←					0	0									1							
PA3639//PA3640	*accA//dnaE*	← ←					0	0									1							
PA3779//PA3780		→ →					0	0									1							
PA3785//PA3786		← ←					0	0									1							
PA4118//PA4119	*//aph*	→ ←					0	0									1							
PA4786//PA4787		→ ←					0	0									1							
PA4873//PA4874		→ ←					0	0									1							
PA0428//PA0429		← ←					1	0							1									
PA2415//PA2416	*//treA*	← →					5	0							1									
PA3621.1//PA3622	*rsmZ//rpoS*	← ←			6	0	1	0			3	0			1									
PA0014//PA0015		← ←					0	0	2	0					1									
PA0407//PA0408	*gshB//pilG*	← →	2	0	2	0	1	0					1	0	1									
PA0668//PA0668.1	*tyrZ//*	→ →			1	0	1	0							1									
PA0845//PA0846	*cerN//*	← ←			1	0	0	0			1	0			1									
PA1013.1//PA1014		→ →					3	0			5	0			1									
PA1142//PA1143		← ←	1	0			0	0							1									
PA1243//PA1244		← ←					2	0							1									
PA1334//PA1335		← ←			2	0	1	0	1	0					1									
PA1348//PA1349		→ →					0	0			1	0			1									
PA1551//PA1552	*//ccoP1*	← ←			1	0	1	0	1	0	2	0			1									
PA1786//PA1787	*nasS//acnB*	← ←					1	0			3	0			1									
PA1941//PA1942		← ←			6	0	1	0							1									
PA1958//PA1959	*//bacA*	← →	2	0	1	0	4	0			1	0			1									
PA2009//PA2010	*hmgA//*	← →			5	1	2	0							1									
PA2069//PA2070		← ←					1	0	1	0					1									
PA2480//PA2481		→ ←					0	0	1	0					1									
PA2509//PA2510	*catB//catR*	← →					1	0							1									
PA2545//PA2546	*xthA//*	→ ←					0	0	1	0	1	0			1									
PA2561//PA2562	*ctpH//*	→ ←					0	0	1	0					1									
PA2583//PA2583.1		← ←					0	0			2	0			1									
PA2875//PA2876	*//pyrF*	← ←			2	0	1	0							1									
PA2952//PA2953	*etfB//*	← →	2	0	3	0	2	0							1									
PA2975//PA2976	*rluC//rne*	← →			3	0	2	0							1									
PA3230//PA3231		← ←					1	0							1									
PA3341//PA3342		← ←			2	0	1	0							1									
PA3418//PA3419	*ldh//*	← →	1	0	1	0	2	0							1									
PA3547//PA3548	*algL//algI*	→ →					0	0			1	0			1									
PA3687//PA3688	*ppc//*	← →	1	0	2	0	3	0							1									
PA4040//PA4041		→ ←					0	0	1	0	6	0	1	0	1									
PA4089//PA4090		→ →	1	0	1	0	2	0							1									
PA4216//PA4217	*phzG1//phzS*	→ →					0	0	1	0					1									
PA4690.5//PA4691		← ←			2	0	1	0					1	0	1									
PA4792//PA4793		← →	1	0			1	0							1									
PA5139//PA5140	*//hisF1*	← ←	1	0			1	0							1									
PA5160.1//PA5161	*//rmlB*	→ →	1	0	2	0	2	0	1	0	2	0			1									
PA5253//PA5254	*algP//fkbZ*	← →	1	0	1	0	2	0							1									
PA5297//PA5298	poxB//	→ →	1	0			1	0							1									
PA5491//PA5492		← →	2	0	2	0	2	0							1									
Total			32	0	97	6	92	19	26	5	40	4	7	2	41	28	19	13	6	4	2	2	1	0

Supplementary Table 8: Description of annotated promoters within each of 88 pathoadaptive regions. Promoters targetted by pathoadaptive mutation cluster are highlighted in red. RpoD promoters are annotated either using computational predictions by BPROM sotware or computational predictions combined with experimental validation using RNA and/or ChIP-seq (Schulz et al. 2015). All other promoters are annotated using computational prediction and experimental validation using RNA and/or ChIP-seq (Schulz et al. 2015).

			Upstream gene											Downstream gene												
Region	**Genes**	**Orientation**	**Promoter**									**Position of mutations**		**Promoter**									**Position of mutations**		**Total promoters**	**Targeted**
			AlgU	RpoN	RpoS	RpoH	FliA	SigX	PvdS	FecI2	RpoD	Before TSS	After TSS	AlgU	RpoN	RpoS	RpoH	FliA	SigX	PvdS	FecI2	RpoD	Before TSS	After TSS		
PA0595//PA0596	*lptD//*	← →									1		1									1	1		2	1
PA0714//PA0714.1	*//phrD*	→ →																				1			1	1
PA0825//PA0826		← ←									1														1	0
PA0842//PA0843	*//plcR*	← ←									1														1	0
PA0979//PA0980		← →									1											1			2	1
PA1163//PA1164	*ndvB//*	← →			1						1											1	1		3	1
PA1191//PA1192		← →																				1			1	1
PA1361//PA1362	*norM//*	← →									1	1										1			2	1
PA2195//PA2196	*hcnC//*	→ →																				1	1		1	1
PA2257//PA2258	*pvcD//ptxR*	→ ←																							0	0
PA2505//PA2506	*opdT//*	← →									1											1			2	1
PA2535//PA2536		→ ←																							0	0
PA3280//PA3281	*oprO//*	← ←									1														1	0
PA3526//PA3527	*motY//pyrC*	← →		1			1					1										1	1		3	1
PA3673//PA3674	*plsB//*	→ →																				1	1		1	1
PA3768//PA3769	*//guaA*	← ←									1														1	1
PA3918//PA3919	*moaC//*	← ←									1														1	1
PA3965//PA3966		→ →																				1		1	1	1
PA4109//PA4110	*ampR//ampC*	← →									1		1									1	1		2	1
PA4209//PA4210	*phzM//phzA1*	← →									1	1										1	1		2	0
PA4568//PA4569	*rplU//ispB*	← →		1							1	1										1	1		3	1
PA4709//PA4710	*phuS//phuR*	← →									1	1									1	1	1		3	1
PA4837//PA4838		← →									1											1			2	1
PA4960//PA4961		← →																				1	1		1	1
PA5492//PA5493	*//polA*	→ ←																							0	0
PA0588//PA0589		← ←									1		1												1	0
PA3005//PA3006	*nagZ//psrA*	← ←	1								1														2	0
PA1709//PA1710	*popD//exsC*	→ →													1							1		1	2	1
PA0114//PA0115	*senC//*	→ ←																							0	0
PA0574.1//PA0575		← ←																							0	0
PA0820//PA0821		→ ←																							0	0
PA0831//PA0832	*oruR//*	→ →																					1		0	0
PA0976.1//PA0977		→ ←																							0	0
PA0977//PA0978		← ←																							0	0
PA0980//PA0981		→ →																							0	0
PA0989//PA0990		← ←																							0	0
PA1375//PA1376	*pdxB//aceK*	→ ←																							0	0
PA1841//PA1842		→ ←																							0	0
PA2418//PA2419		→ →																							0	0
PA2813//PA2814	*yliJ//*	← ←																							0	0
PA2855//PA2856	*//apeA*	← ←																							0	0
PA3639//PA3640	*accA//dnaE*	← ←										1													0	0
PA3779//PA3780		→ →																							0	0
PA3785//PA3786		← ←																							0	0
PA4118//PA4119	*//aph*	→ ←																							0	0
PA4786//PA4787		→ ←																							0	0
PA4873//PA4874		→ ←																							0	0
PA0428//PA0429		← ←									1														1	0
PA2415//PA2416	*//treA*	← →	1	1									1	1	1			1							5	0
PA3621.1//PA3622	*rsmZ//rpoS*	← ←									1	1													1	0
PA0014//PA0015		← ←										1													0	0
PA0407//PA0408	*gshB//pilG*	← →									1	1												1	1	0
PA0668//PA0668.1	*tyrZ//*	→ →																				1			1	0
PA0845//PA0846	*cerN//*	← ←																							0	0
PA1013.1//PA1014		→ →													1	1						1	1		3	0
PA1142//PA1143		← ←																							0	0
PA1243//PA1244		← ←		1							1	1													2	0
PA1334//PA1335		← ←									1														1	0
PA1348//PA1349		→ →																							0	0
PA1551//PA1552	*//ccoP1*	← ←									1		1												1	0
PA1786//PA1787	*nasS//acnB*	← ←		1																					1	0
PA1941//PA1942		← ←									1	1													1	0
PA1958//PA1959	*//bacA*	← →	1								1	1		1								1	1		4	0
PA2009//PA2010	*hmgA//*	← →									1		1									1	1		2	0
PA2069//PA2070		← ←									1														1	0
PA2480//PA2481		→ ←																							0	0
PA2509//PA2510	*catB//catR*	← →																				1			1	0
PA2545//PA2546	*xthA//*	→ ←																							0	0
PA2561//PA2562	*ctpH//*	→ ←																							0	0
PA2583//PA2583.1		← ←																							0	0
PA2875//PA2876	*//pyrF*	← ←									1														1	0
PA2952//PA2953	*etfB//*	← →									1	1										1	1		2	0
PA2975//PA2976	*rluC//rne*	← →									1	1										1			2	0
PA3230//PA3231		← ←									1														1	0
PA3341//PA3342		← ←									1	1													1	0
PA3418//PA3419	*ldh//*	← →			1						1														2	0
PA3547//PA3548	*algL//algI*	→ →																							0	0
PA3687//PA3688	*ppc//*	← →			1						1	1				1								1	3	0
PA4040//PA4041		→ ←																							0	0
PA4089//PA4090		→ →								1	1												1		2	0
PA4216//PA4217	*phzG1//phzS*	→ →																							0	0
PA4690.5//PA4691		← ←									1	1													1	0
PA4792//PA4793		← →																		1			1		1	0
PA5139//PA5140	*//hisF1*	← ←									1														1	0
PA5160.1//PA5161	*//rmlB*	→ →													1	1							1		2	0
PA5253//PA5254	*algP//fkbZ*	← →									1	1										1			2	0
PA5297//PA5298	poxB//	→ →																				1	1		1	0
PA5491//PA5492		← →									1											1			2	0
Total												18	6										19	4	92	19

Supplementary Table 9: Activities of the lux transcriptional fusions with the intergenic mutations relative to that of their wild type. Luminescence production of each transcriptional fusion in PAO1 laboratory reference strain was measured at exponential growth (OD600 = 0.15) in Luria-Bertani (LB) and ABTGC minimal media and normalized by the cell density. Mean luminescence was calculated for three biological replicates of fusions with mutated and wild type regions and the relative fold change caused by the mutation was accordingly calculated. Statistical analysis of the difference between two means was performed by a two-tailed student t test and the * denotes p-value < 0.05.

Region no.	Fusion no.	Downstream gene	Origin	Fold Change LB	Fold change MM	Mutation within putative element	Mutation not in putative element	No known element
1	1	PA1349	DK2-CF211-2006b	1,2	1,0		1	
2	2	*ppC*	DK2-CF211-2006b	-1,0	-2,3		1	
2	3	PA3688	DK2-CF211-2006b	1,2	-1,3		1	
3	4	*rplU*	DK2-CF211-2006b	1,3	1,1		1	
3	5	*ispB*	DK2-CF211-2006b	-1,2	-1,3	1		
4	6	PA0428	DK2-CF211-1997a	1,6	1,5			1
5	7	PA1958	DK2-CF211-1997a	1,5	1,1		1	
5	8	*bacA*	DK2-CF211-1997a	1,3	-1,4		1	
6	9	PA5491	DK2-CF211-1997a	4,2 *	2,6		1	
6	10	PA5492	DK2-CF211-1997a	-2,2 *	-1,5		1	
7	11	PA2069	DK2-CF222-2001	1,0	1,2		1	
8	12	PA1142	DK2-CF222-2001	1,8	1,2		1	
9	13	PA2419	DK2-CF222-2001	-2,5 *	-2,5 *			1
10	14	*ndvB*	DK2-CF222-2001	-1,5	-1,7	1		
11	15	*etfB*	DK2-CF206-2002	-1,2	1,0		1	
11	16	PA2953	DK2-CF206-2002	-1,3	-1,1		1	
12	17	*cerN*	DK2-CF206-2002	-2,0	-2,7 *		1	
13	18	*exsC*	DK2-CF224-2002b	1,8	2,2 *	1		
14	19	PA3780	DK2-CF240-2002	-1,9 *	-5,0 *			1
15	20	PA3966	DK2-CF243-2002	-1,9 *	-1,0		1	
16	21	PA0588	DK2-CF243-2002	1,2	-1,1		1	
17	22	PA1551	DK2-CF243-2002	-1,4	-1,0		1	
18	23	PA5139	DK2-CF243-2002	-2,3 *	-2,4		1	
19	24	*motY*	DK2-CF243-2002	-3,1 *	-2,8 *		1	
19	25	*pyrC*	DK2-CF243-2002	-2,3 *	-1,1	1		
20	26	*norM*	DK2-CF243-2002	-1,1	-1,3		1	
21	27	*hmgA*	DK2-CF243-2002	2,1 *	4,2 *		1	
22	28	*rluC*	DK2-CF66-2008	-1,4	-2,0 *		1	
23	29	PA4793	DK2-CF66-2008	4,2 *	4,6 *		1	
24	30	PA4837	DK2-CF173-2002	22,1 *	23,4 *	1		
25	31	*ampC* 1	DK2-CF173-1995	1,6	1,1	1		
25	32	*ampC* 2	DK1-CF243-2002	1,6	-1,3	1		
25	33	*ampR* 1	DK2-CF173-1995	2,8 *	1,0		1	
25	34	*ampR* 2	DK1-CF243-2002	4,3 *	1,4	1		
					Sum	8	23	3

Supplementary Table 10: Primers used in this study

Name	Sequence
*ampR*i-F-*Xba*I	5'-ATATTCTAGATAGGAGCGCAGCAGGGTGT-3'
*ampC*i-F-*Sac*I	5'-GCTAGAGCTCGAACACTTGCTGCTCCATGAG-3'
PA1349-F-*Pst*I	5'-TCAACTGCAGCCTGAATCCCTACGCACC-3'
PA1349-R-*Xho*I	5'-TAATCTCGAGCAGCTTCGCTTCGTCGAA-3'
ppC-F-*Pst*I	5'-TAATCTGCAGGCGGACAAGCTCACGGAT-3'
ppC-R-*Xho*I	5'-TAATCTCGAGAGTTGGTGGACGTCCTCG-3'
PA3688-F-*Pst*I	5'-TAATCTGCAGCGCATCGATCTCCGGCAT-3'
PA3688-R-*Xho*I	5'-TAATCTCGAGGCGGACAAGCTCACGGAT-3'
rplU-F-*Pst*I	5'-GATTCTGCAGTGAAATCTTCCGCCACCA-3'
rplU-R-*Xho*I	5'-TAATCTCGAGGCTTGCCACCGGTAACAA-3'
ispB-F-*Pst*I	5'-TTATCTGCAGGCTTGCCACCGGTAACAA-3'
ispB-R-*Xho*I	5'-TAATCTCGAGTGAAATCTTCCGCCACCA-3'
PA0428-F-*Pst*I	5'-GATTCTGCAGGAGATCCGCCAGCATTTG-3'
PA0428-R-*Xho*I	5'-TAATCTCGAGTAGCCCGCAGCCTCCAC-3'
PA1958-F-*Pst*I	5'-TAATCTGCAGCACGACGCCCAGGATGAA-3'
PA1958-R-*Xho*I	5'-TAATCTCGAGGCGGCGAAGAGTTCAAGG-3'
bacA-F-*Pst*I	5'-TAATCTGCAGGCGGCGAAGAGTTCAAGG-3'
bacA-R-*Xho*I	5'-TAATCTCGAGCACGACGCCCAGGATGAA-3'
PA5491-F-*Pst*I	5'-TAATCTGCAGGCCGACGATGGGGTTCTT-3'
PA5491-R-*Xho*I	5'-TAATCTCGAGCTGCTTCCGGGTCCTGC-3'
PA5492-F-*Pst*I	5'-TAATCTGCAGCTGCTTCCGGGTCCTGC-3'
PA5492-R-*Xho*I	5'-TAATCTCGAGGCCGACGATGGGGTTCTT-3'
PA2069-F-*Pst*I	5'-ATATCTGCAGCTGTTCGGCCGCCTCAG-3'
PA2069-R-*Xho*I	5'-ATATCTCGAGGGCCAGGTCGTTGTTGGT-3'
PA1142-F-*Pst*I	5'-ATCCCTGCAGGCCGCGTCGAACCGAAG-3'
PA1142-R-*Xho*I	5'-TAATCTCGAGCGAGGTCGAAGAGGGCAA-3'
PA2419-F-*Pst*I	5'-AAATCTGCAGAGAACGGGCGCTTCATCC-3'
PA2419-R-*Xho*I	5'-TAATCTCGAGCGTTGAAGTTGGCGGGAG-3'
etfB-F-*Pst*I	5'-TAATCTGCAGGCATGCGGCGGACAGAC-3'
etfB-R-*Xho*I	5'-TAATCTCGAGCGCGGACCTTGACGTTG-3'
PA2953-F-*Pst*I	5'-TAATCTGCAGCGCGGACCTTGACGTTG-3'
PA2953-R-*Xho*I	5'-TAATCTCGAGCATGCGGCGGACAGACC-3'
cerN-F-*Pst*I	5'-TTCACTGCAGGCGAGGAAGGCGAGAAGG-3'
cerN-R-*Xho*I	5'-TAATCTCGAGGCAAGAGCGCGGTGAATG-3'
exsC-F-*Pst*I	5'-ATATCTGCAGGAGGACGACTGGGAGGAC-3'
exsC-R-*Xho*I	5'-TAATCTCGAGACCACGGAAGCGCTCATC-3'
PA3780-F-*Pst*I	5'-TTAACTGCAGCTGGACCGAGATGGCCTT-3'
PA3780-R-*Xho*I	5'-TAATCTCGAGCCCTTGAGCAATGACGGC-3'
PA3966-F-*Pst*I	5'-TTTTCTGCAGGTGCTGGCGAGTACCGAA-3'
PA3966-R-*Xho*I	5'-TAATCTCGAGGACCGGTCATCATCCACT-3'
PA0588-F-*Pst*I	5'-TACCCTGCAGCCCGCGGATACCAGCAG-3'
PA0588-R-*Xho*I	5'-TAATCTCGAGCGAAGCGTTCCTGGAAGT-3'
PA1551-F-*Pst*I	5'-TATACTGCAGATACAGCCTGTCGCACGG-3'
PA1551-R-*Xho*I	5'-TAATCTCGAGGGCGGGGTGACGTCTTG-3'
PA5139-F-*Pst*I	5'-ATATCTGCAGGGTATCGTGGTGCGCTGA-3'
PA5139-R-*Xho*I	5'-ATATCTCGAGCGCATAGCAGGGCCAGG-3'
motY-F-*Pst*I	5'-TATACTGCAGGGGTGAGACGGTCGGACA-3'
motY-R-*Xho*I	5'-TAGTCTCGAGTAATAGAAGAAGGCGCGG-3'
pyrC-F-*Pst*I	5'-CATTCTGCAGTAATAGAAGAAGGCGCGG-3'
pyrC-R-*Xho*I	5'-TTTTCTCGAGGGGTGAGACGGTCGGACA-3'
norM-F-*Pst*I	5'-TAATCTGCAGTGTGCGGTTATTGCGGGA-3'
norM-R-*Xho*I	5'-TAATCTCGAGCAAGCCACGGGAAAGGGG-3'
PA2975-F-*Pst*I	5'-ATATCTGCAGTCGGGTTGAGTCGCGTTGAT-3'
PA2975-R-XhoI	5'-ATATCTCGAGACGTTGACCAGCCAGTTCCG-3'
PA4837-F-*Pst*I	5'-ATATCTGCAGTCTCGCGGACATGGTCGAGC-3'
PA4837-R-*Xho*I	5'-ATATCTCGAGGAGGACAAGCGACACACTGA-3'
ndvB-F-*Pst*I	5'-TATACTGCAGGAAGCGCTGTTCATCCACC-3'
ndvB-R-*Xho*I	5'-GGGCCTCGAGATCTTGCGTGAAGACATAGA-3'
PA4793-F-*Pst*I	5'-GGGACTGCAGGGATCGCAATACTTCGATTC-3'
PA4793-R-*Xho*I	5'-ATTACTCGAGAGGCCGAGCAGCAGGATT-3'
PA2009-F-*Pst*I	5'-GTTCCTGCAGCCTTGAGGATATCGGTAC-3'
PA2009-R-*Xho*I	5'-TTATCTCGAGGATTGATAGGCGAGGGCAGT-3'
ampC-F-*Pst*I	5'-ATATCTGCAGCAGCGGCAAATGGGGTCGAA-3'
ampC-R-*Xho*I	5'-ATATCTCGAGGCACAGGCAGGGGAATCTGG-3'
ampR-F-*Pst*I	5'-ATATCTGCAGTCGACCAGTGCCTTCAGGCG-3'
ampR-R-*Xho*I	5'-AGATCTCGAGCAGCGGCAAATGGGGTCGAA-3'

Adaptive mutation in a bacterial intergenic region cause pleiotropic effects on gene expressions

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Abstract

Bacterial adaptation to natural environments may be established through different genetic changes. While rewiring global regulatory networks through structural mutations within transacting factors leads to systemic remodeling of transcriptional networks, mutations within *cis*-regulatory elements are proposed to locally modulate transcription of genes. However, the global effects of these mutations in transcription of other genes are unknown. Here we analyze pleiotropic effects of a promoter mutation in *pseudomonas* heme uptake receptor (*phuR*) selected during long-term adaptation of *Pseudomonas aeruginosa* in chronic airway infections. We had previously shown that this mutation confers a growth advantage for *P. aeruginosa* in the presence of hemoglobin through overexpression of *phu* system genes. Microarray analysis revealed significantly altered expression for 118 genes in adapted *P. aeruginosa* DK2-CF30-1979-M2 strain with the mutation in LB medium. The effect was absent in *P. aeruginosa* laboratory PAO1 strain containing the mutation demonstrating that epistatic interaction with other mutations is essential. Nonetheless, PA4711 a gene located downstream of *phuR* with a separated operon was consistently upregulated in strain with the mutation in all genetic backgrounds and tested conditions. Moreover, the mutation conferred three additional phenotypes for *P. aeruginosa* DK2-CF30-1979-M2 including slower growth rate during anoxic condition, changed pigmentation in minimal medium surface agar and increased inhibition of *S. aureus*. Our results propose that *cis*-regulatory intergenic mutations confer pleiotropic effects to optimize bacterial adaptation in highly selective environments such as the CF airways.

Introduction

Understanding the molecular mechanism of adaptation to the human host is critical for invention of treatment strategies against infections caused by bacterial pathogens. Adaptation is defined as transition of an organism towards advantageous phenotypes in the present environment[1]. Its success leads to enhanced fitness or reproductive success of the individuals in the new environment. Changes in metabolic performance, growth rate, stress resistance, production of biofilm-like structure are among major phenotypic alterations associated with bacterial fitness[2]. By natural selection, organisms acquire and spread adaptive mutations essential for fitness in the environment[3]. Adaptation to unique environments is dependent on changes in gene expression. Such changes may be established through mutations targeting *cis*- and *trans*- regulatory elements. *cis*-regulatory (CRE) mutations target binding sites of transacting factors (TAF) in intergenic regions and they are recognized as a frequent cause of phenotype divergence in higher eukaryotes[4–6]. CRE mutations have also been shown to contribute to adaptive traits in bacteria[7–11]. Non-synonymous mutations in *trans*-regulatory elements (TRE) can modify the functionality of TAF and affect their pairing with binding sites in promoters or change their affinity with the core RNA polymerase[12].

CRE mutations are presumed to occur more frequently than TRE mutations as they do not pose deleterious effects by altering protein structure and function[1,2,4]. However, mutations in TRE may accommodate for more radical phenotype advances essential for quick adaptation in response to environmental changes[13]. Accordingly, adaptive mutations in global regulators of gene expression are frequently discovered in both experimentally and naturally evolved isolates of bacteria[2,14,15].

Cystic fibrosis (CF) is a recessive genetic disorder prompted by polymorphisms in Cystic fibrosis transmembrane conductance regulator (CFTR) gene[16]. As a consequence of loss of CFTR function, the lungs can no longer eradicate inhaled microorganisms through mucociliar clearance[17]. The opportunistic pathogen *Pseudomonas aeruginosa* is the prevalent culprit behind airway infections leading to mortality and morbidity in CF patients[18]. Regular sampling of *P. aeruginosa* from CF patients provides unique prospects to study bacterial within-host evolution.

In a previous study on adaptation of *Pseudomonas aeruginosa* to the CF host environment, we observed a series of mutations within the intergenic region upstream of *phuR* and *phuSTUVW*[8]*.* These genes encode proteins of the *Pseudomonas* heme utilization system (*phu*). The mutations significantly increased the transcription of all these genes, and we furthermore demonstrated that the presence of this mutation confers a growth advantage in the presence of hemoglobin. As *phuR* intergenic mutations altered the transcription of genes from the *phu* system, the simple conclusion was that the primary selection factor for this mutation was the expression of the *phu* system[8]. However, given the constitutive expression of the *phu* system and the relative high expression of the *phuR* receptor gene (112 folds compared to the wild type), the presence of pleiotropic effects on other systems is conceivable. Here we have investigated this scenario and found that this intergenic mutation leads to pleiotropic effects on expression of many other genes and emergence of new phenotypes in *P. aeruginosa*. Our results indicate that CRE mutations can potentiate considerable pleiotropic effects on expression of other genes and intergenic regions can be target for radical evolutionary changes.

Understanding the role of intergenic mutations with pleiotropic effects is vital for design of treatment strategies against bacterial pathogens.

Results

phuR promoter mutation result in pleiotropic effects on global gene expression

To investigate the effects of *phuR* promoter mutations on global gene expression patterns in *P. aeruginosa*, we used a CF adapted isolate of the epidemic DK2 lineage[19] in which we engineered a *phuR* promoter mutation (strain DK2-CF30-1979-M2)[8], and an isogenic "wild-type" strain without the mutation (strain DK2-CF30-1979). Microarray analysis of the two strains grown in Luria-Bertani (LB) medium demonstrated that the expression of all six genes of the *phu* system (*phuRSTUVW*) is significantly upregulated as a result of the mutation (*Benjamini-Hochberg*, $P < 0.05$). This was in accordance with our previous results using luciferase reporter gene fusions[8]. Surprisingly, our microarray expression study also revealed significant altered expressions of 1507 additional genes in the strain with the mutation compared to wild type (Supplementary Table 1, *Benjamini-Hochberg*, $P < 0.05$). Since these pleotropic effects were mostly subtle in terms of expression fold changes (FC) between mutant and wild type strain, we considered only fold changes below -2 or above 2 as biologically meaningful and the focus of further study. Introducing this criterion, we identified a total of 118 differentially expressed genes (including those of the *phu* system) of which 70 genes were upregulated and 38 were downregulated in the mutant compared to the wild type (Supplementary Table 2). To identify possible patterns among genes with expression changes, we categorized the list of 118 genes by their associated PseudoCap functions[20]. We found an enrichment of

genes from '*translation, post-translational modification, degradation*', '*central intermediary metabolism*', '*energy metabolism*' and '*fatty acid and phospholipid metabolism*' (Binomial, $P < 0.05$, n = 118, Supplementary Table 3).

To elucidate whether the pleiotropic effects on global gene expression was only present in the particular CF adapted DK2 isolate, we constructed the *phuR* promoter mutation in the common laboratory reference strain PAO1[21]. Microarray analysis of PAO1 containing the mutation and the isogenic wild type PAO1 strain showed that only 2 genes in addition to the six *phu* genes were differentially expressed as a consequence of the mutation (Supplementary Table 4, *Benjamini-Hochberg*, $P < 0.05$, FC > 2 or < -2). The highly diminished pleotropic effect observed in PAO1 relative to the DK2 strain suggests that the global gene expression effects of the promoter mutation is highly dependent on the genetic differences between these two strains. Nevertheless, in both strains we observed a consistent higher expression of PA4711 as a result of *phuR* promoter mutation. PA4711 is located downstream of *phuR* and encodes a Rieske-like iron-sulfur protein of unknown function. PA4711 and *phuR* genes are separated by 102 nt in which a predicted Rho-independent transcriptional terminator is located, suggesting that the two genes are not part of the same operon (Figure 1).

PA4711 is co-expressed with phuR

To determine if upregulation of PA4711 was consistent with *phuR* overexpression, we looked at the transcriptomes of two additional isolates of DK2 lineage DK2-CF173-2005 and DK2-CF66-2008[22]. Both isolates had acquired *phuR* promoter mutation leading to largest overexpression of *phuR* promoter[8,22]. In both isolates,

the expression of PA4711 was significantly upregulated compared to their common ancestor isolate (DK2-CF30-1979), which has no *phuR* promoter mutation. In conclusion, it is clear that the *phuR* promoter mutation leads to overexpression of *phuR* promoter and increased expression of PA4711.

Presence of the entire pleiotropic effect is independent of heme import

As the primary function of the *phu* system is import of heme, we speculated that the pleiotropic effects could be a result of the import and subsequent breakdown of heme which is present in LB medium[23]. To test this hypothesis, gene expression analysis of DK2-CF30-1979 with and without *phuR* promoter was performed in ABTGC minimal medium[8] where heme is absent. In this experiment only 8 genes were differentially expressed (Supplementary Table 5, *Benjamini-Hochberg*, $P < 0.05$, FC > 2 or < -2) suggesting that the pleotropic effect is highly dependent on the environmental context. Nonetheless, while the pleiotropic effects were much less pronounced in minimal medium compared to LB medium, PA4711 was still upregulated in the mutant in both conditions. We therefore confirmed that the upregulation of PA4711 occurs even in the absence of heme.

phuR promoter mutation leads to impaired growth during anoxic conditions

Given that *narK1* and *narK2* which encode nitrite/nitrate transport proteins[24] were among the most downregulated genes in DK2-CF30-1979-M2 isolate with *phuR* promoter mutation, we hypothesized that there is decreased activity for anaerobic metabolism through nitrogen assimilation. To test this hypothesis, we developed an assay to measure growth under anaerobic conditions. Briefly, we inoculated starting

cultures of two isogenic strains of DK2-CF30-1979 and PAO1 in LB medium containing 10 mM nitrate. Cultures were grown in vials sealed off with a lid to avoid introduction of oxygen. Vials were left to incubate at 37 °C, with 200 rpm shaking and OD_{600} measurements were performed continually as an indicator of growth. We found no significant difference for growth rates of PAO1 strains with and without *phuR* promoter mutation. However, there was a small yet significant increase in doubling time of DK2-CF30-1979 strain with *phuR* promoter mutation compared to the wild type (Table 1, Student t-test, $P < 0.05$). This indicates that *phuR* promoter mutation decreases the fitness of *P. aeruginosa* during anaerobic conditions.

phuR promoter mutation leads to change in colony pigmentation and interaction with Staphylococcus aureus

While we correlated downregulation of *narK1* and *narK2* with decreased fitness in the presence of nitrate, we still observed hundreds of genes being differentially expressed as a result of this promoter mutation. We therefore hypothesized that other key physiological changes are possibly caused by this mutation but we cannot directly detect them from microarray results. To investigate possible additional phenotypes caused by the *phuR* promoter mutation, we spotted cultures of the strains with and without *phuR* promoter mutation on a range of surface agar plates and incubated them at 37 °C for 48 hours. Interestingly, DK2-CF30-1979 with *phuR* promoter mutation exhibited a yellow/green colony pigment on ABT minimal medium agar plate whereas the isogenic wild type strain remained white (Figure 3). The change in pigment was absent in PAO1 strain with the mutation (data not shown), possibly because PAO1 already has a green pigment from pyoverdine

production that masks the new pigment. Furthermore, the pleiotropic effects by *phuR* promoter mutation in PAO1 were far less than that of CF adapted DK2-CF30-1979 isolate and perhaps such clear changes in phenotypes are absent in this strain. Additionally both strain of DK2-CF30-1979 exhibited similar pigment on LB agar plate. The second phenotype we sought to investigate was interaction of *P. aeruginosa* with other bacteria. For this purpose we selected *Staphylococcus aureus* because previous observations have highlighted synergistic interactions between CF adapted isolates of *P. aeruginosa* and *S. aureus*[25,26]. Similar to previous observations[25], *S. aureus* JE2 WT altered growth activity of *P. aeruginosa* DK2-CF30-1979 strains with and without *phuR* promoter mutation on LB agar plate (data not shown). However, when spotting *P. aeruginosa* strains next to *S. aureus* on Staphylococcal minimal medium[27] agar plate, we saw a seemingly increased inhibition of *S. aureus* by DK2-CF30-1979 with *phuR* promoter mutation. The change in pigment by presence of *phuR* promoter mutation was also confirmed in this minimal medium agar plate (Figure 2). We therefore have shown two additional phenotypes associated with *phuR* promoter mutation.

Discussion

We had previously investigated a series of recurrent mutations in the intergenic region upstream of *phuR* and *phuS* and verified that mutations in this region increase the expression of the *phu* system in *P. aeruginosa*. Furthermore, they confer a growth advantage in the presence of hemoglobin. In this study, we demonstrated that the overexpression of the *phu* system by promoter mutation result in pleiotropic effects on expression of many genes. The effect was most predominant

in a CF adapted background of *P. aeruginosa* and highly dependent on the environmental context. Looking at genes where expression was more radically changed (*Benjamini-Hochberg*, $P < 0.05$, FC > 2 or < -2), we found an enrichment of five functional classes of genes. Interestingly nine genes belonging to '*translation, post-translational modification, degradation*' were upregulated as a result of *phuR* promoter mutation. Moreover, expression of several ribosomal proteins such as *rpmE*, *rpsI*, *rpsU*, *rplM* and *rpsT* is also increased in the strain with the mutation. We hypothesize that the constitutive expression of *phu* system proteins at high levels overloads the translation machinery leading to upregulation of ribosomal proteins and proteins within the same functional class. However, whether the induction of pleiotropic effect on expression of all other genes is exclusively because of the translational stress remains unknown. In both transcriptomes of DK2 and PAO1 strains with *phuR* promoter mutation, the most upregulated gene after those of the *phu* system was PA4711, a gene located right after *phuR* in *P. aeruginosa* chromosome. PA4711 was also found to be upregulated in two clinical isolates (DK2-CF66-2008 and DK2-CF173-2005) where *phuR* promoter mutation occurred naturally. As PA4711 was also upregulated in other genetic backgrounds with *phuR* promoter mutation and in minimal media, we hypothesize the pleiotropic effects on expression of other genes may be partly or completely initiated by upregulation of PA4711. This gene encodes a hypothetical protein proposed to function as a ferrodoxin and have oxidoreductase activity[20,28]. Oxidoreductases mediate electron transfer between molecules and are part of energy metabolism systems in bacteria. Interestingly, genes of '*energy metabolism*' class were also among those with radically changed expression. We therefore propose that the overexpression of

234 PA4711 may trigger a shift in natural redox stability of *P. aeruginosa*. This can explain
235 change in expression of other players in energy metabolism. Moreover, *narK1* and
236 *narK2* encoding key players of nitrogen assimilation pathway were the most
237 downregulated genes in the strain with *phuR* promoter mutation. This led us to
238 come up with a model where overexpression of PA4711 shifts the redox balance of
239 the cell that ultimately results in reduction of anaerobic metabolism activity. We
240 tested this hypothesis and measured the fitness of isogenic strains with and without
241 *phuR* promoter mutation under nitrate limited anoxic condition. Our results
242 demonstrated that CF adapted strain of *P. aeruginosa* with the mutation is slightly
243 less fit to grow in anoxic condition. There is however conflicting data on primary
244 mode of *P. aeruginosa* growth in the CF environment. Some studies suggest that the
245 primary mode of growth is aerobic[29,30], while others suggest that it is anaerobic[31]. In
246 agreement with both models, we have only highlighted a possible reduction in
247 anaerobic activity where it is still active and the cell potentially functions under both
248 conditions. In an effort to discover additional physiological changes to *P. aeruginosa*
249 by *phuR* promoter mutation, we spotted it on surface agar plates alone and next to
250 *S. aureus*. We observed changes in colony pigmentation towards yellow/green and
251 increased inhibition of *S. aureus*. These two phenotypes can also be linked to
252 changes in redox balance and decreased anaerobic activity. In this model, decrease
253 in flux of anaerobic metabolism through nitrogen assimilation can be compensated
254 by other mechanism. Namely *P. aeruginosa* excretes redox active phenazines to
255 react with oxidants and be taken back by the bacteria, thereby acting as electron
256 shuttles. This helps to rebalance the cellular redox state and support survival in
257 anaerobic conditions[32,33]. We found no support for expression of phenazine genes in

transcriptomes of strain with *phuR* promoter mutation. Phenazine production may be affected at the post-transcriptional level by the *phu* mutation through an unknown mechanism. Regulation of phenazine production is immensely complex, including regulation at the post-transcriptional level by sRNA molecules[34]. Phenazines have various effects on gene expression, biofilm formation and maintenance[35] and act as virulence factors affecting host tissues of CF airways[36]. Moreover, phenazines have antibacterial activity against other bacteria such as *Staphylococcus aureus*[37]. Increased inhibition of *S. aureus* may be through increased production of phenazines or through unknown mechanisms such as interspecies competition with *P. aeruginosa*.

Until recently, *cis*-regulatory intergenic mutations were suggested to have possible local effects on expression of genes in bacteria. This study illustrates a new dimension for effect of these mutations on divergence of new phenotypes. These type of mutations can affect the expression of all genes while mutations within coding region are less likely to affect essential genes because of their deleterious nature. Furthermore, to have an intergenic mutation with pleiotropic effect is a complex scenario where additional beneficial phenotypes arise from the same mutation. However, rise of antagonistic pleitropy where expression of a gene is now detrimental calls for accumulation of additional mutations. Our study is limited in its focus on only one cis-regulatory intergenic mutation with pleiotropic effect and it remains to be elucidated on how widespread these types of intergenic mutations are occurring in evolution of bacteria. Nonetheless, the contribution of these specific mutations on adaptive phenotypes calls for considering them as missing piece of the puzzle in investigation of bacterial evolution.

Materials and methods

Bacterial strains and media

Isolates of *P. aeruginosa* DK2-CF30-1979 wild type and with *phuR* promoter mutation derive from a previous study[8]. The *phuR* promoter mutation was constructed in *P. aeruginosa* PAO1 by allelic exchange using pNJ1-*phu*R(CF173-2005) construct[8]. The construct was transferred to PAO1 by triparental mating using *E. coli* HB101/pRK600. Merodiploid isolates were selected on Pseudmonas isolation agar with tetracyclin. Colonies were restreaked on selective plates before being streaked on 6% (wt/vol) sucrose-no salt LB agar plates. Sucrose-resistant tetracycline sensitive colonies were restreaked on 6% sucrose no-salt LB plates, screened for the presence of the mutated allele by PCR verified by sequencing at LGC Genomics. Luria- Bertani (LB) broth was used for routine preparations of bacterial cultures. ABTGC and Staphylococcal minimal media (SMM) were prepared as previously described[8,27].

Gene expression analysis

All *P. aeruginosa* strains were grown at 37 °C 180 rpm in LB or ABTGC medium starting from OD_{600}=0.01 until OD_{600}=0.5. Bacterial cells were immediately mixed with RNAprotect Bacteria Reagent (Qiagen) and RNA was extracted using RNeasy Mini Kit (Qiagen). RNA extraction, processing of cDNA preparation, labeling and hybridization were done as previously described[15]. The raw CEL files were obtained using Affymetrix GeneChip operating system 1.4 and analyzed by BioConductor tools in the R environment[38]. Microarray expression data were normalized using the robust multichip average (*rma*)[39] algorithm and analysis of gene fold change

between wild type and mutant strains were performed using the *limma* package[40]. Strains were tested in triplicates.

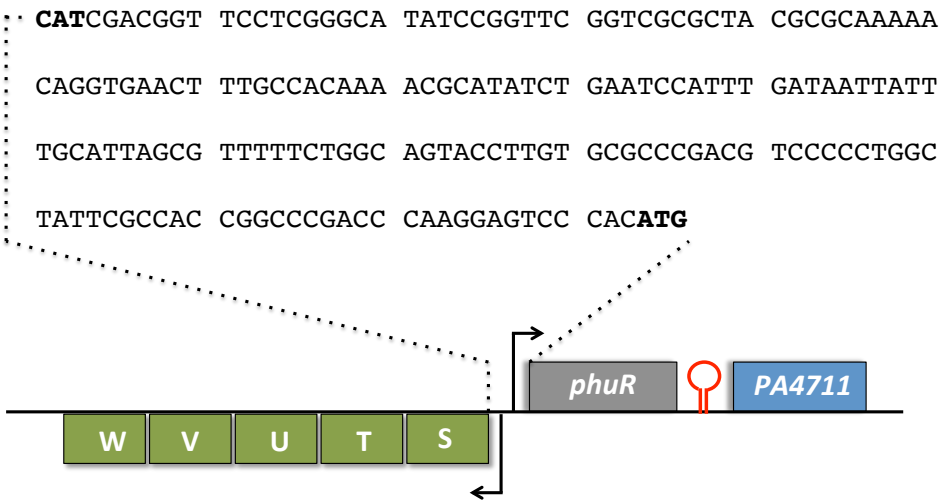
Anoxic growth rate measurements

To examine difference in doubling time *P. aeruginosa* PAO1 and DK2-CF30-1979 with and without *phuR* promoter mutation were propagated overnight in LB media at 37°C, 180 rpm. The overnight cultures were adjusted to an optical density (OD_{600}) of 0.1 and followed until exponential growth to assure that the cultures were in an optimal condition. Subsequently, cultures were adjusted to an optical density (OD_{600}) of 0.05 in glass vials (Schuett Biotec, Germany) with a final volume of 2 mL LB medium containing 10 mM nitrate as alternative electron acceptor. Preparation of cultures in vials was performed inside an anaerobic chamber (Concept 400 Anaerobic Workstation, Ruskinn Technology Ltd, UK) to avoid introduction of oxygen. Furthermore, to create an anoxic environment during growth vials were sealed off with a lid before they were left to incubate at 37°C, 180 rpm. Optical density (OD_{600}) was continually measured as an expression of growth. All media applied for preparation of the vials were equilibrated in the anaerobic chamber 3 days prior to experiment to remove traces of oxygen.

Spot inoculation of Pseudomonas aeruginosa and Staphylococcus aureus

ABTGC and SMM agar plates were made by adding 2% (wt/vol) of agar. Cultures of *P. aeruginosa* PAO1 and DK2-CF30-1979 with and without *phuR* promoter mutation were grown overnight in LB. Cultures were washed with 0.9% NaCl solution three times and the optical density at 600 nm [OD_{600}] was adjusted at 1.0. Five microliters

of each suspension was spotted on ABTGC agar plate and incubated for 48 hours at 37 °C. The morphology and pigment of spots were inspected to observe phenotypes caused by *phuR* promoter mutation. Three biological replicates of each strain were spotted on ABTGC agar plate. To study the interaction of *P. aeruginosa* with *S. aureus*, cell suspensions of *P. aeruginosa* strains and *S. aureus* JE2 WT[41] were prepared similarly and spotted alone or next to each other on SMM agar plate. The interaction zone was inspected after 48 hours growth at 37 °C. The interaction experiment was repeated three times.

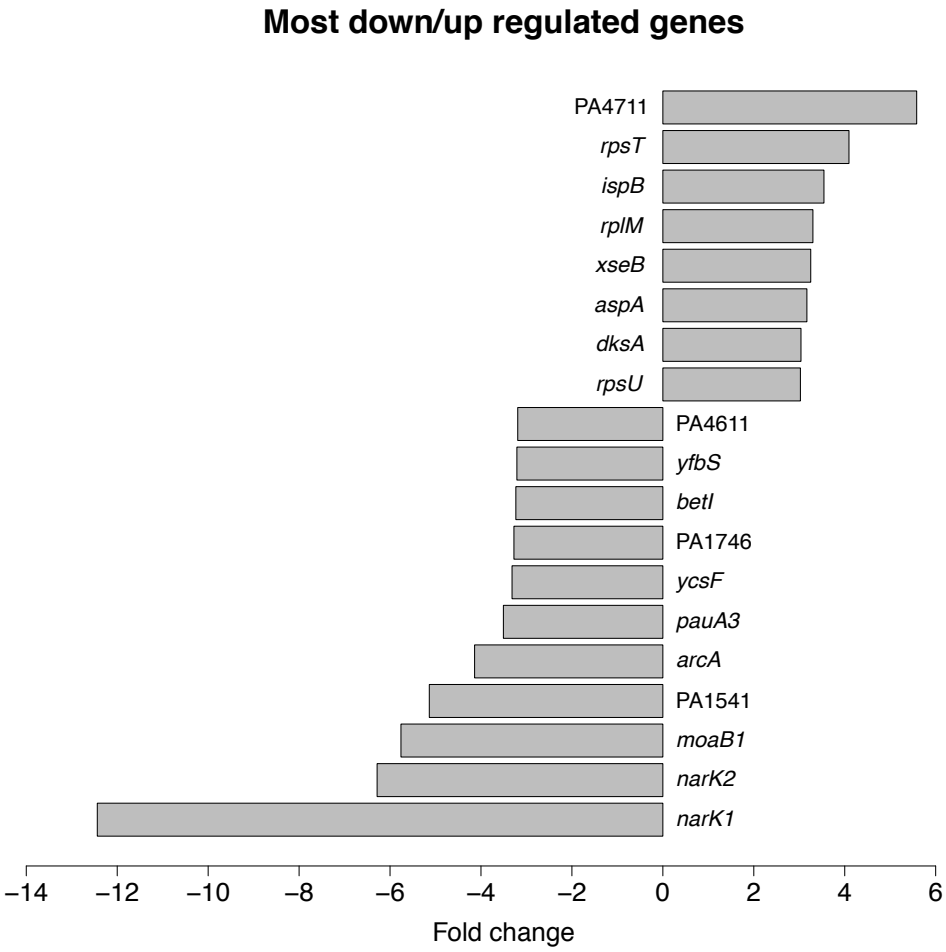


337

338 **Figure 1** Intergenic region between *phuR* and *phuSTUVW* consists of 180 bp. PA4711 is located right downstream

339 of phuR following an intergenic region of 102 bp. A Rho-independent transcriptional terminator is present within

340 this region separating operons of *phuR* and PA4711.



341

342 **Figure 2** Genes most up/down regulated in DK2-CF30-1979-M2 compared to the wild type in LB medium. Eight

343 genes are down-regulated and eleven are up-regulated (FC > 3 or < - 3). The list excludes the *phu* operon genes.

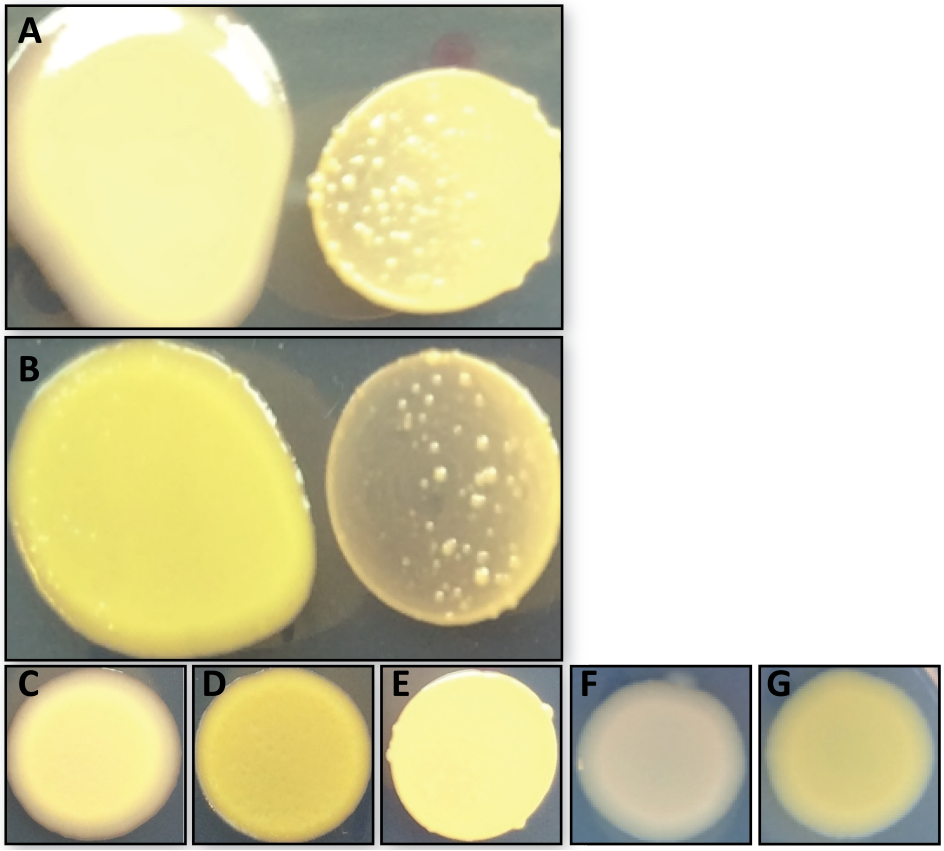


Figure 3 A) Spot inoculation of *Pseudomonas aeruginosa* DK2-CF30-1979 (left) next to *Staphylococcus aureus* JE2 WT (right) on SMM agar plate. **B)** Spot inoculation of *P. aeruginosa* DK2-CF30-1979-M2 containing *phuR* promoter mutation (left) next to the same *S. aureus* strain on the same plate. **C)** Mono spot inoculation of *P. aeruginosa* DK2-CF30-1979 on SMM agar plate **D)** Mono spot inoculation of *P. aeruginosa* DK2-CF30-1979-M2 on the same plate **E)** Mono spot inoculation of *S. aureus* JE2 WT on the same plate. **F)** Mono spot inoculation of *P. aeruginosa* DK2-CF30-1979 on ABTGC agar plate. **G)** Mono spot inoculation of *P. aeruginosa* DK2-CF30-1979-M2 on the same plate as **F**.

Table 1 measurements of doubling time (h) for PAO1 and DK2-CF30-1979 strains with (M2) and without *phuR* promoter mutation (WT) during anoxic growth in LB medium containing 10 mM nitrate.

	Doubling time (h)		*P* value
	WT	M2	
DK2-CF30-1979	2.92 ± 0.04	3.11 ± 0.08	0.02
PAO1	1.15 ± 0.04	1.09 ± 0.04	0.12

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Supplementary Table 1: Overview of significantly altered expressions (adj.p.Val < 0.05) between DK2-CF30-1979-M2 and DK2-CF30-1979 in LB medium. Locus ID, Loc tag, name, synonyms and PseudoCAP function class of each gene is descibed. Calculations of log fold changes and p-values are done using the *limma* package in R.

Locus ID	Locus Tag	Name	Synonyms	log Fold Change	Fold Change	P.Value	adj.P.Val	PseudoCAP Function Class
PA3877_narK1_at	PA3877	narK1		-3,64	-12,44	0,000389008	0,004850801	Membrane proteins; Transport of small molecules
PA3876_narK2_at	PA3876	narK2		-2,65	-6,28	6,03E-07	0,000303998	Membrane proteins; Transport of small molecules
PA3915_moaB1_at	PA3915	moaB1		-2,53	-5,76	2,98E-06	0,000662084	Biosynthesis of cofactors, prosthetic groups and carriers
PA1541_at	PA1541			-2,36	-5,14	1,80E-08	1,66E-05	Membrane proteins; Transport of small molecules
PA5171_arcA_at	PA5171	arcA		-2,05	-4,14	0,000273191	0,004042495	Amino acid biosynthesis and metabolism
PA1566_at	PA1566	pauA3		-1,81	-3,51	0,000579726	0,006258563	Carbon compound catabolism
PA0492_at	PA0492		ycsF	-1,73	-3,32	2,81E-06	0,000648569	Hypothetical, unclassified, unknown
PA1746_at	PA1746			-1,71	-3,28	0,011733588	0,045341003	Hypothetical, unclassified, unknown
PA5374_betI_at	PA5374	betI		-1,69	-3,23	3,39E-07	0,000209268	Transcriptional regulators
PA3839_at	PA3839		yfbS	-1,68	-3,21	0,002077826	0,013975584	Membrane proteins; Transport of small molecules
PA4611_at	PA4611			-1,67	-3,19	0,012105251	0,046421589	Hypothetical, unclassified, unknown
PA5231_at	PA5231		yhiH	-1,57	-2,96	0,002744324	0,016562738	Membrane proteins; Transport of small molecules
PA1540_at	PA1540			-1,52	-2,87	1,98E-05	0,001438517	Membrane proteins
PA0297_at	PA0297	spuA	ycjL	-1,41	-2,66	3,65E-05	0,001684034	Amino acid biosynthesis and metabolism; Carbon compound catabolism
PA1565_at	PA1565	pauB2		-1,40	-2,65	0,002238226	0,014509245	Putative enzymes; Carbon compound catabolism
PA1602_at	PA1602			-1,37	-2,58	3,87E-05	0,00168556	Carbon compound catabolism
PA0132_at	PA0132	bauA	oapT	-1,35	-2,54	4,49E-05	0,001753067	Amino acid biosynthesis and metabolism; Carbon compound catabolism
PA2555_at	PA2555			-1,31	-2,48	0,000385639	0,004819614	Putative enzymes
PA2554_at	PA2554			-1,28	-2,43	0,000373277	0,004706191	Putative enzymes
PA4889_at	PA4889			-1,24	-2,37	5,40E-05	0,001949826	Putative enzymes
PA3584_glpD_at	PA3584	glpD		-1,23	-2,35	0,003644104	0,020001121	Central intermediary metabolism; Energy metabolism
PA2260_at	PA2260		kguE	-1,23	-2,35	6,38E-05	0,002047415	Hypothetical, unclassified, unknown; Carbon compound catabolism
PA5373_betB_at	PA5373	betB		-1,22	-2,33	3,59E-05	0,001684034	Amino acid biosynthesis and metabolism; Adaptation, Protection
PA5172_arcB_at	PA5172	arcB		-1,22	-2,32	0,000121942	0,00270663	Amino acid biosynthesis and metabolism
PA1555_at	PA1555	ccoP2	ccoP; fixP	-1,20	-2,30	0,012769421	0,04804105	Energy metabolism; Central intermediary metabolism
PA4888_at	PA4888	desB	desB	-1,14	-2,21	0,000130142	0,002783815	Fatty acid and phospholipid metabolism
PA1707_pcrH_at	PA1707	pcrH		-1,13	-2,19	0,00012417	0,002720758	Secreted Factors (toxins, enzymes, alginate); Protein secretion/export apparatus
PA1601_at	PA1601			-1,13	-2,19	2,08E-05	0,001438517	Putative enzymes
PA2482_at	PA2482			-1,12	-2,18	0,00024003	0,003827368	Energy metabolism
PA5372_betA_at	PA5372	betA		-1,11	-2,16	1,42E-06	0,000490944	Amino acid biosynthesis and metabolism; Adaptation, Protection
PA2481_at	PA2481			-1,10	-2,14	0,001408127	0,010616439	Hypothetical, unclassified, unknown
PA3582_glpK_at	PA3582	glpK		-1,08	-2,11	0,002157867	0,01422091	Central intermediary metabolism
PA2553_at	PA2553			-1,07	-2,10	0,000708054	0,007079261	Putative enzymes
PA2790_at	PA2790			-1,05	-2,07	1,06E-05	0,001107979	Hypothetical, unclassified, unknown
PA2010_at	PA2010			-1,04	-2,05	7,63E-05	0,002190448	Transcriptional regulators
PA1551_at	PA1551		fixG	-1,03	-2,04	0,002903775	0,017196421	Energy metabolism
PA1137_at	PA1137			-1,01	-2,02	0,001529251	0,011165544	Putative enzymes
PA4063_at	PA4063			-1,00	-2,00	1,69E-05	0,001389481	Hypothetical, unclassified, unknown
PA2009_hmgA_at	PA2009	hmgA		-0,95	-1,94	0,001341089	0,010307068	Carbon compound catabolism
PA0872_phhA_at	PA0872	phhA		-0,95	-1,93	0,005680344	0,027389334	Amino acid biosynthesis and metabolism
PA5002_at	PA5002			-0,94	-1,92	6,88E-06	0,000891574	Membrane proteins
PA1706_pcrV_at	PA1706	pcrV		-0,94	-1,92	9,37E-05	0,002433075	Protein secretion/export apparatus
PA3710_at	PA3710			-0,93	-1,91	4,68E-05	0,001791314	Carbon compound catabolism
PA2318_at	PA2318			-0,92	-1,89	0,002969302	0,017442288	Hypothetical, unclassified, unknown
PA3274_at	PA3274			-0,91	-1,88	0,002673717	0,016277223	Hypothetical, unclassified, unknown
PA2174_at	PA2174			-0,91	-1,88	0,00014109	0,002835467	Hypothetical, unclassified, unknown
PA1809_at	PA1809			-0,91	-1,88	9,49E-06	0,001052699	Transport of small molecules
PA3341_at	PA3341			-0,89	-1,86	0,000252768	0,003914118	Transcriptional regulators
PA3933_at	PA3933	betT3	betT3	-0,89	-1,85	0,000134552	0,002796363	Membrane proteins; Transport of small molecules
PA2024_at	PA2024			-0,88	-1,84	0,001155777	0,009492396	Putative enzymes
PA1708_popB_at	PA1708	popB	pepB	-0,87	-1,83	0,000183259	0,003291616	Protein secretion/export apparatus
PA3973_at	PA3973			-0,87	-1,83	0,000370486	0,004685542	Transcriptional regulators
PA1197_at	PA1197			-0,85	-1,81	0,002815608	0,016894049	Hypothetical, unclassified, unknown
PA1281_cobV_at	PA1281	cobV	cobS	-0,85	-1,80	2,10E-05	0,001438517	Biosynthesis of cofactors, prosthetic groups and carriers
PA5230_at	PA5230		yhhJ	-0,84	-1,79	2,30E-05	0,001534556	Membrane proteins; Transport of small molecules
PA2126_at	PA2126	cgrC		-0,84	-1,79	6,48E-05	0,002047415	Transcriptional regulators
PA3075_at	PA3075			-0,84	-1,79	2,70E-06	0,000648569	Hypothetical, unclassified, unknown
PA2552_at	PA2552		acdB	-0,83	-1,78	0,001073284	0,009162543	Putative enzymes
PA4321_at	PA4321			-0,83	-1,78	3,81E-05	0,00168556	Hypothetical, unclassified, unknown
PA0310_at	PA0310			-0,83	-1,77	7,51E-06	0,000946655	Hypothetical, unclassified, unknown
PA4476_at	PA4476			-0,82	-1,77	6,56E-06	0,000887606	Hypothetical, unclassified, unknown
PA4320_at	PA4320			-0,82	-1,77	7,01E-05	0,00210154	Hypothetical, unclassified, unknown; Membrane proteins
PA3557_at	PA3557	arnE	pmrL; arnE	-0,81	-1,76	2,38E-05	0,001556036	Adaptation, Protection; Cell wall / LPS / capsule; Membrane proteins
PA1178_oprH_at	PA1178	oprH		-0,81	-1,75	0,000186928	0,003313943	Membrane proteins; Adaptation, Protection; Transport of small molecules
PA0835_pta_at	PA0835	pta		-0,81	-1,75	0,000800086	0,007654611	Carbon compound catabolism
PA5458_at	PA5458			-0,80	-1,74	3,46E-05	0,001684034	Membrane proteins; Cell wall / LPS / capsule
PA1180_phoQ_at	PA1180	phoQ		-0,80	-1,74	0,000238215	0,003809384	Two-component regulatory systems
PA1549_at	PA1549		fixI	-0,80	-1,74	7,89E-06	0,000973191	Membrane proteins; Transport of small molecules
PA4072_at	PA4072			-0,80	-1,74	0,000299859	0,004255549	Transport of small molecules
PA4937_rnr_at	PA4937	rnr	vacB	-0,79	-1,73	0,000142054	0,002835467	Transcription, RNA processing and degradation
PA5499_np20_at	PA5499	zur	np20	-0,79	-1,73	3,82E-06	0,000737709	Transcriptional regulators
PA2008_fahA_at	PA2008	fahA		-0,78	-1,72	0,000327628	0,0044559	Carbon compound catabolism
PA2240_at	PA2240	pslJ		-0,78	-1,72	2,13E-05	0,0014394	Cell wall / LPS / capsule
PA2014_at	PA2014	liuB	gnyB	-0,78	-1,72	0,000219845	0,003647122	Carbon compound catabolism
PA3914_moeA1_at	PA3914	moeA1		-0,78	-1,71	0,000963059	0,008582517	Biosynthesis of cofactors, prosthetic groups and carriers
PA0920_at	PA0920			-0,77	-1,71	0,000523402	0,005842549	Membrane proteins
PA1058_at	PA1058	shaE	phaF	-0,77	-1,70	0,000203824	0,003475817	Membrane proteins; Transport of small molecules
PA0130_at	PA0130	bauC		-0,77	-1,70	0,000237657	0,003809384	Putative enzymes; Carbon compound catabolism
PA1718_pscE_at	PA1718	pscE		-0,76	-1,70	0,003488215	0,019433839	Protein secretion/export apparatus; Chaperones & heat shock proteins
PA0131_at	PA0131	bauB		-0,76	-1,69	0,005658009	0,02736891	Carbon compound catabolism
PA3493_at	PA3493		rnfG	-0,76	-1,69	5,17E-05	0,001914115	Hypothetical, unclassified, unknown
PA2006_at	PA2006			-0,76	-1,69	0,000846528	0,00792139	Membrane proteins; Transport of small molecules
PA2013_at	PA2013	liuC	menB; gnyH	-0,76	-1,69	5,91E-05	0,002022898	Carbon compound catabolism
PA1600_at	PA1600			-0,76	-1,69	3,92E-05	0,00168556	Energy metabolism
PA4115_at	PA4115			-0,76	-1,69	0,000503526	0,005678997	Hypothetical, unclassified, unknown
PA4353_at	PA4353		yajB	-0,75	-1,69	6,30E-05	0,002047415	Hypothetical, unclassified, unknown
PA2711_at	PA2711		potF4	-0,75	-1,68	2,87E-05	0,001580082	Transport of small molecules
PA4413_ftsW_at	PA4413	ftsW		-0,75	-1,68	6,91E-06	0,000891574	Cell division
PA1808_at	PA1808			-0,75	-1,68	3,29E-05	0,001642356	Transport of small molecules
PA2526_at	PA2526	muxC	yegO	-0,75	-1,68	0,000119059	0,00268468	Membrane proteins; Transport of small molecules; Antibiotic resistance and susceptibility
PA5473_at	PA5473		yjbB	-0,74	-1,67	0,00539359	0,026494551	Membrane proteins
PA0505_at	PA0505			-0,74	-1,67	3,57E-05	0,001684034	Hypothetical, unclassified, unknown
PA4412_murG_at	PA4412	murG		-0,74	-1,67	1,16E-05	0,00116694	Carbon compound catabolism; Cell wall / LPS / capsule
PA4409_ftsQ_at	PA4409	ftsQ		-0,74	-1,67	0,000160728	0,003054373	Cell division
PA4126_at	PA4126		hpaX	-0,74	-1,67	4,39E-05	0,001749766	Membrane proteins; Carbon compound catabolism; Transport of small molecules
PA2046_at	PA2046			-0,74	-1,67	1,46E-05	0,001289963	Hypothetical, unclassified, unknown
PA4499_at	PA4499			-0,73	-1,66	3,73E-05	0,001684034	Transcriptional regulators
PA4964_parC_at	PA4964	parC		-0,72	-1,65	3,38E-05	0,001675744	DNA replication, recombination, modification and repair
PA4002_rodA_at	PA4002	rodA	mrdB	-0,72	-1,65	0,00040098	0,004924819	Cell wall / LPS / capsule
PA3089_at	PA3089			-0,72	-1,64	0,001485109	0,010964942	Hypothetical, unclassified, unknown
PA1550_at	PA1550			-0,72	-1,64	0,000421811	0,005024095	Hypothetical, unclassified, unknown
PA1338_ggt_at	PA1338	ggt		-0,71	-1,64	1,03E-05	0,001098882	Amino acid biosynthesis and metabolism; Adaptation, Protection; Central intermediary metabolism
PA4410_ddlB_at	PA4410	ddlB		-0,71	-1,64	3,59E-05	0,001684034	Cell wall / LPS / capsule
PA2539_at	PA2539		ynbD	-0,71	-1,64	0,000212622	0,003575272	Membrane proteins
PA3074_at	PA3074			-0,71	-1,63	1,73E-05	0,001389481	Hypothetical, unclassified, unknown
PA3494_at	PA3494		rnfE	-0,71	-1,63	3,72E-05	0,001684034	Hypothetical, unclassified, unknown
PA0129_gabP_at	PA0129	bauD		-0,70	-1,63	0,000260991	0,003935439	Transport of small molecules; Carbon compound catabolism
PA1527_at	PA1527			-0,70	-1,62	1,20E-05	0,001169601	Hypothetical, unclassified, unknown
PA3658_glnD_at	PA3658	glnD	nfrX	-0,69	-1,62	4,66E-05	0,001791314	Amino acid biosynthesis and metabolism
PA4596_at	PA4596	esrC		-0,69	-1,62	3,95E-05	0,00168556	Transcriptional regulators
PA0755_at	PA0755	opdH	opdH	-0,69	-1,62	8,04E-05	0,002248955	Membrane proteins; Transport of small molecules
PA0072_at	PA0072	tagS1		-0,69	-1,62	0,000282141	0,00410918	Membrane proteins; Protein secretion/export apparatus
PA1709_popD_at	PA1709	popD	pepD	-0,69	-1,61	0,000137929	0,002801888	Protein secretion/export apparatus
PA1810_at	PA1810			-0,69	-1,61	5,21E-06	0,000825616	Transport of small molecules
PA1059_at	PA1059	shaF	phaG	-0,68	-1,61	0,000264739	0,003956554	Membrane proteins; Transport of small molecules
PA4220_i_at	PA4220		fptB	-0,68	-1,61	1,09E-05	0,001124211	Hypothetical, unclassified, unknown
PA4003_pbpA_at	PA4003	pbpA	mrdA	-0,68	-1,60	0,00014381	0,002839872	Cell wall / LPS / capsule
PA3459_at	PA3459		asnB	-0,68	-1,60	0,003737929	0,020435239	Amino acid biosynthesis and metabolism
PA2643_nuoH_at	PA2643	nuoH		-0,67	-1,60	6,75E-05	0,002058865	Energy metabolism
PA2408_at	PA2408			-0,67	-1,59	0,000772875	0,007524004	Transport of small molecules
PA4416_murF_at	PA4416	murF		-0,67	-1,59	9,41E-06	0,001052699	Cell wall / LPS / capsule
PA1278_cobP_at	PA1278	cobP	cobU	-0,67	-1,59	1,46E-05	0,001289963	Biosynthesis of cofactors, prosthetic groups and carriers
PA3492_at	PA3492		rnfD	-0,67	-1,59	7,93E-05	0,002245254	Hypothetical, unclassified, unknown
PA4488_at	PA4488	magE		-0,67	-1,59	0,000112262	0,002606459	Hypothetical, unclassified, unknown
PA2007_maiA_i_at	PA2007	maiA		-0,66	-1,58	0,000271365	0,004037015	Carbon compound catabolism
PA5042_pilO_at	PA5042	pilO		-0,66	-1,58	0,000370689	0,004685542	Motility & Attachment
PA0038_at	PA0038			-0,66	-1,58	0,008001656	0,034906597	Hypothetical, unclassified, unknown
PA3890_at	PA3890	opuCB		-0,66	-1,58	0,000103005	0,002492719	Membrane proteins; Transport of small molecules
PA4408_ftsA_at	PA4408	ftsA		-0,66	-1,58	0,000101217	0,002492719	Cell division
PA4117_at	PA4117	bphP		-0,66	-1,58	6,18E-05	0,002047415	Two-component regulatory systems
PA4415_mraY_at	PA4415	mraY	ORF Y	-0,65	-1,57	2,87E-05	0,001580082	Cell wall / LPS / capsule

PA4936_at	PA4936		spoU; yjfH	-0,65	-1,57	0,000165006	0,003114343	Transcription, RNA processing and degradation
PA5010_waaG_at	PA5010	waaG	rfaG	-0,65	-1,57	5,63E-05	0,001962587	Cell wall / LPS / capsule
PA1277_cobQ_at	PA1277	cobQ	cbiP	-0,65	-1,57	6,06E-05	0,002047415	Biosynthesis of cofactors, prosthetic groups and carriers
PA3418_ldh_at	PA3418	ldh		-0,65	-1,57	0,006067936	0,028624717	Amino acid biosynthesis and metabolism
PA4418_ftsI_at	PA4418	ftsI	pbpB	-0,65	-1,57	6,77E-05	0,002058865	Cell division; Cell wall / LPS / capsule
PA2239_at	PA2239	psII		-0,64	-1,56	0,000104219	0,002492719	Putative enzymes; Cell wall / LPS / capsule
PA0754_at	PA0754			-0,64	-1,56	0,000487775	0,005546439	Hypothetical, unclassified, unknown
PA0839_at	PA0839			-0,64	-1,55	0,001159821	0,009492396	Transcriptional regulators
PA1716_pscC_at	PA1716	pscC		-0,64	-1,55	0,000280342	0,004093732	Protein secretion/export apparatus
PA3923_at	PA3923			-0,63	-1,55	0,00153242	0,01117398	Hypothetical, unclassified, unknown
PA4411_murC_at	PA4411	murC		-0,63	-1,55	2,71E-05	0,001564423	Cell wall / LPS / capsule
PA1526_at	PA1526			-0,63	-1,55	2,96E-05	0,001580082	Transcriptional regulators
PA1054_at	PA1054	shaA	phaA	-0,63	-1,55	2,87E-05	0,001580082	Membrane proteins; Putative enzymes; Transport of small molecules
PA1173_napB_at	PA1173	napB		-0,63	-1,55	0,000780514	0,007568922	Energy metabolism
PA3214_at	PA3214			-0,63	-1,55	3,03E-05	0,001587784	Hypothetical, unclassified, unknown
PA1276_cobC_at	PA1276	cobC	cobD	-0,63	-1,55	2,09E-05	0,001438517	Biosynthesis of cofactors, prosthetic groups and carriers
PA2527_at	PA2527	muxB	yegN	-0,63	-1,55	2,93E-05	0,001580082	Membrane proteins; Transport of small molecules; Antibiotic resistance and susceptibility
PA2012_at	PA2012	liuD	accA; mccA; gnyA	-0,63	-1,54	3,62E-05	0,001684034	Carbon compound catabolism
PA4323_at	PA4323			-0,62	-1,54	0,00020406	0,003475817	Hypothetical, unclassified, unknown
PA3560_fruA_at	PA3560	fruA		-0,62	-1,54	0,00022191	0,003659944	Carbon compound catabolism; Transport of small molecules
PA4879_at	PA4879		yhjG	-0,62	-1,54	0,00063475	0,006543734	Hypothetical, unclassified, unknown
PA2144_glgP_at	PA2144	glgP		-0,62	-1,54	0,000527526	0,005852832	Cell wall / LPS / capsule
PA4120_at	PA4120			-0,62	-1,54	0,00273604	0,016538436	Transcriptional regulators
PA5008_at	PA5008		waaX; wapP	-0,62	-1,53	3,47E-05	0,001684034	Hypothetical, unclassified, unknown; Putative enzymes
PA4965_at	PA4965			-0,62	-1,53	7,91E-05	0,002245254	Hypothetical, unclassified, unknown
PA2002_at	PA2002		atoE	-0,61	-1,53	0,001624779	0,011617896	Hypothetical, unclassified, unknown; Membrane proteins
PA2179_at	PA2179			-0,61	-1,53	6,60E-05	0,002047415	Hypothetical, unclassified, unknown
PA3581_glpF_at	PA3581	glpF		-0,61	-1,53	0,007203922	0,032315736	Transport of small molecules
PA3222_at	PA3222			-0,61	-1,53	0,000648622	0,006604048	Membrane proteins
PA2647_nuoL_at	PA2647	nuoL		-0,61	-1,52	0,00012985	0,002783815	Energy metabolism
PA0073_at	PA0073	tagT1		-0,61	-1,52	0,000159311	0,003041892	Transport of small molecules; Protein secretion/export apparatus
PA1179_phoP_at	PA1179	phoP		-0,61	-1,52	0,002990433	0,017504128	Transcriptional regulators; Two-component regulatory systems
PA0077_at	PA0077	icmF1		-0,61	-1,52	4,26E-05	0,001728886	Protein secretion/export apparatus
PA1174_napA_at	PA1174	napA		-0,60	-1,52	0,000197138	0,00342922	Energy metabolism
PA0414_at	PA0414	chpB		-0,60	-1,52	4,46E-05	0,001753067	Chemotaxis
PA5154_at	PA5154			-0,60	-1,52	0,003227733	0,018369939	Membrane proteins; Transport of small molecules
PA0919_at	PA0919			-0,60	-1,52	0,001877716	0,012943412	Hypothetical, unclassified, unknown
PA1717_pscD_at	PA1717	pscD		-0,60	-1,52	0,000245873	0,003859019	Protein secretion/export apparatus
PA2152_at	PA2152			-0,60	-1,51	0,00100598	0,008816382	Putative enzymes
PA1759_at	PA1759			-0,60	-1,51	9,00E-05	0,002378021	Transcriptional regulators
PA4489_at	PA4489	magD		-0,60	-1,51	0,001130384	0,009394647	Adaptation, Protection
PA0587_at	PA0587		yeaH	-0,59	-1,51	0,000174926	0,003218181	Hypothetical, unclassified, unknown
PA5165_at	PA5165	dctB		-0,59	-1,51	3,24E-05	0,001636633	Transport of small molecules; Two-component regulatory systems
PA3659_at	PA3659		dapC	-0,59	-1,50	0,000107834	0,002546258	Putative enzymes
PA5450_wzt_at	PA5450	wzt		-0,59	-1,50	0,000293037	0,004232825	Cell wall / LPS / capsule; Transport of small molecules
PA3257_prc_at	PA3257	prc	tsp	-0,59	-1,50	7,42E-05	0,002164252	Translation, post-translational modification, degradation
PA5009_waaP_at	PA5009	waaP	rfaP	-0,59	-1,50	0,000757409	0,007425553	Cell wall / LPS / capsule
PA2238_at	PA2238	pslH		-0,58	-1,50	8,30E-05	0,002256519	Cell wall / LPS / capsule
PA2644_nuoI_at	PA2644	nuoI		-0,58	-1,50	0,000178128	0,003251418	Energy metabolism
PA1080_flgE_at	PA1080	flgE		-0,58	-1,50	0,002120929	0,014094652	Cell wall / LPS / capsule; Motility & Attachment
PA3802_hisS_at	PA3802	hisS		-0,58	-1,50	0,000900781	0,008207605	Translation, post-translational modification, degradation
PA3972_at	PA3972		aidB	-0,58	-1,50	5,62E-05	0,001962587	Putative enzymes
PA1692_at	PA1692		pscS	-0,58	-1,49	8,07E-05	0,002248955	Protein secretion/export apparatus
PA4116_at	PA4116	bphO		-0,58	-1,49	0,000514617	0,005768908	Hypothetical, unclassified, unknown
PA2259_ptxS_at	PA2259	ptxS		-0,58	-1,49	0,009978341	0,040743056	Transcriptional regulators
PA4283_recD_at	PA4283	recD		-0,58	-1,49	2,47E-05	0,001564423	DNA replication, recombination, modification and repair
PA2080_at	PA2080	kynU		-0,58	-1,49	0,000122556	0,002709418	Amino acid biosynthesis and metabolism
PA0529_at	PA0529			-0,58	-1,49	0,007610925	0,033786419	Hypothetical, unclassified, unknown
PA1637_kdpE_at	PA1637	kdpE		-0,58	-1,49	0,000294444	0,004232825	Transcriptional regulators; Two-component regulatory systems
PA3073_at	PA3073			-0,58	-1,49	0,000260962	0,003935439	Hypothetical, unclassified, unknown
PA5003_at	PA5003			-0,57	-1,49	0,000143182	0,002839872	Antibiotic resistance and susceptibility
PA4414_murD_at	PA4414	murD		-0,57	-1,49	2,68E-05	0,001564423	Cell wall / LPS / capsule
PA4870_at	PA4870		ybil	-0,57	-1,48	0,000568653	0,006187171	Hypothetical, unclassified, unknown
PA1056_at	PA1056	shaC	phaD	-0,57	-1,48	0,000606524	0,006361347	Membrane proteins; Putative enzymes; Transport of small molecules
PA2649_nuoN_at	PA2649	nuoN		-0,57	-1,48	7,99E-05	0,002248955	Energy metabolism; Antibiotic resistance and susceptibility
PA2550_at	PA2550			-0,57	-1,48	0,001761225	0,012246917	Putative enzymes
PA1404_at	PA1404			-0,57	-1,48	0,004666172	0,023908205	Hypothetical, unclassified, unknown
PA1870_at	PA1870			-0,57	-1,48	0,000424588	0,005024095	Hypothetical, unclassified, unknown
PA5364_at	PA5364			-0,57	-1,48	0,013226862	0,048865419	Transcriptional regulators; Two-component regulatory systems
PA1636_kdpD_at	PA1636	kdpD		-0,56	-1,48	7,40E-05	0,002164252	Two-component regulatory systems
PA1964_at	PA1964		ybiT	-0,56	-1,48	0,000575948	0,006229897	Transport of small molecules
PA5500_znuC_at	PA5500	znuC	yebM	-0,56	-1,47	0,001351738	0,010331676	Transport of small molecules
PA1807_at	PA1807			-0,56	-1,47	0,000195747	0,003426505	Transport of small molecules
PA4798_at	PA4798			-0,55	-1,47	0,000119986	0,00268468	Hypothetical, unclassified, unknown
PA0298_at	PA0298	spuB		-0,55	-1,47	0,007657124	0,033883077	Putative enzymes; Carbon compound catabolism
PA0840_at	PA0840			-0,55	-1,47	0,00061299	0,006393765	Putative enzymes
PA4487_at	PA4487	magF		-0,55	-1,46	0,000137311	0,002801888	Hypothetical, unclassified, unknown
PA2232_at	PA2232	pslB		-0,55	-1,46	0,000771186	0,007520762	Cell wall / LPS / capsule
PA2160_at	PA2160		glgX	-0,55	-1,46	0,00056831	0,006187171	Putative enzymes
PA5457_at	PA5457			-0,55	-1,46	0,000177993	0,003251418	Cell wall / LPS / capsule
PA5022_at	PA5022		aefA	-0,55	-1,46	0,000572457	0,006204226	Hypothetical, unclassified, unknown
PA2824_at	PA2824	sagS		-0,55	-1,46	0,000938641	0,008455387	Two-component regulatory systems; Cell wall / LPS / capsule
PA2920_at	PA2920			-0,54	-1,46	0,000394014	0,004880325	Adaptation, Protection; Chemotaxis
PA2525_at	PA2525	opmB	opmB	-0,54	-1,46	0,000351453	0,004588732	Membrane proteins; Transport of small molecules; Antibiotic resistance and susceptibility
PA2961_holB_at	PA2961	holB		-0,54	-1,46	0,001224422	0,009706166	DNA replication, recombination, modification and repair
PA5484_at	PA5484	kinB	kinB	-0,54	-1,46	0,002233556	0,014501484	Two-component regulatory systems
PA4966_at	PA4966			-0,54	-1,45	0,000196624	0,00342922	Hypothetical, unclassified, unknown
PA3356_at	PA3356	pauA5		-0,54	-1,45	0,000233487	0,003766331	Carbon compound catabolism
PA2163_at	PA2163			-0,54	-1,45	9,99E-05	0,002492719	Hypothetical, unclassified, unknown
PA1461_at	PA1461	motD	motB	-0,54	-1,45	4,79E-05	0,001821684	Motility & Attachment
PA2162_at	PA2162			-0,54	-1,45	0,000935033	0,00843658	Putative enzymes
PA5493_polA_at	PA5493	polA		-0,54	-1,45	2,63E-05	0,001564423	DNA replication, recombination, modification and repair
PA3072_at	PA3072			-0,53	-1,45	4,84E-05	0,001826948	Hypothetical, unclassified, unknown
PA2645_nuoJ_at	PA2645	nuoJ		-0,53	-1,45	0,000207264	0,003517142	Energy metabolism
PA1336_at	PA1336	aauS		-0,53	-1,45	0,000120706	0,002689949	Two-component regulatory systems
PA3213_at	PA3213			-0,53	-1,45	0,0001367	0,002801888	Hypothetical, unclassified, unknown
PA5420_purU2_at	PA5420	purU2		-0,53	-1,45	0,000130938	0,002783815	Nucleotide biosynthesis and metabolism
PA4284_recB_at	PA4284	recB	rorA	-0,53	-1,44	0,000106639	0,002528802	DNA replication, recombination, modification and repair
PA3366_amiE_at	PA3366	amiE		-0,53	-1,44	0,00141893	0,010674419	Carbon compound catabolism
PA3267_at	PA3267			-0,53	-1,44	0,000209977	0,003541528	Membrane proteins
PA5452_wbpW_at	PA5452	wbpW		-0,53	-1,44	5,96E-05	0,00202953	Cell wall / LPS / capsule
PA3558_at	PA3558	arnF	pmrM; arnF	-0,53	-1,44	0,000625798	0,006490758	Membrane proteins; Adaptation, Protection; Cell wall / LPS / capsule
PA4119_aph_at	PA4119	aph		-0,53	-1,44	0,000527694	0,005852832	Antibiotic resistance and susceptibility
PA0871_phhB_at	PA0871	phhB		-0,53	-1,44	0,004875868	0,02464134	Amino acid biosynthesis and metabolism
PA0805_at	PA0805			-0,53	-1,44	0,006880858	0,031348012	Hypothetical, unclassified, unknown
PA4322_at	PA4322			-0,53	-1,44	0,000338668	0,00452836	Hypothetical, unclassified, unknown
PA2974_at	PA2974			-0,52	-1,44	0,000711029	0,007083487	Putative enzymes
PA0752_at	PA0752			-0,52	-1,44	0,00253088	0,015757015	Membrane proteins
PA3827_at	PA3827		yjgQ	-0,52	-1,44	0,00037066	0,004685542	Membrane proteins
PA1280_at	PA1280		cobC	-0,52	-1,44	0,00093281	0,008430231	Biosynthesis of cofactors, prosthetic groups and carriers
PA0870_phhC_at	PA0870	phhC	aspC; tyrB	-0,52	-1,44	0,000546569	0,006005761	Amino acid biosynthesis and metabolism
PA1085_flgJ_at	PA1085	flgJ		-0,52	-1,44	0,001713703	0,012052392	Cell wall / LPS / capsule; Motility & Attachment
PA2180_at	PA2180			-0,52	-1,44	0,002144749	0,014192938	Hypothetical, unclassified, unknown
PA4346_at	PA4346			-0,52	-1,43	0,001284423	0,010000535	Hypothetical, unclassified, unknown
PA1990_at	PA1990	pqqH		-0,52	-1,43	0,000183296	0,003291616	Putative enzymes
PA0044_exoT_at	PA0044	exoT		-0,52	-1,43	0,000742318	0,007329398	Secreted Factors (toxins, enzymes, alginate)
PA3102_xcpS_at	PA3102	xcpS		-0,52	-1,43	0,000204202	0,003475817	Protein secretion/export apparatus
PA2153_glgB_at	PA2153	glgB		-0,52	-1,43	0,000110991	0,002587771	Energy metabolism
PA4223_at	PA4223		pchH	-0,52	-1,43	0,000214873	0,003602201	Membrane proteins; Transport of small molecules
PA3983_at	PA3983			-0,52	-1,43	0,000436253	0,005128742	Hypothetical, unclassified, unknown
PA2025_gor_at	PA2025	gor		-0,52	-1,43	0,000298868	0,004252354	Amino acid biosynthesis and metabolism; Biosynthesis of cofactors, prosthetic groups and carriers
PA5077_mdoH_at	PA5077	opgH	mdoH	-0,51	-1,43	0,000222934	0,003659944	Cell wall / LPS / capsule
PA1340_at	PA1340	aatM		-0,51	-1,43	0,000333224	0,00449857	Transport of small molecules; Amino acid biosynthesis and metabolism
PA1480_ccmF_at	PA1480	ccmF	ccl1; cycK	-0,51	-1,43	6,52E-05	0,002047415	Energy metabolism
PA4624_at	PA4624	cdrB		-0,51	-1,43	3,73E-05	0,001684034	Cell wall / LPS / capsule
PA1679_at	PA1679			-0,51	-1,43	0,000881413	0,008097616	Hypothetical, unclassified, unknown
PA2962_tmk_at	PA2962	tmk		-0,51	-1,42	6,96E-05	0,002100031	Nucleotide biosynthesis and metabolism
PA0370_at	PA0370		yhhF	-0,51	-1,42	4,99E-05	0,001872295	Hypothetical, unclassified, unknown
PA4282_at	PA4282		sbcC	-0,51	-1,42	0,000325108	0,004447754	DNA replication, recombination, modification and repair
PA1073_braD_at	PA1073	braD		-0,51	-1,42	0,002692256	0,016345001	Membrane proteins; Transport of small molecules
PA2728_at	PA2728			-0,51	-1,42	0,002785001	0,016743195	Hypothetical, unclassified, unknown
PA2015_at	PA2015	liuA	gnyD; ivd	-0,51	-1,42	0,009212389	0,038639111	Carbon compound catabolism
PA3704_at	PA3704	wspE		-0,51	-1,42	7,45E-05	0,002164252	Two-component regulatory systems; Chemotaxis; Motility & Attachment
PA2234_at	PA2234	pslD		-0,51	-1,42	0,000851399	0,007940188	Cell wall / LPS / capsule; Transport of small molecules
PA0413_at	PA0413	chpA	pilL	-0,51	-1,42	5,80E-05	0,001999004	Two-component regulatory systems; Motility & Attachment; Chemotaxis

PA1335_at	PA1335	aauR		-0,51	-1,42	0,000257464	0,003924905	Transcriptional regulators; Two-component regulatory systems
PA5501_znuB_at	PA5501	znuB	yebI	-0,50	-1,42	0,00029767	0,004246203	Membrane proteins; Transport of small molecules
PA1086_flgK_at	PA1086	flgK		-0,50	-1,42	0,002405111	0,015208398	Cell wall / LPS / capsule; Motility & Attachment
PA3562_at	PA3562	frul	frul; ptsI	-0,50	-1,42	0,002757736	0,016615287	Central intermediary metabolism; Transport of small molecules
PA1055_at	PA1055	shaB	phaC	-0,50	-1,42	0,001092919	0,00923076	Membrane proteins; Transport of small molecules
PA4417_murE_at	PA4417	murE		-0,50	-1,42	0,000491958	0,005582568	Cell wall / LPS / capsule
PA3556_at	PA3556	arnT	pqaB	-0,50	-1,42	0,000370367	0,004685542	Adaptation, Protection; Membrane proteins; Cell wall / LPS / capsule
PA2648_nuoM_at	PA2648	nuoM		-0,50	-1,41	0,002129764	0,014136439	Energy metabolism
PA0201_at	PA0201			-0,50	-1,41	0,003048439	0,017712867	Hypothetical, unclassified, unknown
PA5007_at	PA5007		inaA; wapQ	-0,50	-1,41	0,000355202	0,004603038	Putative enzymes
PA1275_cobD_at	PA1275	cobD	cbiB	-0,50	-1,41	0,001050323	0,009050066	Biosynthesis of cofactors, prosthetic groups and carriers
PA3561_fruK_at	PA3561	fruK		-0,50	-1,41	0,000344023	0,004556045	Central intermediary metabolism; Transport of small molecules
PA1071_braF_at	PA1071	braF		-0,50	-1,41	0,000104142	0,002492719	Transport of small molecules
PA3165_hisC2_at	PA3165	hisC2		-0,50	-1,41	0,000184862	0,003298386	Amino acid biosynthesis and metabolism
PA0071_at	PA0071	tagR1		-0,50	-1,41	0,00078769	0,007568922	Protein secretion/export apparatus
PA5487_at	PA5487			-0,49	-1,41	0,000150723	0,002921111	Hypothetical, unclassified, unknown
PA1460_at	PA1460	motC	motA	-0,49	-1,41	0,00010634	0,002528802	Motility & Attachment
PA1072_braE_at	PA1072	braE		-0,49	-1,41	0,00020203	0,003475817	Membrane proteins; Transport of small molecules
PA3840_at	PA3840		ybiN	-0,49	-1,41	0,001324356	0,010204742	Hypothetical, unclassified, unknown
PA2235_at	PA2235	pslE		-0,49	-1,41	0,000246234	0,003859019	Cell wall / LPS / capsule
PA0995_ogt_at	PA0995	ogt		-0,49	-1,41	0,000115434	0,002648935	DNA replication, recombination, modification and repair
PA4419_ftsL_at	PA4419	ftsL		-0,49	-1,40	0,000416279	0,005024095	Cell division
PA2404_at	PA2404			-0,49	-1,40	0,000670356	0,006775605	Membrane proteins
PA3943_at	PA3943			-0,49	-1,40	0,000116479	0,002648935	Hypothetical, unclassified, unknown
PA1760_at	PA1760			-0,49	-1,40	8,13E-05	0,002248955	Transcriptional regulators
PA2986_at	PA2986			-0,49	-1,40	0,002675226	0,016277223	Hypothetical, unclassified, unknown
PA5004_at	PA5004			-0,48	-1,40	0,000495364	0,005607717	Putative enzymes
PA0751_at	PA0751			-0,48	-1,40	0,000220181	0,003647122	Membrane proteins
PA0861_at	PA0861	rbdA		-0,48	-1,40	0,004099411	0,021809811	Motility & Attachment
PA2151_at	PA2151			-0,48	-1,40	0,000335927	0,004502553	Hypothetical, unclassified, unknown
PA4497_at	PA4497			-0,48	-1,40	0,001452679	0,010834561	Transport of small molecules
PA3920_at	PA3920		yvgX	-0,48	-1,40	0,000179329	0,003262609	Membrane proteins; Transport of small molecules
PA3650_dxr_at	PA3650	dxr	yaeM	-0,48	-1,39	0,001187505	0,009591656	Biosynthesis of cofactors, prosthetic groups and carriers
PA0090_at	PA0090	clpV1		-0,47	-1,39	0,000893639	0,008155927	Translation, post-translational modification, degradation; Chaperones & heat shock proteins; Protein secretion/export apparatus
PA1730_at	PA1730			-0,47	-1,39	0,000328983	0,004463387	Hypothetical, unclassified, unknown
PA5001_at	PA5001			-0,47	-1,39	0,005654032	0,02736891	Hypothetical, unclassified, unknown
PA3889_at	PA3889	opuCC		-0,47	-1,39	7,07E-05	0,00210868	Transport of small molecules
PA0971_tolA_at	PA0971	tolA		-0,47	-1,39	0,000138352	0,002801888	Membrane proteins; Transport of small molecules
PA3191_at	PA3191	gtrS		-0,47	-1,38	0,002095855	0,014019427	Two-component regulatory systems
PA1806_fabI_at	PA1806	fabI	envM	-0,47	-1,38	0,000813584	0,007743697	Fatty acid and phospholipid metabolism
PA3705_at	PA3705	wspD		-0,47	-1,38	0,00013427	0,002796363	Hypothetical, unclassified, unknown; Chemotaxis; Motility & Attachment
PA2903_cobJ_at	PA2903	cobJ	cbiH	-0,47	-1,38	0,000385115	0,004819614	Biosynthesis of cofactors, prosthetic groups and carriers
PA3761_at	PA3761	nagE	nagE	-0,47	-1,38	0,002003344	0,013556775	Transport of small molecules
PA4796_at	PA4796			-0,47	-1,38	0,000419539	0,005024095	Hypothetical, unclassified, unknown
PA2409_at	PA2409			-0,47	-1,38	0,000999504	0,008791918	Membrane proteins; Transport of small molecules
PA1819_at	PA1819		yjdE	-0,47	-1,38	0,000308983	0,004329657	Membrane proteins; Transport of small molecules
PA4224_at	PA4224	pchG		-0,46	-1,38	0,000103098	0,002492719	Transport of small molecules; Membrane proteins
PA2390_at	PA2390	pvdT		-0,46	-1,38	0,000955214	0,008562981	Membrane proteins; Transport of small molecules
PA5448_wbpY_at	PA5448	wbpY		-0,46	-1,38	0,000472306	0,005459771	Cell wall / LPS / capsule
PA1635_kdpC_at	PA1635	kdpC	atkC	-0,46	-1,38	0,008913807	0,037700241	Transport of small molecules
PA1623_at	PA1623			-0,46	-1,38	0,009167307	0,038566631	Hypothetical, unclassified, unknown
PA5451_wzm_at	PA5451	wzm		-0,46	-1,38	0,000420206	0,005024095	Cell wall / LPS / capsule; Membrane proteins; Transport of small molecules
PA0299_at	PA0299	spuC		-0,46	-1,38	0,000441403	0,005167396	Putative enzymes; Carbon compound catabolism
PA5297_poxB_at	PA5297	poxB		-0,46	-1,37	0,009772471	0,040178595	Central intermediary metabolism; Energy metabolism
PA0504_bioD_at	PA0504	bioD		-0,46	-1,37	0,000257431	0,003924905	Biosynthesis of cofactors, prosthetic groups and carriers
PA2261_at	PA2261		kguK; kgk	-0,46	-1,37	0,007887328	0,034489443	Carbon compound catabolism
PA0419_at	PA0419		yggJ	-0,46	-1,37	0,008343874	0,035919437	Hypothetical, unclassified, unknown
PA1279_cobU_at	PA1279	cobU	cobT	-0,46	-1,37	0,0001329	0,002796363	Biosynthesis of cofactors, prosthetic groups and carriers
PA4369_at	PA4369			-0,46	-1,37	0,012580494	0,047564353	Hypothetical, unclassified, unknown
PA2994_nqrF_at	PA2994	nqrF		-0,46	-1,37	0,001646304	0,011722624	Energy metabolism
PA2708_at	PA2708			-0,45	-1,37	0,010506966	0,042248662	Hypothetical, unclassified, unknown
PA4219_at	PA4219	ampO	yfpB	-0,45	-1,37	0,000604554	0,006361347	Membrane proteins; Antibiotic resistance and susceptibility
PA3098_xcpW_at	PA3098	xcpW	pddD	-0,45	-1,37	0,001697869	0,012004574	Protein secretion/export apparatus
PA5006_at	PA5006			-0,45	-1,37	0,005772844	0,027591308	Hypothetical, unclassified, unknown
PA0460_at	PA0460			-0,45	-1,37	0,011260117	0,044063743	Hypothetical, unclassified, unknown
PA0753_at	PA0753			-0,45	-1,37	0,003872778	0,020992323	Membrane proteins
PA4222_at	PA4222		pchI	-0,45	-1,37	0,000185517	0,003299461	Transport of small molecules
PA1998_at	PA1998	dhcR		-0,45	-1,37	0,001278394	0,009991278	Transcriptional regulators
PA1572_at	PA1572			-0,45	-1,37	0,005394103	0,026494551	Hypothetical, unclassified, unknown
PA5419_soxG_at	PA5419	soxG		-0,45	-1,37	0,000828618	0,007806454	Amino acid biosynthesis and metabolism
PA5041_pilP_at	PA5041	pilP		-0,45	-1,37	0,000100459	0,002492719	Motility & Attachment
PA3559_at	PA3559			-0,45	-1,36	0,000589297	0,006312757	Putative enzymes
PA1172_napC_at	PA1172	napC		-0,45	-1,36	0,002415144	0,015228842	Energy metabolism
PA3922_at	PA3922			-0,45	-1,36	0,008592038	0,036590346	Hypothetical, unclassified, unknown
PA3709_at	PA3709			-0,45	-1,36	0,001518166	0,011099217	Membrane proteins; Transport of small molecules
PA2265_at	PA2265		gad	-0,45	-1,36	0,00450793	0,023334426	Carbon compound catabolism
PA1694_pscQ_at	PA1694	pscQ		-0,45	-1,36	0,007364277	0,032928584	Protein secretion/export apparatus
PA2542_at	PA2542		ytfN	-0,45	-1,36	0,000193131	0,003402167	Hypothetical, unclassified, unknown
PA0860_at	PA0860			-0,45	-1,36	0,000347435	0,004571822	Membrane proteins; Transport of small molecules
PA3164_at	PA3164			-0,44	-1,36	0,000496196	0,005607717	
PA2264_at	PA2264			-0,44	-1,36	0,00802714	0,034935371	Hypothetical, unclassified, unknown
PA3424_at	PA3424			-0,44	-1,36	0,00025464	0,003914118	Hypothetical, unclassified, unknown
PA4811_fdnH_at	PA4811	fdnH	fdhH	-0,44	-1,36	0,004153713	0,021972309	Energy metabolism
PA0516_nirF_at	PA0516	nirF		-0,44	-1,36	0,011376886	0,044333104	Energy metabolism; Biosynthesis of cofactors, prosthetic groups and carriers
PA3696_at	PA3696			-0,44	-1,36	0,000477051	0,005492024	Hypothetical, unclassified, unknown
PA4749_glmM_at	PA4749	glmM	mrsA; yhbF	-0,44	-1,36	0,000272711	0,004042495	Cell wall / LPS / capsule
PA1057_at	PA1057	shaD	phaE	-0,44	-1,36	0,007125081	0,032157799	Membrane proteins; Transport of small molecules
PA2585_uvrC_at	PA2585	uvrC		-0,44	-1,35	0,011251196	0,044059904	DNA replication, recombination, modification and repair
PA0866_aroP2_at	PA0866	aroP2		-0,44	-1,35	0,003953084	0,021272406	Transport of small molecules
PA4300_at	PA4300	tadC	tadC	-0,44	-1,35	0,000352413	0,004590469	Membrane proteins; Motility & Attachment
PA3799_at	PA3799		yfgK	-0,44	-1,35	0,001419665	0,010674419	Hypothetical, unclassified, unknown
PA4735_at	PA4735			-0,44	-1,35	0,000128311	0,002770426	Hypothetical, unclassified, unknown
PA5153_at	PA5153			-0,43	-1,35	0,001306597	0,010101449	Transport of small molecules
PA2164_at	PA2164			-0,43	-1,35	0,00043816	0,005140274	Putative enzymes
PA1984_s_at	PA1984	exaC	exaC1	-0,43	-1,35	0,006675312	0,030577507	Putative enzymes
PA5492_at	PA5492		ysxC; yihA	-0,43	-1,35	0,000148375	0,002901296	Hypothetical, unclassified, unknown
PA3192_gltR_at	PA3192	gltR		-0,43	-1,35	0,001029557	0,008954564	Transcriptional regulators; Two-component regulatory systems
PA1803_lon_at	PA1803	lon	lopA; muc; deg; capR	-0,43	-1,35	0,001025866	0,008936466	Adaptation, Protection; Translation, post-translational modification, degradation
PA1483_cycH_at	PA1483	cycH		-0,43	-1,35	0,000529074	0,005852832	Energy metabolism
PA3800_at	PA3800			-0,43	-1,35	0,00017496	0,003218181	Hypothetical, unclassified, unknown
PA5040_pilQ_at	PA5040	pilQ		-0,43	-1,34	0,001566553	0,011330767	Motility & Attachment
PA0074_ppkA_at	PA0074	ppkA	tagE1	-0,42	-1,34	0,002868501	0,017097005	Adaptation, Protection; Translation, post-translational modification, degradation; Protein secretion/export apparatus
PA1506_at	PA1506			-0,42	-1,34	0,000327398	0,0044559	Hypothetical, unclassified, unknown
PA2324_at	PA2324			-0,42	-1,34	0,001295624	0,010055133	Putative enzymes
PA1608_at	PA1608			-0,42	-1,34	0,006917286	0,031462313	Adaptation, Protection; Chemotaxis
PA2266_at	PA2266			-0,42	-1,34	0,000595714	0,006344757	Carbon compound catabolism; Energy metabolism
PA2879_at	PA2879		hpkR	-0,42	-1,34	0,003282932	0,018550904	Transcriptional regulators
PA2236_at	PA2236	pslF		-0,42	-1,34	0,001929871	0,013226212	Cell wall / LPS / capsule
PA4004_at	PA4004		ybeA	-0,42	-1,34	0,000265244	0,003956554	Hypothetical, unclassified, unknown
PA0503_at	PA0503		bioC	-0,42	-1,34	0,000231265	0,003752312	Biosynthesis of cofactors, prosthetic groups and carriers
PA3801_at	PA3801		yfgM	-0,42	-1,34	0,000345773	0,004568322	Hypothetical, unclassified, unknown
PA1866_at	PA1866			-0,42	-1,34	0,003977536	0,021324973	Hypothetical, unclassified, unknown
PA1091_at	PA1091	fgtA		-0,42	-1,34	0,001200248	0,009596793	Hypothetical, unclassified, unknown
PA4496_at	PA4496			-0,42	-1,33	0,00524651	0,02603245	Transport of small molecules
PA1719_pscF_at	PA1719	pscF		-0,41	-1,33	0,005728432	0,027449975	Protein secretion/export apparatus
PA5091_hutG_at	PA5091	hutG		-0,41	-1,33	0,00084409	0,007911918	Amino acid biosynthesis and metabolism
PA2410_at	PA2410			-0,41	-1,33	0,00110933	0,009284575	Hypothetical, unclassified, unknown
PA3205_at	PA3205			-0,41	-1,33	0,006286562	0,029289783	Hypothetical, unclassified, unknown
PA3110_at	PA3110			-0,41	-1,33	0,000367906	0,004685542	Hypothetical, unclassified, unknown
PA4128_at	PA4128		hpaI; hpcH	-0,41	-1,33	0,001375019	0,01044058	Putative enzymes
PA4751_ftsH_at	PA4751	ftsH	tolZ; mrsC; hflB	-0,41	-1,33	0,00193066	0,013226212	Cell division
PA0513_at	PA0513	nirG	nirG	-0,41	-1,33	0,004085048	0,021775151	Biosynthesis of cofactors, prosthetic groups and carriers; Energy metabolism; Transcriptional regulators
PA5228_at	PA5228		ygfA	-0,41	-1,33	0,000664411	0,006740064	Hypothetical, unclassified, unknown
PA3097_xcpX_at	PA3097	xcpX		-0,41	-1,33	0,000486984	0,005546439	Protein secretion/export apparatus
PA5078_at	PA5078	opgG	mdoG	-0,41	-1,33	0,000710214	0,007083487	Hypothetical, unclassified, unknown
PA1573_at	PA1573		yijF	-0,41	-1,33	0,000237124	0,003809384	Hypothetical, unclassified, unknown
PA2895_at	PA2895			-0,41	-1,33	0,000595284	0,006344757	Hypothetical, unclassified, unknown
PA3982_at	PA3982			-0,41	-1,33	0,000529487	0,005852832	Hypothetical, unclassified, unknown
PA3660_at	PA3660		yjcE	-0,41	-1,33	0,00232867	0,015007889	Membrane proteins; Transport of small molecules
PA1693_pscR_at	PA1693	pscR		-0,41	-1,32	0,000248848	0,003873553	Protein secretion/export apparatus
PA3702_at	PA3702	wspR		-0,41	-1,32	0,000506094	0,005696377	Nucleotide biosynthesis and metabolism; Chemotaxis; Motility & Attachment
PA0027_at	PA0027			-0,41	-1,32	0,009531936	0,039620012	Hypothetical, unclassified, unknown
PA2018_at	PA2018	mexY	amrB; mexH	-0,40	-1,32	0,003252336	0,018445514	Transport of small molecules; Membrane proteins; Antibiotic resistance and susceptibility
PA3651_cdsA_at	PA3651	cdsA		-0,40	-1,32	0,00039109	0,004865826	Fatty acid and phospholipid metabolism
PA2407_at	PA2407			-0,40	-1,32	0,002001658	0,013556775	Motility & Attachment

PA4125_hpcD_at	PA4125	hpcD		-0,40	-1,32	0,004373161	0,022849972	Carbon compound catabolism
PA2611_cysG_at	PA2611	cysG		-0,40	-1,32	0,000624733	0,006490758	Biosynthesis of cofactors, prosthetic groups and carriers
PA3856_at	PA3856			-0,40	-1,32	0,00841498	0,036085566	Hypothetical, unclassified, unknown
PA3163_cmk_at	PA3163	cmk		-0,40	-1,32	0,001919126	0,013179741	Nucleotide biosynthesis and metabolism
PA2613_at	PA2613		ycaJ	-0,40	-1,32	0,001223783	0,009706166	Hypothetical, unclassified, unknown
PA5287_amtB_at	PA5287	amtB		-0,40	-1,32	0,004285644	0,022498616	Membrane proteins; Transport of small molecules
PA5065_at	PA5065	ubiB	yigR; aarF	-0,40	-1,32	0,00031705	0,004398278	Putative enzymes; Biosynthesis of cofactors, prosthetic groups and carriers
PA1722_pscI_at	PA1722	pscI		-0,40	-1,32	0,005667125	0,02736891	Protein secretion/export apparatus
PA2873_at	PA2873	tgpA		-0,40	-1,32	0,000320342	0,00442183	Adaptation, Protection; Membrane proteins
PA3851_at	PA3851			-0,40	-1,32	0,008579093	0,036590346	Hypothetical, unclassified, unknown
PA3106_at	PA3106			-0,40	-1,32	0,001284985	0,010000535	Putative enzymes
PA2458_at	PA2458			-0,40	-1,32	0,002957911	0,01743816	Hypothetical, unclassified, unknown
PA2011_at	PA2011	liuE	mvaB; gnyL	-0,39	-1,31	0,002255016	0,01460103	Carbon compound catabolism
PA3099_xcpV_at	PA3099	xcpV	pddC	-0,39	-1,31	0,003207278	0,018328716	Protein secretion/export apparatus
PA1667_at	PA1667	hsiJ2		-0,39	-1,31	0,000406761	0,004982597	Protein secretion/export apparatus
PA2646_nuoK_at	PA2646	nuoK		-0,39	-1,31	0,000783963	0,007568922	Energy metabolism
PA3739_at	PA3739			-0,39	-1,31	0,001216408	0,009698059	Membrane proteins; Transport of small molecules
PA5036_gltB_at	PA5036	gltB	aspB	-0,39	-1,31	0,003097111	0,017901947	Amino acid biosynthesis and metabolism
PA0427_oprM_at	PA0427	oprM		-0,39	-1,31	0,003232122	0,01837607	Antibiotic resistance and susceptibility; Membrane proteins; Transport of small molecules
PA2729_at	PA2729			-0,39	-1,31	0,001682173	0,011921303	Hypothetical, unclassified, unknown
PA2987_at	PA2987		ycfV	-0,39	-1,31	0,001103105	0,009246417	Transport of small molecules
PA5456_at	PA5456			-0,39	-1,31	0,002707295	0,016382528	Cell wall / LPS / capsule
PA4667_at	PA4667			-0,39	-1,31	0,001090697	0,009226036	Hypothetical, unclassified, unknown
PA5149_at	PA5149		mviM	-0,39	-1,31	0,001849086	0,012777807	Hypothetical, unclassified, unknown
PA3228_at	PA3228			-0,39	-1,31	0,001886169	0,012969454	Membrane proteins; Transport of small molecules
PA0899_aruB_at	PA0899	aruB		-0,39	-1,31	0,00161842	0,011587887	Amino acid biosynthesis and metabolism
PA0375_ftsX_at	PA0375	ftsX		-0,39	-1,31	0,000395523	0,004888101	Cell division
PA1547_at	PA1547			-0,39	-1,31	0,002562227	0,015850385	Membrane proteins
PA0368_at	PA0368			-0,39	-1,31	0,001728681	0,012112198	Hypothetical, unclassified, unknown
PA0415_at	PA0415	chpC		-0,38	-1,31	0,00096873	0,008598433	Chemotaxis
PA1811_at	PA1811			-0,38	-1,30	0,0031437	0,018095844	Transport of small molecules
PA2248_bkdA2_at	PA2248	bkdA2		-0,38	-1,30	0,000872675	0,008053586	Amino acid biosynthesis and metabolism
PA3226_at	PA3226			-0,38	-1,30	0,001591407	0,011453591	Putative enzymes
PA3082_at	PA3082	gbt		-0,38	-1,30	0,004949009	0,024920191	Amino acid biosynthesis and metabolism
PA5449_wbpX_at	PA5449	wbpX		-0,38	-1,30	0,000417423	0,005024095	Cell wall / LPS / capsule
PA5251_at	PA5251			-0,38	-1,30	0,00022633	0,003693843	Membrane proteins
PA4756_carB_at	PA4756	carB		-0,38	-1,30	0,013078208	0,048445245	Nucleotide biosynthesis and metabolism; Amino acid biosynthesis and metabolism
PA3096_xcpY_at	PA3096	xcpY		-0,38	-1,30	0,003288077	0,01856108	Protein secretion/export apparatus
PA0927_ldhA_at	PA0927	ldhA	ldhD	-0,38	-1,30	0,001700602	0,012005907	Energy metabolism; Central intermediary metabolism; Carbon compound catabolism
PA0764_mucB_at	PA0764	mucB	algN	-0,38	-1,30	0,000421666	0,005024095	Transcriptional regulators; Cell wall / LPS / capsule
PA5043_pilN_at	PA5043	pilN		-0,38	-1,30	0,000698386	0,007007852	Motility & Attachment
PA0593_pdxA_at	PA0593	pdxA		-0,38	-1,30	0,000348798	0,004573501	Biosynthesis of cofactors, prosthetic groups and carriers
PA5398_at	PA5398	dgcA	dgcA	-0,38	-1,30	0,003025341	0,017652593	Amino acid biosynthesis and metabolism
PA5459_at	PA5459			-0,38	-1,30	0,00076638	0,007487042	Cell wall / LPS / capsule
PA3460_at	PA3460			-0,38	-1,30	0,001713214	0,012052392	Putative enzymes
PA0707_toxR_at	PA0707	toxR	regA	-0,38	-1,30	0,000582373	0,006262772	Transcriptional regulators
PA1339_at	PA1339	aatP		-0,38	-1,30	0,001187254	0,009591656	Transport of small molecules; Amino acid biosynthesis and metabolism
PA2020_at	PA2020	mexZ	amrR; mexZ	-0,38	-1,30	0,000549074	0,006009494	Transcriptional regulators
PA3576_at	PA3576			-0,37	-1,30	0,003873876	0,020992323	Hypothetical, unclassified, unknown
PA0581_i_at	PA0581		ygiH	-0,37	-1,30	0,003212109	0,018337441	Hypothetical, unclassified, unknown
PA5431_at	PA5431			-0,37	-1,30	0,001082412	0,009206318	Transcriptional regulators
PA0494_at	PA0494			-0,37	-1,30	0,001933432	0,01322887	Putative enzymes
PA3305_at	PA3305			-0,37	-1,29	0,000669605	0,006775605	Membrane proteins
PA3430_at	PA3430			-0,37	-1,29	0,009730824	0,040086374	Putative enzymes
PA3023_at	PA3023		yegS	-0,37	-1,29	0,012046566	0,04626048	Hypothetical, unclassified, unknown
PA2241_at	PA2241	pslK		-0,37	-1,29	0,002146816	0,014192938	Membrane proteins; Cell wall / LPS / capsule
PA2897_at	PA2897			-0,37	-1,29	0,002363786	0,015034801	Transcriptional regulators
PA1115_at	PA1115			-0,37	-1,29	0,001090661	0,009226036	Membrane proteins
PA1703_pcrD_at	PA1703	pcrD		-0,37	-1,29	0,013592325	0,049875219	Protein secretion/export apparatus
PA0898_aruD_at	PA0898	aruD	astD	-0,37	-1,29	0,002847634	0,017027504	Amino acid biosynthesis and metabolism
PA4660_phr_at	PA4660	phr		-0,37	-1,29	0,000484019	0,005537774	DNA replication, recombination, modification and repair
PA1104_flil_at	PA1104	flil		-0,37	-1,29	0,004587332	0,023631341	Energy metabolism; Motility & Attachment
PA4996_rfaE_at	PA4996	rfaE		-0,37	-1,29	0,001431804	0,010722109	Cell wall / LPS / capsule
PA5236_at	PA5236		ubiB	-0,37	-1,29	0,000958736	0,008578856	Putative enzymes
PA2995_nqrE_at	PA2995	nqrE		-0,37	-1,29	0,003046822	0,017712867	Energy metabolism
PA3009_at	PA3009			-0,37	-1,29	0,000854426	0,007941725	Hypothetical, unclassified, unknown
PA3978_at	PA3978			-0,37	-1,29	0,002151065	0,014192938	Hypothetical, unclassified, unknown
PA2727_at	PA2727			-0,37	-1,29	0,002336061	0,015025983	Hypothetical, unclassified, unknown
PA0771_era_at	PA0771	era		-0,36	-1,29	0,000759027	0,007428292	Cell division; Translation, post-translational modification, degradation
PA2684_at	PA2684	tse5	rhs; rhsP1	-0,36	-1,29	0,001189984	0,009592625	Hypothetical, unclassified, unknown
PA4664_hemK_at	PA4664	hemK		-0,36	-1,29	0,002176131	0,014290354	Biosynthesis of cofactors, prosthetic groups and carriers
PA3706_at	PA3706	wspC		-0,36	-1,29	0,004300155	0,022532622	Chemotaxis; Adaptation, Protection; Motility & Attachment
PA3760_at	PA3760		nagF	-0,36	-1,28	0,007144692	0,032180111	Transport of small molecules
PA0446_at	PA0446			-0,36	-1,28	0,00211638	0,014094652	Hypothetical, unclassified, unknown
PA5307_at	PA5307			-0,36	-1,28	0,00169825	0,012004574	Hypothetical, unclassified, unknown
PA0897_aruG_at	PA0897	aruG		-0,36	-1,28	0,001374177	0,01044058	Amino acid biosynthesis and metabolism
PA3174_at	PA3174			-0,36	-1,28	0,000732016	0,007240563	Transcriptional regulators
PA3495_nth_at	PA3495	nth		-0,36	-1,28	0,001782608	0,01238009	DNA replication, recombination, modification and repair
PA4474_at	PA4474		tldD	-0,36	-1,28	0,000746097	0,007352777	Hypothetical, unclassified, unknown
PA4466_at	PA4466			-0,36	-1,28	0,00977464	0,040178595	Transport of small molecules
PA2302_at	PA2302	ambE		-0,36	-1,28	0,004390582	0,022897877	Secreted Factors (toxins, enzymes, alginate); Putative enzymes
PA5312_at	PA5312	pauC	kauB	-0,36	-1,28	0,002614495	0,016066263	Putative enzymes; Carbon compound catabolism
PA3601_at	PA3601		ykgM	-0,36	-1,28	0,00174613	0,012168667	Translation, post-translational modification, degradation
PA3703_at	PA3703	wspF		-0,36	-1,28	0,000594425	0,006344757	Transcriptional regulators; Chemotaxis; Motility & Attachment
PA0004_gyrB_at	PA0004	gyrB		-0,36	-1,28	0,009200448	0,038639111	DNA replication, recombination, modification and repair
PA3086_at	PA3086			-0,35	-1,28	0,000718522	0,007132522	Membrane proteins
PA5260_hemC_at	PA5260	hemC	popE	-0,35	-1,28	0,002595377	0,016001939	Biosynthesis of cofactors, prosthetic groups and carriers
PA4477_cafA_at	PA4477	cafA		-0,35	-1,28	0,005840725	0,0278438	Cell division
PA1818_at	PA1818	ldcA	speC; ldcC; adi	-0,35	-1,28	0,002606716	0,016036219	Amino acid biosynthesis and metabolism
PA2904_cobI_at	PA2904	cobI	cbiL	-0,35	-1,28	0,001192831	0,00959278	Biosynthesis of cofactors, prosthetic groups and carriers
PA2231_at	PA2231	pslA		-0,35	-1,28	0,008227756	0,035557492	Cell wall / LPS / capsule
PA3190_at	PA3190		gltB	-0,35	-1,28	0,001166821	0,009509441	Transport of small molecules
PA0426_mexB_at	PA0426	mexB		-0,35	-1,27	0,003219414	0,018341407	Transport of small molecules; Membrane proteins; Antibiotic resistance and susceptibility
PA4102_at	PA4102	bfmS		-0,35	-1,27	0,002234415	0,014501484	Two-component regulatory systems; Cell wall / LPS / capsule; Adaptation, Protection
PA3485_r_at	PA3485	tsi3		-0,35	-1,27	0,004831857	0,02446348	Adaptation, Protection
PA0089_at	PA0089	tssG1		-0,35	-1,27	0,001708007	0,012042862	Protein secretion/export apparatus
PA5453_gmd_at	PA5453	gmd	lpsA; gca	-0,35	-1,27	0,005077431	0,025359734	Cell wall / LPS / capsule
PA1523_xdhB_at	PA1523	xdhB		-0,35	-1,27	0,001126876	0,009394647	Nucleotide biosynthesis and metabolism
PA4662_murI_at	PA4662	murI		-0,35	-1,27	0,00534466	0,026362239	Cell wall / LPS / capsule
PA4812_fdnG_at	PA4812	fdnG	fdhG	-0,34	-1,27	0,001158305	0,009492396	Energy metabolism
PA3803_at	PA3803	gcpE		-0,34	-1,27	0,010299878	0,041687836	Putative enzymes
PA3003_at	PA3003			-0,34	-1,27	0,004093553	0,021799545	Hypothetical, unclassified, unknown
PA1731_at	PA1731			-0,34	-1,27	0,001728755	0,012112198	Hypothetical, unclassified, unknown
PA4750_folP_at	PA4750	folP	dhpS	-0,34	-1,27	0,009712829	0,040041966	Biosynthesis of cofactors, prosthetic groups and carriers
PA4591_at	PA4591			-0,34	-1,27	0,005754871	0,027529121	Hypothetical, unclassified, unknown
PA2016_at	PA2016	liuR	gnyR	-0,34	-1,27	0,009404654	0,039178996	Transcriptional regulators
PA4023_at	PA4023		eutP	-0,34	-1,27	0,001421662	0,010674972	Transport of small molecules
PA4868_ureC_at	PA4868	ureC		-0,34	-1,27	0,0008637	0,008014502	Central intermediary metabolism
PA3109_at	PA3109		cvpA	-0,34	-1,27	0,003798141	0,020662632	Adaptation, Protection
PA2298_at	PA2298			-0,34	-1,27	0,004958907	0,024947395	Putative enzymes
PA3120_leuD_at	PA3120	leuD		-0,34	-1,27	0,006019488	0,028472509	Amino acid biosynthesis and metabolism
PA3649_at	PA3649	mucP		-0,34	-1,26	0,001468608	0,010894792	Hypothetical, unclassified, unknown
PA4663_moeB_at	PA4663	moeB	chlN	-0,34	-1,26	0,009969292	0,040743056	Biosynthesis of cofactors, prosthetic groups and carriers
PA4621_at	PA4621			-0,34	-1,26	0,002851047	0,017029558	Putative enzymes
PA5476_citA_at	PA5476	citA		-0,34	-1,26	0,004531519	0,023391068	Membrane proteins; Transport of small molecules
PA4740_pnp_at	PA4740	pnp		-0,34	-1,26	0,008202001	0,035473814	Transcription, RNA processing and degradation
PA0344_at	PA0344			-0,34	-1,26	0,003526523	0,019563014	Hypothetical, unclassified, unknown
PA0745_at	PA0745			-0,34	-1,26	0,004694757	0,023966153	Putative enzymes
PA2540_at	PA2540			-0,34	-1,26	0,012970003	0,048237633	Hypothetical, unclassified, unknown
PA3587_metR_at	PA3587	metR		-0,34	-1,26	0,010897754	0,043163196	Transcriptional regulators
PA3081_at	PA3081			-0,34	-1,26	0,001495405	0,011002716	Hypothetical, unclassified, unknown
PA2122_at	PA2122			-0,34	-1,26	0,001952127	0,013307561	Hypothetical, unclassified, unknown
PA0078_at	PA0078	tssL1		-0,33	-1,26	0,000999766	0,008791918	Protein secretion/export apparatus
PA2614_lolA_at	PA2614	lolA		-0,33	-1,26	0,000965084	0,008582517	Chaperones & heat shock proteins
PA2615_ftsK_at	PA2615	ftsK		-0,33	-1,26	0,002698726	0,016366371	Cell division; Antibiotic resistance and susceptibility
PA2110_at	PA2110			-0,33	-1,26	0,005663189	0,02736891	Hypothetical, unclassified, unknown
PA4603_at	PA4603			-0,33	-1,26	0,004680166	0,023957787	Hypothetical, unclassified, unknown
PA5045_ponA_at	PA5045	ponA	mrcA	-0,33	-1,26	0,003476716	0,019389242	Cell wall / LPS / capsule
PA4334_at	PA4334		yfeP	-0,33	-1,26	0,001787987	0,012401925	Membrane proteins; Transport of small molecules
PA4668_at	PA4668		lolB	-0,33	-1,26	0,004689961	0,023966153	Cell wall / LPS / capsule
PA4772_at	PA4772			-0,33	-1,26	0,002364513	0,015034801	Energy metabolism
PA2414_at	PA2414			-0,33	-1,26	0,003189439	0,018245565	Carbon compound catabolism
PA5155_at	PA5155			-0,33	-1,26	0,003616525	0,019905129	Membrane proteins; Transport of small molecules
PA3579_at	PA3579			-0,33	-1,25	0,004722099	0,024083572	Putative enzymes
PA3750_at	PA3750			-0,33	-1,25	0,012159555	0,046533358	Hypothetical, unclassified, unknown

PA2853_oprI_at	PA2853	oprI		-0,32	-1,25	0,012139343	0,046488071	Membrane proteins
PA1778_cobA_at	PA1778	cobA		-0,32	-1,25	0,00378098	0,020609686	Biosynthesis of cofactors, prosthetic groups and carriers
PA3303_at	PA3303			-0,32	-1,25	0,009265184	0,03880189	Membrane proteins; Transport of small molecules
PA3662_at	PA3662			-0,32	-1,25	0,002507447	0,015674047	Hypothetical, unclassified, unknown
PA2165_at	PA2165		glgA	-0,32	-1,25	0,002929566	0,017312208	Energy metabolism
PA4491_at	PA4491	magB	pufY; yfaA	-0,32	-1,25	0,004123307	0,021853239	Hypothetical, unclassified, unknown
PA3549_algJ_at	PA3549	algJ		-0,32	-1,25	0,003719448	0,020354258	Cell wall / LPS / capsule; Adaptation, Protection; Secreted Factors (toxins, enzymes, alginate)
PA2973_at	PA2973			-0,32	-1,25	0,005936552	0,02823126	Translation, post-translational modification, degradation
PA5092_hutI_at	PA5092	hutI		-0,32	-1,25	0,003095325	0,017901947	Amino acid biosynthesis and metabolism
PA2944_cobN_at	PA2944	cobN		-0,32	-1,25	0,002968406	0,017442288	Biosynthesis of cofactors, prosthetic groups and carriers
PA4604_at	PA4604		yjiA	-0,32	-1,25	0,004209694	0,02217393	Hypothetical, unclassified, unknown
PA3166_pheA_at	PA3166	pheA		-0,32	-1,25	0,005966677	0,0282742	Amino acid biosynthesis and metabolism
PA4490_at	PA4490	magC		-0,32	-1,25	0,004804246	0,024345898	Hypothetical, unclassified, unknown
PA3642_rnhB_at	PA3642	rnhB		-0,32	-1,25	0,012469577	0,047263443	DNA replication, recombination, modification and repair
PA5222_at	PA5222			-0,32	-1,25	0,00134713	0,010324893	Hypothetical, unclassified, unknown
PA0893_argR_at	PA0893	argR		-0,32	-1,25	0,00395739	0,021272406	Amino acid biosynthesis and metabolism; Transcriptional regulators
PA4592_at	PA4592		opmF	-0,32	-1,25	0,010803903	0,042852651	Hypothetical, unclassified, unknown
PA1843_metH_at	PA1843	metH		-0,32	-1,25	0,011062565	0,04362912	Amino acid biosynthesis and metabolism
PA2992_at	PA2992			-0,32	-1,25	0,003617918	0,019905129	Hypothetical, unclassified, unknown
PA1624_at	PA1624			-0,32	-1,25	0,00667867	0,030577507	Hypothetical, unclassified, unknown
PA5367_pstA_at	PA5367	pstA		-0,32	-1,25	0,002409173	0,01520876	Membrane proteins; Transport of small molecules
PA0260_at	PA0260	tle3		-0,31	-1,24	0,006815057	0,0310993	Membrane proteins; Secreted Factors (toxins, enzymes, alginate)
PA3087_at	PA3087			-0,31	-1,24	0,012821727	0,04804105	Hypothetical, unclassified, unknown
PA0493_at	PA0493			-0,31	-1,24	0,001248946	0,009844319	Putative enzymes
PA0598_at	PA0598			-0,31	-1,24	0,004465659	0,023202194	Hypothetical, unclassified, unknown
PA3080_at	PA3080			-0,31	-1,24	0,009558557	0,039671229	Hypothetical, unclassified, unknown
PA1690_pscU_at	PA1690	pscU		-0,31	-1,24	0,009558469	0,039671229	Protein secretion/export apparatus
PA0502_at	PA0502		bioH	-0,31	-1,24	0,010409839	0,042010326	Biosynthesis of cofactors, prosthetic groups and carriers
PA4021_at	PA4021			-0,31	-1,24	0,008104808	0,035135611	Transcriptional regulators
PA3277_at	PA3277			-0,31	-1,24	0,013375734	0,049349698	Putative enzymes
PA1795_cysS_at	PA1795	cysS		-0,31	-1,24	0,003433249	0,019220772	Amino acid biosynthesis and metabolism; Translation, post-translational modification, degradation
PA5181_at	PA5181			-0,31	-1,24	0,003159919	0,01813277	Putative enzymes
PA0455_dbpA_at	PA0455	dbpA		-0,31	-1,24	0,001167044	0,009509441	Transcription, RNA processing and degradation
PA2443_sdaA_at	PA2443	sdaA		-0,31	-1,24	0,003001435	0,017550015	Amino acid biosynthesis and metabolism
PA2155_at	PA2155		ybhO	-0,31	-1,24	0,003186628	0,018245565	Putative enzymes
PA2001_atoB_at	PA2001	atoB		-0,31	-1,24	0,009807239	0,040178595	Central intermediary metabolism; Fatty acid and phospholipid metabolism
PA4285_recC_at	PA4285	recC		-0,31	-1,24	0,005721272	0,027443667	DNA replication, recombination, modification and repair
PA1530_at	PA1530			-0,31	-1,24	0,006071597	0,028624717	Hypothetical, unclassified, unknown
PA4958_at	PA4958			-0,30	-1,24	0,002210837	0,014432864	Hypothetical, unclassified, unknown
PA1877_at	PA1877			-0,30	-1,23	0,007593352	0,033749559	Protein secretion/export apparatus; Antibiotic resistance and susceptibility
PA3454_at	PA3454			-0,30	-1,23	0,005482262	0,026805691	Putative enzymes
PA5242_ppk_at	PA5242	ppk		-0,30	-1,23	0,002116265	0,014094652	Nucleotide biosynthesis and metabolism; Adaptation, Protection
PA2327_at	PA2327			-0,30	-1,23	0,01118453	0,043860745	Membrane proteins; Transport of small molecules
PA4016_at	PA4016			-0,30	-1,23	0,006277374	0,029271552	Membrane proteins
PA4754_at	PA4754			-0,30	-1,23	0,007078731	0,032039051	Membrane proteins
PA0003_recF_at	PA0003	recF		-0,30	-1,23	0,005133909	0,025526937	DNA replication, recombination, modification and repair
PA3071_at	PA3071			-0,30	-1,23	0,004042889	0,021612711	Hypothetical, unclassified, unknown
PA1715_pscB_at	PA1715	pscB		-0,30	-1,23	0,004625631	0,023744337	Protein secretion/export apparatus
PA5544_at	PA5544			-0,30	-1,23	0,010609601	0,042348958	Membrane proteins
PA1915_at	PA1915			-0,30	-1,23	0,011957153	0,046044581	Hypothetical, unclassified, unknown
PA5223_ubiH_at	PA5223	ubiH	visB	-0,30	-1,23	0,004068418	0,021728248	Energy metabolism; Biosynthesis of cofactors, prosthetic groups and carriers
PA0401_at	PA0401		pyrC'; pyrX	-0,30	-1,23	0,006342504	0,029476178	Nucleotide biosynthesis and metabolism
PA4112_at	PA4112			-0,30	-1,23	0,004123327	0,021853239	Two-component regulatory systems
PA4011_at	PA4011			-0,30	-1,23	0,008530055	0,036410211	Membrane proteins
PA0374_ftsE_at	PA0374	ftsE		-0,30	-1,23	0,002406375	0,015208398	Transport of small molecules; Cell division
PA0928_at	PA0928	gacS	lemA	-0,30	-1,23	0,003254309	0,018445514	Two-component regulatory systems
PA0792_prpD_at	PA0792	prpD		-0,30	-1,23	0,001355986	0,010349881	Carbon compound catabolism
PA1449_flhB_at	PA1449	flhB		-0,29	-1,23	0,013536089	0,049742886	Chemotaxis; Adaptation, Protection; Motility & Attachment
PA3534_at	PA3534			-0,29	-1,23	0,008592032	0,036590346	Putative enzymes
PA0161_at	PA0161			-0,29	-1,23	0,007128148	0,032157799	Hypothetical, unclassified, unknown
PA3970_amn_at	PA3970	amn		-0,29	-1,23	0,007718916	0,034085816	Nucleotide biosynthesis and metabolism
PA0176_at	PA0176	aer2		-0,29	-1,22	0,004130202	0,021868789	Adaptation, Protection; Chemotaxis
PA4559_lspA_at	PA4559	lspA		-0,29	-1,22	0,004474695	0,023212043	Protein secretion/export apparatus; Translation, post-translational modification, degradation
PA0158_at	PA0158	triC	triC	-0,29	-1,22	0,013054476	0,048390658	Antibiotic resistance and susceptibility; Transport of small molecules
PA3111_folC_at	PA3111	folC		-0,29	-1,22	0,003438297	0,019220772	Biosynthesis of cofactors, prosthetic groups and carriers
PA3668_at	PA3668			-0,29	-1,22	0,001977813	0,013449615	Hypothetical, unclassified, unknown
PA4123_hpcC_at	PA4123	hpcC	hpaE	-0,29	-1,22	0,007849376	0,034377419	Carbon compound catabolism
PA5035_gltD_at	PA5035	gltD	aspB	-0,29	-1,22	0,012749326	0,048028519	Amino acid biosynthesis and metabolism
PA5397_at	PA5397			-0,29	-1,22	0,003601226	0,019864021	Hypothetical, unclassified, unknown
PA3052_at	PA3052			-0,29	-1,22	0,011989259	0,046136198	Hypothetical, unclassified, unknown
PA3555_at	PA3555	arnD	amrJ; arnD	-0,29	-1,22	0,010615859	0,042348958	Adaptation, Protection; Antibiotic resistance and susceptibility; Cell wall / LPS / capsule
PA3961_at	PA3961		hrpB	-0,29	-1,22	0,003116772	0,017959471	Putative enzymes
PA4795_at	PA4795			-0,29	-1,22	0,00568239	0,027389334	Hypothetical, unclassified, unknown
PA2284_at	PA2284			-0,29	-1,22	0,002225748	0,014496095	Hypothetical, unclassified, unknown
PA2858_at	PA2858		ybbP	-0,29	-1,22	0,013458593	0,049526193	Membrane proteins
PA3002_mfd_at	PA3002	mfd		-0,29	-1,22	0,005938752	0,02823126	DNA replication, recombination, modification and repair
PA4505_at	PA4505		dppD	-0,29	-1,22	0,00476444	0,024232702	Transport of small molecules
PA0744_at	PA0744			-0,29	-1,22	0,003031091	0,017667565	Putative enzymes
PA1720_pscG_at	PA1720	pscG		-0,28	-1,22	0,006849695	0,03123168	Protein secretion/export apparatus; Chaperones & heat shock proteins
PA2391_at	PA2391	opmQ		-0,28	-1,22	0,005906679	0,028134043	Membrane proteins; Transport of small molecules
PA0782_putA_at	PA0782	putA	pruB; pruA	-0,28	-1,22	0,00340914	0,019152402	Amino acid biosynthesis and metabolism
PA3891_at	PA3891	opuCA		-0,28	-1,22	0,003696751	0,02027003	Transport of small molecules
PA3212_at	PA3212			-0,28	-1,22	0,003414509	0,019157849	Transport of small molecules
PA4606_at	PA4606		cstA	-0,28	-1,22	0,001583316	0,011424995	Adaptation, Protection
PA4407_ftsZ_at	PA4407	ftsZ		-0,28	-1,22	0,005108586	0,025479823	Cell division
PA5011_waaC_at	PA5011	waaC	rfaC	-0,28	-1,22	0,002626507	0,016086633	Cell wall / LPS / capsule
PA3206_at	PA3206			-0,28	-1,22	0,009315622	0,038947731	Two-component regulatory systems
PA3707_at	PA3707	wspB		-0,28	-1,21	0,004198114	0,022143852	Hypothetical, unclassified, unknown; Chemotaxis; Motility & Attachment
PA1908_at	PA1908			-0,28	-1,21	0,011104312	0,043669617	Membrane proteins; Transport of small molecules
PA5113_at	PA5113			-0,28	-1,21	0,01048615	0,042226159	Membrane proteins
PA3095_xcpZ_at	PA3095	xcpZ		-0,28	-1,21	0,002216321	0,014451664	Protein secretion/export apparatus
PA2430_at	PA2430			-0,28	-1,21	0,008017627	0,034935371	Hypothetical, unclassified, unknown
PA3253_at	PA3253			-0,28	-1,21	0,004969682	0,024978955	Membrane proteins; Transport of small molecules
PA3340_at	PA3340			-0,28	-1,21	0,007774117	0,034182708	Membrane proteins
PA5543_at	PA5543			-0,28	-1,21	0,007652286	0,033883077	Hypothetical, unclassified, unknown
PA3841_exoS_at	PA3841	exoS		-0,28	-1,21	0,008317929	0,035851681	Secreted Factors (toxins, enzymes, alginate)
PA3068_at	PA3068	gdhB		-0,28	-1,21	0,005598468	0,027203065	Amino acid biosynthesis and metabolism
PA5021_at	PA5021			-0,28	-1,21	0,0045268	0,023391068	Membrane proteins; Transport of small molecules
PA3400_at	PA3400			-0,28	-1,21	0,003059691	0,017759653	Membrane proteins
PA3084_at	PA3084			-0,28	-1,21	0,003148326	0,018103688	Hypothetical, unclassified, unknown
PA4605_at	PA4605		ybdD	-0,28	-1,21	0,01054115	0,042273049	Hypothetical, unclassified, unknown
PA4380_at	PA4380		colS	-0,27	-1,21	0,012479667	0,0472694	Two-component regulatory systems
PA1419_at	PA1419			-0,27	-1,21	0,006023919	0,028472509	Membrane proteins; Transport of small molecules
PA0296_s_at	PA0296	spuI		-0,27	-1,21	0,009896466	0,040498148	Putative enzymes; Carbon compound catabolism
PA4127_hpcG_at	PA4127	hpcG	hpaH	-0,27	-1,21	0,007026018	0,031878473	Carbon compound catabolism
PA1077_flgB_at	PA1077	flgB		-0,27	-1,21	0,004216784	0,022179085	Cell wall / LPS / capsule; Motility & Attachment
PA0597_at	PA0597			-0,27	-1,21	0,006452231	0,029856087	Putative enzymes
PA2574_at	PA2574	alkB1		-0,27	-1,20	0,003218742	0,018341407	Carbon compound catabolism
PA4629_at	PA4629			-0,27	-1,20	0,005030249	0,025146713	Hypothetical, unclassified, unknown
PA4504_at	PA4504		dppC	-0,27	-1,20	0,010176103	0,041277189	Membrane proteins; Transport of small molecules
PA3751_purT_at	PA3751	purT		-0,27	-1,20	0,01193961	0,046040928	Amino acid biosynthesis and metabolism; Nucleotide biosynthesis and metabolism
PA3598_at	PA3598		ypqQ	-0,27	-1,20	0,007460086	0,033295886	Hypothetical, unclassified, unknown
PA4472_pmbA_at	PA4472	pmbA	tldE	-0,26	-1,20	0,00302426	0,017652593	Translation, post-translational modification, degradation; Adaptation, Protection
PA5477_at	PA5477			-0,26	-1,20	0,012805658	0,04804105	Membrane proteins
PA0512_at	PA0512	nirH	nirH	-0,26	-1,20	0,012887962	0,048093679	Biosynthesis of cofactors, prosthetic groups and carriers; Hypothetical, unclassified, unknown; Energy metabolism
PA2536_at	PA2536		ynbB	-0,26	-1,20	0,010528873	0,042273049	Fatty acid and phospholipid metabolism
PA5567_at	PA5567		thdF	-0,26	-1,20	0,011892524	0,045923186	Putative enzymes
PA2869_at	PA2869			-0,26	-1,20	0,012835573	0,048059781	Hypothetical, unclassified, unknown
PA2874_at	PA2874			-0,26	-1,20	0,003907983	0,021053782	Hypothetical, unclassified, unknown
PA1776_at	PA1776	sigX		-0,26	-1,20	0,008455455	0,036175267	Transcriptional regulators
PA3103_xcpR_at	PA3103	xcpR		-0,26	-1,20	0,003237838	0,018389729	Protein secretion/export apparatus
PA2704_at	PA2704			-0,26	-1,20	0,002444373	0,015343696	Transcriptional regulators
PA0837_slyD_at	PA0837	slyD		-0,26	-1,20	0,007464423	0,033295886	Translation, post-translational modification, degradation; Chaperones & heat shock proteins
PA0594_surA_at	PA0594	surA		-0,26	-1,20	0,006600119	0,030368209	Translation, post-translational modification, degradation; Adaptation, Protection; Chaperones & heat shock proteins
PA2289_at	PA2289			-0,26	-1,20	0,004852306	0,024544619	Hypothetical, unclassified, unknown
PA0304_potI_at	PA0304	spuH	potI	-0,26	-1,20	0,013009751	0,04835213	Membrane proteins; Transport of small molecules
PA2963_at	PA2963		yceG	-0,26	-1,20	0,007724166	0,034085816	Hypothetical, unclassified, unknown
PA0075_at	PA0075	pppA	tagG1	-0,26	-1,20	0,00612576	0,028782252	Putative enzymes; Protein secretion/export apparatus
PA2141_at	PA2141			-0,26	-1,19	0,009708749	0,040041966	Hypothetical, unclassified, unknown
PA0909_i_at	PA0909			-0,26	-1,19	0,011637249	0,045252342	Related to phage, transposon, or plasmid; Membrane proteins
PA1747_at	PA1747			-0,25	-1,19	0,005400133	0,026494551	Hypothetical, unclassified, unknown
PA0457_at	PA0457			-0,25	-1,19	0,010972487	0,043366334	Hypothetical, unclassified, unknown
PA1668_at	PA1668	dotU2		-0,25	-1,19	0,011334958	0,04426297	Protein secretion/export apparatus
PA0670_at	PA0670			-0,25	-1,19	0,00693488	0,03151651	Hypothetical, unclassified, unknown
PA1665_at	PA1665	fha2		-0,25	-1,19	0,006738692	0,030801484	Protein secretion/export apparatus

PA1025_at	PA1025		opdD	-0,25	-1,19	0,005794911	0,027672945	Membrane proteins; Transport of small molecules
PA2640_nuoE_at	PA2640	nuoE		-0,25	-1,19	0,004530718	0,023391068	Energy metabolism
PA2976_rne_at	PA2976	rne	ams	-0,25	-1,19	0,009811137	0,040178595	Transcription, RNA processing and degradation
PA2612_serS_at	PA2612	serS		-0,25	-1,19	0,005644447	0,02736891	Amino acid biosynthesis and metabolism; Translation, post-translational modification, degradation
PA1785_at	PA1785	nasT	nasT	-0,25	-1,19	0,010716307	0,042657668	Hypothetical, unclassified, unknown
PA0231_pcaD_at	PA0231	pcaD		-0,25	-1,19	0,00759654	0,033749559	Carbon compound catabolism
PA4856_at	PA4856	retS	rtsM	-0,25	-1,19	0,00646388	0,029865172	Two-component regulatory systems
PA3101_xcpT_at	PA3101	xcpT	pddA	-0,25	-1,19	0,009580638	0,039733156	Protein secretion/export apparatus
PA5014_glnE_at	PA5014	glnE		-0,24	-1,18	0,007640467	0,03386338	Translation, post-translational modification, degradation
PA2262_at	PA2262		kguT	-0,24	-1,18	0,008885628	0,037609726	Membrane proteins; Transport of small molecules
PA0198_exbB1_at	PA0198	exbB1		-0,24	-1,18	0,006645687	0,030501997	Transport of small molecules
PA3695_at	PA3695			-0,24	-1,18	0,008296277	0,035797853	Hypothetical, unclassified, unknown
PA5412_at	PA5412			-0,24	-1,18	0,011716697	0,045307282	Hypothetical, unclassified, unknown
PA1586_sucB_at	PA1586	sucB		-0,24	-1,18	0,008374057	0,035972788	Energy metabolism
PA0157_at	PA0157	triB	triB	-0,24	-1,18	0,013599064	0,049875219	Antibiotic resistance and susceptibility; Membrane proteins
PA5067_hisE_at	PA5067	hisE		-0,24	-1,18	0,01219377	0,046577095	Amino acid biosynthesis and metabolism
PA4984_at	PA4984			-0,24	-1,18	0,009594156	0,039759502	Transcriptional regulators
PA5377_at	PA5377	cbcW		-0,24	-1,18	0,009789487	0,040178595	Membrane proteins; Transport of small molecules
PA1274_at	PA1274		bluB	-0,24	-1,18	0,010604644	0,042348958	Hypothetical, unclassified, unknown
PA5562_spoOJ_at	PA5562	spoOJ		-0,24	-1,18	0,011093847	0,043669617	Cell division
PA4560_ileS_at	PA4560	ileS		-0,24	-1,18	0,012413722	0,047142448	Amino acid biosynthesis and metabolism; Translation, post-translational modification, degradation
PA0966_ruvA_at	PA0966	ruvA		-0,24	-1,18	0,012784891	0,04804105	DNA replication, recombination, modification and repair
PA2442_gcvT2_at	PA2442	gcvT2		-0,24	-1,18	0,012290685	0,046905785	Central intermediary metabolism; Amino acid biosynthesis and metabolism
PA4618_at	PA4618			-0,24	-1,18	0,007534622	0,03358202	Hypothetical, unclassified, unknown
PA3918_moaC_at	PA3918	moaC		-0,24	-1,18	0,009659155	0,03992116	Biosynthesis of cofactors, prosthetic groups and carriers
PA5375_betT1_at	PA5375	betT1		-0,23	-1,18	0,008793713	0,037271259	Membrane proteins; Transport of small molecules
PA5005_at	PA5005			-0,23	-1,18	0,012288736	0,046905785	Putative enzymes
PA0017_at	PA0017		sun; fmu	-0,23	-1,18	0,011658974	0,045256684	Hypothetical, unclassified, unknown
PA4744_infB_at	PA4744	infB		-0,23	-1,18	0,007926352	0,034632539	Translation, post-translational modification, degradation
PA4447_hisC1_at	PA4447	hisC1	his8	-0,23	-1,17	0,012551934	0,047510698	Amino acid biosynthesis and metabolism
PA5257_at	PA5257		hemY	-0,23	-1,17	0,005693429	0,027400551	Hypothetical, unclassified, unknown
PA1513_at	PA1513			-0,23	-1,17	0,007715561	0,034085816	Membrane proteins
PA1237_at	PA1237			-0,23	-1,17	0,012781617	0,04804105	Transport of small molecules; Antibiotic resistance and susceptibility
PA1046_at	PA1046			-0,23	-1,17	0,013018216	0,04835213	Putative enzymes
PA1458_at	PA1458		cheA	-0,23	-1,17	0,013182147	0,048751281	Chemotaxis; Two-component regulatory systems
PA0232_pcaC_at	PA0232	pcaC		-0,22	-1,17	0,012874486	0,048093679	Carbon compound catabolism
PA2609_at	PA2609			-0,22	-1,16	0,012818087	0,04804105	Hypothetical, unclassified, unknown
PA3548_algI_at	PA3548	algI		-0,22	-1,16	0,010658772	0,042489601	Cell wall / LPS / capsule; Adaptation, Protection; Secreted Factors (toxins, enzymes, alginate)
PA5401_at	PA5401			-0,22	-1,16	0,007050071	0,031935381	Hypothetical, unclassified, unknown
PA2378_at	PA2378			-0,21	-1,16	0,013424572	0,049492661	Putative enzymes
PA1105_fliJ_at	PA1105	fliJ		-0,21	-1,16	0,012810213	0,04804105	Cell wall / LPS / capsule; Motility & Attachment
PA3047_at	PA3047		dacB	-0,21	-1,16	0,007579402	0,033727428	Cell wall / LPS / capsule
PA3187_at	PA3187		gltK	-0,21	-1,15	0,012064551	0,046297506	Transport of small molecules
PA0702_at	PA0702			-0,20	-1,15	0,011245951	0,044059904	Membrane proteins
PA0015_at	PA0015			0,21	1,15	0,01352913	0,049742886	Hypothetical, unclassified, unknown
PA3413_at	PA3413		yebG	0,21	1,16	0,012459255	0,047256601	Hypothetical, unclassified, unknown
PA0181_at	PA0181			0,21	1,16	0,012697812	0,047899496	Transcriptional regulators
PA4993_at	PA4993			0,22	1,16	0,010028954	0,040919608	Hypothetical, unclassified, unknown
PA0034_at	PA0034			0,22	1,17	0,007810415	0,034303955	Transcriptional regulators; Two-component regulatory systems
PA2954_at	PA2954			0,22	1,17	0,009661942	0,03992116	Hypothetical, unclassified, unknown
PA3423_at	PA3423			0,22	1,17	0,010085701	0,041045616	Transcriptional regulators
PA4947_amiB_at	PA4947	amiB		0,22	1,17	0,009797683	0,040178595	Cell wall / LPS / capsule
PA4286_at	PA4286			0,23	1,17	0,013368193	0,049349698	Hypothetical, unclassified, unknown
PA3123_at	PA3123			0,23	1,17	0,013029752	0,048362606	Hypothetical, unclassified, unknown
PA1395_at	PA1395			0,23	1,17	0,006155184	0,028847228	Hypothetical, unclassified, unknown
PA2454_at	PA2454			0,23	1,17	0,004798663	0,024339837	Hypothetical, unclassified, unknown
PA2436_at	PA2436			0,23	1,17	0,00932407	0,038947731	Hypothetical, unclassified, unknown
PA3112_accD_at	PA3112	accD	dedB	0,23	1,17	0,008696363	0,036961038	Fatty acid and phospholipid metabolism
PA2781_at	PA2781			0,23	1,17	0,012317039	0,046974055	Hypothetical, unclassified, unknown
PA3902_at	PA3902			0,23	1,18	0,01316221	0,048723885	Hypothetical, unclassified, unknown
PA3496_at	PA3496			0,24	1,18	0,012600396	0,047564353	Hypothetical, unclassified, unknown
PA4110_ampC_at	PA4110	ampC		0,24	1,18	0,005750271	0,027529121	Adaptation, Protection
PA0658_at	PA0658			0,24	1,18	0,005698986	0,027403527	Putative enzymes
PA3796_at	PA3796			0,24	1,18	0,010615485	0,042348958	Hypothetical, unclassified, unknown
PA0307_at	PA0307			0,25	1,19	0,005306399	0,026243498	Hypothetical, unclassified, unknown
PA2549_at	PA2549		ygjT	0,25	1,19	0,013459272	0,049526193	Membrane proteins
PA3633_at	PA3633	ygbP		0,25	1,19	0,012023627	0,046236385	Biosynthesis of cofactors, prosthetic groups and carriers
PA3656_rpsB_at	PA3656	rpsB		0,25	1,19	0,010243451	0,041489714	Translation, post-translational modification, degradation
PA0791_at	PA0791			0,26	1,19	0,004178712	0,0220835	Transcriptional regulators
PA1288_at	PA1288		ompP1; fadL	0,26	1,20	0,00663194	0,030489341	Membrane proteins; Transport of small molecules
PA0335_at	PA0335			0,26	1,20	0,010089425	0,041045616	Hypothetical, unclassified, unknown
PA0508_at	PA0508			0,26	1,20	0,004571833	0,023577231	Putative enzymes
PA5189_at	PA5189			0,26	1,20	0,00763007	0,033844332	Transcriptional regulators
PA0118_at	PA0118			0,27	1,20	0,003556826	0,019658193	Putative enzymes
PA1469_at	PA1469			0,27	1,20	0,003907811	0,021053782	Hypothetical, unclassified, unknown
PA5438_at	PA5438			0,27	1,20	0,007762979	0,034182708	Transcriptional regulators
PA3657_map_at	PA3657	map		0,27	1,20	0,008699544	0,036961038	Translation, post-translational modification, degradation
PA0461_at	PA0461		yihG	0,27	1,20	0,009163761	0,038566631	Hypothetical, unclassified, unknown
PA3738_xerD_at	PA3738	xerD		0,27	1,20	0,013432321	0,049492661	DNA replication, recombination, modification and repair
PA4234_uvrA_at	PA4234	uvrA		0,27	1,20	0,008399486	0,036046984	DNA replication, recombination, modification and repair
PA5195_at	PA5195		yrfH	0,27	1,21	0,003107474	0,017924505	Chaperones & heat shock proteins
PA3269_at	PA3269			0,27	1,21	0,004655172	0,023873891	Transcriptional regulators
PA2959_at	PA2959		ycfH	0,27	1,21	0,00805214	0,034989291	Hypothetical, unclassified, unknown
PA4015_at	PA4015			0,27	1,21	0,012127748	0,04647574	Hypothetical, unclassified, unknown
PA1170_at	PA1170			0,27	1,21	0,007191981	0,032288269	Membrane proteins
PA1305_at	PA1305			0,27	1,21	0,00544499	0,026691032	Hypothetical, unclassified, unknown; Membrane proteins
PA0159_at	PA0159			0,27	1,21	0,010696412	0,04260904	Transcriptional regulators
PA1182_at	PA1182			0,27	1,21	0,002618447	0,016071281	Transcriptional regulators
PA3475_pheC_at	PA3475	pheC		0,28	1,21	0,002816182	0,016894049	Adaptation, Protection; Amino acid biosynthesis and metabolism
PA0289_at	PA0289	gpuR		0,28	1,21	0,006146716	0,028831889	Transcriptional regulators
PA0554_at	PA0554			0,28	1,21	0,006179666	0,028915579	Hypothetical, unclassified, unknown
PA3712_at	PA3712			0,28	1,21	0,011709642	0,045307282	Hypothetical, unclassified, unknown
PA4457_at	PA4457		kpsF; yrbH; kdsD	0,28	1,21	0,003889583	0,021053782	Secreted Factors (toxins, enzymes, alginate)
PA1859_at	PA1859			0,28	1,21	0,002417843	0,015228842	Transcriptional regulators
PA0704_at	PA0704			0,28	1,21	0,00608997	0,028639796	Putative enzymes
PA1890_at	PA1890			0,28	1,21	0,00625141	0,029224156	Putative enzymes
PA2634_at	PA2634	aceA	aceA	0,28	1,21	0,009496879	0,039533518	Putative enzymes
PA3925_at	PA3925			0,28	1,21	0,005287218	0,026171967	Putative enzymes
PA1203_at	PA1203			0,28	1,21	0,003960064	0,021272406	Hypothetical, unclassified, unknown
PA4537_at	PA4537			0,28	1,21	0,012045159	0,04626048	Hypothetical, unclassified, unknown
PA1234_at	PA1234			0,28	1,21	0,012367967	0,047103306	Hypothetical, unclassified, unknown
PA2337_mtlR_at	PA2337	mtlR		0,28	1,22	0,005954238	0,028263529	Transcriptional regulators
PA1969_at	PA1969			0,28	1,22	0,002091265	0,014019427	Hypothetical, unclassified, unknown
PA2692_at	PA2692			0,28	1,22	0,002327	0,015007889	Transcriptional regulators
PA2063_at	PA2063			0,28	1,22	0,006387714	0,029636641	Hypothetical, unclassified, unknown
PA4324_at	PA4324			0,28	1,22	0,005961443	0,028273543	Hypothetical, unclassified, unknown
PA5340_at	PA5340			0,29	1,22	0,010571758	0,042294655	Hypothetical, unclassified, unknown
PA5110_fbp_at	PA5110	fbp	cfxF; cbbF	0,29	1,22	0,011530271	0,044897402	Central intermediary metabolism; Carbon compound catabolism
PA3091_at	PA3091			0,29	1,22	0,011901985	0,045927756	Hypothetical, unclassified, unknown
PA4232_ssb_at	PA4232	ssb		0,29	1,22	0,009328083	0,038947731	DNA replication, recombination, modification and repair
PA2201_at	PA2201			0,29	1,22	0,012376396	0,047103306	Hypothetical, unclassified, unknown
PA0122_at	PA0122	rahU		0,29	1,22	0,009506914	0,039545628	Adaptation, Protection
PA4047_ribA_at	PA4047	ribA		0,29	1,22	0,009211757	0,038639111	Biosynthesis of cofactors, prosthetic groups and carriers
PA5275_at	PA5275		cyaY	0,29	1,22	0,006045419	0,028624717	Hypothetical, unclassified, unknown
PA3287_at	PA3287			0,29	1,22	0,007038924	0,031910938	Hypothetical, unclassified, unknown
PA1835_at	PA1835			0,29	1,22	0,004590843	0,023631341	Hypothetical, unclassified, unknown
PA0339_at	PA0339			0,29	1,22	0,004123244	0,021853239	Hypothetical, unclassified, unknown
PA4788_at	PA4788			0,30	1,23	0,002587317	0,015977312	Hypothetical, unclassified, unknown
PA5178_at	PA5178			0,30	1,23	0,010402519	0,042010326	Hypothetical, unclassified, unknown
PA0225_at	PA0225			0,30	1,23	0,003436324	0,019220772	Transcriptional regulators
PA1289_at	PA1289			0,30	1,23	0,011015914	0,043476035	Hypothetical, unclassified, unknown
PA5254_at	PA5254		fkl; fkbZ	0,30	1,23	0,00512956	0,025526937	Translation, post-translational modification, degradation; Chaperones & heat shock proteins
PA1121_at	PA1121	yfiR		0,30	1,23	0,006719625	0,030739655	Cell wall / LPS / capsule
PA4456_at	PA4456		yrbF	0,30	1,23	0,003092059	0,017901947	Transport of small molecules
PA3203_at	PA3203			0,30	1,23	0,006456534	0,029856087	Hypothetical, unclassified, unknown
PA1958_at	PA1958			0,30	1,23	0,01136517	0,044333104	Membrane proteins; Transport of small molecules
PA2559_at	PA2559			0,31	1,24	0,00774263	0,034125382	Hypothetical, unclassified, unknown
PA2707_at	PA2707			0,31	1,24	0,01272688	0,047976532	Hypothetical, unclassified, unknown
PA0472_at	PA0472	fiuI	fiuI	0,31	1,24	0,01153788	0,044897402	Transcriptional regulators
PA0733_at	PA0733		rsuA	0,31	1,24	0,012390484	0,047124602	Transcription, RNA processing and degradation
PA3571_mmsR_at	PA3571	mmsR		0,31	1,24	0,003902934	0,021053782	Transcriptional regulators
PA5558_atpF_at	PA5558	atpF	papF; uncF	0,31	1,24	0,010505208	0,042248662	Energy metabolism
PA1773_at	PA1773	cmaX		0,31	1,24	0,006271839	0,029270342	Membrane proteins
PA0424_mexR_at	PA0424	mexR		0,31	1,24	0,002232446	0,014501484	Transcriptional regulators
PA0448_at	PA0448			0,31	1,24	0,001747779	0,012168667	Transcriptional regulators

PA5406_at	PA5406			0,31	1,24	0,006356854	0,029518145	Hypothetical, unclassified, unknown
PA2809_at	PA2809	copR	copR	0,31	1,24	0,005461098	0,026746368	Transcriptional regulators; Two-component regulatory systems; Adaptation, Protection
PA1836_at	PA1836			0,31	1,24	0,003375427	0,019034802	Transcriptional regulators
PA2366_at	PA2366		puuD	0,31	1,24	0,003787925	0,020627277	Hypothetical, unclassified, unknown
PA1752_at	PA1752			0,31	1,24	0,004781136	0,024295349	Hypothetical, unclassified, unknown
PA5080_at	PA5080		pip; pap	0,32	1,24	0,005482872	0,026805691	Translation, post-translational modification, degradation
PA4421_at	PA4421		yabB	0,32	1,24	0,011090517	0,043669617	Hypothetical, unclassified, unknown
PA4090_at	PA4090			0,32	1,24	0,010556067	0,042273049	Hypothetical, unclassified, unknown
PA3605_at	PA3605			0,32	1,25	0,012360408	0,047103306	Membrane proteins
PA3117_asd_at	PA3117	asd		0,32	1,25	0,011694415	0,045284233	Amino acid biosynthesis and metabolism
PA5073_at	PA5073			0,32	1,25	0,002621105	0,016071281	Hypothetical, unclassified, unknown
PA5124_ntrB_at	PA5124	ntrB		0,32	1,25	0,005686162	0,027389334	Two-component regulatory systems
PA3007_lexA_at	PA3007	lexA		0,32	1,25	0,001038399	0,009003244	Adaptation, Protection; Translation, post-translational modification, degradation
PA2544_at	PA2544			0,32	1,25	0,002120292	0,014094652	Hypothetical, unclassified, unknown
PA2030_at	PA2030			0,32	1,25	0,009240279	0,038726818	Hypothetical, unclassified, unknown
PA1552_at	PA1552	ccoP1		0,32	1,25	0,011005373	0,043465347	Energy metabolism; Central intermediary metabolism
PA1065_at	PA1065			0,32	1,25	0,005837752	0,0278438	Hypothetical, unclassified, unknown
PA4715_at	PA4715		yfdZ	0,33	1,25	0,004790179	0,024319032	Putative enzymes
PA0961_at	PA0961			0,33	1,25	0,012879804	0,048093679	Transcriptional regulators
PA3088_at	PA3088		yfjB	0,33	1,25	0,001102956	0,009246417	Hypothetical, unclassified, unknown
PA4781_at	PA4781			0,33	1,26	0,004990937	0,025058849	Transcriptional regulators; Motility & Attachment; Cell wall / LPS / capsule
PA4079_at	PA4079			0,33	1,26	0,001738344	0,012148701	Putative enzymes
PA0479_at	PA0479			0,33	1,26	0,008321673	0,035851681	Transcriptional regulators
PA2885_at	PA2885	atuR		0,33	1,26	0,010050368	0,040976848	Transcriptional regulators
PA0329_at	PA0329			0,33	1,26	0,006410502	0,029717523	Hypothetical, unclassified, unknown
PA1377_at	PA1377		yhhY	0,33	1,26	0,003159146	0,01813277	Hypothetical, unclassified, unknown
PA2866_mttC_at	PA2866	mttC		0,33	1,26	0,011167123	0,043823456	Protein secretion/export apparatus
PA3299_fadD1_at	PA3299	fadD1		0,33	1,26	0,004267306	0,022423563	Fatty acid and phospholipid metabolism
PA2947_i_at	PA2947		cbiG; cobE	0,33	1,26	0,009197943	0,038639111	Biosynthesis of cofactors, prosthetic groups and carriers
PA5127_at	PA5127		yibK	0,33	1,26	0,010139395	0,041171175	Putative enzymes
PA1269_at	PA1269			0,33	1,26	0,010142547	0,041171175	Transcriptional regulators
PA1048_at	PA1048			0,33	1,26	0,003410087	0,019152402	Membrane proteins; Transport of small molecules
PA1770_ppsA_at	PA1770	ppsA		0,33	1,26	0,012595834	0,047564353	Energy metabolism; Carbon compound catabolism; Central intermediary metabolism
PA1968_at	PA1968			0,33	1,26	0,001508696	0,011059122	Hypothetical, unclassified, unknown
PA1075_at	PA1075			0,33	1,26	0,001476584	0,010939339	Hypothetical, unclassified, unknown
PA0955_at	PA0955			0,34	1,26	0,008449284	0,036175267	Hypothetical, unclassified, unknown
PA3134_gltX_at	PA3134	gltX		0,34	1,26	0,008468932	0,036205009	Translation, post-translational modification, degradation
PA3369_at	PA3369			0,34	1,26	0,001344379	0,010318061	Membrane proteins
PA2769_at	PA2769			0,34	1,26	0,00814782	0,035266968	Hypothetical, unclassified, unknown
PA3653_frr_at	PA3653	frr	rrf	0,34	1,26	0,004452683	0,023156457	Translation, post-translational modification, degradation
PA0564_at	PA0564			0,34	1,26	0,00232046	0,014989794	Transcriptional regulators
PA0133_at	PA0133	bauR		0,34	1,26	0,005110658	0,025479823	Transcriptional regulators; Carbon compound catabolism
PA0330_rpiA_at	PA0330	rpiA		0,34	1,26	0,012821913	0,04804105	Energy metabolism
PA0734_i_at	PA0734			0,34	1,26	0,010743278	0,042684832	Hypothetical, unclassified, unknown
PA2900_at	PA2900			0,34	1,27	0,012959548	0,048231073	Membrane proteins; Transport of small molecules
PA1775_at	PA1775	cmpX		0,34	1,27	0,010140688	0,041171175	Membrane proteins
PA4053_ribE_at	PA4053	ribE	ribH	0,34	1,27	0,010918492	0,043214487	Biosynthesis of cofactors, prosthetic groups and carriers
PA1941_at	PA1941			0,34	1,27	0,010740848	0,042684832	Hypothetical, unclassified, unknown
PA4360_at	PA4360			0,34	1,27	0,000885711	0,008110247	Hypothetical, unclassified, unknown
PA1793_ppiB_at	PA1793	ppiB	cypB	0,34	1,27	0,002457435	0,015408255	Translation, post-translational modification, degradation; Chaperones & heat shock proteins
PA5407_at	PA5407			0,34	1,27	0,010556816	0,042273049	Hypothetical, unclassified, unknown
PA3627_at	PA3627	ygbB		0,35	1,27	0,009642764	0,039901342	Biosynthesis of cofactors, prosthetic groups and carriers
PA4462_rpoN_at	PA4462	rpoN	ntrA	0,35	1,27	0,012916065	0,048133811	Transcriptional regulators
PA1653_at	PA1653			0,35	1,27	0,002357079	0,015034801	Transcriptional regulators
PA1994_at	PA1994			0,35	1,27	0,003981655	0,021326454	Hypothetical, unclassified, unknown
PA4372_at	PA4372			0,35	1,27	0,006549052	0,030208385	Hypothetical, unclassified, unknown
PA2779_at	PA2779			0,35	1,27	0,002517637	0,015697042	Hypothetical, unclassified, unknown
PA0312_at	PA0312			0,35	1,27	0,002048654	0,013812854	Hypothetical, unclassified, unknown
PA3678_at	PA3678	mexL		0,35	1,27	0,001559644	0,01129826	Transcriptional regulators
PA2823_at	PA2823			0,35	1,27	0,007154752	0,032199286	Hypothetical, unclassified, unknown
PA4422_at	PA4422		yraL	0,35	1,27	0,003511046	0,019502297	Hypothetical, unclassified, unknown
PA5531_tonB_at	PA5531	tonB1	tonB	0,35	1,28	0,008076596	0,035046217	Transport of small molecules
PA5332_crc_at	PA5332	crc		0,35	1,28	0,000970016	0,008598433	Carbon compound catabolism; Energy metabolism
PA5263_argH_at	PA5263	argH		0,35	1,28	0,011130825	0,043742879	Amino acid biosynthesis and metabolism
PA0081_at	PA0081	fha1		0,35	1,28	0,002042294	0,013786725	Protein secretion/export apparatus
PA3732_at	PA3732		yjfI	0,35	1,28	0,011151482	0,043793045	Hypothetical, unclassified, unknown
PA0179_at	PA0179			0,35	1,28	0,012273323	0,046904043	Chemotaxis; Adaptation, Protection; Two-component regulatory systems
PA4185_at	PA4185			0,35	1,28	0,002393818	0,015163583	Transcriptional regulators
PA3577_i_at	PA3577			0,36	1,28	0,001462096	0,010889211	Hypothetical, unclassified, unknown
PA0395_pilT_at	PA0395	pilT		0,36	1,28	0,000603043	0,006361347	Cell wall / LPS / capsule; Motility & Attachment
PA2638_nuoB_at	PA2638	nuoB		0,36	1,28	0,012902981	0,048117366	Energy metabolism
PA1558_at	PA1558			0,36	1,28	0,0091558	0,038566631	Hypothetical, unclassified, unknown
PA1853_at	PA1853			0,36	1,28	0,005530287	0,026942548	Transcriptional regulators
PA4430_at	PA4430			0,36	1,28	0,012881829	0,048093679	Energy metabolism
PA1878_at	PA1878			0,36	1,28	0,001243138	0,00981248	Hypothetical, unclassified, unknown
PA4395_at	PA4395		yajQ	0,36	1,28	0,003582297	0,01977927	Hypothetical, unclassified, unknown
PA3826_at	PA3826			0,36	1,28	0,001548263	0,011244504	Membrane proteins
PA0654_speD_at	PA0654	speD		0,36	1,28	0,00385536	0,02095337	Central intermediary metabolism
PA5336_gmk_at	PA5336	gmk		0,36	1,28	0,001191083	0,009592625	Nucleotide biosynthesis and metabolism
PA4157_at	PA4157			0,36	1,28	0,00418713	0,022106929	Transcriptional regulators
PA3698_at	PA3698			0,36	1,28	0,002960308	0,01743816	Hypothetical, unclassified, unknown
PA4402_argJ_at	PA4402	argJ		0,36	1,29	0,001096865	0,009238329	Amino acid biosynthesis and metabolism
PA4713_at	PA4713			0,36	1,29	0,007086737	0,032049149	Hypothetical, unclassified, unknown
PA3357_dsdA_at	PA3357	dsdA		0,36	1,29	0,002551742	0,0158208	Amino acid biosynthesis and metabolism
PA5296_rep_at	PA5296	rep		0,36	1,29	0,007231009	0,032384882	DNA replication, recombination, modification and repair
PA1520_at	PA1520			0,36	1,29	0,001194572	0,00959288	Transcriptional regulators
PA2949_at	PA2949			0,36	1,29	0,000788401	0,007568922	Fatty acid and phospholipid metabolism; Putative enzymes
PA1966_at	PA1966			0,37	1,29	0,002148972	0,014192938	Putative enzymes
PA5136_at	PA5136			0,37	1,29	0,005545216	0,026967926	Hypothetical, unclassified, unknown
PA3027_at	PA3027			0,37	1,29	0,003907871	0,021053782	Transcriptional regulators
PA3674_at	PA3674			0,37	1,29	0,013577474	0,049861949	Hypothetical, unclassified, unknown
PA3306_at	PA3306			0,37	1,29	0,001651528	0,011734093	Hypothetical, unclassified, unknown
PA1687_speE_at	PA1687	speE		0,37	1,29	0,00114635	0,009437831	Amino acid biosynthesis and metabolism
PA5229_at	PA5229			0,37	1,29	0,006136847	0,028809952	Hypothetical, unclassified, unknown
PA2921_at	PA2921			0,37	1,29	0,002970438	0,017442288	Transcriptional regulators
PA4931_dnaB_at	PA4931	dnaB		0,37	1,29	0,00443232	0,023072179	DNA replication, recombination, modification and repair
PA4928_at	PA4928		ygiR; ygiQ	0,37	1,29	0,01055874	0,042273049	Hypothetical, unclassified, unknown
PA2849_at	PA2849	ohrR		0,37	1,29	0,001403257	0,010608549	Transcriptional regulators
PA1710_exsC_at	PA1710	exsC		0,37	1,29	0,004605674	0,023663785	Translation, post-translational modification, degradation; Protein secretion/export apparatus
PA3525_argG_at	PA3525	argG		0,37	1,29	0,001325936	0,010204742	Amino acid biosynthesis and metabolism
PA0705_at	PA0705	migA	migA	0,37	1,29	0,004743117	0,024168556	Putative enzymes; Cell wall / LPS / capsule
PA5520_at	PA5520			0,37	1,29	0,002845282	0,017027504	Hypothetical, unclassified, unknown
PA1193_at	PA1193			0,37	1,29	0,009101767	0,038380999	Hypothetical, unclassified, unknown
PA5202_at	PA5202			0,37	1,29	0,001597878	0,011485268	Hypothetical, unclassified, unknown
PA3463_at	PA3463		yheU	0,37	1,29	0,002210758	0,014432864	Hypothetical, unclassified, unknown
PA1580_gltA_at	PA1580	gltA	cisY	0,37	1,30	0,013054751	0,048390658	Energy metabolism
PA0855_at	PA0855			0,37	1,30	0,001647801	0,011722624	Hypothetical, unclassified, unknown
PA5329_at	PA5329			0,37	1,30	0,000817999	0,007745864	Hypothetical, unclassified, unknown
PA4233_at	PA4233		yajR	0,37	1,30	0,006640325	0,030501997	Membrane proteins; Transport of small molecules
PA4712_at	PA4712			0,38	1,30	0,005396295	0,026494551	Hypothetical, unclassified, unknown
PA0398_at	PA0398			0,38	1,30	0,001307054	0,010101449	Hypothetical, unclassified, unknown
PA4446_algW_at	PA4446	algW		0,38	1,30	0,011655949	0,045256684	Translation, post-translational modification, degradation; Adaptation, Protection; Secreted Factors (toxins, enzymes, alginate)
PA0376_rpoH_at	PA0376	rpoH		0,38	1,30	0,001098652	0,009238329	Transcriptional regulators
PA0269_at	PA0269			0,38	1,30	0,010200559	0,041346167	Hypothetical, unclassified, unknown
PA1040_at	PA1040			0,38	1,30	0,008705726	0,036961038	Hypothetical, unclassified, unknown
PA2532_tpx_at	PA2532	tpx		0,38	1,30	0,001057514	0,009069778	Adaptation, Protection
PA1536_at	PA1536			0,38	1,30	0,004755567	0,024209762	Hypothetical, unclassified, unknown
PA4961_at	PA4961			0,38	1,30	0,005637994	0,02736891	Membrane proteins
PA5550_at	PA5550	glmR		0,38	1,30	0,01127864	0,044074064	Transcriptional regulators
PA0848_at	PA0848	ahpB		0,38	1,30	0,010088319	0,041045616	Adaptation, Protection; Putative enzymes
PA0332_at	PA0332			0,38	1,30	0,005503541	0,026860049	Hypothetical, unclassified, unknown
PA0944_purN_at	PA0944	purN		0,38	1,30	0,004353572	0,022769059	Nucleotide biosynthesis and metabolism
PA5133_at	PA5133		yibP	0,38	1,30	0,000946083	0,008508618	Membrane proteins
PA3126_ibpA_at	PA3126	ibpA	hslT	0,38	1,30	0,002670921	0,016277223	Chaperones & heat shock proteins
PA2306_at	PA2306	ambA		0,38	1,30	0,007373663	0,032944006	Membrane proteins; Secreted Factors (toxins, enzymes, alginate)
PA1122_at	PA1122		fms; pdf; def	0,38	1,30	0,012926099	0,048138874	Translation, post-translational modification, degradation
PA0996_at	PA0996	pqsA		0,38	1,30	0,011678756	0,045266859	Biosynthesis of cofactors, prosthetic groups and carriers
PA5481_at	PA5481			0,39	1,31	0,003717732	0,020354258	Hypothetical, unclassified, unknown
PA1734_at	PA1734			0,39	1,31	0,000780161	0,007568922	Hypothetical, unclassified, unknown
PA5527_at	PA5527			0,39	1,31	0,000825482	0,007803405	Hypothetical, unclassified, unknown
PA0562_at	PA0562			0,39	1,31	0,002363187	0,015034801	Putative enzymes
PA2486_at	PA2486			0,39	1,31	0,004691913	0,023966153	Hypothetical, unclassified, unknown
PA3261_at	PA3261			0,39	1,31	0,000843834	0,007911918	Hypothetical, unclassified, unknown
PA1881_at	PA1881			0,39	1,31	0,011952591	0,046044581	Putative enzymes
PA3654_pyrH_at	PA3654	pyrH	smbA	0,39	1,31	0,00128681	0,010000712	Nucleotide biosynthesis and metabolism

PA2770_at	PA2770			0,39	1,31	0,006180191	0,028915579	Hypothetical, unclassified, unknown
PA0576_rpoD_at	PA0576	rpoD	rpoDA	0,39	1,31	0,00438113	0,022870076	Transcriptional regulators
PA4572_fklB_at	PA4572	fklB		0,39	1,31	0,000321717	0,004429797	Translation, post-translational modification, degradation; Chaperones & heat shock proteins
PA2358_at	PA2358			0,40	1,32	0,001616244	0,011587256	Hypothetical, unclassified, unknown
PA4007_proA_at	PA4007	proA	proB	0,40	1,32	0,011662832	0,045256684	Amino acid biosynthesis and metabolism; Biosynthesis of cofactors, prosthetic groups and carriers
PA5344_at	PA5344	oxyR		0,40	1,32	0,001275647	0,00998387	Transcriptional regulators
PA1751_at	PA1751			0,40	1,32	0,001004098	0,008816045	Hypothetical, unclassified, unknown
PA1995_i_at	PA1995			0,40	1,32	0,005511234	0,026873317	Hypothetical, unclassified, unknown
PA2545_xthA_at	PA2545	xthA		0,40	1,32	0,002671924	0,016277223	DNA replication, recombination, modification and repair
PA4378_inaA_at	PA4378	inaA		0,40	1,32	0,001299066	0,010067762	Adaptation, Protection
PA0759_at	PA0759			0,40	1,32	0,012402349	0,047137423	Hypothetical, unclassified, unknown
PA0956_proS_at	PA0956	proS		0,40	1,32	0,00937434	0,039111436	Amino acid biosynthesis and metabolism; Translation, post-translational modification, degradation
PA2972_at	PA2972		yceF	0,40	1,32	0,00396653	0,02128653	Hypothetical, unclassified, unknown
PA3756_at	PA3756		yafK	0,40	1,32	0,00124051	0,009805682	Hypothetical, unclassified, unknown
PA1772_at	PA1772		menG	0,40	1,32	0,002270415	0,014683604	Biosynthesis of cofactors, prosthetic groups and carriers
PA4724_at	PA4724		yadB	0,40	1,32	0,005538953	0,026961096	Putative enzymes
PA1167_at	PA1167			0,40	1,32	0,001132536	0,009394647	Hypothetical, unclassified, unknown
PA3951_at	PA3951			0,40	1,32	0,000465903	0,005408569	Hypothetical, unclassified, unknown
PA0551_epd_at	PA0551	epd	gapB	0,40	1,32	0,005942352	0,02823126	Biosynthesis of cofactors, prosthetic groups and carriers
PA3178_at	PA3178			0,40	1,32	0,002136555	0,01416457	Hypothetical, unclassified, unknown
PA5425_purK_at	PA5425	purK		0,40	1,32	0,004330455	0,022669524	Nucleotide biosynthesis and metabolism
PA2832_tpm_at	PA2832	tpm		0,41	1,32	0,002701722	0,016366656	Nucleotide biosynthesis and metabolism; Adaptation, Protection
PA1333_r_at	PA1333			0,41	1,32	0,000473265	0,005459771	Hypothetical, unclassified, unknown
PA0555_fda_at	PA0555	fda	cfxB; cbbA; fbaA	0,41	1,32	0,002195894	0,014369121	Central intermediary metabolism; Carbon compound catabolism
PA3622_rpoS_at	PA3622	rpoS		0,41	1,33	0,002001887	0,013556775	Transcriptional regulators
PA2031_i_at	PA2031			0,41	1,33	0,00572217	0,027443667	Hypothetical, unclassified, unknown
PA1166_at	PA1166			0,41	1,33	0,00145237	0,010834561	Hypothetical, unclassified, unknown
PA1201_at	PA1201			0,41	1,33	0,001033733	0,008976816	Transcriptional regulators
PA4987_at	PA4987			0,41	1,33	0,000868568	0,008046215	Transcriptional regulators
PA0243_at	PA0243			0,41	1,33	0,001128849	0,009394647	Transcriptional regulators
PA2960_pilZ_at	PA2960	pilZ		0,41	1,33	0,001025127	0,008936466	Motility & Attachment
PA4722_at	PA4722			0,41	1,33	0,003532554	0,019563014	Putative enzymes
PA5200_ompR_at	PA5200	amgR		0,41	1,33	0,003907494	0,021053782	Transcriptional regulators; Two-component regulatory systems; Antibiotic resistance and susceptibility
PA3680_at	PA3680		yhiQ	0,41	1,33	0,003759433	0,020512385	Hypothetical, unclassified, unknown
PA2616_trxB1_at	PA2616	trxB1		0,41	1,33	0,010389071	0,041987585	Nucleotide biosynthesis and metabolism
PA0340_at	PA0340			0,41	1,33	0,003063249	0,017761721	Membrane proteins
PA4312_at	PA4312			0,41	1,33	0,001046011	0,009026927	Hypothetical, unclassified, unknown
PA1743_at	PA1743			0,41	1,33	0,002948686	0,017406658	Hypothetical, unclassified, unknown
PA0358_at	PA0358			0,41	1,33	0,009616442	0,039822116	Hypothetical, unclassified, unknown
PA2449_at	PA2449			0,41	1,33	0,001491526	0,010991337	Transcriptional regulators
PA1754_cysB_at	PA1754	cysB		0,41	1,33	0,008513552	0,036367743	Transcriptional regulators
PA3179_at	PA3179		yciL	0,41	1,33	0,0003057	0,004305405	Hypothetical, unclassified, unknown
PA2529_at	PA2529			0,42	1,34	0,000659022	0,00669764	Hypothetical, unclassified, unknown
PA3345_at	PA3345	hptB		0,42	1,34	0,000902572	0,008210446	Two-component regulatory systems; Motility & Attachment
PA1815_rnhA_at	PA1815	rnhA		0,42	1,34	0,002984547	0,017488122	DNA replication, recombination, modification and repair
PA0336_at	PA0336	ygdP		0,42	1,34	0,006340771	0,029476178	Nucleotide biosynthesis and metabolism
PA0760_at	PA0760			0,42	1,34	0,008375715	0,035972788	Hypothetical, unclassified, unknown
PA5013_ilvE_at	PA5013	ilvE		0,42	1,34	0,001259564	0,00989989	Amino acid biosynthesis and metabolism
PA5335_at	PA5335		yicC	0,42	1,34	0,002679677	0,016286446	Hypothetical, unclassified, unknown
PA5360_phoB_at	PA5360	phoB		0,42	1,34	0,000640449	0,006569039	Transcriptional regulators; Two-component regulatory systems
PA0116_at	PA0116			0,42	1,34	0,003439579	0,019220772	Hypothetical, unclassified, unknown
PA1641_at	PA1641			0,42	1,34	0,000233216	0,003766331	Hypothetical, unclassified, unknown
PA2765_at	PA2765			0,42	1,34	0,004942134	0,024908175	Hypothetical, unclassified, unknown
PA1756_cysH_at	PA1756	cysH		0,42	1,34	0,001258859	0,00989989	Amino acid biosynthesis and metabolism
PA1100_fliE_at	PA1100	fliE		0,42	1,34	0,008295911	0,035797853	Cell wall / LPS / capsule; Motility & Attachment
PA0611_prtR_at	PA0611	prtR		0,42	1,34	0,003508585	0,019502297	Transcriptional regulators
PA1610_fabA_at	PA1610	fabA		0,42	1,34	0,001390219	0,010538695	Fatty acid and phospholipid metabolism
PA1681_aroC_at	PA1681	aroC		0,42	1,34	0,01168177	0,045266859	Amino acid biosynthesis and metabolism
PA0890_aotM_at	PA0890	aotM		0,43	1,34	0,00256066	0,015850385	Membrane proteins; Transport of small molecules
PA5301_at	PA5301	pauR	ycjC	0,43	1,34	0,013187182	0,048751281	Transcriptional regulators; Carbon compound catabolism
PA1295_at	PA1295		ycgL	0,43	1,34	0,008356157	0,035944429	Hypothetical, unclassified, unknown
PA4434_at	PA4434			0,43	1,35	0,000307126	0,00431454	Putative enzymes
PA4863_at	PA4863			0,43	1,35	0,003331099	0,004481138	Hypothetical, unclassified, unknown
PA3263_at	PA3263		yaiD	0,43	1,35	0,003407914	0,019152402	Hypothetical, unclassified, unknown
PA0953_at	PA0953		helX	0,43	1,35	0,000756487	0,007425553	Putative enzymes
PA1563_at	PA1563		ygdE	0,43	1,35	0,002482284	0,015546495	Hypothetical, unclassified, unknown
PA0054_at	PA0054		yjiI	0,43	1,35	0,000518381	0,005799388	Hypothetical, unclassified, unknown
PA3021_at	PA3021			0,43	1,35	0,000217062	0,003617049	Hypothetical, unclassified, unknown
PA4439_trpS_at	PA4439	trpS		0,43	1,35	0,006450427	0,029856087	Amino acid biosynthesis and metabolism; Translation, post-translational modification, degradation
PA0607_rpe_at	PA0607	rpe	dod	0,43	1,35	0,004995344	0,025058849	Energy metabolism
PA4817_at	PA4817			0,43	1,35	0,008077872	0,035046217	Hypothetical, unclassified, unknown
PA3204_at	PA3204			0,44	1,35	0,001220152	0,009700031	Transcriptional regulators; Two-component regulatory systems
PA4061_at	PA4061		ybbN	0,44	1,35	0,001077612	0,009185361	Energy metabolism
PA3530_at	PA3530	bfd	bfd	0,44	1,35	0,000875657	0,008058074	Hypothetical, unclassified, unknown
PA1627_at	PA1627			0,44	1,36	0,002884554	0,017130367	Transcriptional regulators
PA0125_at	PA0125			0,44	1,36	0,001485968	0,010964942	Hypothetical, unclassified, unknown
PA0406_at	PA0406	tonB3		0,44	1,36	0,002603064	0,016031525	Motility & Attachment
PA5303_at	PA5303			0,44	1,36	0,002096688	0,014019427	Hypothetical, unclassified, unknown
PA3898_at	PA3898			0,44	1,36	0,001406933	0,010616439	Transcriptional regulators
PA2365_at	PA2365			0,44	1,36	0,001219506	0,009700031	Hypothetical, unclassified, unknown
PA4701_at	PA4701			0,44	1,36	0,008128271	0,035209817	Hypothetical, unclassified, unknown
PA0655_at	PA0655			0,44	1,36	0,008662376	0,036861599	Hypothetical, unclassified, unknown
PA4854_purH_at	PA4854	purH		0,44	1,36	0,011103632	0,043669617	Nucleotide biosynthesis and metabolism
PA3956_at	PA3956			0,44	1,36	0,001463931	0,010889211	Hypothetical, unclassified, unknown
PA1821_at	PA1821			0,44	1,36	0,000167886	0,003154102	Putative enzymes
PA2884_at	PA2884			0,44	1,36	0,000293731	0,004232825	Membrane proteins
PA2747_at	PA2747			0,44	1,36	0,000162369	0,003075033	Hypothetical, unclassified, unknown
PA3965_at	PA3965			0,44	1,36	0,007771988	0,034182708	Transcriptional regulators
PA3092_fadH1_at	PA3092	fadH1		0,44	1,36	0,000635474	0,006543734	Fatty acid and phospholipid metabolism
PA1164_at	PA1164			0,44	1,36	0,001197554	0,009596793	Hypothetical, unclassified, unknown
PA1790_at	PA1790			0,44	1,36	0,000356448	0,004603038	Hypothetical, unclassified, unknown
PA0039_at	PA0039			0,45	1,36	0,007940076	0,034665208	Hypothetical, unclassified, unknown
PA2854_at	PA2854		erfK	0,45	1,36	0,001955467	0,013313974	Hypothetical, unclassified, unknown
PA3527_pyrC_at	PA3527	pyrC		0,45	1,36	0,002357057	0,015034801	Nucleotide biosynthesis and metabolism
PA1528_zipA_at	PA1528	zipA		0,45	1,36	0,000262579	0,003937974	Cell division
PA3823_tgt_at	PA3823	tgt		0,45	1,36	0,0004855	0,005543291	Transcription, RNA processing and degradation; Translation, post-translational modification, degradation
PA2685_at	PA2685	vgrG4	vgrG1c	0,45	1,36	0,00502567	0,025146476	Protein secretion/export apparatus
PA3034_at	PA3034			0,45	1,36	0,012863712	0,048093679	Transcriptional regulators
PA4925_at	PA4925			0,45	1,37	0,005655329	0,02736891	Hypothetical, unclassified, unknown
PA4827_at	PA4827		nat	0,45	1,37	0,000508629	0,005713323	Adaptation, Protection; Putative enzymes
PA3979_at	PA3979			0,45	1,37	0,002012401	0,013601476	Hypothetical, unclassified, unknown
PA5414_at	PA5414			0,45	1,37	0,001445529	0,010810296	Hypothetical, unclassified, unknown
PA4567_rpmA_at	PA4567	rpmA		0,45	1,37	0,00802098	0,034935371	Translation, post-translational modification, degradation
PA3807_ndk_at	PA3807	ndk		0,45	1,37	0,00657303	0,030293807	Nucleotide biosynthesis and metabolism
PA4275_nusG_at	PA4275	nusG		0,45	1,37	0,000560507	0,006122547	Transcription, RNA processing and degradation
PA5348_at	PA5348			0,45	1,37	0,00571534	0,027443667	DNA replication, recombination, modification and repair
PA1004_nadA_at	PA1004	nadA		0,45	1,37	0,002549522	0,0158208	Amino acid biosynthesis and metabolism; Biosynthesis of cofactors, prosthetic groups and carriers
PA2826_at	PA2826			0,45	1,37	0,012592147	0,047564353	Adaptation, Protection
PA0659_at	PA0659			0,45	1,37	0,005366155	0,026421288	Membrane proteins
PA0832_at	PA0832		ycbL	0,45	1,37	0,001392904	0,010544643	Hypothetical, unclassified, unknown
PA1804_hupB_at	PA1804	hupB		0,46	1,37	0,000253487	0,003914118	DNA replication, recombination, modification and repair
PA4970_at	PA4970		yqiB	0,46	1,37	0,01127696	0,044074064	Hypothetical, unclassified, unknown
PA0582_folB_at	PA0582	folB		0,46	1,37	0,000960077	0,008578856	Biosynthesis of cofactors, prosthetic groups and carriers
PA0500_bioB_at	PA0500	bioB		0,46	1,37	0,003970571	0,037911421	Biosynthesis of cofactors, prosthetic groups and carriers
PA4227_pchR_at	PA4227	pchR		0,46	1,37	0,001143592	0,009437831	Transcriptional regulators
PA1575_at	PA1575			0,46	1,38	0,001674643	0,011883111	Hypothetical, unclassified, unknown
PA5277_lysA_at	PA5277	lysA		0,46	1,38	0,002535775	0,015757015	Amino acid biosynthesis and metabolism
PA3683_at	PA3683			0,46	1,38	0,002336896	0,015025983	Hypothetical, unclassified, unknown
PA0367_at	PA0367			0,46	1,38	0,002534682	0,015757015	Transcriptional regulators
PA1507_at	PA1507			0,46	1,38	0,006078747	0,028634097	Membrane proteins; Transport of small molecules
PA0559_at	PA0559		yhiN	0,46	1,38	0,0053353	0,026362239	Hypothetical, unclassified, unknown
PA1116_at	PA1116			0,46	1,38	0,005343489	0,026362239	Hypothetical, unclassified, unknown
PA2843_at	PA2843			0,46	1,38	0,000478525	0,005497589	Putative enzymes
PA5363_at	PA5363			0,46	1,38	0,000126283	0,002748021	Hypothetical, unclassified, unknown
PA0341_lgt_at	PA0341	lgt	umpA	0,46	1,38	0,000408573	0,004993766	Translation, post-translational modification, degradation; Fatty acid and phospholipid metabolism
PA3940_at	PA3940			0,46	1,38	0,007847012	0,034377419	DNA replication, recombination, modification and repair
PA4637_i_at	PA4637			0,46	1,38	0,000785736	0,007568922	Hypothetical, unclassified, unknown
PA1016_at	PA1016			0,46	1,38	0,000355835	0,004603038	Hypothetical, unclassified, unknown
PA0537_at	PA0537			0,46	1,38	0,004475921	0,023212043	Hypothetical, unclassified, unknown
PA1942_at	PA1942			0,46	1,38	0,002064934	0,013905726	Hypothetical, unclassified, unknown
PA4782_at	PA4782			0,46	1,38	0,003509388	0,019502297	Hypothetical, unclassified, unknown
PA1645_at	PA1645			0,46	1,38	0,003037828	0,017688258	Hypothetical, unclassified, unknown
PA4485_at	PA4485			0,47	1,38	0,000605591	0,006361347	Hypothetical, unclassified, unknown
PA0541_at	PA0541			0,47	1,38	0,000242265	0,003851936	Hypothetical, unclassified, unknown
PA0001_dnaA_at	PA0001	dnaA		0,47	1,38	0,006321959	0,029429992	DNA replication, recombination, modification and repair

PA1041_at	PA1041			0,47	1,38	0,00654461	0,030208385	Membrane proteins; Transport of small molecules
PA2953_at	PA2953			0,47	1,38	0,001317042	0,010164489	Energy metabolism
PA2952_etfB_at	PA2952	etfB		0,47	1,38	0,000418988	0,005024095	Energy metabolism
PA4971_at	PA4971	aspP	yqiE	0,47	1,38	0,004605207	0,023663785	Energy metabolism
PA0066_at	PA0066		yrdA	0,47	1,38	0,000140507	0,002835185	Hypothetical, unclassified, unknown
PA2115_at	PA2115			0,47	1,38	0,007162936	0,032209995	Transcriptional regulators
PA4673_at	PA4673		ychF	0,47	1,39	0,000357526	0,004603038	Hypothetical, unclassified, unknown
PA0929_at	PA0929		pirR	0,47	1,39	0,01038779	0,041987585	Transport of small molecules; Two-component regulatory systems
PA2560_at	PA2560			0,47	1,39	0,001504847	0,011045496	Hypothetical, unclassified, unknown
PA4030_at	PA4030		yeeI	0,47	1,39	0,006909044	0,031450601	Hypothetical, unclassified, unknown
PA2658_at	PA2658			0,47	1,39	0,000454481	0,005289618	Hypothetical, unclassified, unknown
PA3603_dgkA_at	PA3603	dgkA		0,47	1,39	0,00218933	0,014360035	Fatty acid and phospholipid metabolism
PA1157_at	PA1157			0,48	1,39	0,010881137	0,043128165	Transcriptional regulators; Two-component regulatory systems
PA2989_at	PA2989			0,48	1,39	0,000483199	0,005537774	Hypothetical, unclassified, unknown
PA3453_at	PA3453		yceH	0,48	1,39	0,001063426	0,009092377	Hypothetical, unclassified, unknown
PA2968_fabD_at	PA2968	fabD		0,48	1,39	0,000313371	0,004358129	Fatty acid and phospholipid metabolism
PA4568_rplU_at	PA4568	rplU		0,48	1,39	0,009787524	0,040178595	Translation, post-translational modification, degradation
PA4336_at	PA4336			0,48	1,39	0,00158816	0,011445062	Hypothetical, unclassified, unknown
PA1398_at	PA1398			0,48	1,39	0,002193533	0,014369121	Hypothetical, unclassified, unknown
PA0128_at	PA0128		phnA	0,48	1,39	0,002163797	0,01424307	Hypothetical, unclassified, unknown
PA0019_def_at	PA0019	def		0,48	1,40	0,006675646	0,030577507	Translation, post-translational modification, degradation
PA2946_at	PA2946			0,48	1,40	0,000799468	0,007654611	Membrane proteins
PA5308_lrp_at	PA5308	lrp	dadR, dadAX regulator	0,48	1,40	0,000357235	0,004603038	Central intermediary metabolism; Transcriptional regulators
PA1047_at	PA1047			0,48	1,40	0,000410373	0,005004749	Putative enzymes
PA2764_at	PA2764			0,48	1,40	0,000228813	0,003723419	Hypothetical, unclassified, unknown
PA1791_at	PA1791			0,49	1,40	0,009102432	0,038380999	Hypothetical, unclassified, unknown
PA4445_at	PA4445		ybgI	0,49	1,40	0,002673979	0,016277223	Hypothetical, unclassified, unknown
PA4640_mqoB_at	PA4640	mqoB		0,49	1,40	0,002390088	0,015157256	Central intermediary metabolism; Energy metabolism
PA2966_acpP_at	PA2966	acpP		0,49	1,40	0,00236234	0,015034801	Fatty acid and phospholipid metabolism
PA5262_algZ_at	PA5262	fimS	fimS	0,49	1,41	0,001568216	0,011330767	Two-component regulatory systems; Motility & Attachment
PA0548_tktA_at	PA0548	tktA		0,49	1,41	0,003997966	0,021393165	Energy metabolism
PA0121_at	PA0121			0,50	1,41	0,004905577	0,024746408	Hypothetical, unclassified, unknown
PA1442_at	PA1442		fliL	0,50	1,41	0,00515575	0,025589673	Hypothetical, unclassified, unknown; Membrane proteins
PA3699_at	PA3699			0,50	1,41	6,58E-05	0,002047415	Transcriptional regulators
PA0120_at	PA0120			0,50	1,41	0,000911418	0,008277344	Transcriptional regulators
PA3685_at	PA3685			0,50	1,41	0,007140056	0,032180111	Hypothetical, unclassified, unknown
PA4679_at	PA4679			0,50	1,41	0,007727511	0,034085816	Hypothetical, unclassified, unknown
PA5304_dadA_at	PA5304	dadA		0,50	1,41	0,000974065	0,008606822	Energy metabolism; Amino acid biosynthesis and metabolism
PA3057_at	PA3057			0,50	1,41	0,001949824	0,013307561	Hypothetical, unclassified, unknown
PA5461_at	PA5461			0,50	1,41	0,000524345	0,005842549	Hypothetical, unclassified, unknown
PA1840_at	PA1840			0,50	1,41	0,004300243	0,022532622	Hypothetical, unclassified, unknown
PA2039_at	PA2039			0,50	1,41	0,002508299	0,015674047	Membrane proteins
PA5371_at	PA5371		yciA	0,50	1,41	0,007188431	0,032288269	Hypothetical, unclassified, unknown
PA1008_bcp_at	PA1008	bcp		0,50	1,42	0,000280325	0,004093732	Adaptation, Protection
PA1996_ppiC1_at	PA1996	ppiC1		0,50	1,42	0,000100067	0,002492719	Translation, post-translational modification, degradation; Chaperones & heat shock proteins
PA0902_at	PA0902			0,50	1,42	0,000704709	0,007058537	Hypothetical, unclassified, unknown
PA1640_at	PA1640			0,50	1,42	0,00186219	0,012852355	Hypothetical, unclassified, unknown
PA5107_blc_at	PA5107	blc		0,50	1,42	0,000786005	0,007568922	Membrane proteins
PA0290_at	PA0290			0,50	1,42	0,001177169	0,009549871	Hypothetical, unclassified, unknown
PA0318_at	PA0318		hpcE	0,50	1,42	0,002173572	0,014290354	Putative enzymes
PA1744_at	PA1744			0,51	1,42	0,000839319	0,00789387	Hypothetical, unclassified, unknown
PA0400_at	PA0400		metC; metB	0,51	1,42	0,005269659	0,026108336	Amino acid biosynthesis and metabolism
PA2270_at	PA2270			0,51	1,42	0,001272638	0,009974394	Transcriptional regulators
PA2749_endA_at	PA2749	endA		0,51	1,42	0,002873909	0,017110859	DNA replication, recombination, modification and repair
PA3887_nhaP_at	PA3887	nhaP		0,51	1,42	0,001363544	0,010393274	Membrane proteins; Transport of small molecules
PA4874_at	PA4874		psiF	0,51	1,42	4,30E-05	0,001728886	Hypothetical, unclassified, unknown
PA1476_ccmB_at	PA1476	ccmB	helB; cycW; cyt10	0,51	1,42	0,00978351	0,040178595	Membrane proteins; Transport of small molecules
PA1294_rnd_at	PA1294	rnd		0,51	1,43	0,003274128	0,018520014	Transcription, RNA processing and degradation
PA4401_at	PA4401			0,51	1,43	0,002859147	0,017059574	Putative enzymes
PA4268_rpsL_at	PA4268	rpsL	str	0,51	1,43	0,001351532	0,010331676	Translation, post-translational modification, degradation
PA1554_at	PA1554	ccoN1	ccoN; fixN; cytN	0,51	1,43	0,005503672	0,026860049	Energy metabolism; Central intermediary metabolism
PA5108_at	PA5108			0,51	1,43	0,0010608	0,009083921	Hypothetical, unclassified, unknown
PA5039_aroK_at	PA5039	aroK		0,52	1,43	6,34E-05	0,002047415	Amino acid biosynthesis and metabolism
PA1206_at	PA1206			0,52	1,43	0,003544387	0,019608974	Hypothetical, unclassified, unknown
PA2750_at	PA2750			0,52	1,43	0,000618218	0,006436196	Hypothetical, unclassified, unknown
PA2771_at	PA2771			0,52	1,43	0,000454703	0,005289618	Hypothetical, unclassified, unknown
PA4359_i_at	PA4359		feoA	0,52	1,43	0,006090279	0,028639796	Hypothetical, unclassified, unknown
PA4762_grpE_at	PA4762	grpE		0,52	1,43	0,012420663	0,047142448	DNA replication, recombination, modification and repair; Chaperones & heat shock proteins
PA4340_at	PA4340			0,52	1,43	0,000116189	0,002648935	Hypothetical, unclassified, unknown
PA0160_at	PA0160			0,52	1,43	0,000325426	0,004447754	Hypothetical, unclassified, unknown
PA3671_at	PA3671			0,52	1,44	0,009397744	0,039178996	Membrane proteins; Transport of small molecules
PA2586_gacA_at	PA2586	gacA		0,52	1,44	0,000324764	0,004447754	Transcriptional regulators
PA0379_at	PA0379		ygdD	0,52	1,44	0,000965127	0,008582517	Hypothetical, unclassified, unknown
PA3489_at	PA3489		rnfA	0,52	1,44	0,000747336	0,007352777	Membrane proteins
PA5533_at	PA5533			0,53	1,44	0,00113264	0,009394647	Hypothetical, unclassified, unknown
PA3029_moaB2_at	PA3029	moaB2		0,53	1,44	0,003755196	0,020509434	Biosynthesis of cofactors, prosthetic groups and carriers
PA0950_at	PA0950			0,53	1,44	0,001012495	0,00884777	Transport of small molecules; Adaptation, Protection
PA5334_rph_at	PA5334	rph		0,53	1,44	0,000209928	0,003541528	Transcription, RNA processing and degradation
PA5233_at	PA5233			0,53	1,44	5,22E-05	0,001916594	Hypothetical, unclassified, unknown
PA4377_at	PA4377			0,53	1,44	0,009684551	0,039984802	Hypothetical, unclassified, unknown
PA5569_rnpA_at	PA5569	rnpA		0,53	1,44	0,001987794	0,013500942	Translation, post-translational modification, degradation
PA4392_at	PA4392		ybaZ	0,53	1,45	0,00407624	0,021749094	Hypothetical, unclassified, unknown
PA2766_at	PA2766			0,53	1,45	0,002515925	0,015697042	Transcriptional regulators
PA4135_at	PA4135			0,53	1,45	0,002361072	0,015034801	Transcriptional regulators
PA4600_nfxB_at	PA4600	nfxB		0,54	1,45	0,005128085	0,025526937	Transcriptional regulators
PA5337_rpoZ_at	PA5337	rpoZ		0,54	1,45	0,00030435	0,004297291	Transcription, RNA processing and degradation
PA0653_at	PA0653		yhfA	0,54	1,46	0,001172239	0,009523799	Hypothetical, unclassified, unknown
PA3131_at	PA3131		edaB	0,54	1,46	0,011375539	0,044333104	Central intermediary metabolism; Carbon compound catabolism
PA4623_r_at	PA4623			0,54	1,46	0,000571573	0,006204226	Hypothetical, unclassified, unknown
PA3675_at	PA3675			0,55	1,46	0,001280643	0,009994781	Hypothetical, unclassified, unknown
PA0780_at	PA0780	pruR		0,55	1,46	0,005155661	0,025589673	Transcriptional regulators
PA3722_at	PA3722			0,55	1,46	0,000720059	0,007135016	Hypothetical, unclassified, unknown
PA0538_dsbB_at	PA0538	dsbB		0,55	1,46	0,004107251	0,021830592	Translation, post-translational modification, degradation; Chaperones & heat shock proteins
PA0889_aotQ_at	PA0889	aotQ		0,55	1,46	0,004884996	0,024665006	Membrane proteins; Transport of small molecules
PA4643_at	PA4643			0,55	1,46	6,43E-05	0,002047415	Hypothetical, unclassified, unknown
PA2281_at	PA2281			0,55	1,47	0,000151735	0,002923527	Transcriptional regulators
PA2937_at	PA2937			0,55	1,47	0,00274603	0,016562738	Hypothetical, unclassified, unknown
PA2830_htpX_at	PA2830	htpX		0,55	1,47	0,000713326	0,007093633	Adaptation, Protection
PA3831_pepA_at	PA3831	pepA	carP; xerB; phpA	0,55	1,47	0,004404333	0,022948023	Transcription, RNA processing and degradation; Secreted Factors (toxins, enzymes, alginate); Translation, post-translational modification, degradation
PA0947_at	PA0947			0,55	1,47	0,000608736	0,006361347	Hypothetical, unclassified, unknown
PA3637_pyrG_at	PA3637	pyrG		0,55	1,47	0,010452191	0,042119975	Nucleotide biosynthesis and metabolism
PA1315_at	PA1315			0,55	1,47	0,00064797	0,006604048	Transcriptional regulators
PA3262_at	PA3262			0,55	1,47	0,000426519	0,00503565	Translation, post-translational modification, degradation; Chaperones & heat shock proteins
PA1741_at	PA1741			0,56	1,47	0,000311692	0,004356622	Hypothetical, unclassified, unknown
PA5196_at	PA5196			0,56	1,47	0,000276603	0,004065746	Hypothetical, unclassified, unknown
PA4512_at	PA4512	lpxO1		0,56	1,47	0,007326046	0,032784054	Putative enzymes; Cell wall / LPS / capsule
PA2817_at	PA2817			0,56	1,47	0,003265101	0,0184878	Hypothetical, unclassified, unknown
PA0544_at	PA0544			0,56	1,47	0,001425334	0,010688077	Hypothetical, unclassified, unknown
PA4872_at	PA4872			0,56	1,47	0,001513779	0,011081745	Hypothetical, unclassified, unknown
PA2780_at	PA2780			0,56	1,47	0,001007314	0,008816382	Hypothetical, unclassified, unknown
PA1745_at	PA1745			0,57	1,48	0,004999125	0,025058849	Hypothetical, unclassified, unknown
PA2955_at	PA2955			0,57	1,48	0,001606637	0,011533285	Hypothetical, unclassified, unknown
PA4748_tpiA_at	PA4748	tpiA	tpi	0,57	1,48	0,009077747	0,038335174	Central intermediary metabolism; Energy metabolism
PA2985_at	PA2985			0,57	1,48	0,00164739	0,009509441	Membrane proteins
PA5351_at	PA5351	rubA1		0,57	1,48	0,0036204	0,019905129	Carbon compound catabolism
PA5214_gcvH1_at	PA5214	gcvH1		0,57	1,48	0,000295996	0,004243507	Central intermediary metabolism; Amino acid biosynthesis and metabolism
PA1296_at	PA1296			0,57	1,48	0,000805841	0,007683186	Putative enzymes
PA3664_at	PA3664		yffB	0,57	1,48	0,00038065	0,004778793	Hypothetical, unclassified, unknown
PA5183_at	PA5183			0,57	1,48	0,000413757	0,005024095	Membrane proteins
PA0195_pntA_at	PA0195	pntAA		0,57	1,49	0,000930497	0,008423043	Energy metabolism; Transport of small molecules
PA2857_at	PA2857			0,57	1,49	0,003163427	0,018134152	Transport of small molecules
PA3639_accA_at	PA3639	accA		0,57	1,49	0,000254509	0,003914118	Fatty acid and phospholipid metabolism
PA0867_at	PA0867	mliC		0,57	1,49	0,000339947	0,004534537	Hypothetical, unclassified, unknown; Adaptation, Protection
PA1440_at	PA1440			0,57	1,49	1,89E-05	0,001425199	Hypothetical, unclassified, unknown
PA2422_at	PA2422			0,57	1,49	0,000803158	0,007670777	Hypothetical, unclassified, unknown
PA5182_at	PA5182			0,58	1,49	2,52E-05	0,001564423	Membrane proteins
PA1593_at	PA1593			0,58	1,49	0,001732502	0,012123148	Hypothetical, unclassified, unknown
PA2660_at	PA2660			0,58	1,49	0,007225698	0,032384882	Hypothetical, unclassified, unknown
PA3308_hepA_at	PA3308	hepA		0,58	1,49	0,000145146	0,002856074	Transcription, RNA processing and degradation
PA3435_at	PA3435		mioC	0,58	1,49	0,000883796	0,008106094	Hypothetical, unclassified, unknown
PA3270_at	PA3270			0,58	1,49	5,05E-05	0,001879573	Hypothetical, unclassified, unknown
PA2720_at	PA2720			0,58	1,49	0,000246883	0,003859019	Hypothetical, unclassified, unknown
PA2668_at	PA2668			0,58	1,49	0,004211808	0,02217393	Hypothetical, unclassified, unknown
PA5330_at	PA5330			0,58	1,49	0,000334008	0,00449857	Hypothetical, unclassified, unknown
PA3069_at	PA3069			0,58	1,50	0,000149247	0,002905857	Hypothetical, unclassified, unknown

PA3440_at	PA3440			0,58	1,50	0,001184769	0,009591656	Hypothetical, unclassified, unknown
PA4731_panD_at	PA4731	panD		0,58	1,50	0,000124361	0,002720758	Amino acid biosynthesis and metabolism; Biosynthesis of cofactors, prosthetic groups and carriers
PA5201_at	PA5201		yhgF; tex	0,59	1,50	0,00659021	0,030347779	Hypothetical, unclassified, unknown
PA0319_at	PA0319			0,59	1,50	0,006258234	0,029231433	Hypothetical, unclassified, unknown
PA4730_panC_at	PA4730	panC		0,59	1,50	0,00146718	0,010894792	Biosynthesis of cofactors, prosthetic groups and carriers
PA2584_pgsA_at	PA2584	pgsA		0,59	1,50	0,000688056	0,006929262	Fatty acid and phospholipid metabolism
PA3822_at	PA3822		yajC	0,59	1,50	0,000642066	0,006569758	Hypothetical, unclassified, unknown
PA2951_etfA_at	PA2951	etfA		0,59	1,50	0,00109881	0,009238329	Energy metabolism
PA4228_pchD_at	PA4228	pchD		0,59	1,51	0,003399334	0,019150157	Secreted Factors (toxins, enzymes, alginate); Transport of small molecules
PA0778_at	PA0778	icp		0,59	1,51	0,002774032	0,016695341	Hypothetical, unclassified, unknown
PA3832_holC_at	PA3832	holC		0,59	1,51	0,010442556	0,042111733	DNA replication, recombination, modification and repair
PA0250_at	PA0250			0,59	1,51	0,001746583	0,012168667	Hypothetical, unclassified, unknown
PA1748_at	PA1748			0,59	1,51	0,000132867	0,002796363	Putative enzymes
PA1543_apt_at	PA1543	apt		0,59	1,51	0,000141582	0,002835467	Nucleotide biosynthesis and metabolism
PA4631_at	PA4631			0,60	1,51	0,007818471	0,034303955	Hypothetical, unclassified, unknown
PA4890_at	PA4890	desT	yijC; desT	0,60	1,51	0,01052516 3	0,042273049	Transcriptional regulators
PA3655_tsf_at	PA3655	tsf		0,60	1,51	0,001113269	0,00930351	Translation, post-translational modification, degradation
PA4734_at	PA4734			0,60	1,51	0,000138276	0,002801888	Hypothetical, unclassified, unknown
PA5347_at	PA5347			0,60	1,51	0,000148489	0,002901296	Hypothetical, unclassified, unknown
PA5273_at	PA5273			0,60	1,51	0,000225338	0,003688501	Hypothetical, unclassified, unknown
PA3322_at	PA3322			0,60	1,51	0,000181835	0,003286646	Hypothetical, unclassified, unknown
PA4614_mscL_at	PA4614	mscL		0,60	1,51	0,000114053	0,002637	Membrane proteins; Adaptation, Protection; Transport of small molecules
PA3747_at	PA3747			0,60	1,52	0,001375394	0,01044058	Membrane proteins
PA0580_gcp_at	PA0580	gcp	ygjD	0,60	1,52	0,002096977	0,014019427	Translation, post-translational modification, degradation
PA0915_at	PA0915		yehS	0,61	1,52	0,003873857	0,020992323	Hypothetical, unclassified, unknown
PA4291_at	PA4291			0,61	1,52	0,001541294	0,011209229	Hypothetical, unclassified, unknown
PA1026_at	PA1026			0,61	1,52	0,000607957	0,006361347	Hypothetical, unclassified, unknown
PA2983_at	PA2983			0,61	1,53	0,004487801	0,023251922	Transport of small molecules
PA3008_at	PA3008			0,61	1,53	0,00027696	0,004065746	Hypothetical, unclassified, unknown
PA3397_fpr_at	PA3397	fpr		0,62	1,53	0,000642887	0,006569758	Biosynthesis of cofactors, prosthetic groups and carriers; Energy metabolism
PA3958_at	PA3958			0,62	1,54	2,54E-05	0,001564423	Hypothetical, unclassified, unknown
PA4059_at	PA4059			0,62	1,54	0,001237819	0,009798373	Hypothetical, unclassified, unknown
PA4379_at	PA4379			0,62	1,54	0,000422843	0,005024095	Hypothetical, unclassified, unknown
PA3762_at	PA3762			0,62	1,54	0,000543866	0,005987927	Hypothetical, unclassified, unknown
PA0123_at	PA0123			0,62	1,54	0,002091879	0,014019427	Transcriptional regulators
PA1757_thrH_at	PA1757	thrH		0,62	1,54	0,010746208	0,042684832	Amino acid biosynthesis and metabolism
PA4431_at	PA4431			0,62	1,54	0,000118421	0,002682105	Putative enzymes
PA4390_at	PA4390			0,62	1,54	0,000349462	0,004573501	Hypothetical, unclassified, unknown
PA1397_at	PA1397			0,62	1,54	0,001550197	0,011244504	Transcriptional regulators; Two-component regulatory systems
PA4923_at	PA4923			0,62	1,54	0,000424635	0,005024095	Hypothetical, unclassified, unknown
PA4851_at	PA4851			0,63	1,54	0,01096013	0,04334837	Hypothetical, unclassified, unknown
PA3621_fdxA_at	PA3621	fdxA		0,63	1,54	0,003530958	0,019563014	Energy metabolism
PA0750_ung_at	PA0750	ung		0,63	1,54	0,000102679	0,002492719	DNA replication, recombination, modification and repair
PA5128_secB_at	PA5128	secB		0,63	1,55	0,000693869	0,006975143	Protein secretion/export apparatus
PA3717_at	PA3717			0,63	1,55	0,000335801	0,004502553	Translation, post-translational modification, degradation; Chaperones & heat shock proteins
PA5315_rpmG_at	PA5315	rpmG		0,63	1,55	0,002440931	0,015339442	Translation, post-translational modification, degradation
PA2828_at	PA2828			0,63	1,55	0,00997505	0,040743056	Putative enzymes
PA1571_at	PA1571			0,63	1,55	0,000416174	0,005024095	Hypothetical, unclassified, unknown
PA5331_pyrE_at	PA5331	pyrE		0,64	1,55	8,42E-05	0,002273321	Nucleotide biosynthesis and metabolism
PA0356_at	PA0356			0,64	1,55	9,64E-05	0,002452917	Hypothetical, unclassified, unknown
PA5519_at	PA5519			0,64	1,55	0,000597077	0,006347086	Hypothetical, unclassified, unknown
PA2860_at	PA2860			0,64	1,55	8,82E-05	0,002363292	Hypothetical, unclassified, unknown
PA3529_at	PA3529		tsaA	0,64	1,56	0,000159523	0,003041892	Adaptation, Protection; Putative enzymes
PA5472_at	PA5472			0,64	1,56	0,001264043	0,009921039	Hypothetical, unclassified, unknown
PA2759_at	PA2759			0,65	1,57	0,00115968	0,009492396	Hypothetical, unclassified, unknown
PA4952_at	PA4952		yjeQ	0,65	1,57	0,003623028	0,019905129	Hypothetical, unclassified, unknown
PA2380_at	PA2380			0,65	1,57	0,002889534	0,017130367	Hypothetical, unclassified, unknown
PA2855_at	PA2855			0,65	1,57	0,001210963	0,009668536	Hypothetical, unclassified, unknown
PA3224_at	PA3224			0,65	1,57	0,00042927	0,00505737	Hypothetical, unclassified, unknown
PA0342_thyA_at	PA0342	thyA		0,65	1,57	0,000194627	0,00341767	Biosynthesis of cofactors, prosthetic groups and carriers; Nucleotide biosynthesis and metabolism
PA4676_at	PA4676		yadF	0,65	1,57	0,0006311	0,00653353	Putative enzymes; Adaptation, Protection
PA1263_at	PA1263			0,65	1,57	0,006789187	0,031006748	Hypothetical, unclassified, unknown
PA1812_mltD_at	PA1812	mltD	dniR	0,65	1,57	0,008767974	0,037196855	Amino acid biosynthesis and metabolism; Cell wall / LPS / capsule
PA2801_at	PA2801			0,66	1,58	0,000950329	0,008532969	Hypothetical, unclassified, unknown
PA5426_purE_at	PA5426	purE		0,66	1,58	0,000674986	0,006809992	Nucleotide biosynthesis and metabolism
PA5482_at	PA5482			0,66	1,58	0,001637617	0,011680127	Membrane proteins
PA4515_at	PA4515		piuC	0,66	1,58	0,000827199	0,007806341	Hypothetical, unclassified, unknown
PA3787_at	PA3787			0,66	1,58	0,000392931	0,004877793	Hypothetical, unclassified, unknown
PA1962_at	PA1962	azoR2		0,66	1,58	0,000151083	0,002921111	Putative enzymes
PA3030_at	PA3030	mobA	mobA	0,66	1,58	8,69E-06	0,001004748	Biosynthesis of cofactors, prosthetic groups and carriers
PA4032_at	PA4032			0,66	1,58	0,000116464	0,002648935	Transcriptional regulators; Two-component regulatory systems
PA2453_at	PA2453			0,66	1,58	0,003107288	0,017924505	Hypothetical, unclassified, unknown
PA4907_at	PA4907		ydfG	0,66	1,58	1,67E-05	0,001389481	Putative enzymes
PA3539_at	PA3539		yaaA	0,66	1,58	0,000319651	0,00442183	Hypothetical, unclassified, unknown
PA0482_glcB_at	PA0482	glcB	aceB	0,66	1,58	0,000401154	0,004924819	Central intermediary metabolism; Carbon compound catabolism
PA4057_at	PA4057	nrdR	ybaD; nrdR	0,66	1,58	3,08E-05	0,0015998	Nucleotide biosynthesis and metabolism; Transcriptional regulators
PA0937_at	PA0937		yaiL	0,66	1,58	0,006015824	0,028472509	Hypothetical, unclassified, unknown
PA2798_at	PA2798			0,66	1,59	0,000971637	0,008599062	Transcriptional regulators; Two-component regulatory systems
PA5528_at	PA5528			0,67	1,59	3,95E-05	0,00168556	Membrane proteins
PA1293_at	PA1293			0,67	1,59	0,00099743	0,008791918	Hypothetical, unclassified, unknown
PA2116_at	PA2116			0,67	1,59	9,63E-05	0,002452917	Hypothetical, unclassified, unknown
PA2581_at	PA2581			0,67	1,59	0,005357885	0,026404001	Hypothetical, unclassified, unknown
PA4314_purU1_at	PA4314	purU1		0,67	1,59	0,001536248	0,011187194	Nucleotide biosynthesis and metabolism
PA5184_at	PA5184			0,67	1,59	0,001802656	0,012488064	Hypothetical, unclassified, unknown
PA2793_at	PA2793			0,67	1,59	0,003468765	0,019364364	Hypothetical, unclassified, unknown
PA1800_tig_at	PA1800	tig		0,67	1,59	0,002888551	0,017130367	Cell division; Chaperones & heat shock proteins
PA3861_rhlB_at	PA3861	rhl		0,68	1,60	0,008445054	0,036175267	Transcription, RNA processing and degradation
PA1010_dapA_at	PA1010	dapA		0,68	1,60	1,42E-05	0,001289662	Amino acid biosynthesis and metabolism
PA3794_at	PA3794			0,68	1,60	0,000781714	0,007568922	Membrane proteins
PA3815_at	PA3815	iscR		0,68	1,60	0,007120715	0,032157799	Adaptation, Protection
PA1965_at	PA1965			0,68	1,60	0,001171009	0,009523799	Hypothetical, unclassified, unknown
PA4846_aroQ1_at	PA4846	aroQ1	aroD1	0,68	1,60	0,000276642	0,004065746	Amino acid biosynthesis and metabolism
PA0595_ostA_at	PA0595	ostA	imp	0,68	1,61	0,002339602	0,015025983	Adaptation, Protection
PA2970_rpmF_at	PA2970	rpmF		0,68	1,61	0,00236536	0,015034801	Translation, post-translational modification, degradation
PA3574_at	PA3574	nalD		0,69	1,61	7,49E-05	0,002164649	Transcriptional regulators; Antibiotic resistance and susceptibility
PA4789_at	PA4789			0,69	1,62	0,008050185	0,034989291	Hypothetical, unclassified, unknown
PA0384_at	PA0384			0,69	1,62	0,000246523	0,003859019	Hypothetical, unclassified, unknown
PA0024_hemF_at	PA0024	hemF		0,69	1,62	0,010780977	0,042792304	Biosynthesis of cofactors, prosthetic groups and carriers
PA2876_pyrF_at	PA2876	pyrF		0,69	1,62	0,000200846	0,003471951	Nucleotide biosynthesis and metabolism
PA2623_icd_at	PA2623	icd	icdA	0,70	1,62	0,000109828	0,002576747	Carbon compound catabolism; Amino acid biosynthesis and metabolism; Energy metabolism
PA1353_at	PA1353			0,70	1,62	0,000636802	0,006543734	Hypothetical, unclassified, unknown
PA4627_at	PA4627		yjjT	0,70	1,62	0,007887386	0,034489443	Hypothetical, unclassified, unknown
PA0357_mutM_at	PA0357	mutY	fpg	0,70	1,62	0,000136436	0,002801888	DNA replication, recombination, modification and repair
PA2659_at	PA2659			0,70	1,62	0,002979342	0,017476077	Hypothetical, unclassified, unknown
PA3537_argF_at	PA3537	argF		0,70	1,63	0,002359932	0,015034801	Amino acid biosynthesis and metabolism
PA0284_at	PA0284			0,70	1,63	0,007820239	0,034303955	Hypothetical, unclassified, unknown
PA4998_at	PA4998			0,70	1,63	6,30E-05	0,002047415	Hypothetical, unclassified, unknown
PA4154_at	PA4154		ygiM	0,70	1,63	6,47E-05	0,002047415	Hypothetical, unclassified, unknown
PA0316_serA_at	PA0316	serA		0,71	1,63	3,88E-05	0,00168556	Amino acid biosynthesis and metabolism
PA4671_at	PA4671		rplY	0,71	1,64	5,66E-05	0,001962587	Adaptation, Protection; Translation, post-translational modification, degradation
PA3033_at	PA3033			0,71	1,64	4,25E-05	0,001728886	Hypothetical, unclassified, unknown
PA4729_panB_at	PA4729	panB		0,71	1,64	0,000102133	0,002492719	Biosynthesis of cofactors, prosthetic groups and carriers
PA0155_pcaR_at	PA0155	pcaR		0,71	1,64	0,000342221	0,004543023	Carbon compound catabolism; Transcriptional regulators
PA4639_at	PA4639			0,71	1,64	1,26E-05	0,001186712	Hypothetical, unclassified, unknown
PA5490_cc4_at	PA5490	cc4		0,72	1,64	0,000110054	0,002576747	Energy metabolism
PA5046_at	PA5046			0,72	1,64	1,76E-05	0,001392035	Central intermediary metabolism
PA5560_atpB_at	PA5560	atpB	papD; uncB	0,72	1,64	0,000313362	0,004358129	Energy metabolism
PA2387_at	PA2387	fpvI		0,72	1,64	2,07E-05	0,001438517	Transcriptional regulators; Transport of small molecules
PA5316_rpmB_at	PA5316	rpmB		0,72	1,64	0,00184765	0,012777807	Translation, post-translational modification, degradation
PA2813_at	PA2813		yliJ	0,72	1,65	0,000287492	0,004165255	Central intermediary metabolism
PA2582_at	PA2582			0,72	1,65	3,00E-05	0,001587603	Hypothetical, unclassified, unknown
PA2802_at	PA2802			0,72	1,65	0,001040955	0,009011326	Transcriptional regulators
PA1799_at	PA1799	parR		0,72	1,65	0,001057266	0,009069778	Transcriptional regulators; Two-component regulatory systems
PA0350_folA_at	PA0350	folA	tmrA	0,72	1,65	0,000168249	0,003154102	Biosynthesis of cofactors, prosthetic groups and carriers
PA5285_at	PA5285			0,72	1,65	0,000582032	0,006262772	Hypothetical, unclassified, unknown
PA4764_fur_at	PA4764	fur		0,73	1,66	0,000256058	0,003924905	Transcriptional regulators
PA2246_bkdR_at	PA2246	bkdR		0,73	1,66	1,93E-05	0,001428972	Transcriptional regulators
PA5561_atpI_at	PA5561	atpI	uncI	0,73	1,66	4,00E-05	0,001695038	Membrane proteins; Energy metabolism
PA1970_at	PA1970			0,73	1,66	9,38E-05	0,002433075	Hypothetical, unclassified, unknown
PA1574_at	PA1574		yaiE	0,73	1,66	8,15E-05	0,002248955	Hypothetical, unclassified, unknown
PA4109_ampR_at	PA4109	ampR		0,73	1,66	0,000854197	0,007941725	Transcriptional regulators; Antibiotic resistance and susceptibility
PA3742_rplS_at	PA3742	rplS		0,73	1,66	6,10E-05	0,002047415	Translation, post-translational modification, degradation
PA3952_at	PA3952			0,74	1,67	4,27E-05	0,001728886	Hypothetical, unclassified, unknown
PA3385_at	PA3385	amrZ	algZ	0,74	1,67	3,67E-05	0,001684034	Transcriptional regulators
PA0390_metX_at	PA0390	metX	met2	0,74	1,67	0,001144968	0,009437831	Amino acid biosynthesis and metabolism

PA1788_at	PA1788			0,74	1,67	0,00698519	0,031719167	Hypothetical, unclassified, unknown
PA2464_at	PA2464			0,75	1,68	0,000200431	0,003471951	Hypothetical, unclassified, unknown
PA5256_dsbH_at	PA5256	dsbH	dsbB	0,75	1,68	8,44E-05	0,002273321	Translation, post-translational modification, degradation; Chaperones & heat shock proteins
PA3743_trmD_at	PA3743	trmD		0,75	1,68	8,21E-05	0,002254587	Transcription, RNA processing and degradation
PA0381_thiG_at	PA0381	thiG		0,75	1,68	0,000605704	0,006361347	Biosynthesis of cofactors, prosthetic groups and carriers
PA3246_rluA_at	PA3246	rluA	yabO	0,75	1,68	0,001723225	0,01210402	Transcription, RNA processing and degradation
PA3770_guaB_at	PA3770	guaB		0,75	1,69	1,71E-05	0,001389481	Nucleotide biosynthesis and metabolism
PA4451_at	PA4451		yrbA	0,75	1,69	0,000815206	0,007745849	Hypothetical, unclassified, unknown
PA1363_at	PA1363			0,76	1,69	0,000599373	0,006359312	Transcriptional regulators
PA5268_corA_at	PA5268	corA		0,76	1,69	4,29E-05	0,001728886	Membrane proteins; Transport of small molecules
PA1675_at	PA1675			0,76	1,70	0,000606439	0,006361347	Hypothetical, unclassified, unknown
PA4058_at	PA4058			0,76	1,70	1,02E-05	0,001098882	Hypothetical, unclassified, unknown
PA0775_at	PA0775		yecO	0,76	1,70	0,008798946	0,037271259	Hypothetical, unclassified, unknown
PA5143_hisB_at	PA5143	hisB		0,77	1,70	0,000548328	0,006009494	Amino acid biosynthesis and metabolism
PA0888_aotJ_at	PA0888	aotJ		0,77	1,70	0,000363081	0,004662003	Transport of small molecules
PA3050_pyrD_at	PA3050	pyrD		0,77	1,70	0,00092643	0,008399939	Nucleotide biosynthesis and metabolism
PA1159_at	PA1159			0,77	1,70	0,007560436	0,033670029	Transcriptional regulators; Adaptation, Protection
PA1831_at	PA1831			0,77	1,71	2,60E-05	0,001564423	Hypothetical, unclassified, unknown
PA3001_at	PA3001			0,77	1,71	0,000363786	0,004662003	Putative enzymes
PA0667_at	PA0667		yebA	0,78	1,71	0,005020815	0,025144858	Hypothetical, unclassified, unknown
PA4574_at	PA4574		yqhA	0,78	1,71	0,002432172	0,015301723	Hypothetical, unclassified, unknown
PA3645_fabZ_at	PA3645	fabZ	sefA	0,78	1,72	0,000187645	0,003316062	Cell wall / LPS / capsule; Fatty acid and phospholipid metabolism
PA2492_mexT_at	PA2492	mexT		0,78	1,72	0,000173246	0,003215193	Transcriptional regulators
PA4473_at	PA4473		yjgA	0,78	1,72	0,000296717	0,004243507	Hypothetical, unclassified, unknown
PA5274_rnk_at	PA5274	rnk		0,79	1,73	2,16E-06	0,000578762	Transcriptional regulators
PA2622_cspD_at	PA2622	cspD		0,79	1,73	0,001497036	0,011002716	Transcriptional regulators; Adaptation, Protection
PA3055_at	PA3055			0,79	1,73	0,000302402	0,004280686	Hypothetical, unclassified, unknown
PA5570_rpmH_at	PA5570	rpmH		0,79	1,73	0,000817687	0,007745864	Central intermediary metabolism; Translation, post-translational modification, degradation
PA2702_at	PA2702	tse2		0,79	1,73	0,001141917	0,009437831	Secreted Factors (toxins, enzymes, alginate)
PA4666_hemA_at	PA4666	hemA	glutR; hem1	0,79	1,73	0,002878083	0,017117346	Biosynthesis of cofactors, prosthetic groups and carriers; Amino acid biosynthesis and metabolism; Translation, post-translational modification, degradation
PA0023_qor_at	PA0023	qor		0,80	1,74	4,41E-05	0,001749766	Energy metabolism
PA5463_at	PA5463			0,80	1,75	9,32E-05	0,002433075	Hypothetical, unclassified, unknown
PA4438_at	PA4438		yhcM	0,81	1,75	0,001084479	0,009206318	Hypothetical, unclassified, unknown
PA4035_at	PA4035			0,82	1,76	0,002825617	0,016932342	Hypothetical, unclassified, unknown
PA4850_prmA_at	PA4850	prmA		0,82	1,76	9,23E-05	0,002426102	Translation, post-translational modification, degradation
PA5129_grx_at	PA5129	grx		0,82	1,76	0,000136646	0,002801888	Energy metabolism; Nucleotide biosynthesis and metabolism
PA2629_purB_at	PA2629	purB		0,82	1,77	6,17E-05	0,002047415	Amino acid biosynthesis and metabolism; Nucleotide biosynthesis and metabolism
PA0563_at	PA0563			0,82	1,77	0,001937609	0,01324112	Hypothetical, unclassified, unknown
PA3480_at	PA3480		dcd	0,82	1,77	0,000244099	0,003859019	Nucleotide biosynthesis and metabolism
PA1676_at	PA1676			0,83	1,77	5,08E-06	0,000825616	Membrane proteins
PA5286_at	PA5286		yjbQ	0,83	1,78	0,002652666	0,016228935	Hypothetical, unclassified, unknown
PA0082_at	PA0082	tssA1		0,83	1,78	0,000585691	0,006286266	Protein secretion/export apparatus
PA0020_at	PA0020	tsaP		0,83	1,78	4,08E-05	0,001714063	Hypothetical, unclassified, unknown
PA5289_at	PA5289			0,84	1,79	2,51E-05	0,001564423	Hypothetical, unclassified, unknown
PA4389_at	PA4389			0,84	1,79	3,33E-06	0,000693523	Putative enzymes; Amino acid biosynthesis and metabolism
PA1591_at	PA1591			0,84	1,79	0,002920354	0,017276165	Membrane proteins
PA4765_omlA_at	PA4765	omlA	oprX	0,84	1,79	5,88E-06	0,00084537	Membrane proteins; Transport of small molecules
PA1959_bacA_at	PA1959	bacA		0,85	1,80	0,00013421	0,002796363	Cell wall / LPS / capsule; Adaptation, Protection; Antibiotic resistance and susceptibility
PA1774_at	PA1774	crfX	cfrX	0,85	1,80	0,000401157	0,004924819	Hypothetical, unclassified, unknown
PA5479_gltP_at	PA5479	gltP		0,85	1,80	0,000158774	0,003041892	Membrane proteins; Transport of small molecules
PA4276_secE_at	PA4276	secE	prlG	0,85	1,81	0,000119572	0,00268468	Protein secretion/export apparatus
PA4607_at	PA4607			0,85	1,81	8,13E-06	0,000980823	Hypothetical, unclassified, unknown
PA0945_purM_at	PA0945	purM		0,86	1,81	0,000636039	0,006543734	Nucleotide biosynthesis and metabolism
PA3313_at	PA3313			0,86	1,81	2,82E-05	0,001580082	Transport of small molecules
PA4325_at	PA4325			0,86	1,82	0,001881002	0,012949977	Hypothetical, unclassified, unknown
PA0062_at	PA0062			0,87	1,82	0,000283947	0,004124667	Hypothetical, unclassified, unknown
PA4043_ispA_at	PA4043	ispA		0,87	1,83	0,002108915	0,014082275	Biosynthesis of cofactors, prosthetic groups and carriers
PA2957_at	PA2957			0,87	1,83	7,33E-05	0,002164252	Transcriptional regulators
PA2667_at	PA2667	mvaU		0,87	1,83	2,96E-05	0,001580082	Transcriptional regulators
PA3139_at	PA3139		tyrB; aspC	0,87	1,83	4,12E-05	0,001718084	Amino acid biosynthesis and metabolism; Putative enzymes
PA4852_at	PA4852		yhdG	0,87	1,83	0,001085048	0,009206318	Hypothetical, unclassified, unknown
PA1475_ccmA_at	PA1475	ccmA	helA; cycV	0,88	1,85	0,000848143	0,007923143	Transport of small molecules
PA4645_at	PA4645		hpt; hprT	0,88	1,85	2,54E-05	0,001564423	Nucleotide biosynthesis and metabolism
PA5217_at	PA5217			0,90	1,87	0,000374019	0,004706191	Transport of small molecules
PA4642_at	PA4642			0,90	1,87	7,70E-07	0,000328758	Hypothetical, unclassified, unknown
PA4768_smpB_at	PA4768	smpB		0,91	1,88	0,000422921	0,005024095	Translation, post-translational modification, degradation
PA3955_at	PA3955			0,91	1,88	0,00026053	0,003935439	Membrane proteins
PA3745_rpsP_at	PA3745	rpsP		0,92	1,89	5,36E-05	0,001949826	DNA replication, recombination, modification and repair; Translation, post-translational modification, degradation
PA4029_at	PA4029		dedA	0,92	1,90	0,000443324	0,005178956	Hypothetical, unclassified, unknown
PA1106_at	PA1106			0,93	1,90	2,44E-06	0,000614944	Hypothetical, unclassified, unknown
PA3161_himD_at	PA3161	himD		0,93	1,91	5,41E-05	0,001949826	Translation, post-translational modification, degradation; Transcription, RNA processing and degradation; DNA replication, recombination, modification and repair
PA3824_queA_at	PA3824	queA		0,94	1,91	0,001626799	0,011617896	Translation, post-translational modification, degradation
PA2856_tesA_at	PA2856	tesA	apeA	0,94	1,92	0,000171654	0,003196331	Fatty acid and phospholipid metabolism
PA2569_at	PA2569			0,94	1,92	8,25E-05	0,002254587	Hypothetical, unclassified, unknown
PA3049_rmf_at	PA3049	rmf		0,94	1,92	7,19E-05	0,002135025	Translation, post-translational modification, degradation
PA4481_mreB_at	PA4481	mreB	rodY; envB	0,94	1,92	0,000873718	0,008053586	Cell wall / LPS / capsule; Cell division
PA4545_comL_at	PA4545	comL	ypiY	0,94	1,92	9,96E-05	0,002492719	Cell wall / LPS / capsule
PA2851_efp_at	PA2851	efp		0,94	1,92	0,000127815	0,002770426	Translation, post-translational modification, degradation
PA3818_at	PA3818		suhB	0,95	1,93	0,009312464	0,038947731	Translation, post-translational modification, degradation; Adaptation, Protection
PA1596_htpG_at	PA1596	htpG		0,95	1,93	0,001199147	0,009596793	Chaperones & heat shock proteins
PA0094_at	PA0094			0,96	1,94	0,000169862	0,003173608	Hypothetical, unclassified, unknown
PA1189_at	PA1189			0,97	1,96	0,00026009	0,003935439	Hypothetical, unclassified, unknown
PA3244_minD_at	PA3244	minD		0,97	1,96	0,000183939	0,003292502	Cell division
PA3744_rimM_at	PA3744	rimM		0,98	1,98	0,000888079	0,008118536	Transcription, RNA processing and degradation
PA0055_at	PA0055			0,98	1,98	2,07E-05	0,001438517	Hypothetical, unclassified, unknown
PA4354_at	PA4354			0,99	1,98	0,001043047	0,009015372	
PA0422_at	PA0422			1,00	2,00	5,94E-06	0,00084537	Hypothetical, unclassified, unknown
PA3967_at	PA3967			1,00	2,00	6,99E-07	0,000323296	Hypothetical, unclassified, unknown
PA4974_at	PA4974		opmH	1,01	2,01	0,00012454	0,002720758	Protein secretion/export apparatus
PA1852_at	PA1852			1,01	2,01	3,37E-06	0,000693523	Hypothetical, unclassified, unknown
PA5300_cycB_at	PA5300	cycB		1,02	2,02	0,000364928	0,004665861	Energy metabolism
PA2800_at	PA2800	vacJ	vacJ	1,02	2,03	9,46E-05	0,002441172	Antibiotic resistance and susceptibility
PA3975_thiD_at	PA3975	thiD		1,02	2,03	0,000102377	0,002492719	Biosynthesis of cofactors, prosthetic groups and carriers
PA5276_lppL_i_at	PA5276	lppL		1,02	2,03	9,34E-07	0,000370132	Cell wall / LPS / capsule
PA3056_at	PA3056			1,02	2,03	6,42E-05	0,002047415	Hypothetical, unclassified, unknown
PA5028_at	PA5028			1,03	2,04	0,000143568	0,002839872	Hypothetical, unclassified, unknown
PA0706_cat_at	PA0706	cat		1,03	2,04	0,000130463	0,002783815	Antibiotic resistance and susceptibility
PA5298_at	PA5298		xpt	1,04	2,06	0,000872392	0,008053586	Nucleotide biosynthesis and metabolism
PA4672_at	PA4672		pth	1,04	2,06	1,86E-05	0,001425199	Translation, post-translational modification, degradation
PA4853_fis_at	PA4853	fis		1,04	2,06	7,66E-05	0,002190448	Transcriptional regulators; DNA replication, recombination, modification and repair; Transcription, RNA processing and degradation
PA1796_folD_at	PA1796	folD		1,05	2,07	3,23E-05	0,001636633	Translation, post-translational modification, degradation; Nucleotide biosynthesis and metabolism; Biosynthesis of cofactors, prosthetic groups and carriers
PA2755_eco_at	PA2755	eco		1,07	2,10	1,92E-06	0,000578762	Translation, post-translational modification, degradation
PA1354_at	PA1354			1,08	2,11	0,001056103	0,009069778	Hypothetical, unclassified, unknown
PA1750_at	PA1750			1,09	2,13	8,99E-05	0,002378021	Amino acid biosynthesis and metabolism
PA3612_at	PA3612		ypeB	1,09	2,14	1,56E-05	0,001332158	Hypothetical, unclassified, unknown
PA4784_at	PA4784			1,10	2,14	0,000181389	0,003286646	Transcriptional regulators
PA2936_at	PA2936			1,10	2,14	0,000222817	0,003659944	Membrane proteins
PA1064_at	PA1064			1,11	2,16	1,32E-05	0,001221855	Hypothetical, unclassified, unknown
PA1006_at	PA1006		yrkI	1,11	2,16	8,90E-05	0,002374416	Hypothetical, unclassified, unknown
PA4881_at	PA4881			1,12	2,17	0,000204124	0,003475817	Hypothetical, unclassified, unknown
PA0421_at	PA0421			1,12	2,18	1,89E-06	0,000578762	Putative enzymes
PA4317_at	PA4317			1,12	2,18	0,000341486	0,004543023	Membrane proteins
PA0433_at	PA0433			1,13	2,18	6,58E-05	0,002047415	Hypothetical, unclassified, unknown
PA4693_pssA_at	PA4693	pssA		1,13	2,19	0,00244854	0,003859019	Fatty acid and phospholipid metabolism
PA0362_fdx1_at	PA0362	fdx1		1,14	2,21	5,90E-06	0,00084537	Energy metabolism
PA2666_at	PA2666		ptpS	1,15	2,21	0,000472719	0,005459771	Biosynthesis of cofactors, prosthetic groups and carriers
PA1198_at	PA1198			1,16	2,23	1,21E-05	0,001169601	Hypothetical, unclassified, unknown
PA1035_at	PA1035			1,16	2,24	3,86E-06	0,000737709	Hypothetical, unclassified, unknown
PA5192_pckA_at	PA5192	pckA		1,16	2,24	2,66E-05	0,001564423	Carbon compound catabolism; Energy metabolism
PA3611_at	PA3611			1,17	2,25	8,50E-06	0,001002984	Hypothetical, unclassified, unknown
PA0385_at	PA0385			1,17	2,25	0,000133561	0,002796363	Hypothetical, unclassified, unknown
PA1192_at	PA1192		ydaO	1,17	2,26	0,002588503	0,015977312	Hypothetical, unclassified, unknown
PA3295_at	PA3295			1,18	2,26	0,000216905	0,003617049	Putative enzymes
PA3245_minE_at	PA3245	minE		1,18	2,27	6,79E-05	0,002058865	Cell division
PA1009_at	PA1009			1,19	2,28	2,93E-05	0,001580082	Hypothetical, unclassified, unknown
PA0167_at	PA0167			1,19	2,29	5,08E-06	0,000825616	Transcriptional regulators
PA2971_at	PA2971		yceD	1,20	2,29	4,67E-05	0,001791314	Hypothetical, unclassified, unknown
PA5049_rpmE_at	PA5049	rpmE		1,20	2,30	0,000347686	0,004571822	Translation, post-translational modification, degradation
PA4031_ppa_at	PA4031	ppa	ipyR	1,21	2,31	6,49E-06	0,000887606	Central intermediary metabolism
PA0005_at	PA0005	lptA	plsC	1,22	2,33	4,32E-06	0,000799407	Fatty acid and phospholipid metabolism
PA3223_acpD_at	PA3223	azoR3		1,22	2,33	0,000249209	0,003873553	Fatty acid and phospholipid metabolism
PA2491_at	PA2491	mexS		1,23	2,34	1,15E-06	0,000426744	Putative enzymes; Transcriptional regulators
PA5491_at	PA5491			1,23	2,34	5,57E-05	0,001962587	Energy metabolism
PA5462_at	PA5462			1,24	2,36	6,64E-05	0,002048484	Hypothetical, unclassified, unknown
PA3177_at	PA3177			1,25	2,37	1,99E-06	0,000578762	Hypothetical, unclassified, unknown

PA3686_adk_at	PA3686	adk		1,25	2,37	5,54E-05	0,001962587	Nucleotide biosynthesis and metabolism
PA0578_at	PA0578			1,25	2,38	0,000103941	0,002492719	Hypothetical, unclassified, unknown
PA0380_i_at	PA0380			1,25	2,38	0,000104195	0,002492719	Hypothetical, unclassified, unknown
PA4632_at	PA4632			1,26	2,39	0,000262109	0,003937974	Hypothetical, unclassified, unknown
PA4432_rpsI_at	PA4432	rpsI		1,27	2,40	0,000424616	0,005024095	Translation, post-translational modification, degradation
PA4636_at	PA4636			1,27	2,41	0,001483625	0,010964942	Hypothetical, unclassified, unknown
PA1504_at	PA1504			1,28	2,43	1,22E-05	0,001169601	Transcriptional regulators
PA4670_prs_at	PA4670	prs	prsA	1,28	2,43	0,000175147	0,003218181	Carbon compound catabolism; Nucleotide biosynthesis and metabolism
PA4441_at	PA4441			1,31	2,49	1,47E-07	0,000101985	Hypothetical, unclassified, unknown
PA3243_minC_at	PA3243	minC		1,32	2,50	3,14E-05	0,001614521	Cell division
PA5130_at	PA5130		yibN	1,34	2,52	1,90E-05	0,001425199	Hypothetical, unclassified, unknown
PA0363_coaD_at	PA0363	coaD	kdtB	1,34	2,54	2,19E-06	0,000578762	Central intermediary metabolism
PA3684_i_at	PA3684			1,37	2,58	0,00114505	0,009437831	Hypothetical, unclassified, unknown
PA3472_at	PA3472			1,39	2,61	2,62E-05	0,001564423	Hypothetical, unclassified, unknown
PA4602_glyA3_at	PA4602	glyA3		1,42	2,67	9,50E-05	0,002441172	Amino acid biosynthesis and metabolism
PA1674_folE2_at	PA1674	folE2		1,47	2,76	0,002371788	0,015058411	Biosynthesis of cofactors, prosthetic groups and carriers
PA3229_at	PA3229			1,51	2,86	5,44E-06	0,000837751	Hypothetical, unclassified, unknown
PA2619_infA_at	PA2619	infA		1,54	2,90	0,000540126	0,005958569	Translation, post-translational modification, degradation
PA0579_rpsU_at	PA0579	rpsU		1,60	3,03	3,80E-05	0,00168556	Translation, post-translational modification, degradation
PA4723_dksA_at	PA4723	dksA		1,60	3,04	4,32E-07	0,000239941	Transcriptional regulators; Adaptation, Protection; DNA replication, recombination, modification and repair
PA5429_aspA_at	PA5429	aspA		1,66	3,17	1,56E-05	0,001332158	Amino acid biosynthesis and metabolism
PA4042_xseB_at	PA4042	xseB		1,70	3,25	5,50E-05	0,001962587	DNA replication, recombination, modification and repair
PA4433_rplM_at	PA4433	rplM		1,72	3,30	2,32E-05	0,001534556	Translation, post-translational modification, degradation
PA4705_at	PA4705	phuW	phuW	1,74	3,33	8,93E-09	1,24E-05	Hypothetical, unclassified, unknown
PA4569_ispB_at	PA4569	ispB	cel	1,82	3,54	1,87E-05	0,001425199	Energy metabolism; Biosynthesis of cofactors, prosthetic groups and carriers
PA4563_rpsT_at	PA4563	rpsT		2,03	4,09	4,54E-06	0,000812775	Central intermediary metabolism; Translation, post-translational modification, degradation
PA4711_at	PA4711			2,48	5,58	4,83E-06	0,000825616	Hypothetical, unclassified, unknown
PA4706_at	PA4706	phuV	phuV	2,63	6,19	5,32E-10	1,47E-06	Transport of small molecules
PA4707_at	PA4707	phuU	phuU	2,79	6,91	4,62E-08	3,67E-05	Membrane proteins; Transport of small molecules
PA4709_at	PA4709	phuS	phuS	3,96	15,60	1,23E-08	1,36E-05	Putative enzymes; Transport of small molecules
PA4708_at	PA4708	phuT	phuT	4,11	17,22	4,35E-09	8,05E-06	Transport of small molecules
PA4710_at	PA4710	phuR		7,26	152,95	1,81E-10	1,00E-06	Transport of small molecules

Supplementary Table 2: Genes from Supplementary Table 1 with consideration of fold change criterion (FC > -2 or FC < -2)

Locus ID	Locus Tag	Name	Synonyms	log Fold Change	Fold Change	P.Value	adj.P.Val	PseudoCAP Function Class
PA3877_narK1_at	PA3877	narK1		-3,64	-12,44	0,000389008	0,004850801	Membrane proteins; Transport of small molecules
PA3876_narK2_at	PA3876	narK2		-2,65	-6,28	6,03E-07	0,000303998	Membrane proteins; Transport of small molecules
PA3915_moaB1_at	PA3915	moaB1		-2,53	-5,76	2,98E-06	0,000662084	Biosynthesis of cofactors, prosthetic groups and carriers
PA1541_at	PA1541			-2,36	-5,14	1,80E-08	1,66E-05	Membrane proteins; Transport of small molecules
PA5171_arcA_at	PA5171	arcA		-2,05	-4,14	0,000273191	0,004042495	Amino acid biosynthesis and metabolism
PA1566_at	PA1566	pauA3		-1,81	-3,51	0,000579726	0,006258563	Carbon compound catabolism
PA0492_at	PA0492		ycsF	-1,73	-3,32	2,81E-06	0,000648569	Hypothetical, unclassified, unknown
PA1746_at	PA1746			-1,71	-3,28	0,011733588	0,045341003	Hypothetical, unclassified, unknown
PA5374_betI_at	PA5374	betI		-1,69	-3,23	3,39E-07	0,000209268	Transcriptional regulators
PA3839_at	PA3839		yfbS	-1,68	-3,21	0,002077826	0,013975584	Membrane proteins; Transport of small molecules
PA4611_at	PA4611			-1,67	-3,19	0,012105251	0,046421589	Hypothetical, unclassified, unknown
PA5231_at	PA5231		yhiH	-1,57	-2,96	0,002744324	0,016562738	Membrane proteins; Transport of small molecules
PA1540_at	PA1540			-1,52	-2,87	1,98E-05	0,001438517	Membrane proteins
PA0297_at	PA0297	spuA	ycjL	-1,41	-2,66	3,65E-05	0,001684034	Amino acid biosynthesis and metabolism; Carbon compound catabolism
PA1565_at	PA1565	pauB2		-1,40	-2,65	0,002238226	0,014509245	Putative enzymes; Carbon compound catabolism
PA1602_at	PA1602			-1,37	-2,58	3,87E-05	0,00168556	Carbon compound catabolism
PA0132_at	PA0132	bauA	oapT	-1,35	-2,54	4,49E-05	0,001753067	Amino acid biosynthesis and metabolism; Carbon compound catabolism
PA2555_at	PA2555			-1,31	-2,48	0,000385639	0,004819614	Putative enzymes
PA2554_at	PA2554			-1,28	-2,43	0,000373277	0,004706191	Putative enzymes
PA4889_at	PA4889			-1,24	-2,37	5,40E-05	0,001949826	Putative enzymes
PA3584_glpD_at	PA3584	glpD		-1,23	-2,35	0,003644104	0,020001121	Central intermediary metabolism; Energy metabolism
PA2260_at	PA2260		kguE	-1,23	-2,35	6,38E-05	0,002047415	Hypothetical, unclassified, unknown; Carbon compound catabolism
PA5373_betB_at	PA5373	betB		-1,22	-2,33	3,59E-05	0,001684034	Amino acid biosynthesis and metabolism; Adaptation, Protection
PA5172_arcB_at	PA5172	arcB		-1,22	-2,32	0,000121942	0,00270663	Amino acid biosynthesis and metabolism
PA1555_at	PA1555	ccoP2	ccoP; fixP	-1,20	-2,30	0,012769421	0,04804105	Energy metabolism; Central intermediary metabolism
PA4888_at	PA4888	desB	desB	-1,14	-2,21	0,000130142	0,002783815	Fatty acid and phospholipid metabolism
PA1707_pcrH_at	PA1707	pcrH		-1,13	-2,19	0,00012417	0,002720758	Secreted Factors (toxins, enzymes, alginate); Protein secretion/export apparatus
PA1601_at	PA1601			-1,13	-2,19	2,08E-05	0,001438517	Putative enzymes
PA2482_at	PA2482			-1,12	-2,18	0,00024003	0,003827368	Energy metabolism
PA5372_betA_at	PA5372	betA		-1,11	-2,16	1,42E-06	0,000490944	Amino acid biosynthesis and metabolism; Adaptation, Protection
PA2481_at	PA2481			-1,10	-2,14	0,001408127	0,010616439	Hypothetical, unclassified, unknown
PA3582_glpK_at	PA3582	glpK		-1,08	-2,11	0,002157867	0,01422091	Central intermediary metabolism
PA2553_at	PA2553			-1,07	-2,10	0,000708054	0,007079261	Putative enzymes
PA2790_at	PA2790			-1,05	-2,07	1,06E-05	0,001107979	Hypothetical, unclassified, unknown
PA2010_at	PA2010			-1,04	-2,05	7,63E-05	0,002190448	Transcriptional regulators
PA1551_at	PA1551		fixG	-1,03	-2,04	0,002903775	0,017196421	Energy metabolism
PA1137_at	PA1137			-1,01	-2,02	0,001529251	0,011165544	Putative enzymes
PA4063_at	PA4063			-1,00	-2,00	1,69E-05	0,001389481	Hypothetical, unclassified, unknown
PA3967_at	PA3967			1,00	2,00	6,99E-07	0,000323296	Hypothetical, unclassified, unknown
PA4974_at	PA4974		opmH	1,01	2,01	0,00012454	0,002720758	Protein secretion/export apparatus
PA1852_at	PA1852			1,01	2,01	3,37E-06	0,000693523	Hypothetical, unclassified, unknown
PA5300_cycB_at	PA5300	cycB		1,02	2,02	0,000364928	0,004665861	Energy metabolism
PA2800_at	PA2800	vacJ	vacJ	1,02	2,03	9,46E-05	0,002441172	Antibiotic resistance and susceptibility
PA3975_thiD_at	PA3975	thiD		1,02	2,03	0,000102377	0,002492719	Biosynthesis of cofactors, prosthetic groups and carriers
PA5276_lppL_i_at	PA5276	lppL		1,02	2,03	9,34E-07	0,000370132	Cell wall / LPS / capsule
PA3056_at	PA3056			1,02	2,03	6,42E-05	0,002047415	Hypothetical, unclassified, unknown
PA5028_at	PA5028			1,03	2,04	0,000143568	0,002839872	Hypothetical, unclassified, unknown
PA0706_cat_at	PA0706	cat		1,03	2,04	0,000130463	0,002783815	Antibiotic resistance and susceptibility
PA5298_at	PA5298		xpt	1,04	2,06	0,000872392	0,008053586	Nucleotide biosynthesis and metabolism
PA4672_at	PA4672		pth	1,04	2,06	1,86E-05	0,001425199	Translation, post-translational modification, degradation
PA4853_fis_at	PA4853	fis		1,04	2,06	7,66E-05	0,002190448	Transcriptional regulators; DNA replication, recombination, modification and repair; Transcription, RNA processing and degradation
PA1796_folD_at	PA1796	folD		1,05	2,07	3,23E-05	0,001636633	Translation, post-translational modification, degradation; Nucleotide biosynthesis and metabolism; Biosynthesis of cofactors, prosthetic groups and carriers
PA2755_eco_at	PA2755	eco		1,07	2,10	1,92E-06	0,000578762	Translation, post-translational modification, degradation
PA1354_at	PA1354			1,08	2,11	0,001056103	0,009069778	Hypothetical, unclassified, unknown
PA1750_at	PA1750			1,09	2,13	8,99E-05	0,002378021	Amino acid biosynthesis and metabolism
PA3612_at	PA3612		ypeB	1,09	2,14	1,56E-05	0,001332158	Hypothetical, unclassified, unknown
PA4784_at	PA4784			1,10	2,14	0,000181389	0,003286646	Transcriptional regulators
PA2936_at	PA2936			1,10	2,14	0,000222817	0,003659944	Membrane proteins
PA1064_at	PA1064			1,11	2,16	1,32E-05	0,001221855	Hypothetical, unclassified, unknown
PA1006_at	PA1006		yrkI	1,11	2,16	8,90E-05	0,002374416	Hypothetical, unclassified, unknown
PA4881_at	PA4881			1,12	2,17	0,000204124	0,003475817	Hypothetical, unclassified, unknown
PA0421_at	PA0421			1,12	2,18	1,89E-06	0,000578762	Putative enzymes
PA4317_at	PA4317			1,12	2,18	0,000341486	0,004543023	Membrane proteins
PA0433_at	PA0433			1,13	2,18	6,58E-05	0,002047415	Hypothetical, unclassified, unknown
PA4693_pssA_at	PA4693	pssA		1,13	2,19	0,000244854	0,003859019	Fatty acid and phospholipid metabolism
PA0362_fdx1_at	PA0362	fdx1		1,14	2,21	5,90E-06	0,00084537	Energy metabolism
PA2666_at	PA2666		ptpS	1,15	2,21	0,000472719	0,005459771	Biosynthesis of cofactors, prosthetic groups and carriers
PA1198_at	PA1198			1,16	2,23	1,21E-05	0,001169601	Hypothetical, unclassified, unknown
PA1035_at	PA1035			1,16	2,24	3,86E-06	0,000737709	Hypothetical, unclassified, unknown
PA5192_pckA_at	PA5192	pckA		1,16	2,24	2,66E-05	0,001564423	Carbon compound catabolism; Energy metabolism
PA3611_at	PA3611			1,17	2,25	8,50E-06	0,001002984	Hypothetical, unclassified, unknown
PA0385_at	PA0385			1,17	2,25	0,000133561	0,002796363	Hypothetical, unclassified, unknown
PA1192_at	PA1192		ydaO	1,17	2,26	0,002588503	0,015977312	Hypothetical, unclassified, unknown
PA3295_at	PA3295			1,18	2,26	0,000216905	0,003617049	Putative enzymes
PA3245_minE_at	PA3245	minE		1,18	2,27	6,79E-05	0,002058865	Cell division
PA1009_at	PA1009			1,19	2,28	2,93E-05	0,001580082	Hypothetical, unclassified, unknown
PA0167_at	PA0167			1,19	2,29	5,08E-06	0,000825616	Transcriptional regulators
PA2971_at	PA2971		yceD	1,20	2,29	4,67E-05	0,001791314	Hypothetical, unclassified, unknown
PA5049_rpmE_at	PA5049	rpmE		1,20	2,30	0,000347686	0,004571822	Translation, post-translational modification, degradation
PA4031_ppa_at	PA4031	ppa	ipyR	1,21	2,31	6,49E-06	0,000887606	Central intermediary metabolism
PA0005_at	PA0005	lptA	plsC	1,22	2,33	4,32E-06	0,000799407	Fatty acid and phospholipid metabolism
PA3223_acpD_at	PA3223	azoR3		1,22	2,33	0,000249209	0,003873553	Fatty acid and phospholipid metabolism
PA2491_at	PA2491	mexS		1,23	2,34	1,15E-06	0,000426744	Putative enzymes; Transcriptional regulators
PA5491_at	PA5491			1,23	2,34	5,57E-05	0,001962587	Energy metabolism
PA5462_at	PA5462			1,24	2,36	6,64E-05	0,002048484	Hypothetical, unclassified, unknown
PA3177_at	PA3177			1,25	2,37	1,99E-06	0,000578762	Hypothetical, unclassified, unknown
PA3686_adk_at	PA3686	adk		1,25	2,37	5,54E-05	0,001962587	Nucleotide biosynthesis and metabolism
PA0578_at	PA0578			1,25	2,38	0,000103941	0,002492719	Hypothetical, unclassified, unknown
PA0380_i_at	PA0380			1,25	2,38	0,000104195	0,002492719	Hypothetical, unclassified, unknown
PA4632_at	PA4632			1,26	2,39	0,000262109	0,003937974	Hypothetical, unclassified, unknown
PA4432_rpsI_at	PA4432	rpsI		1,27	2,40	0,000424616	0,005024095	Translation, post-translational modification, degradation
PA4636_at	PA4636			1,27	2,41	0,001483625	0,010964942	Hypothetical, unclassified, unknown
PA1504_at	PA1504			1,28	2,43	1,22E-05	0,001169601	Transcriptional regulators
PA4670_prs_at	PA4670	prs	prsA	1,28	2,43	0,000175147	0,003218181	Carbon compound catabolism; Nucleotide biosynthesis and metabolism
PA4441_at	PA4441			1,31	2,49	1,47E-07	0,000101985	Hypothetical, unclassified, unknown
PA3243_minC_at	PA3243	minC		1,32	2,50	3,14E-05	0,001614521	Cell division
PA5130_at	PA5130		yibN	1,34	2,52	1,90E-05	0,001425199	Hypothetical, unclassified, unknown
PA0363_coaD_at	PA0363	coaD	kdtB	1,34	2,54	2,19E-06	0,000578762	Central intermediary metabolism
PA3684_i_at	PA3684			1,37	2,58	0,00114505	0,009437831	Hypothetical, unclassified, unknown
PA3472_at	PA3472			1,39	2,61	2,62E-05	0,001564423	Hypothetical, unclassified, unknown
PA4602_glyA3_at	PA4602	glyA3		1,42	2,67	9,50E-05	0,002441172	Amino acid biosynthesis and metabolism
PA1674_folE2_at	PA1674	folE2		1,47	2,76	0,002371788	0,015058411	Biosynthesis of cofactors, prosthetic groups and carriers
PA3229_at	PA3229			1,51	2,86	5,44E-06	0,000837751	Hypothetical, unclassified, unknown
PA2619_infA_at	PA2619	infA		1,54	2,90	0,000540126	0,005958569	Translation, post-translational modification, degradation
PA0579_rpsU_at	PA0579	rpsU		1,60	3,03	3,80E-05	0,00168556	Translation, post-translational modification, degradation
PA4723_dksA_at	PA4723	dksA		1,60	3,04	4,32E-07	0,000239941	Transcriptional regulators; Adaptation, Protection; DNA replication, recombination, modification and repair
PA5429_aspA_at	PA5429	aspA		1,66	3,17	1,56E-05	0,001332158	Amino acid biosynthesis and metabolism
PA4042_xseB_at	PA4042	xseB		1,70	3,25	5,50E-05	0,001962587	DNA replication, recombination, modification and repair
PA4433_rplM_at	PA4433	rplM		1,72	3,30	2,32E-05	0,001534556	Translation, post-translational modification, degradation
PA4569_ispB_at	PA4569	ispB	cel	1,82	3,54	1,87E-05	0,001425199	Energy metabolism; Biosynthesis of cofactors, prosthetic groups and carriers
PA4563_rpsT_at	PA4563	rpsT		2,03	4,09	4,54E-06	0,000812775	Central intermediary metabolism; Translation, post-translational modification, degradation
PA4711_at	PA4711			2,48	5,58	4,83E-06	0,000825616	Hypothetical, unclassified, unknown
PA4705_at	PA4705	phuW	phuW	1,74	3,33	8,93E-09	1,24E-05	Hypothetical, unclassified, unknown
PA4706_at	PA4706	phuV	phuV	2,63	6,19	5,32E-10	1,47E-06	Transport of small molecules
PA4707_at	PA4707	phuU	phuU	2,79	6,91	4,62E-08	3,67E-05	Membrane proteins; Transport of small molecules
PA4709_at	PA4709	phuS	phuS	3,96	15,60	1,23E-08	1,36E-05	Putative enzymes; Transport of small molecules
PA4708_at	PA4708	phuT	phuT	4,11	17,22	4,35E-09	8,05E-06	Transport of small molecules
PA4710_at	PA4710	phuR		7,26	152,95	1,81E-10	1,00E-06	Transport of small molecules
PA1414_at	PA1414			-1,56	-2,95	0,037853798	0,102865194	Hypothetical, unclassified, unknown
PA2753_at	PA2753			-2,38	-5,21	0,039917681	0,107006383	Hypothetical, unclassified, unknown
PA1556_at	PA1556	ccoO2	ccoO; fixO	-1,01	-2,01	0,044380962	0,116165076	Energy metabolism; Central intermediary metabolism
PA3337_rfaD_at	PA3337	rfaD		-2,22	-4,66	0,048646212	0,12485561	Cell wall / LPS / capsule
PA2501_at	PA2501			-1,42	-2,68	0,05004659	0,127389234	Membrane proteins
PA2127_at	PA2127	cgrA		-1,15	-2,22	0,052866573	0,132321431	Transcriptional regulators
PA4587_ccpR_at	PA4587	ccpR		-1,29	-2,44	0,068186228	0,157981368	Energy metabolism

PA5232_at	PA5232	yhiI	-1,59	-3,01	0,071344675	0,163456483	Hypothetical, unclassified, unknown
PA3278_at	PA3278		-1,73	-3,31	0,081187903	0,181210739	Membrane proteins
PA3309_at	PA3309	uspK	-1,60	-3,03	0,08197465	0,182242522	Hypothetical, unclassified, unknown
PA1789_at	PA1789		-1,34	-2,54	0,086152759	0,188264004	Hypothetical, unclassified, unknown
PA5170_arcD_at	PA5170	arcD	-2,26	-4,79	0,087501297	0,190484385	Membrane proteins; Amino acid biosynthesis and metabolism; Transport of small molecules
PA2567_at	PA2567		-1,04	-2,06	0,095212577	0,202427045	Hypothetical, unclassified, unknown
PA0141_at	PA0141		-1,25	-2,37	0,09815526	0,20669972	Hypothetical, unclassified, unknown
PA5475_at	PA5475		-1,92	-3,79	0,10666369	0,219946792	Hypothetical, unclassified, unknown
PA0200_i_at	PA0200		-1,67	-3,18	0,130634923	0,252400135	Hypothetical, unclassified, unknown
PA1196_at	PA1196		-1,38	-2,61	0,134292556	0,25708033	Transcriptional regulators
PA4610_at	PA4610		-1,04	-2,06	0,138813398	0,263883366	Hypothetical, unclassified, unknown
PA4352_at	PA4352		-1,02	-2,03	0,150254553	0,279831017	Hypothetical, unclassified, unknown
PA2119_at	PA2119	adh	-1,31	-2,48	0,190540738	0,328357316	Putative enzymes
PA4577_at	PA4577		-1,01	-2,02	0,194157736	0,332832383	Hypothetical, unclassified, unknown
PA5427_adhA_at	PA5427	adhA	-1,17	-2,25	0,207817231	0,348588385	Energy metabolism; Carbon compound catabolism
PA1673_at	PA1673		-1,27	-2,41	0,234067529	0,379189526	Hypothetical, unclassified, unknown

Supplementary Table 3: Analysis of PseudoCap function class enrichment among genes from Supplementary Table 2 (n=118). P(X≥x) ~ binom(X; p), where P(X≥x) is the probability of observing ≥x of the 118 genes to belong to a functional class of genes.

	Total genes	% of total no. of genes (*p*)	Genes present (*x*)	% of genes	Fold enrichment	P(X≥x)~ binom(X; p)
Translation, post-translational modification, degradation	198	3,6	9	7,6	2,1	0,0259
Central intermediary metabolism	108	1,9	6	5,1	2,6	0,0284
Energy metabolism	206	3,7	9	7,6	2,1	0,0321
Fatty acid and phospholipid metabolism	64	1,2	4	3,4	2,9	0,0484
Carbon compound catabolism	193	3,5	8	6,8	1,9	0,0543
Amino acid biosynthesis and metabolism	246	4,4	9	7,6	1,7	0,0796
Nucleotide biosynthesis and metabolism	86	1,5	4	3,4	2,2	0,1119
Biosynthesis of cofactors, prosthetic groups and carriers	160	2,9	6	5,1	1,8	0,1268
Cell division	30	0,5	2	1,7	3,1	0,1342
DNA replication, recombination, modification and repair	88	1,6	3	2,5	1,6	0,2883
Putative enzymes	472	8,5	11	9,3	1,1	0,4217
Antibiotic resistance and susceptibility	74	1,3	2	1,7	1,3	0,4678
Transcription, RNA processing and degradation	55	1,0	1	0,8	0,9	0,6913
Hypothetical, unclassified, unknown	1923	34,7	37	31,4	0,9	0,8015
Protein secretion/export apparatus	142	2,6	2	1,7	0,7	0,8076
Transcriptional regulators	487	8,8	8	6,8	0,8	0,8225
Adaptation, Protection	208	3,7	3	2,5	0,7	0,8230
Transport of small molecules	607	10,9	10	8,5	0,8	0,8432
Secreted Factors (toxins, enzymes, alginate)	104	1,9	1	0,8	0,5	0,8927
Membrane proteins	675	12,2	9	7,6	0,6	0,9580
Cell wall / LPS / capsule	193	3,5	1	0,8	0,2	0,9847

Supplementary Table 4: Overview of significantly altered expressions (adj.p.Val < 0.05) between PAO1-M2 and PAO1 in LB medium. Locus ID, Loc tag, name, synonyms and PseudoCAP function class of each gene is descibed. Calculations of log fold changes and p-values are done using the *limma* package in R.

Locus ID	Locus Tag	Name	Synonyms	log Fold Change	Fold Change	P.Value	adj.P.Val	PseudoCAP Function Class
PA4710_at	PA4710	phuR		6,96	124,89	8,07E-12	4,48E-08	Transport of small molecules
PA4705_at	PA4705	phuW	phuW	3,36	10,27	4,38E-10	8,10E-07	Hypothetical, unclassified, unknown
PA4706_at	PA4706	phuV	phuV	3,78	13,75	4,28E-10	8,10E-07	Transport of small molecules
PA4711_at	PA4711			3,50	11,34	8,50E-10	1,18E-06	Hypothetical, unclassified, unknown
PA4709_at	PA4709	phuS	phuS	4,40	21,08	2,34E-09	2,59E-06	Putative enzymes; Transport of small molecules
PA4708_at	PA4708	phuT	phuT	4,27	19,24	3,52E-09	3,26E-06	Transport of small molecules
PA4707_at	PA4707	phuU	phuU	3,85	14,46	3,20E-08	2,54E-05	Membrane proteins; Transport of small molecules
PA4712_at	PA4712			2,55	5,87	1,55E-07	0,00010741	Hypothetical, unclassified, unknown
PA0091_at	PA0091	vgrG1	vgrG1a	0,99	1,99	1,69E-06	0,00104354	Protein secretion/export apparatus
PA0075_at	PA0075	pppA	tagG1	0,59	1,51	1,56E-05	0,00865313	Putative enzymes; Protein secretion/export apparatus
PA3908_at	PA3908			0,58	1,50	3,51E-05	0,01768733	Hypothetical, unclassified, unknown
PA3877_narK1_at	PA3877	narK1		-0,74	-1,67	4,87E-05	0,0225392	Membrane proteins; Transport of small molecules
PA1920_at	PA1920	nrdD	nrdD	-0,51	-1,43	8,07E-05	0,02983554	Nucleotide biosynthesis and metabolism
PA3615_at	PA3615			-0,45	-1,37	7,56E-05	0,02983554	Hypothetical, unclassified, unknown
PA4713_at	PA4713			0,76	1,70	7,25E-05	0,02983554	Hypothetical, unclassified, unknown
PA1197_at	PA1197			-0,62	-1,54	0,0001384	0,0452694	Hypothetical, unclassified, unknown
PA4577_at	PA4577			-0,45	-1,37	0,00013869	0,0452694	Hypothetical, unclassified, unknown
PA3914_moeA1_at	PA3914	moeA1		-0,95	-1,93	0,00014802	0,04563217	Biosynthesis of cofactors, prosthetic groups and carriers

Supplementary Table 5: Overview of significantly altered expressions (adj.p.Val < 0.05) between DK2-CF30-1979-M2 and DK2-CF30-1979 in ABTGC medium. Locus ID, Loc tag, name, synonyms and PseudoCAP function class of each gene is descibed. Calculations of log fold changes and p-values are done using the *limma* package in R.

Locus ID	Locus Tag	Name	Synonyms	log Fold Change	Fold Change	P.Value	adj.P.Val	PseudoCAP Function Class
PA1632_kdpF_at	PA1632	kdpF		-1,03	-2,04	2,75E-05	0,01905455	Transport of small molecules
PA4220_i_at	PA4220		fptB	-0,91	-1,88	7,59E-05	0,04209642	Hypothetical, unclassified, unknown
PA1911_at	PA1911	femR		-0,60	-1,52	0,00010625	0,04670253	Membrane proteins; Transcriptional regulators
PA4223_at	PA4223		pchH	-0,56	-1,47	0,00011812	0,04681751	Membrane proteins; Transport of small molecules
PA1634_kdpB_at	PA1634	kdpB	atkB	-0,51	-1,42	0,00010941	0,04670253	Transport of small molecules
PA3126_ibpA_at	PA3126	ibpA	hslT	0,54	1,46	0,00010122	0,04670253	Chaperones & heat shock proteins
PA1546_hemN_at	PA1546	hemN		0,60	1,51	5,36E-05	0,03304577	Biosynthesis of cofactors, prosthetic groups and carriers
PA4705_at	PA4705	phuW	phuW	1,20	2,30	8,56E-07	0,00079172	Hypothetical, unclassified, unknown
PA4706_at	PA4706	phuV	phuV	1,32	2,50	1,18E-06	0,00093482	Transport of small molecules
PA4707_at	PA4707	phuU	phuU	1,58	2,98	6,33E-07	0,00070303	Membrane proteins; Transport of small molecules
PA4708_at	PA4708	phuT	phuT	1,89	3,71	1,44E-07	0,00026672	Transport of small molecules
PA4709_at	PA4709	phuS	phuS	2,24	4,73	2,37E-08	8,73E-05	Putative enzymes; Transport of small molecules
PA4711_at	PA4711			2,60	6,06	2,03E-07	0,00028104	Hypothetical, unclassified, unknown
PA4710_at	PA4710	phuR		4,24	18,88	3,15E-08	8,73E-05	Transport of small molecules