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Gravistimulation of Pisum Sativum and Expression of the Cell Membrane Expansin Proteins

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GRAVISTIMULATION OF PISUM SATIVUM AND EXPRESSION OF THE CELL MEMBRANE EXPANSIN PROTEINS

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Advisor_____

Department of _____

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Dean of the Graduate College

ABSTRACT

GRAVISTIMULATION OF PISUM SATIVUM AND EXPRESSION OF THE CELL MEMBRANE EXPANSIN PROTEINS

By Sherrine A. Ibrahim

This thesis research investigates the regulation of gene expression in pea stems (*Pisum sativum*) after changes in orientation to gravity (gravistimulation). Specifically, this project examines the changes in extracellular soluble proteins due to gravistimulation in pea seedlings. This change in gravity alters the cellular growth pattern, which can be directly linked to enzymatic activity resulting in the expression of cell wall loosening proteins called expansins. This loosening of the cell wall matrix subsequently increases elasticity in growing cells, thus leading to an upward curvature of the stems. These changes in enzymatic activity and gene expression are crucial for the survival of plants when variations in environmental conditions occur.

The methods utilized in this project were performed in order to determine whether or not expansin genes are up-regulated during gravistimulation, and if the pea expansin gene family is highly conserved throughout the plant kingdom. Proteins were first extracted from the cell walls of peas at various times during gravistimulation (0 hrs. – 48 hrs.) and analyzed using SDS PAGE. Standard molecular techniques were also utilized in which, 1) the probe used to identify the expansin genes was created using RT-PCR, 2) a DNA library for *Pisum sativum* was created and screened for the presence of expansin genes, 3) standard Northern blotting techniques were applied to pea RNA extracted at various times during gravistimulation (0 hrs. – 48 hrs.) The results of this thesis found that the cell wall expansin genes were probably up-regulated during specific times of gravistimulation (i.e. 24 hours). However, due to the degeneracy of the expansin probe there is the possibility that more than one gene is being hybridized, thus decreasing our confidence in these results. In conclusion, these experiments must be repeated with a more specific probe to determine whether expansins do indeed exist in *Pisum sativum* and if they are being up-regulated.

DEDICATION

I would like to dedicate this work to my parents, Adel and Maureen Ibrahim. Both of my parents have always been supportive of my academic pursuits, and have given me all the love and respect I have needed to succeed. Without their devotion, understanding and support, this would not be possible. Thank you so much mom and dad, I could not have done this without all your love and encouragement. I gladly dedicate my Masters thesis to both of you.

ACKNOWLEDGMENTS

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CHAPTER I

Introduction

A terminally differentiated higher plant cell expresses an array of genes required for fulfilling its normal, stable metabolic role. As plant cells mature, they transform themselves into a variety of differentiated cell types with unique shapes, sizes and structural properties fitting to their final station in life. This transformation requires a major remodeling of the cell wall, in which new structural polymers are added to the old, while the wall is reshaped by selective yielding to the mechanical forces generated by cell turgor pressure (Cosgrove, 2000a). Similarly, when changes in environmental conditions occur, the cell can respond in a rapid and specific manner by selectively increasing or decreasing the expression of specific genes. Genes whose expression is increased during times of gravistimulation presumably are critical to the adaptation of the organism to a changing environment. External to the plant cell membrane exists a complex matrix (cell wall) composed of carbohydrate and protein polymers, which contains numerous soluble proteins. These soluble proteins may represent precursors to cell wall matrix and enzymes, which are responsible for altering cell wall structure in the response to environmental influences (Carpita et al., 1993).

Plants use the gravity vector as a directional guide to growth, thereby positioning roots and shoots below and above ground (Sedbrook et al., 1999). Furthermore, roots and shoots grow in opposite directions within the gravity field in an environmentally regulated manner (Masson, 1995). This response of a plant to the gravitational field of the earth, or a plant's response to specific directions of gravity by altering its pattern of growth and development is called gravitropism. However, to date, very few details of the underlying mechanisms have been resolved.

This thesis research investigates the changes in gene expression after gravistimulation (change in orientation to gravity) in pea stems (*Pisum sativum*). Gravistimulation alters the pattern of cellular growth along the stem resulting in a characteristic upward gravitropic curvature (MacDonald, 1983). In other words, these plant stems and roots

alter their direction of growth to maintain a set angle with the gravity vector (MacDonald, 1983). These changes in cell growth are necessarily linked to enzymatic activities in the cell wall. Gravistimulation of plant stems changes the elasticity of cell wall matrix, which in turn alters cell growth required for upward curvature. In this project, changes in extracellular soluble proteins due to gravistimulation were evaluated in pea seedlings. The wall-loosening proteins that disrupt binding between microfibrils and the cell wall matrix are referred to as expansins (Zhang et al., 2000). These proteins unlock a network of wall polysaccharides, permitting turgor-driven cell enlargement facilitating increase elasticity in growing cells (Cosgrove, 2000).

In order to analyze changes in the plant's cell walls at the molecular level a variety of molecular techniques were utilized. Pea seedlings were initially sterilized, rinsed, soaked, planted, allowed to germinate in the dark for at least six days, and exposed to dim green light periodically. For DNA and RNA extraction the growing pea stem segments were cut and flash frozen with liquid nitrogen ground and centrifuged so that the cell wall fluid could be extracted and used for biochemical analysis. The enzymatic activities of the proteins influenced by gravistimulation were evaluated by studying the results of 1 – D SDS polyacrylamide gel electrophoresis (SDS – PAGE). This system is a powerful and widely used method for the analysis of complex protein mixtures extracted from cells, tissues, or other biological samples (Berkelman et al., 1998). This technique separates proteins according to their molecular weights (MW) (Berkelman et al., 1998). Results from these gels provided us with evidence suggesting that specific proteins are "turned on" during exposure to a change in the orientation to gravity.

After studying previous gravistimulation research (McQueen – Mason, 1992) it was hypothesized that the highly conserved expansin gene family could be linked to the increase of certain proteins during gravistimulation. It is believed that these expansins are extracellular proteins that facilitate cell wall extension, possibly by disrupting hydrogen bonding between hemicellulose wall components and cellulose microfibrilst (McQueen – Mason, 1992). Comparison of expansin sequences from GenBank demonstrate how expansins are not only highly conserved within a species, but also conserved from species to species (Fig. 1.1)

The main significance of this project is that it directly corresponds to NASA's Space Biology Program by addressing the poorly understood transduction of plant's responses to gravity. NASA's interest is primarily due to experimental findings of gene expression due to gravistimulation, and their implications regarding changes in the plants at the molecular level. The concepts presented in this project represent the basic information in the evaluation of plant requirements for growth in a closed environment under microgravity or near gravity conditions.

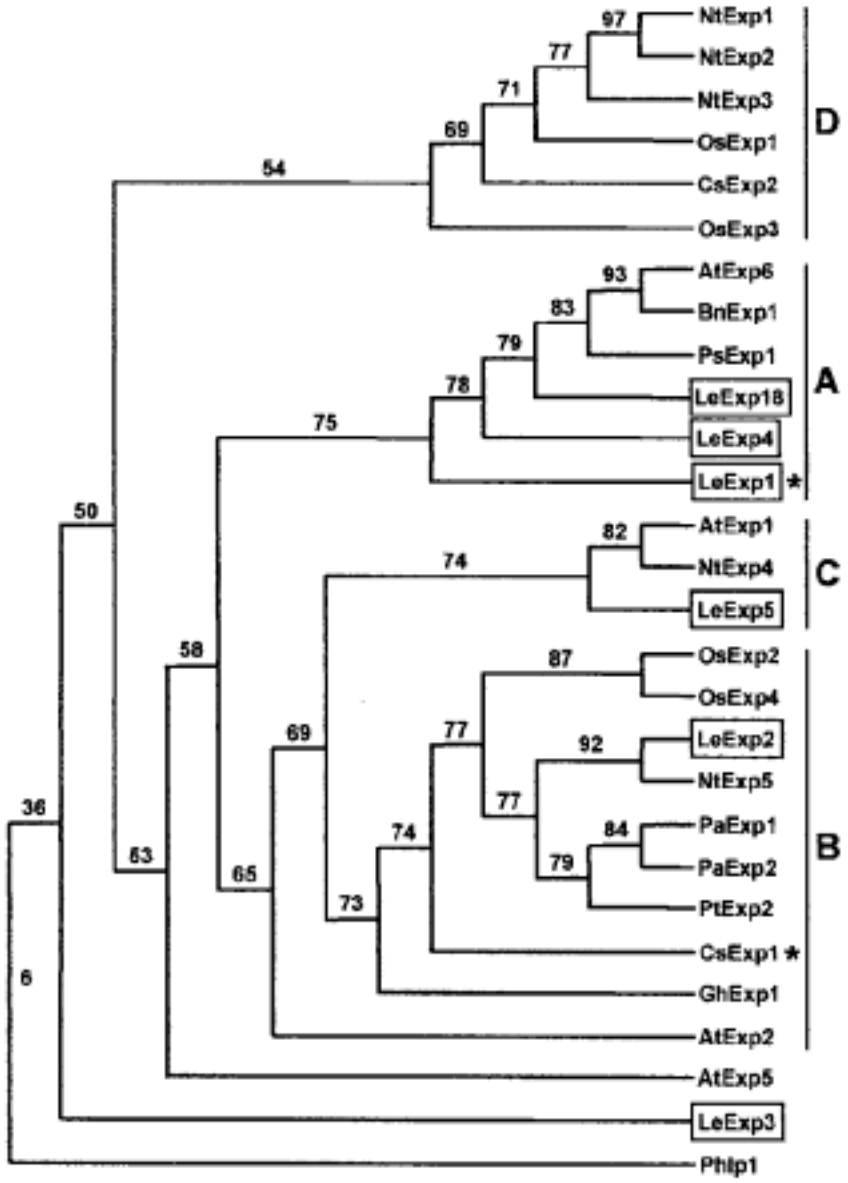


FIGURE 1.1 PHYLOGENTIC ANALYSIS OF EXPANSIN GENES. THE TREE WAS GENERATED BASED ON AN ALIGNMENT OF THE DEDUCED AMINO ACID SEQUENCES OF 27 EXPANSIN GENES TOGETHER WITH A POLLEN ALLERGEN (PHLP1), USING CLUSTALW FROM BIOLOGY WORKBENCH. THIS TREE WAS GENERATED BY ROSE ET AL. (2000) USING CLUSTALW FROM BIOLOGY WORKBENCH.

CHAPTER II

Review of Literature

The Cell Wall

The plant cell wall is a complex rigid polymeric structure consisting of a variety of polymers including cellulose, xyloglucan, xylan, and polygalacturonan. The cell wall is secreted by growing cells, and aids the plant by providing support, shape, mechanical strength, and a barrier against pathogens. The typical cell wall of a higher plant cell contains crystalline cellulose microfibrils embedded in a hydrophilic gel – like matrix of mixed linked polysaccharides (hemicelluloses) and proteins (pectins) and is placed under considerable tensile stress by the internal hydrostatic pressure of the cell (McQueen-Mason S. et al., 1994; Cosgrove, D.J., 2000c).

Gravitropism

Gravitropic responses of plants are probably the fastest growing area of NASA plant biology research. This research deals with changes of plants at the molecular level when exposed to different gravitational stimuli. Gravitropism consists of a series of sequential events allowing plant organs to direct their growth for shoots and downward growth for roots (Sedbrook et al., 1999). Gravitropism has attracted a great deal of attention since it's discovery by Knight (Knight, 1806), and its characterization by Darwin (Darwin, C. 1880). This interest is because a change in the direction of the gravity vector is one of the few methods that cause changes in plant development in a fast, reversible, but noninvasive way (Barlow, 1995). The graviresponses of plants are a powerful system with which to determine the mechanisms controlling plant development, information that will be of fundamental value in agricultural sciences (Barlow, 1995).

Another set of important results found that terminally differentiated higher plant cell expresses an array of genes required for fulfilling its normal, stable metabolic role. However, these plants can rapidly respond to changes in the environment, by selectively increasing or decreasing the expression of specific genes. Genes whose expression is

increased during times of gravistimulation presumably are critical to the adaptation of the organism to a changing environment. For example, the invertase gene has been identified as under regulation in the gravistimulated oat pulvinus (Wu et al., 1993). The breakdown of sucrose catalyzed by invertase provides substrate for starch synthesis and cell wall biosynthesis important in gravitropism in higher plants. Wu *et al.* found that invertase is under transcriptional regulation with increased mRNA levels induced in the lower portion of the gravistimulated oat pulvinus (Wu et al., 1993). The pulvinus is a group of cells at the base of a leaf that bring about changes in the position of the leaves by rapidly losing water.

Changes in enzymatic activity and gene expression are crucial for the survival of plants when variations in environmental conditions occur. If biologists are able to manipulate these mechanisms based on this research, then they are that much closer to controlling a plant's responses to gravity. However, few details of the underlying mechanisms must still be resolved, thus providing a need for further research in this field.

Studying gravitropism has greatly enhanced the knowledge underlying plant mechanisms. Some recent studies have concentrated on the effects of gravistimulation on pea seedlings, *Pisum sativum*, which are found to possess gravitropic mutants (Space Science Board, 1998). Specifically, pea stems alter the angle at which their organs grow in response to the direction of gravity (negatively gravitropic). The results of these experiments have found changes in extracellular soluble proteins after changes in orientation to gravity. These changes in cell growth have been linked to expression of expansins in the cell wall, which facilitate loosening of the matrix and increase elasticity in growing cells (Cosgrove, 1996).

The molecular basis to cell wall loosening is considered essential in stimulating cellular growth in plants. Enlargement in plant cells results from vacuolation and turgor – driven extension of cell walls (Carpita, et al., 1993). Many studies support the acid growth hypothesis for auxin action, which predicts that auxin induces growth by secreting protons into the wall (Rayle et al., 1992). The lowered cell wall pH was thought necessary to activate hypothetical enzymes or processes that initiate wall loosening and thereby result in cell enlargement (Zhang et al., 2000). However, the molecular basis of

acid – induced growth remained unclear until the discovery of expansins (Cosgrove, 1989; McQueen – Mason et al., 1992).

Expansins

Expansins are extracellular proteins that are thought to function primarily by loosening plant cell walls. However, these proteins have also been found to function in cell enlargement, pollen tube invasion of the stigma (in grasses), wall disassembly during fruit ripening, abscission and other cell separation events (Cosgrove, 2000a). Structural analysis indicates that one expansin region resembles the catalytic domain of family-45 endoglucanases but glucanase activity has not been detected. The diagram in Figure 2.1 demonstrates the proposed structure of the expansin protein. In this diagram, signal peptide (gray) directs the nascent polypeptide into the ER/Golgi secretory pathway. This part of the protein (typically 22-25 amino acids) is removed as the protein enters the ER. The mature protein is ~25-27 kDa and may consist of two domains, a cysteine-rich region with limited sequence similarity to family-45 endoglucanases (EG45-like domain (green)) and a tryptophan-rich carboxy-terminus (pink) that may function as a polysaccharide-binding domain (Cosgrove, 1998). Expansins are encoded by two multigene families and each gene is often expressed in highly specific locations and cell types (Cosgrove, 2000a). At present these two families of expansin genes are termed alpha-expansins and beta-expansins. These two families of expansins that have been recognized, share only ~20-25% amino acid identity. These regions of identity are spread throughout the protein backbone and include 6 of the 8 conserved cysteines in the cysteine-rich region, the tryptophans near the carboxy terminus, and the short segments outlined in BLUE in Figure 2.1 (Cosgrove, 1998). These conserved cysteines and the conserved BLUE segments are also found in family-45 glycosyl hydrolases. Alpha-expansins are not glycosylated, unlike beta- expansins (Cosgrove, 1998).

Expansins are relatively conserved proteins with the following features:

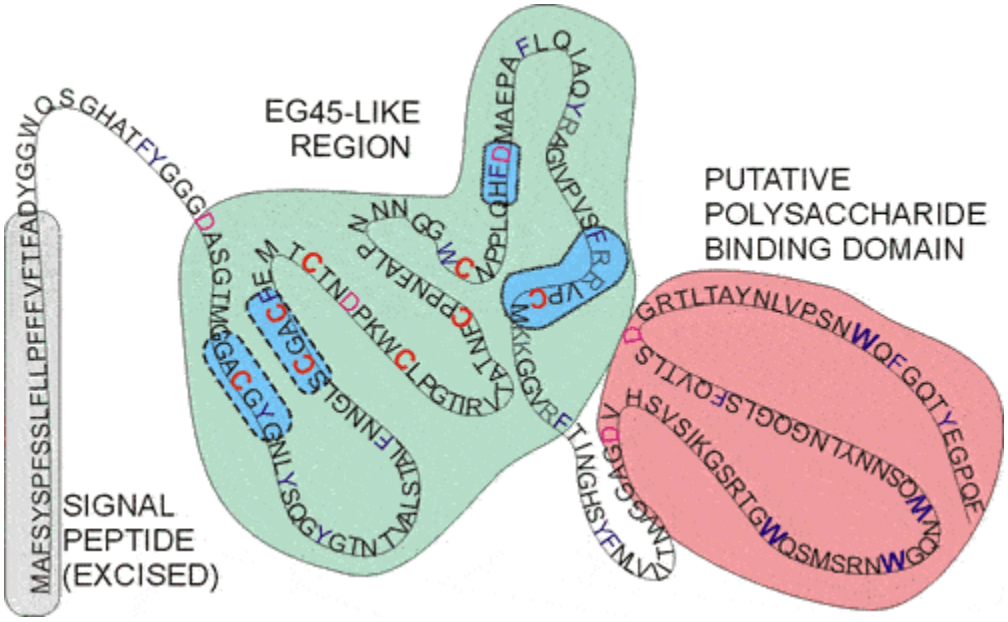


FIGURE 2.1 PROPOSED STRUCTURE OF EXPANSIN PROTEIN (PICTURE COURTESY OF DANIEL COSGROVE, HTTP://WWW.BIO.PSU.EDU/EXPANSINS/).

Alpha Expansins:

Alpha-expansins are a highly conserved group of proteins hypothesized to control cell wall enlargement, and perhaps other developmental processes including cell wall disassembly and cell separation (Cosgrove, 2000b). *Arabidopsis* contains 26 genes encoding for alpha-expansins, designated EXP1-EXP26. Most alpha-expansin genes have a conserved intron structure as indicated in Figure 2.2 (www.bio.psu.edu/expansins).

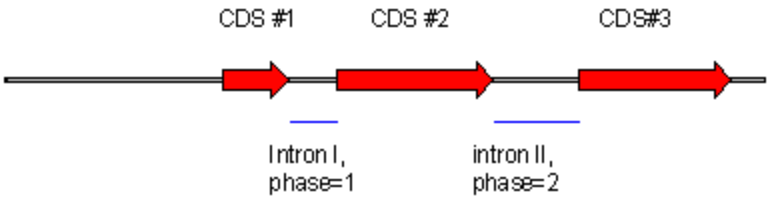


FIGURE 2.2 INTRON LENGTHS RANGE FROM ABOUT 90 BP TO 500 BP. SOME ALPHA-EXPANSIN GENES, E.G. EXP17 - EXP26, ARE MISSING INTRON #2. EXP10 HAS AN ADDITIONAL INTRON IN THE 5' UNTRANSLATED REGION. (PICTURE COURTESY OF DANIEL COSGROVE, HTTP://WWW.BIO.PSU.EDU/EXPANSINS).

Beta Expansins:

The first beta-expansins to be discovered were previously known as group-1 grass pollen allergens. They are profusely secreted by grass pollen and have potent wall loosening effects on grass cell walls. Their biological function seems to be to soften the stigma and stylar tissues to speed penetration of the pollen tubes through the maternal tissues to the ovule (www.bio.psu.edu/expansins). The presence of many other beta-expansins in young grass seedlings and non-pollen tissues hints that this expansin class has a variety of developmental functions related to their wall-loosening action (Cosgrove, 2000b).

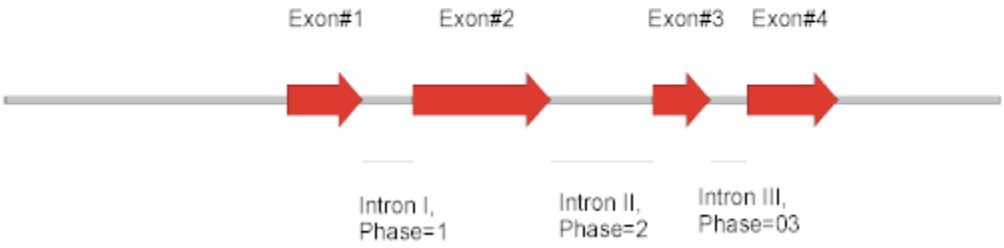


FIGURE 2.3 *ARABIDOPSIS* HAS 5 BETA-EXPANSIN GENES, DESIGNATED EXPB1-EXPB5. THEY TYPICALLY CONTAIN THREE INTRONS, TWO OF WHICH ARE CONSERVED WITH

ALPHA-EXPANSINS, AND A THIRD ONE THAT IS NOT FOUND IN ALPHA-EXPANSINS AND MAY BE FOUND BEFORE OR AFTER INTRON II. BELOW IS THE STRUCTURE OF EXPB1, IN WHICH THE INTRON III IS LOCATED AFTER THE INTRON II. (PICTURE COURTESY OF DANIEL COSGROVE, HTTP://WWW.BIO.PSU.EDU/EXPANSINS).

Expansin Mechanism of Action

Expansins have been found in a variety of plants such as cucumber, *Arabidopsis*, pine, rice, oat, etc. and range in size from 25 – 26 kDa (Zhang et al., 2000). However, the mode of action of expansins in cell wall loosening is not well understood. Extension induced by expansins is almost identical to acid – induced elongation (McQueen – Mason et al., 1992; Caderas et al., 2000). Biochemical and biophysical data indicate that expansin proteins bind to the surface of cellulose microfibrils and that they allow the cell wall to extend without steadily weakening the tensile strength of the cell wall (Caderas et al., 2000). Unlike previous hypothesis involving cell wall extension, it is believed that expansins weaken the non-covalent binding between wall polysaccharides, thereby allowing turgor-driven polymer creep (Cosgrove, 2000c) (Fig. 2.4). In this scheme, expansin would make use of the mechanical strain energy in the wall to catalyze an inchworm-like movement, or reptation, of the wall polymers. Expansin movement may be confined to lateral diffusion along the surface of the cellulose microfibril, as been observed for other polysaccharide-binding proteins (Cosgrove, 2000c). Such contained diffusion would enable expansin to search the microfibril surface, locally loosening its attachment to the matrix, and allowing chain movement and stress relaxation (Cosgrove, 2000c).

This feature is different from the effect of wall hydrolases, which progressively weaken the cell wall (McQueen – Mason, 1995; Shieh and Cosgrove, 1998). Specifically, ethylene-induced growth inhibition is accompanied by peroxidase activation (Britz and Galston, 1982) and red light-inhibited growth in etiolated corn mesocotyls is accompanied by an increase in acidic cell wall peroxidase levels (Britz and Galston, 1982). Cell wall enzymes such as glucanases and xylanases clip portions of the cell wall matrix releasing biologically active cell wall fragments, which may be important in signaling cell wall enzyme activation, cell elongation, or ethylene biosynthesis (Shieh and Cosgrove, 1998; Bandurski et al., 1984). Also, cell wall polymer structure (which

includes polysaccharides and glycoproteins) is altered in the upper and lower sides of gravistimulated pea stems (Field, 1981; MacDonald et al., 1983).

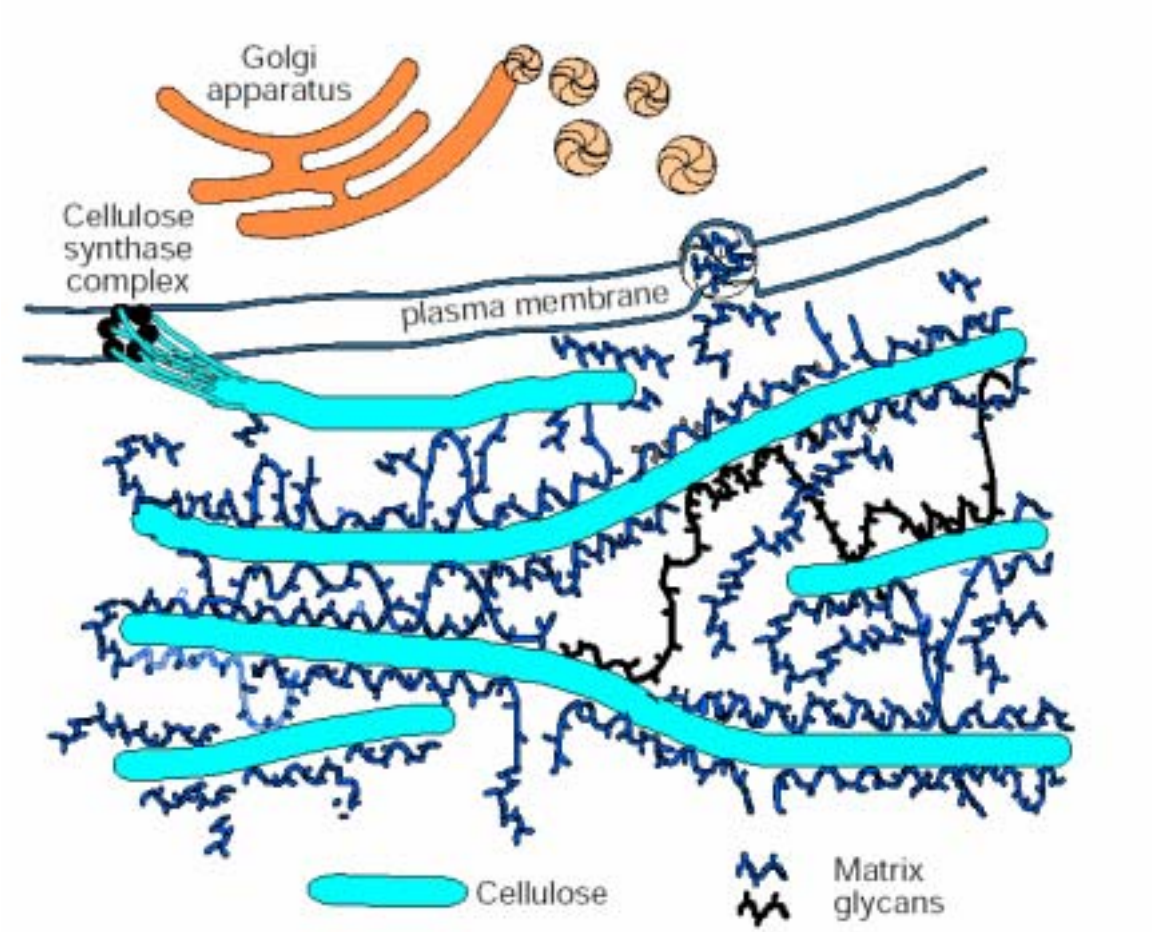


FIGURE 2.4 SIMPLIFIED MODEL OF HOW EXPANSINS MIGHT INTERACT WITH OTHER WALL COMPONENTS. (PICTURE COURTESY OF DANIEL COSGROVE, HTTP://WWW.BIO.PSU.EDU/EXPANSINS/).

The image in Figure 2.4 demonstrates how wall polysaccharides are secreted to the cell surface and then assemble into a load-bearing structure. The action of expansins is hypothesized to cause a transient release of short segment of matrix glycans attached to cellulose microfibrils, with the result that the cellulose and matrix polymers slide relative to one another (Cosgrove, 2000c). Wall hydrolases such as endoglucanase cut matrix glucans into shorter segments, which may lead to weakening, but not creep, of the cell wall (Cosgrove, 2000c). Transglycosylases, such as XET, can recombine glycans into

shorter or longer pieces, depending upon conditions within the wall. Proton-ATPases in the plasma membrane (star symbol) may lower the wall pH, thereby activating expansins and other enzymes with acidic optima and inactivating wall enzymes with neutral pH optima. For graphical simplicity, pectins and structural proteins are not shown in this figure, but one should image them filling the space between microfibrils.

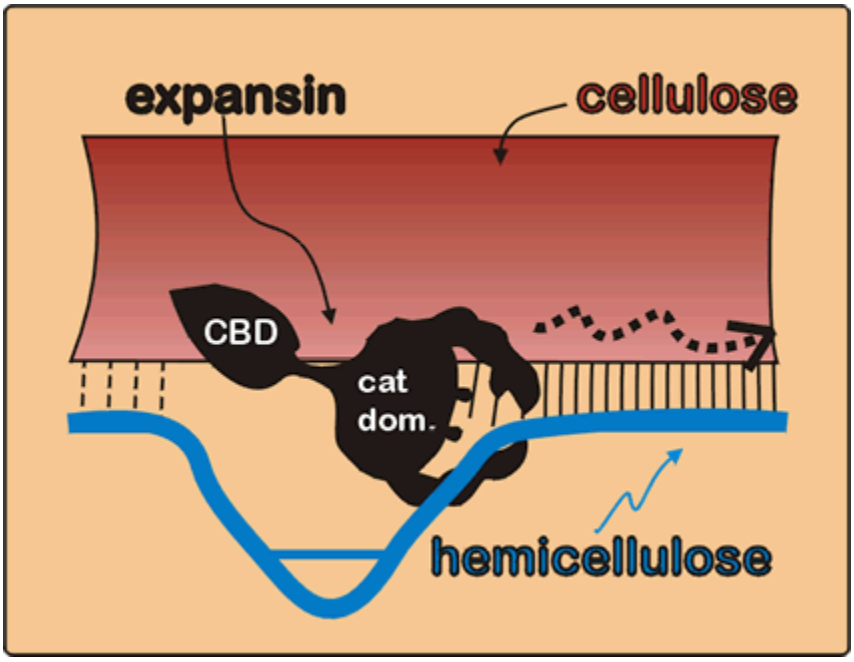


FIGURE 2.5 ANOTHER SIMPLIFIED MODEL OF HOW EXPANSIN MIGHT ACT. ONE DOMAIN (HERE LABELED AS CBD-LIKE) MAY RESTRICT EXPANSIN MOBILITY BY BINDING TO THE SURFACE OF CELLULOSE MICROFIBRILS. THE MOVEMENT OF EXPANSIN ALONG THE CELLULOSE SURFACE MAY DISRUPT LOOSELY-BOUND MATRIX POLYMERS, WITH THE RESULT THAT THE WALL POLYMERS MOVE, OR CREEP, RESULTING IN TURGOR-DRIVEN WALL EXTENSION. (PICTURE COURTESY OF DANIEL COSGROVE, HTTP://WWW.BIO.PSU.EDU/EXPANSINS/).

Objectives

The objectives of this project were to investigate the changes in gene expression after gravistimulation (change in orientation to gravity) in plant stems. Gravistimulation alters the pattern of cellular growth along the stem resulting in a characteristic upward gravitropic curvature. These changes in cell growth are necessarily linked to enzymatic activities in the cell wall, which facilitate loosening of the matrix and increase elasticity in growing cells. The major objectives involved in this study included:

(1) analysis of the soluble cell wall and cytoplasmic proteins during gravitropic curvature

(2) characterization of novel cell wall and cytoplasmic enzymes, which respond to a change in orientation to gravity.

(3) identification and isolation of the specific genes (i.e. expansins) involved in the gravitropic response using Lambda ZAP® II Predigested EcoR I/CIAP (Stratagene, catalog no. 236612).

CHAPTER III

Materials and Methods

Plant Material

Seedlings of Pisum sativum L. cv. Alaska were surface sterilized in 0.5 % NAOCI (10 % commercial bleach) for 10 minutes, and then rinsed thoroughly using distilled water for approximately 30 minutes. The seedlings were then soaked for 2-4 hours in distilled water. Seeds were then placed in sterile plastic trays containing water soaked Kimwipes and allowed to germinate at 23° C in darkness for 3 days. The germinated seeds were then wrapped individually with Kimwipes and transferred to tubes where they are again allowed to germinate at 23° C in darkness for approximately 4 more days. After germination, seedlings receive 45 minutes of dim green light daily after germination. For protein extractions, etiolated seedlings were harvested under green light by excising the growing region of the hypocotyls (upper 4 cm).

RNA Isolation

Total RNA was isolated from the plant stems using the Qiagen RNA Plant mini kit with Qiashredder® columns (Qiagen, catalog no. 74903). RNA concentrations were then determined by spectroscopy. This technique produced total RNA and was used to isolate RNA from individual stem segments. This RNA was subsequently used for reverse transcription polymerase chain reaction (RT-PCR), dot blotting and northern blotting. Figure 3.1 is a detailed schematic describing Qiagen's RNA extraction method.

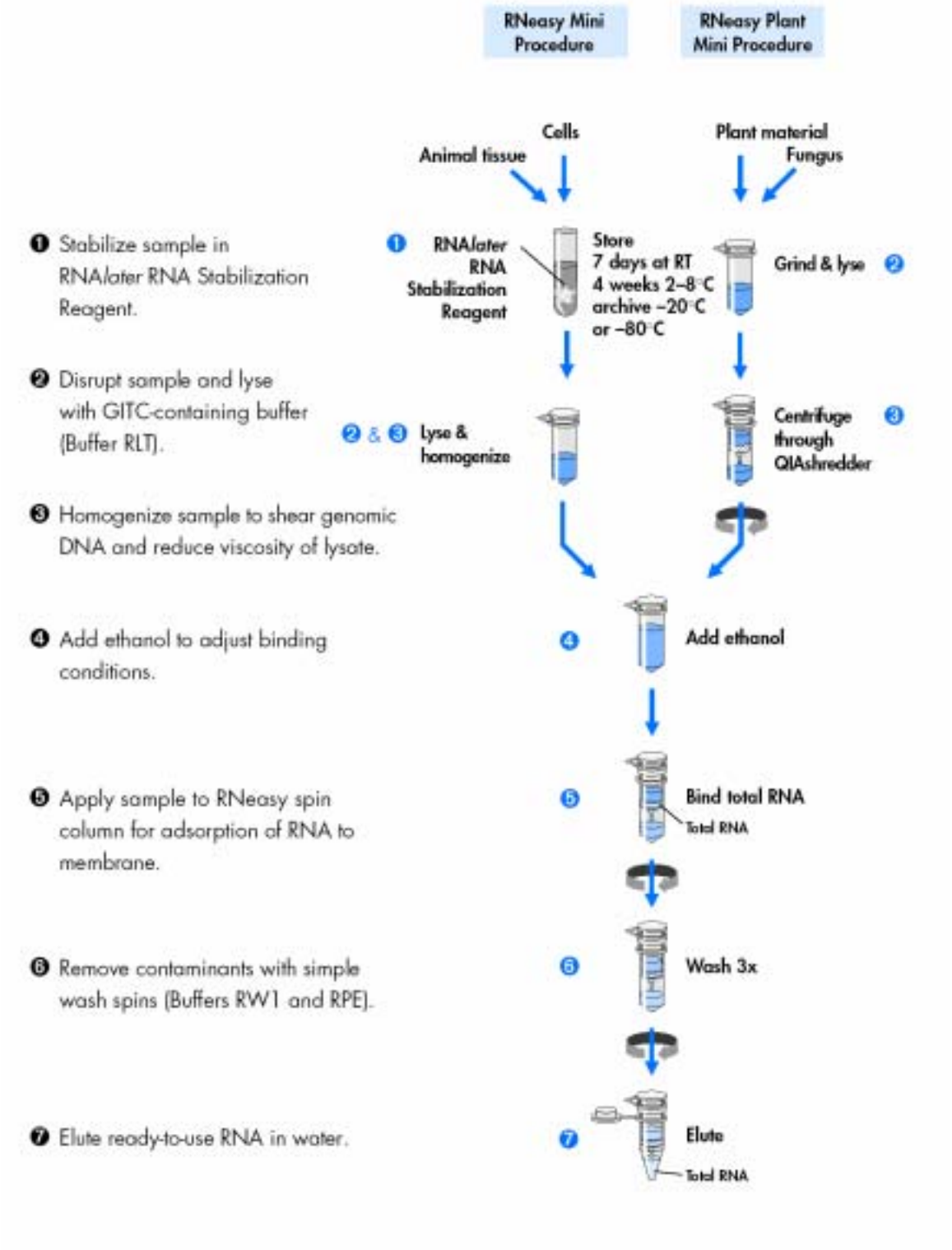


FIGURE 3.1 ISOLATION OF PEA DNA USING THE RNEASY MINI PLANT AND FUNGUS KIT FROM QIAGEN.

RT-PCR

Total RNA was isolated from a six day old pea seedling as described above. Using this RNA, a RT-PCR reaction mixture was then created (Table 3.1), and after reverse transcription, PCR amplification was performed (Table 3.2). The primers used in amplification of the expansin gene fragment were designed by Rose et al (1997) and have the following sequences, BG(GC) (N)CA (TC)GC (N)AC (N)TT (CT)TA (CT)GG (N)G, (forward 22-mer, also known as the degenerate 5' expansin), and B(TC)T GCC A(AG)T T(TC)T G(N)C CCC A(AG)T T (reverse 21-mer, also known as the degenerate 3' expansin), where B = Biotin. For more information on the primers please see Appendix L. The PCR product was then quantified using a Spectronic Genesys 5 spectrophotometer and gel electrophoresis. The resulting PCR fragment (~545 bp) was subsequently used as a probe for screening a library, a dot blot, and a Northern Blot. For complete instructions on how to perform RT-PCR please refer to Appendix C.

Table 3.1 RT-PCR Reaction

Components	Reaction volume (µl)
H_2O	10.0 µl
$MgSO_4$ (25 mM)	2.0 µl
AMV 5 X Buffer	10.0 µl
DNTP's	1.0 µl
Tf1 polymerase	1.0 µl
Master Mix	24 µl
Upstream Primer (50 picomoles)	5.0 µl
Downstream Primer (50 picomoles)	5.0 µl
RNA	0.5 – 1.0 µg
AMV RT	1.0 µl
Additional Water	Bring final volume up to 50 µl

Table 3.2 Temperature and time settings for each step of the RT-PCR reaction used in the amplification of target sequences. Pre-cycling conditions, number of cycles, and post-cycling conditions given below.

Expansin Probe	Time	Temperature (°C)
1) Pre-cycling (RT step)	45:00	48
2) Denaturing	0:30	94
3) Annealing	1:00	61.5
4) Extension	2.00	68
5) Cycles (repeat 2-6)	40	
6) Post-cycling	7:00	68
7) Hold	Indefinitely	4

Northern Blotting

Equal amounts (3 µg) of total RNA isolated from the curved stems of *Pisum sativum* of plants gravistimulated for various durations, or from vertical controls were separated on formaldehyde-containing agarose (1% [w/v]) gels according to Fourney et al. (1988). RNA was transferred to a Hybond – N hybridization nylon transfer membrane (Amersham Life Sciences, catalog no. RPN 303N) overnight in RNase free water and baked for 2 hours at 80 °C using a vacuum oven. Blots were prehybridized for 30 min. at 50 °C and were hybridized at the same temperature overnight. Prehybridization and hybridization was carried out using the North2South Chemiluminescent Nucleic Acid Hybridization and Detection Kit (Pierce Biotechnology, catalog no. 17075). Blots were probed with a biotylinlated cDNA coding for expansin generated by RT PCR using degenerate 5' and 3' primers (Appendix L). Blots were washed three times (approx. 15 - 20 min. each) at 50 °C in 1 X wash buffer (2X SSC/0.1% SDS). Blots were then placed in a blocking buffer and incubated at room temperature with gentle agitation. Streptavidin-HRP was then added to the blocking buffer containing the blot using a 1:300 dilution factor. The final washes were carried out in a .1 X SSC / 0.1% (w/v) SDS solution for 4 X 5 min. at room temperature with gentle agitation. Hybridization was visualized by autoradiography using KODAK Films BioMax® Light-2 Autoradiography

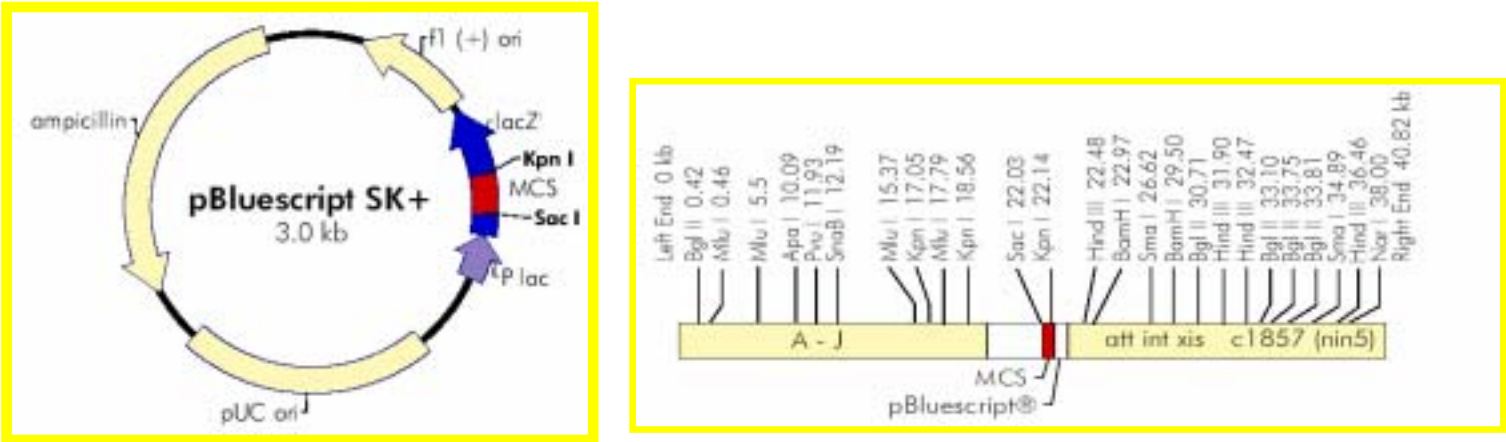
Film, 20.3x25.4cm (8x10"), product number 8761520001EA). For full procedure, please refer to Appendix G.

DNA Extraction

Genomic DNA was previously isolated from seedlings of *P. sativum* (Murray and Harrison) and used for subsequent experiments. Ten micrograms of total DNA aliquots were digested with *Eco*RI for vary time periods (5 min., 10 min, 15 min., 20 min., 25 min., 30 min., etc.) and electrophoresed on a 0.8 % agarose gel. The sample that appeared to have been the most clearly cut was used to construct the DNA library.

Construction and Screening DNA Library

A DNA library was constructed by inserting the cut DNA from the previously described DNA extraction into a lambda phage vector (Figure 3.7). The resulting phage library was then treated with the Lambda Zap II Predigested EcoRI/CIAP - Treated Vector Kit with Gigapack Gold Packaging extract (Stratagene Cat. no. 236612). A detailed schematic of the procedure is demonstrated in Figures 3.2 – 3.7 (www.stratagene.com).



A B

FIGURE 3.2 MAPS OF: (A) THE PBLUESCRIPT SK (+/-) PHAGEMID (B) LAMBDA ZAP II INSERTION VECTOR.

Screening a Lambda library using conventional laboratory methods

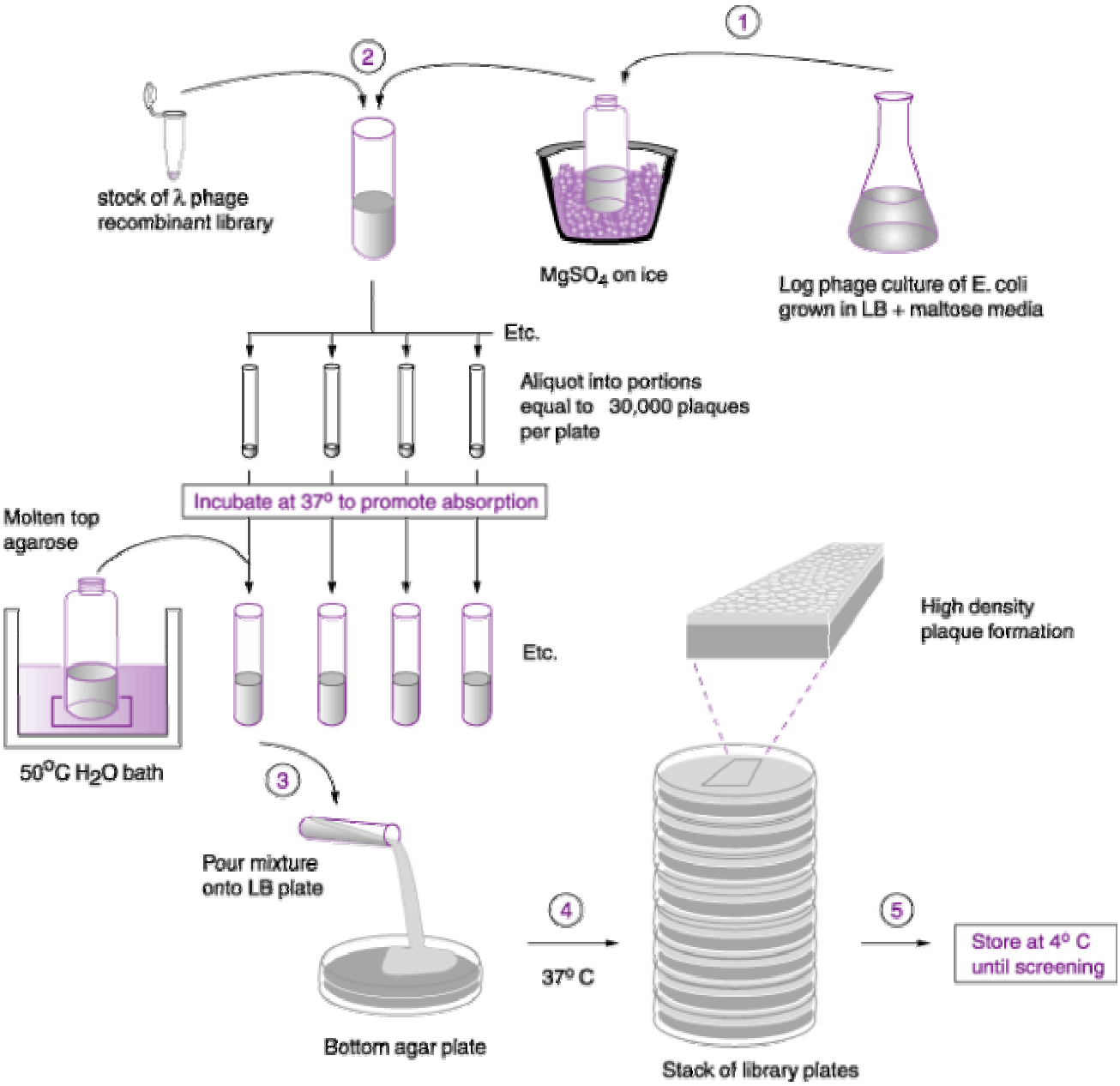


FIGURE 3.3 STEP 1: SPREAD ALL THE PHAGE OUT BY "PLATING THE LIBRARY" USING LARGE PLATES AND AN ACCURATE COUNT OF PHAGE TITER.

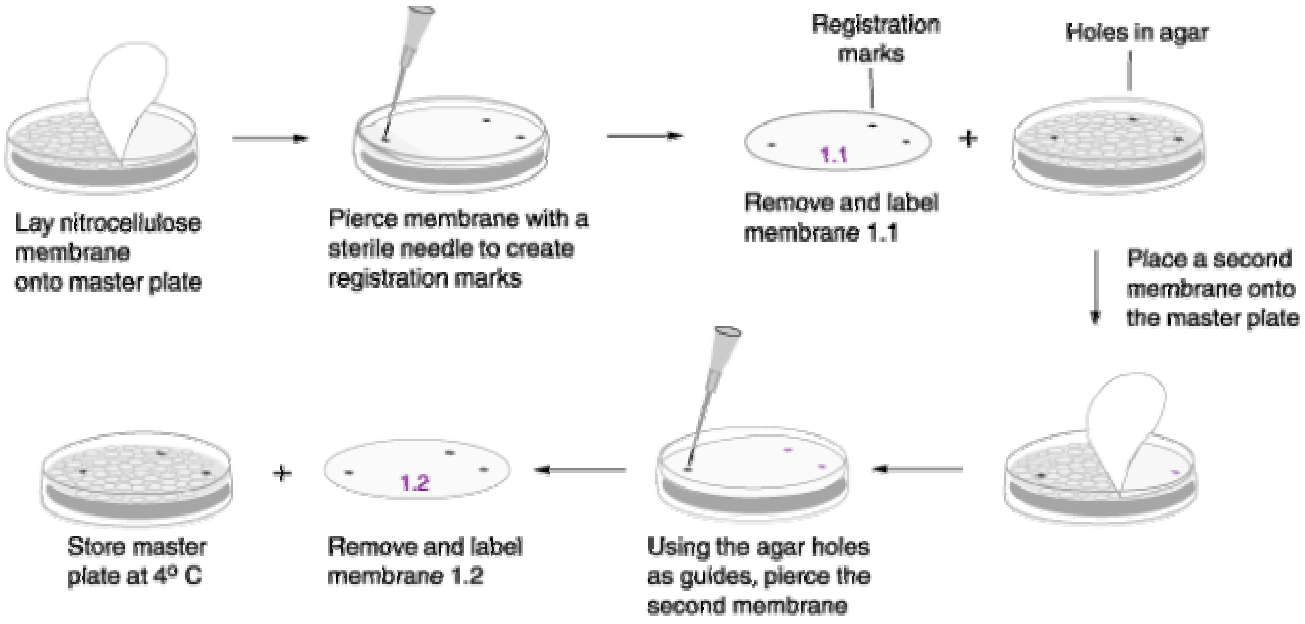


FIGURE 3.4 STEP 2: "LIFT THE PHAGE" OFF THE TOP AGAR USING NITROCELLULOSE FILTERS THAT ARE CAREFULLY LAID DOWN ON THE MASTER PLATE, REGISTERED WITH A NEEDLE PUNCTURE, AND THEN CAREFULLY REMOVED TO CAPTURE A SMALL FRACTION OF THE PHAGE IN THE PLAQUE. ABOUT 20% OF THE PHAGE PARTICLES IN THE PLAQUE ADHERE NON-COVALENTLY TO THE NITROCELLULOSE, LEAVING BEHIND ~80% OF THE VIABLE PHAGE FOR PROPAGATION LATER.

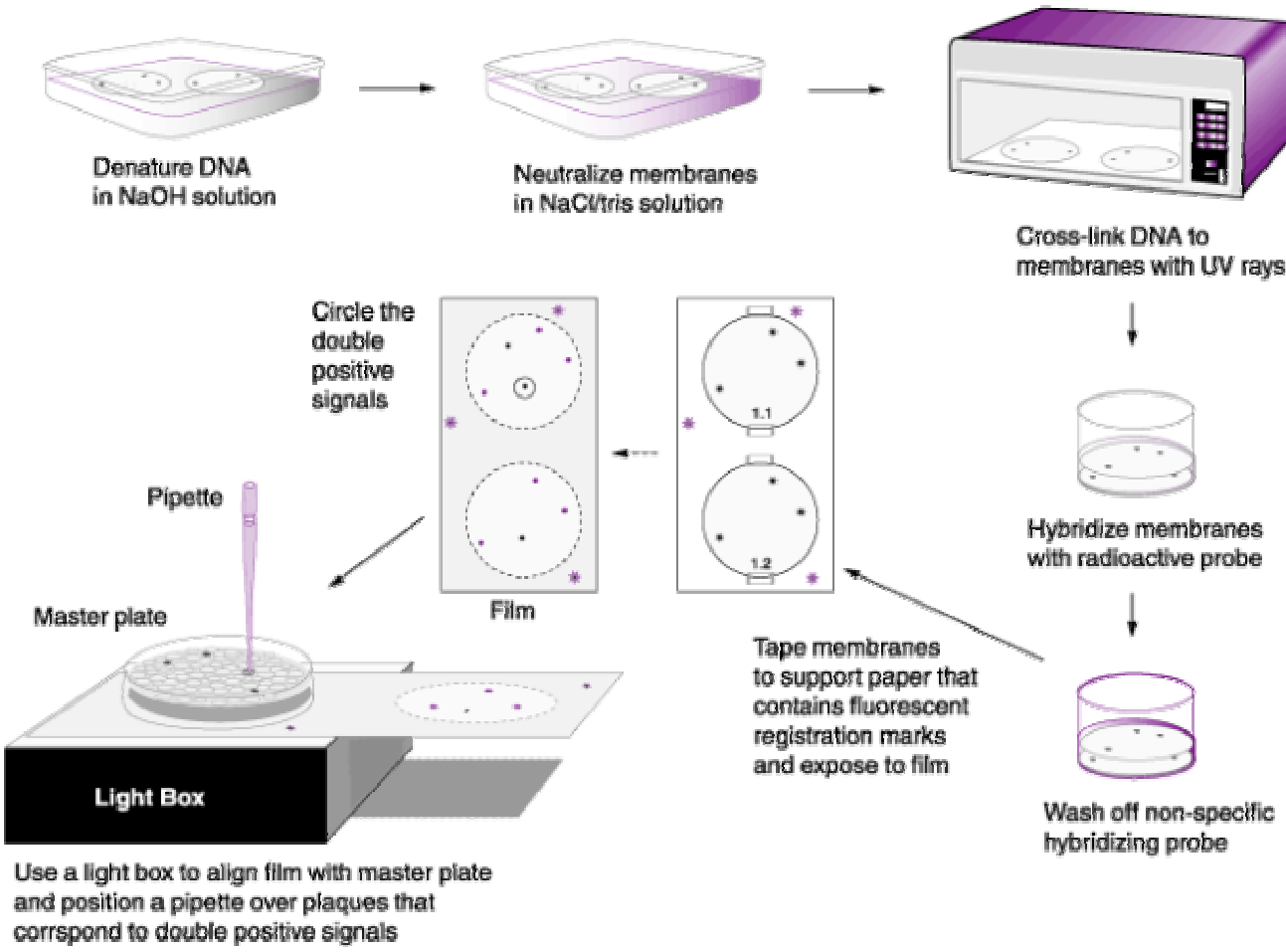


FIGURE 3.5 STEP 3: DENATURE THE DNA AND COVALENTLY LINK IT TO THE NITROCELLULOSE BY BAKING THE MEMBRANE FOR 2 HOURS AT 80 DEGREES CELSIUS. THE FILTERS ARE THEN PROCESSED BY HYBRIDIZATION TO THE LABELED PROBE. THE DEVELOPED FILM IS RE-ALIGNED WITH THE MASTER PLATES AND AGAR PLUGS ARE REMOVED FROM THE AREA OF THE PLATE CORRESPONDING TO THE POSITIVE SIGNAL. THIS LAST STEP IS DEPENDENT ON ACCURATE REGISTRATION MARKS AND WELL PRESERVED MASTER PLATES.

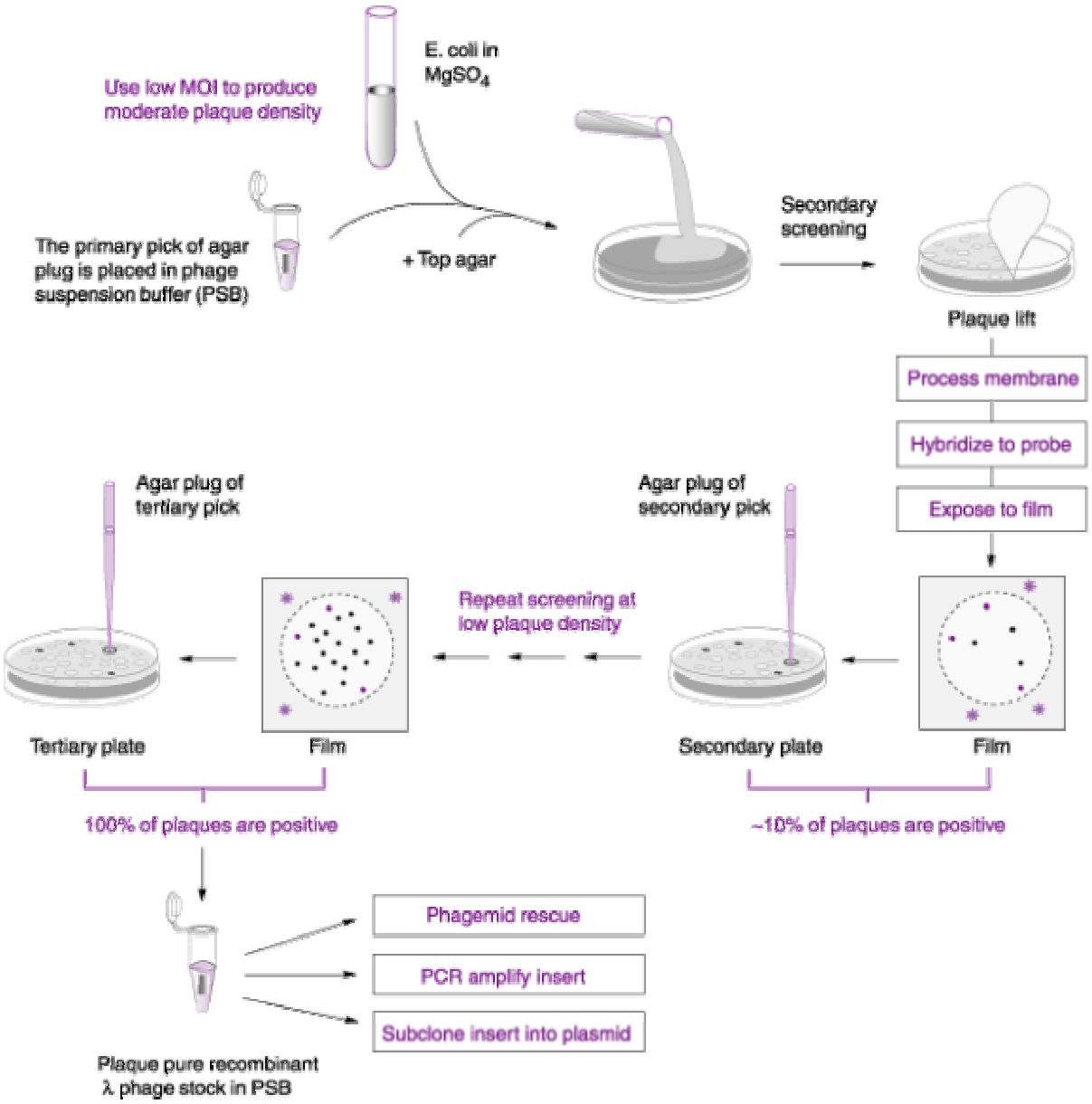
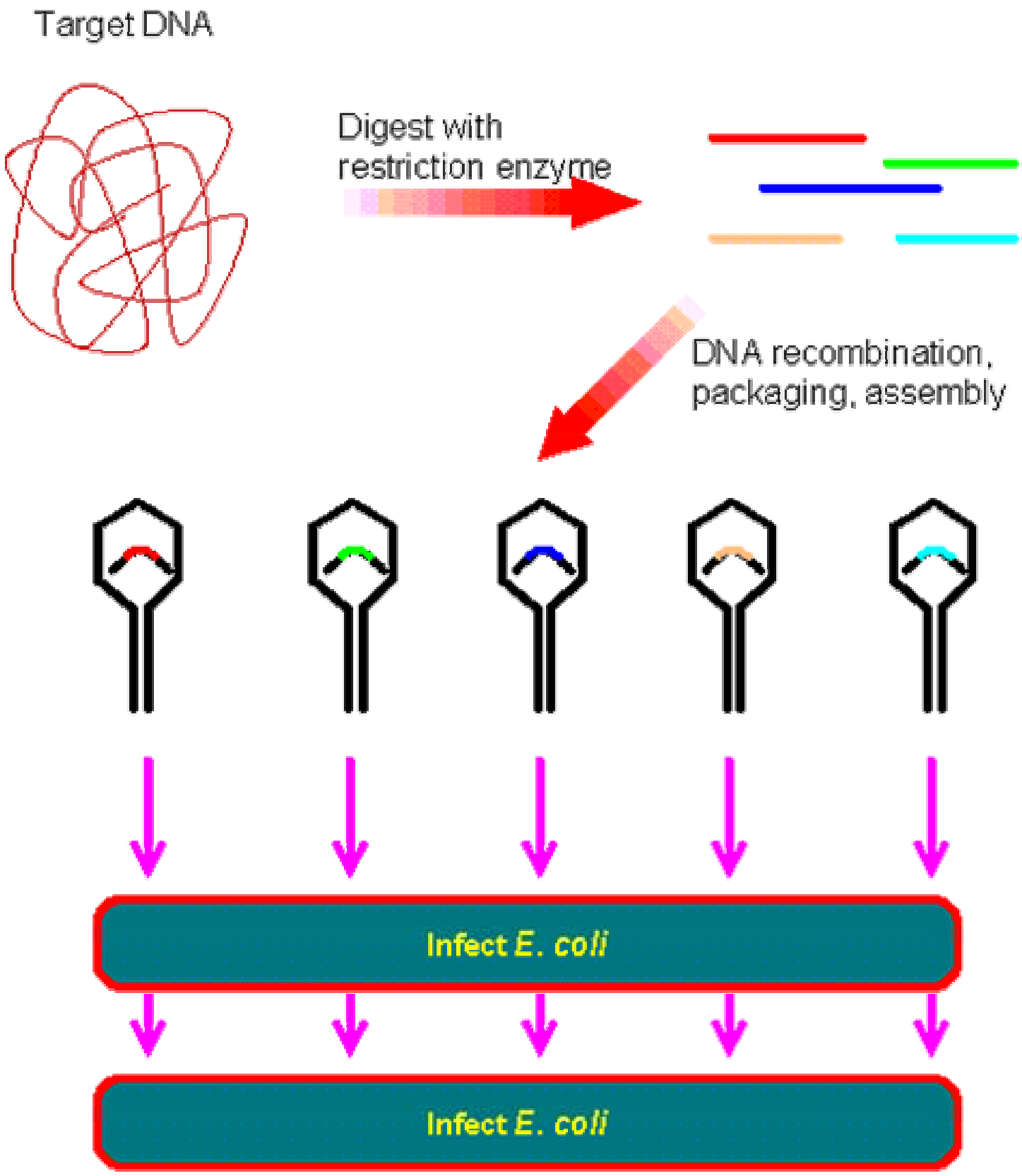


FIGURE 3.6 FINAL STEP IN LIBRARY SCREENING: PURIFY THE CANDIDATE PHAGE BY LIMITING DILUTION. IT IS AT THIS STEP OF PLAQUE PURIFICATION WHERE POSITIVE SIGNALS ARE CONFIRMED AND FALSE-POSITIVES PHAGE ARE DISCARDED. THE PURIFIED L PHAGE IN THE FINAL STAGE ARE CALLED "**PLAQUE PURE**", MEANING THAT 100% OF THE PHAGE IN THE TERTIARY PLATING HYBRIDIZE TO THE PROBE.


Target DNA
Digest with
restriction enzyme
DNA recombination,
packaging, assembly
Infect E. coli
Infect E. coli

Cell Wall Protein Extraction and Analysis

Cell wall fluid is collected from 2-cm sub apical pea stem segments as described by Terry and Bonner (Terry and Bonner, 1980). The segments are cut at daily intervals ranging from one, two, four, or seven days. The cut tissue is then homogenized in ice-cold 50 mM Tris-HCl buffer (pH 8.65), containing 20 mM KCl and 10 mM $MgCl_2$. Cell wall fluid was then obtained by mild centrifugation at 1,300 g for 5 minutes, and the supernatant was subsequently collected for protein analysis. This procedure allows the collection of cell wall fluid with little cytoplasmic contamination (Terry and Bonner, 1980). Cell wall proteins were then separated by SDS-Poly Acrylamide Gel Electrophoresis (SDS-PAGE) according to Laemmli (1970), silver stained, and then fixed in 10% acetic acid and 50% methanol.

Phylogenetic Alignments (Rose et al.)

The deduced amino acid sequences used to generate the phylogenetic tree in Figure 1 were aligned using the default parameters of CLUSTALW for Biology Workbench (http://workbench.sdsc.edu/) software version 3.2. A complete list of the GenBank accession numbers can be found in Appendix M: Arabidopsis AtExp1, U30476; A*tExp2*, U30481; *AtExp5*, U30478; *AtExp6*, U30480; rape *BnExp1,* AJ000885; cotton *GhExp1*, AF043284; cucumber *CsExp1*, U30482; *CsExp2*, U30460; pea *PsExp1*, X85187; *Phleum* pollen allergen *Phlp1*, X78813; pine *PtExp2*, U64890; rice *OsExp1*, Y07782; *OsExp2*, U30477; *OsExp3*, U30479; *OsExp4*, U85246; tobacco *NtExp1*, AF049350; *NtExp2*, AF049351; *NtExp3*, AF049352; *NtExp4*, AF049353; *NtExp5*, AF049354; tomato *LeExp1*, U82123; *LeExp2*, AF096776; *LeExp3,* AF059487; *LeExp4*, AF059488; *LeExp5*, AF059489; *LeExp18*, AJ004997; and apricot *PaExp1*, U93167, *PaExp2*, AF038815.

Data Analysis of Sequences

After the sequences were obtained for all samples, they were aligned using the default settings in CLUSTALW version 3.2 from Biology Workbench (http://workbench.sdsc.edu/). The alignment of the sequences was done by software to help maximize the repeatability of the study, as the computer program uses algorithms and will align the same sequences in the same manner every time where as a human may not. For further information about the CLUSTALW program and its use, please see Appendix J. In order to find homology between sample sequences generated, and known sequences found in GenBank, the default parameters of BLAST were utilized (http://www.ncbi.nlm.nih.gov/).

CHAPTER IV

Results

Gravistimulation of *Pisum sativum*

When pea plants were exposed to a change in their orientation to gravity (placed on their sides) a characteristic upward curvature was observed to take place. Curvatures can be observed to occur as early as 1 hour, but 2 hours is when a significant increase in the amount of curvature occurs. Figure 4.1 demonstrates gravistimulation of the pea stems over a time course ranging from 0 to 48 hours.

A B C

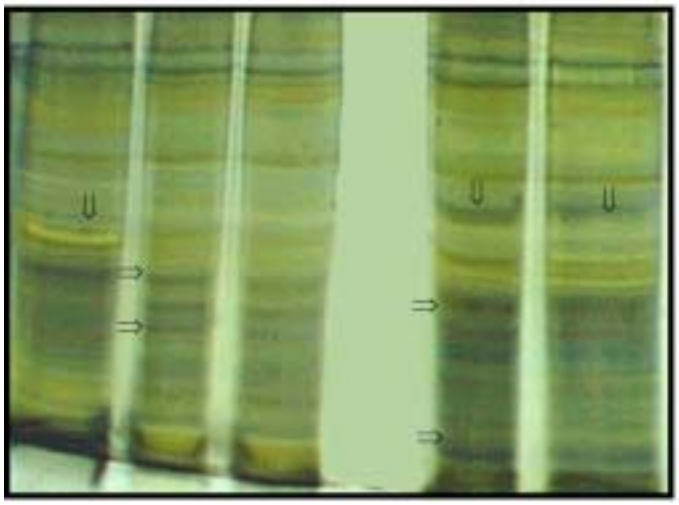
D E F

G

FIGURE 4.1 MORPHOLOGICAL EXAMPLE OF A GRAVISTIMULATED PEA STEM. (A) 0 HOURS (VERTICAL CONTROL) (B) 2 HOUR CURVE (C) 4 HOUR CURVE (D) 6 HOUR CURVE (E) 8 HOUR CURVE (F) 24 HOUR CURVE (G) 48 HOUR CURVE

Protein Analysis

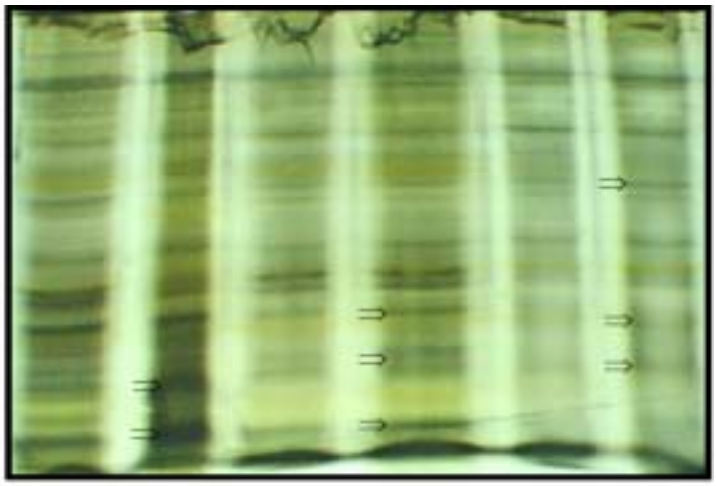
Qualitative changes of protein patterns in pea stems exposed to gravistimulation were analyzed by one – dimensional electrophoresis. This procedure revealed 23 extracellular proteins ranging from 10-150 kD in size, consistent with findings of Morrow and Jones (Morrow and Jones, 1986). Specifically, pea seedlings subjected to short - term (2-21 hours) and long-term (2 –7 days) gravistimulation showed specific enhancement of low molecular weight proteins ranging from 7 to 30 kD. A novel 26 kD protein is induced at 6 hrs and remains stable in the 24 hour treatment (Figure 4.2). Several proteins showed significant enhancement uniformly at long-term gravistimulation (2 –7 days) (Figure 4.3). The molecular weight of these proteins were determined by comparing the unknown proteins to a know molecular weight protein standard (Kaleidoscope Prestained Standard, Bio-Rad Cat.# 161-0324). It is apparent from our studies gravistimulation enhances the production of specific proteins. The proteins appeared as a rapid response by pea seedlings to gravistimulation. These soluble proteins may act as precursors to cell wall matrix which is responsible for altering cell wall structure in the response to environmental influences.



control 2 hr. 4 hr. 6 hr. 24 hr.

⇒= enhancements from control to experimental
⇓ = band present in control that disappears with environmental stress

FIGURE 4.2 SDS – PAGE EXAMINING GRAVISTIMULATION OF SEVEN DAY OLD SEEDLING PEA STEMS AT HOURLY INTERVALS OF 2, 4, 6, & 24 HRS.



2 d. c.* 2 d. 4 d.c.* 4d. 7 d.c.* 7 d.

*d.c. = Controls
⇒= enhancements from control to experimental
⇓ = band present in control that disappears with environmental stress

FIGURE 4.3 SDS – PAGE EXAMINING GRAVISTIMULATION OF SEVEN DAY OLD PEA STEMS AT HOURLY INTERVALS OF 2, 4, & 7 DAYS

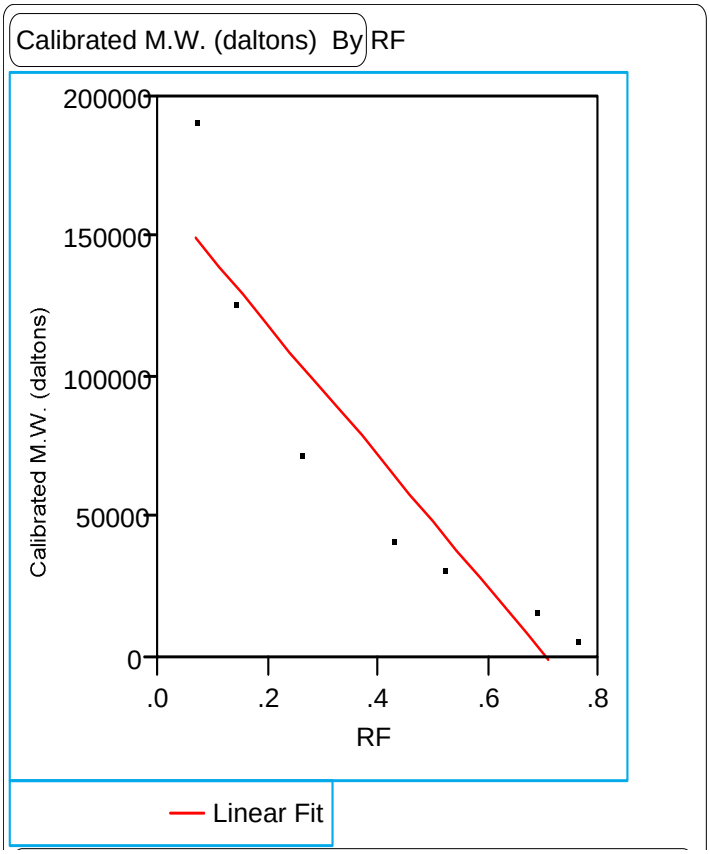


FIGURE 4.4 PRESTAINED PROTEIN MOLECULAR WEIGHT STANDARD CURVE

RT-PCR Analysis

The use of RT-PCR was of primary importance in this research for several reasons. This procedure was first used to determine if the primers originally constructed for tomato expansins (Rose et al., 1997) would work using the RNA of peas. From figure 4.5 it can be seen that a strong band approximately 545 base pairs in size was created using RT-PCR. The size of the product was determined using the Biolabs 100 bp ladder as a reference. Although, it was determined that the primers did in fact work on the pea RNA, it had to be determined if the resulting RT-PCR product (cDNA) would act as an appropriate probe for expansins during hybridization with either RNA or DNA. It also has to be determined, which band is it that is actually hybridizing to the RNA. This step of this thesis was probably the most critical step in the entire process, because it would ideally allow us to generate a probe that could be used to screen for expansins.

However, some problems arose when attempting this procedure. Because RNA is easily degradable, there were several instances where the RT-PCR product was not intense enough to have confidence in the result. Also, as time progressed the primers used for this procedure also began degrade. There is also the possibility that the dilution of the primers could have been carried out incorrectly (not properly thawed and mixed), so continual testing of these primers is suggested before new primers are ordered. However, the significant question that this procedure brought up is demonstrated in figure 4.5 (second gel). As can be seen, more than one band was generated in the RT-PCR product. Other than the assumed expansin band, a larger band (more the size of the ribosomal RNA) can be faintly seen, as well as a very bright primer dimer band. Therefore, there is the possibility that another “probe” is actually doing the hybridizing instead of the expansin probe.

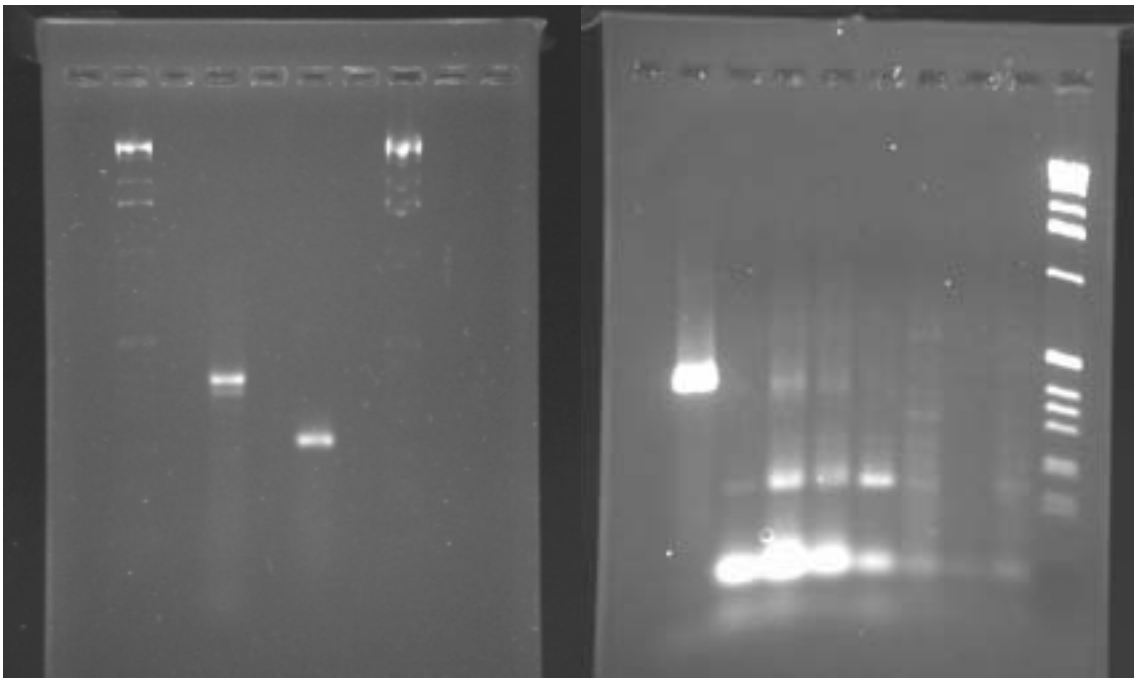


FIGURE 4.5 RT-PCR PRODUCTS GEL 1: (A) 100 BP LADDER (B) EXPANSIN PROBE (C) TUBULIN CONTROL (D) 100 BP LADDER. GEL 2: (1) BLANK (2) 18S RIBOSOMAL CONTROL (3) 0 HR. (4) 2 HR. (5) 4 HR. (6) 6 HR. (7) 8 HR. (8) BLANK (9) O HR. (10) 1 KB LADDER

Northern Blot Analysis

Several attempts were made in order to create a suitable RNA gel for Northern hybridization. After several failed attempts due to improper reagents, RNA degradation and improper pipetting, several modifications were made to the original protocol (Fourney et al., 1988) to obtain a successful result. Specifically, although DEPC water was recommended, it was found that using very sterile water worked just as well. It was also discovered that it was not necessary to add formaldehyde to the gel. Adding formaldehyde to the sample buffer appears to be enough to denature the RNA. This not only saves reagents, but also limits exposure to formaldehyde, which is a very toxic substance.

After the RNA denaturing gel procedure was optimized several successful gels were generated (Figure 4.6 – 4.8). These gels were then subjected to standard Northern analysis previously described in materials and methods. The gels in figures 4.6 and 4.7

were subjected to standard northern blot analysis (Fourney et al., 1988), but problems arose. Specifically, there were limitations to the size of the water bath used, thus limitations to the size of the container hybridization took place in. Because of these problems, hybridization of the probe to the membrane failed. Therefore, optimization of current Northern techniques were implemented and the gel in figure 4.8 was used to obtain a successful hybridization blot (Figure 4.9 – 4.10). As seen in these figures there is one bright band that appears to be up-regulated over time, being expressed the most at 24 hours. However, there also appears to be two smaller lighter bands that can be seen starting at 6 hours. Although, it was initially assumed that the bright band was the expansin gene, when the actual size of the bright band was determined more questions arose. The size of the band was predicted using a ruler, and comparison to the initial Northern gel rRNA bands (25S and 18S rRNA sizes were described by Hamby et al., 1991). The size of the bright band was found to be larger than 2.2 Kb, which is larger than most other expansin genes (usually 1 -2 Kb). The smaller bands, however, seem to fall in the expected range for expansins (~1.5 –1.9 Kb). However, since little is know about pea expansins, and the size of different expansin genes are so varied, it is possible that they may be bigger than other expansin genes. Therefore, after using RT-PCR to create a probe and optimizing northern blot techniques to accommodate limitations in the lab, it was discovered that expression of what is believed to be the expansin gene reaches its maximum in approximately 24 hours (Figure 4.10). However, as stated before there was the possibility that it may not have been expansin, and therefore optimization of RT-PCR is required so that a know expansin probe is generated to screen with. One final result should be noted here. After carefully examining the Northern gel after previous analysis, it was determined that overloading occurred at the 24 hour time slot. Therefore, results leading to the conclusion of increased expression during 24 hours was premature. Several more Northern blots will have to be performed in order to determine the validity of this assumption.

Lane: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

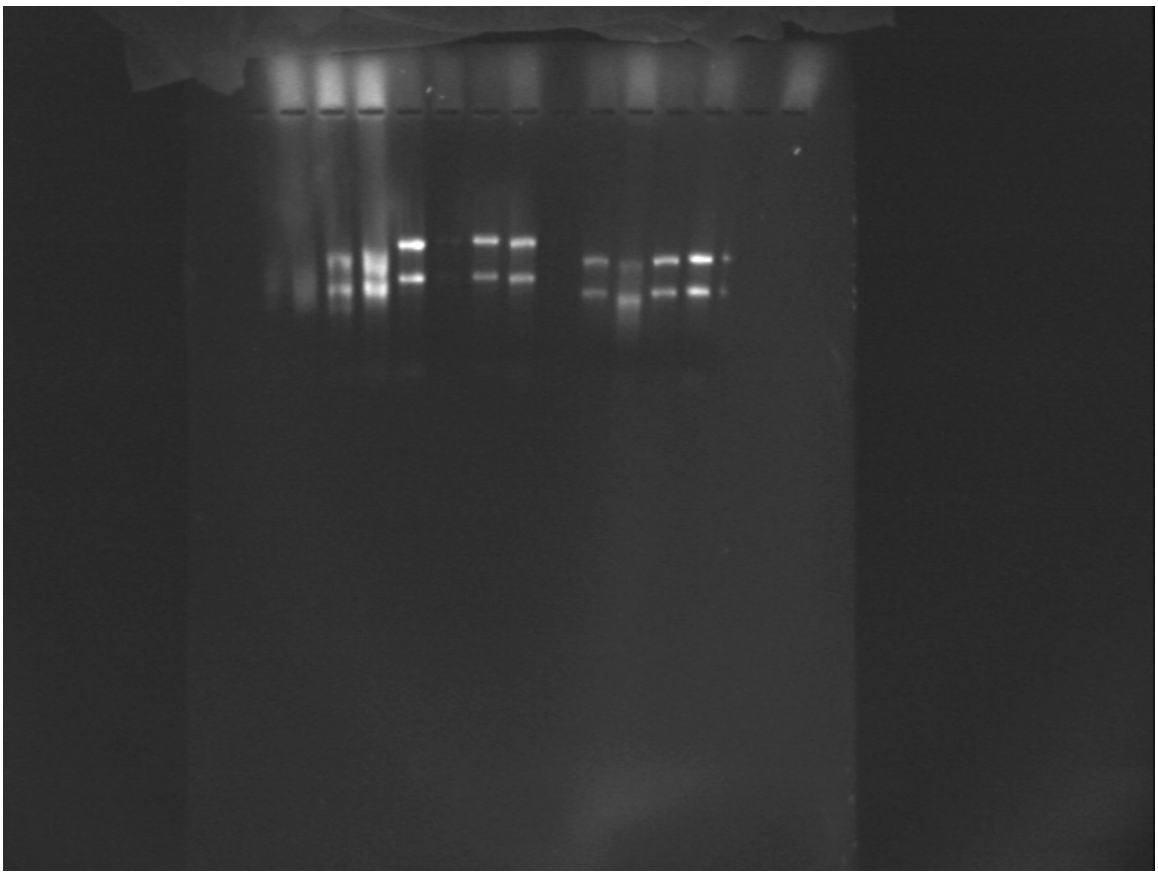


FIGURE 4.6 RNA DENATURING GEL USED FOR NORTHERN BLOT ANALYSIS. THE FIRST BAND IS 25S RRNA APPROX. 2151 BP KB IN SIZE AND THE SECOND BAND IS APPROX. 1755 LANE: (1) BLANK (2) 0 HR. (3) 2 HR. (4) 4HR. (5) 6 HR. (6) 8 HR. (7) 24 HR. (8) 48 HR (9) BLANK (10) 0 HR. (11) 2 HR. (12) 4 HR. (13) 6 HR. (14) 8 HR. (15) BLANK

Lane: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

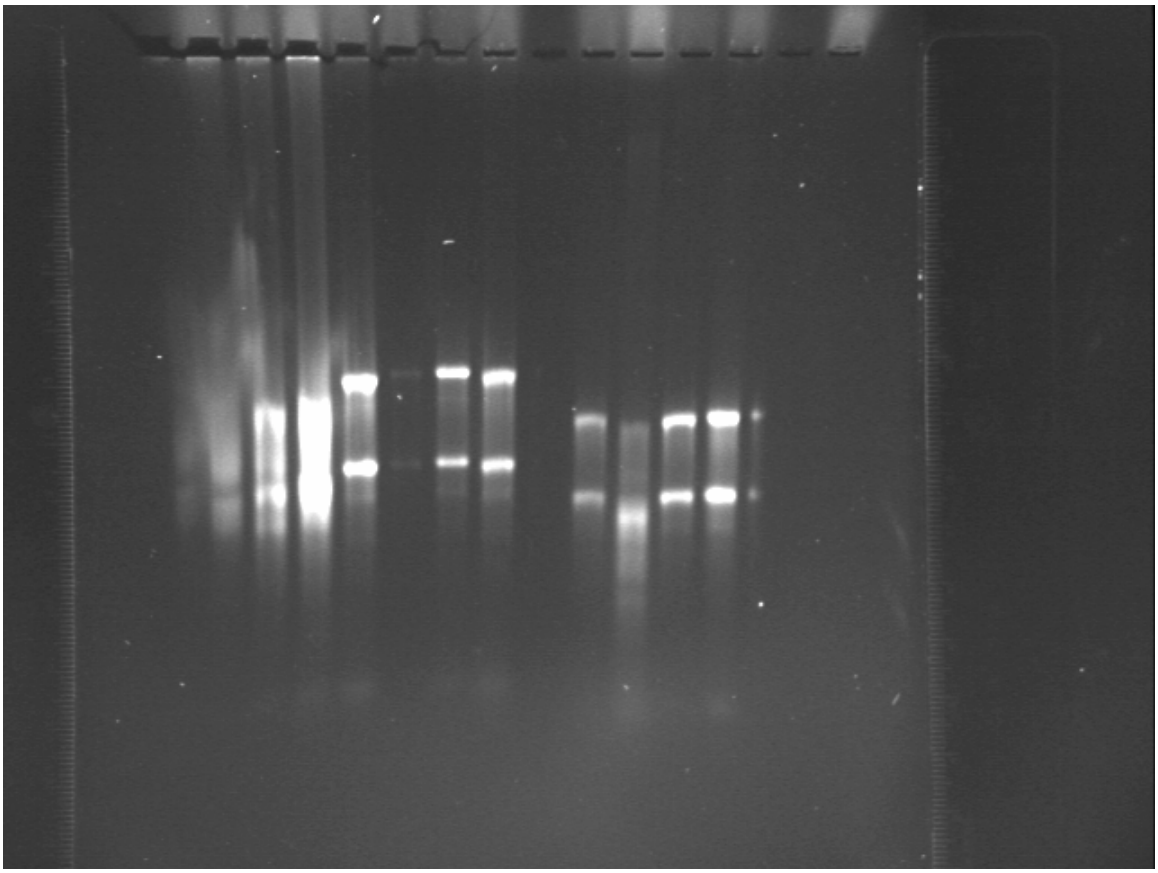


FIGURE 4.7 RNA DENATURING GEL USED FOR NORTHERN BLOT ANALYSIS. LANE: (1) BLANK (2) 0 HR. (3) 2 HR. (4) 4HR. (5) 6 HR. (6) 8 HR. (7) 24 HR. (8) 48 HR (9) BLANK (10) 0 HR. (11) 2 HR. (12) 4 HR. (13) 6 HR. (14) 8 HR. (15) BLANK

Lane: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

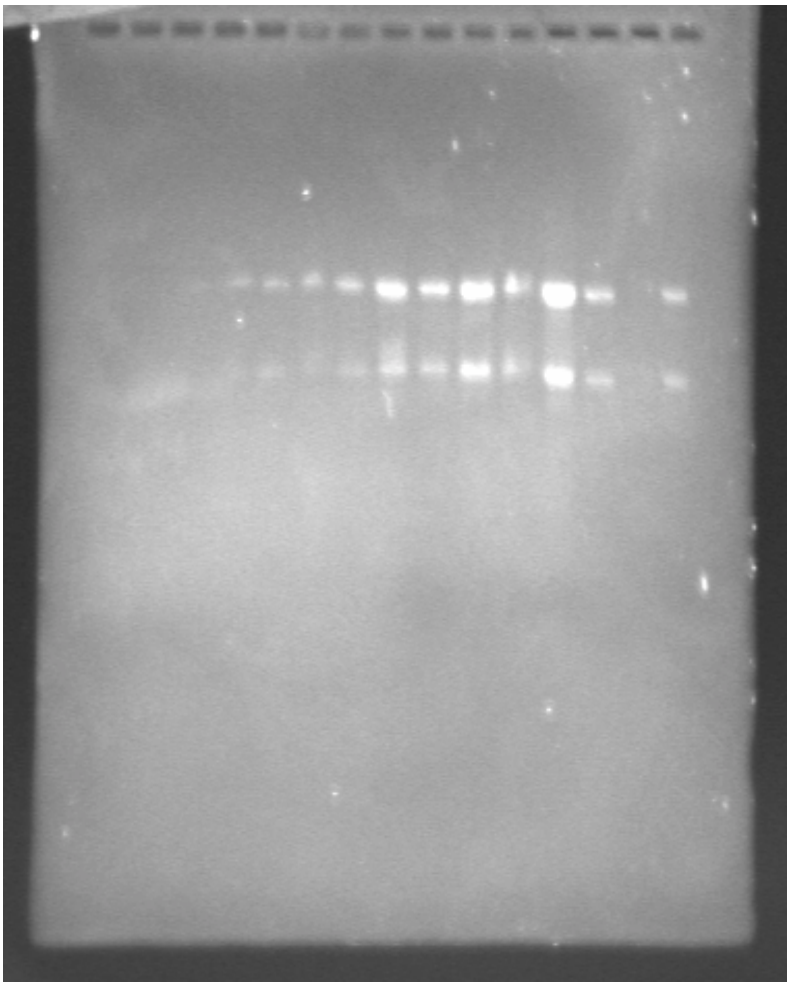


FIGURE 4.8 RNA DENATURING GEL USED FOR NORTHERN BLOT ANALYSIS. LANE: (1) BLANK (2) 0 HR. (3) 2 HR. (4) 4HR. (5) 6 HR. (6) 8 HR. (7) 0 HR. (8) 2 HR (9) 4 HR. (10) 6 HR. (11) 8 HR. (12) 24 HR. (13) 48 HR. (14) BLANK (15) 6 HR. REPEATED

Lane: 7 8 9 10 11 12 13 14 15

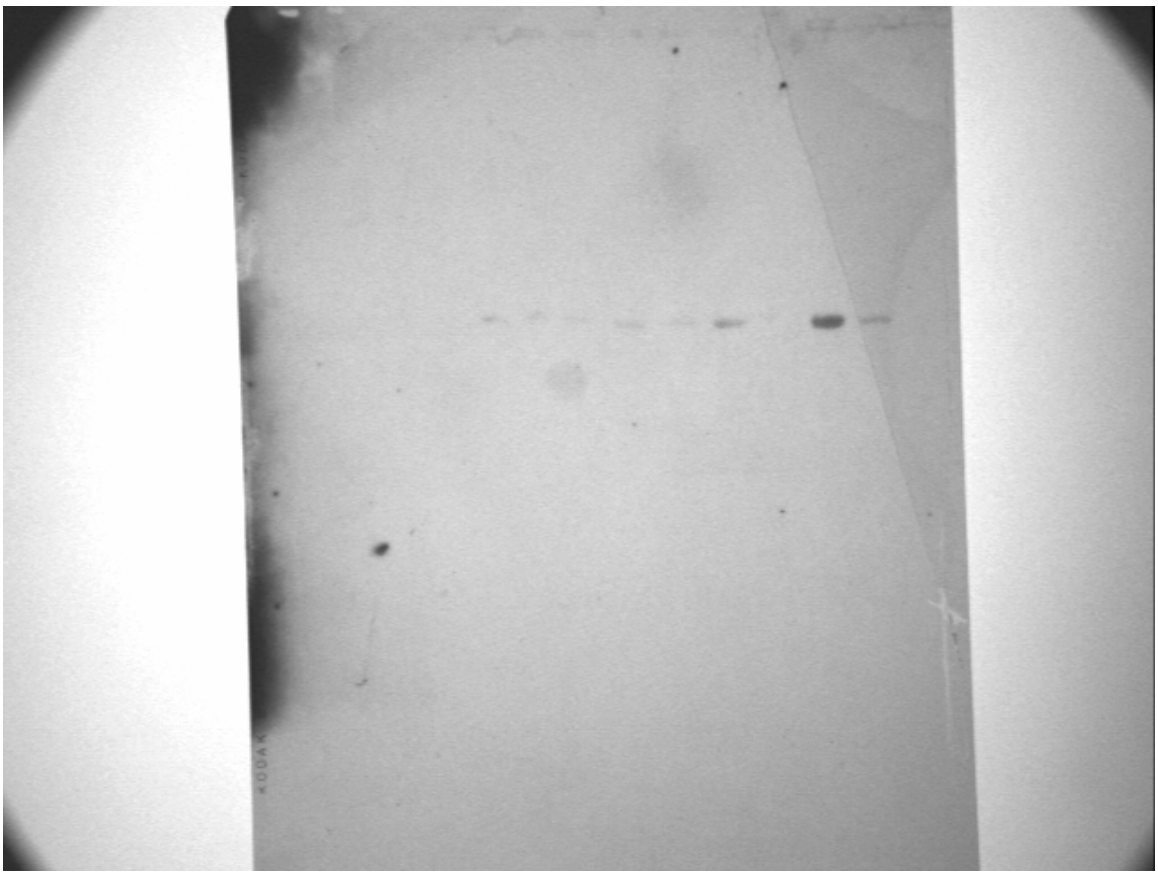


FIGURE 4.9 RESULTS OF NORTHERN BLOT HYBRIDIZATION (10 MIN. EXPOSURE)

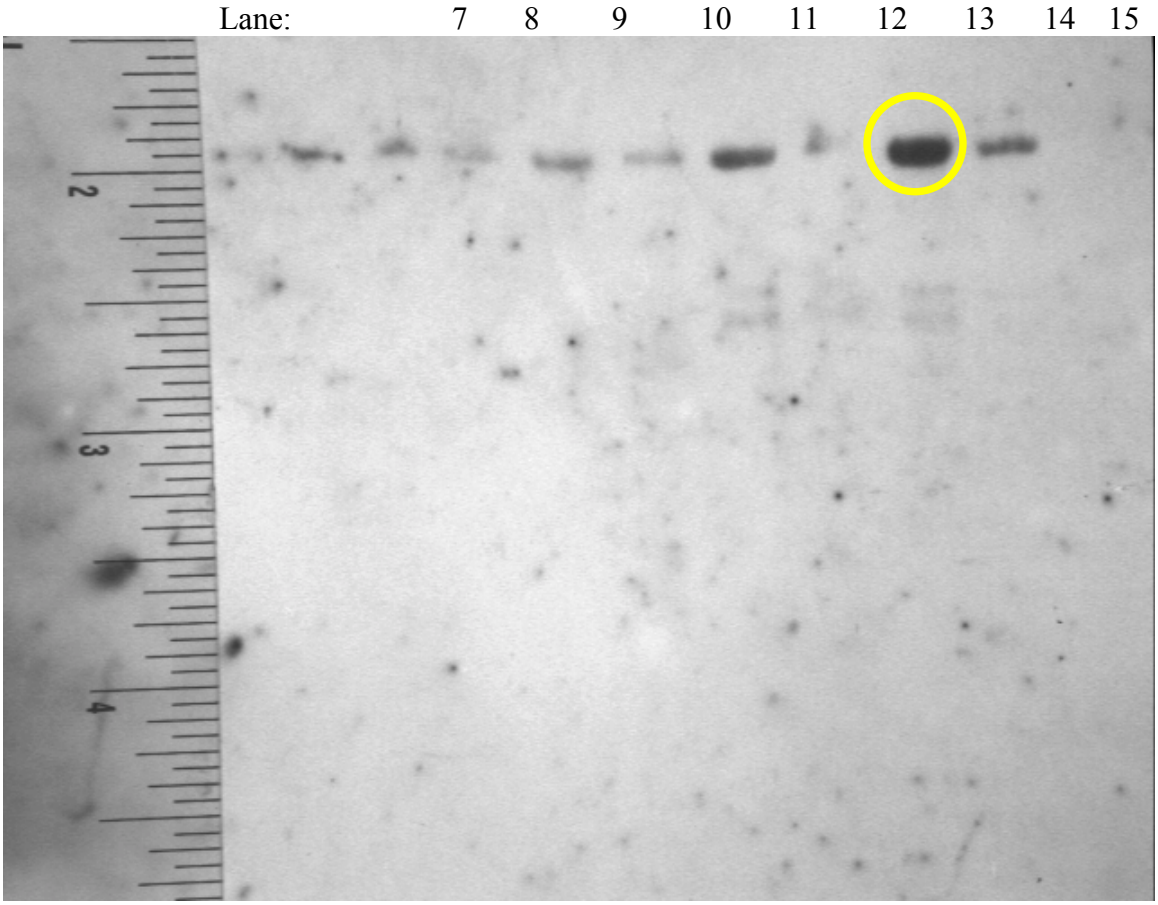


FIGURE 4.10 RESULTS OF NORTHERN BLOT HYBRIDIZATION (45 MIN. EXPOSURE) (7) 0 HR. (8) 2 HR (9) 4 HR. (10) 6 HR. (11) 8 HR. (12) 24 HR. (13) 48 HR. (14) BLANK (15) 6 HR. REPEATED. THE GREATEST INTENSITY OF HYBRIDIZATION OCCURS AT THE 24 HOUR TREATMENT AND THEN DROPS AGAIN DURING THE 48 HOUR TREATMENT. BAND INTENSITY MAY BE DUE TO OVERLOADING.

Semi - Quantitative Analysis of Bands and Their Intensities

Using BioRad's Gel Doc system, semi-quantitave data was gathered about the intensity of the bands present after the Northern blot analysis (Figure 4.10). A total of 384 points of data were gather for each lane, starting at 1 at the well of the gel, and ending at 384 at the end of the marked lane, and then compiled into Figure 4.11.

A typical Northern blot analysis allow the examination of a specific gene of interest, and gives a small notion as to what degree the gene is being expressed. Using BioRad's Gel Documentation imaging system, data was collected allowing for semi-quantitative analysis of the intensities of the band. The higher the intensity the band seen in the Northern blot, it is assumed the more expression of the gene specific for the probe that was present at the time the sample was taken.

The X-ray film from the Northern analysis was placed in the Gel-Doc system on top of a White light transiluminator. An image was acquired, and then lanes were marked using BioRad's Quantity One software program. A 'marked' lane is designated as a lane that has a line drawn down the middle of the bands / wells that make up that lane. Once the lanes were identified, the software computes an average background noise for all lanes and this is used in future calculations.

After having established a background, the computer collected data along the 'marked' line for the length of the lane. The computer measured the intensity (RF value) of the pixel at each data point and records the value as a number. For the samples represented in this study, 384 points of data were taken for each lane. When these points are taken and entered into a Microsoft Excel spreadsheet, a graph was generated Figure 4.12.

The first and most prominent band seen in the Northern blot analysis, is the band of interest to this study. While the figure 4.11 may seem overwhelming, looking only at the data from the first band (Figure 4.12), which shows a better picture of what is actually being expressed. It is evident from figure 4.12 that there is a gradual increase in the amount of expansin being expressed over time, with peak expression at 24 hours. After 24 hours there appears to be a decline in the production of what is thought to be the

expansin gene. However, it is likely that overloading in the 24 hour lane occurred, thus creating questions about the validity of these results.

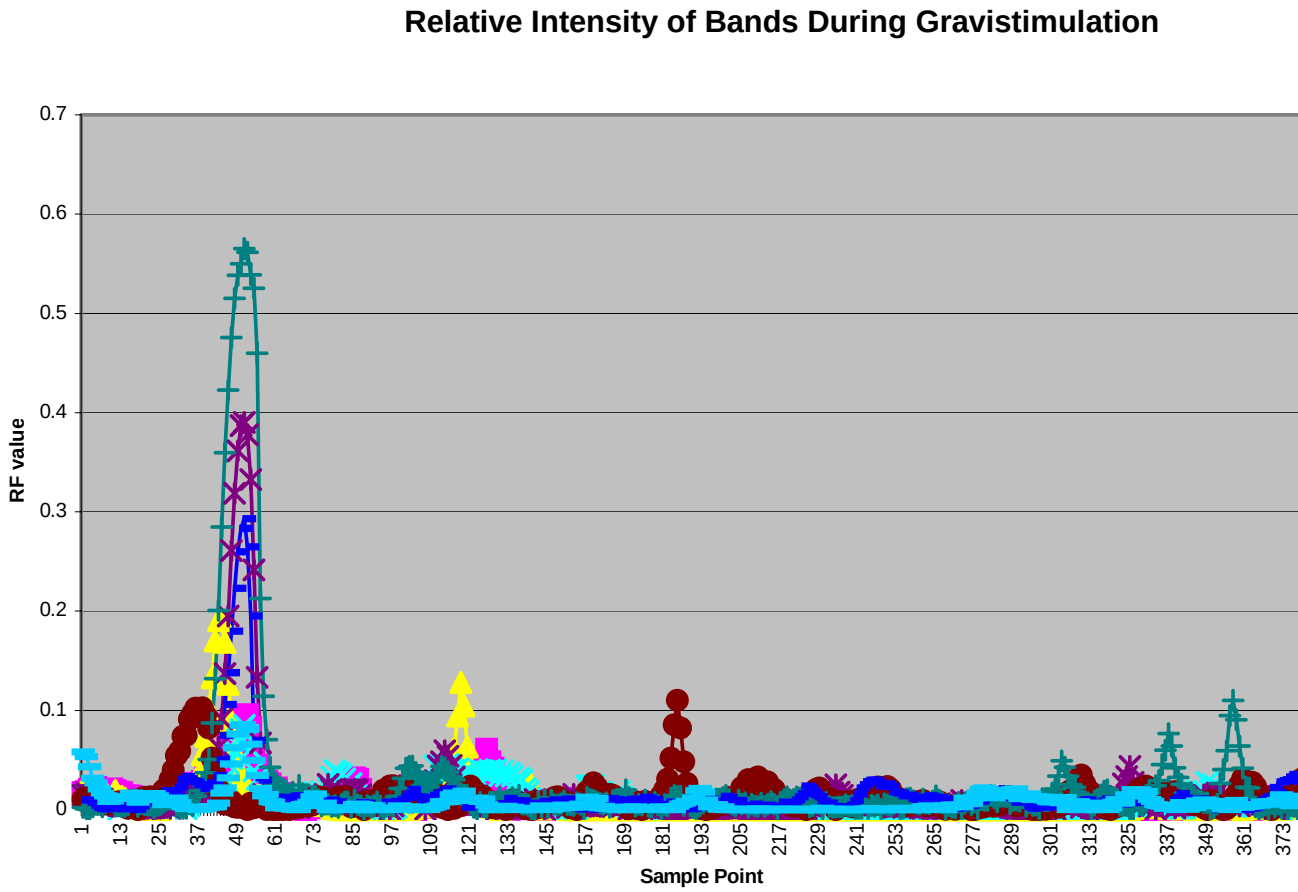


FIGURE 4.11 GRAPHICAL REPRESENTATION BANDS AND THEIR INTENSITIES FOLLOWING NORTHERN BLOT ANALYSIS.

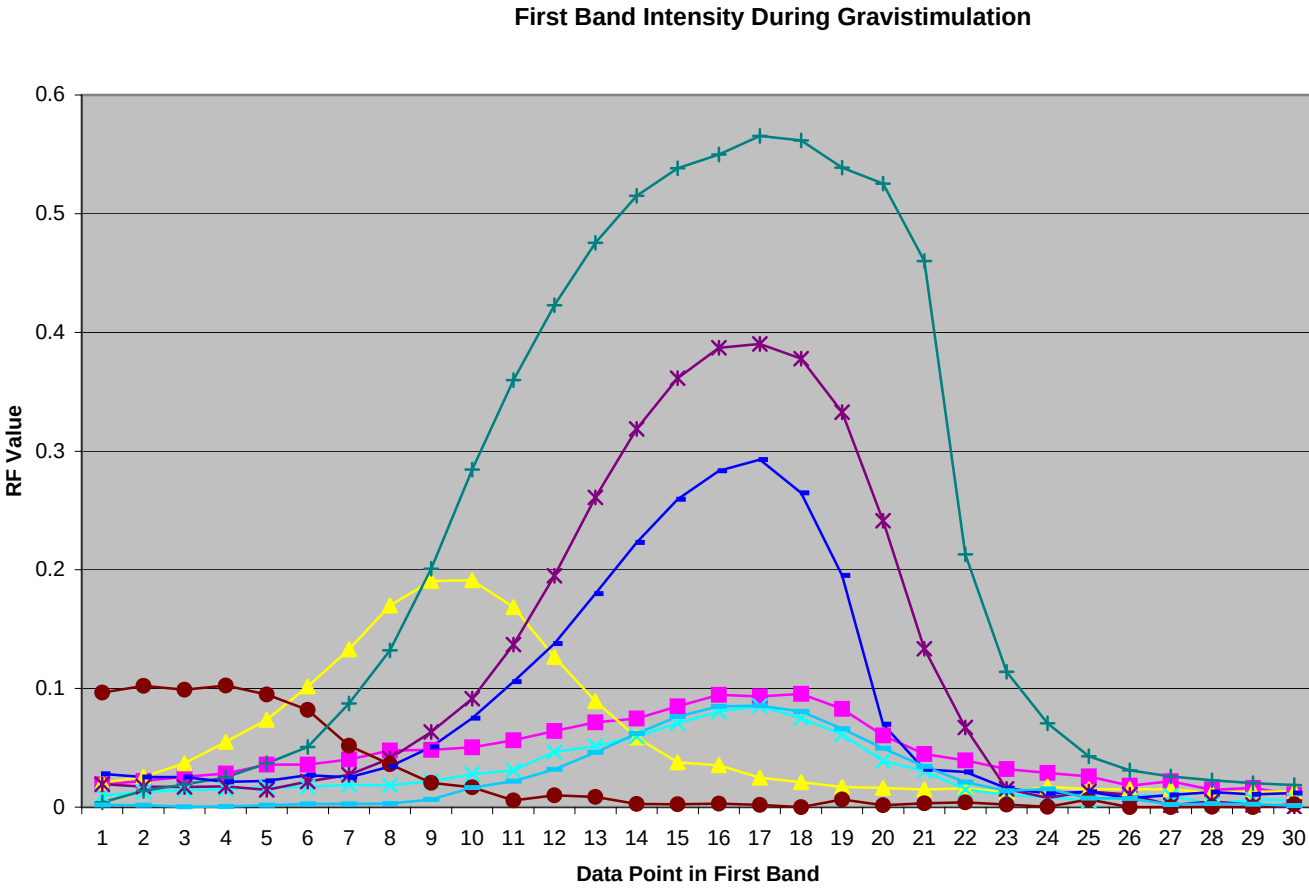


FIGURE 4.12 GRAPHICAL REPRESENTATION OF THE FIRST BAND AND ITS INTENSITIES FOLLOWING NORTHERN BLOT ANALYSIS.USING BIORAD'S GEL DOC SYSTEM, SEMI-QUANTITAVE DATA WAS GATHERED ABOUT THE INTENSITY OF THE FIRST BAND AFTER A SOUTHERN BLOT ANALYSIS. 384 TOTAL POINTS OF DATA WERE GATHERED FOR EACH LANE. THIS FIGURE REPRESENTS POINT 36 THROUGH POINT 64 OF THE 384 POINTS.

Library Screening Analysis

Results from the pea DNA library screening using Stratagene's Lambda Zap II/ EcoRI/CIAP Vector kit are still in the process of being analyzed. Several screenings (Figure 4.13 –4.14) were performed in order to ensure proper hybridization with the expansin cDNA probe. Several plaques were isolated after the second round of screening (Figure 4.14) and excision of the pBluescript phagemid was performed. Six of these released phagemids were randomly selected for minipreps (Promega Wizard plus minipreps) and purified using phenol extraction. These samples were then each subjected to EcoRI digestion to insure that there was in fact an insert (Figure 4.15). Results from the digestion found that there was indeed an insert that appeared to have an EcoRI cut site in the middle. Figure 4.15 demonstrates this digestion, and it can be seen that the phagemid was around 3000 bp in size, which was expected because the phagemid is known to be 2958 bp in size. The first cut band appears to be approximately 1700 bp, while the second band is approximately 1500 bp long. These sizes were determined using BioRad's Quantity One software analysis using Biolabs 1 Kb ladder as a standard to compare against. These six samples were then sent to the DNA Core Facility of Marshall University. However, only four samples were clean enough to be sequenced. These sequences can be found in Appendix K.

Sequencing involved using M13 primers (700 strand is the M13 forward primer and the 800 strand stands for M13 reverse). After the sequences were received, they were entered into the NCBI's BLAST software that looks for homology among sequences. However, unlike what was expected no expansin genes were found to be homologous with our sequences. Instead, we received several hits for ethylene response elements in several plants (i.e. *Arabidopsis*). These results can be construed several different ways. For instance, this may imply that one of the bands generated during the RT-PCR actually hybridizes some section related to these ethylene response elements. There is also the chance that the expansin gene may be very close to something that resembles an ethylene response element. Of course there is always the chance that a "fluke" was created, and that we really didn't get anything at all.

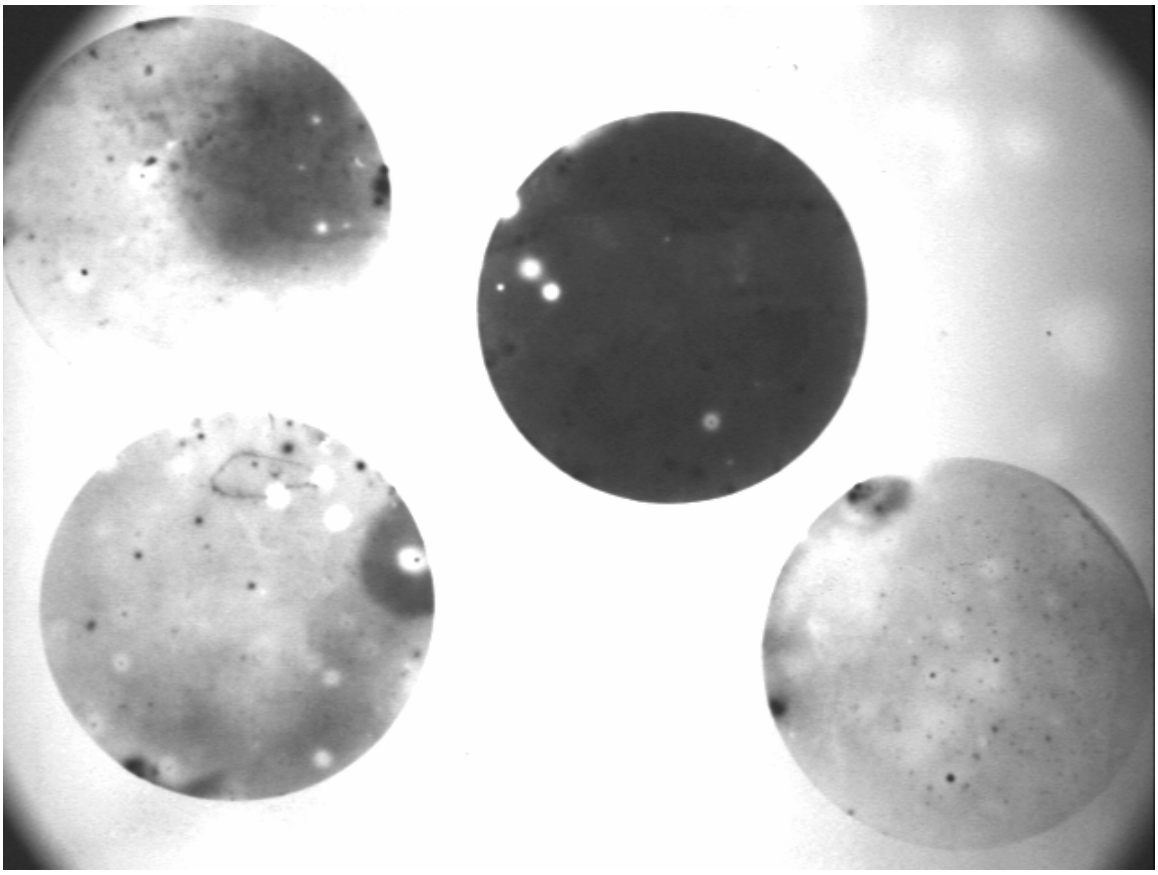


FIGURE 4.13 FIRST ROUND SCREENING OF LAMBDA ZAP II LIBRARY.

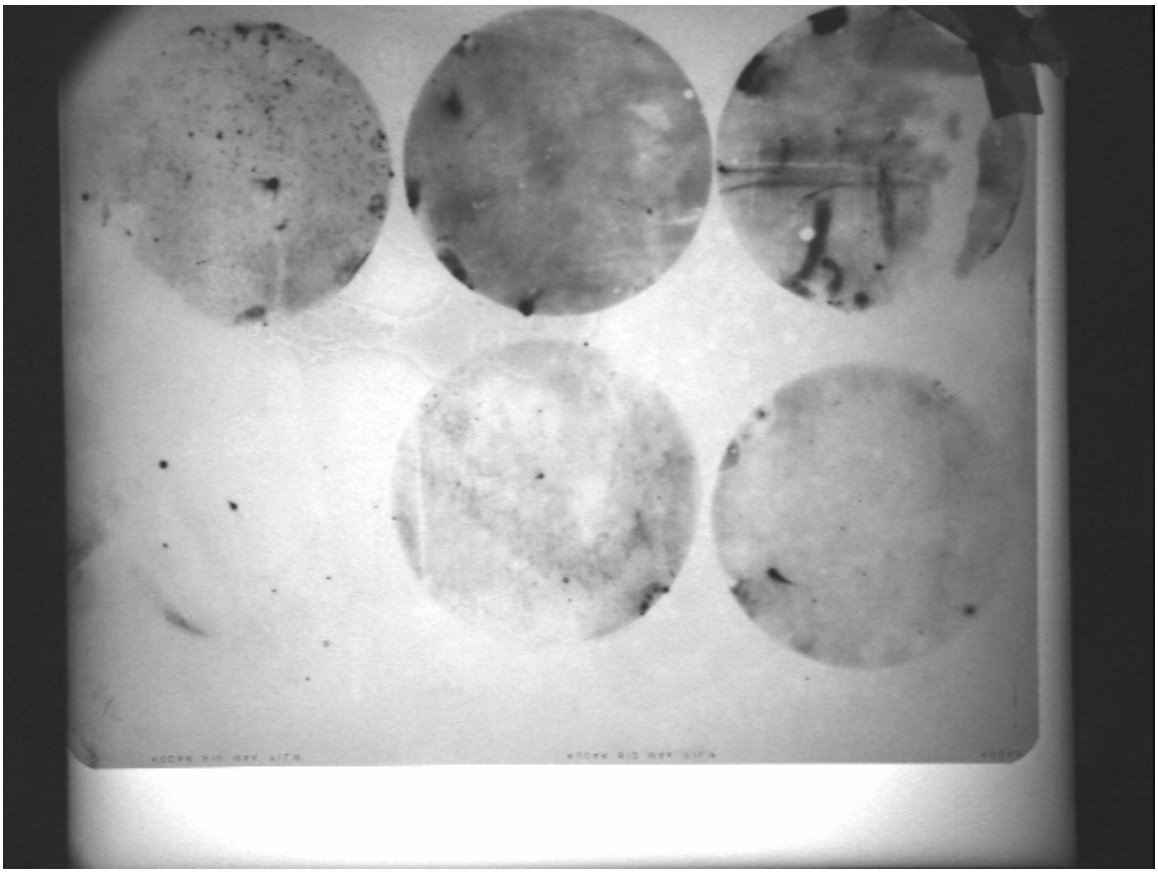


FIGURE 4.14 SECOND SCREENING OF LAMBDA ZAP II LIBRARY

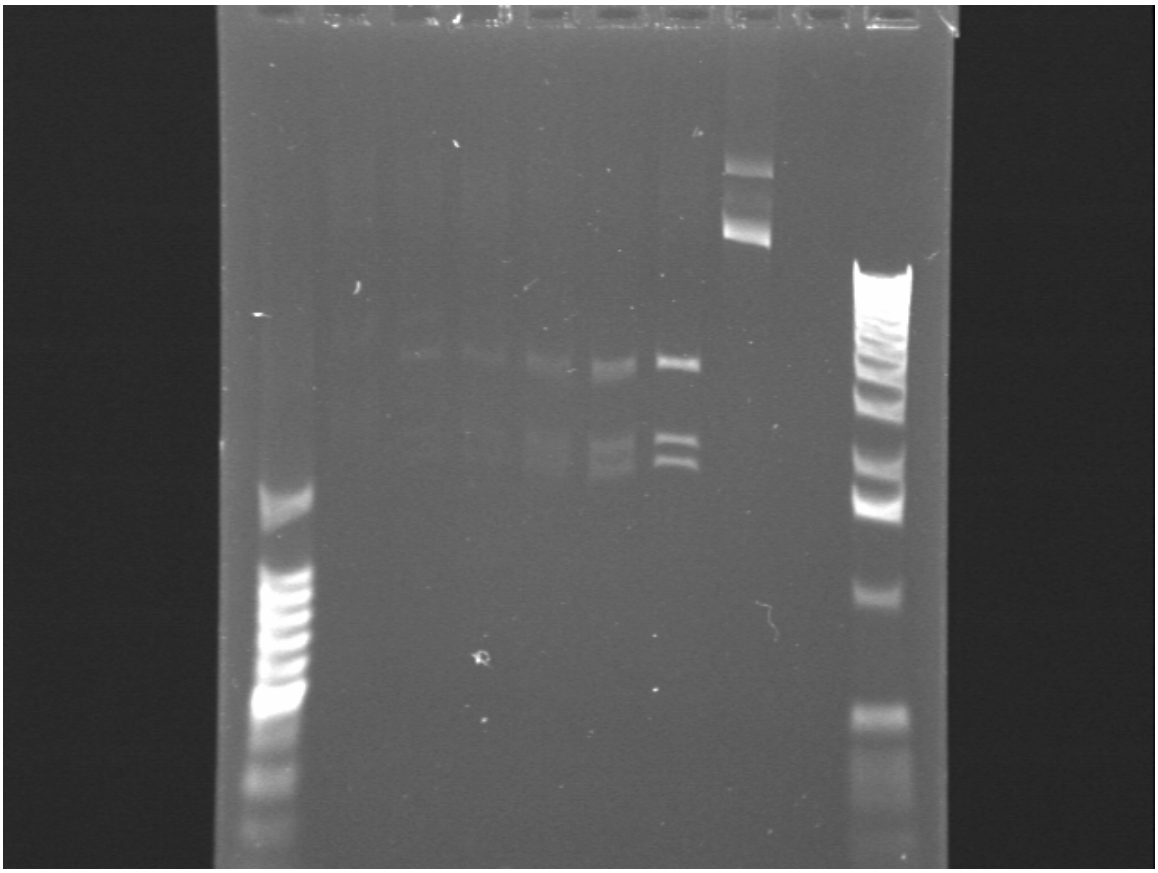


FIGURE 4.15 PLASMID ECORI DIGESTION ON 0.8 % AGAROSE GEL. (1) 100 BP LADDER (2) PLASMID 1 CUT WITH ECORI (3) PLASMID 2 CUT WITH ECORI (4) PLASMID 3 CUT WITH ECORI (5) PLASMID 4 CUT WITH ECORI (6) PLASMID 5 CUT WITH ECORI (7) PLASMID 6 CUT WITH ECORI (8) UNDIGESTED PLASMID 6 (9) BLANK (10) 1 KB LADDER

CHAPTER V

Summary and Conclusion

As stated before, expansins are a highly conserved protein family that induce extension in isolated plant cell walls *in vitro* and have been proposed to disrupt noncovalent interactions between hemicellulose and cellulose microfibrils (Rose et al., 1997). Because the plant primary cell wall acts as a constraint to cell enlargement, this process may be integral to plant cell expansion, and studies of expansins have focused on their role in growth (Rose et al., 1997). SDS PAGE was first performed to determine whether or not there were any differences in the expression of proteins during gravistimulation. It was found that there were indeed enhancements proteins in gravistimulated tissue. Because silver staining is nanogram sensitive and what is know about expansins, there is some degree of confidence that the protein enhancements seen could be possibly related to an up-regulation in expansin genes.

In order to better understand the roles of expansins during gravistimulation, degenerate primers specific for expansins were created (Rose et al. 1997) for purposes of RT-PCR. However, because of their degeneracy, it was determined that there were over 4000 combinations of possible probes, it is difficult to determine how specific the probe used was. In fact, after running the cDNA product on an agarose gel, more than one band could be seen. Therefore, it is probable that more than one probe exists that can hybridize to the RNA. These probes were used in subsequent experiments including Northern and Dot Blotting, and for screening a DNA library. Therefore, due to the degeneracy of the probes any and all results are subject to speculation.

The results from these experiments were mixed as described in the previous chapter, but several questions could be answered by simply repeating the experiments several more times. In particular, optimization of the RT-PCR procedure is critical to ensure that an expansin cDNA probe is indeed being created. Specificity of the probe is critical if confident screening for expansins are to be performed. Thus, it is concluded that although a great deal of preliminary work has been performed, there is still a great deal of work that can be done. First and foremost, optimization and repetition of the aforementioned experiments should be carried out to ensure that expansins are indeed

being screened. Once a very specific probe is created future results can be compared to the findings of this thesis research to verify consistent and reliable results. Several future experiments may also aid in verifying that expansins due indeed exist in *Pisum sativum*, and that they function in the cell wall loosening during gravistimulation. Several of these future experiments have been outlined in Chapter six.

CHAPTER VI

Future Experiments

Re-amplify Phagemid

The quickest and most definable experiment that can be performed immediately is to amplify the phagemid using PCR. This will do several things. First, if finding a gene from the library was a fluke the first time, and then it should not amplify or hybridize when PCR is run using the previous primers described in Appendix L. Second, if the phagemid is amplified with these primers, then the new product can be compared to previous products. Since previous products resulted in at least two bands (three with the primer dimers), this procedure should allow us to see which of the bands is hybridizing to our phagemid. In other words, if the phagemid re-amplifies then it's size can be compared to the size of the other RT PCR products, to see which one it is closer to.

Southern Blot on EcoRI Cut Phagemid

If re-amplification of the phagemid works, then the next logical step would be to perform a Southern Blot. In other words, a gel similar to Figure 4.15 where the phagemid was cut with EcoRI would be created and then hybridized using the product from the PCR reaction described above. This should give us a better idea of where exactly the probe is found giving us a better picture of whether or not it is indeed an expansin gene, or some other gene up-regulated during gravistimulation.

Real Time PCR with Taqman™ Probes.

Real time PCR is a variation of PCR that allows quantitative analysis of expression. In this procedure, cDNA is made, and a Taqman probe is designed for the gene of interest. The Taqman™ Probe has a fluorescent molecule and a repressor molecule incorporated into the probe, which is essentially a 'third primer'. If the Taqman™ Probe has not been incorporated into a PCR product yet the repressor molecule silences the fluorescent molecule, therefore there is no fluorescence. During

the extension phase of the PCR process, the fluorescent molecule is cleaved from the Taqman™ Probe, and the molecule now fluoresces. This process allows the quantification of the amount of cDNA that contains the gene of interest as it is being made. This type of experiment would suit the question of expansin activity over a time course of 48 hours very well. Please refer to Figure 6.1.

Use of the pBluescript Plasmid

"The polylinker of the pBluescript phagemid has 21 unique cloning sites flanked by T3 and T7 promoters and a choice of 6 different primer sites for DNA sequencing. The phagemid had the bacteriophage f1 origin of replication, allowing rescue of single-stranded DNA, which can be used for DNA sequencing or site-directed mutagenesis. Unidirectional deletions can be made with exonuclease III and mung bean nuclease by taking advantage of the unique positioning of 5' and 3' restriction sites. Transcripts made from the T3 and T7 promoters generate riboprobes useful in Southern and Northern blotting, and the *lacZ* promoter may be used to drive expression of fusion proteins suitable for Western blot analysis or protein purification (Stratagene)."

Because of these unique features of the pBluescript phagemid, a relatively straightforward experiment could be constructed. The first several steps would resemble what has been initially done in this thesis. A probe would be constructed to screen the DNA library using RT-PCR. Once a purified sequence was isolated, and then a riboprobe could be constructed from the T3 and T7 promoters. This riboprobe could then be used on Northern or Southern Blot analysis with pea RNA or DNA. This method should allow you to perform blot analysis with much more certainty. Instead of assuming the probe created is hitting the right gene, the sequence of the probe would already be known. Thus, experiments could e carried out with more confidence.

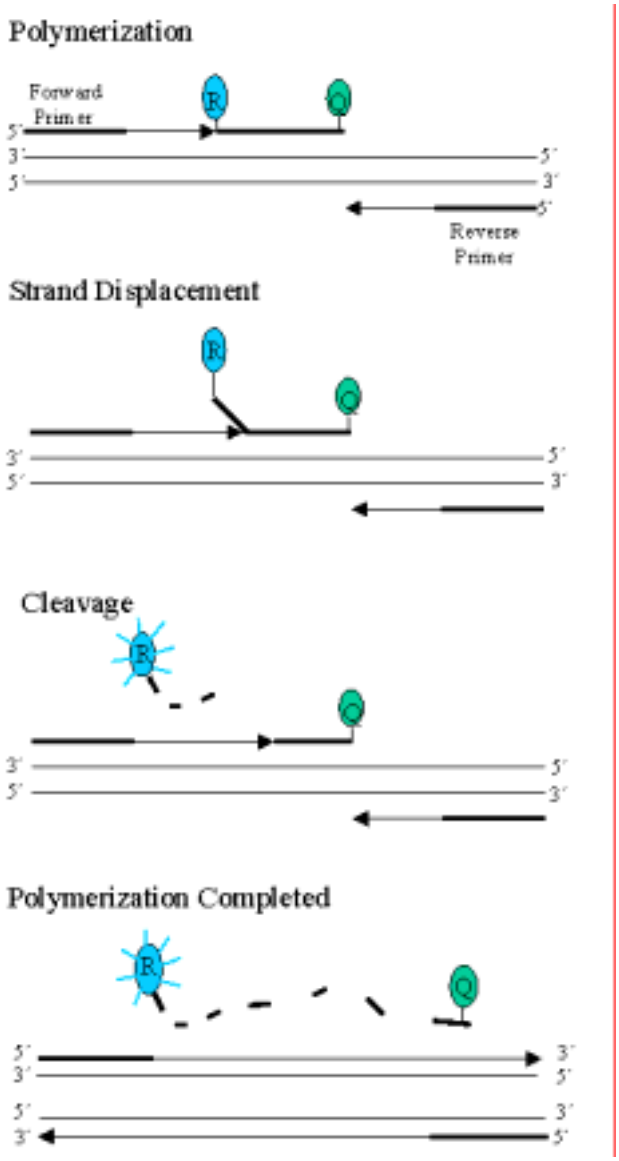


FIGURE 6.1 SCHEMATICE OF HOW TAQMAN AND REAL TIME PCR WORKS

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Appendix

Appendix A

Recipes and Formulas

DNA Gel Electrophoresis: Agarose Gels

1x TBE (Tris, Boric Acid and EDTA) Buffer

Tris Base	10.8 g
Boric Acid	5.5 g
EDTA	0.74 g

Add distilled water to bring final volume to 1 L

PH = 8.0

Stored at room temperature

OR:

10X TBE Buffer*

NaOH	1 g
Tris base	108 g
Boric acid	55 g
EDTA (disodium salt)	7.4 g

Add distilled water to bring final volume to 1 L.

Dilute to 1X TBE before using the buffer in gels or for electrophoresis.

*This is the stock solution.

1X TBE Buffer

10X TBE	100 mL
distilled water	900 mL

Mix well

Save the TBE buffer after running the gel; it can be reused.

0.8 % Agarose Gel

	for a 35 mL gel	**for a 100 mL gel**
Agarose	0.28 g	0.8 g
1X TBE buffer	35 mL	100 mL

Ethidium Bromide (EtBr)

10mg/ml concentration

H_2O	10mL
Ethidium bromide	0.1 gram

Stored at 4 °C

RNA Gel Electrophoresis: Agarose Gels

10X MOPS/EDTA Buffer

MOPS	20.93 g (0.2 M final conc.)
EDTA (0.5 M)	10 ml (10 mM final conc.)
Sodium Acetate (3 M)	8.33 ml (50 mM final conc.)

Add DEPC-treated water to bring final volume to 500 ml

pH = 7.0 (with approx. 2 g NaOH)

Stored at 4 °C

1X MOPS/EDTA Buffer

10X MOPS/EDTA	100 ml
Sterile Water	900 ml

Mix well

Save the TBE buffer after running the gel; it can be reused.

0.8 % Agarose Gel

	for a 35 mL gel	**for a 100 mL gel**
Agarose	0.28 g	0.8 g
1X TBE buffer	35 mL	100 mL

Ethidium Bromide (EtBr)

10mg/ml concentration

H_2O	10mL
Ethidium bromide	0.1 gram

Stored at 4 °C

Northern Blot

***Important Note : It is crucial all glassware, plastic ware, water, etc. is RNASE free. This can be done either by treating everything with DEPC, or by autoclaving frequently.**

10 X MOPS/ EDTA Buffer

MOPS (0.2 M)	41.85 g
Sodium Acetate (50 mM)	16.6 mL
EDTA (0.5 M)	20.0 mL

Fill to 1 Liter with DEPC treated water

Autoclave

Electrophoresis Sample Buffer

Deionized formamide	0.750 mL
10 X MOPS	0.150 mL
Formaldehyde	0.240 mL
Deionized RNase – free water	0.100 mL
Glycerol	0.100 mL
10% (w/v) Bromophenyl Blue	0.080 mL

Freshly prepare prior to loading

Store at –20 C in small aliquots

1 X MOPS/ EDTA Running Buffer

10 X MOPS/EDTA Buffer	200 mL

Bring up to 2 Liters with sterile water

RNA Denaturing Gel

Agarose	1.25 g
10 X MOPS/EDTA	12.5 mL
Sterile water	102.5 mL

Cool to 50 C before pouring

Formaldehyde (37%)	5.1 mL**

** This step can be excluded

SDS/ PAGE Electrophoresis

Acyrlamide/Bis (30% T, 2.67% C)

Acrylamide	87.6 g	(29.2 g/100 ml)
N'N'-bis-methylene-acrylamide	2.4 g	(0.8 g/100 ml)

Make to 300 ml with distilled water.

Filter and store in the refrigerator (30 days max.)

1.5 M Tris-HCl

Tris Base	27.23 g	(18.15 g/100 ml)
HCl (1 N)	Variable	

Dissolve in around 20 ml distilled water and adjust pH using HCl.

Add distilled water to bring final volume to 100 ml.

PH = 8.8

Store in the refrigerator (30 days max.)

0.5 M Tris-HCl

Tris Base	6.0 g
HCl (1 N)	Variable

Dissolve in around 60 ml distilled water and adjust pH using HCl.

Add distilled water to bring final volume to 100 ml.

PH = 6.8

Store in the refrigerator.

10% w/v SDS

SDS	10 g

Dissolve and bring to volume to 100 ml distilled water.

Add distilled water to bring final volume to 100 ml.

Store at room temperature.

5X Running Buffer

Tris Base	15 g
Glycine	72 g
SDS	5 g

Add distilled water to bring final volume to 1 L

pH 8.3 (1 liter)

Dilute to 1X before use.

Store at room temperature until use.

Sample Buffer

Distilled water	4.0 ml
Tris-HCL (0.5 M)	1.0 ml
Glycerol	1.0 ml
10% SDS	1.6 ml
2-beta-mercaptoethanol	0.4 ml
0.05% (w/v) bromophenol blue	0.2 ml
Total volume	8.2 ml

- Dilute samples at least 1:4 with sample buffer, heat at 95 °C for 4 minutes prior to loading.

Plant DNA Extraction

2x CTAB Buffer

For 50ml

1.0 g CTAB Powder (= 2%)*

5.0 ml 1M Tris-HCl pH 8 (= 100mM)

2.0 ml 0.5 M EDTA (= 20mM)

14.0 ml 5 M NaCl (= 1.4M)

Make the solution up to 50 ml with sterile water

*CTAB = Cetyltrimethylammonium bromide

Use immediately

Lambda Zap II Library

LB Phage Broth

LB	2.0 g
Maltose (0.2 %)	0.2 g
MgSO4 (10 mM)	0.1 g

Add distilled water to bring final volume to 100 ml

Autoclave

LB Top Agar

LB	2.0 g
Maltose (0.2 %)	0.2 g
MgSO4 (10 mM)	0.1 g
Agar (0.7 %)	0.7 g

Add distilled water to bring final volume to 100 ml; Autoclave

LB Agar Plates

LB 15.0 g

Agar 11.25 g

Add distilled water to bring final volume to 750 ml

Autoclave

SM Buffer (gelatin)

NaCl 5.8 g

$MgSO_4$ * $7H_2O$ 2.0 g

1 M Tris-HCl (pH 7.5) 50 ml

Gelatin (2 %) 5.0 ml

Add distilled water to bring final volume to 1 L

Autoclave

20 X SSC Buffer

NaCl 175.3 g

Sodium Citrate 88.2 g

Dissolve in around 800 ml distilled water and adjust pH using 10 N NaOH.

PH = 7.0

Add distilled water to bring final volume to 1l.

Primers

100µM Stock
10µM use
1/9 One part primer/ Nine part H_2O

Store in freezer

U.S. Dept Commerce/NOAA/NMFS/NWFSC/Molecular Biology Protocols

Appendix B

RNeasy Mini Protocol for Isolation of Total RNA from Plant Cells and Tissues and Filamentous Fungi (Modified Qiagen Protocol)

RNeasy Plant Mini Kits (20)
Catalog No. 74903
Preparations per kit 20
RNeasy mini spin columns (pink) 20
QIAshredder spin columns (lilac) 20
Collection tubes (1.5 ml) 20
Collection tubes (2 ml) 20
Buffer RLT*† 18 ml
Buffer RLC† 18 ml
Buffer RW1† 18 ml
Buffer RPE‡ 5 ml
RNase-free water 10 ml
Handbook 1
* Buffer RLT is also available separately. See ordering information (page 108).
† Not compatible with disinfecting reagents containing bleach. Contains a guanidine salt, which is an irritant.
Take appropriate safety measures and wear gloves when handling.
‡ Buffer RPE is supplied as a concentrate. Before using for the first time add 4 volumes of ethanol (96–100%)
as indicated on the bottle to obtain a working solution.

• Fresh or frozen tissue can be used. To freeze tissue for long-term storage, flash-freeze in liquid nitrogen, and immediately transfer to –70°C. Tissue can be stored for several months at –70°C. To process, do not allow tissue to thaw during weighing or handling prior to disruption in Buffer RLT. Homogenized lysates (in Buffer RLT, step 4) can also be stored at –70°C for several months. To process frozen lysates, thaw

samples and incubate for 15–20 min at 37°C in a water bath to dissolve salts. Continue with step 5.

• β-Mercaptoethanol (β-ME) must be added to Buffer RLT or Buffer RLC before use. β-ME is toxic; dispense in a fume hood and wear appropriate protective clothing. Add 10 µl β-ME per 1 ml Buffer RLT. Buffer RLT is stable for 1 month after addition of β-ME.

• Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%), as indicated on the bottle, to obtain a working solution.

• Buffer RLT may form a precipitate upon storage. If necessary, redissolve by warming, and then place at room temperature.

• All steps of the RNeasy protocol should be performed at room temperature. During the procedure, work quickly.

• All centrifugation steps are performed at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.

1. Determine the amount of plant material. Do not use more than 100 mg.
Weighing tissue is the most accurate way to determine the amount.

2. Immediately place the weighed sample in liquid nitrogen, and grind thoroughly with a mortar and pestle. Decant powder and liquid nitrogen into an RNase-free, liquid nitrogen –cooled, 2 ml microcentrifuge tube (not supplied). Allow the liquid nitrogen to evaporate, but do not allow the tissue to thaw. Continue immediately with step 3.
RNA in plant material is not protected after harvesting until the sample is flash frozen in liquid nitrogen. Frozen tissue should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible.

3. Add 450 µl Buffer RLT or Buffer RLC to a maximum of 100 mg tissue powder. Vortex vigorously. Pipet the lysate directly onto a QIAshredder spin column (lilac) placed in 2 ml collection tube, and centrifuge for 2 min at maximum speed. Carefully transfer the supernatant of the flow-through fraction to a new microcentrifuge tube (not supplied) without disturbing the cell-debris pellet in the collection tube. Use only this supernatant in subsequent steps.

It may be necessary to cut the end off the pipette tip in order to pipette the lysate onto the QIAshredder spin column. Centrifugation through the QIAshredder spin column removes cell debris and simultaneously homogenizes the lysate. While most of the cell debris is retained on the QIAshredder spin column, a very small amount of cell debris will pass through and form a pellet in the collection tube. Be careful not to disturb this pellet when transferring the lysate to a new microcentrifuge tube (not supplied).

5. Add 0.5 volume (usually 225 µl) ethanol (96–100%) to the cleared lysate, and mix immediately by pipetting. Do not centrifuge. Continue without delay with step 6.
If some lysate is lost during step 4, adjust volume of ethanol accordingly.
A precipitate may form after the addition of ethanol, but this will not affect the RNeasy procedure.

6. Apply sample (usually 650 µl), including any precipitate that may have formed, to an RNeasy mini column (pink) placed in a 2 ml collection tube (supplied). Close the tube gently, and centrifuge for 15 s at ≥8000 x g (≥10,000 rpm). Discard the flow-through.* Reuse the collection tube in step 7.
If the volume exceeds 700 µl, load aliquots successively onto the RNeasy column, and centrifuge as above. Discard the flow-through after each centrifugation step.*

7. Add 700 µl Buffer RW1 to the RNeasy column. Close the tube gently, and centrifuge for 15 s at ≥8000 x g (≥10,000 rpm) to wash the column. Discard the flow-through and collection tube.*

8. Transfer the RNeasy column into a new 2 ml collection tube (supplied). Pipet 500 µl Buffer RPE onto the RNeasy column. Close the tube gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the column. Discard the flow-through.
Reuse the collection tube in step 9.
Note: Buffer RPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RPE before use.

9. Add another 500 µl Buffer RPE to the RNeasy column. Close the tube gently, and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to dry the RNeasy silica-gel membrane. Continue directly with step 10, or, to eliminate any chance of possible Buffer RPE carryover, continue first with step 9a.
Note: Following the centrifugation, remove the RNeasy mini column from the collection tube carefully so the column does not contact the flow-through as this will result in carryover of ethanol.

9a. Discard flow-through and re-centrifuge in a microcentrifuge at full speed for 1 min.

10. To elute, transfer the RNeasy column to a new 1.5 ml collection tube (supplied). Pipet 30–50 µl RNase-free water directly onto the RNeasy silica-gel membrane. Close the tube gently, and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute.

11. If the expected RNA yield is >20 µg, repeat the elution step (step 10) as described with a second volume of RNase-free water. Elute into the same collection tube.
To obtain a higher total RNA concentration, this second elution step may be performed by using the first elute (from step 10). The yield will be 15–30% less than the yield obtained using a second volume of RNase-free water, but the final concentration will be higher.

Appendix C

Setting up and running a Reverse Transcription PCR

Wear Gloves at all times

1. Pull needed ingredients out of the freezer and start them thawing.
2. Prepare an ice bucket to keep the master mix in while mixing.
3. Measure out the following ingredients in the respective proportions. All ingredients are listed in µl.

H_2O	10.0 µl
$MgSO_4$ (25 mM)	2.0 µl
AMV 5 X Buffer	10.0 µl
Primer 1	5.0 µl (50 picomoles)
Primer 2	5.0 µl (50 picomoles)
DNTP's	1.0 µl
Tf1 polymerase	1.0 µl
AMV RT	1.0 µl
RNA	0.5 µg

4. Label a 1.5 ml microcentrafugue tube "master mix" in which the H_2O, $MgSO_4$, 5 X AMV RT Buffer, DNTP's, and *Tf1* polymerase will be placed.
5. Label all thin wall tubes that will be placed in the thermocycler.
6. Starting with the largest volume, add all ingredients except for the AMV Reverse Transcriptase , which will be added at the very end. Be sure to change pipette tips between each ingredient.
7. Add AMV RT, be sure to pipette up and down to make sure that you have all of the AMV RT out of the pipette tip and into the master mix.
8. Vortex master mix for 5 seconds.
9. Pipette 25 µl out of the master mix tube into each of the waiting thin-walled tubes that are going into the thermocycler.
10. Add enough template RNA to deliver 0.5 –1.0 µg of RNA to the each of the corresponding thin-walled tubes, and close the lid on the tube. Tap the side gently to knock down anything that may be on the sidewall. When adding the template RNA be sure that you get the tip of the pipette all the way down to the bottom of the thin-walled tube.

11. Place the thin-walled tubes into the thermocycler and press start.
12. When the thermocycler is finished remove the thin-walled tubes from the machine and secure them in a rack and place the rack in a refrigerator.

Appendix D

How to prepare for and run an agarose gel electrophoresis

0.8% Agarose Gel

An 0.8% agarose gel means that there are 0.8 grams of agarose per 100 mL of buffer. The amount of agarose you will add, depends upon the size of the gel. The mini-gels generally need between 30-50 mL of solution per gel.

Preparing and Casting an Agarose Gel

1. Weigh 1 gram of agarose on a folded piece of weighing paper and add to empty 250 ml bottle or flask.

2. Add 125 ml of 1X TBE buffer solution to agarose. Note: The container should never be filled more than half-way in order to prevent the solution from boiling over.

3. If a bottle is used, cap the bottle loosely to release air during boiling. If you using a flask, cover the opening and neck of flask with a kim wipe.

4. Mix solution by swirling. Microwave the agarose solution at high heat until powder is completely dissolved. The length of time required will vary depending on the microwave oven. The molten agarose solution should look clear (no floating particles or "lens" are visible).

5. To keep the agarose liquefied (for example, during several biology classes), store the bottle or flask of agarose in a hot water bath. Be sure that the bottle or flask is covered to prevent evaporation.

6. Cool to 55 °C before pouring gel solution into the casting tray.
7. While the mixture cools, pace the plastic comb in the slots on the side of the gel tray. The comb teeth should not touch the bottom of the tray.

8. Pour the agarose mixture into the gel tray until the comb teeth are immersed about 6 mm or 1/4" into the agarose. Push any bubbles to the side farthest from the wells.

9. Allow the agarose gel to cool until solidified. The gel will appear a cloudy white color and will feel cool to the touch (about 20 minutes). Gels can be stored, wrapped in plastic wrap, in the refrigerator for a few days.

10. If there is agarose left over in the container, you can let the agarose solidify and store it at room temperature until next use. Be sure that the container is well covered.

- Do not let the solution boil over in the microwave.
- Use gloves. The glass flasks get hot!
- The gel solution can be remelted in the microwave if it solidifies in the flask.

Gels can be made 1-2 days ahead of time and store in the refrigerator wrapped in plastic wrap.

http://biotech.biology.arizona.edu/labs/Electrophoresis_dyes_teach.html

Loading and Running the Gel

1. Remove the comb from the wells by pulling straight up on the comb. Be careful not to tear the wells as you remove the comb. Remove the tape from both ends of the gel tray.

2. Place the gel tray in the gel box with the wells closest to the negative (black) electrode.

3. Add enough 1X TBE buffer to fill the buffer tank and submerge the gel about 1/4 inch.

4. On a piece parafilm, place N dots of 1-3 µl of loading dye, (N is the number of samples that are going to be examined.)

5. With a new tip each time, add x µl (dependant on the sample and experiment) of your sample to one of the dots of loading dye.

6. Mix the solution by pipetting the dye up and down into the 10 uL DNA sample. The solution should turn blue in color. The gel loading dye contains glycerol, which makes the DNA more dense so that it will sink into the wells. It also contains dye molecules, which are smaller and travel faster through the gel than the DNA molecules. The dye molecules provide a visual tracking method so you know how far the DNA has traveled through the gel.

7. Rinse the pipette or capillary tube with clean water by gently pipetting up and down two or three times.

8. Make a sketch of the gel; include all of the wells and the positive (red) and negative (black) electrodes. Indicate under each well the name of the sample to be loaded.

9. Pipette 10 - 12 uL of the first sample, usually a reference sample such as a 1 kb ladder, into the first well. Keep the tip of the pipettor ABOVE the well. The DNA will sink into the well because it has been mixed with loading dye. If you puncture the bottom of the well your DNA run out the bottom of the gel into the buffer tank.
    - *Molecular biologists often use a size standard called a 1 kb DNA ladder. The DNA ladder produces several different sized fragments or bands and can be used to estimate the size of an unknown DNA fragment.*

10. Rinse the pipette or capillary tube in the buffer tank by gently pipetting up and down two or three times.

11. Load the next sample into the next well. Repeat steps #7-8 until all of the samples and controls have been loaded into the gel. Record on the sketch the order the samples were loaded (including standards!)

- *A concentration standard is used to estimate how much DNA is present in a sample.*

12. Close the top of the box. Plug the leads into the gel box. The black lead is the negative lead and should be plugged in closest to the wells. The red lead is the positive lead and should be plugged in furthest from the wells.
    - *Remember: Red ahead.*

13. Plug the other end of the leads into the power source and turn it on. Run the gel at between 80-120 volts until the loading dye has traveled 1/2 - 3/4 of the way down the gel (about 30-45 minutes).

14. Turn off the power supply. Unplug the leads and the power supply before opening the gel box.

15. The gels may be wrapped in Syran Wrap and stored overnight until they can be stained.

Staining the DNA

The DNA must be stained in order to be seen. DNA can be stained with fluorescent or chemical dyes. Ethidium bromide, an ultraviolet (UV) fluorescent stain, was used because it shows very small amounts of DNA and is faster to use. Ethidium bromide, however, is a carcinogen and a mutagen. Extreme caution must be used when dealing with this compound.

ALWAYS WEAR GLOVES WHEN WORKING WITH ETHIDIUM BROMIDE.

1. Place the gel in a plastic container, which is a little larger than the gel and immerse it in 1X TBE buffer. Add 1-4 μg ethidium bromide to the buffer and place gel in the dark. Stain the gel for approximately 10-30 minutes.

2. Wearing gloves, carefully transfer gel to another plastic container, containing 1X TBE buffer only. Allow gel to be destained for a short period of time (10 min.) to help remove background color.

3. Place gel in the bio-rad gel documentation system, and turn on the UV light tray.

4. Look at your results, zoom and focus on gel until you get a clear picture, save image.

Appendix E

Restriction Digest of DNA

Protocol

1. Prepare the reaction for restriction digestion by adding the following reagents in the order listed to a microcentrifuge tube:

sterile ddH20	q.s (where "q.s." means quantity sufficient)
10X assay buffer	one-tenth volume
DNA	x ul
restriction enzyme*	y ul (1-10 units per ug DNA)
Total volume	z ul

	Ecor I	PST I	e+p	BAM I	Hind III	b+h
Final Volume	200 ul	200 ul	200 ul	200 ul	200 ul	200 ul
10X Buffer	20	20	20	20	20	20
pea DNA	5	5	5	5	5	5
BSA 100 X	2	2	2	2	2	2
Enzyme	6	6	6	6	6	6
water	167	167	167	167	167	167
NH4AC	20	20	20	20	20	20
100% ETOH	400	400	400	400	400	400
blue-orange dye	2	2	2	2	2	2
each well	12	12	12	12	12	12

2. Gently mix by pipetting and incubate the reaction at the appropriate temperature (typically 37degC) for 1-3 hours.

3. Inactivate the enzyme(s) by heating at 70-100degC for 10 minutes or by phenol extraction (see the vendor's catalog to determine the degree of heat inactivation for a given enzyme). Prior to use in further protocols such as dephosphorylation or ligation, an aliquot of the digestion should be assayed by agarose gel electrophoresis versus non-digested DNA and a size marker, if necessary.

4. Run DNA on an 0.8% agarose gel.

5. Double digestion with 2 different restriction enzymes, both enzymes can be added at the same time if they use the same buffer. If not, digest with one enzyme, precipitate the DNA. Then resuspend the DNA and digest with the second enzyme in its appropriate buffer.

Precipitation of DNA

Isopropanol precipitation

1) Add to the DNA solution 1/2 vol. of 7.5M NH4OAc and 2 vol. isopropanol (1 vol. isopropanol is sufficient to ppt. the DNA).

2) Incubate at room temperature for 10 minutes.

3) Spin for 10 minutes. Wash with 80% ethanol.

4) Air-dry DNA and resuspend in water or buffer of choice.

Appendix F

SDS/Polyacrylamide Gel Electrophoresis

Standard SDS-polyacrylamide gel electrophoresis (Laemmli)--gel preparation. Volumes given are sufficient for small (8 cm X 10 cm X 1.5 mm) gel format (10 ml of monomer). Scale up volumes as needed.

1. Pour the Separating Gel

Set up your gel apparatus, prepare separating gel monomer. Add TEMED just prior to pouring gel (I "pour" the gels using a Pasteur pipette and a rubber bulb). Allow to polymerize before adding stacking gel by overlaying gently with water or n-butanol. With higher % gels, one can immediately pour the stacking gel on the unpolymerized separating gel. Be careful not to mix the two layers.

Separating Gels, in 0.375 M Tris, pH 8.8				
	7%	**10%**	**12%**	**15%**
distilled H_2O	5.1 ml	4.1 ml	3.4 ml	2.4 ml
1.5 M Tris-HCl, pH 8.8	2.5 ml	2.5 ml	2.5 ml	2.5 ml
20% (w/v) SDS	0.05 ml	0.05 ml	0.05 ml	0.05 ml
Acrylamide/Bis-acrylamide (30%/0.8% w/v)	2.3 ml	3.3 ml	4.0 ml	5.0 ml
10% (w/v) ammonium persulfate	0.05 ml	0.05 ml	0.05 ml	0.05 ml
TEMED	0.005 ml	0.005 ml	0.005 ml	0.005 ml
Total monomer	**10.005 ml**	**10.005 ml**	**10.005 ml**	**10.005 ml**

2. **Pour the Stacking Gel**

After the separating gel has polymerized, decant the overlay, prepare the stacking monomer, add the TEMED, and pour. Insert the comb and allow to polymerize completely before running.

Stacking Gels, 4.0% gel, 0.125 M Tris, pH 6.8	
distilled H_2O	3.075 ml
0.5 M Tris-HCl, pH 6.8	1.25 ml
20% (w/v) SDS	0.025 ml
Acrylamide/Bis-acrylamide (30%/0.8% w/v)	0.67 ml
10% (w/v) ammonium persulfate	0.025 ml
TEMED	0.005 ml
Total Stack monomer	**5.05 ml**

For best results:

- Make ammonium persulfate solution fresh daily.
- Degas solutions for 15 minutes or more (around 30 in. Hg vacuum) before adding TEMED for 15 min at room temperature.

3. **Running the gel**

Gels are usually run at a constant current, 25-50 mA, depending on gel size in 1X Running Buffer.

Appendix G

Northern Blotting: Efficient RNA Staining and Transfer

(original protocol by R. M. Fourney, S. Miyakoshi, R. S. Day III, and M. C. Peterson (Focus 10:1), modifications of this protocol were carried out by Elizabeth Murray and Sherrine Ibrahim)

Methods

Glassware should be silanized and baked at 200oC for > 4 hours. Plastic ware should be DEP-treated and autoclaved.

Buffers

All solutions should be DEPC-treated and autoclaved except SDS and Denharts, which should be made with DEP-treated, autoclaved H20. The pH of the 37% formaldehyde solution should be adjusted to 7.0.

10x MOPS/EDTA Buffer: 0.2 M Mops[3-(N-morpholino) propanesulfonic acid], 50 mM sodium acetate, 10 mM EDTA adjusted to pH 7.0 and autoclaved.

Electrophoresis Sample Buffer (freshly prepared prior to loading or stored at -20oC in small aliquots): 0.75 ml deionized formamide, 0.15 ml 10x MOPS, 0.24 ml formaldehyde, 0.1ml deionized RNase-free H2O, 0.1 ml glycerol, 0.08 ml 10% (w/v) bromophenol blue.

Electrophoresis buffer: 1x MOPS/EDTA buffer.

Other solutions required: 37% formaldehyde (pH 7.0), 10x SSC, 1.0 mg/ml ethidium bromide in deionized RNase-free H20.

Sample Preparation

1. RNA was isolated using the qiagen RNeasy Mini Protocol for Isolation of Total RNA from Plant Cells and Tissues and Filamentous Fungi (catalog # 74103).
2. Elute the sample in 60 μl RNase free water.
3. Add 5-10 μg total RNA to an RNase-free micro -centrifuge tube.
4. Adjust volume to 5 μl with DEPC-treated, autoclaved water. If necessary, concentrate dilute samples by lyophilization.
5. Add 25 μl Electrophoresis Sample Buffer and 1μl ethidium bromide solution and heat at 65º C for 15 minutes and do not put on ice.
6. Load the sample on the gel.

Gel Preparation and Electrophoresis

1. Add 1.0-1.5 g agarose, 10 ml 10x MOPS and 87 ml diethyl pyrocarbonate (DEPC)-treated autoclaved H20 to an RNase-free flask (we prefer the sterile orange cap flasks).
2. Dissolve agarose and let cool to 50 °C.
3. In a fume hood, introduce 5.1 ml 37% formaldehyde into the agarose solution (optional), gently mix, and than pour the gel into an RNase-free 11 x 14-cm gel tray.
4. Allow the gel to sit for 1 h before use (if waiting longer than an hour to load gel cover gel in saran wrap).
5. Prior to loading the gel, flush sample wells by pipetting electrophoresis buffer in and out of the wells.
6. Load wells and electrophorese the gel at 30-60 V (constant voltage) at room temperature for 2-6 hours. Bromophenol blue migrates ~10 cm into the gel.

Transfer Preparation

1. Prepare the gel for transfer by soaking it for three 20-minute periods in sterile water at room temperature with gentle shaking, changing the water in between soaks.
2. During the gel washing procedure, pre-wet the membrane in distilled water for 5 minutes.
3. Transfer the RNA in Sterile water by capillary action using a sponge to enhance capillary action (it was found that not using a sponge worked just as well).
4. Fix the RNA to the membrane by baking for 2 h at 80oC (optional).

Comments

1. If a thick gel or a high concentration of agarose (>1.3%) has been used, it is beneficial to allow the gel to soak for 10-20 minutes in very sterile water
2. The authors recommend using a charge-modified nylon membrane because it has a higher binding capacity than nitrocellulose and will withstand multiple stripping and reprobing. We found that Hybond N+ work really well.
3. The RNA bound to the membrane can be viewed for transfer efficiency or photographed under UV transillumination: no staining is required.

Hybridization and Autoradiography

Membranes were prehybridized overnight, and then hybridized for 12-36 h at 42oC with gentle shaking.

1. Wash the membranes: two 20-minute washes in 2x SSC, 1% SDS at room temperature followed by two 20-minute washes in 1x SSC, 0.5% SDS at 50-55oC.

2. Autoradiograph with Kodak XAR film at -70oC for 2-3 days using Dupont Cronex Lightning Plus screens.

Comments:

1. We routinely use the following prehybridization and hybridization solution: 50% deionized formamide, 0.47x Denharts solution, 4.7x SSPE, 0.1% SDS, 0.18 mg/ml denatured salmon sperm carrier DNA, and 5% dextran sulfate. Note that the addition of fat-free mild powder to the prehybridization solution (0.34% final concentration) will decrease the background on membranes, which have higher binding capacities.
2. We use DNA probes labeled to high specific activity (>1x10^8cpm/ug) using [a-^32P]CTP by the random primer labeling procedure (1).

(1) Feinberg, A.P. and Vogelstein, B. 1983. *Anal. Biochem.* 132, 6.

Appendix H

Wild Type Lambda Packaging

Preparing the bacteria:
Streak the bacteria on a plate of LB-Tetracycline Agar (12.5 mg/ml) XL1 Blue MFR strain of bacteria. Plate out bacteria for subsequent steps.

Inoculate media with a single colony and grow the bacteria to OD600 < 1.0(LB with 0.2% maltose with 10 mM MgSO4). This is 4-6 hrs with shaking at 37 degrees C OR longer at 30 degrees C at 200 rpm.
Centrifuge the bacteria at 500 X G for 10 minutes using sterile blue tubes and a cold rotor. Gently resuspend the cells in half the original volume of sterile 10 mM MgSO4. Dilute the cells until the OD is 0.5 with more sterile 10 mM Mg SO4.

Use the bacteria IMMEDIATELY after dilution.

Don't add antibiotic to this media. The antibiotic will bind to the cell wall and inhibit the ability of the phage to bind to it. Overgrowing the bacteria will give you dead bacteria that will still bind phage particles, and this will reduce your titer by taking some of the phage non-productively out of the solution.

Packaging Reaction:

Thaw out the frozen wild type control DNA. Place on ice and mix well.

Thaw out the packaging extract between your fingers until it just begins to thaw.

Add 1 ul of the control DNA (about 0.2 ug) through very gentle stirring or pipetting up and down with NO BUBBLES introduced.

Spin down the tube for 3-5 seconds.

Incubate the tube at room temperature for 2 hours. Do not exceed 2 hours.

Add 1000 ml SM buffer to the tube.

Add 200 ul chloroform to the tube and mix contents gently.

Spin the tube briefly to sediment the debris.

The supernatant carrying the phage is ready for tittering. The supernatant may be stored for up to 1 month at 4 degrees centigrade.

Titering the phage:

Prepare two consecutive 1/100 dilutions of packaging reaction in sterile SM buffer.
Take 10 ul and add to 990 ul. Mix well. This is 1/100 dilution
Take 10 ul of the first dilution and add to 990 ul. Mix well. This is now 1/10,000 dilution. Add 10 ul of this to 200 ul of the prepared bacteria in the 10 mM $MgSO_4$.

Incubate at 37 degrees C for 15 minutes.

Add this to 3 ml of to agar melted and cooled to 48 degrees C.

Quickly pour this onto dried, pre-warmed LB agar plates. Let set. Place in incubator right side up. Then after about 1 hour, turn plates over upside-down. .

Incubate the plates at least 12 hours and count plates.

Dilutions:
10 ul full strength
*10 ul/90 ul = 1/10 dilution
**10 of the */90 = 1/100 dilution
10 of the ** /90 = 1/1000 dilution

Timeline:
Night before-
Pour plates.
Plate bacteria at 37 degrees C.

Next day:
Make top agar and dispense while still hot into sterile disposable tubes. Keep tubes at 48 degrees C.

About 11:00 grow a single colony of bacteria in sterile media with maltose and MgSO4.

About 12:00 grow a single colony of bacteria in sterile media as above.

About 1:00 grow a single colony of bacteria in the shake in sterile media.

Put LB plates upside down in incubator to dry out and warm up.

3:00: DNA thawed out.
3:15: Packaging extract thawed out and mixed with DNA. Incubate 2 hrs.
3:15-5:00 Monitor the growing bacteria and pick the flask closest to OD < 1.00.
Centrifuge the bacteria at 500 X G for 10 minutes using sterile blue tubes and a cold rotor.

Gently resuspend the cells in half the original volume of sterile 10 mM MgSO4. Dilute the cells until the OD is 0.5 with more sterile 10 mM Mg SO4.

5:15: Add Chloroform to phage and centrifuge.

5:20: Dilute the phage in sterile SM buffer.

5:30: Begin plating phage mixed with bacteria.

Number Description

17095 North2South® Chemiluminescent Nucleic Acid Hybridization and Detection Kit,

Appendix I

Procedure for Nucleic Acid Hybridization and Detection

Pierce Biotechnologies North2South® Chemiluminescent Hybridization and Detection Kit.

A. Pre-Hybridization and Hybridization

Notes: Heat incubator to appropriate temperature (e.g., 55 °C for DNA hybrids or 65 °C for RNA:RNA hybrids).

- Quantitate the probe using a spectrophotometer.

1. Equilibrate the North2South® Hybridization Buffer to room temperature (RT).
Note: This buffer has been specifically optimized for use with the North2South® Chemiluminescent Hybridization and Detection Kit.

2. Place the blot in a container such as a 50 ml centrifuge tube and add sufficient Hybridization Buffer to completely cover the blot. Use at least 0.1 ml per cm^2 of membrane.

3. Seal the container and pre-hybridize the membrane with shaking or rotating for at least 30 minutes. For DNA hybrids incubate at 55°C; for RNA:RNA hybrids incubate at 65°C.

4. While pre-hybridizing, denature the biotinylated DNA probe. Heat DNA probe at 100°C for 10 minutes and place on ice for 5 minutes.

5. After the pre-hybridization, add the denatured biotinylated probe.
For biotinylated RNA probes, add 3-5 ng of probe per ml of hybridization solution; for biotinylated DNA probes, add ~30 ng of probe per ml of hybridization solution.

6. Incubate overnight with shaking or rotating at 55°C for DNA hybrids or 65°C for RNA:RNA hybrids.

B. Stringency Washes

1. On the following day, equilibrate the North2South® Hybridization Stringency Wash Buffer (2X) to RT. Once the Wash Buffer is fully in solution, add an equal volume of sterile ultra pure water.

The resulting 1X buffer contains 2X SSC/0.1% SDS.

Note: Other wash conditions may be used to increase or decrease stringency if required. In general, stringency increases with increasing temperature and decreasing ionic strength.

2. Wash the membrane three times for 15-20 minutes per wash with agitation. Use 0.2 ml of 1X Stringency Wash Buffer per cm^2 of membrane and perform washes at 55□C for DNA hybrids or 65□C for RNA:RNA hybrids.

C. Probe Detection

Note: Use clean forceps to handle only the corners of the membrane. For optimal results, rinse forceps with ethanol and
allow them to dry between steps.

1. Decant the Stringency Wash Buffer and add sufficient North2South® Blocking Buffer to generously cover the membrane. Use at least 0.25 ml/cm^2 of membrane. Incubate with shaking or rotating for 15 minutes at RT.

2. Determine the amount of Streptavidin-HRP to add to obtain a 1:300 final dilution in the tube with the blot but do not add it yet. Decant a portion of the Blocking Buffer solution from the tube containing the membrane into a separate tube.

3. Add the Streptavidin-HRP conjugate from step 2 to the separated buffer. Add the buffer/Streptavidin-HRP mix to the tube containing the membrane and incubate for 15 minutes at RT with agitation.
Note: Performing steps 2 and 3 as described will prevent the undiluted conjugate from coming in direct contact with the
membrane, which would produce undesirable results.

4. Dilute North2South® Wash Buffer (4X) to 1X with sterile ultra pure water. Wash the membrane 4 x 5 minutes with 1X North2South® Wash Buffer at RT with agitation. Place membrane into a clean wash container for the next step.

5. Add North2South® Substrate Equilibration Buffer to container with the membrane. Use 0.25 ml/cm2 of membrane. Incubate for 5 minutes at RT with agitation.

D. Substrate Development
1. Prepare the Substrate Working Solution by mixing equal volumes of the North2South® Luminol/Enhancer Solution and North2South® Stable Peroxide Solution. Prepare enough solution to completely cover the membrane (i.e., approximately 0.1 ml/cm^2).
Note: The Working Solution is stable for 6 hours at room temperature. Exposure to the sun or any other intense light can harm the Working Solution. For best results keep the Working Solution in an amber bottle and avoid prolonged exposure to any intense light. Typical laboratory lighting will not harm the Working Solution.
2. Place the moist membrane on a tray or a piece of plastic wrap and cover with the Substrate Working Solution. Incubate for 5 minutes at RT. Make sure the membrane is fully covered with substrate.

3. Drain the substrate from the membrane surface and transfer the moist membrane to a sheet protector or wrap in clear plastic wrap. Remove any trapped air bubbles or wrinkles within the plastic wrap and blot any substrate that may have leaked out the edges.

4. Expose the blot to film for 1 minute. Shorter or longer exposures may be required to obtain desired signal.

5. Develop the film according to the manufacturer's instructions.
Note: For images that have been overexposed, or that show high background and/or speckling, use the Erase-It™ Background Eliminator Kit (Product No. 21065).
Note: For best results, use a new membrane for each hybridization procedure. If stripping and reuse of the membrane is required, test the stripping protocol by incubating the stripped membrane in Substrate Working Solution, placing it in a sheet protector and exposing it to film for at least 30 minutes. If no bands are visible upon developing the film, the membrane can be rinsed with Wash Buffer and reprobed.

Appendix J

Using ClustalX for sequence alignment 1.81

1. Open notepad. You cannot use Microsoft Word for the preparation of the input file. The file needs to be saved as a *.txt file.
2. Each sequence needs to entered as follows:

Start:	Each sequence starts with the ">" symbol.
Label:	The next ten character spaces directly after the start symbol are assigned to the sequence as the name or label. I suggest that you do not use anything longer than 8 characters.
Space:	This space separates the data label from any other information that you may want to include.
Extra data:	It is important to note that this information will not be shown anywhere other than the input file. PAUP, and CLUSTALX will not use this data. This is the chance to enter any other data that you would like to see.
Hard Return:	Enter a hard return. This separates the label information from the actual sequence itself.
Sequence data:	Here is the sequence data
Hard return:	Hard return and start again for the next sequence.

There is no symbol or character needed to indicate the end of a file. Please see the example input file below.

>123asdf Location data
GTCGTCGTCGTCGTCGTCGTCGTCGTCGT……..etc.

>234asdf location data
CTGCTGCTGCTGCTGCTGCTGCTGCTGCT……..etc.

3. Once the input file has been created, save the file as *.txt.
4. Open CLUSTALX 1.81. Open the "File" menu, and select "load sequences". This will bring up a browsing window, find your file and click open.
5. Open the "Alignment" menu and select "Output format options". Select the nexus format, and click close

Open the "Alignment" menu and select "Do complete alignment" At this point CLUSTALX will generate the alignment and save it to the disk. The file generated can be used right away by PAUP.

Appendix K

Phagemid DNA Sequences

Phagemid DNA "raw data" sequences used or generated for this thesis are listed in the following appendix. The following sequences were further analyzed using the Marshall University DNA Core Facility Chromas software that can be downloaded at: http://www.microbiology.adelaide.edu.au/www/FTP/MOLBIOL/CHROMAS/CHROMAS.HTM (results not shown). After further analysis it is hoped these sequences will be submitted to GenBank, a public database for sequences, that can be found at the following address: http://www.ncbi.nlm.nih.gov/

```
Name:            1_700
Report run date: Sat Jun 22 14:05:23 EDT 2002
Sample remarks:
Statistics:      169 As, 92 Cs, 99 Gs, 99 Ts, 54 Ambigs, 0
Gaps

   1 aWAGgNgatD gGaCNVGCCc cTgaGGCGCG GTATCGVTAA GCTTGATATC
  51 GAATTCATGA TCCAATACTT GATTGTGCAG AAAACAGCTT AGGAATGCCA
 101 AACAAAGGAC AGAGAAGTAG AGATTAATAG ATTCAGCAGT GAACAACAAG
 151 ATTCAAAATA CTGTAACAAA GTGAAAATTA GAAAAATCGA CCTTCAATAC
 201 AAGAGTGTGG CCTTGTTGCT TTCGAAACAC AATAGCGAAA CAAAGCTCGG
 251 ACCTCGAACA GAACAGAGCC AATTGACCAT GAACCCGACT ACGACTACGA
 301 ATCAGGATCC AGAAAAAGAC TATACCAACT CGACTATATt WRGACAACCG
 351 TTTCGGGTGT AAAARGGTGG TTTCAATAGA GGTTTGTGGC TGGATTTCGA
 401 GCTATTTcGC AGCTTGTTTC ACTACTtttD GtCAGAtTTt tCaGCAGATt
 451 CGCAGCTGAt TWWRCWGCWC WCWHcCGMAg AAAAAAaRRA GGAgAAAARC
 501 aAAAMAVAAa RCA
```

```
Name:            1_800
Report run date: Sat Jun 22 14:05:36 EDT 2002
Sample remarks:
```

Statistics: 151 As, 75 Cs, 99 Gs, 95 Ts, 35 Ambigs, 0 Gaps

```
   1 HWcACWAAAG RGMACAMAAG CTGGAGCTCC ACCGCGGTGG CGGCCGCTCT
  51 AGAACTAGTG GaTCCcCCGG GCTGCAGGAA TTCAATCGTT CAGTCAAAAA
 101 AGACAAGTTA GCATGTTTGA tCAGATACCA AGAAGTTCGT CGTCCTCAGA
 151 AACTAATTCC TCAAAAGGAA GTAGTGCTGA CGWagaGSYg aCATCATATT
 201 CCATCAAGAG AAAAGACGCA AATACAGACA AAGAAGAATT AAAAGATGAA
 251 GAAAAGCATT ATATAGGaGT TAGAAAAAGG CCATGGGGGA AATATGCAGC
 301 AGAAATAAGA GATTCAACAA GAAATGGAAT TAGGGTATGG TTAGGTACAt
 351 TTRATACTGT TGAAGAAGCT GCTTTAGCTT ATGAtCAAGC TGCATTTTCA
 401 ATgCGTGGtG CTTTAGCATT TcTCAaTttT cCAaTGGgAA AHSTCCAAGt
 451 tTCAC
```

Name: 3_700
Report run date: Sat Jun 22 14:05:26 EDT 2002
Sample remarks:
Statistics: 153 As, 88 Cs, 100 Gs, 122 Ts, 61 Ambigs, 0 Gaps

```
   1 atAGggMGaW tggGTACRVG SCCCCcTcGN GgtcGaCGGT ATcgaTAAGC
  51 TTGATATCGA ATTCATGaTC CAATACTTGA TTGTGCAGAA AACAGCTTAG
 101 GAATGCCAAA CAAAGGACAG RGAAGTAGAG ATTAATAGAT TCAGCAGTGA
 151 ACAACAAGAT TCAAAATACT GTAACAAAGT GAAAATTAGA AAAATCGACC
 201 TTCAATACAA GAGTGTGGCC TTGTTGCTTT CGAAACACAA TAGCGAAACA
 251 AAGCTCGGaC CTCGAACAGA ACAGAGCCAA TTGACCATGA ACCCGACTAC
 301 GACTACGAAT cAGGATCCAG AAAAAGACTA TACCAACTMG ACTATATWWR
 351 GACAACCGtT TcGGGTGTAA AARGGtGGtT TCAATAGAGG TTTgTgGCTG
 401 GaTTTcGAGC TATTtHGCAG CTTGTTTCAC TACTTTtDGT CAGATTTTtc
 451 AGCAGATTCG CMGCTGATTT TDCTGCTCTC TAACGcTGAA TTTtaggtGG
 501 TGTTttRCAA TTMTDTtTBC TGCA
```

Name: 3_800
Report run date: Sat Jun 22 14:05:40 EDT 2002
Sample remarks:
Statistics: 137 As, 63 Cs, 96 Gs, 85 Ts, 75 Ambigs, 0 Gaps

```
   1 AMMWMAMWAa AGRGACAaAA GCTGGAGCTC CACCGCGGTG GCGGCCgcTC
  51 TAGAACTAGT GGATCCYCGG GCTGCAGGAA TTCAATMGTT CAGTcAAAAA
 101 AGACAAGTTA GCATGTTTGA tCAGATACCA AGAAGtTCGT CGTCCTCAGA
 151 AACTAATTCC TCAAAAGGAA GTAGTGCTGA CGMAGAGGTG ACAWaAtATt
 201 cCMtCaMGaG AAAAGaCGCA AATACAGACA AAGAAGAaTT AAAAGATGAA
 251 GAAAAGCATT ATATAGGAGT TAGAAAAAgG CCATGGGKgG AaATATGMAG
 301 CAGAaaTAAG AGATTCARCA AGAAaTGGcA TTaGGGTATG GtTAGGtACA
```

```
 351 TTTKATACTR tTGAAGAAGc TgCTTtAGCT tATGMtCARg CTGCATTWtM
 401 AATGCGtRGT GCTTTAGcAT TTCTcAaTTt TYCAATGGta aaWgtCCaAG
 451 YWTYtC
```

```
Name:            5_700
Report run date: Sat Jun 22 14:05:28 EDT 2002
Sample remarks:
Statistics:      174 As, 121 Cs, 151 Gs, 178 Ts, 56 Ambigs,
0 Gaps

   1 WTAGGGCGaA TTGGGTACgg GCCCCCCTCG AGGTCGACGG TATCGaTAAG
  51 CTTGATATCG AATTCATGAT CCAATACTTG ATTGTGCAGA AAACAGCTTA
 101 GGAATGCCAA ACAAAGGACA GAGAAGTAGA GATTAATAGA TTCAGCAGTG
 151 AACAACAAGA TTCAAAATAC TGTAACAAAG TGAAAATTAG AAAAATCGAC
 201 CTTCAATACA AGAGTGTGGC CTTGTTGCTT TCGAAACACA ATAGCGAAAC
 251 AAAGCTCGGA CCTCGAACAG AACAGAGCCA ATTGACCATG AACCCGACTA
 301 CGACTACGAA TCAGGATCCA GAAAAAGACT ATACCAACTC GACTATATTt
 351 VGACAACCGT TTCGGGTGTA AAAgGGTGGT TTCAATAGAG GTTTGTGGCT
 401 GGATTTCGAG CTATTTCGCA GCTTGTTTCA CTACTTTYcG TCAGATTTYH
 451 CAGCAGATTC GCAGCTGATT TTSCTGCTCT CTAACGCTGA ATTTYSGGTG
 501 GtGTTTtGCA ATTCTgTtTg CTGCATTTYC CAACTgATTt tHGCAGTTTT
 551 tHGCTTGTGC TTGGgGGCTG TTTCGGGGCT GATTTGGGTG GTGTTtWMtG
 601 GHGGTtCATG GCTGCATTTT cGGGCAGATT TTYaGCTTCT TTtTtSGaGA
 651 atYYBAgGtC GWCTYTgGGA CTaGAtaKSC
```

```
Name:            5_800
Report run date: Sat Jun 22 14:05:42 EDT 2002
Sample remarks:
Statistics:      189 As, 93 Cs, 128 Gs, 133 Ts, 29 Ambigs, 0
Gaps

   1 AACCtcACTA AAGGGAACAa AAGCTGGAGC TCCACCGCGG TGGCGGCCGC
  51 TCTAGAACTA GTGGAYCCCC GGGCTGCAGG AATTCAATCG TTCAGTCAAA
 101 AAAGACAAGT TAGCATGTTT GATCAGATAC CAAGAAGTTC GTCGTCCTCA
 151 GAAACTAATT CCTCAAAAGG AAGTAGTGCT GACGAAGAGG TGACATcATA
 201 TTCCATCAAG AGAAAAGACG CAAATACAGA CAAAGAAGAA TTAAAAGATG
 251 AAGAAAAGCA TTATATAGGA GTTAGAAAAA GGCCATGGGG GAAATATGCA
 301 GCAGAAATAA GAGATTCAAC AAGAAATGGA ATTAGGGTAT GGTTAGGTAC
 351 ATTTRATACT GTTGAAGAAG CTGCTTTAGC TTATGAtCAA GCTGCATTTT
 401 CAATGCGTGG TGCTTTAGCA TTTCTCAATT tTCCAATGGA AAAMgTCCAA
 451 GAMTCACTTC AAAAAATtAA GATGAaTTcG ACAATGGGTT TGAGTCGGTG
 501 ATTGTaAAAC TTcCTTTTaT CGaTCTTgAT TcGCGTATAA DtKcCGGAaC
 551 TATGGRCCTa GCTGAaCCGG WG
```

```
Name:            6_700
Report run date: Sat Jun 22 14:05:30 EDT 2002
Sample remarks:
Statistics:      162 As, 108 Cs, 138 Gs, 163 Ts, 71 Ambigs,
0 Gaps

   1 tRTaGGGCGA aTTGGGTACg gGGCCCCCCt NcGAGGTCGA CGgTATCGAT
  51 AAGCTTGATA TCGAATTCAT GATCCAATAC TTGATTGTGC AGAAAACAGC
 101 TTAGGAATGC CAAACAAAGG ACAGAGAAGT AGAGATTAAT AGATTCAGCA
 151 GTGAACAACA AGATTCAAAA TACTGTAACA WAGTGAAAAT TAGAAAAaTC
 201 GACCTTCAAT ACAAGAGTGT GGCCTTGTTG CTTTCGAAAC ACAATAGCGA
 251 AACAWAGCTC GGACCTCGAA CAGAACAGAG CCAATTKACC ATGAaCCCGA
 301 CTACGACTAC GAATcARGaT CCAGAAAAAg ACTATACCAA CTCGACTATA
 351 TTtDGaCAAC CGTTTCGGGT GTAAAARGGT GGTTTcAATA GaGGtTTGTg
 401 GCTGGaTTTc GAGCTATTtB GCAGCTTGTT TcACTACTTt tKGtCAGAtT
 451 TtKHAGCAGA TTCGCAGCTG ATTTTBCTGC TCTCTWACgc TGAATTTBgG
 501 GTGGBGTTTt GCAATTCTgT YTGCtGCATT TDCCAACTgA TTttNGCAGT
 551 TTTtHGCTTG TGCTTGGgGG CTGTtTCGGG GCTGATTTgg GTGGTGTTTa
 601 AtGGaGGTTC ATGGcTGCWT TtBGGGCAGA TTTtKAGCTT CT




Name:            6_800
Report run date: Sat Jun 22 14:05:44 EDT 2002
Sample remarks:
Statistics:      144 As, 78 Cs, 108 Gs, 112 Ts, 120 Ambigs,
0 Gaps

   1 aacCcBcACt AAAGGGAACA aAAGCTGGAG CTcCACCGCG GTGGCGGCCG
  51 CTCTAGAACT AGTGGATCCC CCGGGCTGCA GGAATTCAAT CGTTCAGTCA
 101 AAAAAGACAA GTTAGCATGT TTGATCAGAT ACCAAGAAGT TCGTCGTCCT
 151 CAGAAACTAA TTYCTCAAAA GGaAGTAGTG CTGACGAAGA GGTGNaCATc
 201 ATATTcCATC AAGAGAaAaG ACGCAAATAC AGACAAAGAA GAATTAaWaK
 251 ATGAAGAaAA GCATTATATA GKagTTAGAa AAaKgCCATG GGGGAAATAT
 301 gCAGCAGAAA TAaGaGaTTC AaCAMGAMAT GGVATBVGGG tATGGBTAGG
 351 WACAtttDAt ACTRTtGAAG AAGCTGCTTT AGCTTATgWt CANgCTGCAT
 401 TTTcAATGCG TGGTGCTTTa GCATTTcTcA HTTttcCAAT GGAAAANgtC
 451 CAAGaNTCAC TWCAaMRcat tVVGMtGRST KcaGaCAMTG GGtTTGYGTC
 501 GGWGATTGTt MHtcTTCCTT TTYTCgctCT YKMTTcSMGT ATTaRtDaMV
 551 RtAcTWttKA aM
```

Appendix L

Primer Information

Expansin primers

Degenerate 5' Expansin

Length : 22
Sequence: BG(GC) (N)CA (TC)GC (N)AC (N)TT (CT)TA (CT)GG (N)G
B = Biotin

Td : 59.1° (nearest neighbor method)
Tm: 65.7 (%GC method)
Tm: 60.0° (2 * (A + T) + 4*(GC))
Nmol/OD: 4.87 (nearest neighbor extn. coeff.)
μg/OD: 33.4

Composition: A + T = 6 (27.3%)
C + T = 8 (36.1%)

O.D. = 12.5 A_{260}

Degenerate 3' Expansin

Length : 21
Sequence: B(TC)T GCC A(AG)T T(TC)T G(N)C CCC A(AG)T T
B = Biotin

Td : 68.4° (nearest neighbor method)
Tm: 65.0° (%GC method)
Tm: 58.0 ° (2 * (A + T) + 4*(GC))

Nmol/OD: 5.39 (nearest neighbor extn. coeff.)
μg/OD: 34.5

Composition: A + T = 8 (38.1%)
C + T = 8 (38.1%)

O.D. = 12.7 A_{260}

Appendix M

List of GenBank Accession Numbers for Various Expansins

Plant Genus	GenBank Accession Number	Name(s)	Comments
Cucumis (cucumber)	U30382	Cs-EXP1	cDNA, complete cds
	U30460	Cs-EXP2	cDNA, complete cds
Oryza (rice) (unpublished data provided by Yi Lee and Hans Kende, Michigan State University)	Y07782	Os-EXP1	cDNAs from seedlings, older plants
	AF394543	Os-EXP1	genomic DNA; contact Kende
	U30477	Os-EXP2	cDNAs from seedlings, older plants
	AF394544	Os-EXP2	genomic DNA; Contact Kende
	U30479	Os-EXP3	cDNAs from seedlings, older plants
	U85246	Os-EXP4	cDNAs from seedlings, older plants
	AF394545	Os-EXP4	genomic DNA; contact Kende
	AF247162	Os-EXP5	expressed in internodes, leaves, coleoptiles, and roots
	AF394546	Os-EXP5	genomic DNA; contact Kende
	AF247163	Os-EXP6	expressed in internodes and leaves
	AF247164	Os-EXP7	expressed in internodes and leaves
	AF394547	Os-EXP7	genomic DNA; contact Kende
	AAD38296	Os-EXP8	predicted from genome (TIGR)
	AC007789	contains Os-EXP8	BAC genomic clone
	AAD38297	Os-EXP9	predicted from genome (TIGR)
	AC007789	contains Os-EXP9	BAC genomic clone
	AF247165	Os-EXP10	expressed in internodes, leaves, coleoptiles, and roots

	BAA88200	Os-EXP11	predicted from genome (JRGP); corresponds to EST AU078708
	AP000837	contains Os-EXP11	BAC genomic clone
	AF394548	Os-EXP12	genomic DNA; contact Kende
	AF394549	Os-EXP13	genomic DNA; contact Kende
	AF394550	Os-EXP14	genomic DNA; contact Kende
	AF394551	Os-EXP15	genomic DNA; contact Kende
	AF394552	Os-EXP16	genomic DNA; contact Kende
	AP000616	contains Os-EXP17	BAC genomic clone
	BAA85432	Os-EXP17	predicted cds
	AF394553	Os-EXP18	genomic DNA; contact Kende
	AF394554	Os-EXP19	genomic DNA; contact Kende
	AF394555	Os-EXP20	genomic DNA; contact Kende
	AF394556	Os-EXP21	genomic DNA; contact Kende
	AF394557	Os-EXP22	genomic DNA; contact Kende
	AF394558	Os-EXP23	genomic DNA; contact Kende
	AF394559	Os-EXP24	genomic DNA; contact Kende
	AF394560	Os-EXP25	genomic DNA; contact Kende
	AF394561	Os-EXP26	genomic DNA; contact Kende
	AC087550	contains Os-EXP27	BAC genomic clone
	AC087550	contains Os-EXP28	BAC genomic clone
	AF394562	Os-EXP29	genomic DNA; contact Kende
Pisum (pea)	X85187	(Ps-EXP1)	cDNA from flower petals
Lycopersicum (tomato)	U82123	Le-EXP1	cDNA from ripening fruit
	AF096776	Le-EXP2	cDNA
	AJ239068	Le-EXP2	gene for EXP2
	AF059487	Le-EXP3	cDNA, complete cds
	AF059488	Le-EXP4	cDNA, complete cds
	AF059489	Le-EXP5	cDNA, complete cds
	AF059490	Le-EXP6	partial (498 bp)
	AF059491	Le-EXP7	partial (486 bp)
	AF184232	Le-EXP8	unpublished;
	AJ243340	Le-EXP9	cDNA, complete cds
	AF184233	Le-EXP10	unpublished;

	AF218775 AI781569 AI775584 AF218775 AI781569 AI775584	Le-EXP11	partial; ; contact Monika Werner EST EST
	AJ004997 AJ270960	Le-EXP18	cDNA expressed in shoot meristem partial cDNA from fruit
Rumex acetosa	AF167365	Ra-EXP1	mRNA, partial cds
	AF167357	Ra-EXP2	mRNA, partial cds
	AF167358	Ra-EXP3	mRNA, partial cds
	AF167359	Ra-EXP4	mRNA, partial cds
	AF167361	Rp-EXP2	partial cds
	AF167362	Rp-EXP3	partial cds
	AF167363	Rp-EXP4	partial cds
	AF167364	Rp-EXP5	partial cds
	AF167356	Rp-EXP6	partial cds
Brassica	AJ000885	(Bn-EXP1)	cDNA from young seedlings
Gossypium (cotton)	AF043284, D88415	(Gh-EXP1)	cDNAs from cotton fibers (ovule trichomes)
Fragaria x ananassa (strawberry)	AF163812	Fa-EXP1	mRNA, partial cds; sequence update expected ~22 Jan 2000
	AF159563	Fa-EXP2	mRNA, complete cds;
	AF226700	Fa-EXP3	partial cds
	AF226701	Fa-EXP4	partial cds
	AF226702	Fa-EXP5	partial cds
	AF226703	Fa-EXP6	partial cds
	AF226704	Fa-EXP7	partial cds
Pinus taeda (pine)	U65891-5	-	PCR cDNAs from hypocotyls
	AC556861	-	EST from Pinus taeda xylem
	AF085330	-	expansin cDNA, complete cds
Nicotiana (tobacco)	AF049350	Nt-EXP1	cDNAs from BY2 cell culture
	AF049351	Nt-EXP2	cDNAs from BY2 cell culture
	AF049352	Nt-EXP3	cDNAs from BY2 cell culture
	AF049353	Nt-EXP4	cDNAs from BY2 cell culture

	AF049354	Nt-EXP5	cDNAs from BY2 cell culture
	AF049355	Nt-EXP6	cDNAs from BY2 cell culture
Prunus armeniaca (apricot)	U93167	Pa-EXP1	cDNA from fruit
	AF038815	Pa-EXP2	cDNA from fruit
Marsilea quadrifolia (a fern)	AF202119	Mq-EXP1	complete cDNA from rachis
Regnellidium diphyllum (a fern)	AF202120	Rd-EXP1	complete cDNA from rachis
Striga asiatica (a parasitic plant)	AF291657	Exp1	partial cDNA
	AF291658	Exp2	partial cDNA
	AF291659	Exp3	complete cDNA
Zea mays (maize)	AF332174 L14271	Zm-EXPB1 Zea m1	complete cDNA pollen allergen (incomplete sequence)
	AF332175	Zm-EXPB2	partial cDNA
	AF332176	Zm-EXPB3	partial cDNA
	AF332177	Zm-EXPB4	complete cDNA
	AF332178	Zm-EXPB5	partial cDNA
	AF332179	Zm-EXPB6	complete cDNA
	AF332180	Zm-EXPB7	complete cDNA
	AF332181	Zm-EXPB8	complete cDNA

Some beta-expansins:

Plant Genus	GenBank	Name	Comments
Oryza (rice) Unpublished data provided by Yi Lee and Hans Kende, Michigan State University	AF261270 AY039023	Os-EXPB1	complete cDNA genomic DNA; contact Kende
	U95968 AC037426	Os-EXPB2	complete cDNA genomic DNA containing EXPB2
	AF261271 AC037426	Os-EXPB3	complete cDNA genomic DNA containing EXPB3
	AF261272 AC069300	Os-EXPB4	complete cDNA genomic DNA containing EXPB4

	AF261273 AY039024	Os-EXPB5	complete cDNA genomic DNA ; contact Kende
	AF261274 AC037426	Os-EXPB6	complete cDNA genomic DNA containing EXPB6
	AF261275	Os-EXPB7	complete cDNA
	AF261276	Os-EXPB8	complete cDNA
	AF261277 AC020666	Os-EXPB9	complete cDNA genomic DNA containing EXPB9
	AF261278 AF391111	Os-EXPB10	complete cDNA genomic DNA; contact Kende
	AF391103	Os-EXPB11	genomic DNA; contact Kende
	AF391104 AF391105	Os-EXPB12	5' genomic; contact Kende 3' genomic; contact Kende
	AF391106	Os-EXPB13	genomic DNA; contact Kende
	AF391107	Os-EXPB14	genomic DNA; contact Kende
	AF391108	Os-EXPB15	genomic DNA; contact Kende
	AF391109	Os-EXPB16	genomic DNA; contact Kende
	AF391110	Os-EXPB17	genomic DNA; contact Kende
Glycine (soybean)	U03860	CIM1	cytokinin induced mRNA
Cynodon (Bermuda grass)	S83343	Cyn d1	pollen allergen
Phleum (timothy grass)	X78813	Phl p1	pollen allergen
Lolium (rye grass)	M57476	Lol p1	pollen allergen
Zea (maize)	AF332174 L14271	Zm-EXPB1 Zea m1	complete cDNA pollen allergen (incomplete sequence)
	AF332175	Zm-EXPB2	partial cDNA
	AF332176	Zm-EXPB3	partial cDNA
	AF332177	Zm-EXPB4	complete cDNA
	AF332178	Zm-EXPB5	partial cDNA
	AF332179	Zm-EXPB6	complete cDNA
	AF332180	Zm-EXPB7	complete cDNA
	AF332181	Zm-EXPB8	complete cDNA
	-	Zm-EXPB9	contact Cosgrove
Holcus (velvet grass)	Z27084	Hol-l1	pollen allergen
Oryza (rice)	U31771	Ory s1	pollen allergen
Phalaris (canary grass)	Q41260	Pha a1	pollen allergen

Poa pretense (timothy grass)	AJ131850	Poa p1	pollen allergen
Tricicum aestivum (wheat)	U91981	-	cDNA from roots

Appendix N

DNA Sequences

All sequences used or generated for this thesis are listed in the following appendix. These sequences were found using GenBank, a public database for sequences, and can be found at the following address :

http://www.ncbi.nlm.nih.gov/

Oryza sativa alpha-expansin OsEXP7 mRNA, complete cds

>Os-EXP7, mRNA |7407662|gb|AF247164.1|AF247164 Oryza sativa alpha-expansin OsEXP7 mRNA, complete cds

GCTCGCCGTTGCCACTCGCACTCACAATGTCGCCGGCGCCGCGGGTGTTGGTGTTGGTG
GTGGCCACAGTGGTGGCGCTGCAGGTGTCGCCGGCGGCGGGGCGGATCCCGGGGGCGTA
CGGGGGCGGGGAGTGGCAGAGCGCGCACGCAACGTTCTACGGGGGCAGCGACGCGTCAG
GGACGATGGGCGGGGCGTGCGGGTACGGGAACCTGTACAGCCAGGGGTACGGGGTGAAC
AACGCGGCGCTGAGCACGGCGCTGTTCAACTCCGGGCAGAGCTGCGGCGCGTGCTTCGA
GATCAAGTGCGTGAACCAGCCCGGGTGGGAGTGGTGCCACCCGGGGAGCCCCTCCATCC
TCATCACCGCCACCAACTTCTGCCCGCCCAACTACGCCCTCCCCTCCGACAACGGCGGC
TGGTGCAACCCTCCTCGCCCCCACTTCGACCTCGCCATGCCCATGTTCCTCCACATCGC
CGAGTACCGCGCCGGCATCGTCCCCGTCTCCTACCGCCGGGTGCCGTGCAGGAAGAAGG
GAGGGGTTCGGTTCACGATAAACGGGTTCAGGTACTTCAACCTGGTGCTGATCACGAAC
GTGGCCGGGGCCGGGGACATCGTGAGGGCGAGCGTGAAGGGGACGAGCACCGGGTGGAT
GCCCATGTCGCGGAACTGGGGCCAGAACTGGCAGTCCAACTCCGTCCTCGTCGGCCAGG
CGCTCTCGTTCCGCGTCACCGGCAGCGACCGCCGCACCTCCACATCCTGGAACGCCGCA
CCCGCCGGATGGCACTTCGGCCAGACCTTCGAGGGCAAGAACTTCCGGGTCTGAATTGA
ATGAAGCAAAACTGCAATATACCCTCTTATTTACTCATCTAGTAATTACTGTCATTATA
GCGAGCAGGTGTGTTTTTTACCTTGGTTAAGTTTGGGTTCTTCTTCGGTTGGTGGCCTT
GTCAAAGTGAGGGGCCGGGGGAAAACAGGTGGTAAAGACTAGTACTAGTGTCAATTAGG
GTTAAACTGGTAAATTGCTAGTACTGCTGTTACTCATCATCATCATCGCTAGTGGTCAC

TGCTAGTAGTAGTTGTGCTAGTGCATGTATGGTTATGGATACTGCATCATGTTGGTTGG
TGAAAAAAAAAAAAAAAAAAAAA

Oryza sativa alpha-expansin OsEXP6 mRNA, complete cds; expressed in internodes and leaves

>Os-EXP6, mRNA |7407660|gb|AF247163.1|AF247163 Oryza sativa alpha-expansin OsEXP6 mRNA, complete cds
CCCACGCGTCCGCCAATGGCGCCACCCCTGCTCCTCCTCCTCGCTTCTCTCCTCCTCGT
CGCAGCGCGGCGAGCCCTCGGCCTCGGCCTCGGCCAGTGGCAGCCCGGCCACGCCACGT
TCTACGGCGGCGGCGACGCCTCCGGCACGATGGGTGGCGCGTGCGGGTACGGGAACCTG
TACAGCCAGGGGTACGGCACGAGCACGGCGGCGCTGAGCACGGCGCTGTTCAACAGGGG
CCTGAGCTGCGGCTCCTGCTACGAGCTCCGGTGCGCGGGCGACCACCGCCGGTCGTGCC
TCCCCGGCGGCGCCACCGTGACGGTGACGGCGACCAACTTCTGCCCCCCGAACTACGCG
CTCCCGAGCGACGGCGGCGGCTGGTGCAACCCACCGCGGCGGCACTTCGACCTCGCCGA
GCCGGCGTTCCTCCGCATCGCGCGGCACGCCGCCGGGATCGTCCCGGTCTCCTTCCGCC
GCGTGGCGTGCGCCAGGAAGGGCGGCGTCAGGTTCACCGTCAACGGCCACGCCTACTTC
AACCTGGTGCTCGTCACCAACGTCGGCGGCGCCGGCGACGTGCGCTCCCTCGCCGTGAA
RGGGTCGGGGTCCGGGTCGCGCGTGGGGGGGCGGTGGCAGCCGATGTCGCGCAACTGGG
GGCAGAACTGGCAGAGCAACGCGTACCTCGACGGCAAGGCGCTCTCCTTCCGCGTCACC
GCCGGCGACGGCCGCTCCCTCACCTGCGCCGACGTGGCGCCCGCCGGCTGGCAGTTCGG
CCAGACCTTCGAGGGCAGGCAGTTCTAGATTAGTTTAGCCAAGAGGATTATTATCTCTT
AATTCTTACTAGTATCTTTTGAGTTTAATTGCACACCCAATCGGTGTTTAGTGAGGTCT
AGTCGTCTAGTAGATGCTGAGGTTGCTGTGGCTCCTGCAAGAGAAGGCAAGGCATTTCG
CGCCCGCGTGTCAGAGGCCGGATGGTTTGCAGTTTGCTGCTCTGCTCTTAGTTCAGATT
TGTACAGGGCATGTGGCATGAGTAAATGAGTTGATTAGTTAGGTCCAGAGAGTGGCTGG
CTATAGTGCGAGCGGTGATCGGTGAGCCAGCCTCTCTGAAAAAGAGTGAGCTGTCTGTA
CAAATGCAGTTGCTTGTTTGCTACTCACTCCATTTAAAATATAGCAATTTATATTATTT
CTAAAAAAAAAAAAAA

Oryza sativa alpha-expansin (OsEXP5) mRNA, complete cds; expressed in internodes, leaves, coleoptiles, and roots

>Os-EXP5, mRNA |7407658|gb|AF247162.1|AF247162 Oryza sativa alpha-expansin OsEXP5 mRNA, complete cds

CTCGTCGCAGCGCACCCCGTCGCGTGCGACGACTCCATCCCCGTCACAACAATGTCGTC
CCGCCGAGACGTCCTCGCTGTTGTCCTCGTGGCCGCGCTGCTCCCGCCGGCGTTGTCCC
GTGGGCTTTGGCTGGGGCACCACGGGCTGGGGCACGGGCACGGGCGATGGCGCGCGCCG
CATGTCGGCGGACACGGCCAGGGACAGGGGCCGCAGCAGCACGCGCCGCTCGGCGGCGG
CGGGTGGTCGTCGGCGCACGCCACGTTCTACGGCGGGGGTGACGCGTCGGGCACCATGG
GAGGGGCGTGCGGGTACGGGAACCTCTACAGCCAGGGGTACGGGACGAACACGGCGGCG
CTGAGCACGGCGCTGTTCAACAACGGCCTCAGCTGCGGCGCGTGCTTCGAGGTGCGCTG

CGACGCCGGCGGCGGCGGGAGCCACTCGTGCCTGCCCGGCTCGGTCGTCGTCACGGCCA
CCAACTTCTGCCCGCCGAACAACGCGCTCCCGTCCGACGACGGCGGCTGGTGCAACCCT
CCGCGCGCGCACTTCGACATGTCCCAGCCCGTCTTCCAGCGCATCGCTCTCTTCAAGGC
CGGCATTGTCCCCGTCTCCTACCGCCGGGTGGCTTGCCAGAAGAAGGGCGGGATCCGGT
TCACCATCAACGGGCACTCCTACTTCAACCTGGTGCTGGTGACCAACGTGGGCGGCGCC
GGCGACGTGCACGCGGTGGCCGTGAAGTCGGAGCGCTCGGCGGCGTGGCAGGCCCTGTC
GCGCAACTGGGGCCAGAACTGGCAGAGCGCCGCGCTCCTCGACGGTCAGGCGCTCTCCT
TCCGCGTCACCACCGGCGACGGCCGCTCCGTCGTCTCCAACAACGCCGTCCCCCGTGGC
TGGTCCTTCGGCCAGACCTTCAGCGGGGCCCAGTTCAACTGATTCCGCTCAATTTTACT
CGAGCATCGTCGTGGTAGTTGCAGTAGTACTCCCAACTGGACCGTGCTGGGAAGTGCCA
TTGTTTTATGATTGGTACTCAGTCTCGCCTTGGTTCTTGGGAAAGTATTTAGTGGGTAG
TTTGGCCTAGTATCAACATGGGCTTTGTGCTGGTGTCACTGCTAGTGGTAGTTTGTTAC
AAGTACCATTAGTTGAGCTTTTGTGGTATCTTTTGGCTAGGGATTGGAAGTGGTGGTAA
GGCTGTGGCTTGATTGACAGAGGCCGCCTTGTATCACCCGCCAAAATAGCAAAATTGGG
CCTAATGGGAATGGCCTTCTGCTAGTAATGGTTTTGCCTGGTTGTTGTCTGTACCGGAC
CCCAGTACCGGACCTCATTTTGGGGGTGCCAAACAGTTTAGGCATCTGGGGTATGCCCT
GTGTGCTGCTTATGAACAATTTCTTTTTAAAAAAAAAAAAAAAAAAAAAAAAA

Oryza sativa expansin (Os-EXP4) mRNA, complete cds (cDNAs from seedlings, older plants)

>gi Oryza sativa expansin (Os-EXP4) mRNA
|1815680|gb|U85246.1|OSU85246 Oryza sativa expansin (Os-EXP4) mRNA, complete cds
ACCTCCGCTTCAAGTGAGCTAGTCTAGCCAGCGTTTAGACACCATCACCAGCCATGGCG
ATCGCTGGCGTTCTCTTCCTCCTCTTCTTGGCACGGCAAGCCTCCGCCGCCGGGTACGG
CGGTTGGCAGAGCGCCCACGCCACGTTCTACGGCGGCGGCGATGCTTCCGGAACCATGG
GCGGGGCTTGCGGTTACGGCAACCTGTACAGCCAAGGGTATGGCACGAACACGGCGGCG
CTGAGCACGGCGCTGTTCAACGACGGCGCGGCGTGCGGGTCGTGCTACGAGCTGCGGTG
CGACAACGCCGGGAGTTCGTGCCTGCCGGGCTCCATCACCGTGACGGCCACCAACTTCT
GCCCGCCCAACTACGGCCTCCCCAGCGACGACGGCGGCTGGTGCAACCCGCCCCGCCCT
CACTTCGACATGGCCGAGCCGGCCTTCCTCCACATCGCCCAGTACCGCGCCGGCATCGT
CCCCGTCTCCTTCCGCAGGGTGCCGTGCGTGAAGAAGGGTGGGATCAGGTTCACCGTGA
ACGGGCACTCCTACTTCAACCTGGTGCTGGTGACCAACGTGGCCGGCGCCGGCGACGTC
CGCTCCGTCTCCATCAAGGGGTCGCGCACCGGGTGGCAGCCCATGTCGCGGAACTGGGG
TCAGAACTGGCAGAGCAACGCGTTCCTCGACGGCCAGAGCCTCTCCTTCCAGGTCACCG
CCAGCGACGGCCGCACCGTCACCAGCAACAACGTCGCCCACCCCGGCTGGCAGTTCGGC
CAGACCTTCGAGGGCGGCCAGTTCTAGCCGCCACCGACATCGCCGTCTCCGACACCCAC
ATATAATAGCACGGTGTCCTTCTTCGTCTTCGTTCTCGTCGTCGTCTTTCTTCCTTGAG
AGATTATTTATTTTGAGCAAGAGAGAAAGAAAAGCCTCTTCGTTATAGTGGAGAAGAGG
TTTTGGGCTATGCTGAGGCTGCTAATTAGCACCCGCTTAGACGTTTTCCTTCTCTGGAC
TTCGTCCAATAATGACTGGACCGGTCCACCATCATCAAGTGTAGTGGTAGTAGTAAAGG
GGAGTACTAAGCTTAGTACTACTTGAAGAAAAAATCAATAATAATTGTATTGATTAAAT
TTCTACTTTTGAGCGTTTTCTTGTACCGGAGTGATGGCCTTGCAACGGAATTAAAAACC
GCGAGGCCGTGTTTTATTTATCAAGGGGACTATTGATGT

Oryza sativa expansin Os-EXP3 (Os-EXP3) mRNA, complete cds (cDNAs from seedlings, older plants)

>gi Oryza sativa expansin Os-EXP3 (Os-EXP3) mRNA, complete cds
|1041711|gb|U30479.1|OSU30479 Oryza sativa expansin Os-EXP3 (Os-EXP3) mRNA, complete cds
AAGAATCACCAGCCTTGCAAACCAATCAACCTGTCTAGATTAATTTTTGCATGCTCTCT
GGAATGGAGAAACAACCTGCAATGCTTCTCGTTCTTGTGACACTCTGCGCCTTCGCATG
CAAGCNATCCGTCGCACAGTCTGCCTTTGCCACGTTCTACGGTGGCAAGGACGGGTCGT
GCACCATGGGTGGCGCGTGTGGGTACGGTAACCTGTACAACGCCGGGTACGGGCTGTAC
AACGCGGCGCTGAGCTCGGCGCTGTTCAACGACGGCGCCATGTGCGGCGCGTGCTACAC
CATCACCTGCGACACCAGCCAGACCAAGTGGTGCAAGCCCGGCGGCAACTCCATCACCA
TCACGGCCACCAACTTATGCCCGCCTAACTGGGCGCTGCCCAGCAACAGCGGCGGGTGG
TGCAACCCACCGCTGCAGCACTTCGACATGTCACAGCCGGCCTGGGAGAACATCGCCGT
CTATCAAGCCGGCATTGTCCCCGTCAACTACAAGAGGGTTCCGTGCCAGAGGAGCGGGG
GGATCCGGTTCGCGATCAGCGGTCACGACTACTTTGAGCTTGTGACCGTGACGAACGTG
GGCGGCAGCGGCGTGGTGGCGCAGATGTCGATCAAGGGGTCCAACACGGGCTGGATGGC
GATGAGCAGGAATTGGGGCGCCAATTGGCAGAGCAACGCGTACCTCGCCGGGCAGAGCC
TGTCCTTCATTGTGCAGCTCGACGACGGCCGCAAGGTCACGGCCTGGAACGTCGCCCCG
TCCAACTGGTTCTTCGGTGCCACCTACTCTACCTCTTGGGTGCAGTTCTGAAGTACTGC
AACGTACGTACAGGTTTAGTTGATAAATTATACACATGCTGTTCATGGACATAATTAAT
TAATTACTAGCCATCCCGGCCGTTTGTGCATGTTCTGCTAGGTTGTGTGTTGCGTGGCA
TTAACTTGTTTTTCTCTGTTACCAGTCGTTTTTGTCAAACGCGTTGGTTGTGGCAAGCG
CGATTGAATGTTTTGCCCACCAATTAATGTAACTGATCGTATGGGTGTTGAGTTAATAA
ATTATGTTTAATTATGCGGATGCC

Oryza sativa expansin Os-EXP2 (Os-EXP2) mRNA, complete cds (cDNAs from seedlings, older plants)

>gi Oryza sativa expansin Os-EXP2 (Os-EXP2) mRNA, complete cds |1041709|gb|U30477.1|OSU30477 Oryza sativa expansin Os-EXP2 (Os-EXP2) mRNA, complete cds
ATCGATTCGATCCTAGCTTAAGCCGAGTGAAGTAGCTAGCAGGACATTGTTGGTAGTGT
GATCTTCTCCCTGTTTTGCAACAATGGCCTCACGCAGTAGTGCCCTGCTCCTCCTCTTC
TCGGCCTTCTGCTTCCTCGCCCGGCGAGCCGCCGCCGACTACGGCTCCTGGCAGAGCGC
CCACGCCACGTTCTACGGCGGCGGCGATGCGTCCGGCACGATGGGCGGGGCGTGTGGGT
ATGGGAACCTGTACAGCACCGGGTACGGCACCAACACGGCGGCGCTGAGCACGGTGCTG
TTCAACGACGGCGCGGCGTGCCGGTCGTGCTACGAGCTGCGGTGCGACAACGATGGGCA
GTGGTGCCTGCCGGGCAGCGTCACCGTCACCGCCACCAACCTGTGCCCGCCGAACTACG
CGCTCCCCAACGACGACGGCGGCTGGTGCAACCCGCCGCGCCCCCACTTCGACATGGCC
GAGCCGGCCTTCCTCCAGATCGGCGTGTACCGCGCCGGCATCGTGCCCGTCTCCTACAG
AAGGGTGCCGTGCGTGAAGAAGGGCGGGATCAGGTTCACCATCAACGGGCACTCCTACT
TCAACCTGGTTCTTGTGACCAACGTGGCCGGNCCAGGCGACGTGCAGTCCGTGTCGATC
AAGGGGTCCAGCACCGGGTGGCAGCCCATGTCCCGCAACTGGGGCCAGAACTGGCAGAG

CAACTCCTACCTCGACGGCCAGAGCCTCTCCTTCCAGGTCGCCGTCAGCGACGGCCGCA
CCGTCACCAGCAACAACGTCGTGCCGGCCGGCTGGCAGTTCGNCCAGACCTTCGAGGGC
GGCCAGTTCTGATCGAGTACTCGGCTAATTCCGTTTTTCAGTTCACTTTTCTGCAGAAA
TGTCAGTGTGAGATTGAGATTGAGGTGGAGGAGGAGGGAGGCCTCTTTGCTATAGTAAA
AGAGAAGAGGCTTTGGCTATTCTGAGGCTGCTTATTAATTAGCACCCGCTTAGGCCTTT
GCTTTCTCCCATAGGTCCAGTAGACATAAATAAGTTATCATGGAGTTGGGCATTTTTTT
TTTCTGCAATTGTTGTAGCCTTGGTTTAAAATTTGGCAAGGCAATTTAAGGATTATATT
GTAGAACAGCAGGGAGGTGGTGTCTTGTAACTTTTGTTGTTACATTGTAACCCCCCGAA
TCAGGCCACATGGGCCGGAGGAGATTGTGGGGGAGCACCGGACGCTTGTGCTTTTGGAC
CATGAACTATGCTAGTTTATGGGTCTATGTATATGATATGTTTGTGATTTGATTATACT
TTCTTTTGTCGCCTTATCAAGTACC

Oryza sativa expansin (Os-EXP-1) mRNA, complete cds (cDNAs from seedlings, older plants)

>gi Oryza sativa expansin (Os-EXP-1) mRNA, complete cds |2924246|emb|Y07782.1|OSEXPANSI Oryza sativa expansin (Os-EXP-1) mRNA, complete cds
ACGCGCGCACACATGGCCGGCTCGTCAGCCGCAACGTCGTGTGCTCGGTTCCTGGCATT
GCTGGCGACATGCCTCCTCTGGAACGAGGCCGCATCGTTCACGGCGTCCGGCTGGAACA
AGGCGTTCGCCACCTTCTACGGCGGCAGTGACGCTTCAGGAACGATGGGTGGGGCGTGT
GGGTACGGGGACCTGTACTCGACGGGGTACGGGACGAACACGGCGGCGCTGAGCACGGT
GCTGTTCAACGACGGGGCGTCGTGCGGGCAGTGCTACCGGATCATGTGCGACTACCAGG
CGGACAGGCGGTTCTGCATCTCCGGCACGTCGGTGACCATCACGGCGACCAACCTCTGC
CCGCCGAACTACGCGCTCCCCAACGACGCCGGCGGGTGGTGCAACCCGCCGCGGCAGCA
CTTCGACATGGCCGAACCGGCTTGGCTCAAGATCGGCGTCTACGTCGGCGGCATCGTGC
CGGTGATGTACCAGCGGGTGCCGTGCGCCAAGCAGGGCGGGGTGAGGTTCACCATCAAC
GGCAGGGACTACTTCGAGCTGGTGCTCGTCTCCAACGTCGGCGGCGTCGGCTCCATCCA
GTCGGTGTCGATCAAGGGGTCGAGGACCGGGTGGATGGCCATGTCCAGGAATTGGGGCG
TCAACTGGCAGTCCAACGCCTACCTCGACGGCCAGAGCCTGTCGTTCAAGGTCACCAGC
AGCGACGGCCAGACGCTCACCTTCCTCGACGTCGCCCCGGCGGGCTGGACGTTCGGCCA
GACCTTCTCGACCTCACAGCAGTTCTCTTAAGTAATTAAATATTAATGAGTTATTACTG
GTCCATTCATATATGTGTTTAATTCCGAGCGTATATTATCATCGGCGTCGGCGTGTAAA
GACTAAAGATATATTACTAGTAGCTGTTTGGGTTGCCCACAAGTTTGGAGCATGCGCGC
ACAAGTGTTTGGTGTGGCGAGCTATATATATGATTTGCAAGCTAAGGACACCTGCTTTT
TTCAATGCGAATTAAAACGGTATATATATGTACAGTTTGCGAGGTGCTTGCCAATCGAT
GATCGGCATGCTTTACTGT

Pisum sativum mRNA for pollen allergen-like protein (cDNA from flower petals)

>gi Pisum sativum mRNA for pollen allergen-like protein |732904|emb|X85187.1|PSPALPR P. sativum mRNA for pollen allergen-like protein

GTCTCTCTCTCTCTTGCAATGGCTGCAATTCTTCTCCTCACCATTGCATCACTAACCTC
ACTTTTTGCTCCAACCACCGCTCGAATCCCTGGAGTTTACACCGGCGGCCCTTGGACCA
GTGCTCATGCTACTTTCTACGGCGGCAGTGATGCCTCCGGAACAATGGGTGGTGCTTGT
GGGTACGGAAACTTATACAGCCAAGGCTACGGCGTCAACACCGCAGCACTAAGCACAGC
ACTATTCAACAATGGCTTAAGTTGCGGTGCATGTTTTGAGCTCAAGTGTGACCAAGATC
CACGGTGGTGTAACCCTGGAAACCCTTCCATCTTAATCACTGCAACAAACTTTTGTCCT
CCAAATTTCGCTGAACCTAGTGACAATGGTGGCTGGTGTAACCCACCTCGACCACACTT
TGATCTTGCCATGCCCATGTTCCTTAAGATCGCTCAATACCGCGCCGGAATCGTCCCCG
TTGCTTACCGCCGTGTGCCATGCAGAAAGGCAGGAGGCATCAGATTCACAATCAACGGT
TTCCGTTACTTCAACTTGGTTCTAATCACCAACGTCGCGGGTGCAGGGGATATCGTGCG
CGTGAGTGTAAAAGGAACCAACACAGCGTGGATGACAATGAGCCGTAACTGGGGGCAGA
ACTGGCAATCCAACGCAGTTTTTGTGGGCCAGGCTCTCTCTTTTAGGGTCACCGGCAGT
GACCGTCGTACATCCACGTCATGGAACGTGGCACCACCTCATTGGCAGTTCGGACAAAC
CTTCACAGGAAAGAATTTCCGCGTCTGAAAAAATTATTAAAAAAAAAAAAAAAAA

Lycopersicon esculentum mRNA for expansin18; cDNA expressed in shoot meristem partial cDNA from fruit

>Le-EXP18, mRNA |3355779|emb|AJ004997.1|LEAJ4997
Lycopersicon esculentum mRNA for expansin18
TTTTTCTTTTTCCTTCTGTTTTTTAGTAGAAATGGGGTTTTTGAATGTGGCCTTCTGTG
TTGCTTCACTTTTCACTAATTTTACTAACAGCTGAAGCTAGAATCCCTGGTGTTTTTAC
TGCCGGAGCATGGCAGAGTGCTCATGCTACCTTTTATGGCGGAAGCGACGCTTCTGGTA
CTATGGGTGGAGCGTGTGGGTATGGTAACCTATACAGTCAGGGATACGGAGTGAACAAT
GGAGCACTTAGCACAGCGCTATTCAACAATGGACTGAGTTGTGGAGCGTGTTTTGAGAT
CAAATGTGACAACTACCCACAATGGTGCCATCCCGGAAGTCCTTCCATCTTCATTACTG
CTACGAACTTCTGCCCTCCCAACTTTGCTCTCCCAAACGACAACGGCGGTTGGTGTAAC
CCTCCTCGCCCTCATTTCGACCTCGCCATGCCTATGTTCCTCCATATCGCGGAGTACCG
TGCCGGAATTGTCCCCGTCGTTTATCGCCGGGTGCCATGCAGGAAGCAAGGTGGAATAA
GGTTCACAATTAACGGTTTCCGTTACTTCAACTTGGTTTTGGTAACGAACGTGGCGGGT
GCAGGGGATATTGTGAGTCTTAGTATTAAAGGTTCAAAAACTAATTGGATAAGTATGAG
CAGGAACTGGGGGCAAAATTGGCAAACAAATTCAGTTTTGTTTGGTCAGTCACTTTCAT
TTAGGGTTAGAGCTGGTGATAGAAGGAGCTCTACTTCATGGAATATAACACCTGCACAT
TGGCAATTTGGTCAAACTTTTGTTGGGAAGAATTTCAGAGTTTAAATAGTGTAGTAGTA
ATTTTATTTTATGTGTCTGTTTGACTATGGTTGTTAATGGGTATTTTGGGAGATTGTGA
AAGTAGTTAGAAAAAGAAAATGCTTTTTCTGGAATTGGGATTATGGAAAAGTAAAGTTT
TTCTGGTAATGGGGTTCTTTTTTTTATATATTTTTGGACCTTTTATTGTTGGAGGTGAA
AGGGGAAGGATTGATGAATTTTGTGGAGAAATTGTAATAGGGATTTGGGAAGGGATGAA
GTGTAGATGATAGAAGAGCTGAAGCGGCTGCAAAAACATGTAGCCCGCAGCTTCTATTA
GGACTATATAATTTAGTATGAAATCTATTTTACGATTATTCTAAAAAAAAAAAAAAAAA
AAAA

Lycopersicon esculentum (LE-EXP-9) mRNA, for expansin 9 (cDNA, complete cds)

>gi Lycopersicon esculentum (LE-EXP-9) mRNA, for expansin 9 (cDNA, complete cds) |5419926|emb|AJ243340.1|LES243340 Lycopersicon esculentum mRNA for expansin9 (exp9 gene)
AAAAAAAAAAAAATTAAAAATGGAGATTTTGAAGTTTTTTCTAATAATTATTTTAGTAA
TTTTAGTAGGAGTAGAAGGTAGAATTCCAGGTGTTTATAGTGGTGGATCATGGCAAAAT
GCTCATGCTACTTTCTATGGTGGTAGTGATGCTTCTGGTACTATGGGCGGGGCATGTGG
ATATGGCAATCTTTATAGCCAAGGGTATGGTGTAAACAATGCAGCATTAAGCACAGCAT
TATTCAACAATGGACTAAGTTGTGGAGCTTGTTTTGAGATCAAATGTGACAATCAACCA
CAATGGTGTCATCCTGGAAGTCCTTCTATTTTAATTACTGCAACTAACTTTTGTCCTCC
AAATTATGCATTGCCTAATGATAATGGTGGTTGGTGTAACCCTCCTCGTACTCATTTCG
ATCTCGCCATGCCTATGTTCCTCAAGATCGCGGAGTATCGTGCTGGTATCGTCCCTGTT
GTTTATCGTAGGGTGCCATGTAGGAAGCAAGGTGGAATAAGGTTCACTATCAATGGTTT
TAGTTACTTCAACTTGATTTTAGTCACGAATGTCGCGGGTGCAGGTGACATTACTAAGG
TTATGGTGAAGGGTACGAGGACTAACTGGATAACGTTGAGTCGTAATTGGGGTCAGAAC
TGGCAAACCAACTCCGTTTTGGTTGGTCAGTCGTTGTCATTCAGGGTTACAGCTAGCGA
TAAACGCAAGTCTACGTCGTGGAACATAGCCCCATCACACTGGCAATTTGGTCAGACAT
TTGTTGGAAAGAACTTTAGAGTCTAAATAGGGAAATGAGGCGATCATAACATAGTATGT
AAGACTCGTGGTTCATGTACTTTGAGCTTTTATTTTGTTTAGACCCTAATGAATTCGTT
TTTAACCTGTGGTTTTTTGTATCTGGTGGTGCATGTAGAAGAGCTGAAGCGGCTGCAGA
AATATGCAGCCCGCAGCTGTTCTAAGAACAATGTAGTAGTATCAGTATAATGTTTTGTA
GTACTTGA

Lycopersicon esculentum expansin (LE-EXP8) mRNA, complete cds

>gi Lycopersicon esculentum expansin (LE-EXP8) mRNA, complete cds |11191998|gb|AF184232.1|AF184232 Lycopersicon esculentum expansin (Exp8) mRNA, complete cds
CAAAAAGTAATTTCTTAATTTTCCACTAAGAAATTAAAATGGCAAATAATGTCAATTTA
GCATTGGGATTTATAATTGGTTTGTGTACATTTTTCTCCAGTGCAAATGGTTTCTCAGC
AGATTCTGGATGGACAAGTGCTCATGCCACATTTTATGGTGGAGCTGATGCTTCTGGCA
CAATGGGAGGTGCTTGTGGATATGGTAATTTGTATTCAACAGGATATGGTACTAGAACA
GCAGCATTGAGTACAGCATTGTTCAATGATGGAGGATCATGTGGTCAATGTTACAAAAT
AATTTGTGATTACAAATTAGATCCTCAATGGTGCAAAAAGGGAGTATCAGTTACAATTA
CATCTACAAATTTTTGTCCACCAAATTATAATCTTCCTAGTAACAATGGAGGATGGTGC
AACCCTCCACGTCCACATTTTGATATGGCTCAACCAGCTTGGGAAAAAATTGGCATTTA
CAAAGGTGGCATTGTTCCTGTTTTATACAAAAGGGTACCTTGCAAGAAGCATGGTGGAG
TTAGATTCACAATAAATGGAAGAGACTATTTTGAACTAGTGTTGGTAAGCAATGTAGGA
GGGGCAGGATCTGTTGAATCAGTTCAAATCAAAGGCTCAAACACAAATTGGCTAACAAT
GTCAAGAAATTGGGGAGCAAGTTGGCAATCAAATGCATATCTTGATGGACAATCAATAT
CATTCAAAGTTACTACTAGTGATGGTGTCACAAAAACATTCTTAAATATTGTTCCATCT
AGTTGGAAATTTGGCCAAACATTTTCAAGCAAAACTCAATTCTGATTTGTTAAATGTTC
GGTGAATACGTTTTTAATCAGAGGTCTTGAGTTTATAGGGCAGCGGCATGCTTACTTTT

TACATCAAGTAGCCCTCCCCAAAAAAAGAATCATTCTTATTGAGGTATATTTTTGAGGA
GTTGTTGGTTAAGTAAGCACATGAATGAACTATAGTCCAAATAAAGATAGTGAAAAAAT
TAGAAATAACTAATTATTGGTGTAAGTAGGGGTAGTTCAAAAAATTGTATTTTTTTACT
ATATGAAAGGATGAATAAGATAAGTTTGTTTAAGCTACATC

Lycopersicon esculentum expansin (Exp6) mRNA, partial cds; partial (498 bp)

>Le-Exp6, mRNA, partial cds |4138917|gb|AF059490.1|AF059490
Lycopersicon esculentum expansin (Exp6) mRNA, partial cds
ATGGGGGGGGCGTGTGGCTACGGGAATCTGTACAGCCAAGGGTACGGAGTAAACAACGC
GGCATTGAGTACGGCACTTTTCAACAATGGTCTGAGCTGCGGCGCTTGCTTCGAGATAA
AGTGTACTGACAGTAAGAAGGAATATTGCAACCCTGGAAACCCTTCCATCTTGGTAACA
GGAACCAATTTCTGCCCACCAAACTACGCCTTGCCTAACGATAATGGAGGCTGGTGTAA
CCCACCTCGCCCACATTTCGACCTCGCCACGCCCATGTTCCTCAAAATTGCTGTGTACC
GTGCTGGAATTGTTGCTGTCAACTACCGAAGGGTTCCATGCAGGAAACAAGGAGGAATC
AGATTCACAGTCAACGGTTTCCAATACTTTAACTTAGTGTTAGTCACCAACGTTGCAGG
TGCAGGGGACATTCAAAAGGTTTATGTTAAAGGCACAAACACGCCATGGATTTCAATGA
CTCGTAACTGGGGCGCGAACTGGCAG

Lycopersicon esculentum expansin (Exp7) mRNA, partial cds; partial (486 bp)

>Le-Exp7 mRNA, partial cds |4138919|gb|AF059491.1|AF059491
Lycopersicon esculentum expansin (Exp7) mRNA, partial cds
ATGGGGGGGGCGTGCGGGTACGGAGACACAATCAAACAAGGGTATGGCCTAGGAACAAC
AGCACTAAGCACAGCACTCTTTAACAAAGGATCTACCTGTGGAGCTTGCTATCAAATAA
AGTGTGTCAATGCTCCTAAAGCATGCCATCCAGACCAAGTCATAATTGTTACTGCCACC
AATTTATGCCCTCCAAATTCCAAAAAAACTAATGACGATTGGTGCAATCCACCACAAAA
ACACTTTGATCTGACAATGCCTATGTTCATAAAGATTGCAGAGCAAACAGCAGGGGTTG
TACCAGTTGTTTACAGAAGAGTCACTTGCCAGAAAAAAGGAGGTCTCAAGTTTGAGATC
GCCGGGAATCCCAATTGGATTCTTGTTCTTGTTTTCAACGTGGGAGGTGTTGGAGATGT
TGTCAATGTCAAAATCAAAGGATCTAAGACTCAATGGATACCAATGTCGCGGAACTGGG
GCGAAAACTGGCAG

Lycopersicon esculentum expansin (Exp8) mRNA, complete cds

>Le-EXP8, mRNA |11191998|gb|AF184232.1|AF184232
Lycopersicon esculentum expansin (Exp8) mRNA, complete cds

CAAAAAGTAATTTCTTAATTTTCCACTAAGAAATTAAAATGGCAAATAATGTCAATTTA
GCATTGGGATTTATAATTGGTTTGTGTACATTTTTCTCCAGTGCAAATGGTTTCTCAGC
AGATTCTGGATGGACAAGTGCTCATGCCACATTTTATGGTGGAGCTGATGCTTCTGGCA
CAATGGGAGGTGCTTGTGGATATGGTAATTTGTATTCAACAGGATATGGTACTAGAACA
GCAGCATTGAGTACAGCATTGTTCAATGATGGAGGATCATGTGGTCAATGTTACAAAAT
AATTTGTGATTACAAATTAGATCCTCAATGGTGCAAAAAGGGAGTATCAGTTACAATTA
CATCTACAAATTTTTGTCCACCAAATTATAATCTTCCTAGTAACAATGGAGGATGGTGC
AACCCTCCACGTCCACATTTTGATATGGCTCAACCAGCTTGGGAAAAAATTGGCATTTA
CAAAGGTGGCATTGTTCCTGTTTTATACAAAAGGGTACCTTGCAAGAAGCATGGTGGAG
TTAGATTCACAATAAATGGAAGAGACTATTTTGAACTAGTGTTGGTAAGCAATGTAGGA
GGGGCAGGATCTGTTGAATCAGTTCAAATCAAAGGCTCAAACACAAATTGGCTAACAAT
GTCAAGAAATTGGGGAGCAAGTTGGCAATCAAATGCATATCTTGATGGACAATCAATAT
CATTCAAAGTTACTACTAGTGATGGTGTCACAAAAACATTCTTAAATATTGTTCCATCT
AGTTGGAAATTTGGCCAAACATTTTCAAGCAAAACTCAATTCTGATTTGTTAAATGTTC
GGTGAATACGTTTTTAATCAGAGGTCTTGAGTTTATAGGGCAGCGGCATGCTTACTTTT
TACATCAAGTAGCCCTCCCCAAAAAAAGAATCATTCTTATTGAGGTATATTTTTGAGGA
GTTGTTGGTTAAGTAAGCACATGAATGAACTATAGTCCAAATAAAGATAGTGAAAAAAT
TAGAAATAACTAATTATTGGTGTAAGTAGGGGTAGTTCAAAAAATTGTATTTTTTTACT
ATATGAAAGGATGAATAAGATAAGTTTGTTTAAGCTACATC

Lycopersicon esculentum expansin precursor (LE-EXP5) mRNA, complete cds

>gi Lycopersicon esculentum expansin precursor (LE-EXP5) mRNA, complete cds |4138915|gb|AF059489.1|AF059489 Lycopersicon esculentum expansin precursor (Exp5) mRNA, complete cds
CTTCATTTCCTAACTATACCCTCGTTCCTCAAGCAAACACATAGAAACTTTATTTTTTT
GTTCAAAAATGGCTCTTTTAGCTATCCTTTTAATGGGAATTTCCCTCATGTTTCAATCA
GCCCATGGCTATGGTGGTTGGATCAATGCACATGCCACTTTTTACGGTGGAGGAGATGC
TTCTGGAACTATGGGTGGTGCTTGTGGTTACGGAAATTTATACAGCACGGGATACGGAA
CGAACACTGCTGCGTTGAGTACTGCTCTGTTCAACAACGGGTTGAGCTGTGGAGCGTGT
TTTCAGCTAATGTGTGTGAATGCGGGGCAGTACTGCCTGCCAGGCATAATTACGGTAAC
AGCCACCAATTTCTGTCCACCAGGAGGATGGTGTGATCCTCCACGCCCTCATTTTGATC
TCTCTCAACCTATCTTCTTGCGCATTGCTCAATATAGAGCTGGCATAGTTCCTGTTGCC
TATAGAAGGGTACCCTGCAGAAGAAGCGGAGGAATTAGATTTACAATCAATGGCCACTC
TTACTTCAACTTGGTTCTTGTAACAAATGTTGGAGGTTCAGGGGATGTACATTCAGTAT
ACATAAAGGGTTCAAGAACTCAATGGCAACCAATGTCAAGAAATTGGGGCCAAAATTGG
CAGAATAACGCTTACCTTAACGGCCAAAGCTTGTCCTTTAAGGTCACTACAGGCGATGG
TCGCACTGTTGTTTCATACAATGCAGCTCCTAGTTCTTGGTCATTTGGTCAGACATTTT
CTGGAGGACAATTCCGTTAAAACGCAACATATTCTTTAAGAAAAAAAAATCAGAATTTT
CAAAGTATACTACTTGCTAATATATTATTCTAAGAGTTTTGCCTTAAATTTGAGGGTTT
TACTCACCAAAATATTTGGGGAAAAGCGCAGTTTGGCACTTTTTGTCCATTGTAAAATT
GGTCATTCAATTGTGGAAGTAAAAAGAATTGATTTGGCCTACTCTTTTTTGGGTGGTTA
TTAACGTTTAGGGGGCGGCAGTTGTGAGCTTTTACTGCTCAATTTAATAGTATAAATAG

CATTTACGGTATTGTGTGCATAGAAGAAAAATATCTATTTTTTTTTTTTACTGCAAAAG
AGCTAAGCACTAATTATGGTGAGTATGTATGTACCAATTGTCTCTAGCTATTTGTTTGT
AAGAAAATGTTACTATAATAATAAAAGGAAACTCCAAGTTGTACT

Lycopersicon esculentum expansin precursor (LE-EXP4) mRNA, complete cds

>gi Lycopersicon esculentum expansin precursor (LE-EXP4) mRNA, complete cds |4138913|gb|AF059488.1|AF059488
Lycopersicon esculentum expansin precursor (Exp4) mRNA, complete cds
CACCTCCAAAAAAAAACTTGTTTTCCATTTCTCTCTCTCTACTTTCTTTCTCTTAACAC
TGAGCAGTAAGCGAGTGAGTATGAGAAAAATGGCTGCCAATATGATGCTCTACATTACT
ATTACTGTTCTTCTCTGTTTTCTCACTGCCGTCAATGCCAGAATCCCCGGCGTTTATAC
CGGCGGACCATGGCAAACCGCCCACGCCACCTTCTACGGTGGCTCTGACGCATCTGGAA
CTATGGGTGGAGCTTGTGGATATGGCAATTTATACAGCCAAGGTTACGGAGTGAATAAT
GCAGCGTTAAGCACAGTGCTATTTAACAATGGACTAAGCTGCGGAGCGTGCTTTGAATT
AAAGTGTGATAACGATGGCAAATGGTGTCTTCCTGGTAATCCATCCATTTTCGTGACGG
CGACAAATTTTTGCCCGCCGAACTTCGCTTTACCAAACGATGACGGCGGGTGGTGTAAC
CCTCCTCGTCCTCATTTCGATCTCGCCATGCCTATGTTCCTCAAAATCGGACTGTACCG
TGCCGGAATTGTCCCCGTCACATACCGCCGAGTACCATGCAGAAAACAAGGAGGAATTC
GATTCACAATAAACGGTTTCCGTTACTTCAATTTGTTATTGGTAACAAACGTTGCGGGT
GCAGGGGATATACAGAAGGTCTTAATTAAAGGAACAAACACACAATGGATAGCAATGAG
TCGTAATTGGGGGCAAAATTGGCAAACTAATTCACCTTTAGTGGGTCAAGCCCTTTCTA
TTCGGGTTAAAGCTAGTGATCATCGTAGTGTCACGAATGTCAACGTGGCACCCTCTAAT
TGGCAGTTTGGACAACTTTTGAAGGCAAGAATTTCCGGGTTTAGATCCATAAACCCATT
TCAACTGACCCAACCCAAAAAAACAGAATTACTTTAGTATTATATACAACCACAAAAAC
AAGATTTTTCCTAGACTTTAATTGTTTCTCTTTTTTACTGAGAAAGTATTGAAGTCTAA
GGGGAATTAGTATCTTTTATTATTTTTTTTGAACTTGAGTATTGTTTTTTTTTTTTTTA
CTATTTGGTGTTGTGAATTGGGCTGAAGAGGTTGCAAAAGCACCCAAAAAAAATGATTT
TAAAGGGAAAAAGCATGTAGCCCGCAGCTCTATTTGGCATGTTGATGTATTTTCTATGA
ACGAATACCAAAAGATATATAAGGTAATTATATATAATATACTCTAATGTTTTTATAT

Lycopersicon esculentum expansin precursor (LE-EXP3) mRNA, complete cds

>gi Lycopersicon esculentum expansin precursor (LE-EXP3) mRNA, complete cds |4138911|gb|AF059487.1|AF059487
Lycopersicon esculentum expansin precursor (Exp3) mRNA, complete cds
GGGGAGAAGTAATAAAATTTCCCAAATTCCCAAAATTCTCAGTACTATCGAAAATGTTG
CTACAATTATTACTAACACTCACACTGTGTAGCACATTCTCACTAGCTCACTCCCACTA
CAATTGGTCACCGTCTTCCTCCTCCACCGTCGCCGCCGCCGCCGCTCACCCATCGGAGT
GGCGCCCGGGACGGGCAACCTACTATGCTCCAGCAGATCCACGGGATGTAGTAGGTGGC

GCGTGTGGGTACGGTGATTTAGAGAGAAGTGGTTATGGTAAATCAACAGCTGGATTAAG
CACTGTGCTATTCGAAAAGGGTCAGATTTGCGGAGCTTGCTTTGAAGTTCGATGTGTTG
AGGAACTCCGATGGTGTATTCCGGGTACTTCGATTATTGTTACTGCAACGAATTTTTGT
GCTCCGAATTATGGATTTGAACCTGATGGCGGTGGACATTGTAACCCTCCAAATGCACA
TTTTGTTCTGCCGATTGAAGCTTTTGAGAAAATTGCTATTTGGAAAGCTTCGAATATGC
CTGTTCAGTATCGAAGAATTAAGTGCAGAAAGGAAGGAGGAGTTCGATTCACGATCAAT
GGTGCTGGAATATTCTTGTCTGTTTTAATCAGTAACGTTGCAGGCGCGGGTGATATAGT
GGCAGCAAAAGTTAAGGGTTCAAGAACAGGATGGCTTCCCATGGGTAGGATTTGGGGCC
AAAACTGGCATATAAGTGCGGATTTGAAGAATCAACCACTTTCTTTCGAGATAACAAGC
AGTGATGGAGTCACCTTAACATCTTACAATGTTGCTCCGAAGATCTGGAATTTTGGACA
GACTTTTCAAGGTAAGCAGTTCGAATCGTAGGCTGTTCTGGACCTTCCTTGAGGTATTC
ATCAAGATTGAGTTGTGCTTGTTTCTGATGGGGAACTACCAGATGTAGATTTTGGAGTG
CAAAGTTGTAAGATCACTTGAATGTATTAGTATGTTTCATTGTTTATACATGTGTTGTG
TATTGTATTGTGTTGGTTGCTATATGAAGAATAATGTGCACAT

Lycopersicon esculentum (LE-EXP2) gene for expansin2 (gene for EXP2)

>gi Lycopersicon esculentum (LE-EXP2) gene for expansin2
|4884432|emb|AJ239068.1|LES239068 Lycopersicon esculentum
exp2 gene for expansin2
GGGGGCTGTTCCCCGAGTTTATATTACCCATCCTTCCCTTTCAAAATTATATATAGTAA
TGGCATTTTTTTAAAAAAAAATTTAAAGATGTGCACAATTCTAAGTACGGTTCTGGAGT
TCAAATCCTATTTCTTTTTCAGTTTTTTTCTTTTTAAAATTAGGTAATACATCTCTAAG
GGATTATTGATAATATTTTTGACGTTTTAGCTATTATTATTTTATTTTATATTTATAAT
TTATTTATTTGTTTTAATCTTTAAAAACTTTTAAGCTTTGTCACACTACAAAAATCCTA
TTTTCTAGCGCTATAAACTTTTTACATGATTAAATCAAATATGTACATTAAAACTAGAT
AGTAATACTGAATGATATAGCATATTGTCAATGACTACAACCGCTCTTGATATCATTTC
GACGCAAGTGGTAAAAAAGAAAATATCATTTGAATAAATAAGAATTATTTTTAGATCTG
TAATTTTAAATTTTTTAACAGTTATGGTAATTATAAGAACTCTAAGTCAAATGGGTCAA
AGTTTAGTCTTAGATCTATACTCCCTCCGTCCCTTTTAACATGTTTAATTTTAATTTTT
TATTTGTCTTTTTTTTTAATTTGTCATTTTTGATAAATTCAAAAAAGACAATATTTTCT
AATTTATTATACTCTCAATTTATTTAAAAGTTAATGTTTTTGAAAAAAAGTAAATATCT
TCAATAAGTAAATTGATTTTTGAAATTCTATCGGTTAATAAAGATAAGTGGTGAACTCT
ATGTCAAAAGTATTAATTTTTTAATAGGTGTGCCAAGTAAAATAATGAACGAGTAATTA
TGCGCAGAAGGAATATTTCCGTAATAGACCGACCTTCAAAATCTTGAATTTGTCTTTGT
CTCGCGAGGATTAATAAAATAAAGCTTAGACTCCCCCCCCCCTCCCCACCTCCCTTCCC
CCCCTTACTTGTACCTTTACTTGTACTCTTCTCTTTGAGGGTTTTTTTTTTGTTTTTTT
TATCAATAAAAATCAGCCCTAATTATCACGCCTAACATGTCAAATTTGTATCTTAGATC
TATTTTTTTTAATTGTAATTATAATGCACTCATTCTTTCGAAACTCTGAACATGTTTG
CCTCGTTAAAAATAAGACCTAAACCGACTTATTTATGCTTCTCTTTTTCAGATTCTTTT
TGAGATTCCCTTTTTATATATTAAGTAAAATTAGCCTTAGCCAACCTTGAAAAAAAAAT
TGTTCTATGGTAGTAGAATGGTATAGTTACAATTAAAAAGTTTGTTGAGCAAGCTTCCA
ACTTCCTACGTTGTCACCTACCTGAGTGGCGCAACTTTTTAGGGGAAATAAGTGGTGAA
AACAAATGAATGAATGAAAGGAAAGCGTAAAAATAGGGTTGAGAAATATTGCATGTGCT
AAGTAGGGTCAATCAAATCTCAAGTGTAACAATTGCATGTGCGTTTTGGTAGTTGTTTT

ATTTTATGACTTAAAAAACTAATGGTTCTAATACTCTTTCTTTCTTTCTTTCTCTTGTG
TATTTTCACACGATGTCTGTATTATAATTCTAATTAAATTCAAATCATGTGCGGTAGAG
TCTATTTGAGGGTGACACTGCCAACAAGATCATATCGAGATCTTTAATTAAGAGCGAAA
CAATTTTACCAACTGCATTACAACTCATGTTGGTCTAGTACTCTTTCTTTTCTATTTTG
GTTTCTTACTTGATGTTCGGAGTCTGAATTGAAGCCTCGACTAAATCTGAATGACGCAC
TTCAGGGCTCATGCAGGAGTGTGGCGTTCCCAACATAATTTTCTTCATACTCAAAATTT
AAACCTGAGATTTATGGTTAATAATAAAGCAATCTCGCAATTGCACCAGAACTCATGTG
TCTTTCTTTCAAAACGTGGACACTTTAAACTTGCCACTTTCTTCTATAATTAATTAGCA
TATCTTATGCCTAAAATTCAGCTTCTATTTTTTTTGTGTATAAATAGAACTTTGTCAAT
AACCCTTGGCTCATATCAATTCCCACTCCCACTCATCTCTTATCCTCTCCTTAGTTTTA
ATTTACTCAATGGCTTCACTTCCACTTGTTTTCTTCTTTTTAAGCTTTTGCTTCTATTC
TACTTTTGCTGATTATGGAGGATGGCAAACTGCTCATGCCACTTTCTATGGAGGGGGTG
ATGCCTCTGGCACAATGGGTATATCCACTTGTACTGTCTTACTTTCCATCAAATCGAC
TCAAATATATAAGTGGTCTAAAGCAAAATCTAAATAGAGGCCTATATATTTTTGTAAAG
TCTATTATAACCAATTATTTAATTTTTCTTGAAACATAGTATTCAACATTTGTCTAACT
TATATTACTTCTTTTACAAAATAATACTTGATATTTTTAACATATATACATTTTTTTAA
GAAAACGAGGCGCTCAAATTTGGGGGCTAAGACAATTGAGCGGCTCCTGCTTTCCATAT
AGTTTTTCACATTTAAATCAGGTTAACTATACATTAATTATTAATAAAATTAACTTATA
AGTGTAAAGAATTTGTACATTACTAGTGGACTTTTTGACTTAGTTTTTAACTTATACCA
TGTCATATTTTCTAGTATGGACGCCGTTAGTGTATAAAACTTAAACTTGTTGTAGTATA
ATAACATATATGGTTGTCCAAACAACTGCAGGGGGTGCTTGTGGATATGGAAATTTGTA
TAGCCAAGGGTATGGAACTAACACTGCAGCACTAAGTACAGCACTATTCAACAATGGTT
TAACTTGTGGTGCTTGTTATGAGCTCACTTGCAACAATGCAGCTCAATGGTGTCTCCAA
GGGACTATTACTGTCACTGCAACTAATTTTTGTCCTCCGAACCCGTCTCTACCTAACAA
TAATGGTGGTTGGTGCAATCCTCCTCTCCAACATTTTGATTTAGCACAACCTGCTTTCT
TGCAAATTGCTAAATACAAAGCCGGTATCGTCCCTGTATCTTTTCGAAGGTAAACATTA
GTATTGACTTAATTATTATTTATAAAAAAAAGTAACGAGTTCAGTTGAACACGTAATAA
TATGTGAATTTTGTTTGCAGGGTGCCCTGTATGAGAAAAGGAGGAATAAGGTTTACAGT
AAATGGACACTCATTTTTCAACTTGGTTTTAGTGACAAATGTTGGAGGTGCTGGTGATA
TTCAATCAGTTTCAATTAAAGGGTCTAATACTGGATGGCAAGCAATGTCAAGAAATTGG
GGCCAAAATTGGCAAAGCAATTCTAATCTAAATGGTCAAAGTCTTTCATTTCAAGTCAC
CACAAGTGATGGAAGGACACTCATTAGCAACAATGCTGCACCAAATAATTGGCAATTT
GGACAAACTTTTGAAGGGGCTCAATTTTAATTTATTTACTACAAAGCACAAATTGAGGG
GTTTTCAACTTGTAATTTGTATATGGCATAAAAGGGGTGAGTAGGTTTGGGAGAAAACT
CCTTATGGTGGGGGGTTTGGCTCTATTGCTGTGGTGGCTGGTTTGCACCCGCTTAGGCC
TATTAGTAATTTTATTTTATTTTTACAATTTTAGAGATATTTGATTAGCGGCCATTTAT
GGATTTGAGGGTCACTTGAATATCACATAGATTTGTCTATTAGTAGTTCCTAATTCATT
GTGTAAGCAGAATTAGGGCAACTTAATGTCATCATGTTATCCTTCTTTATAATTGCTTT
GGTTCATTTTCTTCTTCTATATCTTTCTTTGGGTTTGCCTTTCTGATCGATGGCTCGAC
AGTGTTAAAAAAAAGATATGTCTTAAACCCCCAAATTTAAGAAGGGCTCATTTTTAATA
GTAATAGGTTATAAGTTATTATTTTTTAATAAATATTGAATATTATTTGTAAAAAAAGT
AAACTTTTCATGAAAATGAAGAAATCCTTAGAAGAAAATTGATATATTTGAAGTACTAT
ATCTCATGAAAAATAATTTTAAATTAAGGATAGTTATATTATCAATTGAAAATTATAAC
AAATCAATTATATAAAAGTTCTAAACAATTCAAATTTAAGGCCCCATTTAATTTTTGTT
TTAGGTCACTAATATGCTTGAGTCTCTCCTGACTTTTCTCATTTGACTAATTTGCTTCT
ATTGGCAAGGCAAGTGAACAAGATCAATATAACACTTACACTAATCCTTTCAAACCTAT
AAGTTGGTCAAACTAGTTTTTAAGTATTGTTTGATATACACTAAGAGATCGTACGGTAC

AAAGATCAATACTTCAGGGAGTAGTAATACATTTTTTGGTAATGCAGAGATTATTTTTA
TCAAGTGTTTGGTTCATTGTTTTTACCTAATTTTGTGTGTGGTTTAAAGTTTACAAAAA
ATAATTCTTTCCAATTATACGCTAAAGTTATTATGAGATTTTATATTTCATGTAATTGG
GTCAAAATAGATAATTGACCGATAATATTATTTTTTATAACATTTTTAACTAGCTAGG
AGAATAAAAATTTTATTGTCATGTTCACTATTTTCTCACTTAAATTATTTGAGAGTAAA
TAATTGTCATCTTAATAAATTAGAGTTAATATCTCAAAAAGTCACAGAACTACAATGAT
TTATAGAGAAAATTTATTGAACTTTGTTTTGTATCAATAAGCTTTAAAAAAACTCTTTA
TCCTAAAATAAAATAAAATAAATATAGCAAAATAAATTAAACTATTTTTATACGAGAGA
TGTGTGTATGTTTGCATATATATATATATATATATACACACACACACACACACACACAC
TCTCTTGTTTTAAACTACTTGTGTAATGTATAACTTATGAATTGTTCTTAAATTCATAC
ATCAATTAGTCCGATTATAATATTTTATATTAATAATAAATAAATTAAATAACTCATAT
TTTAGAAACAAAAAAATTATATCTAA

Lycopersicon esculentum expansin (Le-EXP2) mRNA, complete cds

>gi Lycopersicon esculentum expansin (Le-EXP2) mRNA, complete cds |3747131|gb|AF096776.1|AF096776 Lycopersicon esculentum expansin (LeEXP2) mRNA, complete cds
GATATCAATTCCCACTCCCACTCATCTCTTATCCTCTCCTTAGTTTTAATTTACTCAAT
GGCTTCACTTCCACTTGTTTTCTTCTTTTTAAGCTTTTGCTTCTATTCTACTTTTGCTG
ATTATGGAGGATGGCAAACTGCTCATGCCACTTTCTATGGAGGGGGTGATGCCTCTGGC
ACAATGGGGGGTGCTTGTGGATATGGAAATTTGTATAGCCAAGGGTATGGAACTAACAC
TGCAGCACTAAGTACAGCACTATTCAACAATGGTTTAACTTGTGGTGCTTGTTATGAGC
TCACTTGCAACAATGCAGCTCAATGGTGTCTCCAAGGGACTATTACTGTCACTGCAACT
AATTTTTGTCCTCCGAACCCGTCTCTACCTAACAATAATGGTGGTTGGTGCAATCCTCC
TCTCCAACATTTTGATTTAGCACAACCTGCTTTCTTGCAAATTGCTAAATACAAAGCCG
GTATCGTCCCTGTATCTTTTCGAAGGGTGCCCTGTATGAGAAAAGGAGGAATAAGGTTT
ACAGTAAATGGACACTCATTTTTCAACTTGGTTTTAGTGACAAATGTTGGAGGTGCTGG
TGATATTCAATCAGTTTCAATTAAAGGGTCTAATACTGGATGGCAAGCAATGTCAAGAA
ATTGGGGCCAAAATTGGCAAAGCAATTCTAATCTAAATGGTCAAAGTCTTTCATTTCAA
GTCACCACAAGTGATGGAAGGACACTCATTAGCAACAATGCTGCACCAAATAATTGGCA
ATTTGGACAAACTTTTGAAGGGGCTCAATTTTAATTTATTTACTACAAAGCACAAATTG
AGGGGTTTTCAACTTGTAATTTGTATATGGCATAAAAGGGGTGAGTAGGTTTGGGAGAA
AACTCCTTATGGTGGGGGGTTTGGCTCTATTGCTGTGGTGGCTGGTTTGCACCCGCTTA
GGCCTATTAGTAATTTTATTTTATTTTTACAATTTTAGAGATATTTGATTAGCGGCCAT
TTATGGATTTGAGGGTCACTTGAATATCACATAGATTTGTCTATTAGTAGTTCCTAATT
CATTGTGTAAGCAGAATTAGGGCAACTTAATGTCATCATGTTATCCTTCTTTATAATT
GCTTTGGTTCATTTTCTTAAAAAAAAA

Lycopersicon esculentum expansin (Le-EXP1) mRNA, complete cds (cDNA from ripening fruit)

>gi Lycopersicon esculentum expansin (Le-EXP1) mRNA, complete cds |2062420|gb|U82123.1|LEU82123 Lycopersicon esculentum expansin (LeEXP1) mRNA, complete cds
GAACTTCAATTCCATTAAATCTTAAGAATGGGTATCATAATTTTCATCCTTGTTCTTCT
TTTTGTAGACTCATGTTTCAACATTGTTGAAGGAAGAATCCCTGGTGTTTACTCTGGTG
GTTCATGGGAAACTGCACATGCTACATTTTACGGCGGAAGTGATGCTTCTGGAACAATG
GGCGGTGCGTGTGGTTATGGAAATTTATACAGCCAAGGATACGGAGTTAACACAGCAGC
ACTGAGTACTGCTTTGTTTAACAATGGATTAAGTTGTGGAGCCTGTTTTGAACTTAAAT
GTACAAATACTCCTAATTGGAAATGGTGTCTTCCTGGAAACCCTTCCATTTTAATCACA
GCTACCAATTTCTGCCCACCAAATTACGCGTTGCCAAATGACAATGGTGGCTGGTGTAA
CCCTCCTCGCCCTCACTTTGACCTCGCTATGCCTATGTTTCTCAAACTTGCTCAGTACC
GCGCTGGCATTGTTCCTGTAACTTATCGCAGGATCCCATGCCGAAAGCAAGGAGGAATC
AGATTTACCATCAATGGATTCCGTTACTTCAACTTAGTGTTGATCACGAATGTAGCAGG
TGCAGGGGATATTATTAAGGTTTGGGTAAAAGGAACAAAGACAAATTGGATTCCATTGA
GCCGTAATTGGGGACAAAATTGGCAATCAAATGCGGTTTTAACTGGTCAATCACTCTCT
TTCAGAGTTAAAGCTAGTGACCATCGATCTTCTACCTCATGGAATATGGTTCCTTCTCA
TTGGCAATTTGGCCAAACTTTCATCGGAAAGAATTTCAAAATATAAAATTAGTAAGGGT
ATTGTTATTTTTAATTTGTGGGAAAACTAGGATATTTCAGAGTGTTGTTCACCTTAGGA
AAAGAAATCGAGTCCTCACTGAAAATTCAGATAGATAATTAATTAAATTACTAAAATTT
TTCGATATTTTTGAGTGTGTATCAACATTTTAACCTAAGTATGGTTAAATGGAGAGAAA
GGTTGAAGTGGCTGCAAAATCATGCAGCCCGCAGCTGTTTTTTTTTTTTTACAATATAC
ATCACAAG

Brassica napus cDNA for Expansin ((Bn-EXP1) cDNA from young seedlings)

>gi Brassica napus cDNA for Expansin |2828240|emb|AJ000885.1|BNJ000885 Brassica napus cDNA for Expansin
GAATTCGGCACGAGTGAAAAAATGGCAATGTTGGGTTTGGTGTTATGTCTTCTTACGAT
TCTTGCTGATTTCACAACAATTTCTGAAGCAAGAATCCCAGGAGTTTACAGTGGAGGTG
GTTGGGAAACTGCTCATGCTACTTTCTATGGTGGTTCTGATGCTTCTGGCACCATGGGA
AGTGCTTGTGGGTACGGGAACTTGTACACCAAAGGCTACGGTGTGAACACGGCGGCTCT
AAGCACGGCTCTGTTCAACAACGGTTTCAGCTGCGGTGCTTGTTTCGAGCTCAAATGCG
CGAGTGATCCTAAGTGGTGCCACTCAGGTAGCCCTTCCATCTTCGTCACTGCCACCAAC
TTCTGTCCTCCAAACTTTGCTCAGCCAAGTGACAATGGCGGATGGTGCAACCCTCCTAG
GCCTCACTTCGATCTCGCGATGCCTATGTTCCTCAAGATCGCCGAGTATCGCGCCGGAA
TCGTCCCCGTCTCTTTCCGCCGAGTGCCATGCCGGAAGAGAGGAGGAATGAGGTTCACG
ATCAACGGTTTCCGTTACTTTAACTTGGTTCTGGTTACAAACGTCGCCGGCGCCGGAAA
CATCGTGCGGCTGAGCGTGAAGGGAACAAGAACATCGTGGATAAGCATGAGCCGAAACT
GGGGACAAAACTGGCAATCGAACTCTGTTCTGGTTGGTCAGGCACTTTCGTTTAGAGTC
ACAGCCAGTGACCGTAGATCATCCACGTCATGGAACATTGCTCCGACCCATTGGCAGTT
TGGTCAGACTTTCATGGGAAAGAATTTCAGAGTCTAAGGGCAATTTCGGGAACTCACTA
TCAAAATATCAGTTTTTATTAGTATTCCTAATCCTACTTATTCTGATTACTAAGGCCGA
AGGCAGCTAGTAATTTGGGAAATTATATATATTAAATATTATATTTTCCCATGTTTCTT
AGGACCACTTGGAAATTCCAATATTCTACCTCTGAAAAAAAAAAAAAAAAAACTCGAG

Gossypium hirsutum expansin (Gh-EXP1) mRNA, complete cds (cDNAs from cotton fibers (ovule trichomes))

>gi Gossypium hirsutum expansin (Gh-EXP1) mRNA, complete cds |2811277|gb|AF043284.1|AF043284 Gossypium hirsutum expansin (GhEX1) mRNA, complete cds

TTAGCTAGCTCTTACTCAAATGGCAACCAAAACGATGATGTTGCAAATATTTCCACTTT
TCTTCTTTTTGTTCAGTGTCTGCAACTCCATTTTCCTTGGTGCTAATGGAGATGACAAT
GGTGGTTGGCAAACTGCCCATGCCACCTTCTACGGTGGTGCTGATGCTACCGGCACAAT
GGGGGGAGCTTGTGGTTATGGAAACCTGTACAGTCAAGGGTATGGAACGAGCACAGCAG
CTTTGAGCACTGCACTTTTCAACAATGGCTTGAGCTGCGGTGCCTGCTACGAGCTCCGG
TGCAACAATGATCCTCAATGGTGCATTAGTCGAACCATAACCGTGACAGCCACCAACTT
TTGTCCACCTAACTATGCTTTATCTAGTGACAATGGCGGGTGGTGCAATCCCCCACGAG
AACACTTTGATTTGGCCGAACCGCGATTCTTGCGGATAGCAGAATATCGAGCTGGAATC
GTCCCTGTTATGTTCAGAAGGGTGTCATGTGTGAAGAAAGGAGGCATCAGGTACACCAT
GAATGGACATTCGTACTTCAACATGGTGTTGATAACGAAGCTGGGAGGGGCAGGGGATA
TAACGTCAGTGTCCATCAAGGGTTCCAGAACAGGATGGCTACCTATGTCCAGAAATTGG
GGCCAAAACTGGCAGAGCAATGCTTACCTTAACGGACAAAGCCTCTCTTTTAAAGTGAC
TGCCAGCGATGGCAGGACTATCACAGCCTACAATGTAGTGCCTGCTGGTTGGCAATTCG
GACAAACTTTTGAAGGAGGCCAGTTTTAAGACAATATTATAGTGTCTGTCTAATATAAA
ACTGGAATTGACATATTACTTATATAAGGCACATGAGCGTTTTATGCCGAGGTAGCAAA
ATGGCCGCCGCTGGCTTTATGTGTGAAATAGGCGAGCAAGTGCCATTAGCCTATAATCT
ATACATTTCTTATAGTGAACCAAACTATTAAGTTTGAACTCTAGAGGATATATCCATAA
TGTCTGAATTTGTTTGTTGATGATTGACCATGATATTTATGGTTTTCATTATTGAATAC
CTTTTTTTATAATTTAAAAAAAAAAAAAAAAAAAAAAA

Fragaria x ananassa expansin (Fa-EXP2) mRNA, complete cds

>gi Fragaria x ananassa expansin (Fa-EXP2) mRNA, complete cds |6646884|gb|AF159563.1|AF159563 Fragaria x ananassa expansin (Exp2) mRNA, complete cds

TCTTCTCCTTCTAGCTAGCTAGCTCTCACTTTCTTTCTCACACAATGGCTTTTACTTCA
TGCTTGGCTATTACTCTTCTGGTATCTGTCCTCAACCTCTGCATCAGAGGCACCTATGC
CGACTACGGCGCCGGTTGGGTTGGTGGCCATGCCACTTTCTATGGAGGTGGTGATGCTT
CTGGCACAATGGGAGGTGCATGTGGATATGGAAACTTGTACAGCCAAGGGTATGGAACC
AACACTGCAGCACTAAGCACAGCTCTGTTCAACGATGGCTTGAGCTGCGGGTCTTGCTA
CGAAATGCGATGTGACAATGACCCTAGATGGTGCCTTCCCGGAAGCATCATCGTCACCG
CCACCAACTTCTGCCCTCCCAACTTTGCTCAGGCCAATGACAACGGTGGCTGGTGCAAC
CCTCCCCTCCAGCACTTCGATTTGGCCGAGCCTGCGTTCTTGCAAATCGCTCAGTACCG
CGCTGGTATCGTCCCCGTCTCATTCAGAAGAGTTGCTTGTGTGAAAAAGGGAGGGATCA
GATTCACAATCAACGGGCACTCCTACTTCAACTTGGTTTTGATCACAAACGTTGCAGGA
GCAGGAGATGTGCACTCGGTTTCGATCAAAGGCTCCAAGGGTGGTTGGCAATCCATGTC
AAGGAACTGGGGACAGAACTGGCAGAGCAACAACTACCTCAACGGACAAGCCCTGTCTT
TTCAGGTCACAACCAGTGACGGCAGGACTGTGACCAGCAACAACGTTGCCCCTGGTAAC

TGGCAGTTTGGTCAAACGTTTTCAGGCGGTCAATTCTAGACTTTTTTCACCGAGTTACT
ATTTGCGGTGAAAATGATGATTTGTATATGTAATATTCTGATTGGGAGAGAGGAGGGGG
ATCGAGATTTGTGAGGGTAGATTAGGGAGAGGCAACGTGCTGAGGTGGCTCATTGGCAC
CCGCTAGCTAGGCCTATATATATATATAAATATATATATATCATGATATAAATATATAT
TATTTATAGTGGAAATTGTGAGTTTATAGTTTTTCTAGAGACAACAATTTTCCATTTGA
TGATGCATTTTGCTTGAAGCAAGGAAATGCAAGTAGTATGTTTGAAGGTTTGTAAAAC
CAGTTTGGGGCAGTTGCTTTGTTCTGTCACCTTAATCAAATACTTAGTTGATTTCGCAG
T

Fragaria x ananassa expansin (Fa-EXP1) mRNA, partial cds (mRNA, partial cds; sequence update expected ~22 Jan 2000)

>gi Fragaria x ananassa expansin (Fa-EXP1) mRNA, partial cds |5524699|gb|AF163812.1|AF163812 Fragaria x ananassa expansin (Exp1) mRNA, partial cds
GTGGTGATGCTTCTGGCACAATGGGAGGTGCGTGTGGATATGGAAATTTGTACAGCCAA
GGGTATGGAACGAACACTGCAGCACTAAGCACAGCTCTGTTCAACGATGGCTTGAGCTG
CGGGTCTTGCTACGAAATGCGATGTGACAATGACCCTAGATGGTGCCTTCCCGGAAGCA
TCATCGTCACCGCCACCAACTTCTGCCCTCCCAACTTTGCTCAGGCCAATGACAACGGT
GGCTGGTGCAACCCTCCCCTCCAGCACTTCGATTTGGCCGAGCCTGCGTTCTTGCAAAT
CGCTCAGTACCGCGCTGGTATCGTCCCCGTCTCATTCAGAAGAGTTGCTTGTGTGAAAA
AGGGAGGGATCAGATTCACAATCAACGGGCACTCCTACTTCAACTTGGTTTTGATCACA
AACGTTGCAGGAGCAGGAGATGTGCACTCGGTTTCGATCAAAGGCTCCAAGGGTGGTTG
GCAATCCATGTCAAGG

Prunus armeniaca expansin (Pa-EXP2) mRNA, complete cds (cDNA from apricot fruit)

>gi Prunus armeniaca expansin (Pa-EXP2) mRNA, complete cds |3510539|gb|AF038815.1|AF038815 Prunus armeniaca expansin (Exp2) mRNA, complete cds
GTCAATAGTCAAGCAAGTCTTTCTCTCCCCTATCTCCCATTCTTTCTCACAACACAAAA
AATATACACAACAATGGCTTTTACCTCACACTTAGCCATTGCTCTTCTGTTCTCTGTTC
TCAATCTATGTCTTCAAGGCACTTATGGTGACTATGGAGGAGGATGGGAAGGTGGTCAT
GCCACATTTTATGGTGGCGGTGATGCCTCTGGCACAATGGGAGGTGCTTGTGGATATGG
AAATTTGTATAGCCAAGGGTATGGAACCAACACTGCAGCTCTCAGCACAGCTCTGTTCA
ACGATGGCTTGAGCTGTGGGTCTTGTTATGAGATGAGATGTGACAGTGACCCCAAATGG
TGCCTCCCCGGCAGCATCATCGTCACTGCCACAAACTTCTGCCCTCCTAACTTAGCTCA
GTCTAATGACAATGGTGGCTGGTGCAACCCTCCTCTCCAGCACTTTGATTTGGCTGAGC
CTGCCTTCTTACAAATTGCTCAATACCGAGCTGGAATTGTCCCCATCTCCTTCAGAAGG
GTTTCTTGTGTGAAAAAGGGAGGGATAAGATTCACCATCAACGGTCACTCTTACTTCAA
CTTGGTTTTGATCACAAACGTTGGAGGTGCAGGGGATGTGCACTCTGTTTCAATCAAGG
GGTCCAAAACAGGGTGGCAAGCCATGTCAAGGAACTGGGGCCAGAACTGGCAGAGTAAC
TCTTACCTCAATGGCCAGGCTCTATCTTTCCAAGTCACCACCAGTGACGGTAGAACTGT
GACCAGCAATGCTGTGCCAGCTGACTGGCAGTTTGGTCAAACATTTTCGGGCGGTCAAT

TCTAGACTCTTTTTATAAACAGTAAAAGGTCTGGAAGAAATGGCGCGGGGTGATTTGTA
TATGTAATATTTGATTGCTTGGGAAAGGTGAGAGAGGAGAAGGAGACAGACAGGGTAGA
TTAGGAGAGAGGCAAATGTTATTGCTGAGGTGGCTAATTTGGCACCCGCAAGGCCTGCT
ATATATATATATATTATTTATAGTAACGGTGGAGATTGTGAGTTATATAGTTCGCAGAA
ATCGTCCATTTTGAAGATGCATTTTGTTTGTTTTGAAGCAAAATGCACATTAATATCT
TTGAAGGTGGTAGGTAGGTATGCAGATTTATTACTCATACCATACCTAACTAGGTACTG
AGCCCAGCTGGGGCAACTTTGTACTGGGCATCTTAATTATATACTTTGTGAATTTCTAT
GAAAAAAAAAAAAA

Prunus armeniaca expansin (Pa-EXP1) mRNA, complete cds (cDNA from apricot fruit)

>gi Prunus armeniaca expansin (Pa-EXP1) mRNA, complete cds |3510537|gb|U93167.1|PAU93167 Prunus armeniaca expansin (PA-Exp1) mRNA, complete cds
AAACACCTCCCCTGCATAAAACCGAAGCTCCTCCTCTCTCTTCCTTTCTCCTATTGAAA
AATCATATCAAAACATGGCACCTCAGGCATTGTCTTTAGCTCCCCTAGCCCTCTCTCTT
GTTCTCTTCAATCTTCATCTTCATGGTGCTTTTGCTGATTATGGTGGCTGGGAAGGCGC
TCATGCCACTTTTTATGGTGGCGGTGATGCCTCGGGAACCATGGGGGGAGCATGTGGGT
ATGGGAACTTGTACAGCCAAGGGTATGGAACCAACACTGCAGCTTTAAGCACAGCCTTG
TTTAACAATGGCTTGAGCTGTGGCTCTTGTTATGAAATGAGATGCAACAATGACCCTAG
ATGGTGTCGTCCTGGAAGCATCATTGTTACTGCCACAAACTTTTGCCCACCTAACTTTG
CTCAGTCCAACGACAATGGCGGCTGGTGCAATCCTCCCCTCCAGCACTTTGATTTGGCT
GAGCCTGCCTTTCTTCAAATTGCCCAATACCGCGCTGGGATTGTGCCTGTTACCTTCAG
AAGAGTGCCCTGTATGAAGAAGGGAGGAATCAGATTCACCATCAATGGCCACTCCTACT
TCAACCTGGTTTTGATCACCAACGTGGGTGGTGCAGGAGACGTCCACTCAGTTTCAATC
AAGGGGTCCAGAACAGGGTGGCAACCCATGTCAAGAAACTGGGGGCAAAACTGGCAGAG
CAACAATTACCTCAATGGCCAGAGCCTCTCCTTCCAAGTGACCACCAGTGACGGAAGAA
CTGTTACAAGCTACAACGTGGCCCCTGGTAATTGGCAGTTTGGTCAGACTTTCTCAGGG
GGTCAATTTTAGAGTTATTCCACTAGATTATTAGTAAAAGTTTGTATATCTATCTGCTG
TCCTTTTTCTTTTTTACCGAGTGTAAAAGCCAAGAGTAGTTGTAAGGTGAGGGTTTGCT
GAGGTGAGCTAAAAGCACCCGCTGGGCCTTTCACATTTGAGATTTCTGGAGGAGAAATT
AAATTTACCTCTTTTAGGTTACAACCCATTTCTAAGTTGTAACATTAATAATATATATT
TATAAAATCAAAGAGGTTTATAAAAAAAAAAAAAAAAAAAAAAAAA

Zea mays beta-expansin 8 (Zm-EXPB8) mRNA, complete cds

>gi Zea mays beta-expansin 8 (Zm-EXPB8) mRNA, complete cds |14193774|gb|AF332181.1|AF332181 Zea mays beta-expansin 8 (expB8) mRNA, complete cds
GCACGAGCTCGCACTCACTCCACCGCTGCCCACTTCTTGTTTGTATTGTATCCAGACTC
CAGAGGAAGCCGAGAGGGCCAATGAGGCAAGGCATGAGCTCCCCTTCCGCCGCCGCGGC
GCTCGTGCTCCTCCTGGCCCTGCTCGCCGGAGGCCAGTGCCGAGAGGCCCAGCTCGACG
CCGCGGACGCCGGCACCGGCACGGAGAACTACACCGCCAGCGACGCCGCCGTGTACTGG
GGCCCCTGGCAGAGGGCCCGGGCCACCTGGTACGGCCAGCCCAACGGCGCCGGCCCCGA

CGACAACGGTGGCGCGTGCGGATTCAAGCACACCAACCAGTACCCCTTCATGTCCATGG
GCTCCTGCGGCAACCAGCCATTGTTCAAGGACGGCAAGGGCTGCGGCTCCTGCTACAAG
ATTCGGTGCAGGAAGGACCCGTCCTGCTCCGGGCGGACGGAGACGGTGATCATCACCGA
CATGAACTACTACCCGGTGTCCAAGTACCACTTCGACCTCAGCGGCACTGCGTTCGGCA
GGCTGGCCAAGCCCGGCCTCAACGACAAGCTCCGCCACTCCGGCATCATCGACATCGAG
TTCACCAGGGTGCCGTGCGAGTTCCCGGGCCTGAAGATCGGGTTCCACGTGGAGGAGTA
CTCGAACCCCGTCTACTTCGCGGTGCTGGTGGAGTACGAGGACGGCGACGGCGACGTGG
TGCAGGTGGACCTGATGGAGTCCAAGACGGCGCGCGGCCCGCCGACGGGGCGGTGGACG
CCGATGCGCGAGTCCTGGGGCTCCGTCTGGCGCATGGACACCAACCACCGCATGCAGCC
GCCCTTCTCCATCCGCATCCGCAACGAGTCCGGCAAGACGCTCGTCGCCAACAACGTCA
TCCCGGCCAACTGGCGCCCCAACACCTTCTACCGCTCCTTCGTCCAGTACAGCTAACCG
ATCGGGATCCGTGCCCTGCCCGCAATTGGAGCTGGCTGGCTGGGGCGCTACTCGGCTCG
GTCACCATCGTCTACAGTACTATACTATGCTCTACTACCACTAGACTGCTGCTGTTACT
ACTAGTCTACTACCACCAAGTACGGCGGAACGGAACAGCTAGTCGTCGTCGGCGGCCT
TGGGAGGGGAGGTGTCTCCGGTCACCGTGCCGTTGCAGTTGTATCGTGATCATGGTCCT
GGGAGTTGTTGCATCCGGGGCGCGCTTTTTAGGTCAGGCGTCGTGTGCGATGTTTGTGG
GGTCAGGCTGGCATGTCATGCCGTGCGTGACCCTGATTGCGAGCCGGCAAGCAAGAAGA
CAAAAAAACTAAAGCCGTGTGGGAAAATGGAGGAGGCAGGCGTACAAGCTACGCTCTCC
CGCCCACTGTTGCTTTTATAATTTATCATCATCATCATCTTCATTCCGATCGGTGATTA
ATCGAAAAGTATATTGTAATGTATAAACGCCATTTTCCTTTCGTCAAAAAAAAAAAAAA
AAAAA

Zea mays beta-expansin 7 (Zm-EXPB7) mRNA, complete cds

>gi Zea mays beta-expansin 7 (Zm-EXPB7) mRNA, complete cds
|14193772|gb|AF332180.1|AF332180 Zea mays beta-expansin 7
(expB7) mRNA, complete cds
ACACAGAACTTCTCCTGTCGGCCTACTAATACAGCTAGCTAGCTGCCCTCTTAGGTATA
CTGTGATGGCCACAACCTTGTCCTCCACAGTAGTAGTTGCACTTGGTGCACCTCTCTTC
TTGCTCCTTGTAACGTGTGGCTCGTGCGCGAGGCCGGTGAGCTTTAACGCCTCCGACCT
CACCGCCGATCCCGGCTGGGATGCTGCCAGGGCCACCTGGTACGGTGCGCCCACCGGCG
CCGGCCCTGATGACGACGGTGGTGCCTGTGGATTCAAGAACGTGAATCTGCCGCCGTTC
TCGGCAATGACGTCGTGCGGCAACGAGCCCCTGTTCAAGGACGGCAAGGGCTGCGGCTC
CTGCTACCAGATACGATGCCAAAACCACCCGGCCTGCTCCGGCAACCCAGAGACGGTGA
TCATCACTGACATGAACTACTACCCCGTGGCCAAGTACCACTTCGACCTCAGCGGCACG
GCGTTCGGCGCCATGGCCAAGCCCGGCCGCAACGACGAGCTCCGCCACGCCGGCATCAT
CGACATCCAGTTCAAGAGGGTGCCCTGCAACTACCCCGGGCAGAAGGTGACGTTCCACG
TCGAGGAGGGCTCCAACCCCGTCTACTTCGCGGTGCTGGTCGAGTTCGAAGACGGCGAC
GGCGACGCGGTGCAGGTGGACCTCATGGAGGCCAACTCTGCGTCGTGGACGCCGATGCG
CGAGTCCTGGGGATCCATCTGGAGGCTCGACTCCGGCCACCGCCTCACCGCGCCATTCT
CCCTGCGCATTACGAACGAGTCCGGCAAGACGCTGGTGGCTGACCATGTCATCCCCGCC
AACTGGGTGCCCAACACCTACTACCGTTCTATCGTCCAGTATTAGCCGCTCCCAGCTGC
TGCGCCGGCCGGATAATAATATACAATTCTGTGTCATTGTGCTTTACTATTAGTTTGGT
ATTAGTATTGTATTTCTGCATGGGACTGATGAGGCACAGAGCTGAAGTGAGGTGCCTCA
CTCCATTGCTAATTTGCTACTAGGTGTTAAGTTGGTGTGCGGCTTCAGGGAGTTGAAGA

CTGGGAAGTGGAGGAAGCAGTGCTTTTTCCCGCCCATTGTCGTGAATGCATGTACCAGT
GCGATTAAGATTATATAAATTATATTGACAGTAAAAAAAAAAAAAAAAAAAA

Zea mays beta-expansin 6 (Zm-EXPB6) mRNA, complete cds

>gi Zea mays beta-expansin 6 (Zm-EXPB6) mRNA, complete cds
|14193770|gb|AF332179.1|AF332179 Zea mays beta-expansin 6
(expB6) mRNA, complete cds
TCGAAACACAAACCAGCAGCAGGCAGAGCAACGACTAGCTGCCAGGCAAGCCGGCCGGG
GGTGCTGCAAGAGTGCAACAAACAATGGCCACCGCGCTCTCCTTCAAGGCCTTGGCACT
GGCCGCGCTCCTCTCCATGGTCGTCGCCTCTGGCGCTCGCGCCGAGCAACAGTTCAAGC
GCAACGCCACCACCGCCTCGCAGGACAGATCCTTGTTGTCCTACAGCAGTGGCTGGCTG
CCGGCCAAGGCCACCTGGTACGGCGCCCCCACCGGCGCCGGCCCCCTCGACAACGGTGG
TGCGTGCGGGTTCAAGGGCACCAACCAGTACCCGTTCTCGTCCATGACGTCCTGTGGCA
ACGAGCCCATCTTCAAGGACGGCAAGGGTTGCGGCTCATGCTACCAGATACGGTGCCTG
AAGAGCAACCACCCGGCCTGCTCCGGCGTGGCTCAGACGGTGATCATCACGGACATGAA
CTACTACCCGGTGGCCAAGTACCACTTCGACCTGAGCGGCACGGCGTTCGGGTCCATGG
CCAGCTGGGGCCTGAACGACAAGCTCCGGCACGCCGGGATCATCGACATGCAGTTCAGG
CGGGTGCCGTGCAACTTCCCGGGCCTGACCATCACCTTCCGCGTCCAGCACGGCTCCAA
CCCCATGTACCTGGCCGTGCTCATCGAGCACGAGAACAAGGACGGCGACGTGGTGCAGG
CGGACATCATGCAGAGCAACTCCGGCCACTGGGAGCCCATGCACGAGTCCTGGGGCTCC
ATCCCCAACCGCCCGCTCCGTGGCCCCTTCTCCATGCGCATCACCAACGAGTCCGGCAG
GCAGCTGGTGGCCAAGAACGTCATCCCGGCCAACTACATCCCCGACGTCGACTACCGCT
CCTTCGTCCAGTACTAATGATCGAGCTAGCTTGGTCATTGCTTAAGCGTTTAATTGTTT
CTTCTTTATTTAATTAGTGGCTGCCGTTGCCGACATGCATGTGGGGGAAATTGGAGGAG
GCAAGCATCAATGCAATGTGTGCTCTCCCGCCCACTGTCACTATACCGTTATTAATTAC
CAGTGTGTAACCAGCAAATCATACTACATATATGGTTATATAATTTCATACTAAAAAAA
AAAAAAAAA

Zea mays beta-expansin 5 (Zm-EXPB5) mRNA, partial cds

>gi Zea mays beta-expansin 5 (Zm-EXPB5) mRNA, partial cds
|14193768|gb|AF332178.1|AF332178 Zea mays beta-expansin 5
(expB5) mRNA, partial cds
CAAGGGATGTGGCTCCTGCTACCAAATACGATGCCAGAACCACCCTGCCTGCTCCGGTA
ACCCGGAGACGGTGATCATCACCGACATGAACTACTACCCGGTGGCCAAGTACCACTTC
GACCTTAGCGGCACGGCGTTCGGCGCCATGTCCAAGCCCGGCCGCAACGACGAGCTCCG
CCACGCCGGCATCATCGACATCCAGTTCAAGAGGGTGCCCTGCAACTACCCCGGGCGGA
AGGTGACGTTCCACGTGGAGGAGGGCTCGAACGCCGTCTACCTGGCGGTGCTCGTCGAG
TTCGAGGACGGCGACGGCGACGTGGTGCAGGTGGACCTCATGGAGGCCAACTCCGGCTA
CTGGACGCCGATGCGCGAGTCCTGGGGGTCCATCTGGAGGCTGGACGCCAACCACAGGC
TACAGGCGCCCTTCTCGCTGCGCGTCACCAACGAGTCCGGCAGGAAGCTGGTGGCCACT
CGGGTCATCCCTGCCAACTGGGCGCCCAACACCTACTACCGCTCCATCATTCAGTACTA
GCTCTCTGCTACTGCGCCTCGACCGATTCGGTGTCATTTGGCTTCATTTGAACTGTATG

TAATTGTATCTTCACGTGCACAGGATTACGGTGCCTTAACTAGTTAAAAAAAAAAAAAA
A

Zea mays beta-expansin 4 (Zm-EXPB4) mRNA, complete cds

>gi Zea mays beta-expansin 4 (Zm-EXPB4) mRNA, complete cds
|14193766|gb|AF332177.1|AF332177 Zea mays beta-expansin 4
(expB4) mRNA, complete cds
GGCACGAGGAACAATCGAGCTACTAATAAGGTCGTATACATATCTTCTATATACTCCTC
TGAAAGTTGTGAACTCCGGTCGAGCTTAAAAACAGCAGCAATGGCGAAGCTTTGGACAT
TGCTGCTGGCTGCAGTGGTGGTCCTCTCACTCCTAGTGAGCCCCATTGCTTGCACCCGA
AAGCTCAACAAACCCAAGCCGAAGCCGGGCAGCTACAGGCGGCCGGTCAAGCCGAAGCC
AAAACCGGTCACGGGCAGCTACAAGCCGGCGCCTGTGGCCGCCAGAAGAAACCACACAG
CTACACCCACGCCATCGCCGACTGTCTACGGCCCCGGTGGCTGGCTGTCAGGCGCCGGC
GCCACGTACTACGGCGCGACCAACGGCGACGGGAGCGACGGCGGCGCGTGCGGCTACCA
GACGGCCGTCGGAAAGAAGCCATTCGACTCGATGATCGCCGCCGGGAGCACGCCACTGT
ACAGGGGAGGCGAGGGCTGCGGCGCCTGCTACGAGGTGAAATGCACGACCAACGCCGCG
TGCTCCGGCCAGCCCGTGACCATCGTAATCACCGACCAGTCCCCTGGCGGGCTGTTCCC
CGGCGAGGTCGAGCACTTTGACATGAGCGGCACCGCCATGGGCGCCATGGCCCGGCCCG
GCATGGCCGACAAGCTCCGCGCTGGCGGCGTGCTCAGGATCCTGTACAGGAGGGTGCCG
TGCAAGTACACCGGCGTCAACATCGCGTTCAAGGTGGATCAGGGCGCGAACCCGTACTA
CTTCGACGTGCTCATCGAGTTCGAGGACGACGACGGCGACCTCAGCGCCGTGGACCTCA
TGGAGGCCGGCAGCGGCGTCTGGACTCCTATGGCGCACAACTGGGGCGCCACGTGGCGC
CTCAACAACGGCAGGAAGCTCAAAGCGCCGTTCGGGCTCCGGCTCACCTCCGACTCCCG
CAGGGTGCTCGTCGCCAACAACGCCATCCCGGCCGCGTGGAAGCCCGGCAAGACCTACC
GCTCCTTGGTCAACTACCCCTGAAAAGAGAAATACCGACAAGTGGATGGCGTGTATTGT
GCGTCCGGGTGTTGCGAGTGGCGGCGGTGTACTACTGGTGTCGGAAAACAGAAGAGAA
TGAAAGAGGAGGTTGAAGAAGAGAATAATGTCCTTCTTCCCTCCCTGGACGGTCTCTGC
AGTCCCCAAAAGTGATGTGTGACGGTGTTAGTCAAATCATGCCGGTAATTTGATACTTC
ATCTCGATTTGAGTTTTAAAAAAAAAAAAAAAAA

Zea mays beta-expansin 3 (Zm-EXPB3) mRNA, partial cds

>gi Zea mays beta-expansin 3 (Zm-EXPB3) mRNA, partial cds
|14193764|gb|AF332176.1|AF332176 Zea mays beta-expansin 3
(expB3) mRNA, partial cds
CGGACGCGTGGGCGGCGGCGCGTGCGGCTTCAAGGGCGTGAACATGCCACCTTTCTCGG
CGATGACGTCGTGCGGCAACGAGCCAATCTTCAAGGACGGCAAAGGATGCGGGTCCTGC
TACCAGATCCGGTGCAAGGCCCACCCTGCGTGCTCCGGCGTGGCGGAGACGGTGATCAT
CACGGACATGAACTACTACCCGGTGGCCCCCTACCACTTCGACCTGAGCGGCACTGCCT
TCGGGGCCATGGCAAAAGACGAGCACAACGACGAGCTAAGGCACGCAGGAATCATCGAC
ATCCAGTTCAAGAGGGTTCCCTGCCAGTACCCTGGTCTGACGGTGACCTTCCACATCGA
GCGTGGCTCCAACCCCAACTACCTGGCGGTGCTGGTGGAGTACGAGAACGGCGACGGCG
ACGTGGTGCAGGTGGACCTGATGGAGTCCAGCCCCGACGACGGCGAGCCCACCGGCGTC
TGGGAGCCCATGCGCGAGTCCTGGGGCTCCATATGGAGGATGGACACCCGCCGGCCGCT

GCAAGGGCCCTTCTCGCTGCGCGTCACCAACGAGTCCGGCAAGACGCTGGTGGCGGACC
AGGTCATTCCGGCAGACTGGCAGCCCGACAACGTCTACAGCTCCATCGTCCAGTTCGAG
TAGCAGTATATGCCATGCGTCGTACATATAGATATATATACACTACACACACAACGCGG
TGTCAGCAGTAATTTAAGCAAGTATATGCATCATCATTAATTCCATGGACGGATCGGAT
CGATGATATATATGTACGTAA

Zea mays beta-expansin 2 (Zm-EXPB2) mRNA, partial cds

>gi Zea mays beta-expansin 2 (Zm-EXPB2) mRNA, partial cds
|14193762|gb|AF332175.1|AF332175 Zea mays beta-expansin 2
(expB2) mRNA, partial cds
ATTCGGTGCAGGAAGGACCCGTCCTGCTCCGGGCGGACGGAGACGGTGATCATCACCGA
CATGAACTACTACCCGGTGTCCAAGTACCACTTCGACCTCAGCGGCACGGCGTTCGGCA
GGCTGGCCAAGTCCGGCCTCAACGACAAGCTCCGCCACTCGGGCATCATCGACATCGAG
TTCACCAGGGTGCCGTGCGAGTTCCCTGGCCTCAAGATCGGGTTCCACGTGGAGGAGTA
CTCGAGCCCCGTCTACTTCGCGGTGCTGGTGGAGTACGAGGACG
GCGACGGCGACGTGGTGCAGGTGGACCTGATGGAGTCCAAGACGGCGCGCGGGCCGCCG
ACGGGGCGCTGGGCGCCGATGCGCGAGTCCTGGGGCTCCGTCTGGCGCATGGACACCAA
CCACCGCATGCAGCCGCCCTTCTCCATCCGCATCCGCAACGAGTCCGGCAAGACGCTCG
TCGCCAGGAACGTCATCCCGGCCAACTGGAGGCCCAACACCTTCTACCGCTCCTTCGTC
CAGTACAGCTAGCTAGCTAGCTGGTTTGCGCCCCTAGTTCACCACCCACCACTACTACC
ACCGCCACCCACTAGACTACTGCTTCTGCTACCAAATACTACGGCGGAACGGAACGGCT
GGTTGCCGCCGCCGCCGTCGTCCTTGGAAAGGTTGAGGCGTCTCTTGGTCATCCGTATC
GTTACCGTTGTCATGGTCCTTTGAGTCGTTGCAACCCTGATTGCAAGCCGGCAAGGGGG
AAAAAACCAACAAAGCCGTGTGGGAAAATGGAGGAGGCAGGCGTACAATGTACGCTCTC
CCGCCCACTGTTGCTTTATAATCTCTATATCATCATCATCTTCTTCTTCTCCATTCCGA
TCGGTGATTAATCGAAAAGTATATTGTAATGTAAAAA

Zea mays beta-expansin 1 (Zm-EXPB1) mRNA, complete cds (complete cDNA pollen allergen (incomplete sequence))

>gi Zea mays beta-expansin 1 (Zm-EXPB1) mRNA, complete cds
|14193760|gb|AF332174.1|AF332174 Zea mays beta-expansin 1
(expB1) mRNA, complete cds
GGCATTTCGAATAACACAGGTGAGCGCGACGATGGGATCCCTCGTTAATAACATCATGG
TCGTGGGCGCCGTCCTTGCGGCGCTCGTCGCCGGCGGGTCGTGCGGGCCCCCGAAGGTG
CCACCCGGCCCCAACATCACCACCAACTACAACGGCAAGTGGCTCACCGCTAGGGCCAC
CTGGTACGGTCAGCCCAACGGTGCCGGCGCTCCTGACAACGGCGGTGCGTGCGGGATCA
AGAACGTGAACCTGCCACCCTACAGCGGCATGACGGCGTGCGGCAACGTCCCCATCTTC
AAGGACGGCAAGGGCTGCGGCTCATGCTACGAGGTGAGATGCAAGGAAAAACCTGAGTG
CTCGGGCAATCCAGTCACGGTGTACATCACTGACATGAACTACGAGCCTATCGCTCCCT
ACCACTTCGACTTGAGCGGCAAGGCCTTCGGCTCCCTGGCAAAGCCCGGGCTCAACGAC
AAGATTCGCCACTGCGGCATCATGGACGTCGAGTTCAGAAGGGTGCGATGCAAGTACCC
CGCCGGGCAGAAGATCGTGTTCCACATCGAGAAGGGCTGCAACCCCAACTACCTGGCCG
TGCTGGTGAAGTATGTGGCGGACGACGGCGACATCGTGCTGATGGAAATCCAGGACAAG

TTGTCGGCTGAGTGGAAGCCCATGAAGCTCTCTTGGGGCGCCATCTGGAGGATGGACAC
TGCCAAGGCGCTCAAGGGCCCCTTCTCCATCCGCCTCACCAGCGAGTCCGGCAAGAAGG
TCATCGCCAAAGACGTCATCCCGGCGAACTGGAGACCCGATGCCGTCTACACTTCCAAC
GTCCAATTCTACTAGACTTTGAATTCCCTTCGATTCATCCGGCACAGCGGGCTATGGAC
CTTCAGCAGCAAGCTAATTAAGTTGGCAGCATGCACCGCTAACCTTATATACTACTGAG
ACTTCCAAATTCTAGTATATGTAATCCTTTTGTTCGGGTTCATGATCGAATTCCAAAGA
GTGGAAAACAAGCAAAAGGTTAAATATACATGCCATTTTTGGAGGCATTTTTTTCATGA
AAAAAAAAAAAAAAAAAA

Rumex acetosa expansin (EXP1) mRNA, partial cds

>Ra-EXP1 mRNA, partial cds |5734351|gb|AF167365.1|AF167365
Rumex acetosa expansin (EXP1) mRNA, partial cds
GGCGGAGCTTGTGGGTATGGAAATCTGTACAGCCAAGGGTACGGAACGAACACAGCAGC
TCTGAGCACAGCTATGTTCAACAATGGGTTAGCATGCGGCTCCTGCTACGAAATCACCT
GTGTGGCTGACCGGAAATGGTGCATTCCGGGCAGCATTATGGTGACCGCGACCAACTTC
TGCCCGCCGAACAACGCCTTGCCGAACAACGCCGGCGGCTGGTGTAACCCCCCTCTTCA
CCACTTTGACCTGGCTCAGCCTGTTTTCCAGCACATTGCACAGTACAAAGCCGGAATAG
TCCCTGTTGCTTACAGAAGGGTGCCATGTAGAAGAAGGGGAGGAATGAGGTTCACAATC
AATGGGCACTCCTACTTCAATTTGGTTCTTATCACTAACGTTGGAGGAGCTGGAGATGT
GCACGCGGTGGCCATTAAAGGCTCAAGAACAAGGTGGCAAGCAATGTCCAGGAACT

Rumex acetosa expansin (EXP2) mRNA, partial cds

>Ra-EXP2 mRNA, partial cds |5734335|gb|AF167357.1|AF167357
Rumex acetosa expansin (EXP2) mRNA, partial cds
GGAGGTGTATGTGGATACGGAAACCTATACAGCCAAGGGTACGGGACCAACACGGCAGC
GCTAAGCACGGCCCTGTTCAACGACGGGCTTAGCTGCGGGGCCTGCTTTGAGATGCGAT
GCAGCGGTGACCCGAGATGGTGCATTGGTGGAGTCATAACTGTGACCGCCACCAACTTC
TGTCCCCCCAACTTCGCCCTAAGCAACGACAACGGCGGGTGGTGCAACCCACCCCTCCA
ACACTTCGACCTCGCCGAGCCTGCCTTCCTTCAGATCGCTCAGTACCACGCTGGCATTG
TACCCGTCTCATTCCGAAGAGTTCCTTGCGGAAGAAAAGGAGGAATAAGGTTCACAATA
AACGGGCATTCCTACTTCAACCTAGTCCTGATCACCAACGTCGGAGGCGCAGGCGACGT
CCACTCGGTCTCGATCAAGGGCTCGCGTGGAGGGTGGCAGGCGATGTCCCGTAACT

Curriculum Vitae

Sherrine Adele Ibrahim

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sherrine@aol.com

EDUCATION:

2000 MARSHALL UNIVERSITY, Huntington, WV
Graduate School for Biological Sciences
Degrees Earned
Master of Science in Biology

1999 UNIVERSITY OF PENNSYLVANIA, Philadelphia, PA
Degrees Earned
Bachelor of Arts in Biological Basis and Behavior (BBB) and Psychology
Minor in English

1995 HUNTINGTON HIGH SCHOOL, Huntington, WV
Member of: National Honor Society, Mu Alpha Theta, Beta Club, Phoenix Club, Latin Honorary, Girl's State
***Activities*:** Peer Counselor, Student Council, President of Senior Class, Treasurer of Mu Alpha Theta, Captain of Cheerleading Team, Dance

AWARDS / HONORS:

2001 MARSHALL UNIVERSITY, Huntington, WV

Recipient of a Marshall Summer Thesis Research Scholarship of $500
Researcher: The Graduate College of Marshall University awarded me a summer thesis research grant for the period covering the end of the spring semester to June 30, 2001. The criteria for selection included quality and significance of the thesis research, and the likelihood that the research would eventuate in a completed thesis.

2000-2001 MARSHALL UNIVERSITY, Huntington, WV
Recipient of a NASA Student Scholarship of $1000
Researcher: Received this scholarship based on current research involving the regulation of gene expression in gravistimulated pea stems. The major studies I am examining include (1) analysis of the soluble cell wall and cytoplasmic proteins during gravitropic curvature (2) amino acid sequence analysis of novel proteins associated with gravistimulation, and (3) characterization of novel cell wall and cytoplasmic enzymes which respond to a change in orientation to gravity. As a requirement of the scholarship, this research shall be presented at the Sigma Xi Society April 2001.

2000 MARSHALL UNIVERSITY, Huntington, WV
Dean's List

PRESENTATIONS:

2001 **Ibrahim, S.A**., Valluri, J., and Deraimo, D. Regulation of gene expression in gravistimulated pea stems, *Pisum sativum*. The 62nd Meeting of the Association of Southeastern Biologists, New Orleans, Louisiana. (Published abstract in *Southeastern Biology* April 2001).

2001 **Ibrahim, S.A**., Valluri, J., and Deraimo, D. Regulation of gene expression in gravistimulated pea stems, *Pisum sativum*. Sigma Xi Society, Huntington, West Virginia.

2000 **Ibrahim, S.A**., Ibrahim, A. A., and Heaberlin, B. Management of Chronic Venous Stasis Disease in the Twentieth Century. Marshall University School of Medicine, Huntington, West Virginia.

EXPERIENCE:

1995 – Present **OFFICE OF DR. ADEL IBRAHIM AND DR. TARA SHARMA**
Huntington, WV
Responsibilities: Assistant Business Manager:

- Included various secretarial duties (i.e. answer phones, schedule appointments and surgeries, send faxes, etc.), assisting in the billing department, and helping patients fill out forms and explain procedures to them.

- Allowed to assist the doctors in several minor procedures and answered any questions the patients might have.

1995 – 1999 **PENN DANCE**
University of Pennsylvania, Philadelphia, PA
Responsibilities:

- Co-Chairperson: January – December 1996 –1998. Organized and prepared for bi – annual performances. Worked with the Treasurer and Student Activities Council in dispersing University finances to other student groups. Also worked with the Performing Arts Council to create space for performing arts groups, and organizing several charity fund-raisers and memorials (The Emily Sacs Benefit).
- Publicist: September – September 1995-1996. Responsible for publicizing our Bi – annual performances, which included creating posters, banners, painting the bookstore wall, and preparing the program. These duties overlapped with a semester of the Co-chair position, thus was also required to delegate responsibility to other dancers. Participated as a dancer and choreographer.

RESEARCH EXPERIENCE:

2000 – Present **PLANT AND CELL BIOLOGY RESEARCH UNDER DR. JAGAN VALLURI**
Marshall University, Huntington WV
Program Description: *The research being pursued in conjunction with*
Dr. Jagan Valluri emphasizes three areas of research. 1) Genetic engineering of plant drought and salt tolerance, 2) changes in gene expression during gravistimulation and 3) production of economically important secondary metabolites from plant cell cultures. This research will eventually lead to my masters thesis.
Responsibilities: My responsibilities include keeping the lab well stocked, performing experiments, preparing presentation material based on research, bio-reactor experiments, and specific research for the masters thesis.

1999 – 2000 **MARSHALL UNIVERSITY SCHOOL OF MEDICINE'S 13TH ANNUAL RESEARCH CONFERENCE**
Marshall University, Huntington, WV
Program Description: *The program consisted of a series of faculty, residents, Medical students, and graduate students highlighting current clinical care, investigations, and basic research being conducted. The objectives of this program was to compare different approaches to medical investigation, compare and contrast the importance of basic research and cellular mechanisms as it relates to human disease, discuss and review research related*

to current and future improvements in the clinical management of patients, and interpret and analyze data for medical investigation.
Responsibilities:

- Worked in conjunction with a physician and medical student to determine the effectiveness of the physician's novel modality for treating Chronic Venous Stasis Disease.
- Compiled six years of patient information, researching the current techniques for treating the disease, researching the biological mechanism that causes the disease, comparing a control group with a study group, and providing sufficient evidence for the success of the treatment.
- Presented the study at the research conference in March.

1998 – 1999 **THE PENN RESILIENCY PROJECT**
University of Pennsylvania, Philadelphia, PA

Program Description: *A National Institute of Mental Health funded project under Drs. Martin Seligman, Karen Reivich and Jane Gillham at the Department of Psychology. The study investigates the effectiveness of school-based programs in preventing depression in adolescents.*
Responsibilities: 10 – 15 hours of office hours required which included: data entry, photocopying, putting together and labeling various packets which are to be administered to children, teachers, and parents in the study, coding tapes for reliability purposes, running errands, and preparing a poster presentation based on the research collected during the year.

CLINICAL EXPERIENCE:

2001 - Present "**SHADOWING" STEPHEN SHY, D.O.**
Tri - State Physicians Network, Coal Grove, OH
Responsibilities:

- To follow, observe, and learn from a practicing Family Practice Physician in a clinical setting.
- To gain insight into diagnosing and treating patients at the primary care level.

1999 – 2000 "**SHADOWING" BONNIE BEAVER, M.D., F.A.C.S.**
Marshall University School of Medicine, Huntington, WV
Responsibilities:

- To follow, observe, and learn from a practicing Pediatric Surgeon.
- Observed her in a clinical setting (office and surgery), and learned about various symptoms and diseases that commonly affect children between infancy and 15 years of age.

1998 – 1999 **THE PENN RESILIENCY PROJECT**
University of Pennsylvania, Philadelphia, PA
Clinical Interviewer
Responsibilities:

- Administration of the Child Depression Rating Scale (CDRS), a structured clinical interview, to project participants (children) evidencing above average levels of depressive symptomatology on a paper-and-pencil measure (CDI).
- Conducted interviews with students to assess the severity of suicidal ideation if such thoughts are reported during the CDRS.
- Received 20 hours of training and coded other interviews for reliability purposes.

1993 – 1995 **HUNTINGTON HIGH SCHOOL COUNSELER'S OFFICE**
Huntington High School, WV
Responsibilities:

- Conducted peer sessions that allowed high school students to discuss problems with people of their own age.
- Received a semester of training to cope with various problems and situations that may arise in counseling sessions.

COMMUNITY SERVICE:

1999 – 2000 **SHiNE (Seeking Harmony in Neighborhoods Everyday)**
Philadelphia, PA
Program Description: *SHiNE is a national non-profit organization dedicated to preventing youth violence by implementing artistic and creative programs that teach expression through the mind instead of through violence.*
Responsibilities:

- Volunteer
- Promoted the organization by passing out flyers at events.
- Worked to educate people about the program and at Woodstock 1999 and Earth Day 2000.

1997 – 1999 **SPECIAL OLYMPICS**
Philadelphia, PA
Program Description: *This program supports and offers daily encouragement to athletes with mental retardation, and provides families a place where they can come together with opportunities for sports, social interaction, and fun.*
Responsibilities:

- Volunteer
- Performed several tasks including: acting as a greeter, athlete escort, and "cheerleader" at events.
- Took part in recreational and social outings with athletes

1994 – 1999 **HUNTINGTON CITY MISSION**

Huntington, WV
Responsibilities:

- Volunteer
- Prepared, served, and cleaned up meals for the local homeless.

TEACHING EXPERIENCE:

2001 - Present **MARSHALL UNIVERSITY TEACHING ASSISTANT**
Marshall University, Huntington, WV
Responsibilities: Currently teaching introductory Biology and cellular biology laboratory classes to Marshall University undergraduates.

1997 - 1998 **WEST PHILADELPHIA TUTORING PROJECT**
Philadelphia, PA
Responsibilities:

- Tutoring and encouraging inner-city middle school students.
- Taught children study skills and provided them with insight into high school and college life.

COMPUTER SKILLS:

IBM Compatibles, Macintosh, Windows '95, '98, and 2000 Microsoft Office, Microsoft Publisher, Web Page Creation
Statistics Packages: Systat and JMP IN
Database Programs: Alpha Five for Windows
Word Processing Programs: Microsoft Word (all versions), Microsoft Works (all versions), Power Point, Microsoft Excel
Email Programs: Telnet, AOL, and Outlook Express
Internet Programs: Netscape, Internet Explorer, and AOL.

COLLEGE ACTIVITIES:

Alpha Phi Sorority, Kite and Key, Penn Dance

LANGUAGE SKILLS:

Proficient in Latin

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