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MANIPULATION OF GENES INVOLVED IN SECONDARY CELL WALL
DEVELOPMENT DURING WOOD FORMATION IN POPLAR

By

Kavitha Satish Kumar

A DISSERTATION

Submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

In Biological Sciences

MICHIGAN TECHNOLOGICAL UNIVERSITY

2018

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This dissertation has been approved in partial fulfillment of the requirements for the
Degree of DOCTOR OF PHILOSOPHY in Biological Sciences.

Department of Biological Sciences

Dissertation Advisor: *Dr. Chandrashekhar P. Joshi*

Committee Member: *Dr. Guiliang Tang*

Committee Member: *Dr. Rupali Datta*

Committee Member: *Dr. Ashutosh Tiwari*

Department Chair: *Dr. Chandrashekhar P. Joshi*

Dedication

To my family

Avinash Satish Kumar, Usha Satish Kumar, and Satish Kumar

With me, my family graduates!

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Preface

Chapters included in this dissertation have been written and formatted with the purpose of publishing them in peer-reviewed journals.

Chapter 2: Study was designed by Kavitha Satish Kumar along with Dr. Chandrashekhar P. Joshi and conducted by Kavitha Satish Kumar with help from Surattana Boonsai and Dane Wouri.

Chapter 3: Study was designed by Kavitha Satish Kumar along with Dr. Chandrashekhar P. Joshi and conducted by Kavitha Satish Kumar with help from Surattana Boonsai and Dane Wouri.

Chapter 4: Study was designed by Kavitha Satish Kumar along with Dr. Chandrashekhar P. Joshi and Dr. Guiliang Tang and conducted by Kavitha Satish Kumar with help from Surattana Boonsai and Dane Wouri.

None of the above chapters have been published or submitted for publication yet.

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List of abbreviations

PRX	Peroxidase
LAC	Lacasse
PCW	Primary cell wall
SCW	Secondary cell wall
PAL	phenylalanine ammonia-lyase
C4H	cinnamate 4-hydroxylase
C3H	4-hydroxycinnamate 3-hydroxylase
CCoAOMT	caffeoyl CoA 3-O-methyltransferase
COMT	caffeic acid 3-O-methyltransferase
F5H	ferulate 5-hydroxylase
4CL	hydroxycinnamate CoA ligase
CCR	hydroxycinnamoyl CoA:NADPH oxidoreductase
CAD	hydroxycinnamoyl alcohol dehydrogenase
SDX	Stem-differentiating xylem
miRNA	micro-ribonucleic acid
NREL	National Renewable Energy Laboratory
STTM	Short Tandem Target Mimic
H ₂ O ₂	Hydrogen peroxide
HRP-C	Horseradish peroxidase isoenzyme C
CWPO-C	Cationic cell wall peroxidase
Tyr	tyrosine

PCD	Programmed cell death
ROS	Reactive oxygen species
AIR	Alcohol insoluble residue
S/G	Syringyl/guaiacyl
HD-ZIPIII	Class III HOMEODOMAIN-LEUCINE ZIPPER
HB	Homeobox

Abstract

Lignins are second most abundant components of vascular plant cell walls. They provide plants with structural rigidity and are polymers of monolignols. Lignin polymerization is catalyzed by peroxidases and/or laccases. These enzymes are suggested to share functional overlap and mechanism by which they coordinate this process is not clearly understood. There are about 100 peroxidases and 50 laccase genes known in poplar genome out of which some stem differentiating xylem-specific (SDX) enzymes were selected for our study. The main objective was to genetically manipulate genes expressed in the SDX region in the cell wall to see the effects on lignin content in the wood without affecting yield of the transgenic poplar trees. We also aimed to understand the role these enzymes play during lignin polymerization. We also adopted the short tandem target mimic (STTM) strategy to manipulate miRNAs that might be involved in wood development of poplar trees. Our results indicate that peroxidases, laccases and miRNAs under investigation play some specific roles in secondary cell wall biosynthesis. Therefore, manipulation of expression of these genes may prove beneficial towards future genetic engineering of the poplar trees for improved downstream applications.

1 Chapter 1

Introduction

Plant cell walls are classified into primary and secondary cell walls that provide cellular elasticity and structural rigidity, respectively. The primary cell walls (PCW) are synthesized during cytokinesis, separated by a pectin-rich layer that forms the middle lamella and remodeled during cell expansion. Programmed cell death of specialized cells such as xylem and sclerenchyma that are rich in lignin and hydroxycinnamic acids, gives rise to the secondary cell wall (SCW). Polymers such as polysaccharides and polyphenols are deposited in the secondary cell wall in the form of cellulose, hemicellulose and lignin (Mellerowicz and Sundberg 2008) (Zhong and Ye 2014) (Bashline et al. 2014).

Cellulose are the most abundant plant biomass. They are polymers of β (1, 4)-linked D-glucopyranoses comprising of 15-30% and 40-50% of the primary and secondary cell wall, respectively, making it the first choice for biomass conversion to biofuel such as bioethanol. There have been a lot of studies to genetically manipulate to quantitatively improve and increase the cellulose content in the wood or biomass (Harris, Stork, and Debolt 2009) (Sahoo et al. 2013) and reduce the lignin content owing to its recalcitrant nature to biological degradation (Chen and Dixon 2007). Cellulose is polymerized by cellulose synthase (CESA) enzyme and alteration in this enzyme has shown enhanced saccharification for fermentable sugars (Joshi and Mansfield 2007). Saccharification involves physical, chemical or biological pre-treatment of lignocellulosic material that results in delignified pulp. This pulp is subjected to cellulolytic enzymes that hydrolyze

sugar polymers and release simple sugars (monomers) which are further fermented to obtain bioethanol.

Lignins are second most abundant components of vascular plant cell walls after cellulose. Lignins constitute a colossal reservoir of fixed carbon in land plants. Lignins are synthesized from the oxidative coupling of three major monolignols – p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol. They provide structural stability, rigidity and hydrophobicity to vascular plants. The proportions of these monomers differ between varied plant species or even within the same species in different organs and cells. This variation is easily observed when comparing gymnosperms and angiosperms. The lignin in gymnosperms comprises of guaiacyl lignin (G units) and p-hydroxyphenyl lignin (H units) polymerized from coniferyl alcohol and p-coumaryl alcohol. The lignin in angiosperms comprises of guaiacyl lignin and syringyl lignin (S units) polymerized from coniferyl alcohol and sinapyl alcohol. Lignin also functions to provide resistance to pathogen infection, biotic and abiotic stresses which pose an obstruction to microbial degradation to convert plant cell wall biomass to biofuel or other bioproducts (also known as recalcitrance). The importance of understanding more about lignin and its biosynthesis increased with the desire to generate transgenic plants with less or more easily digestible lignin for reducing the processing cost involved in producing biofuels (Boudet et al. 2003) (Chen and Dixon 2007) (Nookaraju et al. 2013) (Barros et al. 2015).

Studies have been performed to understand the difference in lignin composition and content by developing a network of reactions involved in the phenylpropanoid metabolism. The core enzymes involved in the biosynthesis of lignin from phenylalanine are

phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H), 4-hydroxycinnamate 3-hydroxylase (C3H); caffeoyl CoA 3-O-methyltransferase (CCoAOMT); ferulate 5-hydroxylase (F5H) and caffeic acid 3-O-methyltransferase (COMT), hydroxycinnamate CoA ligase (4CL), hydroxycinnamoyl CoA:NADPH oxidoreductase (CCR) and hydroxycinnamoyl alcohol dehydrogenase (CAD). These enzymes have been functionally characterized and act as catalysts for monolignol production from phenylalanine via a series of intermediary reactions. Downregulation or upregulation of synthesis of one or more of these enzymes in different plants has been attempted to understand the role they play in the biosynthesis process. Results suggest that the regulatory metabolic network for the cell wall components is linked and may play a concerted role in cell wall biosynthesis (Boudet 1998). These modifications in the lignin biosynthesis pathway have also shown to improve saccharification efficiency in the several plant species (Nookaraju et al. 2013). Intensive research has been carried out to understand the regulatory mechanism involved in lignin biosynthesis to bring about a change in the lignin content/composition (Liang, Davis, et al. 2006) (Wang et al. 2008) (Marjamaa, Kukkola, and Fagerstedt 2009) (Li and Chapple 2010) (Wang et al. 2013) (Trumbo, Zhang, and Stewart 2015). This understanding will help generate transgenic trees with altered lignin that can be used for improved biofuel production via saccharification.

Figure 1.1 Schematic representation of intermediary reactions involved in lignin biosynthesis and polymerization pathway

After synthesis, the monolignols are suggested to be transported from the cytosol through the plasma membrane to the cell wall. Co-expression analysis and transcript profiling suggest that ATP-binding cassette (ABC) transporters could be involved in transport of monolignols for free radical based oxidative polymerization catalyzed by laccase and/or peroxidase (Li and Chapple 2010). Polymerization event creates two topographic zones of lignifying cell walls based on the amounts of polymerizing enzyme and substrates. The PCW of xylem parenchyma cells is categorized by increased levels of peroxidase enzyme and substrates such as monolignols and hydrogen peroxide (H_2O_2). The second zone created by the SCW thickening of xylem vessels is categorized by lower levels of peroxidase and unaltered substrates. This gave rise to two types of polymerization event

namely, rapid “bulk polymerization” and gradual “end-wise polymerization”. As the name suggests, the former is a fast process and facilitates the C-C coupling of monolignols. This forms highly branched and cross-linked polymers rich in p-coumaryl residues with β -5, β -1, β - β , 5-5 and 5-*O*-4 inter-unit bonds. The latter is a relatively slow process facilitates β -*O*-4 coupling of monolignols at pH 5.5 forming linear polymers of lignin in the SCW (Grabber, Hatfield, and Ralph 2003) (Barceló et al. 2004) (Grabber 2005).

The two gene families supposed to be involved in this final step of polymerization of lignin monomers are peroxidases (PRX) and/or laccases (LAC). Monolignol radicals formed by oxidation of monomers are catalyzed by these enzymes. They are large gene families which are said to exhibit overlapping functions. PRXs uses H₂O₂ whereas LAC uses O₂ as electron acceptor to form these radicals that bind with each other/oligomers to form the large lignin macromolecule. Several studies have been performed on a wide range of plants – *Arabidopsis*, *Nicotiana*, switchgrass, poplar, and others, to understand the molecular mechanism behind lignin polymerization from monolignol units. Some studies suggest that these enzymes share a functional overlap while others suggest their functions are non-redundant with each other (Zhao et al. 2013b).

However, the mechanism by which plants regulate the polymerization process is not clearly understood and leaves these open questions:

1. Which enzyme drives lignin polymerization? Is it PRX or LAC or a combinatorial action of both these enzymes?
2. Do they play separate non-redundant functions in lignification?
3. What are the effects of PRX and/or LAC genetic manipulations on lignin content in transgenic wood and changes in saccharification efficiency?

Recent studies suggest regulatory role of miRNAs in SCW and wood development and it seemed interesting to look at certain miRNA that will help provide insights to our understanding of cell wall synthesis. Plant microRNAs (miRNAs) are 21nt non-coding RNAs that play a modulatory role in gene expression at the post-transcriptional and translational stages of development. Two miRNAs – miR397a and miR166, stood out to be interesting candidates to study these functions. (Lu et al. 2013) (Trumbo, Zhang, and Stewart 2015). The overexpression of miR397a has been shown to negatively regulate laccases thereby affecting the lignin content in poplar. This led to a coordinated repression of specific laccases expressed in the xylem area and also an upregulation of PRXs in these plants. The miR166 regulates a class of transcription factors called the class III HOMEODOMAIN-LEUCINE ZIPPER (HD-ZIP III) thereby regulating the expression of homeobox (HB) genes that are involved in cambial and wood development. These findings involving the regulation exerted by these two miRNAs in cell wall biosynthesis led to these questions:

1. Can regulation of miRNA gene expression help to understand the role of its target genes?
2. Can this help to provide further insights into the role of miRNA in wood development?

Based on these proposed questions for lignin biosynthesis and bioenergy production, the current study has been designed to understand how the PRX and LAC enzymes, and miRNAs function with respect to lignin polymerization during SCW formation. This will help to elucidate whether either of the enzymes, one or none is the rate limiting step in lignin biosynthesis.

The broad objectives of this study include:

1. Impact of genetic manipulation of PRXs and LACs on lignin content and composition of wood from transgenic trees
2. Explore functional redundancy (if any) between the lignin polymerizing enzymes, PRXs and LACs in poplar
3. Regulation of specific miRNAs to decipher their role(s) in secondary cell wall biosynthesis.

Specific objectives of each chapter to achieve the broader objectives included:

Availability of the entire sequence of the genome, amenable plant transformation procedures and being a woody tree species made poplar, the model plant to work on for this project.

Chapter 2: We aimed to manipulate and overexpress specific stem-differentiating xylem (SDX)-specific PRXs in a tissue-specific manner to alter lignin content in poplar trees.

Chapter 3: We adopted the same method as chapter 2, to manipulate and overexpress specific SDX LACs in tissue-specific manner with an aim to alter lignin content in poplar trees. We also aimed at manipulating the expression of miR397a which is supposed to regulate the laccase activity during lignin polymerization and to achieve this, we used the short tandem target mimic (STTM) strategy.

Chapter 4: In this chapter, using STTM strategy, we aimed to understand the regulation exerted by miR166 during cambial and wood development which could be an alternative approach to manipulate wood for bioenergy purposes.

Approaches

Approach 1: There are 93 peroxidases and 49 laccase genes in poplar genome (Lu et al. 2013) (Ren et al. 2014). Based on the experimental and *in-silico* expression profile in tissues, we selected two SDX PRX and two LAC genes, respectively from these gene families in poplar. We overexpressed the selected genes under developing xylem-specific promoter, DX15 (Ko et al. 2012).

Figure 1.2: Graphical representation of selected of PRX and LAC genes for the current study

Approach 2: We adopted STTM strategy developed by Dr. Guiliang Tang's group to study loss-of-function of miR397a and miR166 where the expression of these miRNAs was suppressed and the downstream effects on wood development was studied.

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2 Chapter 2

Genetic manipulation of two peroxidase genes in poplars

Abstract

The growing importance of conversion plant biomass into value-added products makes it important to understand in the process of plant cell wall biosynthesis. Over years, significant understanding of lignin biosynthesis has helped pave a way for efficient use of plant biomass for saccharification purposes. But the process of polymerization of lignin still caters to several unanswered questions mainly due to the suggested involvement of two gene families namely, peroxidase (PRX) and laccase (LAC) in this process. Our study aimed at understanding the role PRXs and LACs play in lignin polymerization and to specifically can suggest which of these enzymes are the rate limiting step during this process. For this, we selected and overexpressed two out of six stem differentiating-xylem (SDX)-specific PRX genes – PRX25 and PRX36 using tissue-specific promoter (DX15) to manipulate the expression of PRXs and to study its downstream effects on lignin formation. We transformed our gene constructs into *Agrobacterium* and performed poplar leaf-disc transformation to generate transgenic poplar plants expressing the PRX genes. The overexpression of PRX25 led to its co-suppression in the PRX25 transgenic lines. The PRX36 gene was overexpressed in PRX36 transgenic lines. We determined that every PRX in SDX has an essential role in lignin polymerization. We were also able to delineate functional characterization of PRXs and LACs in lignin polymerization. Our study could also demonstrate that PRXs and LACs played some specific role in this process and that they did not compensate for each other's functions. Overall, we observed that manipulation

of PRXs did not significantly change the lignin content but affected the S/G lignin ratio. These alterations brought about structural changes in the plant cell wall leading to improved sugar release but did not affect the plant growth yield. These characteristics are highly desirable for lignocellulosic biomass to be used for downstream bioenergy applications.

2.1 Introduction

Peroxidases are heme-containing enzymes present in all living organisms that utilize hydrogen peroxide (H_2O_2) as an electron acceptor to oxidize the donor molecule. Peroxidases in higher plants are categorized into four types: glutathione peroxidase, catalase, ascorbate peroxidase (intracellular class I peroxidase) and classical secretory class III plant peroxidases (PRXs) (Passardi, Penel, and Dunand 2004) (Shigeto and Tsutsumi 2016). Among, these we will focus here on PRXs that are plant-specific heme-oxidoreductases that are also part of a large gene family. All categories of plant peroxidase enzyme have the similar structural arrangement that comprise of ten α -helices and ferriprotoporphyrin IX as a prosthetic group. In addition to this structure, presence of three extra α -helices, highly conserved amino acids and four disulfide bridges distinguishes class III PRXs from other three types of plant peroxidases. They are present in all land plants throughout their life cycle. They share their involvement in plant developmental and physiological processes such as germination, cell growth, stress tolerance, cell wall cross-linking, lignification, senescence to name a few (Passardi, Penel, and Dunand 2004).

It is speculated that mechanistically, H_2O_2 oxidizes the active site of peroxidase enzyme, the substrate binds to this site and gets oxidized to a phenoxy radical (Barceló et al. 2004).

PRXs are highly substrate specific, exclusively coniferyl alcohol (Guaicyl or G units), and some are specific to sinapyl alcohol (Syringyl or S units). They are further classified into

acidic or basic peroxidases due to differences in their isoelectric points and substrate specificity. Acidic peroxidases or anionic peroxidases are poor catalysts for oxidizing sinapyl alcohol as their activity is hindered because of hydrophobic interactions between methoxy atoms of sinapyl alcohol and conserved regions – I-138 and P-139 in the class III peroxidases. This makes them specific for oxidizing coniferyl alcohol (Aoyama et al. 2002). Basic peroxidases or cationic peroxidases can oxidize almost all the three types of monolignols making them less substrate specific compared to acidic peroxidases. The suggested reason for this may be the substitution of the I-138 by L-138 in most basic peroxidase and thereby reducing the hydrophobic interactions exhibited by sinapyl alcohol. (Østergaard et al. 2000) (Passardi et al. 2005). It is still unknown whether this is the only factor that makes them different in their capability of oxidizing monolignols. Lignin polymers rich in coniferyl and sinapyl alcohol are suggested to be oxidized by basic peroxidases. This has been demonstrated in previous studies that explains the existence of a redox shuttle mechanism exhibited by coniferyl alcohol or p-coumaric acid. The guaiacyl radicals – ($G^{\cdot}$) from coniferyl alcohol or (p-coumaric acid radicals – ($p-CA^{\cdot}$) from p-coumaric acid) are capable of withdrawing $H^{\cdot}$ atom from sinapyl alcohol that are catalyzed by either acidic or basic peroxidases. This reaction regenerates coniferyl alcohol (G) or p-coumaric acid (p-CA) and sinapyl alcohol-free radical ($S^{\cdot}$). But, guaiacyl radicals – $G^{\cdot}$ or p-coumaric acid radicals – $p-CA^{\cdot}$ radicals are incapable of withdrawing a $H^{\cdot}$ atom from sinapyl oligomers. This reaction and thus, forming condensed polymers are catalyzed by only basic peroxidase. This ability of basic peroxidases to oxidize S oligomers makes them

an integral part of the lignification process (Fournand, Cathala, and Lapierre 2003) (Barceló et al. 2004) (Marjamaa, Kukkola, and Fagerstedt 2009).

Modifications in peroxidases

For the past several decades, researchers have been trying to categorize the exact functions of PRXs using reverse genetics. In one such approach it was observed that constitutive and increased expression of two anionic PRXs led to induced wilting of leaves through loss of turgor pressure in transgenic tobacco (Lagrimini, Bradford, and Rothstein 1990). Another study demonstrated the effects of heterologous expression of chimeric tobacco anionic PRX (TobAnPOD) in tomato plants. The findings of this study suggested involvement of TobAnPOD in wound-induced phenolic metabolism, lignification and thereby increasing lignin levels in wounded-tissues. But it was also observed that elevated levels of this peroxidase did not help to improve the plants resistance to pathogens (Lagrimini et al. 1993). In 1998, Christensen et al. characterized five xylem-specific anionic peroxidases in poplar and suggested PXP3-4 and PXP5 (PRX7 and PRX22 respectively, according to the new nomenclature) to be likely involved in lignin polymerization (Christensen et al. 1998). Antisense suppression of lignin-specific cationic peroxidase in tobacco (NtPrx60) and anionic peroxidase in aspen (PrxA3a) led to vascular tissue modification and reduction in lignin content and composition (Blee et al. 2003; Li et al. 2003).

PRX isozymes function was mostly dependent on the spatiotemporal regulation of PRX gene expression. This was since these isozymes shared high amino acid sequence similarity and other structural traits. Over the years, biochemical strategies were adopted to identify

and develop a network of function and biochemical properties pertaining to PRX isozymes. These strategies considered characteristics such as substrate specificity, selectivity and mode of action. As stated earlier, lignin polymerization step requires an enzyme that can catalyze the oxidation of four lignin precursors, the three monolignols and growing lignin polymer (Sasaki et al. 2004). One of the most commonly studied PRX protein, horseradish peroxidase isoenzyme C (HRP-C) are found in non-lignified tissues. An *in vitro* study performed demonstrated that HRP-C could actively oxidize coniferyl alcohol i.e. involved in the formation of G-lignin. But it failed to oxidize sinapyl alcohol and other lignin oligomers as these polymers could not fit into the heme pocket of the enzyme owing to their large size. This implies that HRP-C and many similar PRX isoenzymes cannot be involved in lignin polymerization due to lower oxidation activity for sinapyl alcohol. Similar studies have determined PRXs with higher oxidation activity for both coniferyl and sinapyl alcohol. These PRXs play a critical role in dehydrogenative polymerization of sinapyl alcohol and its dimer to lignin polymers (Marjamaa et al. 2006). Cationic cell wall peroxidase (CWPO-C) – a peroxidase isoenzyme from poplar callus can oxidize sinapyl alcohol, synthetic lignin polymers and ferrocyclochrome *c*. Studies indicate that the heme pockets in this isoenzyme are similar in size to other plant PRXs. Instead, these isoenzymes display two tyrosine (Tyr) residue – Tyr-177 and Tyr-74 near the heme-pocket on its protein surface. This site is unique and active oxidation site for this isoenzyme as modifications made to these residues suppresses the oxidation activity (Sasaki et al. 2008). PRXs that are like CWPO-C have been identified in other plant species, for example ZePrx from *Zinnia elegans*, BPX1 from *Betula pendula* exhibiting the same oxidation property. CWPO-C and ZePrx play an essential role in lignification of cell walls over the radical

mediator theory like their putative *Arabidopsis* orthologs (AtPrx71 and AtPrx4, 52 and 72 respectively) were involved in lignification (Aoyama et al. 2002) (Sasaki et al. 2004) (Pham et al. 2011) (Shigeto et al. 2013) (Shigeto et al. 2014) (Herrero, Carrasco, and Zapata 2014) (Fernández-Pérez, Pomar, et al. 2015) (Fernández-Pérez, Vivar, et al. 2015). A suggestive ability of CWPO-C, AtPrx2, 25 and 71 in oxidation of large lignin polymers was demonstrated by their capability to oxidize ferrocyanochrome c (Shigeto et al. 2014). In addition, AtPrx71 also negatively regulates *Arabidopsis* growth and cell wall alterations promotes accumulation of reactive oxygen species (ROS) that affects cell expansion (Raggi et al. 2015). Enhanced activity of apoplastic peroxidase from barley, HvPrx8 in tobacco plants revealed that an increase in peroxidase activity can affect lignification of cell walls, programmed cell death (PCD) and elevated expression of the antioxidant ascorbate peroxidase (Burbridge et al. 2014).

Numerous similar studies have been performed to understand the role played by peroxidases in cell wall lignification. The most interesting study demonstrated that the alteration of PRXs could lead to increase in saccharification by 200-300% in tobacco plants (Kavousi et al. 2010) (Cook et al. 2012). But it is still unclear whether the peroxidase activity is the rate limiting step for monolignol polymerization. The current study was designed to alter the lignin content in the developing xylem with an aim to comprehend the role of peroxidases in monolignol polymerization and changes in saccharification efficiency in poplar trees. For this purpose, we selected two stem-differentiating xylem (SDX)-specific peroxidase genes from the 93 known peroxidases in poplar trees (Ren et al. 2014) that were overexpressed under a tissue-specific promoter, DX15 (Ko et al. 2012)

that will ensure the changes caused by the manipulation are only seen in the developing xylem.

2.2 Materials and Methods

2.2.1 Gene selection and vector construction

For this study, out of the 93 peroxidase genes present in *Populus trichocarpa*, we selected two peroxidase genes (PRX25 and PRX36) based on their expression in SDX (Ren et al. 2014) (see Appendix B.1). The full-length amplification of the coding region of these genes was performed using gene specific primers (see Appendix A.1). We used QIAquick PCR purification kit (Qiagen) for clean-up of PCR reactions residual components to purify the amplified gene product. The purified gene products were digested using restriction enzymes *XbaI/PacI* for PRX25 gene and *BamHI/SacI* for PRX36 gene. Digested fragments were cloned using NEB® 5-alpha competent *E. coli* (high-efficiency) cells into modified pBI101 vector with tissue specific promoter DX15 to replace GUS gene. Cloned plasmid was then transformed into *Agrobacterium* strain C58C1 using freeze thaw method (Boerjan et al. 1997) (Wu, Joshi, and Chiang 2000). The transformed plasmids were selected by antibiotic resistance. Verification PCR for the gene of interest on the selected clones were carried out before transforming the plants. The sequences of the selected clones for DX15, PRX25 and PRX36 genes from plasmid and agrobacterium transformation was also confirmed by sanger sequencing (see Appendix A.2, B.2, B.3, B.4, B.5).

2.2.2 Plant transformation and growth conditions

The positively verified *Agrobacterium* clones were used to transform *Populus tremula* x *alba* clone 717-1B4 genotype (routinely referred as 717) using leaf-disc transformation protocol as previously described (Boerjan et al. 1997) (Wu, Joshi, and Chiang 2000). First, the *Agrobacterium* clone is allowed to infect the leaf discs at the wound site (where the leaves have been cut) through several initial steps. Later these infected leaves are allowed to grow on callus induction medium (CIM) for three to four weeks to develop callus (unorganized mass of plant cell). Once callus is formed, they were moved to shoot induction medium (SIM) and then to shoot elongation medium (SEM) for three to four weeks each. Shoots generating from separate calli were considered independent lines. Keeping track of each of these independent lines, they were further moved to root induction medium (RIM) until the shoots developed roots. The callus and shoots were grown in a growth chamber (Thermo Scientific Precision model 818 incubator) with a light and dark cycle of 16 hrs and 8 hrs at 25°C. Plants that rooted were selected and genotypically verified for the presence of the gene of interest using genomic PCR. Verified transgenic lines were acclimatized and, moved and maintained in the green house for approximately 3 months before harvesting. The green house growth conditions were kept similar to the growth chamber with light and dark cycle of 16hrs and 8hrs, temperature of 25°C and relative humidity ranging from 40-70%. Upon harvest of the 13 weeks old plants, all leaf material from each plant was weighed before and after drying and stored in paper bags. The stem was cut into approximately four to five pieces (with ten to fifteen internodes each). The bark was cut open with a sharp blade and developing xylem on the wood material was

scraped in liquid N₂, collected in aluminum foil and stored in liquid nitrogen until use. The remaining wood material from each plant was air-dried and stored for further analysis.

2.2.3 RNA extraction and qRT-PCR analysis

Total RNA was extracted from SDX (1-10 internodes counted from the base of the plants with 40-50 internodes) using TRIzol (Ambion, Life Technologies) method. First-strand cDNA was synthesized from 1 µg of total RNA using high capacity cDNA reverse transcription kit (Applied Biosystems). qRT-PCR was performed using PowerUp SYBR green master mix (Applied Biosystems). Primers were designed for each PRX gene as listed in Appendix A.1. Each reaction mixture was 12 µl containing 6 µl of SYBR, 1 µl of each primer (1 µM), 1 µl of cDNA template and 3 µl of RNase-free water. The reaction for each gene was conducted in triplicates with thermal cycling conditions as follows: 95°C for 10 mins, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The primer specificity was confirmed by melt curve analysis. The relative gene expression was calculated using the $2^{-\Delta\Delta C_t}$ method. Actin gene was used as an internal control and values were normalized to actin and comparing the normalized values to those of WT plants.

2.2.4 Peroxidase enzyme assay

For peroxidase purification and enzyme assay, we followed (Gafur, Schutzendubel, and Polle 2007) with modifications, where half a gram of SDX was homogenized in 5 ml of cold extraction buffer (10mM Tris-HCl, 10mM NaHCO₃, 10mM MgCl₂, 0.1mM Na-EDTA, 10mM Mercaptoethanol, 1mM PMSF, 2% polyvinylpolypyrrolidone (PVPP), 0.1% [v/v] Triton X-100) at 4°C. After several centrifugation steps, the supernatant was

finally subjected to column chromatography using Sephadex G-25 column for purifying total peroxidase enzyme from each transgenic line for PRX25 and PRX36. The rest of the assay was conducted in triplicates for each sample. The reaction mixture included potassium phosphate buffer pH 5.25, guaiacol as substrate, purified enzyme and H₂O₂. The addition of H₂O₂ initiated the reaction and the change in optical density of peroxidase enzyme using guaiacol was monitored at 436 nm using spectrophotometer every minute starting at time zero for a duration of five mins.

2.2.5 Lignin analysis

Dried wood samples from previously harvested poplar plants were used for the analysis. These wood samples were milled to mesh 20 using Willey-mill. Each sample weighing 100 mg was placed in microfuge tubes and sent to National Renewable Energy Laboratory (NREL) for pyrolysis/molecular beam mass spectrometry (Py-MBMS) (Kelley et al. 2004). Each sample was analysed in duplicates for this procedure.

2.2.6 Microscopic image analysis

For microscopy, stems of poplar plants were cut at the tenth internode from the apex of the plant with 40-50 internodes and were fixed in cold FAA (formaldehyde – acetic acid – ethanol) solution. The internode was vacuum infiltrated for 15-20 mins and stored with fresh cold FAA solution overnight at 4°C. After fixation, these internodes were dehydrated and embedded in wax. Seven-micrometer-thick consecutive sections were cut with a microtome, mounted on a slide and de-waxed using xylene and ethanol series. Lignin

autofluorescence from de-waxed sections was photographed using fluorescent light imaging.

2.2.7 Saccharification assay

For performing saccharification assay to estimate glucose release, we followed (Kumar and Turner 2015) with some modifications. Ten milligrams of dried and milled wood samples from previously harvested poplar plants were used for the analysis. Alcohol insoluble residue (AIR) samples were prepared, treated with acetonitrile solution (acetic acid: nitric acid: water 8:2:1) at 100°C for 30 mins. These samples were further subjected to sulfuric acid swelling. Sugar release was measured by anthrone method by monitoring the reactants' solution absorbance at 620 nm. Using 10-250 $\mu\text{g } \mu\text{l}^{-1}$ of glucose, the standard curve regression line was generated and the glucose release in each sample was calculated.

2.2.8 Growth data analysis

Growth data for height, leaf count and stem girth were measured for all the generated transgenic lines every week (for 13 weeks) until the day of harvest.

2.3 Results

2.3.1 Tissue level expression of PRXs

We tested and compared tissue level expression of all SDX PRXs with *in-silico* data from (Ren et al. 2014) as well as data obtained from Poplar eFP browser provided in Bio-Analytic Resource for plant biology (BAR) (see Appendix B.1) Out of 93 poplar PRXs, only six were SDX PRXs. Of which, two PRXs – PRX25 and PRX36 were selected for the

experiments as their expression was highest in the developing xylem (DX). The full-length coding region of these PRXs expressed under tissue-specific promoter DX15 (PtrDX15 – Potri.009G012200) were amplified, cloned and transformed into poplar 717 WT plants (Phytozome v12.1 accession numbers: PtrPRX25 – Potri.004G015300, PtrPRX36 – Potri.005G195600). The 13-week old harvested transgenic trees were tested for change in gene expression of PRX25 and PRX36 compared to WT plants.

2.3.2 Gene expression studies in PRX25 and PRX36 transgenic lines

The relative gene expression of PRX25 and PRX36 were quantified using qRT-PCR. It was observed that the attempted over-expression of the PRX25 led to the major down regulation of this gene (co-suppression) in transgenic poplar lines when compared to WT (Figure 2.1 a). We also tested the relative expression of other SDX PRXs – PRX36, PRX12, PRX33, PRX22, PRX23 in these lines. As shown in Figure 2.2 (a-e), an increase in the expression of PRX36, decrease in the expression PRX33 and no significant changes in PRX12, PRX22 and PRX33 was observed in PRX25 transgenic lines.

Figure 2.1: Relative gene expression of PRX25 and PRX36 in developing xylem of harvested poplar transgenic lines. (a) relative expression of PRX25 gene in PRX25 transgenic lines (b) relative expression of PRX36 gene in PRX36 transgenic lines, compared to WT plants. Significant compared to WT, ** P-value < 0.009, *** P-value < 0.0001.

The attempted over-expression of PRX36 lines led to a significant increase in the expression of this gene in the six independent lines (Figure 2.1 b). Similarly, we tested the expression of other five SDX PRXs in PRX36 lines (Figure 2.3 a-e). We observed an increase in PRX25 gene expression, decrease in the expression of PRX23 and PRX33 and no significant change to PRX12 and PRX22 gene expression. To further support these findings, we next tested the total enzyme activity in DX of all lines in PRX25 and PRX36.

Figure 2.2: Relative gene expression of other SDX PRXs in developing xylem of harvested PRX25 transgenic lines. (a) relative expression of PRX36 gene in PRX25 transgenic lines. (b) relative expression of PRX12 gene in PRX25 transgenic lines. (c) relative expression of PRX33 gene in PRX25 transgenic lines. (d) relative expression of PRX22 gene in PRX25 transgenic lines. (e) relative expression of PRX23 gene in PRX25 transgenic lines, compared to WT plants. Significant compared to WT, * P-value < 0.04, ** P-value < 0.0076, *** P-value < 0.0007.

Figure 2.3: Relative gene expression of other SDX PRXs in developing xylem of harvested PRX36 transgenic lines. (a) relative expression of PRX25 gene in PRX36 transgenic lines. (b) relative expression of PRX12 gene in PRX36 transgenic lines. (c) relative expression of PRX33 gene in PRX36 transgenic lines. (d) relative expression of PRX22 gene in PRX36 transgenic lines. (e) relative expression of PRX23 gene in PRX36 transgenic lines, compared to WT plants. Significant compared to WT, * P-value < 0.04, ** P-value < 0.0076, *** P-value < 0.0007

2.3.3 Effect on total peroxidase enzyme activity

Purified peroxidase enzyme was extracted from the DX from WT and transgenic PRX25 and PRX36 lines. The enzyme activity was tested for a period of five minutes using guaiacol as substrate for peroxidase. A decrease in total peroxidase enzyme activity was observed in PRX25 lines when compared to WT as shown in Figure 2.4 (a). Down

regulation of PRX25 gene can be suggested as a reason for the decrease in enzyme activity. The increase in PRX36 gene in PRX25 lines did not seem to contribute to increase the activity. As shown in Figure 2.4 (b), an increase in peroxidase activity was observed in PRX36 lines on comparison with WT. The overall increase in total enzyme activity in these lines can be suggested to be due to the increase in expression of PRX36 and PRX25.

Figure 2.4: Quantification of peroxidase activity of purified SDX proteins from WT and transgenic poplar lines using guaiacol as substrate. (a) decrease in peroxidase activity in PRX25 lines (b) increase in peroxidase activity in PRX36 lines, compared to WT plants. Significant compared to WT, *** P-value < 0.0001.

2.3.4 Effect on xylem formation

If peroxidase enzyme plays a significant role in lignin polymerization, the changes in gene expression could also lead to changes in the development of xylem. We, therefore, looked at the stem sections of the tenth internode from the apex in transgenic lines and compared it to WT. Autofluorescence images revealed a significant decrease in the DX region in PRX25 lines as shown in Figure 2.5 (a and b). We also observed decreased autofluorescence in the phloem and sclerenchyma region when compared to wild type (Figure 2.5 a and b). We also calculated the area of xylem region in these lines and an apparent decrease in this region was observed (Figure 2.5 d).

In contrast to the gene expression and enzyme activity findings, the xylem region was significantly reduced in the PRX36 lines (Figure 2.5 a and c). A drastic reduction in the DX rays with reduced to no autofluorescence in the phloem and sclerenchyma of these lines. The area of the xylem region was significantly reduced when compared to WT (Figure 2.5 e).

Figure 2.5: Autofluorescence of lignin in stem sections and estimated xylem area of harvested WT and transgenic poplar lines. Cross section of (a) WT, (b) PRX25 transgenic lines, (c) PRX36 transgenic lines, (d) area of xylem in PRX25 transgenic lines, (e) area of xylem in PRX36 transgenic lines, compared to WT plants. Significant when compared to WT, *** P-value < 0.0001.

2.3.5 Effect of gene manipulation on lignin content

The entire experiment was designed to increase the lignin content in poplar trees and to understand how PRXs plays a role in lignin polymerization. To evaluate the lignin content, we used dried and milled wood between the first and second internode from the base of the tree. Lignin analysis was carried out by Py-MBMS for WT and transgenic lines. The PRX25 lines showed no significant change in lignin content but a significant decrease in

syringyl/guaiacyl (S/G) lignin ratio was observed in these lines as seen in Figure 2.6 (a) and (c), respectively. In PRX36 transgenic lines, there was an overall decrease in lignin content between 3-6% when compared to WT with no significant change in S/G lignin ratio in these lines (Figure 2.6 b and d). These changes could possibly explain the changes in the xylem region respective to the PRXs as mentioned in the previous section. Since, we observed a change in lignin content/composition, we further decided to test the sugar release in these trees.

Figure 2.6: Estimation of lignin content and S/G lignin ratio in WT and transgenic poplar lines. Lignin content in (a) PRX25 transgenic lines (b) PRX36 transgenic lines, compared to WT. S/G lignin ratio in (c) PRX25 transgenic lines (d) PRX36 transgenic lines, compared to WT. Significant when compared to WT, * P-value < 0.02, ** P-value < 0.0094.

2.3.6 Saccharification assay in PRX25 and PRX36 lines

We performed the saccharification assay using the anthrone method to test the sugar release in PRX25 and PRX36 lines. With changes in lignin in these lines, we expected to observe

a change in the sugar yield. As expected, there was an overall increase in the sugar release by 40-65% in both PRX25 and PRX36 lines as shown in Figure 2.7 (a) and (b).

Figure 2.7: Glucose release assay of WT and transgenic poplar lines using anthrone method. Percent change of glucose release in (a) PRX25 transgenic lines (b) PRX36 transgenic lines, compared to WT. Significant when compared to WT, *** P-value < 0.0001.

2.3.7 Effect on biomass and growth of PRX25 and PRX36 lines

Apart from molecular and biochemical analysis, we also studied several phenotypic characteristics in all transgenic lines and compared them to WT. Change in leaf biomass, stem girth, plant height and leaf count were the four traits that we analyzed for this study.

Upon harvest, we weighed the leaf weight that accounted as wet biomass. These leaves were then placed in paper bags, dried and weighed again which accounted as dry biomass. This was done for all controls, transgenics and their biological replicates. We observed slight or no significant change in the wet/dry leaf biomass in PRX25 and PRX36 lines as compared to wild type (Figure 2.8 a and b).

Figure 2.8: Estimated wet/dry leaf biomass of WT and transgenic poplar lines. Leaf biomass change in (a) PRX25 transgenic lines (b) PRX36 transgenic lines, compared to WT.

The other traits were monitored weekly (week 0 – week 13, until harvest). Significant changes in height and leaf count of PRX25 lines was observed with no significant change in stem girth. No significant changes were observed in PRX36 transgenic lines when compared to WT (Figure 2.9 a-f). The changes in gene expression and lignin content did not seem to affect the plant growth rate and was like that of WT for the 13 week-period.

Figure 2.9 a: Estimated height (in cm) of WT and PRX25 transgenic lines over a period of 13 weeks until harvest.

Figure 2.9 b: Estimated leaf count of WT and PRX25 transgenic lines over a period of 13 weeks until harvest.

Figure 2.9 c: Estimated stem girth (in mm) of WT and PRX25 transgenic lines over a period of 13 weeks until harvest.

Figure 2.9 d: Estimated height (in cm) of WT and PRX36 transgenic lines over a period of 13 weeks until harvest.

Figure 2.9 e: Estimated leaf count of WT and PRX36 transgenic lines over a period of 13 weeks until harvest

Figure 2.9 f: Estimated stem girth (in mm) of WT and PRX36 transgenic lines over a period of 13 weeks until harvest.

2.3.8 Correlation analysis

Data from all the experiments were correlated to establish a relationship pattern between different factors using the correlation data analysis feature on Microsoft Excel. In summary, the correlation analysis for PRX25 lines suggested a positive correlation between gene expression and enzyme activity, gene expression and lignin content, lignin content and glucose release and, S/G lignin ratio and lignin content. A negative correlation was observed between lignin content and enzyme activity and, S/G lignin ratio and glucose release when compared to WT. For PRX36 lines, a positive correlation was observed between gene expression and enzyme activity and, S/G lignin ratio and lignin content. A strong negative correlation was observed between gene expression and lignin content, lignin content and enzyme activity, lignin content and glucose release and, S/G lignin ratio and glucose release when compared to WT (Figure 2.10 (a) and (b), Figure 2.11 (a) and (b)).

Figure 2.10: Correlation analysis of relative gene expression, peroxidase enzyme activity and lignin content in WT and transgenic poplar lines. (a) PRX25 transgenic lines (b) PRX36 transgenic lines, compared to WT

Figure 2.11: Correlation analysis of lignin content, S/G lignin ratio and glucose release content in WT and transgenic poplar lines. (a) PRX25 transgenic lines (b) PRX36 transgenic lines, compared to WT.

2.4 Discussion

Lignins are heteropolymers belonging to the phenylpropanoid family. They have an essential role in maintaining cellular plasticity and rigidity in plant cell wall. About ten different enzymes participate for lignin biosynthesis and produce lignin monomers, termed as monolignols (Nookaraju et al. 2013). Oxidative coupling of the three monolignols (p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol) catalyzed by either peroxidase

and laccase enzymes gives rise to lignin polymers. Lignin polymers form complexes with other polysaccharides within the plant cell wall giving plants its tensile structure. The sustainable nature of the wood obtained from plants makes it desirable for paper and pulp industry, building construction and as biomass for energy production, to name a few. Making the wood readily available for such purposes i.e. increasing cellulose accessibility and reducing or altering lignin/hemicellulose content) involves the use of chemicals and bleaching agents that are considered as environmental pollutants. Through several decades, efforts have been made to engineer plants with less lignin to decrease the innate biomass recalcitrance and making the process more cost effective. Though lignin is credited as a hindrance for wood degradability, there are several other beneficial applications of lignin for generation of value-added products, for instance, energy conversion from biomass into chemicals and fuels, production of carbon fiber and polymers, use as additives and its applications in pharmaceutical sciences (Lange, Decina, and Crestini 2013) (Ragauskas et al. 2014).

Owing to the growing importance of lignin, the current study aimed to interpret the role PRXs during lignin polymerization and the impact of the changes in expression of SDX PRXs on lignin quantity and sugar releases from the plant cell wall. Peroxidases are speculated to function at several developmental stages in plants from germination to senescence. For the past several decades attempts have been made to delineate the exact role Class III heme-PRXs play in lignin polymerization. Also, this understanding will help to generate plants with improved lignin characteristics for the above stated applications. Peroxidases are classified based on their isoelectric point into acidic (anionic) and basic

(cationic) peroxidases with the capability to consume H_2O_2 and release reactive oxygen species (Passardi, Penel, and Dunand 2004). Previous studies have demonstrated competence of basic PRXs over acidic PRXs due to substrate specificity to catalyze the polymerization of SA and other synthetic lignin oligomers. The substitution of I-138 with L-138 or the presence two Tyr residue (Tyr-74 and Tyr-177) near the heme pockets are the speculated the reasons for this competency.

We attempted the overexpression of PRX25 and PRX36 using xylem-specific promoter in poplar trees. Overexpression of PRX25 led to the co-suppression of this gene in PRX25 transgenic lines which is a known phenomenon called post-transcriptional gene silencing (PTGS) and first defined in plants in the year 1990 as co-suppression (Van der Krol et al. 1990) (Napoli, Lemieux, and Jorgensen 1990). Co-suppression leads to sequence-specific degradation of endogenous mRNA and acts as an epigenetic regulation that has been studied in several plant species (Atanassova et al. 1995) (Burch-Smith et al. 2004) (Jung et al. 2012). The expression analysis of PRX25 corroborated with the total peroxidase enzyme activity where we observed a severe reduction in the activity (Figure 2.4). The co-suppression of PRX25 seems to have affected the xylem development evident by the reduction in the xylem area and reduced autofluorescence in the phloem fibers in these lines that are rich in S-lignin units (Figure 2.5). This reduction in autofluorescence correlates with the decreased S/G lignin ratio though the lignin content was unaffected. The reduction in S/G lignin ratio also makes us to speculate the importance of the presence of this gene to oxidize S-lignin monomers or oligomers. For instance, the expression of PRX36 gene was high in PRX25 transgenic lines but this did not help to maintain the S/G

ratio nor help with xylem formation. We strongly believe that the changes observed in these lines are strictly due to these changes in gene expression of PRX25 because none of the six SDX PRX share gene sequence similarity (see Appendix B.6, B.7, B.8) and therefore would fail to replace any function carried out by the other PRXs. These findings suggest that PRX25 could be a basic peroxidase and it would be interesting to demonstrate either substitution of I-138 with L-138 or the presence two Tyr residue (Tyr-74 and Tyr-177) near the heme pockets in PRX25 to confirm this finding.

The genetic manipulation of PRX36 gene led to significant increase in the expression of PRX36 gene with an increase in the total peroxidase enzyme activity in the PRX36 transgenic lines. With this increase in gene expression of PRX36, we expected to see an increase in the xylem area and lignin content. But contrary to our expectations, we observed a severe reduction in the xylem area and the phloem fibers almost non-existing in these lines (Figure 2.5). The lignin content in these lines showed a downward trend but was not statistically significant. The S/G lignin ratio showed reduction in a few plants with significant increase in glucose release about 40-60%. To sum-up these findings, it can be said that modification of PRX36 did not affect the lignin content. But, the overexpression of PRX36 could be detrimental for lignin production and xylem intactness in SCW.

Studies have demonstrated alteration to the S/G ratio have improved pulpability and improved downstream bioconversion of biomass. We further speculate, the changes in S/G lignin ratio brought about structural changes within the cell wall that led to increased sugar release thereby improving saccharification efficiency in these transgenic PRX lines (Figure 2.7). Previous studies have demonstrated genetically engineered plants with increased

saccharification efficiency but resulting in developmental deformities in plants which limits the use of these plants for bioenergy purposes. We succeeded in generation of plants with improved saccharification efficiency, exhibiting normal phenotype like WT and displays no growth penalty.

We also performed gene expression analysis of SDX LACs in the PRX25 and PRX36 lines which revealed decreased expression of LAC18 and LAC14 in these lines. The expression of LAC27 seemed to be high in both the PRX25 and PRX36 lines (Figure 2.12 a-f). Though previous gene expression studies suggest that LAC27 is one of the highly expressed SDX LAC in poplar developing xylem, our study suggests that LAC27 does not replace the function of these PRXs. This is evident by the reduction in xylem area and autofluorescence of phloem fibers and sclerenchyma, we observed in PRX25 and PRX36 lines. Therefore, suggesting specific role of PRXs and LACs in lignin polymerization with no functional overlap.

Figure 2.12: Relative gene expression of SDX LACs in developing xylem of harvested PRX25 and PRX36 transgenic lines. (a) relative expression of LAC27 gene in PRX25 transgenic lines. (b) relative expression of LAC18 gene in PRX25 transgenic lines. (c) relative expression of LAC14 gene in PRX25 transgenic lines. (d) relative expression of LAC27 gene in PRX36 transgenic lines. (e) relative expression of LAC18 gene in PRX36 transgenic lines. (f) relative gene expression of LAC14 in PRX36 transgenic lines, compared to WT plants.

Conclusion

In conclusion, this study establishes an effort in characterizing the role of PRXs in polymerization of lignin. Based on the gene expression analysis, enzyme assay and wood analysis, it provides proof that explains proportional differences in expression of PRX/LAC. Thus, suggesting that each of these enzymes has a unique role to play in lignin

polymerization and that they do not take over each other's functions i.e. they could be non-redundant. Nevertheless, this study also stands out as a promising approach to genetically engineer lignin for saccharification purposes without yield penalty as the manipulation of PRXs did not show any morphological defects in plants.

Summary of manipulation of PRXs

2.5 Acknowledgements

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3 Chapter 3

Genetic manipulation of secondary wall-specific laccase genes in poplars

Abstract

Lignification takes place in the xylem tissue during plant cell wall differentiation that provides plants with structural stability. In this study, we aimed at understanding the molecular mechanism behind the functioning of laccase gene family which are said to be involved in the lignin polymerization process. We selected and overexpressed two SDX LAC – LAC27 and LAC18 genes using tissue-specific promoter to manipulate the process of lignin formation. To gain comprehensive knowledge about laccase activity, we also adopted the STTM technology to study the effect of loss-of-gene function of miR397a which are suggested to be negative regulators of laccase gene expression. Poplar leaf-disc transformation helped to generate transgenic plants with gene of interest. Gene expression analysis suggested that manipulation of LACs did not significantly change the lignin content but affected the S/G lignin ratio. The manipulation of miR397a led to suppression of miR397a gene in poplars and seemed to increase the S/G lignin ratio. These changes brought about minor structural changes in the plant cell wall leading to improved sugar release but did not affect the plant growth yield making the wood highly desirable for bioconversion of lignocellulosic material with enhanced saccharification efficiency.

3.1 Introduction

Laccases are copper-containing oxidoreductases with highly conserved histidine motifs. They are broadly classified as fungal and plant laccases depending upon their origin. They can oxidize a broad range of aromatic/non-aromatic compounds as they are not substrate specific. Oxygen acts as the electron acceptor and gets reduced to water in the process.

Plant laccases are highly glycosylated. They are active in the pH range of 5-7.5. Though little is known about plant laccases, polymerization of phenols is assumed to be an act of defense in plants (Ranocha et al. 1999) (Berthet et al. 2012). Earlier studies suggested that laccases do not play a role in lignification in plants (Harkin and Obst 1973). However, later studies suggested their role in lignification where, in the absence of hydrogen peroxide (H_2O_2), they catalyze a reaction to polymerize monolignols under *in-vitro* conditions in maple (Driouich et al. 1992) (O'Malley et al. 1993). A similar *in-vitro* study in sycamore maple suspension cells and loblolly pine xylem suggested a combined role of peroxidase and laccase in dehydrogenation of lignin that contributes to lignin heterogeneity in plant cell walls (Sterjiades, Dean, and Eriksson 1992) (Bao et al. 1993). Therefore, it became increasingly important to understand how laccases could oxidize lignin monomers. A comparison analysis of encoded nucleotide sequences of cDNA clones for laccase genes from sycamore maple and tobacco stem helped to discern this function. The conserved copper-binding domains identified from both the species shared only 48% sequence similarity suggestive of the physical and functional divergence exhibited by laccases from

different species (LaFayette, Eriksson, and Dean 1995) (Kiefer-Meyer et al. 1996). Overexpression of a laccase (*Ltlacc2.1-2.4*) in yellow poplar (*Liriodendron tulipifera*) and tobacco (*Nicotiana tabacum*) cells showed no effect on lignification (LaFayette, Eriksson, and Dean 1999). Downregulation of laccases in poplar with the help of antisense RNA technology led to increased levels of soluble phenols and irregular xylem fibers, but lignin content or composition was unaltered. This study also suggested that a clear connection could not be derived between laccase expression and lignification process, though laccase could be involved to maintain the xylem integrity in the plant cell wall (Ranocha et al. 2002). Mutant analysis of laccase gene in *Arabidopsis* (*AtLAC15*) showed decrease in laccase activity as well as in lignin content (Liang, Haroldsen, et al. 2006). Another study generated transgenic poplar with heterologous overexpression of cotton laccase (*GaLAC1*) that showed a 2.1–13.2-fold increase in laccase activity as well as 2–20% increase in lignin compared to WT (Wang et al. 2008). An extensive study on polymerization of different lignins gave insights on the capability of laccases to oxidize lignin without mediators. It was also determined that the laccase activity is pH dependent, they are capable of polymerizing different lignins and this efficiency was measured by analyzing the degree of polymerization (Mattinen et al. 2008). *Arabidopsis* have been identified with 17 laccases gene family members and classified into six groups based on sequence similarity (McCaig, Meagher, and Dean 2005). In a few studies using laccase double mutants (*lac4-2 lac11-1*, *lac 4-2 lac17-1* and *lac11-1 lac17-1*) and triple mutant (*lac4*, *lac11* and *lac17*) in *Arabidopsis*, growth defects in these mutants were observed due to monolignol toxicity, extensive loss of lignin causing vascular function impairment and deposition of G units was affected. Some studies also suggested that they function during early xylem

development and peroxidases function in the later stages of xylem development. That study also suggested that laccase and peroxidase serve non-redundant functions in lignification of vascular tissues of stem and roots wherein laccase and peroxidase are involved in the initial and later stages of monolignol polymerization respectively (Zhao et al. 2013a). Poplar has 49 laccases when compared to 17 laccases in *Arabidopsis*, making it difficult to characterize the role of each laccase. Few recent studies stated below helps to provide an understanding of how laccase could be involved with the lignification process. A unique system comprising of fluorescently tagged monolignol analogs (γ -nitrobenzofuran, NBD-tagged coniferyl alcohol, CA) and VND-7 induced protoxylem system was used to demonstrate that laccase (LAC4 and LAC17) were the essential participant to direct lignification of protoxylem tracheary elements (TE) in *Arabidopsis* (Schuetz et al. 2014). Mutant analysis of LAC5 and LAC6 in *Brachypodium distachyon* showed reduced lignin, an increase S-lignin due to change in S/G lignin ratio and improved saccharification efficiency thus revealing the reduced recalcitrance of grass lignocelluloses (Wang et al. 2015). In a recent study, using RNAi to downregulate PdLAC2 from *Populus deltoides* showed an increase in the S/G lignin ratio with unaltered lignin content when compared to control lines. These RNAi mediated knockdown lines also exhibited reduced recalcitrance to pre-treatment procedures with an increase in the release of five and six carbon sugar (Bryan et al. 2016). These studies suggest that the involvement of laccase may or may not affect the lignin content in the cell walls with an improvement in the saccharification efficiency. Though, it is still a puzzle as to how each enzyme within the gene family functions during this process.

Role of miR397 in lignin biosynthesis

MicroRNAs (miRNAs) are a class of 20-21 nucleotide (nt) long, non-coding upstream gene regulators that are found in almost all eukaryotic organisms. They play an essential role in modulating gene expression at several developmental stages of an organisms at pre- and post-transcriptional level, translational modifications and epigenetic factor regulation. Thousands of miRNAs have been identified in plants and animals and functions of many such regulators are still unknown (Gielen et al. 2012) (Zhou and Luo 2013).

One such plant miRNA was identified to play a role in lignin biosynthesis is miR397a as it specifically targets laccase genes involved in lignin polymerization. Computational analysis suggested that miR397 targets laccases such as LAC2, LAC4 and LAC17 and regulates the lignin biosynthesis in *Arabidopsis thaliana* (Berthet et al. 2011). Significant reduction of lignin in poplar was observed due to constitutive overexpression of a regulatory miRNA (miR397a) leading to general silencing of thirteen stem differentiating xylem (SDX) laccases in transgenic poplar (Lu et al. 2013). It was determined that *Ptr*-miR397a and *Ath*-miR397b were negative regulators of laccase gene expression and affected the lignin content in *Populus* and *Arabidopsis*, respectively (Lu et al. 2013) (Wang et al. 2014). These studies helped to provide an insight to understand plant laccases, to a certain extent and the role they play in lignification by exploring the post-transcriptional regulation exerted by miR397 in different plant species.

In poplar, seventeen out of the total forty-nine laccases are abundantly expressed in the SDX. Studies performed on these SDX laccases suggested that the expression of thirteen

PtrLACs (*PtrLAC2*, 7, 11, 12, 14, 15, 18, 23, 24, 26, 30, 40, and 49) was regulated by miR397a. The other four – *PtrLAC17*, 19, 25, and 27 were not targets of this miRNA. For the current study, we selected *PtrLACs* based on their expression level in the SDX (Lu et al. 2013), their phylogenetic similarity with previously studied *AtLACs* and whether these *LACs* were targets of miR397a that indirectly affected the lignification. We selected and overexpressed *PtrLAC27*, which is the most abundant laccase present in the SDX, it belonged to clade one and closely related with *AtLAC4* and *AtLAC11* and it was not a target of miR397a. Next, we also selected *PtrLAC18* which was a target of miR397a but was expressed at lower levels compared to *PtrLAC27*. We also used the short tandem target mimic (STTM) technology to target miR397a which has been successfully used in previous studies to analyze the regulatory functions exhibited by several miRNAs in plants and animals (Tang et al. 2012) (Yan et al. 2012). The STTM designed to target miR397a will block and knockdown the expression of these miRNAs in poplar trees and in turn will not control expression of laccases. Due to loss of function of this specific miRNA, we expect to see alterations in laccase gene regulation and consequently in lignin polymerization. This study was also developed to demarcate the functional role of laccase and peroxidase enzymes involved in lignin polymerization as an extended hypothesis from chapter one. We expect this study to help provide additional information on the significant role played by these two gene families in wood development in poplar.

3.2 Materials and Methods

3.2.1 Gene selection and vector construction

For this study, out of the forty-nine laccase genes present in *Populus trichocarpa*, we selected two laccase genes (LAC27 and LAC18) based on their expression in SDX (Lu et al. 2013) (see Appendix C.1). The full-length amplification of the coding region of these genes was performed using gene specific primers (see Appendix A.1). We used QIAquick PCR purification kit (Qiagen) for clean-up of PCR reactions residual components to purify the amplified gene product. The purified gene products were digested using restriction enzymes *Bam*HI/*Sac*I for LAC27 and LAC18. Digested fragments were cloned using NEB® 5-alpha competent *E. coli* (high-efficiency) cells into modified pBI101 with tissue specific promoter DX15 to replace GUS gene.

Cloned plasmid was then transformed into *Agrobacterium* strain C58C1 using freeze thaw method (Boerjan et al. 1997; Wu, Joshi, and Chiang 2000). The transformed plasmids were selected for antibiotic resistance. Verification PCR for the gene of interest on the selected clones were carried out before transforming the plants. The sequences of the selected clones for LAC27, LAC18 and miR397a genes from plasmid and agrobacterium transformation was also confirmed by sanger sequencing (see Appendix C.2, C.3, C.4, C.5).

3.2.2 Plant transformation and growth conditions

The positively verified *Agrobacterium* clones were transformed into *Populus tremula* x *alba* clone 717-1B4 genotype (routinely referred as 717) using leaf-disc transformation

protocol previously described in (Boerjan et al. 1997; Wu, Joshi, and Chiang 2000). First, the *Agrobacterium* clone is allowed to infect the leaf discs at the wound site (where the leaves have been cut) through several initial steps. Later these infected leaves are allowed to grow on callus induction medium (CIM) for three to four weeks to develop callus (unorganized mass of plant cell). Once callus is formed, they were moved to shoot induction medium (SIM) and then to shoot elongation medium (SEM) for three to four weeks each. Shoots generating from each callus was considered as an independent line. Keeping track of each of these independent lines, they were further moved to root induction medium (RIM) until the shoots developed roots. The callus and shoots were grown in a growth chamber (Thermo Scientific Precision model 818 incubator) with a light and dark cycle of 16 hrs and 8 hrs at 25°C. Plants that rooted were selected and genotypically verified for the presence of the gene of interest using genomic PCR. Verified transgenic lines were acclimatized and, moved and maintained in green house for approximately 3 months before harvesting. The green house growth conditions were kept similar to the growth chamber with light and dark cycle of 16hrs and 8hrs, temperature of 25°C and relative humidity ranging from 40-70%. Upon harvest of the 13-week old (LAC27 and LAC18) and 16-week old (STTM:miR397a) plants, all leaf material from each plant was weighed before and after drying and stored in paper bags. The stem was cut into approximately four to five pieces (with ten to fifteen internodes each). The bark was cut open with a sharp blade and developing xylem on the wood material was scraped in liquid N₂, collected in aluminum foil and stored in liquid nitrogen until use. The remaining wood material from each plant was air-dried and stored for further analysis.

3.2.3 RNA extraction and RT-PCR analysis

Total RNA was extracted from SDX (1-10 internodes counted from the base of the plants with 40-50 internodes) using TRIzol (Ambion, Life Technologies) method. First-strand cDNA was synthesized from 1 µg of total RNA using high capacity cDNA reverse transcription kit (Applied Biosystems). qRT-PCR was performed using PowerUp SYBR green master mix (Applied Biosystems). Primers were designed for each LAC gene as listed in Appendix A.1. Each reaction mixture was 12 µl containing 6 µl of SYBR, 1 µl of each primer (1 µM), 1 µl of cDNA template and 3 µl of RNase-free water. The reaction for each gene were conducted in triplicate with thermal cycling conditions as follows: 95°C for 10 mins, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The primer specificity was confirmed by melt curve analysis. The relative gene expression was calculated using the $2^{-\Delta\Delta C_t}$ method. Actin gene was used as an internal control and values were normalized to actin and comparing the normalized values to those of WT plants.

3.2.4 Laccase enzyme assay

For laccase purification and enzyme assay, we followed (Berthet et al. 2011) (Lu et al. 2013) with modifications, where one gram of SDX was homogenized in 10 ml of extraction buffer [25 mM Tris, pH 7.0, 200 mM CaCl₂, 10% (vol/vol) glycerol, 4 µM sodium cacodylate, 1 mM PMSF and 1/200 (vol/vol) protease inhibitor cocktail (P-9599); Sigma-Aldrich]. After several extraction and centrifugation steps, the supernatant was finally subjected to affinity column chromatography using Concanavalin-A Sepharose beads. Methyl- α -glucopyranoside was used to elute proteins from each transgenic line for LAC27,

LAC18 and STTM:miR397a as stated in (Berthet et al. 2011). The rest of the experiment was conducted in triplicates for each sample. The generation of stable cationic radical by laccase enzyme during the oxidation of ABTS was measured at 420 nm for 5 mins using a spectrophotometer.

3.2.5 Lignin analysis

Dried wood samples from previously harvested poplar plants were used for the analysis. These wood samples were milled to mesh 20 using Willey-mill. Each sample weighing 100 mg were placed in microfuge tubes and sent to NREL for pyrolysis-molecular beam mass spectrometry (Py-MBMS) (Kelley et al. 2004). Each sample was analyzed in duplicates for this procedure.

3.2.6 Microscopic image analysis

For microscopy, stems of poplar plants were cut at tenth internode from the apex of the plant with 40-50 internodes and were fixed in cold FAA (formaldehyde – acetic acid – ethanol) solution. The internode was vacuum infiltrated for 15-20 mins and stored with fresh cold FAA solution overnight at 4°C. after fixation, these internodes were dehydrated and embedded in wax. Seven-micrometer-thick consecutive sections were cut with a microtome, mounted on a slide and de-waxed using xylene and ethanol series. Lignin autofluorescence from de-waxed sections was photographed using fluorescent light imaging.

3.2.7 Saccharification assay

For performing saccharification assay to estimate glucose release, we followed (Kumar and Turner 2015) with some modifications. Ten milligrams of dried and milled wood samples from previously harvested poplar plants were used for the analysis. Alcohol insoluble residue (AIR) samples were prepared, treated with acetonitrile solution (acetic acid: nitric acid: water 8:2:1) at 100°C for 30 mins. These samples were further subjected to sulfuric acid swelling. Sugar release was measured by anthrone method by monitoring the reactants' solution absorbance at 620 nm. Using 10-250 $\mu\text{g } \mu\text{l}^{-1}$ of glucose, the standard curve regression line was generated and the glucose yield in each sample were calculated.

3.2.8 Growth data analysis

Growth data for height, leaf count and stem girth were measured for all the generated transgenic lines every week (for 13-weeks – LAC27 and LAC18 lines, for 16-weeks – STTM:miR397a lines) until the day of harvest.

3.3 Results

3.3.1 Tissue level expression of LACs

We compared the tissue level expression of all SDX LACs with in-silico data from (Lu et al. 2013) as well as data obtained from Poplar eFP browser provided in Bio-Analytic Resource for plant biology (BAR) (see Appendix C.1). Out of the 17 SDX LACs, we verified the presence of six SDX LACs in six different regions of WT poplar – apex, young leaf (YL), mature leaf (ML), young stem (YS), mature stem (MS) and developing xylem (DX). We selected LAC27 and LAC18 after the expression analysis for the following

reasons. First, they are among the highly expressed LACs (see Appendix C.1), they belong to different clades in the phylogenetic tree – LAC27 in clade 1 and LAC18 in clade 5 (Lu et al. 2013). Lastly, among the two LACs, LAC27 is not a target of miR397a whereas LAC18 is a target of miR397a. The miR397a is said to act as a negative regulator of SDX LACs and indirectly play a role in suppressing lignin polymerization. The full-length coding region of these LACs expressed in modified pBI101 under DX15 promoter were then amplified, cloned and transformed into poplar 717 plants (Phytozome v12.1 accession numbers: PtrLAC27 – Potri.010G193100.1, PtrLAC18 – Potri.008G073700.1). The 13-weeks and 16-weeks old harvested transgenic trees were tested for change in gene expression of LAC27, LAC18 and compared to WT plants whereas STTM:miR397a was compared to vector control.

3.3.2 Gene expression studies in LAC27, LAC18 and STTM:miR397a transgenic lines

We attempted to overexpress previously selected LAC27 and LAC18 genes in poplar 717 lines and quantified the relative expression by qRT-PCR. The overexpression of LAC27 led to significant increase in LAC27 gene expression in transgenic lines compared to WT (Figure 3.1 a). Similarly, we also tested the relative expression of other SDX LACs in these lines. Downregulation of LAC18 and LAC14 genes was observed whereas the expression of other LACs (LAC11, LAC15, and LAC23) remained undetermined (i.e. even after 40 cycles of amplification the presence of these genes could not be detected in the sample) in these lines (Figure 3.2 (a) and (b)).

Figure 3.1: Relative gene expression of LAC27, LAC18 and miR397a in developing xylem of harvested poplar transgenic lines. Relative expression of (a) LAC27 gene in LAC27 transgenic lines (b) LAC18 gene in LAC18 transgenic lines, compared to WT plants. Significant compared to WT, *** P-value < 0.0001, ** P-value < 0.006. Relative expression of (c) miR397a gene in STTM:miR397a transgenic lines, compared to vector control. Significant compared to vector control, * P-value < 0.01.

A significant increase in LAC18 gene expression was observed in LAC18 transgenic lines as shown in Figure 3.1 (b). Among the other SDX LACs, relative expression of LAC27 was significantly increased but a decrease in LAC14 expression was observed in LAC18 lines (Figure 3.2 (c) and (d)). Like LAC27 lines, the other three SDX LACs tested remained undetermined.

Figure 3.2: Relative gene expression of other SDX LACs in developing xylem of harvested LAC27, LAC18 and STTM:miR397a transgenic lines. Relative expression of (a) LAC18 gene in LAC27 transgenic lines. (b) LAC14 gene in LAC27 transgenic lines. (c) LAC27 gene in LAC18 transgenic lines. (d) LAC14 gene in LAC18 transgenic lines, compared to WT plants. Significant compared to WT, *** P-value < 0.0006, ** P-value < 0.004, * P-value < 0.02 Relative expression of (e) LAC27 gene in STTM:miR397a transgenic lines. (f) LAC18 gene in STTM:miR397a transgenic lines. (g) LAC14 gene in STTM:miR397a transgenic lines, compared to vector control plants. *** P-value < 0.0001.

The STTM strategy has been proven to be prudent for functional characterization of miRNAs in several plant species (Tang et al. 2012) (Yan et al. 2012) (Wong et al. 2014) (Zhang et al. 2017). With this aim, we designed a construct with the STTM cassette with miR397a sequence in pBI121 vector under CaMV 35S promoter and studied the changes in gene expression for miR397a. The relative gene expression of miR397a and its target genes were quantified using qRT-PCR. For STTM lines, all transgenic lines were compared to vector control which had the empty STTM cassette with no specific miRNA region. The STTM:miR397a lines showed significant downregulation of miR397a in transgenic lines when compared to control (Figure 3.1 c). The miR397a has been previously demonstrated to be a negative regulator of laccase genes present in developing xylem. So, we also tested the target SDX laccase genes in the STTM:miR397a lines. An increase in LAC27 and LAC14 gene expression was observed in these lines while LAC18 expression was not significant when compared to the vector control (Figure 3.2 (e) – (g)). The other SDX LACs that were tested – LAC11, LAC15 and LAC23 could not be determined. We next analyzed total laccase enzyme activity due to changes in gene expression pattern for laccase in these lines.

3.3.3 Effect on total laccase enzyme activity

Total laccase enzyme was extracted from DX of WT and LAC27 and LAC18 transgenic lines which were purified using affinity chromatography. To test the total laccase enzyme activity, ABTS was used as a substrate and monitored for a period of 5 mins. There was an overall increase in total laccase activity in LAC27 and LAC18 lines when compared to WT

(Figure 3.3 (a) and (b)). This extends a correlation with the observation made from gene expression analysis.

Since laccase genes are targets of miR397a, we also tested the total enzyme activity in the STTM lines. There was an overall increase in the total enzyme activity in the STTM lines when compared to control (Figure 3.3 (c)) which also correlates with the gene expression data.

Figure 3.3: Quantification of total laccase enzyme activity of purified SDX proteins from WT and transgenic poplar lines using ABTS as substrate. (a) increase in total laccase activity in LAC27 transgenic lines (b) increase in total laccase activity in LAC18 transgenic lines, compared to WT plants. Significant compared to WT, *** P-value < 0.0002 (c) increase in total laccase activity in STTM:miR397a transgenic lines, compared to vector control plants. Significant compared to vector control, *** P-value < 0.0002.

3.3.4 Effect on xylem formation

As laccases are suggested to play a role in monolignol polymerization, the changes in gene expression in transgenic lines could possibly bring about a change in xylem development.

Stem sections of the tenth internode from the apex was used to study any such changes in development. On comparing autofluorescence images of LAC27 lines with WT, an increase in the DX region in LAC27 lines was observed as shown in Figure 3.4.1 (a) and (b). We also calculated the area of xylem region in these lines and an evident increase in this region was observed (Figure 3.4 (d)).

Figure 3.4-1: Autofluorescence of lignin in stem sections and estimated xylem area of harvested WT and transgenic poplar lines. Cross section of (a) WT, (b) LAC27 transgenic lines, (c) LAC18 transgenic lines, (d) area of xylem in LAC27 transgenic lines, (e) area of xylem in LAC18 transgenic lines, compared to WT plants.

With an increase in gene expression and enzyme activity in LAC18 lines, we expected an increase in xylem region like LAC27 lines. Contradicting the previous findings, the xylem region was reduced in the LAC18 lines when compared to WT (Figure 3.4 (a) and (c)). Irregular xylem vessels and reduced DX rays were observed in these sections. The area of the xylem region was also significantly reduced when compared to WT (Figure 3.4 (e)). A

drastic reduction in autofluorescence in the phloem and sclerenchyma was also observed in these lines.

Stem sections of STTM:miR397a lines were also observed to study the effect of gene expression changes on xylem development. An increase in xylem region with irregular xylem vessels were observed in STTM lines (Figure 3.4.2 (f) and (g)). The area of xylem region in these lines were significantly increased when compared to vector control (Figure 3.4 (h)). Next, we analyzed the lignin content/composition to assess the changes caused by the manipulation of laccase genes and miR397a.

Figure 3.4-2: Autofluorescence of lignin in stem sections and estimated xylem area of harvested vector control and transgenic poplar lines. Cross section of (f) vector control, (g) STTM:miR397a transgenic lines, (h) area of xylem in STTM:miR397a transgenic lines, compared to vector control plants.

3.3.5 Effect of gene manipulation on lignin content and composition

Using Py-MBMS, we evaluated the lignin content/composition in dried and milled wood from transgenic LAC and WT lines. An overall 5-8% decrease in lignin content was observed in LAC27 lines but there was no change in the S/G lignin ratio (Figure 3.5 (a) and 2.6 (a)). In LAC18 lines, no change in lignin content was observed whereas a decrease in S/G lignin ratio was observed as shown in Figure 3.5 (b) and 3.6 (b). These changes in lignin content and composition could possibly explain difference in the observation made in DX region of LAC27 and LAC18 lines. A six percent decrease in lignin content and an increase in S/G lignin ratio was estimated in the STTM lines (Figure 3.5 (c) and 2.6 (c)). Owing to these changes, we also further analyzed the glucose release in LAC and STTM lines.

Figure 3.5: Estimation of lignin content in WT, vector control and transgenic poplar lines. Lignin content in (a) LAC27 transgenic lines, significant when compared to WT (b) LAC18 transgenic lines, compared to WT. Lignin content in (c) STTM:miR397a transgenic lines, compared to vector control plants. Significant when compared to vector control, *** P-value < 0.0001.

Figure 3.6: Estimation of syringyl/guaiacyl (S/G) lignin ratio in WT, vector control and transgenic poplar lines. S/G lignin ratio in (a) LAC27 transgenic lines (b) LAC18 transgenic lines, compared to WT. Significant when compared to WT, ** P-value < 0.008, * P-value < 0.04. S/G lignin ratio in (c) STTM:miR397a transgenic lines, compared to vector control plants. Significant when compared to vector control, *** P-value < 0.0001.

3.3.6 Saccharification assay in LAC27, LAC18 and STTM:miR397a lines

Saccharification assay showed an increase in glucose release in both LAC27 and LAC18 lines compared to WT (Figure 3.7 (a) and (b)). In LAC27 lines, there was an overall 28-45% increase in glucose release whereas the release was between 32-76% in LAC18 lines. This increase could be attributed to the lignin content and S/G lignin ratio changes observed in these lines. An increase in glucose release in the range of 56-76% was observed in the STTM:miR397a lines (Figure 3.7 (c)).

Figure 3.7: Glucose release assay of WT, vector control and transgenic poplar lines using anthrone method. Percent change of glucose release in (a) LAC27 transgenic lines (b) LAC18 transgenic lines, compared to WT. Significant when compared to WT, *** P-value < 0.0002, ** P-value < 0.002. Percent change of glucose release in (c) STTM:miR397a lines, compared to vector control. Significant when compared to vector, *** P-value < 0.0002.

3.3.7 Effect on biomass and growth of LAC27 and LAC18 lines

We measured four phenotypic traits – wet/dry biomass, stem girth, height and leaf count in the transgenic LAC lines. There was no significant change in the wet/dry biomass in LAC27 and LAC18 lines when compared to WT (Figure 3.8 (a) and (b)). Similarly, no significant change in other traits - stem girth, height or leaf count was observed in these lines (Figure 3.9 (a) – (f)). In the 13-week period, there was no detectable change in growth rate of these lines and WT.

Figure 3.8: Estimated wet/dry leaf biomass of WT, vector control and transgenic poplar lines. Leaf biomass change in (a) LAC27 transgenic lines (b) LAC18 transgenic lines, compared to WT. Leaf biomass change in (c) STTM:miR397a transgenic lines, compared to vector control.

Figure 3.9 a: Estimated height (in cm) of WT and LAC27 transgenic lines over a period of 13 weeks until harvest.

Figure 3.9 b: Estimated leaf count of WT and LAC27 transgenic lines over a period of 13 weeks until harvest.

Figure 3.9 c: Estimated stem girth (in mm) of WT and LAC27 transgenic lines over a period of 13 weeks until harvest.

Figure 3.9 d: Estimated height (in cm) of WT and LAC18 transgenic lines over a period of 13 weeks until harvest.

Figure 3.9 e: Estimated leaf count of WT and LAC18 transgenic lines over a period of 13 weeks until harvest.

Figure 3.9 f: Estimated stem girth (in mm) of WT and LAC18 transgenic lines over a period of 13 weeks until harvest.

We analyzed the four same phenotypic traits in STTM lines. There was a slight to significant increase in wet/dry biomass in these lines (Figure 3.8 c). There was no significant change in the stem girth, height and leaf count traits in these lines (Figure 3.9 (g) – (i)).

Figure 3.9 g: Estimated height (in cm) of vector control and STTM:miR397a transgenic lines over a period of 16 weeks until harvest.

Figure 3.9 h: Estimated leaf count of vector control and STTM:miR397a transgenic lines over a period of 16 weeks until harvest.

Figure 3.9 i: Estimated stem girth (in mm) of vector control and STTM:miR397a transgenic lines over a period of 16 weeks until harvest.

3.3.8 Correlation analysis

Correlation data analysis using Microsoft Excel was used to summarize the findings for this chapter. In LAC27 lines, the only positive correlation was established between gene expression and enzyme activity. The other factors – gene expression and lignin content, lignin content and enzyme activity, lignin content and glucose release, S/G lignin ratio and lignin content and, glucose release and S/G lignin ratio – correlates negatively with each other (Figure 3.10.1 (a) and 3.10.2 (a)). For LAC18 lines, a positive correlation was observed between gene expression and enzyme activity, gene expression and lignin content and, S/G lignin ratio and lignin content. lignin content and enzyme activity, lignin content and glucose release and, glucose release and S/G lignin ratio correlates negatively with each other in LAC18 lines on comparison with WT (Figure 3.10.1 (b) and 3.10.2 (b)). In the STTM:miR397a lines, we observed a positive correlation between gene expression and lignin content and, S/G lignin ratio and glucose release. A negative correlation existed between gene expression and enzyme activity, lignin content and enzyme activity, lignin content and glucose release and, S/G lignin ratio and lignin content (Figure 3.10.1 (c) and 3.10.2 (c)).

Figure 3.10-1: Correlation analysis of relative gene expression, laccase enzyme activity and lignin content in WT and transgenic poplar lines. (a) LAC27 transgenic lines (b) LAC18 transgenic lines, compared to WT. (c) STTM:miR397a transgenic lines, compared to vector control.

Figure 3.10-2: Correlation analysis of lignin content, S/G lignin ratio and glucose release content in WT and transgenic poplar lines. (d) LAC27 transgenic lines (e) LAC18 transgenic lines, compared to WT. (f) STTM:miR397a transgenic lines, compared to vector control.

3.4 Discussion

The development and regulation of primary and secondary plant cell wall plays a critical role in vascular plants. The inelastic nature of the secondary cell wall provides structural rigidity and protects the plant from mechanical stress and pathogens. Cellulose, hemicellulose, lignin, pectins and other proteins builds a strong intricate network of polymers within the secondary cell wall. Due to its abundance, cellulose is considered the most essential component for bioconversion of plant biomass for production of several wood-based products. Recalcitrant to enzymatic and microbial degradation, lignin and hemicellulose are major hindrances for accessibility of cellulose and other fermentable sugars to be used for bioconversion. Extensive research has been carried out to manipulate the biosynthesis pathway these three components individually to understand the structural and functional complexity of cell wall. This understanding has helped to create new methods for generating plants with less or easily digestible lignin/hemicellulose for downstream applications (Boudet et al. 2003) (Chen and Dixon 2007) (Nookaraju et al. 2013).

Recent findings suggest plant laccases play significant role at different developmental stages like peroxidases, especially during lignin polymerization. This family of copper-containing oxidoreductases can oxidize a broad range of substrates in presence of oxygen. Their expression during lignin biosynthesis and wood formation has been demonstrated to be regulated by miR397a (Lu et al. 2013). It has also been suggested that LAC and PRXs share non-redundant functions and have a combinatorial participation in lignin polymerization (Zhao et al. 2013a). Our study is aimed at expanding this knowledge by

manipulating certain SDX laccases and miR397a gene expression with an attempt to determine the impact caused by these changes and for functional characterization of laccase and peroxidase.

The two SDX LACs selected for this study were LAC27 and LAC18 based on their tissue gene expression profile, their sequence similarity with each other and if they were targets of miR397a. An attempted overexpression of these LACs in poplar using a xylem-specific promoter led to the overexpression of LAC27 and LAC18 in their respective transgenic lines. The overexpression of LAC27 brought about an increase in total laccase enzyme activity, decreased the lignin content, increased the xylem area but did not affect the S/G ratio in LAC27 transgenic lines. We speculate the changes observed in LAC27 lines were dependent mostly on the expression profile of LAC27. We believe this to be true because the expression of the other tested SDX LACs were significantly low in these lines. This could be suggested to be either due to the regulation exerted by miR397a or redundancy among the laccase gene family due to overexpression of LAC27 (Figure 3.2 (a) and (b)).

On the other hand, though the overexpression of LAC18 saw an increase in the total laccase enzyme activity, it did not affect the lignin content, severely reduced the xylem area and significantly decreased the S/G ratio in LAC18 transgenic lines. This reduction in S/G lignin ratio also correlates with the reduced autofluorescence observed in the phloem fibers which are otherwise rich in S-lignin units. The gene expression analysis suggests that the increase in expression of LAC27 did not seem to contribute to maintain the S/G ratio neither the xylem development in LAC18 lines. The possible reason could be that they do

not share high sequence similarity and therefore do not share a functional overlap (see Appendix C.6, C.7, C.8, C.9, C.10).

Figure 3.11: Relative gene expression of SDX PRXs in developing xylem of harvested LAC27 transgenic lines. Relative expression of (a) PRX25 gene in LAC27 transgenic lines. (b) PRX12 gene in LAC27 transgenic lines. (c) PRX33 gene in LAC27 transgenic lines. (d) PRX22 gene in LAC27 transgenic lines. (e) PRX23 gene in LAC27 transgenic lines, compared to WT plants, *** P-value < 0.0009.

Similarly, the gene expression analysis of the six SDX PRXs in LAC27 (Figure 3.11 (a) – (e)) and LAC18 (Figure 3.12 (a) – (e)) lines were tested. Though we observed an increase in PRX25 expression, we do not believe it participated in the changes observed in LAC27 lines because these two enzymes are among the highly expressed LACs and PRXs in SDX and could exhibit a level of competition to polymerize lignin in this region. On the other hand, the expression of PRX25 was significantly higher in LAC18 lines and we believe PRXs did not compensate for the function exhibited by LAC18.

Therefore, it would be appropriate to say that each enzyme in the laccase gene family is different from the other and PRX gene family in their effect they display during the cell wall biosynthesis. This is evident by the difference in effect seen by overexpression of LAC27 and LAC18 which demonstrated contrary impact on the cell wall. could possibly exist a level of cross talk between the functioning of LACs and PRX25 or competition for substrates (monolignols) due to the difference in substrate specificity.

Figure 3.12: Relative gene expression of SDX PRXs in developing xylem of harvested LAC18 transgenic lines. Relative expression of (a) PRX25 gene in LAC18 transgenic lines. (b) PRX12 gene in LAC18 transgenic lines. (c) PRX33 gene in LAC18 transgenic lines. (d) PRX22 gene in LAC18 transgenic lines. (e) PRX23 gene in LAC18 transgenic lines, compared to WT plants, *** P-value < 0.0002, ** P-value < 0.009, * P-value < 0.02.

We used STTM technology to manipulate the expression of miR397a that negatively regulates expression laccases in SDX. So, suppression of miR397a expression should ideally emancipate the negative regulation exerted on laccase activity and which in turn should increase lignin polymerization if these LACs are involved in this process. Using this approach, we clearly observed a reduction in the miR397a gene expression with an

increase in laccase gene activity in these lines. Gene expression analysis of the SDX LACs that are targets of miR397a, displayed an increase only in LAC14 expression whereas the other tested LACs – LAC18, LAC11, LAC23 and LAC30 were either reduced or undetermined in the STTM lines. We believe that the change in miR397a expression did bring about a change in the expression pattern of LACs but it is undetectable due to the differential expression of these genes in the xylem. This is evident by the increase in xylem area and increase in autofluorescence in the phloem fibers. The manipulation of miR397a led to an increase in the S/G lignin ratio with a 6% decrease in lignin with no significant changes to the xylem formation in STTM:miR397a lines. Thus, the increase in S-lignin would reduce the branching within the lignin backbone that will help to make the lignocellulosic biomass easy to digest making the wood obtained from these lines more desirable for bioenergy purposes.

The manipulations of LACs and miRNA also witnessed a significant increase in sugar release. We believe the reason for this to be the changes within the internal structure of SCW brought about by changes in lignin content and/or composition. This could have resulted in improved saccharification efficiency in LAC and STTM lines which again was not as significant as demonstrated by (Kavousi et al. 2010; Cook et al. 2012). But making this approach beneficial for cell wall deconstruction by producing wood with easily digestible lignin when subjected to acid pre-treatment as evident with increased sugar release. Successful attempts to engineer lignin have been performed in the past but it has been evident that these plants suffer severe growth abnormalities and therefore, limiting their use for bioenergy purposes (Nookaraju et al. 2013). In our current study with the

manipulation of LAC or miR397, we did observe changes in lignin content and composition, but this did not affect the growth characteristics of the LAC or STTM lines.

Conclusion

Overall, the manipulation of laccases and miR397a help to ascertain the role they play in lignin polymerization. We did not detect any co-suppression phenomena exhibited by LAC27 or LAC18 lines as observed during the overexpression of PRXs (Chapter 2). We were able to decipher a relation between LACs and PRXs with respect to polymerization. We could draw conclusions that LACs and PRXs share proportional differences in their expression and they act independently of the other with non-redundant functions. This study was novel in its own way due to the application of STTM technology to study the regulation of miRNA in trees. This study provides a better platform to manipulate lignocellulosic biomass efficiently with no yield penalty which is important due to the growing demand for plant-based bioproducts.

Summary of LAC and miR397a manipulation

3.5 Acknowledgements

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4 Chapter 4

Secondary cell wall regulation by miRNA166

Abstract

Plant miRNAs regulate target gene expressions at transcriptional or post-translational level for most of the biological process that takes place in plants. Over the years, understanding the role of miRNAs in secondary cell wall biosynthesis has been gaining importance. The functional characterization of the mechanism behind miRNAs regulating wood development will prove to be beneficial to design and generate plants with wood characteristics essential for bioconversion of lignocellulosic biomass. We used STTM strategy to manipulate the regulation of miR166 which is suggested to be involved in cambial and wood development. We performed leaf disc-transformation of poplar plants and generated transgenic lines with reduced expression of miR166. This is the first study to use STTM technology for studying regulation of miR166 in trees. The study revealed that miR166 acts as positive and negative regulator of the target HD-ZIPIII homeobox (HB) genes. The changes in these regulations exerted by miR166 led to changes in the growth characteristics of the transgenic plants. One of the significant finding was the pleiotropic effect seen on the leaf polarity exhibiting the curled-leaf phenotype. Improved saccharification efficiency with no growth penalty were other significant findings of this study. Overall, the study was helpful to understand wood development regulated by miR166 and generated trees with superior wood quality that can be used for bioenergy applications.

4.1 Introduction

Plant miRNAs belong to a large class of endogenous small non-coding RNAs with potential regulatory functions. It has been shown that they play a modulatory role in gene expression either by transcriptional modification causing methylation of target DNA or post-transcriptional cleavage or translational inhibition of target mRNAs (Baulcombe 2004) (Kume et al. 2014). They are involved in several biological processes such as developmental stages, environmental stress responses, and secondary cell wall biosynthesis. They can act as positive or negative regulators and the effects of this regulation on their targets can either be studied by silencing or constitutive overexpression of miRNAs, or creating artificial target mimics against miRNAs (Gielen et al. 2012) (Zhou and Luo 2013) (Tang and Tang 2013).

In the 20-21nt (nucleotide) long mature miRNA sequence, the second to eighth position of the miRNA is called as the seed region. This region is suggested to be involved in recognizing the binding site on the target genes. The miRNA cutting region is believed to be between the tenth and the eleventh nucleotide. This characteristic being almost universal for all plant miRNAs identified so far, the next step was to characterize their function in plants (Baulcombe 2004) (Kume et al. 2014). A method called short tandem target mimic (STTM) was pioneered and designed by Dr. Guiliang Tang's group. These STTM structures were capable to block and knockdown specific miRNAs and helped to reveal effects caused due to their loss of function in plants. A weak stem-loop structure linked with two miRNA-binding sites on either end defines the functional STTM, artificially designed non-coding RNA sequence. There exists a partial complementarity between the

miRNA-binding site in the STTM structure and its target gene. There exists a 3-nt bulge that corresponds to the tenth and eleventh nucleotide of the target miRNA. This bulge also prevents the cleavage of STTM by the targeted miRNA. The efficacy of STTMs to knockdown miRNA activity has been successfully demonstrated in several plant and animal species (Yan et al. 2012) (Tang et al. 2012; Tang and Tang 2013).

With recent developments in understanding the role of miRNA in regulation of lignin biosynthesis and secondary cell wall formation, we decided to adopt the STTM strategy for our current study. Previous studies suggested that overexpression of several miRNAs such as miR156, miR166 and miR397 in poplar led to the decrease in lignin, enhanced plant biomass for sustainable biofuel and reduce recalcitrance (Lu et al. 2013) (Trumbo, Zhang, and Stewart 2015) (Chen et al. 2016). We have attempted to suppress miRNA166 formation using STTM strategy.

Role of miR166 in cambial and vascular tissue development

In one of our previous studies, we used the STTM strategy to characterize the role of miR397a in lignin polymerization (Chapter 2). Similarly, for this study we selected miR166 due to its suggested involvement in active cambial cells and vascular tissue development. miR166 family comprises of 7 family members – miR166a to miR166g. This family of miRNAs regulates the class III *HOMEODOMAIN-LEUCINE ZIPPER* (HD-ZIP III) transcription factor family and binds to GTAAT(G/C)ATTAC sequence within the promoter region (Sessa et al. 1998) (Zhong and Ye 2007). They exert their regulation by epigenetic silencing of a few enzymes involved in secondary cell wall formation and by

increased expression in tension wood when compared to opposite wood in *Populus*, *Arabidopsis* and *Acacia* (Yan et al. 2012) (Ong and Wickneswari 2012) (Ding, Zeng, and He 2014). The HD-ZIPIII homeobox (HB) genes present in *Populus trichocarpa* shares a high sequence similarity with the five known HB genes in *Arabidopsis*. But due to the duplication event of poplar genome, there are eight HB proteins in poplar. They share high sequence similarity with genes within a specific group but dissimilar between groups (see Appendix D.2, D.3). The role played by each HB protein, therefore seems to be independent of the other HB protein. An inverse correlation between developmental and seasonal regulation of *PtaHB1* and *PtamiR166* was demonstrated in a study (Ko, Prassinis, and Han 2006). The PHB, PHV and REV genes regulate polarity patterning of lateral organs and vascular bundles. PopREV, a transcription factor is suggested to be negatively regulated by miR165/166 that functions in the initiation of cambium development and patterning of secondary vascular tissue (Robischon et al. 2011). Studies also suggest that miR166 might have prospective application in biofuel production, recalcitrance and bio-confinement due to its participation in shoot and floral development (Trumbo, Zhang, and Stewart 2015).

A detailed miRNA profiling is necessary to understand plant vascular development and wood formation. For this purpose, in our study, we used the STTM strategy with an aim to suppress the activity of miR166 to study the changes occurring in the vascular development. We expect the target HD-ZIPIII HB genes to be free from the regulation exerted by this miRNA and would help provide insights to understand their functions during wood development in poplar trees.

4.2 Materials and methods

4.2.1 Vector construction

STTM constructs were generated as previously stated in (Tang et al. 2012). Modifications were made to facilitate successful transformation in poplar. The final STTM-pFGC5941 plasmid was digested with *PacI* to generate the STTM fragment. This fragment was incorporated into pBI121 vector with Cauliflower mosaic virus (CaMV) 35S promoter for STTM:miR166. Cloned plasmid was then transformed into *Agrobacterium* strain C58C1 using freeze thaw method (Boerjan et al. 1997) (Wu, Joshi, and Chiang 2000). The transformed plasmids were selected for antibiotic resistance. Verification PCR for the gene of interest on the selected clones were carried out before transforming the plants. The sequences of the selected clones for STTM:miR166 genes from plasmid and agrobacterium transformation was also confirmed by sanger sequencing (see Appendix D.1).

4.2.2 Plant transformation and growth conditions

The verified clones were transformed into *Populus tremula* x *alba* clone 717-1B4 genotype (routinely referred as 717) using leaf-disc transformation protocol previously described in (Boerjan et al. 1997) (Wu, Joshi, and Chiang 2000). First, the *Agrobacterium* clone is allowed to infect the leaf discs at the wound site (where the leaves have been cut) through several initial steps. Later these infected leaves are allowed to grow on callus induction medium (CIM) for three to four weeks to develop callus (unorganized mass of plant cell). Once callus is formed, they were moved to shoot induction medium (SIM) and then to shoot elongation medium (SEM) for three to four weeks each. Shoots generating from each callus was considered as an independent line. Keeping track of each of these independent

lines, they were further moved to root induction medium (RIM) until the shoots developed roots. The callus and shoots were grown in a growth chamber (Thermo Scientific Precision model 818 incubator) with a light and dark cycle of 16 hrs and 8 hrs at 25°C. Plants that rooted were selected and genotypically verified for the presence of the gene of interest using genomic PCR. Verified transgenic lines were acclimatized and, moved and maintained in green house for approximately 4 months before harvesting. The green house growth conditions were kept similar to the growth chamber with light and dark cycle of 16hrs and 8hrs, temperature of 25°C and relative humidity ranging from 40-70%. Upon harvest of the 16-week old STTM:miR166 transgenic plants, all leaf material from each plant was weighed before and after drying and stored in paper bags. The stem was cut into approximately four to five pieces (with ten to fifteen internodes each). The bark was cut open with a sharp blade and developing xylem on the wood material was scraped in liquid N₂, collected in aluminum foil and stored in liquid nitrogen until use. The remaining wood material from each plant was air-dried and stored for further analysis.

4.2.3 RNA extraction and qRT-PCR analysis

Total RNA was extracted from SDX (1-10 internodes counted from the base of the plants with 40-50 internodes) using TRIzol (Ambion, Life Technologies) method. Primers were designed for each miRNA and target gene are as listed in (see Appendix A.1). Stem-loop qRT-PCR was performed to measure the targeted miRNA expression level and detection of miRNA target gene expression was performed by traditional qRT-PCR. For both, first-strand cDNA synthesis from 1µg of total RNA was performed using high capacity cDNA reverse transcription kit (Applied Biosystems). The analysis was performed with an

Applied Biosystems Step-one instrument using PowerUp SYBR green master mix (Applied Biosystems). The reaction for each gene were conducted in triplicate with thermal cycling conditions as follows: 95°C for 10 mins, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The primer specificity was confirmed by melt curve analysis. The relative gene expression was calculated using the $2^{-\Delta\Delta C_t}$ method. Actin gene was used as an internal control and values were normalized to actin and comparing the normalized values to those of control plants.

4.2.4 Lignin analysis

Dried wood samples from previously harvested poplar plants were used for the analysis. These wood samples were milled to mesh 20 using Willey-mill. Each sample weighing 100 mg were placed in microfuge tubes and sent to NREL for pyrolysis/molecular beam mass spectrometry (Py-MBMS) (Kelley et al. 2004). Each sample was analyzed in duplicates for this procedure.

4.2.5 Microscopic image analysis

For light microscopy, stems of poplar plants were cut at tenth internode from the apex of the plant with 40-50 internodes and were fixed in cold FAA (formaldehyde – acetic acid – ethanol) solution. The internode was vacuum infiltrated for 15-20 mins and stored with fresh cold FAA solution overnight at 4°C. after fixation, these internodes were dehydrated and embedded in wax. Seven-micrometer-thick consecutive sections were cut with a microtome, mounted on a slide and de-waxed using xylene and ethanol series. Lignin

autofluorescence from de-waxed sections was photographed using fluorescent light imaging.

4.2.6 Saccharification assay for STTM:miR166 lines

For performing saccharification assay to estimate glucose release, we followed (Kumar and Turner 2015) with some modifications. Ten milligrams of dried and milled wood samples from previously harvested poplar plants were used for the analysis. Alcohol insoluble residue (AIR) samples were prepared, treated with acetonitrile solution (acetic acid: nitric acid: water 8:2:1) at 100°C for 30 mins. These samples were further subjected to sulfuric acid swelling. Sugar release was measured by anthrone method by monitoring the reactants' solution absorbance at 620 nm. Using 10-250 $\mu\text{g } \mu\text{l}^{-1}$ of glucose, the standard curve regression line was generated and the glucose release in each sample were calculated.

4.2.7 Growth data analysis

Growth data for height, leaf count and stem girth were measured for all the generated transgenic lines (for STTM:miR166) every week (for 16 weeks) until the day of harvest.

4.3 Results

4.3.1 Gene expression study of miR166 by STTM strategy

In this study, all transgenic lines were compared to vector control which had the empty STTM cassette with no specific miRNA region. The relative gene expression of miR166 and its target genes were quantified using qRT-PCR. A significant downregulation of miR166 was observed in the STTM lines when compared to vector control (Figure 4.1). It

has been previously demonstrated that miR166 exhibits a regulation over the HD-ZIPIII family genes. Since the expression profile of the miR166 changed, we decided to test the expression of its target genes. It was observed that there was a significant decrease in the expression of HB1 and HB5 whereas a significant increase in the expression of HB4 and HB7 in STTM:miR166 lines (Figure 4.2 (a) – (d)).

Figure 4.1: Relative gene expression of miR166 in developing xylem of harvested poplar transgenic lines. Relative expression of miR166 gene in STTM:miR166 transgenic lines, compared to vector control. Significant compared to vector control, ** P-value < 0.002, * P-value < 0.07

Figure 4.2: Relative gene expression of target HB gene in developing xylem of harvested STTM:miR166 transgenic lines. Relative expression of (a) HB1 gene in STTM:miR166 transgenic lines. (b) HB4 gene in STTM:miR166 transgenic lines. (c) HB5 gene in STTM:miR166 transgenic lines. (d) HB7 gene in STTM:miR166 transgenic lines, compared to vector control plants. *** P-value < 0.0007, ** P-value < 0.003.

4.3.2 Effect on xylem formation

The HD-ZIPIII genes are suggested to play a role in cambial development. Stem sections of the tenth internode from the apex of the transgenic STTM lines were compared to vector control. The lignin autofluorescence images of the STTM lines demonstrated a decrease of cells in the cambial region whereas a slight increase around xylem region. (Figure 4.3 (a) and (b)). We calculated the xylem area in these lines but did not show any significant change in this region (Figure 4.3 (c)).

Figure 4.3: Autofluorescence of lignin in stem sections and estimated xylem area of harvested vector control and transgenic poplar lines. Cross section of (a) vector control, (b) STTM:miR166 transgenic lines, (c) area of xylem in STTM:miR166 transgenic lines, compared to vector control plants.

4.3.3 Effect of gene manipulation on lignin content and saccharification assay in STTM:miR166 lines

Evaluation of lignin content/composition in dried and milled wood from transgenic STTM and vector lines was carried out using Py-MBMS. An overall decrease of only 2-4% in lignin content was observed in the STTM lines. The S/G lignin ratio was significantly higher in these lines as shown in Figure 4.4 (a) and (b). Glucose release had also increased in transgenic STTM lines and calculated to be in the range of 55-57% when compared to vector controls (Figure 4.4 (c)).

Figure 4.4: Estimation of lignin content, S/G lignin ratio and glucose release assay in vector control and transgenic poplar lines. (a) Lignin content, (b) S/G lignin ratio, (c) percent change of glucose release, in STTM:miR166 transgenic lines, compared to vector control plants. Significant when compared to vector control, *** P-value < 0.0005.

4.3.4 Effect on growth and biomass of STTM:miR166 lines

As demonstrated in previous studies, the leaves of transgenic STTM:miR166 lines exhibited curled leaf phenotype and loss of leaf polarity in the early stages of development

(Figure 4.5 (a – (i-v))). It was observed that once the plants were moved to the green house, the leaf polarity gradually shifted to normal and did not exhibit this curled leaf phenotype. The growth characteristics such as height and leaf count showed a significant increase in these lines when compared to control (Figure 4.5 (b) and (c)). There was no significant difference in the stem girth of these lines (Figure 4.5 (d)). The wet and dry leaf biomass also exhibited a significant increase in the STTM lines. (Figure 4.5 (e)).

Figure 4.5 a: Comparison of phenotypes between vector control and STTM:miR166 transgenic lines. (i – iii) Altered leaf and root development was observed after knock down of miR166, (iv & v) curled leaf and loss of leaf veins and polarity observed in STTM:miR166 lines, compared to vector control

Figure 4.5 b: Estimated height (in cm) of vector control and STTM:miR166 transgenic lines over a period of 16 weeks until harvest.

Figure 4.5 c: Estimated leaf count of vector control and STTM:miR166 transgenic lines over a period of 16 weeks until harvest.

Figure 4.5 d: Estimated stem girth (in mm) of vector control and STTM:miR166 transgenic lines over a period of 16 weeks until harvest.

Figure 4.5 e: Estimated wet/dry leaf biomass change in STTM:miR166 transgenic lines, compared to vector control

4.3.5 Correlation analysis

Correlation analysis helped to summarize the findings of this study. Here, a positive correlation was observed between gene expression of miR166 and lignin content and, S/G lignin ratio and glucose release in STTM lines. Negative correlation was exhibited by STTM lines for lignin content and glucose release and, S/G lignin ratio and lignin content (Figure 4.6 (a) and (b)).

Figure 4.6: (a) Correlation analysis of relative gene expression and lignin content in vector control and STTM:miR166 transgenic lines. (b) Correlation analysis of lignin content, S/G lignin ratio and glucose release content in vector control and STTM:miR166 transgenic lines.

4.4 Discussion

A class of small non-coding RNAs, called miRNAs that are ~21 nt long are said to play a significant role in gene, epigenetic and chromatin regulation. They are believed to be a class of post-transcriptional negative regulators active during plant development and stress responses. Functional genomics is making it easier to ascertain the exact relation between miRNA and its target genes and how it affects downstream processes in an organism. The discovery of miRNA has made it possible to believe that the hidden puzzles of plant cell wall biosynthesis can be determined. These miRNAs are highly conserved between plant species and are involved in the regulation of same or similar processes in these species. This makes it easier to translate the findings and exhibit similar results in another plant species. There have been several studies stating HD-ZIP III proteins have a regulatory role in wood formation and vascular differentiation (Du et al. 2011) (Robischon et al. 2011) (Zhu et al. 2013) (Zhu et al. 2017). We used STTM strategy for the first time in poplars to understand the mechanism behind the regulation exerted by miR166 on their target genes in poplar trees.

The application of STTM technology to study the regulation of miR166 on wood development was successful as suggested by gene expression analysis that demonstrated downregulation of miR166 gene in the STTM:miR166 lines. Gene expression analysis of the miR166 target genes (homeobox HB) in our study suggested that miR166 can act as positive and negative regulator of HB genes in poplar. It positively regulates the expression of *PtrHB1* and *PtrHB5* genes whereas it negatively regulates the expression of *PtrHB4* and *PtrHB7* genes (Figure 4.2). The suppression of miR166 caused pleiotropic changes to

the morphology of these lines and exhibited the curly leaf phenotype during tissue culture phase. This finding corroborates with the increased expression of *PtrHB7* gene in STTM lines which has previously been demonstrated to be the reason for exhibiting this severe phenotype (Zhu et al. 2013). Interestingly, this curled leaf phenotype was reversed once these plants were grown in the green house for a period of 16-weeks. The reason for the reversal of phenotype is still unclear. In 2017, Zhu et al demonstrated the importance of HB4 in enhancing cambial activity (Zhu et al. 2017). Our study demonstrated an increase in the cambial region but no ectopic effects of cambial or phloem activity. In contrast, the expression of HB1 was reduced with the downregulation of miR166, the expression of which was previously stated to be inversely correlated (Ko, Prassinis, and Han 2006). It is not clear how HB5 is involved in cambial and wood development in poplar. We believe a concerted regulation of many factors control the process of cambial and wood development. We tested only four out of eight HB genes controlled by miR166 as they shared high sequence similarity.

The downregulation of miR166 using STTM strategy saw a reduction in lignin content which was not a significant change when compared to previous studies. The increase in S/G lignin ratio leading to structural changes within the plant cell wall was another significant observation of this study. The STTM lines also exhibited increase in growth characteristics such as height and leaf count thereby increasing the biomass content. The increase in release of sugars helped to improve saccharification efficiency in these transgenic lines. These phenotypic and genotypic changes did not affect the plant growth

and help generate plants without any growth compensation increasing their value for wood production.

Conclusion

Overall, this study helped to understand the regulation miR166 exerts on its targets in poplar trees. We predict that the HB gene family in poplar plays a concerted role in cambial development as evident with reversal of leaf polarity, no ectopic effects in the cambial and wood development, increase in plant growth without growth penalty and improved saccharification efficiency. Thus, making it a desirable approach for generating trees to be used in the downstream application such as paper and pulp or biofuel production.

Summary of manipulation of miR166

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5 Chapter 5

Conclusion and future perspectives

Understanding plant cell wall development with respect to lignin biosynthesis and polymerization has been a challenging task. Several attempts have been made over the decades to manipulate and engineer lignin to understand the functions of every gene and enzyme involved in its synthesis. We, now have a better understanding of how lignin biosynthesis occurs and how its alterations could be beneficial for its downstream applications such as lignin valorization. Despite the progress made, the mechanism behind lignin polymerization still needs to be deciphered. It is now known that peroxidase and laccase gene families catalyze this process but their exact role and how they function during the process is still unclear. Current research on lignin mostly aims on improving lignin degradability for sugar accessibility (saccharification) from lignocellulosic biomass. Understanding lignin polymerization in plants, especially in trees like poplars (*Populus* species) might enable genetic improvement of lignin for better wood utilization in the future due to the growing importance of lignin-based bioproducts.

The first step towards this goal was to identify specific SDX-PRXs and -LACs from the entire gene family in *Populus*. In the current study, we identified SDX-PRXs and -LACs each based on previous *in-silico* studies. We verified the expression of these SDX genes in different parts of the poplar plant. We selected two SDX genes for PRX and LAC and attempted to overexpress these genes in transgenic poplar plants. We further decided to manipulate these genes using a tissue-specific, in this case developing xylem-specific

promoter (DX15) rather than using a constitutive promoter (CaMV35S) that typically produces many growth abnormalities in transgenic trees. This was mainly to observe the downstream changes only within the expected developing xylem region where wood development occurs and to avoid ectopic expression of these genes in different parts of the plant.

The overexpression of PRX25 and its expression analysis indicated that this gene was co-suppressed in PRX25 transgenic lines that altered the S/G lignin ratio. The overexpression of PRX36 was detrimental for lignin production and xylem intactness. Manipulation of PRXs increased the sugar release and improved the saccharification. Expression analysis of the six SDX-PRXs in these lines also suggests that they do not share redundant functions and each enzyme seems to play a significant role in lignin polymerization. The study also implies that SDX LACs do not compensate for the alterations in PRX expression. Changes within the cell wall due to PRXs manipulation did not affect the plant physiology and generated transgenics with no growth abnormalities.

The overexpression of LAC27 and LAC18 and its expression analysis indicated that these genes were overexpressed in their respective transgenic lines with no or less significant changes observed in lignin content and S/G ratio. This study also implies that there exists a level of cross talk in functioning between LACs and PRXs but they do not override other genes function. This is evident in LAC18 transgenic lines where PRX25 is expressed at higher levels than LAC18 and LAC27 but we see a reduction in the S/G lignin ratio and xylem formation. The manipulation of miR397a using STTM strategy also helped to understand the role it plays in regulating lignin polymerization. The suppression of

miR397a increased the S/G lignin ratio and improved saccharification efficiency of these plants.

We also attempted to understand the changes in cambial and wood development via manipulation of miR166 using STTM strategy. This is the first study to demonstrate the effect of changes in cambial development owing to the regulation of all the Homeobox (HB) genes. miR166 acts as a positive and negative regulator of HB genes which in turn affects the xylem and cambial formation. These changes improved growth characteristics and biomass and, increased S/G lignin ratio and improved saccharification efficiency. This study also suggests that this approach could be adapted for downstream application to procure improved biomass for bioenergy production.

In conclusion, we could decipher that there exists a communication between the PRXs and LACs which is strongly sensed by the plant system and regulates its function. Each enzyme in the pathway plays a specific role which is unknown and how they balance their function is still unclear. Each enzyme is different in their effect that they have on the plant cell wall as seen with the manipulation of PRXs/LACs. Their manipulation affects the expression of the other gene but the net effect is not lignin increase. The most significant finding was that none of our transgenic lines showed any growth penalty and/or ectopic expression of lignin and exhibited a normal phenotype. To lay emphasis over again, any minor alterations in expression of these genes led to structural changes within the cell wall and this is evident by the increased improved saccharification efficiency. Thus, making it highly desirable to use of wood efficiently for future applications.

Future goals

Both PRXs and LACs were shown to exhibit non-redundant functions during lignin polymerization. An in-depth study of SDX-PRXs/-LACs to validate their role during this process can be performed using gene editing techniques (CRISPR/Cas9). This study was performed by homologous expression of poplar genes in polar plants. To take a step further to understand the function of PRXs and LACs, our group is also working on heterologous expression of these genes i.e. expressing *Arabidopsis* PRX and LAC in poplar plants. It will also be interesting to group HB genes that were manipulated positively and negatively by miR166 in poplar and to study the changes in cambial and wood development. Looking at the larger picture, being able to answer these proposed questions and changes could strengthen the knowledge about lignin polymerization, improve the commercially available lignin in economically important tree species making it beneficial to produce value-added bioproducts from lignocellulosic biomass.

A Appendix A

A.1 The entire list of primers used for all experiments includes primers for full-length amplification of genes, genomic PCR verification primers, stem-loop PCR primers for miRNAs and qRT-PCR primers for all genes

Table A 1: Highly expressed LAC and PRX in Ptr for full-length DNA amplification

Highly expressed LAC and PRX in Ptr for full-length DNA amplification		
Gene	Primer name	Primer sequence 5' - 3' with adaptors
<i>PtrLAC18</i>	PtrLAC18_BamHI_F	GCGGATCCATGGAGGTTATCAACC
<i>PtrLAC18</i>	PtrLAC18_SacI_R	GCGAGCTCTTAACATATTGGCAGA
<i>PtrLAC27</i>	PtrLAC27_BamHI_F	GCGGATCCATGGAGTACTATCAGG
<i>PtrLAC27</i>	PtrLAC27_SacI_R	GCGAGCTCCTAGCACGTTGGAAGG
<i>PtrPRX36</i>	PtrPRX36_BamHI_F	GCGGATCCATGGCCAGAGCTGCTA
<i>PtrPRX36</i>	PtrPRX36_SacI_R	GCGAGCTCTCATATTAATCCGGAC
<i>PtrPRX25</i>	PtrPRX25_XbaI_F	GCTCTAGAATGGATGCCAAAGCTC
<i>PtrPRX25</i>	PtrPRX25_PacI_R	GGCTTAATTAAGTAGTGGTGCTTGT

Gene	Primer name	Semi-quant primers
<i>PtrLAC18</i>	LAC18_M_3'_F	CACAGCTACAGTCACAGCCTT

<i>Ptr</i> LAC27	LAC27_M_3'_F	CATGGATACTGTAGTTGCTGT
<i>Ptr</i> PRX36	PRX36_M_3'_F	CAGCTCTTTCCTGACCGC
<i>Ptr</i> PRX25	PRX25_M_3'_F	CAGGAAGAGCAGAGCAGATGT
<i>Ptr</i> DX15	DX15_3'_F	GATCCTCTCTTCACCAGACCAC
	DX15_M_5'_R	CTCCGAGTATTCCTACATACGAG

Gene	Primer name	STTM Primers
	STTM F_B4	AACCTCCTCGGATTCCATTGCCCAGC
	STTM R_B5	CCTTGTCTTCCTCCTTCCTTCCGTCC
	STTM seq F_B6	CGCACAATCCCACTATCCTTCGC
	STTM seq R_B7	CGGGAACTACTCACACATTATTTATGG

Gene name	Seq Name	quantitative-PCR primers (Seq 5' to 3')
<i>Ptr</i> _LAC11	qRT_LAC11_F	GGCCTTCCAGGGCCTTTATACAAT
	qRT_LAC11_R	CCTGGACTGATGAGGAGCGTGTC
<i>Ptr</i> _LAC18	qRT_LAC18_F	GGTCAACCTGGTGATCTTTATAACTGC
	qRT_LAC18_R	AACTGTAACTTATGGTTGGCTATG
<i>Ptr</i> _LAC27	qRT_LAC27_F	GGTGGACAATGGTAAGGGCCCA
	qRT_LAC27_R	CTATACTCCTCCCTCCCGCATC
<i>Ptr</i> _LAC2	qRT_LAC2_F	AGTTTTGAGTTTCACCAATATTACCTG
	qRT_LAC2_R	CCAGAGGCAGCTCATAGCAA
<i>Ptr</i> _LAC14/15	qRT_LAC14_F	TGCCTCTTCCCTGCTTTGGTCC
	qRT_LAC14/15_R	GCCATTGACGGTAACAATCGGC
	qRT_LAC15_F	TCTTGCTGTCTGCCTCCTCCCC
<i>Ptr</i> _LAC30	qRT_LAC30_F	GCAGATCCAGAGGCGATCATTAGT
	qRT_LAC30_R	TGCCCTCAATGACGAGCTCTTCTT

Ptr_PRX12	qRT_PXP12_F	CTTAGCTGCTTGCTCATTTTGCAGC
	qRT_PXP12_R	GCTGGTGATCTGCTTGCTGGTTACT
Ptr_PRX22	qRT_PRX22_F	GTGAACAACATCATTCGTGGAGTCC
	qRT_PRX22_R	CAGGACAAGCATTCTCTACTGCAGC
Ptr_PRX23	qRT_PRX23_F	GGCAGTGGCAATCCTGATCCA
	qRT_PRX23_R	AACACTCTGATTTCCAGCTTGAGGA
Ptr_PRX25	qRT_PRX25_F	CCGAGAGCTTCAAAGCAAGGTCAG
	qRT_PRX25_R	TGCAAAGGCCAGCCTGACC
Ptr_PRX33	qRT_PRX33_F	GAGGCTGTGATGCCTCTGTTCTGA
	qRT_PRX33_R	CAGCGCATGATACCACTCCCGG
Ptr_PRX36	qRT_Prx36_F	CAAAACCATGGCCAGTGCTGC
	qRT_Prx36_R	CGAAGCAAGCCAGCAGCTTG
Ptr_HB1	qRT_HB1_F	CGCAGGTGTCGAGAGAAGCAG
	qRT_HB1_R	CTGCCGCAGGTGCAGTTTGTAG
Ptr_HB5	qRT_HB5_F	CGCCGCCAGCAGCTCATT
	qRT_HB5_R	TCCTATTCACCGCTTGGAGGC
Ptr_HB7	qRT_HB7_F	GGTACACACCAGAGCAAGTGGAAG
	qRT_HB7_R	CTGATCAACTGTTGGCGACGCG
Ptr_HB4	qRT_HB4_F	ACCAGGGTGGTCGGTGTTG
	qRT_HB4_R	CCTCTCCAATGCCTCAACTTGCTC
Ptr_Actin	qRT_Actin_F	ATGGCCGATTCTGAGGATATTGAG
	qRT_Actin_R	TCTGCCCCATTCCAACCATGACA

Gene	Primer name	STTM Primers for Stem loop PCR
	Real-common-R	CTGGTGATTTACGCGTACCGAA

	Origin-del-PacI-F	TCCCTTAATTAAGTTTGCAAGCAGCAGATTACGCG
	SLC_U_RP	TGGTGTCGTGGAGTCG
miR397a	F RTP_397	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCATCAACG
	FFP_397	ACACTCCAGCTGGGTCATTGAGTGCAGCG
miR166	F RTP_166	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGGGGGAATG
	FFP_166	ACACTCCAGCTGGGTCGGACCAGGCTTCA

A.2 Sequencing data comparison of plasmid clones with full length CDS region of DX15 promoter

CLUSTAL 2.1 Multiple Sequence Alignments

```
Sequence type explicitly set to DNA
Sequence format is Pearson
Sequence 1: DX15_Promoter      1025 bp
Sequence 2: DX15_HSbf_3_joined 1027 bp
Sequence 3: DX15_HSbf_5_joined 1027 bp
Sequence 4: DX15_Sbf_6_joined  1027 bp
Sequence 5: DX15_HSbf_8_joined 1027 bp
Start of Pairwise alignments
Aligning...
```

```
Sequences (1:2) Aligned. Score: 98.1463
Sequences (1:3) Aligned. Score: 98.1463
Sequences (1:4) Aligned. Score: 98.1463
Sequences (1:5) Aligned. Score: 98.1463
Sequences (2:3) Aligned. Score: 100
Sequences (2:4) Aligned. Score: 100
Sequences (2:5) Aligned. Score: 100
Sequences (3:4) Aligned. Score: 100
Sequences (3:5) Aligned. Score: 100
Sequences (4:5) Aligned. Score: 100
Guide tree file created: [clustalw.dnd]
```

```
There are 4 groups
Start of Multiple Alignment
```

```
Aligning...
Group 1: Sequences: 2      Score:19513
Group 2: Sequences: 3      Score:19513
Group 3: Sequences: 4      Score:19513
Group 4: Sequences: 5      Score:19268
Alignment Score 67916
```

```
CLUSTAL-Alignment file created [clustalw.aln]
```

clustalw.aln

CLUSTAL 2.1 multiple sequence alignment

```
DX15_Sbf_6_joined      TTCCCCCTTTTGGTTCAATGCCTTTTATTCTTCCAAATTAATTTCAATATTTGTATCCG
DX15_HSbf_8_joined     TTCCCCCTTTTGGTTCAATGCCTTTTATTCTTCCAAATTAATTTCAATATTTGTATCCG
DX15_HSbf_5_joined     TTCCCCCTTTTGGTTCAATGCCTTTTATTCTTCCAAATTAATTTCAATATTTGTATCCG
DX15_HSbf_3_joined     TTCCCCCTTTTGGTTCAATGCCTTTTATTCTTCCAAATTAATTTCAATATTTGTATCCG
DX15_Promoter          TTCCCCCTTTTGGTTCAATGCCTTTTATTCTTCCAAATTAATTTCA-TATTTTGTATCCG
                        ***** * *****
DX15_Sbf_6_joined      GAGGAACATATTTGTTTCAAAGGTGTCAGAAAATCAAAGCCCATTGAAAATATATAAAC
DX15_HSbf_8_joined     GAGGAACATATTTGTTTCAAAGGTGTCAGAAAATCAAAGCCCATTGAAAATATATAAAC
DX15_HSbf_5_joined     GAGGAACATATTTGTTTCAAAGGTGTCAGAAAATCAAAGCCCATTGAAAATATATAAAC
DX15_HSbf_3_joined     GAGGAACATATTTGTTTCAAAGGTGTCAGAAAATCAAAGCCCATTGAAAATATATAAAC
DX15_Promoter          GAGGA-CATATTTGTTTCAAAGGTGTCAGAAAATCAAAGCCCATTGAAAATATATAAAC
                        *****
```

DX15_Sbf_6_joined DX15_HSbf_8_joined DX15_HSbf_5_joined DX15_HSbf_3_joined DX15_Promoter	ATATATAGATATAAAAACTCAAGGATTCAATCCAAAAATATAAGAACAACTGATTGAATT ATATATAGATATAAAAACTCAAGGATTCAATCCAAAAATATAAGAACAACTGATTGAATT ATATATAGATATAAAAACTCAAGGATTCAATCCAAAAATATAAGAACAACTGATTGAATT ATATATAGATATAAAAACTCAAGGATTCAATCCAAAAATATAAGAACAACTGATTGAATT ATATATAGATATAAAAACTCAAGGGTTCAATCCAAAAATATAAGAACAACTGATTGAATT *****
DX15_Sbf_6_joined DX15_HSbf_8_joined DX15_HSbf_5_joined DX15_HSbf_3_joined DX15_Promoter	AATTTGTTATTTTAAGAACACTGTCTATATGTTTATATAGTGGTAGGTAGTGTGTTTTAA AATTTGTTATTTTAAGAACACTGTCTATATGTTTATATAGTGGTAGGTAGTGTGTTTTAA AATTTGTTATTTTAAGAACACTGTCTATATGTTTATATAGTGGTAGGTAGTGTGTTTTAA AATTTGTTATTTTAAGAACACTGTCTATATGTTTATATAGTGGTAGGTAGTGTGTTTTAA AATTTGTTATTTTAAGAACACTGTCTATATGTTTATATAGTGGTAGGTAGTGTGTTTTAA *****
DX15_Sbf_6_joined DX15_HSbf_8_joined DX15_HSbf_5_joined DX15_HSbf_3_joined DX15_Promoter	ATCATATACTAACTTATTATAAAAAATAATCATAAAAAAGGAACCTCAAGCATCCCCTGG ATCATATACTAACTTATTATAAAAAATAATCATAAAAAAGGAACCTCAAGCATCCCCTGG ATCATATACTAACTTATTATAAAAAATAATCATAAAAAAGGAACCTCAAGCATCCCCTGG ATCATATACTAACTTATTATAAAAAATAATCATAAAAAAGGAACCTCAAGCATCCCCTGG ATCATATACTAACTTATTATAAAAAATAATCATAAAAAAGGAACCTCAAGCATCCCCTGG *****
DX15_Sbf_6_joined DX15_HSbf_8_joined DX15_HSbf_5_joined DX15_HSbf_3_joined DX15_Promoter	TAAGCTCGTATGTAGGAATACTCGGAGATCAAATGTCCGAATGTCAAATGTTAAGGCAAG TAAGCTCGTATGTAGGAATACTCGGAGATCAAATGTCCGAATGTCAAATGTTAAGGCAAG TAAGCTCGTATGTAGGAATACTCGGAGATCAAATGTCCGAATGTCAAATGTTAAGGCAAG TAAGCTCGTATGTAGGAATACTCGGAGATCAAATGTCCGAATGTCAAATGTTAAGGCAAG TAAGCTCGTATGTAGGAATACTCGGAGATCAAATGTCCGAATGTCAAATGTTAAGGCAAG *****
DX15_Sbf_6_joined DX15_HSbf_8_joined DX15_HSbf_5_joined DX15_HSbf_3_joined DX15_Promoter	TGAAATATCCCTGACTTTTTAGCAAGCAAATTGTTGAGTAGCTAAAAATGAATTATTTTAA TGAAATATCCCTGACTTTTTAGCAAGCAAATTGTTGAGTAGCTAAAAATGAATTATTTTAA TGAAATATCCCTGACTTTTTAGCAAGCAAATTGTTGAGTAGCTAAAAATGAATTATTTTAA TGAAATATCCCTGACTTTTTAGCAAGCAAATTGTTGAGTAGCTAAAAATGAATTATTTTAA TGAAATATCCCTGACTTTTTAGCAAGCAAATTGTTGAGTAGCTAAAAATGAATTATTTTAA *****
DX15_Sbf_6_joined DX15_HSbf_8_joined DX15_HSbf_5_joined DX15_HSbf_3_joined DX15_Promoter	TATTTTAAATCATTTTAAATATATTAATATTAATAAAAAAATTAATATTTTTTTAATACA TATTTTAAATCATTTTAAATATATTAATATTAATAAAAAAATTAATATTTTTTTAATACA TATTTTAAATCATTTTAAATATATTAATATTAATAAAAAAATTAATATTTTTTTAATACA TATTTTAAATCATTTTAAATATATTAATATTAATAAAAAAATTAATATTTTTTTAATACA TATTTTAAATCATTTTAAATATATTAATATTAATAAAAAAATTAATATTTTTTTAATACA *****
DX15_Sbf_6_joined DX15_HSbf_8_joined DX15_HSbf_5_joined DX15_HSbf_3_joined DX15_Promoter	TTTTCAATAACAAACACTTTAAATATAATCTTTGTCACACTCTTAAACAGTAACAGCAG TTTTCAATAACAAACACTTTAAATATAATCTTTGTCACACTCTTAAACAGTAACAGCAG TTTTCAATAACAAACACTTTAAATATAATCTTTGTCACACTCTTAAACAGTAACAGCAG TTTTCAATAACAAACACTTTAAATATAATCTTTGTCACACTCTTAAACAGTAACAGCAG TTTTCAATAACAAACACTTTAAATATAATCTTTGTCACACTCTTAAACAGTAACAGCAG *****
DX15_Sbf_6_joined DX15_HSbf_8_joined DX15_HSbf_5_joined DX15_HSbf_3_joined DX15_Promoter	AAAGCATATGTGAGTGATATAGCTATAGTTGCTGTTTGACACGGACAATCTCCATCTAAA AAAGCATATGTGAGTGATATAGCTATAGTTGCTGTTTGACACGGACAATCTCCATCTAAA AAAGCATATGTGAGTGATATAGCTATAGTTGCTGTTTGACACGGACAATCTCCATCTAAA AAAGCATATGTGAGTGATATAGCTATAGTTGCTGTTTGACACGGACAATCTCCATCTAAA AAAGCATATGTGAGTGATATAGCTATAGTTGCTGTTTGACACGGACAATCTCCATCTAAA *****
DX15_Sbf_6_joined DX15_HSbf_8_joined DX15_HSbf_5_joined DX15_HSbf_3_joined DX15_Promoter	TTCATGAATAATAAGTTTTGCCTACACACCCACTTGAAATCTCCTCCTAGTTTTCTGA TTCATGAATAATAAGTTTTGCCTACACACCCACTTGAAATCTCCTCCTAGTTTTCTGA TTCATGAATAATAAGTTTTGCCTACACACCCACTTGAAATCTCCTCCTAGTTTTCTGA TTCATGAATAATAAGTTTTGCCTACACACCCACTTGAAATCTCCTCCTAGTTTTCTGA TTCATGAATAATAAGTTTTGCCTACACACCCACTTGAAATCTCCTCCTAGTTTTCTGA *****
DX15_Sbf_6_joined DX15_HSbf_8_joined DX15_HSbf_5_joined DX15_HSbf_3_joined DX15_Promoter	TTTGCCATGCTAACTACAAGAACAAGATGCTAGCTAGTATCTTGTCTGTCTCTCGCTCT TTTGCCATGCTAACTACAAGAACAAGATGCTAGCTAGTATCTTGTCTGTCTCTCGCTCT TTTGCCATGCTAACTACAAGAACAAGATGCTAGCTAGTATCTTGTCTGTCTCTCGCTCT TTTGCCATGCTAACTACAAGAACAAGATGCTAGCTAGTATCTTGTCTGTCTCTCGCTCT TTTGCCATGCTAACTACAAGAACAAGATGCTAGCTAGTATCTTGTCTGTCTCTCGCTCT *****

DX15_Sbf_6_joined	CTCTCTATCTCTCCAGTTGATAGTTGATAGTTGATAGTTGATAGCTGATACACTCCCACC
DX15_HSbf_8_joined	CTCTCTATCTCTCCAGTTGATAGTTGATAGTTGATAGTTGATAGCTGATACACTCCCACC
DX15_HSbf_5_joined	CTCTCTATCTCTCCAGTTGATAGTTGATAGTTGATAGTTGATAGCTGATACACTCCCACC
DX15_HSbf_3_joined	CTCTCTATCTCTCCAGTTGATAGTTGATAGTTGATAGTTGATAGCTGATACACTCCCACC
DX15_Promoter	CTCTCTATCTCTCCAGTTGATAGTTGATAGTTGATAGTTGATAGCTGATACCTCCCACC

DX15_Sbf_6_joined	TTTCCCAGAAAGATGATTGAGGAAGTACTGCTGTTTCGTGTAACATAACTGTTTCATG
DX15_HSbf_8_joined	TTTCCCAGAAAGATGATTGAGGAAGTACTGCTGTTTCGTGTAACATAACTGTTTCATG
DX15_HSbf_5_joined	TTTCCCAGAAAGATGATTGAGGAAGTACTGCTGTTTCGTGTAACATAACTGTTTCATG
DX15_HSbf_3_joined	TTTCCCAGAAAGATGATTGAGGAAGTACTGCTGTTTCGTGTAACATAACTGTTTCATG
DX15_Promoter	TTTCCCAGAAAGATGATTGAGGAAGTACTGCTGTTTCGTGTAACATAACTGTTTCATG

DX15_Sbf_6_joined	GCACCTAACTTGATCCTCTCTTCACCAGACCACTATAAAAAACCTATCTGTCTCCTCAT
DX15_HSbf_8_joined	GCACCTAACTTGATCCTCTCTTCACCAGACCACTATAAAAAACCTATCTGTCTCCTCAT
DX15_HSbf_5_joined	GCACCTAACTTGATCCTCTCTTCACCAGACCACTATAAAAAACCTATCTGTCTCCTCAT
DX15_HSbf_3_joined	GCACCTAACTTGATCCTCTCTTCACCAGACCACTATAAAAAACCTATCTGTCTCCTCAT
DX15_Promoter	GCACCTAACTTGATCCTCTCTTCACCAGACCACTATAAAAAACCTATCTGTCTCCTCAT

DX15_Sbf_6_joined	AATCATCTCACTACACCCAACACTTCTGCAAGCACAACTCCATTCAAGAACATCAAGAGT
DX15_HSbf_8_joined	AATCATCTCACTACACCCAACACTTCTGCAAGCACAACTCCATTCAAGAACATCAAGAGT
DX15_HSbf_5_joined	AATCATCTCACTACACCCAACACTTCTGCAAGCACAACTCCATTCAAGAACATCAAGAGT
DX15_HSbf_3_joined	AATCATCTCACTACACCCAACACTTCTGCAAGCACAACTCCATTCAAGAACATCAAGAGT
DX15_Promoter	AATCATATCACTACACCCAACACTTCTGCAAGCACAACTCCATTCAAGAACATCAAGAGT

DX15_Sbf_6_joined	ATAGGCCGCCACTGCAACAAAACAGCACTCCTAGCTACTTCAAGATGAGGCCACAATCTT
DX15_HSbf_8_joined	ATAGGCCGCCACTGCAACAAAACAGCACTCCTAGCTACTTCAAGATGAGGCCACAATCTT
DX15_HSbf_5_joined	ATAGGCCGCCACTGCAACAAAACAGCACTCCTAGCTACTTCAAGATGAGGCCACAATCTT
DX15_HSbf_3_joined	ATAGGCCGCCACTGCAACAAAACAGCACTCCTAGCTACTTCAAGATGAGGCCACAATCTT
DX15_Promoter	ATAGGCCGCCGCTGCAACAAAACAGCACTCCTAGCTACTTCAAGATGAGGCCACAATCTT

DX15_Sbf_6_joined	TCATCTT
DX15_HSbf_8_joined	TCATCTT
DX15_HSbf_5_joined	TCATCTT
DX15_HSbf_3_joined	TCATCTT
DX15_Promoter	TCATCTT

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DX15_Promoter:0.01854,
DX15_HSbf_3_joined:0.00000)
:0.00000,
DX15_HSbf_5_joined:0.00000)
:0.00000,
DX15_Sbf_6_joined:0.00000,
DX15_HSbf_8_joined:0.00000);
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B Appendix B

B.1 Complete list of the ninety-three peroxidases in *Populus trichocarpa* with tissue gene expression profile comparison

Table B 1: Complete list of 93 PRX genes in *Populus trichocarpa* and their expression profile in difference parts of the tree

	Gene Model (Ren et al & Phytozome)	Alias	Old nomeclature (Christensen et. al)	Expression (Gene Atlas)	Protein Homolog	Ortholog in <i>Arabidopsis thaliana</i>	Ortholog gene expression	Expressi on (Poplar eFP Browser)	Absolute Value (Poplar eFP browser)
Ptr PRX1	Potri.001G145800.1	POPTR_0001s02870		Roots 32.201		AT2G35380.1		Roots	
Ptr PRX2	Potri.001G011000.1	POPTR_0001s04820		immature leaf	Potri.001G011300.1 (97.7%), Potri.001G013000.1 (95.0%)			Light grown seedling	
Ptr PRX3	Potri.001G011200.1	POPTR_0001s04840		Leaf and root tip	Potri.001G011000.1 (90.1%), Potri.001G011300.1 (89.2%),			Light grown seedling	

					Potri.001G013000.1 (87.2%)				
Ptr PRX4	Potri.001G011300.1	POPT R_000 1s048 50		Root 12.119	Potri.001G011000.1 (97.7%), Potri.001G013000.1 (94.2%)			Not in db	
Ptr PRX5	Potri.001G011400.1	Gene not found	PXP22					Not in db	
Ptr PRX6	Potri.001G011500.1	POPT R_000 1s048 70		Roots 1.148				Male catkins	
Ptr PRX7	Potri.001G013000.1	POPT R_000 1s050 50	PXP3-4	Leaf, stem node	Potri.001G011000.1 (95.0%), Potri.001G011300.1 (94.2%)			Not in db	
Ptr PRX8	Potri.001G013100.1	POPT R_000 1s050 60	PXP11	not expressed in any sample				Not in db	

Ptr PRX9	Potri.001 G182400 .1	POPT R_000 1s182 70		leaf				Female Catkins	
Ptr PRX10	Potri.001 G218500 .1	POPT R_000 1s226 10		roots	Potri.001G2 18600.1 (94.8%)			Male catkins	
Ptr PRX11	Potri.001 G329200 .1	POPT R_000 1s336 80		not expressed in any sample	Potri.017G0 64100.1 (89.5%)			Not in db	
Ptr PRX12	Potri.001 G351000 .1	POPT R_000 1s346 60		Stem internode 30.355		At3g28200,At 5g40150; similar to Atperox P63/ATP26a	At3g28200 - top of Stem 365.92, Whole stem top 227.62 bottom of Stem 117.64, whole stem bottom 171.09, At5g40150 SAM pWUS (Rib Meristem) 780.28, pFIL (PZ) 700.66, pLAS (organ	Xylem	2034.23

							boundaries) 241.35		
Ptr PRX13	Potri.001 G458700 .1	POPT R_000 1s462 90		not expressed in any sample	Potri.001G4 58900.1 (99.4%)			Male catkins	
Ptr PRX14	Potri.001 G458900 .1	POPT R_000 1s463 10		not expressed in any sample	Potri.001G4 58700.1 (99.4%)	similar to Peroxidase 4 precursor (EC 1.11.1.7) (Atperox P4) (ATP46).; [ortholog of At1g14540,]		Male catkins	
Ptr PRX15	Potri.002 G018000 .1	POPT R_000 2s019 60		Roots		similar to Peroxidase 64 precursor (EC 1.11.1.7) (Atperox P64) (PRXR4) (ATP17a).; [co-ortholog		Roots	

						(1of3) of At5g42180,]			
Ptr PRX16	Potri.002 G031200 .1	POPT R_000 2s032 60		roots		AT1G44970. 1		Dark etiolated seedling	
Ptr PRX17	Potri.002 G065300 .1	POPT R_000 2s065 90		Roots 145.177	Potri.005G1 95600.1 (86.7%)	At1g71695.1		Light grown seedling	
Ptr PRX18	Potri.003 G053700 .1	POPT R_000 3s052 60		not expressed in any sample				Not in db	
Ptr PRX19	Potri.003 G156100 .1	POPT R_000 3s154 80		roots				Roots	
Ptr PRX20	Potri.003 G215000 .1	POPT R_000 3s216 00		roots				Male catkins	

Ptr PRX21	Potri.003 G214900 .1	POPT R_000 3s216 10		roots				Roots	
Ptr PRX22	Potri.003 G214800 .1	POPT R_000 3s216 20		Stem internode 21.256, stem node 15.564	Potri.003G2 14700.1 (95.4%)			Xylem	554.03
Ptr PRX23	Potri.003 G214700 .1	POPT R_000 3s216 40	PXP1	Stem internode 36.71, stem node 22.162	Potri.003G2 14800.1 (94.1%)			Xylem	1078.93
Ptr PRX24	Potri.003 G214500 .1	POPT R_000 3s216 60		Root				Not in db	
Ptr PRX25	Potri.004 G015300 .1	POPT R_000 4s015 10		Stem Internode 962.818, Stem Node 892.617, Root 715.983, Leaf 558.952	Potri.004G0 15300.2 (100%)	similar to Peroxidase 42 precursor (EC 1.11.1.7) (Atperox P42) (PRXR1) (ATP1a/ATP 1b).; [ortholog of At4g21960,]	At4g21960 - Top of stem 6430.3, Bottom of stem 7021.52, whole stem 6309.13, Stem internode 4985.81	Highest in leaf, Xylem	25852.3
Ptr PRX26	Potri.004 G023100 .1	POPT R_000 4s022 30		Root	Potri.004G0 23200.1 (97.9%)			Roots	

Ptr PRX27	Potri.004 G023200 .1	POPT R_000 4s022 40		Root (191.027)	Potri.004G0 23100.1 (97.9%)			Roots	
Ptr PRX28	Potri.004 G052100 .1	POPT R_000 4s051 40		Stem internode 2.278, Stem node 1.415				Male catkins	
Ptr PRX29	Potri.004 G134800 .1	POPT R_000 4s142 40		Root 26.161, Stem internode 21.043, Stem node 15.901, leaf 14.62	Potri.T0455 00.1 (90.2%)	similar to peroxidase; putative; similar to class 3 peroxidase ATP32 (Arabidopsis thaliana) gi 17530547 g b AAL40837 ; similar to cDNA class 3 peroxidase ATP32 GI:17530546; [ortholog of At4g33420,]	roots	Roots	
Ptr PRX30	Potri.004 G144600 .1	POPT R_000 4s152 00		Root 34.623				Male catkins	

Ptr PRX31	Potri.005 G072800 .1	POPT R_000 5s073 90		Stem internode 16.7, Stem node 14.599	Potri.007G0 96200.1 (94.2%)	similar to Peroxidase 17 precursor (EC 1.11.1.7) (Atperox P17) (ATP25a).; [co-ortholog (1of2) of At2g22420,]	trichomes	Not in db	
Ptr PRX32	Potri.005 G089400 .1	POPT R_000 5s091 70		not expressed in any sample				Male catkins	
Ptr PRX33	Potri.005 G108900 .1	POPT R_000 5s110 70		Stem internode 156.854, Stem node 92.599				Xylem	5947.86
Ptr PRX34	Potri.005 G118700 .1	POPT R_000 5s120 70		Roots 19.545	Potri.007G0 19300.1			Roots	
Ptr PRX35	Potri.005 G135300 .1	POPT R_000 5s141 90		Roots 26.983				Not in db	

Ptr PRX36	Potri.005 G195600 .1	POPT R_000 5s217 40		Roots 134.633, Stem internode 70.742, Stem node 47.371		similar to peroxidase precursor. [ORG:Euphor bia characias]; [co-ortholog (2of3) of At1g71695, AAS97959,]	Cotyledon and top epidermal peel 542.85, pFIL (PZ) 803.42	Xylem	11006.93
Ptr PRX37	Potri.005 G195700 .1	POPT R_000 5s217 50		Roots 92.811		similar to peroxidase precursor. [ORG:Euphor bia characias]; [co-ortholog (3of3) of At1g71695, AAS97959,]		Light grown seedling	
Ptr PRX38	Gene not found							Not in db	
Ptr PRX39	Potri.006 G069600 .1	POPT R_000 6s068 90		Roots 6.448	Potri.018G1 31600.1	similar to Peroxidase 25 precursor (EC 1.11.1.7) (Atperox P25).; [ortholog of At2g41480,]		Female Catkins	
Ptr PRX40	Potri.006 G107000 .1	POPT R_000		Roots 7.609				Male catkins	

		6s107 60							
Ptr PRX41	Potri.006 G129900 .1	POPT R_000 6s131 90		Roots 149.211		similar to Peroxidase 21 precursor (EC 1.11.1.7) (Atperox P21) (PRXR5) (ATP2a/ATP 2b).; [ortholog of At2g37130,		Not in db	
Ptr PRX42	Potri.006 G267400 .1	POPT R_000 6s283 20		not expressed in any sample				Dark etiolated seedling	
Ptr PRX43	Potri.007 G132800 .1	POPT R_000 7s015 80		Roots				Light grown seedling	
Ptr PRX44	Potri.007 G122300 .1	POPT R_000 7s025 60		Roots 6.734				Not in db	
Ptr PRX45	Potri.007 G122200 .1	POPT R_000 7s025 70		Root 2.665				Dark etiolated seedling	

Ptr PRX46	Potri.007 G122100 .1	POPT R_000 7s025 80		Roots 202.075	Potri.017G0 37900.1 (90.5%)	similar to peroxidase precursor. [ORG:Glycin e max]; [ortholog of At1g05260,AA AD11482,AA D11481,]		Roots	
Ptr PRX47	Potri.007 G122000 .1	POPT R_000 7s025 90		not expressed in any sample				Not in db	
Ptr PRX48	Potri.007 G096200 .1	POPT R_000 7s051 00		Stem node 11.925	Potri.005G0 72800.1 (94. 2%)	similar to Peroxidase 17 precursor (EC 1.11.1.7) (Atperox P17) (ATP25a).; [co-ortholog (2of2) of At2g22420,	Trichomes	Not in db	
Ptr PRX49	Potri.007 G074700 .1	POPT R_000 7s073 30		Root 1.446				Male catkins	
Ptr PRX50	Potri.007 G067200 .1	POPT R_000 7s082 40		Leaf 0.187	Potri.007G0 67200.2 (96.0%)			Male catkins	

Ptr PRX51	Potri.007 G053400 .1	POPT R_000 7s100 40		Root 54.263		similar to Peroxidase 50 precursor (EC 1.11.1.7) (Atperox P50) (PRXR2) (ATP9a).; [ortholog of At4g37530,At 4g37520		Male & female catkins	
Ptr PRX52	Potri.007 G019300 .1	POPT R_000 7s134 20		Root 50.025	Potri.005G1 18700.1 (90.4%)	similar to Peroxidase 49 precursor (EC 1.11.1.7) (Atperox P49) (ATP31).; [ortholog of At4g36430,]		Not in db	
Ptr PRX53	Potri.T1 60100.1	POPT R_000 8s023 30		Root 5.485	Potri.008G0 22700.1(99. 4%), Potri.T1600 00.1 (99.4%), Potri.008G0 22600.1 (97.7%)				
Ptr PRX54	Potri.008 G022700 .1	POPT R_000 8s023 40		Root 3.543				Roots	

Ptr PRX55	Potri.008 G103200 .1	POPT R_000 8s102 70		not expressed in any sample		similar to peroxidase 40 (PER40) (P40); similar to SP O23474 Pe roxidase 40 precursor EC (1 11.1.7.Atpero x) (P40); [ortholog of At4g16270,]		Young leaf	
Ptr PRX56	Potri.008 G110600 .1	POPT R_000 8s110 00		Root 2.969		similar to Peroxidase 11 precursor (EC 1.11.1.7) (Atperox P11) (ATP23a/AT P23b).; [co- ortholog (2of2) of At1g68850,		Not in db	
Ptr PRX57	Potri.009 G106400 .1	POPT R_000 9s109 60		Root 21.363				Not in db	
Ptr PRX58	Potri.010 G036100 .1	POPT R_001 0s045 50		Root 0.131		similar to Peroxidase 6 precursor (EC 1.11.1.7) (Atperox P6).; [ortholog of At1g24110,]		Male catkins	

Ptr PRX59	Potri.010 G134500 .1	POPT R_001 0s144 60		Root 13.805		similar to Peroxidase 11 precursor (EC 1.11.1.7) (Atperox P11) (ATP23a/AT P23b).; [co- ortholog (1of2) of Atlg68850,		Not in db	
Ptr PRX60	Potri.010 G147800 .1	POPT R_001 0s157 60		not expressed in any sample				Not in db	
Ptr PRX61	Potri.010 G175100 .1	POPT R_001 0s182 50		Root 2.071				Dark etiolated seedling	
Ptr PRX62	Gene not found								
Ptr PRX63	Potri.010 G236900 .1	POPT R_001 0s243 40		Root 1.18	Potri.T1631 00.1 (99.4%), Potri.T1630 00.1 (97.8%), Potri.T1632 00.1 (96.2%)			Male catkins	
Ptr PRX64	Potri.011 G027300 .1	POPT R_001 1s002 80		Roots 391.632	Potri.T0993 00.1 (98.2%), Potri.019G0			Roots	

					63200.1 (97.9%)				
Ptr PRX65	Potri.011 G062300 .1	POPT R_001 1s060 80		Stem node 6.642, Stem internode 2.652				Not in db	
Ptr PRX66	Potri.012 G006800 .1	POPT R_001 2s007 00		Roots 28.444	Potri.012G0 06800.2 (95.0%)			Male catkins	
Ptr PRX67	Potri.012 G042800 .1	POPT R_001 2s039 80		Roots 253.787				Roots	
Ptr PRX68	Potri.012 G076500 .1	POPT R_001 2s078 00		not expressed in any sample		similar to Peroxidase 41 precursor (EC 1.11.1.7) (Atperox P41).; [ortholog of At4g17690,		Male catkins	
Ptr PRX69	Gene not found							Not in db	
Ptr PRX70	Potri.013 G066800 .1	POPT R_001 3s063 00		Roots 18.235				Male catkins	

Ptr PRX71	Potri.013 G083600 .1	POPT R_001 3s081 30		Stem node 4.582				Male catkins	
Ptr PRX72	Potri.013 G154400 .1	POPT R_001 3s150 50		not expressed in any sample				Light grown seedling	
Ptr PRX73	Potri.013 G156400 .1	POPT R_001 3s152 40		root 0.318				Male catkins	
Ptr PRX74	Potri.013 G156500 .1	POPT R_001 3s152 50		Stem node 19.253, Stem node 10.412	Potri.013G1 56500.2 (92.3%)			Dark etiolated seedling, 3 hr light seedling, male catkins, roots	
Ptr PRX75	Potri.013 G156800 .1	POPT R_001 3s152 70		Roots 9.226				Male catkins	
Ptr PRX76	Gene not found							Not in db	
Ptr PRX77	Potri.014 G143200 .1	POPT R_001 4s140 00		Roots 1.184	Potri.014G1 43200.2 (99. 4%)			Light grown seedling	

Ptr PRX78	Potri.015 G003500 .1	POPT R_001 5s005 80		Stem node 11.96	Potri.015G0 03500.2 (99.7%)			Light grown seedling	
Ptr PRX79	Potri.015 G003600 .1	POPT R_001 5s005 90		Roots 37.547				Light grown seedling	
Ptr PRX80	Potri.015 G110200 .1	POPT R_001 5s121 70		not expressed in any sample				Male catkins	
Ptr PRX81	Gene not found							Not in db	
Ptr PRX82	Potri.016 G125000 .1	POPT R_001 6s132 80		Roots 12.019				Light grown seedling, Female and male catkins	
Ptr PRX83	Potri.016 G132700 .1	POPT R_001 6s140 30		Stem node 11.533, Stem internode 10.728				Roots	
Ptr PRX84	Potri.016 G132800 .1	POPT R_001 6s140 40		Roots 6.416				Roots	

Ptr PRX85	Potri.016 G132900 .1	POPT R_001 6s140 50		Roots 5.315				Dark etiolated, 3 hr light & light grown seedling	
Ptr PRX86	Potri.017 G037900 .1	POPT R_001 7s065 50		Stem internode 53.057, Stem node 52.311	Potri.007G1 22100.1 (90 .2%)			Roots	
Ptr PRX87	Potri.017 G038100 .1	POPT R_001 7s065 70		Root 5.511	Potri.017G0 38100.2 (96.3%)			Female & male catkins	
Ptr PRX88	Potri.017 G064100 .1	POPT R_001 7s096 40		Root 47.977				Roots	
Ptr PRX89	Potri.018 G015500 .1	POPT R_001 8s029 10		Root tip 4.773	Potri.006G2 67400.1 (91.3%)			Male catkins	
Ptr PRX90	Potri.018 G089900 .1	POPT R_001 8s097 10		Root 161.904		similar to Peroxidase 16 precursor (EC 1.11.1.7) (Atperox P16) (ATP22a).; [ortholog of At2g18980,At 4g30170,		Female catkins	

Ptr PRX91	Potri.018 G131600 .1	POPT R_001 8s131 50		Root 1.157	Potri.006G0 69600.1 (95.5%)			Roots	
Ptr PRX92	Potri.018 G136900 .1	POPT R_001 9s003 40		Root 2.994	Potri.T0455 00.1 (99.2%), Potri.004G1 34800.1 (94.5%)			3 hr light seedling	
Ptr PRX93	Potri.019 G063200 .1	POPT R_001 9s091 50		Root 19.433	Potri.T0993 00.1 (99.7%), Potri.011G0 27300.1 (97.9%)	similar to Peroxidase 27 precursor (EC 1.11.1.7) (Atperox P27) (PRXR7) (ATP12a).; [ortholog of At3g01190,]		Roots	

B.2 Sequencing data comparison of plasmid clones with full length CDS region of PRX25_F

CLUSTAL 2.1 Multiple Sequence Alignments

```
Sequence type explicitly set to DNA
Sequence format is Pearson
Sequence 1: DXMF          766 bp
Sequence 2: DXMFP25      766 bp
Start of Pairwise alignments
Aligning...
```

```
Sequences (1:2) Aligned. Score: 98.3029
Guide tree file created: [clustalw.dnd]
```

```
There are 1 groups
Start of Multiple Alignment
```

```
Aligning...
Group 1: Sequences: 2      Score:14373
Alignment Score 5323
```

```
CLUSTAL-Alignment file created [clustalw.aln]
```

clustalw.aln

CLUSTAL 2.1 multiple sequence alignment

```
DXMF      ACTACACCCAACACTTCTGCAAGCACAACTCCATTCAAGAACATCAAGAGTATAGGCCGC
DXMFP25   ACTACACCCAACACTTCTGCAAGCACAACTCCATTCAAGAACATCAAGAGTATAGGCCGC
*****

DXMF      CGCTGCAACAAAACAGCACTCCTAGCTACTTCAAGATGAGGCCACAATCTTTCATCTTCC
DXMFP25   CACTGCAACAAAACAGCACTCCTAGCTACTTCAAGATGAGGCCACAATCTTTCATCTTCC
*  *****

DXMF      TGCAGGTCGACTCTAGAATGGATGCCAAAGCTCTTCTTCTTTGCCTTGTTGTCCTTCT
DXMFP25   TGCAGGTCGACTCTAGAATGGATGCCAAAGCTCTTCTTCTTTGCCTTGTTGTCCTTCT
*****

DXMF      CAGCTGTGTCGGTCAGGCCGGCATTAGCAGAAAATGAAGAAGACCCTGGTCTTGTTATGA
DXMFP25   CAGCTGTGTCGGTCAGGCTGGCCTTTGCAGAAAATGAAGAAGACCCTGGTCTTGTTATGA
*****

DXMF      ACTTTTACAAGGATACATGCCCTCAAGCTGAGGACATTGTCAAAGAACAAGTTAGACTCC
DXMFP25   ACTTTTACAAGGATACATGCCCTCAAGCTGAGGACATTGTCAAAGAACAAGTTAGACTCC
*****

DXMF      TTTACAAGAGACACAAAACACTGCATTTTCTTGGCTAAGAAACATCTTCCATGACTGTG
DXMFP25   TTTACAAGAGACACAAAACACTGCATTTTCTTGGCTAAGAAACATCTTCCATGACTGTG
*****
```

DXMF DXMFP25	CTGTTCAGTCATGTGATGCTTCACTGCTGCTGGACTCAACAAGGAGGACCTTGTCCGAGA CTGTTCAGTCATGTGATGCTTCACTTCTGCTGGACTCAACAAGGAGGACCTTGTCTGAGA *****
DXMF DXMFP25	AGGAGACAGACAGGAGCTTTGGCCTCAGGAACCTTAGATACTTTGACGATATCAAAGAAG AGGAGACAGACAGGAGCTTTGGCCTCAGGAACCTTAGATACCTTGACGAAATCAAAGAAG *****
DXMF DXMFP25	CTGTTGAAAGAGAGTGTCTGGAGTCGTTTCCTGTGCTGATATTCTTGCTCCTGTCTGCTA CTGTTGAAAGGGAGTGTCTGGAGTTGTTTCCTGTGCTGATATTCTTGCTCCTCTCTGCTA *****
DXMF DXMFP25	GAGATGGCATTGTTTCGCTAGGAGGACCTCATATCCCTCTCAAACTGGAAGAAGGGATG GAGATGGCATTGTTTCGCTAGGAGGACCTCATATCCCTCTCAAACTGGAAGAAGGGATG *****
DXMF DXMFP25	GCAGGAAGAGCAGAGCAGATGTGATCGAGGACTATCTCCAGACCACAATGAAAGCATAT GCAGGAAGAGCAGAGCAGATGTGATCGAGGACTATCTCCAGACCACAATGAAAGCATAT *****
DXMF DXMFP25	CTGTTGTTCTTGACAGGTTTGCTTCCATGGGTATTGACACCCCTGGACTGGTTGCCTTGC CTGTTGTTCTTGACAGGTTTGCTTCCATGGGTATTGACACCCCTGGAGTGGTTGCCTTGC *****
DXMF DXMFP25	TAGGAGCTCACAGTGTTGGGAGAACTCACTGTGTGAAGCTGGTGCA TAGGAGCTCACAGTGTTGGGAGAACTCACTGTGTGAAGCTGGTGCA *****

clustalw.dnd

(DXMF:0.0084856,DXMFP25:0.0084856);

B.3 Sequencing data comparison of plasmid clones with full length CDS region of PRX25_MF

CLUSTAL 2.1 Multiple Sequence Alignments

```
Sequence type explicitly set to DNA
Sequence format is Pearson
Sequence 1: P25MF          834 bp
Sequence 2: DXP25MF       834 bp
Start of Pairwise alignments
Aligning...
```

```
Sequences (1:2) Aligned. Score: 97.8417
Guide tree file created: [clustalw.dnd]
```

```
There are 1 groups
Start of Multiple Alignment
```

```
Aligning...
Group 1: Sequences: 2      Score:15639
Alignment Score 5679
```

```
CLUSTAL-Alignment file created [clustalw.aln]
```

clustalw.aln

CLUSTAL 2.1 multiple sequence alignment

```
P25MF          AAGCATATCTGTTGTTCTTGACAGGTTTGCTTCCATGGGTATTGACACCCCTGGACTGGT
DXP25MF        AAGCATATCTGTTGTTCTTGACAGGTTTGCTTCCATGGGTATTGACACCCCTGGAGTGGT
*****

P25MF          TGCCTTGCTAGGAGCTCACAGTGTTGGGAGAACTCACTGTGTGAAGCTGGTGCACCGTTT
DXP25MF        TGCCTTGCTAGGAGCTCACAGTGTTGGGAGAACTCACTGTGTGAAGCTGGTGCACCGTTT
*****

P25MF          GTACCCGGAAGTTGACCCAGCACTAAACCCTGACCATGTTGAGCACATGCTTTACAAGTG
DXP25MF        GTACCCGGAAGTTGACCCAGCACTAAACCCTGACCATGTTGAGCACATGCTTTACAAGTG
*****

P25MF          CCCTGATTCAATCCCAGACCCTAAAGCTGTCCAATATGTGAGGAATGACAGAGGCACACC
DXP25MF        CCCTGATTCAATCCCAGACCCTAAAGCTGTCCAATATGTGAGAAATGACAGAGGCACACC
*****

P25MF          CATGGTTCTAGACAACAACACTACTACAGAAACATATTGGACAACAAGGGCTTGTGTATAGT
DXP25MF        CATGGTTCTAGACAACAACACTACTACAGAAACATATTGGACAACAAGGGCTTGTGTATAGT
*****

P25MF          GGATCACCAACTAGCCACTGACAAGAGAACTAAGCCTTACGTGAAGAAAATGGCCAAGAG
DXP25MF        GGATCACCAACTAGCCAATGACAAGAGAACTAAGCCTTATGTGAAGAAAATGGCCAAGAG
*****
```

P25MF DXP25MF	TCAGGATTACTTCTTTAAGGAGTTTTCTAGGGCTATCACCATTCTTTCTGAGAACAACCC TCAGGATTACTTCTTTAAGGAGTTTTCTAGGGCTATCACCATTCTTTCTGAGAACAACCC *****
P25MF DXP25MF	ACTCACCGGTACCAAGGGTGAGATCAGAAAGCAGTGCCTGTTGCAAACAAGCACCCTA ACTCACCGGTACCAAGGGGGAGATCAGAAAGCAGTGCCTGTTGCAAACAAGCACCCTA *****
P25MF DXP25MF	GTTAATTAATCGAGAGCTCGAATTTCCCCGATCGTTCAAACATTTGGCAATAAAGTTTCT GTTAATTAATCGAGAGCTCGAATTTCCCCGATCGTTCAAACATTTGGCAATAAAGTTTCT *****
P25MF DXP25MF	TAAGATTGAATCCTGTTGCCGGTCTTGCGATGATTATCATATAATTTCTGTTGAATTACG TAAGATTGAATCCTGTTGCCGGTCTTGCGATGATTATCATATAATTTCTGTTGAATTACG *****
P25MF DXP25MF	TTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTTATGAGATGGGTTTTTATGA TTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTTATGAGATGGGTTTTTATGA *****
P25MF DXP25MF	TTAGAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATAGCGCGCAAAT TTAGAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATAGCGCGCAAAT *****
P25MF DXP25MF	AGGATAAATTATCGCGCGCGGTGTCATCTATGTTACTAGATCGGGAA-TTCACTGGCC-G AGGATAAATTATCGCGCGCGGTGTCATCTATGTTACTAGATCGGGAAATTCCTGGCCCG *****
P25MF DXP25MF	TCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACCTTAATCGCCTT TCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACCTTAATCGCC-- *****

clustalw.dnd

(P25MF:0.010791,DXP25MF:0.010791);

B.4 Sequencing data comparison of plasmid clones with full length CDS region of PRX36_F

CLUSTAL 2.1 Multiple Sequence Alignments

```
Sequence type explicitly set to DNA
Sequence format is Pearson
Sequence 1: DXMF          611 bp
Sequence 2: DXMFP36      611 bp
Start of Pairwise alignments
Aligning...
```

```
Sequences (1:2) Aligned. Score: 97.3813
Guide tree file created: [clustalw.dnd]
```

```
There are 1 groups
Start of Multiple Alignment
```

```
Aligning...
Group 1: Sequences: 2      Score:11400
Alignment Score 4350
```

```
CLUSTAL-Alignment file created [clustalw.aln]
```

clustalw.aln

CLUSTAL 2.1 multiple sequence alignment

```
DXMF          ACTACACCCAACACTTCTGCAAGCACAACTCCATTCAAGAACATCAAGAGTATAGGCCGC
DXMFP36       ACTACACCCAACACTTCTGCAAGCACAACTCCATTCAAGAACATCAAGAGTATAGGCCGC
*****

DXMF          CGCTGCAACAAAACAGCACTCCTAGCTACTTCAAGATGAGGCCACAATCTTTCATCTTCC
DXMFP36       CACTGCAACAAAACAGCACTCCTAGCTACTTCAAGATGAGGCCACAATCTTTCATCTTCC
*  *****

DXMF          TGCAGGTCGACTCTAGAGGATCCATGGCCAGAGCTGCTATGTCCTTTACCTCTCTTCTTC
DXMFP36       TGCAGGTCGACTCTAGAGGATCCATGGCCAGAGCTGCTAAGTCCCTTACCTCTCTTCTTC
*****

DXMF          TGATTTCTTCTCTCTTTCTAGCTTCTTGTTTTGTGCCACTGAAGCACAAAGGCACCATCC
DXMFP36       TGATTTCTTCTCTCTTTCTAGCTTCTTGTTTTGTGCCACTGAAGCACAAAGGCACCATCC
*****

DXMF          CTATAGAGAATGGCCTGTCATGGACATTCTACAAGTCTAAATGCCCTAAAGTTGAATCCA
DXMFP36       CTATAGTGAATGGCCTTTTCATGGACATTCTACAAGTCTAAATGCCCTAAAGTTGAATCCA
*****

DXMF          TTATAAGGAAACAGCTGGGAAAGGTGTTCAAGAAGGATATCGAACAAGCTGCTGGCTTGC
DXMFP36       TTATAAGGAAACAGCTGGGAAAGGTGTTCAAGAAGGACATCGAGCAAGCTGCTGGCTTGC
*****
```

DXMF	TTCGTCTCCACTTCCATGATTGCTTTGTTTCAGGGATGTGATGGTTCGGTATTGCTTGATG
DXMFP36	TTCGTCTCCACTTCCATGATTGCTTTGTTTCAGGGATGTGATGGCTCTGTATTGCTTGATG
	***** ** *****
DXMF	GATCGGCGAGTGGTCCAAGCGAGCAGGACGCCCCCTCCCAACCTGACCTTGAGAGCAAAGG
DXMFP36	GATCGGCGAGTGGTCCGAGCGAGCAGGACGCCCCCTCCCAACCTGACCTTGAGAGCAAAGG
	***** *****
DXMF	CGTTTGAGATCATCGAAGACCTACGCCGGCGCGTGGAAAAGGCTTGCGGCCTTGTCGTCT
DXMFP36	CATTTGAGATCATCGAAGACCTACGCCGGCGCGTGGAAAAGGCTTGCGGCCTTGTCGTCT
	* *****
DXMF	CCTGTTCTGATATTCTTGCGCTTGCAAGCCGTGACTCTGTTTACCTGTCTGGTGGCCCAG
DXMFP36	CCTGTTCTGATATTCTTGCGCTTGCAAGCCGTGACTCTGTTTTCCTGTCTGGTGGCCCAG
	***** *****
DXMF	ATTATAATGTT
DXMFP36	ATTATGATGTT

[clustalw.dnd](#)

(DXMF:0.013093,DXMFP36:0.013093);

B.5 Sequencing data comparison of plasmid clones with full length CDS region of PRX36_MF

CLUSTAL 2.1 Multiple Sequence Alignments

```
Sequence type explicitly set to DNA
Sequence format is Pearson
Sequence 1: P36MF          575 bp
Sequence 2: DXP36MF       575 bp
Start of Pairwise alignments
Aligning...
```

```
Sequences (1:2) Aligned. Score: 97.7391
Guide tree file created: [clustalw.dnd]
```

```
There are 1 groups
Start of Multiple Alignment
```

```
Aligning...
Group 1: Sequences: 2      Score:10783
Alignment Score 3765
```

```
CLUSTAL-Alignment file created [clustalw.aln]
```

[clustalw.aln](#)

CLUSTAL 2.1 multiple sequence alignment

```
P36MF      AATCTCAAGGAAGTTTGCCCCACAAGAGACTTCAATAACACAACCGTGTGGATATTCGA
DXP36MF    AATCTCAAG-AAGTTTGCCCCACAAGAGACTTCAATAACACAACCGTGTGGATATTCGA
*****

P36MF      TCTCCTAATAAATTTGACAACAAGTACTATGTTGATCTCATGAATCGCCAAGGCCTGTTT
DXP36MF    TCTCCTAATAAATTTGACAACAAGTACTACGTCGATCTCATGAATCGCCAAGGCCTGTTT
*****

P36MF      ACTTCAGACCAGGACTTGTACACGAACAAGAAGACAAGGGGCATTGTCACTAGCTTTGCT
DXP36MF    ACTTCAGACCAGGACTTGTACACGAACAAGAAGACAAGGGGCATTGTCACTAGCTTTGCT
*****

P36MF      GTTAATCAAAGTTTGTCTTTGATAAGTTTGTGGTTGCAATGATCAAAATGTCACAGCTC
DXP36MF    GTCAATCAAAGTTTGTCTTTGATAAGTTTGTGGTTGCAATGATCAAAATGTCACAGCTC
**

P36MF      AAGGTCTTGACAGGAAATCAAGGTGAAATCCGCGCCAGTTGCGAGGAGAGAAATTCAGGC
DXP36MF    AAGGTCTTGACAGGAAATCAAGGTGAAATCCGCGCCAGTTGCGAGGAGAGAAATTCAGGC
*****

P36MF      TACAGTTACTTGGAGTCTGTGGTGGAAGAGGGTCTCGACGCACTGTCCGGATTAATATGA
DXP36MF    TACAGTTACTTGGAGTCTGTGGTGGAAGAGGGTCTCGACGCACTGTCCGGATTAATATGA
*****
```


P36MF	GAGCTCGAATTTCCCGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCC
DXP36MF	GAGCTCGAATTTCCCGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCC

P36MF	TGTTGCCGGTCTTGCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAAT
DXP36MF	TGTTGCCGGTCTTGCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAAT

P36MF	AATTAACATGTAATGCATGACGTTATTTATGAGATGGGTTTTATGATTAGAGTCCCGCA
DXP36MF	AATTAACATGTAATGCATGACGTTATTTATGAGATGGGTTTTATGATTAGAGTCCCGCA

P36MF	ATTATACATTTAATACGCGATAGAAAACAAAATAT -
DXP36MF	ATTATACATTTAATACGCGATAGAAAACAAAATATA

clustalw.dnd

(P36MF:0.011304,DXP36MF:0.011304);

B.6 Sequence similarity of the CDS region for PRX25 and PRX36

CLUSTAL 2.1 Multiple Sequence Alignments

Sequence type explicitly set to DNA
Sequence format is Pearson
Sequence 1: Ptr_PRX25_CDS 996 bp
Sequence 2: Ptr_PRX36_CDS 1065 bp
Start of Pairwise alignments
Aligning...

Sequences (1:2) Aligned. Score: 36.1446
Guide tree file created: [clustalw.dnd]

There are 1 groups
Start of Multiple Alignment

Aligning...
Group 1: Sequences: 2 Score:10680
Alignment Score 2769

CLUSTAL-Alignment file created [clustalw.aln]

clustalw.aln

CLUSTAL 2.1 multiple sequence alignment

```
Ptr_PRX25_CDS  -----ATGGATGCCAAAGCTCTCTTCTTTGCCTTGTTGCTCTCTCAGC
Ptr_PRX36_CDS  ATGGCCAGAGCTGCTATGTCTTTACCTCTCTTCTGATTTCTCTCTCTTCT-AGC
                  * * * * *
Ptr_PRX25_CDS  TGTGTCGGTCAGGCCGGCATTAGCAGAAAATGAAGA-----AGACCTGGTCTTGTTAT
Ptr_PRX36_CDS  TTCTTGTTTTGTGCCACTGAAGCACAAGGCACCATCCCTATAGAGAATGG-CCTGTCAT
                  * * * * *
Ptr_PRX25_CDS  GAAC-TTTTACAAGGATACATGCCCTCAAGCTGAGGACATTGTCAAAGAACAAGTTAGAC
Ptr_PRX36_CDS  GGACATTCTACAAGTCTAAATGCCCTAAAGTTGAATCCATTATAAGGAAACAGCTGGGAA
                  * * * * *
Ptr_PRX25_CDS  TCCTTTACAAGAGACACAAAAACACTGCATTTTCTTGGCTAAGAAACATCTTCCATGACT
Ptr_PRX36_CDS  AGGTGTTCAAGAAGGATATCGAACAAGCTGCTGGCTTGCTTCGCTCCACTTCCATGATT
                  * * * * *
Ptr_PRX25_CDS  GTGCTGTTCAAGTCATGTGATGCTTCACTGCTGCTGACTCAACAAGGAGGACCTTGTCGG
Ptr_PRX36_CDS  GCTTTGTTCAAGGATGTGATGGTTCGGTATTGCTTGATGGATCGGCGAGTGGTCCAAGCG
                  * * * * *
Ptr_PRX25_CDS  AGAAGGAGACA-----GACAGGAGCTTTGGCCTCAGGAACCTTTAGATACTTTGACGATA
Ptr_PRX36_CDS  AGCAGGACGCCCCCTCCCAACCTGACCTTGAGAGCAAAGGCGTTTGAGATCATCGAAGACC
                  * * * * *
Ptr_PRX25_CDS  TCAAAGAAGCTGTTGAAAGAGAGTGTCTGAGTCGTTTCTGTGCTGATATTCTTGTC
Ptr_PRX36_CDS  TACGCCGGCGCGTGGAAAAGGCTTGCAGCCTTGTGCTCTCTGTTCTGATATTCTTGCGC
                  * * * * *
```

Ptr_PRX25_CDS	TGTCTGCTAGAGATGGCATTGTTTCGCTAGGAGGACCTCATATCCCTCTCAAAACTGGAA
Ptr_PRX36_CDS	TTGCAGCCCGTGACTCTGTTTACCTGTCTGGTGGCCAGATTATAATGTTCCCTTGGGAA
	* * * * * * * * * * * * * * * *
Ptr_PRX25_CDS	GAAGGGATGGCAGGAAGAGCAGAGC--AGATGTGATCGAGGACTATCTCCCAGACCACA
Ptr_PRX36_CDS	GGCGAGACGGTCTAAAATTGCAACTCAAAATGAAACCTTAGACAACCTCCCGCCACCCT
	* * * * * * * * * * * * * * * *
Ptr_PRX25_CDS	ATGAAAGCATATCTGTTGTTCTTGACAGTTTGCTTCCATGGGTATTGACACCCCTGGAC
Ptr_PRX36_CDS	TTGCTAACGCCGACACAATTCTCTCATCTCTCGCCACCAAGGGCTTCGATGCCACCGACG
	* * * * * * * * * * * * * * * *
Ptr_PRX25_CDS	TGGTTGCCTTGCTAGGAGCTCACAGTGTGGGAGAAGTCACTGTGTGAAGCTGGTGCACC
Ptr_PRX36_CDS	TGGTAGCCCTGTCCGGTGGCCACACCATTGGTATAAGTCACTGC---AGCTCTTTCACT
	* * * * * * * * * * * * * * * *
Ptr_PRX25_CDS	GTTTGTACCCGGAAGTTGACCCAGCACTAAACCCTGACCATGTTGAGCACATGCTTTACA
Ptr_PRX36_CDS	GACCGCCTCT-----ATCCAACCCAAGATCCT---ACAATGGACAAAACCTTTGCC
	* * * * * * * * * * * * * * * *
Ptr_PRX25_CDS	AGTGCCCTGATTCAATCCCAGACCCTAAAGCTGTCCAATATGTGAGGAATGACAGAGGCA
Ptr_PRX36_CDS	AACAATCTCAAGGAAGTTTGCCCCACAAGAGACTTCAATAACACAACCGTGTGGATATT
	* * * * * * * * * * * * * * * *
Ptr_PRX25_CDS	CA---CCCATGGTTCTAGACAACAACACTACTACAGAAACATATTGGACAACAAGGGCTTG
Ptr_PRX36_CDS	CGATCTCCTAATAAATTTGACAACAAGTACTATGTTGATCTCATGAATCGCCAAGGCCTG
	* * * * * * * * * * * * * * * *
Ptr_PRX25_CDS	TTGATAGTGGATCACCAACTAGCCACTGACAAGAGAACTAAGCCTTACGTGAAGAAAATG
Ptr_PRX36_CDS	TTTACTTCAGACCAGGACTTGACACGAACAAGAAGACAAGGGGCATTGTCACTAGCTTT
	* * * * * * * * * * * * * * * *
Ptr_PRX25_CDS	GCCAAGAGTCAGGATTACTTCTTTAAGGAGTTTCTAGGGCTATCACCATTCTTTCTGAG
Ptr_PRX36_CDS	GCTGTTAATCAAAGTTTGTCTTTGATAAGTTTGTTGCAATGATCAAAATGTCACAG
	* * * * * * * * * * * * * * * *
Ptr_PRX25_CDS	AACAACCCACTCACCGGTACCAAGGGTGAGATCAGAAAGCAGTGCCTGTTGCAAA--CA
Ptr_PRX36_CDS	CTCAAGGTCTTGACAGGAAATCAAGGTGAAATCCGCGCCAGTTGCGAGGAGAGAAATTCA
	* * * * * * * * * * * * * * * *
Ptr_PRX25_CDS	AGCACCACTAG-----
Ptr_PRX36_CDS	GGCTACAGTTACTTGAGTCTGTGGTGGAAGAGGGTCTCGACGCACTGTCCGGATTAATA
	* * * * *
Ptr_PRX25_CDS	---
Ptr_PRX36_CDS	TGA

clustalw.dnd

(Ptr_PRX25_CDS:0.31928,Ptr_PRX36_CDS:0.31928);

B.7 Sequence similarity of the CDS region for all six SDX PRXs

CLUSTAL 2.1 Multiple Sequence Alignments

```
Sequence type explicitly set to DNA
Sequence format is Pearson
Sequence 1: Ptr_PRX12_Potri.001G351000    963 bp
Sequence 2: Ptr_PRX22_Potri.003G214800   1050 bp
Sequence 3: Ptr_PRX23_Potri.003G214700   1065 bp
Sequence 4: Ptr_PRX25_Potri.004G015300    996 bp
Sequence 5: Ptr_PRX33_Potri.005G108900   1137 bp
Sequence 6: Ptr_PRX36_Potri.005G195600   1065 bp
Start of Pairwise alignments
Aligning...
```

```
Sequences (1:2) Aligned. Score: 31.7757
Sequences (1:3) Aligned. Score: 31.568
Sequences (1:4) Aligned. Score: 32.2949
Sequences (1:5) Aligned. Score: 33.541
Sequences (1:6) Aligned. Score: 35.0987
Sequences (2:3) Aligned. Score: 93.8095
Sequences (2:4) Aligned. Score: 32.0281
Sequences (2:5) Aligned. Score: 33.1429
Sequences (2:6) Aligned. Score: 38.4762
Sequences (3:4) Aligned. Score: 31.1245
Sequences (3:5) Aligned. Score: 33.0516
Sequences (3:6) Aligned. Score: 35.3991
Sequences (4:5) Aligned. Score: 31.3253
Sequences (4:6) Aligned. Score: 36.1446
Sequences (5:6) Aligned. Score: 32.3005
Guide tree file created: [clustalw.dnd]
```

```
There are 5 groups
Start of Multiple Alignment
```

```
Aligning...
Group 1: Sequences: 2      Score:19013
Group 2: Sequences: 3      Score:11575
Group 3: Sequences: 4      Score:10384
Group 4: Sequences: 2      Score:9783
Group 5: Sequences: 6      Score:9787
Alignment Score 39873
```

```
CLUSTAL-Alignment file created [clustalw.aln]
```

clustalw.aln

CLUSTAL 2.1 multiple sequence alignment

```
Ptr_PRX22_Potri.003G214800  -----ATGATGGTAGATAAAGCAATGCATCC
Ptr_PRX23_Potri.003G214700  ATGGAAAGAGTATTTTCCTTCAAAATGATGATAGATAAAGCCTTGCATCC
Ptr_PRX36_Potri.005G195600  ATGGCCAGAGCTGCTATGTCCTTACCTCTCTTCTTGATTTCTTCTCT
Ptr_PRX25_Potri.004G015300  -----ATGGATGCCAAAGCTCTCTTCTTCTTTGC
Ptr_PRX12_Potri.001G351000  -----ATGGCAATGCC
Ptr_PRX33_Potri.005G108900  -----ATGGC--TTTT
```

Ptr_PRX22_Potri.003G214800	GTTACTAGCATCTCTTTTTCTTGAATCTGGTTTGAGGATCACTTCCCT
Ptr_PRX23_Potri.003G214700	TTTAGTTGCATCTCTTTTTTTTGAATCTGGTTTGAGGATCACTTCCCT
Ptr_PRX36_Potri.005G195600	CTTTCTAGCTTCTTGGTTTTGTGCCA-CTGAAGCACAAGGCACCATCCCT
Ptr_PRX25_Potri.004G015300	CTTGTGTCCCTCTCAGCTGTGTCGGTCAGGCCGGCATTAGCAGAAAAATG
Ptr_PRX12_Potri.001G351000	AAAGGAGCGATTTTCTTATTCTCATTTCTCTCTCTCATATACC
Ptr_PRX33_Potri.005G108900	GTTGCTGC--TTTATGCTTGAGCTCGGTTC-TCGTATTCTCG-ATATCTT
	* *
Ptr_PRX22_Potri.003G214800	ATGCCTATGCTCAGCTGACTCCCACATTCTACGATGGAACATGCCCGAAT
Ptr_PRX23_Potri.003G214700	ATGCCTATGCTCAGCTGACTCCCACATTCTACGATGGAACATGCCCGAAT
Ptr_PRX36_Potri.005G195600	ATAG--AGAATGGCCTGTCTATGGACATTCTACAAGTCTAAATGCCCTAAA
Ptr_PRX25_Potri.004G015300	AAGAAGACCCTGGTCTTGTATGAACTTTTACAAGGATACATGCCCTCAA
Ptr_PRX12_Potri.001G351000	CCTCCCAGTCCAGACTCTCTACAACACTACTCCTCAAATCCTGTCCAAAC
Ptr_PRX33_Potri.005G108900	CAGGAGCAGATGCACCTGAGCTTGAATTACTATGAGAAAACATGCCCTGAT
	** * * * *
Ptr_PRX22_Potri.003G214800	GTGAGCACCATCATTCGAGGAGTCCTTGTTGAGGCTTTGCAGACAGATCC
Ptr_PRX23_Potri.003G214700	GTGAGCACCATCATTCGAGGAGTCCTTGTTGAGGCTTTGCAGACAGATCC
Ptr_PRX36_Potri.005G195600	GTTGAATCCATTATAAGGAAACAGCTGGGAAAGGTGTTCAAGAAGGATAT
Ptr_PRX25_Potri.004G015300	GCTGAGGACATTGTCAAAGAACAAGTTAGACTCCTTTACAAGAGACACAA
Ptr_PRX12_Potri.001G351000	TTCAACAAAATCATTCAAGAAACAGTAACCAAGCAGCAGATACCAGCCC
Ptr_PRX33_Potri.005G108900	GTCGATTCCATTGTT--ACGAATGCTGTCAATCATGCA-ATGATGAAAGA
	** * * *
Ptr_PRX22_Potri.003G214800	CAGGATTGGAGCCA--GCCTCATTCGACTTCATTTCCATGATTGTTTTG
Ptr_PRX23_Potri.003G214700	CAGGATTGGAGCCA--GCCTCATTCGACTTCATTTCCATGATTGTTTTG
Ptr_PRX36_Potri.005G195600	CGAACAAGCTGCTG---GCTTGCTTCTCTCCACTTCCATGATTGCTTTG
Ptr_PRX25_Potri.004G015300	AAACACTGCATTTT---CTTGGCTAAGAAACATCTTCCATGACTGTGCTG
Ptr_PRX12_Potri.001G351000	CAGCACCGCAGCTG--GCACT-CTCCGCCTCTTCTCCATGACTGCTTAC
Ptr_PRX33_Potri.005G108900	CAAAACGGTCCCGCAGCGCTACTCCGGATGCATTTCCATGACTGTTTCA
	* * * *
Ptr_PRX22_Potri.003G214800	TTGATGGTTGTGATGGATCGATTCTTTTGGACAACACTGATACTATAGAG
Ptr_PRX23_Potri.003G214700	TTGATGGTTGTGATGGATCAATTCTTTTGGACAACACTGATACTATAGAG
Ptr_PRX36_Potri.005G195600	TTGATGGTTGTGATGGATCGATTCTTTTGGACAACACTGATACTATAGAG
Ptr_PRX25_Potri.004G015300	TTGATGGTTGTGATGGATCGATTCTTTTGGACAACACTGATACTATAGAG
Ptr_PRX12_Potri.001G351000	TTGATGGTTGTGATGGATCGATTCTTTTGGACAACACTGATACTATAGAG
Ptr_PRX33_Potri.005G108900	TTGATGGTTGTGATGGATCGATTCTTTTGGACAACACTGATACTATAGAG
	** * * * *
Ptr_PRX22_Potri.003G214800	AGTGAGAAAGAAGCTGCTCCAAATAATAATTC---AGCAAGGGGTTTTGA
Ptr_PRX23_Potri.003G214700	AGTGAGAAAGAAGCTGCTCCAAATAATAATTC---AGCAAGGGGTTTTGA
Ptr_PRX36_Potri.005G195600	AGCGAGCAGGACGCCCTCCCAACCTGACCTTGAGAGCAAAGGCGTTTGA
Ptr_PRX25_Potri.004G015300	TCCGAGAAGGAGACAGACAGGAGCTTTGGCCT----CAGGAACTTTAGA
Ptr_PRX12_Potri.001G351000	GCCGAGCGTGATGCTGACATCAACCTTTCCTTACCCGGCGAGCGCTTCGA
Ptr_PRX33_Potri.005G108900	GCAGAGAAAGATGGCCACCTAACATCTCTTTGC-----ATGCGTTTTTA
	** * * *
Ptr_PRX22_Potri.003G214800	TGTTGTTGATGACATGAAGGCTGCA-GTAGAGAATGCTTGCTCGGATC
Ptr_PRX23_Potri.003G214700	TGTTGTTGATAACATGAAGGCTGCA-GTAGAGAATGCTTGCCCTGGGATA
Ptr_PRX36_Potri.005G195600	GATCATCGAAGACCT-ACGCCGGCGCGTGGAAAAGGCTTGCGGCTTGTC
Ptr_PRX25_Potri.004G015300	TACT-TTGACGATATCAAAGAAGCT-GTTGAAAGAGAGTGCTCGGAGTC
Ptr_PRX12_Potri.001G351000	CCTTGTCACTCGCGCAAGACCGCC-CTCGAGCTCTCTGCCCAACACC
Ptr_PRX33_Potri.005G108900	TGTCATTGACAACGCAAGAAAGAA-GTGAAGCTTCATGCCCAGGAGTG
	* * * *
Ptr_PRX22_Potri.003G214800	GTCTCCTGTGCTGATATTCTCGCCATTGCAGCTGAAGAGTCTGTTCTGTTT
Ptr_PRX23_Potri.003G214700	GTTTCTTGCTGATATTCTCGCCATTGCAGCTGAAGAGTCTGTTCTGTTT
Ptr_PRX36_Potri.005G195600	GTCTCCTGTGCTGATATTCTTGCGCTTGACGCCGTGACTCTGTTTACCT
Ptr_PRX25_Potri.004G015300	GTTTCTTGCTGATATTCTTGCTGCTGCTGCTAGAGATGGCATTGTTTC
Ptr_PRX12_Potri.001G351000	GTCTCCTGCGCCGACATCCTCACCATGCCACCCGTGATCTCGTAACCAT
Ptr_PRX33_Potri.005G108900	GTATCATGCGCTGATATTCTGGCTTTAGCTGCAAGAGATGCAGTTGACT
	** * * * *
Ptr_PRX22_Potri.003G214800	GGCAGGAGGACCTCATGGACAGTTTCTTTGGGAAGAAGAGACAGCTTAA
Ptr_PRX23_Potri.003G214700	GGCAGGAGGACCTCATGGACAGTTTCTTTGGGAAGAAGAGACAGCTTAA
Ptr_PRX36_Potri.005G195600	GTCTGGTGGCCAGATTATAATGTTCCCTTGGGAAGCGAGACGGTCTAA
Ptr_PRX25_Potri.004G015300	GCTAGGAGGACCTCATATCCCTCTCAAACATGGAAGAAGGGATGGCAGGA

Ptr_PRX12_Potri.001G351000
Ptr_PRX33_Potri.005G108900

GGTCGGTGCCCTTACTACAATGTCTCCTCGGGCGCAAAGATTACCGCA
TTCCGGAGGTCCAACATGGGATGTCCCAAAAGGAAGAAAGATGGGAGAA
* * * * * * * * *

Ptr_PRX22_Potri.003G214800
Ptr_PRX23_Potri.003G214700
Ptr_PRX36_Potri.005G195600
Ptr_PRX25_Potri.004G015300
Ptr_PRX12_Potri.001G351000
Ptr_PRX33_Potri.005G108900

TA---GCAAACAGAAGTGGTGCTAATTCTGCCCTTCCGTCTCCCTTCGCG
TA---GCAAACAGAAGTGGTGCTAATTCTTCCATTCCGGCTCCCTCCGAG
AATTGCAACTCAAATGAAACCTTAGACAACCTCCCGCCACCTTTGCT
AGA--GCAGAGCAGA-TGTGATCGAGGACTATCTCCAGACCAACAATGAA
TCA--GTAAATCTT-CTTATGTTGAAGGAAATCTGCCAAGACCCACCATG
-CG--TCAAGGG--CTAGTGAAACAACACGGTTGCCTTCTCTACCTTT
* * * * *

Ptr_PRX22_Potri.003G214800
Ptr_PRX23_Potri.003G214700
Ptr_PRX36_Potri.005G195600
Ptr_PRX25_Potri.004G015300
Ptr_PRX12_Potri.001G351000
Ptr_PRX33_Potri.005G108900

AGCCTTGATGTTCTCAAATCGAAGTTTGACGCCGTGGGCTCAACACCAG
AGCCTTGCTGTTCTCAAATCCAAGTTTGACGCCGTGGGCTCAACACCAG
AACGCCGACACAATTCTCTCATCTCTGCCACCAAGGGCTTCGATGCCAC
AGCATATCTGTTGTTCTTGACAGGTTTGCTTCCATGGGTATTGACACCCC
CCTATGCTAAAATTATCAGCTTGTTTGCTGTTAAAGGTTTCAAGTGCCA
AACATAGCTCAGCT--GCAGCAGAGTTTCTCTCAGAGAGGTCTGTCCCTG
* * * * *

Ptr_PRX22_Potri.003G214800
Ptr_PRX23_Potri.003G214700
Ptr_PRX36_Potri.005G195600
Ptr_PRX25_Potri.004G015300
Ptr_PRX12_Potri.001G351000
Ptr_PRX33_Potri.005G108900

TAGTGACCTGGTTGCCCTTTCAGGTGCTCACACATTTGGAAGGGCTCAAT
TAGTGACCTGGTTGCCCTTTCAGGTGCTCACACATTTGGAAGAGCTCAAT
C---GACGTGGTAGCCCTGTCCGGTGGCCACACCATTTGGTATAAGTCACT
T---GGACTGGTTGCCCTTGCTAGGAGCTCACAGTGTGGGAGAACTCACT
AG-AAA--TGGTGGCACTGAGCGGTGCTCATACAATTGGTTTCTCTCATT
GA-TGACCTTGTGGCTCTTTCAGGTGGCCATACTTTAGGGTTTTCCCACT
* * * * *

Ptr_PRX22_Potri.003G214800
Ptr_PRX23_Potri.003G214700
Ptr_PRX36_Potri.005G195600
Ptr_PRX25_Potri.004G015300
Ptr_PRX12_Potri.001G351000
Ptr_PRX33_Potri.005G108900

GTTCAAGTTTCAATCTTAGATTGTATAATTTTAGTGGCAGTGGAATCCT
GTTTAAATTTTCATCAGTAGATTGTATAATTTTAGTGGCAGTGGAATCCT
GCAGCTCTTTCAGTACCCTCTATC-----CAACCCA
GTGTGAAGCTGGTGACCGTTTGTACC-----CGGAAGT
GCAAGGAGTTCAAGAGTT--ATTGTGA----TAACGACACGCAT-----T
GCTCATCCTTCCAAAGCAGAATCCGCAATTTCAATGCCACTCATGA-CAT
* * * * *

Ptr_PRX22_Potri.003G214800
Ptr_PRX23_Potri.003G214700
Ptr_PRX36_Potri.005G195600
Ptr_PRX25_Potri.004G015300
Ptr_PRX12_Potri.001G351000
Ptr_PRX33_Potri.005G108900

-GATCCAACACTGAACACAACCTAT-CT-AGCAGAACTTCAACAATTATG
-GATCCAACACTGAACACAACCTAT-CT-AGCAGCACTTCAACAATTATG
AGATCCTACAATGGACAAAACCTTTGCC-AACA--ATCTCAAGGAAGTTT
TGACCCAGCACTAAACCTGACCATGTTGAGCACATGCTTTACAAGTGCC
ATAATCAGAGATTTGTTTCAAGGCGTTGCG----AAATGCTTGCGCGATTA
AGACCCATCCATGCATCCATCGTTTGCA----GCCAGCTTAAGGAGTGTT
* * * * *

Ptr_PRX22_Potri.003G214800
Ptr_PRX23_Potri.003G214700
Ptr_PRX36_Potri.005G195600
Ptr_PRX25_Potri.004G015300
Ptr_PRX12_Potri.001G351000
Ptr_PRX33_Potri.005G108900

TC--CTCAAGCTGGGAATGAGAGTGAGAGTGTTGTAACAAATCTTGATCC
TC--CTCAAGTGGAATCG-----GAGTGTTTTAAACAACTTGATCG
GC--CCCACAAGAGACTTCA-----ATAACACAACCGTTTGGATAT
CTGATTCAATCCCAGACCTAAAGCTGTCCAATATG-TGAGGAATGACAG
TC--CAAAGAACCAACTCTG----TCG-GTGTTCATGATA-TAATGA-
TG--TCCAAAATCGAACAGGG----CAAAGAATGCAGGAACAACCATGGA
*

Ptr_PRX22_Potri.003G214800
Ptr_PRX23_Potri.003G214700
Ptr_PRX36_Potri.005G195600
Ptr_PRX25_Potri.004G015300
Ptr_PRX12_Potri.001G351000
Ptr_PRX33_Potri.005G108900

CACTACACCCGATACTTTTCGATGGCAACTATTTTCCAATCTTCAAACCA
CACTACACCCGATACTTTTCGATGGCAACTATTTTCCAATCTTCAAACCA
TCGATCTCCTAATAAATTTGACAACAAGTACTATGTTGATCTCATGAATC
AGGCACACCCATGTTTCTAGACAACAACCTACTACAGAAACATATTGGACA
-----CACCAATAACTTTGATAATAAGTATTTTGATAATTTGGGTAAAG
TCCCTCGTCGACAACCTTTGATAATACATATTTCAAGTCGATCCTCCAGA
* * * * *

Ptr_PRX22_Potri.003G214800
Ptr_PRX23_Potri.003G214700
Ptr_PRX36_Potri.005G195600
Ptr_PRX25_Potri.004G015300
Ptr_PRX12_Potri.001G351000
Ptr_PRX33_Potri.005G108900

ATGAAGGCCTGCTTCGTAGTGATCAAGAGTTGTTTTCTACTACAGGTGCC
ATGAAGGCCTGCTTCAGAGTGATCAAGAGTTGTTCTCTACTACAGGTGCC
GCCAAGGCCTGTTTACTTCAGACCAGGACTTGTACACGA--ACAAG---
ACAAGGCCTGTTGATAGTGGATCACCACCTAGCCACTG--ACAAG---
GGTTGGGGCTATTAGAGTCAGATCATGGGTATATAACAACCCAAT---
AAAGGGGTCTATTCTCTCAGACCAATCTCTACTCAGCACTCCAAA---
* * * * *

Ptr_PRX22_Potri.003G214800
Ptr_PRX23_Potri.003G214700

GATACCATTTGACATTGTCAACAATTTTAGTAGTAATCAGACTGCATTCTT
GATACCATTTGCCATTGTCAACAATTTTAGTAGTAATCAGACTGCATTCTT

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Ptr_PRX36_Potri.005G195600      AAGACAAGGGGCATTGTCACTAGCTTTGCTGTTAATCAAAGTTTGTCTT
Ptr_PRX25_Potri.004G015300      AGAACTAAGCCTTACGTGAAGAAAATGGCCAAGAGTCAGGATTACTTCTT
Ptr_PRX12_Potri.001G351000      --GACAAATCCTTTTGTGGAGATTATGCCAAGGATGAGAAGAAATTCCT
Ptr_PRX33_Potri.005G108900      --GACTAAAGATTGGTCAACCAAGTTTGTAGCTCCAAGCCAATTTCAA
                                ** *      **      *      *      ***

Ptr_PRX22_Potri.003G214800      TGAAAGCTTTGTGGTGTCCATGATTAGAATGGGGAATAT--TAGCCCTT
Ptr_PRX23_Potri.003G214700      TGAAAGCTTTGTGGTGTCCATGATTAGAATGGGGAATAT--TAGCCCTT
Ptr_PRX36_Potri.005G195600      TGATAAGTTTGTGGTTGCAATGATCAAAATGTCACAGCT--CAAGGTCTT
Ptr_PRX25_Potri.004G015300      TAAGGAGTTTCTAGGGCTATCACCATTCTTTCTGAGAA--CAACCCACT
Ptr_PRX12_Potri.001G351000      TCAGGATTTTGAAGAGCCATGGAGAAGCTAAGTGT----TATGGGATC
Ptr_PRX33_Potri.005G108900      TAAGGCTTTTGTATCATCCATGATCAAGATGAGTAGCATCACAGCGGTC
                                * *      ***      * *      *      *      *

Ptr_PRX22_Potri.003G214800      AA---CCGGGACAGATGGAGAGAT--CAGATTGAATTGCAGAAGAGTCA
Ptr_PRX23_Potri.003G214700      AA---CTGGGACAGATGGAGAGAT--CAGATTGAATTGCAGAATAGTCA
Ptr_PRX36_Potri.005G195600      GA---CAGGAAATCAAGGTGAAAT--CCGCGCCAGTTGCGAGGAGAGAA
Ptr_PRX25_Potri.004G015300      CA---CCGGTACCAAGGTGAGAT--CAGAAAGCAGTGCACTGTTGCAA
Ptr_PRX12_Potri.001G351000      AAGA--CTGGGAGGAGAGGCGAGAT--TAGGCGCAGGTGTGATGCCATTA
Ptr_PRX33_Potri.005G108900      AGGAGGTTAGGAAGGATTGTAGGGTGGTAAACTAAGGTTCTGTTAGCTTG
                                * *      *      *      *

Ptr_PRX22_Potri.003G214800      ACGACAACCTCAACCGGATCAAATGCTCTCCTGGTTAG---CTCAATTTA
Ptr_PRX23_Potri.003G214700      AC---AACTCAACTGGATCAAATGCTCTTCTGGTTAG---CTCAATTTA
Ptr_PRX36_Potri.005G195600      ATTCAAGGCTACAGTTACTTGGAGTCTGTGGTGGAAAGAGGGTCTCGACGCA
Ptr_PRX25_Potri.004G015300      AC--AAGCACCAC TAG-----
Ptr_PRX12_Potri.001G351000      ATTAA-----
Ptr_PRX33_Potri.005G108900      GTTCGTTACTTTTAAACTGTGAAATGGCAATCCACCAGATGTATCGATAG

Ptr_PRX22_Potri.003G214800      A-----
Ptr_PRX23_Potri.003G214700      A-----
Ptr_PRX36_Potri.005G195600      CTGTCCGGATTAATATGA-----
Ptr_PRX25_Potri.004G015300      -----
Ptr_PRX12_Potri.001G351000      -----
Ptr_PRX33_Potri.005G108900      CAAGAAC TAGACATCTGGTTTGCTAATAGCTATGAAGGCCATCTATCTTA

Ptr_PRX22_Potri.003G214800      -----
Ptr_PRX23_Potri.003G214700      -----
Ptr_PRX36_Potri.005G195600      -----
Ptr_PRX25_Potri.004G015300      -----
Ptr_PRX12_Potri.001G351000      -----
Ptr_PRX33_Potri.005G108900      TATTGTAATAGATATTTTGAAGAATAATACATCCAGTGACATTTTCCTGG

Ptr_PRX22_Potri.003G214800      -----
Ptr_PRX23_Potri.003G214700      -----
Ptr_PRX36_Potri.005G195600      -----
Ptr_PRX25_Potri.004G015300      -----
Ptr_PRX12_Potri.001G351000      -----
Ptr_PRX33_Potri.005G108900      TAATGACTTTTAGATCAT

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(
(
(
Ptr_PRX12_Potri.001G351000:0.32839,
Ptr_PRX33_Potri.005G108900:0.33620)
:0.01275,
Ptr_PRX25_Potri.004G015300:0.33686)
:0.00923,
(
Ptr_PRX22_Potri.003G214800:0.02560,
Ptr_PRX23_Potri.003G214700:0.03630)
:0.29907,
Ptr_PRX36_Potri.005G195600:0.30060);

```

B.8 **Phylogenetic tree of all six SDX PRXs**

C Appendix C

C.1 Complete list of the forty-nine laccases in *Populus trichocarpa* with tissue gene expression profile comparison

Table C 1: Complete list of all 49 LAC genes in *Populus trichocarpa* and their expression profile in difference parts of the tree

Laccase name	Gene model (Lu et. al)	Phytozome (Alias)	Expression (Gene Atlas)	Protein Homolog	Ortholog in At	Expression (Lu et. al)	Gene model number (Ren et. al)	Expression	Absolute value
Ptr LAC1	POPTR_0001s14010	Potri.001G054600.1	Stem	Potri.001G401300.1 (92.8%), Potri.001G401100.1 (95.0%)	At5g60020 (Lac 17)	Young stem (S)	Potri.001G054600.1	not in db	
Ptr LAC2	POPTR_0001s18500	Potri.001G184300.1	Stem	Potri.009G156800.1 (99.0%), Potri.009G156600.1 (98.0%)		Stem differentiating xylem (X)	Potri.001G184300.1	Xylem	1848.83
Ptr LAC3	POPTR_0001s21380	Potri.001G206200.1	not expressed	No Homologs	At5g09360 (lac 14)	Young Leaf (YL)	Potri.001G206200.1	Male catkins	

Ptr LAC4	POPTR_ 0001s255 80	Potri.001G 248700.1	Roots and Stem internode	Potri.009G 042500.1 (95%)		Roots [R]	Potri.001 G248700 .1	not in db	
Ptr LAC5	POPTR_ 0001s357 40	Potri.001G 341600.1	Stem internode			X	Potri.001 G341600 .1	not in db	
Ptr LAC6	POPTR_ 0001s411 60	Potri.001G 401100.1	Stem internode	Potri.001G 054600.1 (95%), Potri.001G 401300.1 (92.1%)		X	Potri.001 G401100 .1	not in db	
Ptr LAC7	POPTR_ 0001s411 70	Potri.001G 401300.1	Stem internode	Potri.001G 054600.1 (92.8%), Potri.001G 401100.1 (92.1%)		X	Potri.001 G401300 .1	not in db	
Ptr LAC8	POPTR_ 0004s163 70	Potri.004G 156400.1	Roots and Stem internode	Potri.004G 156400.2 (93.3%), Potri.009G 102700.1 (93.8%)		S	Potri.004 G156400 .1	Male catkins, xylem	305.37
Ptr LAC9	POPTR_ 0005s222 40	Potri.005G 200600.1	Roots	Potri.005G 200500.1 (96.1%), Potri.011G 071100.1 (92.4%), Potri.005G		All tissues	Potri.005 G200600 .1	not in db	

				200500.2 (89.4%)					
Ptr LAC10	POPTR_ 0005s222 50	Potri.005G 200700.1	roots			R	Potri.005 G200700 .1	Female catkins	
Ptr LAC11	POPTR_ 0006s087 40	Potri.006G 087100.1	Stem internode			X	Potri.006 G087100 .1	Xylem	16137.26
Ptr LAC12	POPTR_ 0006s087 80	Potri.006G 087500.1	Stem internode			X, Phloem (P)	Potri.006 G087500 .1	Xylem	9639.45
Ptr LAC13	POPTR_ 0006s095 20	Potri.006G 094100.1	Roots		At3g0922 0 (Lac 7)	R	Potri.006 G094100 .1	not in db	
Ptr LAC14	POPTR_ 0006s098 30	Potri.006G 096900.1	Stem internode	Potri.006G 097100.1 (99.1%), Potri.006G 097000.1 (96.8%), Potri.016G 112000.1 (97.3%), Potri.016G 112100.1 (94.6%)		X,R	Potri.006 G096900 .1	not in db	

Ptr LAC15	POPTR_ 0006s098 40	Potri.006G 097000.1	Stem internode 10.952FPK M	Potri.006G 097100.1 (97.7%), Potri.006G 096900.1 (96.6%), Potri.016G 112000.1 (94.8%), Potri.016G 112100.1 (92.3%)		X	Potri.006 G097000 .1	Xylem	687.56
Ptr LAC16	POPTR_ 0007s130 50	Potri.007G 023300.1	stem internode, leaf, root			X, S, R	Potri.007 G023300 .1	Female catkins	
Ptr LAC17	POPTR_ 0008s064 30	Potri.008G 064000.1	Stem internode 18.444 FPKM, stem node 6.048 FPKM	Potri.010G 193100.1 (87.8%)		X	Potri.008 G064000 .1	Xylem	10716.7
Ptr LAC18	POPTR_ 0008s073 70	Potri.008G 073700.1	Stem internode 33.574, Stem node 17.707, root 28.21	Potri.010G 183600.1	At2g4037 0 (Lac 5)	X, P, R	Potri.008 G073700 .1	Xylem	6541.96

Ptr LAC19	POPTR_ 0008s073 80	Potri.008G 073800.1/.2 /.3/.4/.5	Root, Stem internode 4.898	Potri.008G 073800.2 (92.8%), Potri.008G 073800.4 (92.8%), Potri.010G 183500.1 (93.1%), Potri.010G 183500.2 (86.8%)		R, P, X	Potri.008 G073800 .1	Roots, Xylem	7161.13, 6299.89
Ptr LAC20	POPTR_ 0009s039 40	Potri.009G 034500.1	Stem internode 9.025, roots	Potri.009G 156800.1 (97.2%), Potri.009G 156600.1 (97.0%), Potri.001G 184300.1 (96.9%)		X, P	Potri.009 G034500 .1	Xylem	1848.83
Ptr LAC21	POPTR_ 0009s047 20	Potri.009G 042500.1	Stem internode 19.539, stem node, roots 20.951	Potri.001G 248700.1 (95.1%)		R, P, X	Potri.009 G042500 .1	not in db	
Ptr LAC22	POPTR_ 0009s105 50	Potri.009G 102700.1	Leaf	Potri.004G 156400.1 (94.3%)		Mature Leaf (ML)	Potri.009 G102700 .1	dark etiolated seedling	

Ptr LAC23	POPTR_ 0009s158 40	Potri.009G 156600.1	Stem internode 3.437	Potri.009G 156800.1 (99.5%), Potri.001G 184300.1 (98.8%), Potri.009G 034500.1 (97.0%)		X	Potri.009 G156600 .1	not in db	
Ptr LAC24	POPTR_ 0009s158 60	Potri.009G 156800.1	Stem internode 4.941	Potri.009G 156600.1 (99.5%), Potri.001G 184300.1 (99.0%), Potri.009G 034500.1 (97.2%)		X	Potri.009 G156800 .1	not in db	
Ptr LAC25	POPTR_ 0010s190 80	Potri.010G 183500.1/.2	Stem internode 8.118, Stem node 6.976, root	Potri.010G 183500.2 (92.8%)		X	Potri.010 G183500 .1	Xylem	1462.66
Ptr LAC26	POPTR_ 0010s190 90	Potri.010G 183600.1/.2	Root, Stem internode 7.754, Stem node 4.764	Potri.008G 073700.1 (93.7%)		X, R	Potri.010 G183600 .1	Xylem	1015.36
Ptr LAC27	POPTR_ 0010s200 50	Potri.010G 193100.1 /. 2	Stem internode 13.176, Stem node 4.157	Potri.010G 193100.2 (92.4%)		X	Potri.010 G193100 .1	Xylem	75190.83

Ptr LAC28	POPTR_0011s06880	Potri.011G071100.1	Root 10.97	Potri.005G200500.1 (95.6%), Potri.005G200600.1 (93.1%), Potri.005G200500.2 (89.0%)		R	Potri.011G071100.1	Male catkins	
Ptr LAC29	POPTR_0011s12090	Potri.011G120200.1	Stem internode 2.193, Stem node 1.009	Potri.011G120300.1 (95.2%)		X	Potri.011G120200.1	Xylem	3110.03
Ptr LAC30	POPTR_0011s12100	Potri.011G120300.1	Stem internode 11.458, Stem node 4.494	Potri.011G120200.1 (95.2%)		X	Potri.011G120300.1	Xylem	3110.03
Ptr LAC31	POPTR_0012s04620	Potri.012G048900.1	root 5.813	Potri.015G040400.1 (91.4%), Potri.015G040600.1 (91.4%)	At1g18140 (Lac 1)	R	Potri.012G048900.1	Male catkins	
Ptr LAC32	POPTR_0013s14890	Potri.013G152700.1	Root 16.217	Potri.019G121700.1 (91.8%), Potri.019G124300.1 (91.7%)	At2g30210 (Lac3)	R	Potri.013G152700.1	not in db	

Ptr LAC33	POPTR_ 0014s096 10	Potri.014G 100600.1	Leaf immature, root, stem internode 2.53, stem node 2.086			S, R, X	Potri.014 G100600 .1	dark etiolated seedling	
Ptr LAC34	POPTR_ 0015s043 40	Potri.015G 040700.1	not expressed	Potri.015G 040600.1 (94.8%), Potri.015G 040400.1 (94.3%)			Potri.015 G040700 .1	not in db	
Ptr LAC35	POPTR_ 0015s043 50	Potri.015G 040600.1	root 0.66	Potri.015G 040400.1 (97.9%)		ML	Potri.015 G040600 .1	not in db	
Ptr LAC36	POPTR_ 0015s043 70	Potri.015G 040400.1	root 11.831	Potri.015G 040600.1 (97.9%)		R	Potri.015 G040400 .1	not in db	
Ptr LAC37	POPTR_ 0016s115 00	Potri.016G 106000.1	root 17.317	Potri.016G 107500.1 (95.8%), Potri.016G 107900.1 (93.7%), Potri.016G 106100.1 (93.7%), Potri.016G 106300.1 (90.3%)		R	Potri.016 G106000 .1	Roots	

Pt LAC38	POPTR_0016s115_20	Potri.016G106100.1	root	Potri.016G107900.1 (96%), Potri.016G107500.1 (93.7%), Potri.016G106000.1 (93.5%), Potri.016G106300.1 (91.7%)		R	Potri.016G106100.1	Roots	
Pt LAC39	POPTR_0016s115_40	Potri.016G106300.1	root	Potri.016G106100.1 (92.9%), Potri.016G107900.1 (92.9%), Potri.016G107500.1 (91.5%), Potri.016G106000.1 (91.3%)			Potri.016G106300.1	not in db	
Pt LAC40	POPTR_0016s119_50	Potri.016G112000.1	Stem internode	Potri.006G096900.1 (97.5%), Potri.006G097100.1 (97.3%), Potri.006G097000.1 (95.2%), Potri.016G		X	Potri.016G112000.1	Male catkins	

				112100.1(9 5.2%)					
Ptr LAC41	POPTR_ 0016s119 60	Potri.016G 112100.1	stem internode	Potri.016G 112000.1 (94.0%), Potri.006G 097100.1 (94.0%), Potri.006G 096900.1(9 3.6%), Potri.006G 096900.1(9 1.5%)		S, X	Potri.016 G112100 .1	not in db	
Ptr LAC42	POPTR_ 0019s118 10	not in db				R			
Ptr LAC43	POPTR_ 0019s118 20	Potri.019G 088500.1	root	Potri.019G 088600.1 (97.2%), Potri.019G 088900.1(9 7.5%), Potri.019G 088800.1 (96.8%), Potri.019G 088700.1(9		R, P	Potri.019 G088500 .1	Continuous light grown seedling	

				6.5%), Potri.019G 088700.2 (96.3%)					
Pt LAC44	POPTR_0019s11830	Potri.019G 088600.1	root	Potri.019G 088700.1 (98.6%), Potri.019G 088700.2 (98.4%), Potri.019G 088900.1(9 8.2%), Potri.019G 088500.1 (97.2%), Potri.019G 088800.1 (97.0%)		R	Potri.019 G088600 .1	Continuous light grown seedling	

Ptr LAC45	POPTR_ 0019s118 50	Potri.019G 088800.1	root	Potri.019G 088900.1 (97.9%), Potri.019G 088600.1 (97.0%), Potri.019G 088500.1 (96.8%), Potri.019G 088700.1 (96.8%), Potri.019G 088700.2 (96.6%)		R	Potri.019 G088800 .1	Continuous light grown seedling	
Ptr LAC46	POPTR_ 0019s118 60	Potri.019G 088900.1	root	Potri.019G 088600.1 (98.2%), Potri.019G 088800.1 (97.9%), Potri.019G 088700.1 (97.9%), Potri.019G 088700.2 (97.7%), Potri.019G 088500.1 (92.1%)		R	Potri.019 G088900 .1	Continuous light grown seedling	
Ptr LAC47	POPTR_ 0019s145 30	Potri.019G 121700.1	root	Potri.019G 124300.1 (99.8%)		R	Potri.019 G121700 .1	Roots	

Ptr LAC48	POPTR_ 0091s002 70	Potri.016G 107900.1	root	Potri.016G 106100.1 (96.0%), Potri.016G 107500.1 (93.7%), Potri.016G 106000.1 (93.5%), Potri.016G 106300.1 (91.7%)		R	Potri.016 G107900 .1	Roots	
Ptr LAC49	POPTR_ 0958s002 00	Potri.006G 097100.1	stem internode 5.026	Potri.006G 097000.1 (97.7%), Potri.006G 096900.1 (98.9%), Potri.016G 112000.1 (97.0%), Potri.016G 112100.1 (94.8%)		X	Potri.006 G097100 .1	Xylem	687.56

C.2 Sequencing data comparison of plasmid clones with full length CDS region of LAC27_F

CLUSTAL 2.1 Multiple Sequence Alignments

```
Sequence type explicitly set to DNA
Sequence format is Pearson
Sequence 1: DX15MF          725 bp
Sequence 2: DX15MFL27      725 bp
Start of Pairwise alignments
Aligning...
```

```
Sequences (1:2) Aligned. Score: 97.7931
Guide tree file created: [clustalw.dnd]
```

```
There are 1 groups
Start of Multiple Alignment
```

```
Aligning...
Group 1: Sequences: 2      Score:13594
Alignment Score 5089
```

```
CLUSTAL-Alignment file created [clustalw.aln]
```

clustalw.aln

CLUSTAL 2.1 multiple sequence alignment

```
DX15MF          TATCACTACACCCAACACTTCTGCAAGCACAACTCCATTCAAGAACATCAAGAGTATAGG
DX15MFL27       TCTCACTACACCCAACACTTCTGCAAGCACAACTCCATTCAAGAACATCAAGAGTATAGG
* ****
DX15MF          CCGCCGCTGCAACAAAACAGCACTCCTAGCTACTTCAAGATGAGGCCACAATCTTTCATC
DX15MFL27       CCGCCACTGCAACAAAACAGCACTCCTAGCTACTTCAAGATGAGGCCACAATCTTTCATC
*****
DX15MF          TTCCTGCAGGTCGACTCTAGAGGATCCATGGAGTACTATCAGGCTCGGACAATGCTGCTA
DX15MFL27       TTCCTGCAGGTCGACTCTAGAGGATCCATGGAGTACTATCAGGCTCGGACAATGCTGCTA
*****
DX15MF          GTGATTTTCATTTTCCGGCATTGGTCGAGTGCAAGGTCCGTCTTTATAATTTACAGGGTA
DX15MFL27       CTGATTTTCATTTTCCGGCATTGGTCGAGTGCAAGGTCCGTCTTTATAATTTACAGGGTA
*****
DX15MF          GTCCTGACGAACACAACAAAGCTTTGTTCAACTAAGTCCATCCCCACCATCAATGGAAAG
DX15MFL27       GTCCTGACGAACACAACAAAGCTTTGTTCAACTAAGTCCATCCCCACCATCAATGGAAAG
*****
DX15MF          TTTCCGGGACCCACTATTTATGCAAGGGAAGGTGATAATGTCAATATAAGGCTCACTAAC
DX15MFL27       TTTCCGGGACCCACCATTATGCAAGGGAAGGTGATAATGTCAATATAAGGCTCGCTAAC
*****
```

DX15MF	CAGGTCCAATACAATGTTACCGTCCATTGGCATGGAGTGAGGCAGTTGCGTACGGGCTGG
DX15MFL27	CAGATCCAATACAATGTTACCGTCCACTGGCATGGAGTGAGGCAGTTGCGTACGGGCTGG
	*** ****
DX15MF	GCCGATGGGCCAGCATACATTACACAGTGTCCAATACAGCCTGGGCAAAGTTATCTCTAC
DX15MFL27	GCCGATGGGCCAGCATACATTACACAGTGTCCAATACTGCCTGGGCAAAGTTATCTCTAC

DX15MF	AATTTCACTCTTACAGGCCAGAGAGGCACACTTCTCTGGCATGCACATATTTTCATGGTTA
DX15MFL27	AATTTCACTCTTACAGGCCAGAGAGGCACACTTCTCTGGCATGCACATATTTTCATGGTTA

DX15MF	AGGGCAACGATACATGGTGCCATTGTCATCTTTCCTAAGAAAGGGGTTCTTACCCATTT
DX15MFL27	AGGGCAACGATACACGGTGCCATTGTCATCTTTCCTAAGAAAGGCGTTCTTACCCATTT

DX15MF	CCAAAACCTGACAAGGAAAAGATCATCATATTGAGTGAATGGTGGAAAGGCTGATGTTGAA
DX15MFL27	CCAAAACCTGACAAGGAAAAGATCATCATATTGAGTGAATGGTGGAAAGGCTGATGTTGAA

DX15MF	GCTGTGGTCAACCAAGCAACAATGACTGGCTTGCCTCCAAATATATCAGATGCACACACC
DX15MFL27	GCTGTGATCAACCAAGCAACAATGACTGGCTTGCCTCCAAATATATCAGATGCACACACC

DX15MF	GTTAA
DX15MFL27	GTTTA
	*** *

clustalw.dnd

(DX15MF:0.011034,DX15MFL27:0.011034);

C.3 Sequencing data comparison of plasmid clones with full length CDS region of LAC27_MF

CLUSTAL 2.1 Multiple Sequence Alignments

```
Sequence type explicitly set to DNA
Sequence format is Pearson
Sequence 1: LAC27MF      744 bp
Sequence 2: DXL27MF      744 bp
Start of Pairwise alignments
Aligning...
```

```
Sequences (1:2) Aligned. Score: 93.2796
Guide tree file created: [clustalw.dnd]
```

```
There are 1 groups
Start of Multiple Alignment
```

```
Aligning...
Group 1: Sequences: 2      Score:13616
Alignment Score 4907
```

```
CLUSTAL-Alignment file created [clustalw.aln]
```

clustalw.aln

CLUSTAL 2.1 multiple sequence alignment

```
LAC27MF      TGC GCTATAAGGGAACCCCTTGCATTCTCCCCACCTGTCCTAACCACCACTCCTGCTATAA
DXL27MF      TGC GCTATA--GGGACCCCTTGCATTCTCCCCACCTGTCCTAACCACCACTCCTGCTATAA
*****      ** *****

LAC27MF      ACGCAACTCCGGCAACTTCAACTTTTCATGGACAAACTTCGAAGCCTGAATTCAAAGAAGT
DXL27MF      ACGCAACTCCGGCAACTTCAACTTTTCATGGACAAACTTCGAAGCCTGAATTCAAAGAAGT
*****

LAC27MF      ACCCAGCCAATGTCCCATTAACAGTAGACCATGATCTATACTTCACGATCGGTGTCGGGA
DXL27MF      ACCCAGCCAATGTCCCATTAACAGTAGACCATGATCTATACTTCACGATCGGTGTCGGGA
*****

LAC27MF      TTGACCCTTGTGCAACATGTACTAATGGTAGCAAGGCTGTGGCAGATATAAATAATGTTT
DXL27MF      TTGACCCTTGTGCAACATGTACTAATGGTAGCAAGGCTGTGGCAGATATAAATAATGTTT
*****

LAC27MF      CTTTATAATGCCAACTACAGCTTTACTTCAAGCACACTACTATAACATAAGTGGGGTCT
DXL27MF      CTTTATAATGCCAACTACAGCTTTACTTCAAGCACACTACTATAACATAAGCGGGGTCT
*****

LAC27MF      TCACAGATGATTTCCCAGCAAAACCACCGATTTCTTTAATTATACGGGAAATAATACGG
DXL27MF      TCACAGATGATTTCCCAGCAAAACCACCGATTTCTTTAATTATACGGGAAATAATACGG
*****
```


LAC27MF	CAATGAATCTAAAGACCACAAATGGGACTAGAGCTTATAGGCTGGCTTTTAATTCAGCAG
DXL27MF	CAATGAATCTAAAGACCACAAATGGGACTAAAGCTTATAGGCTGGCTTTTAATTCAGCAG

LAC27MF	TTCAAGTGGTGCTGCAAGGAACCACTATAATAGCACCTGAGAGTCATCCATTCCATCTTC
DXL27MF	TTCAAGTGGTGTTGCAAGGAACCACTGTAATAGCACCTGAGAGTCATCCATTCCATCTTC

LAC27MF	ATGGATTTAATTTCTTTGTTGTTGGCAAGGGAATAGGAAATTTTGATCCAGATAATGATC
DXL27MF	ATGGATTTAATTTCTTTGTTGTTGGCAAGGGAATAGGAAATTTTGATCCAGATAATGATC
	***** *
LAC27MF	CAAAGAAATTTAATCTGGCTGATCCTGTTGAAAGGAACACAGTCAGTGTACCTACTGCCG
DXL27MF	CAAAGAAATTTAATCTGGCCGATCCTGTTGAAAGGAACACAGTCAGTGTACCTACTGCCG

LAC27MF	GATGGATTGCAATCAGATTCAA-GGCAGATAATCCAGGTGTTTGGTTTTTGATTGCCAT
DXL27MF	GATGGATTGCAATCAGATTCAAAGGCAGATAATCCAGGTGTTTGGTTTTTGATTGCCAT

LAC27MF	TTGGAAGTACACACGACATGGGGACTAAAGATGGCATTGTGGTGACAAATGGTAAGGGC
DXL27MF	TTGGAAGTACACACGACATGGGGACTCA-GATGGCATTGTGGTGAC--ATGGTAAGG-C
	***** * ***** *
LAC27MF	CCAAATGAGTCAATACTACCACTC----
DXL27MF	CCAAACGAGTCAATACTACCCCTCTAGTGA
	***** *

clustalw.dnd

(LAC27MF:0.033602,DXL27MF:0.033602);

C.4 Sequencing data comparison of plasmid clones with full length CDS region of LAC18_F

CLUSTAL 2.1 Multiple Sequence Alignments

```
Sequence type explicitly set to DNA
Sequence format is Pearson
Sequence 1: DXL18          774 bp
Sequence 2: DXl18S        774 bp
Start of Pairwise alignments
Aligning...
```

```
Sequences (1:2) Aligned. Score: 97.5452
Guide tree file created: [clustalw.dnd]
```

```
There are 1 groups
Start of Multiple Alignment
```

```
Aligning...
Group 1: Sequences: 2      Score:14479
Alignment Score 5371
```

```
CLUSTAL-Alignment file created [clustalw.aln]
```

clustalw.aln

CLUSTAL 2.1 multiple sequence alignment

```
DXL18      AGCACAACTCCATTCAAGAACATCAAGAGTATAGGCCGCCACTGCAACAAAACAGCACTC
DXl18S     -GCACAACTCCATTCAAGAACATCAAGAGTATAGGCCGCCGCTGCAACAAAACAGCACTC
           *****

DXL18      CTAGCTACTTCAAGATGAGGCCACAATCTTTCATCTTCCTGCAGGTCGACTCTAGAGGAT
DXl18S     CTAGCTACTTCAAGATGAGGCCACAATCTTTCATCTTCCTGCAGGTCGACTCTAGAGGAT
           *****
```

DXL18	CCATGGAGGTTATCAACCGTATCTTTGCCAATCGCCATTGTTCTTTCTTCTTGCTCCTGC
DXL18S	CCATGGAGGTTATCAACCGTATCTTTGCTAATCGCCATTGTTCTTTCTTCTTGCTCCTGC

DXL18	TTCTGGCCTCAGCAATGTCATTAGCTATTGCAAAAACCCACCACCATGATTTTATTGTTT
DXL18S	TTCTGGCCTCAGCAATGTCATTAGCTATTGCAAAAACCCACCACCATGATTTTACTGTTT

DXL18	AAGCAGCAAAAGTTAAAAGGCTTTGCAAAACCCACAACAGCATCACAGTAAACGGAATGT
DXL18S	AAGCAACAAAAGTGAAAAGGCTATGCAAAACCCACAACAGCATCACAGTAAACGGAATGT

DXL18	TCCCGGGGCCAACCTTGGAAGTGAAGAACGGAGACACTCTAGTTGTAAAAGTTGTGAACA
DXL18S	TCCCGGGGCCAACCTTGGAAGTGAAGAACGGAGACACTCTAGTTGTAAAAGTTGTGAACA

DXL18	GAGCCCGATATAATGTTACCATTCACTGGCACGGTATAAGGCAAATGAGAACGGGCTGGG
DXL18S	GAGCCCGATATAATGTTACCATTCACTGGCACGGTATTAGGCAAATGAGAACGGGCTGGG

DXL18	CAGATGGGCCAGAATTTGTGACACAATGCCCAATTAGACCAGGTGGAAGTTACACCTACA
DXL18S	CAGATGGGCCAGAATTTGTGACACAATGCCCAATTAGACCAGGTGGAAGTTACACCTACA

DXL18	GGTTTACTATTGAAGGACAAGAAGGAACACTCTGGTGGCACGCTCATAGCTCCTGGCTTA
DXL18S	GGTTTACTATTGAAGGACAAGAAGGAACACTTTGGTGGCATGCTCATAGCTCCTGGCTTA

DXL18	GAGCCACTGTTTATGGTGCTCTAATCATCCATCCAAGAGAAGGATCATCGTATCCATTCT
DXL18S	GAGCCACTGTTTATGGTGCTCTAATCATCCATCCAAGAGAAGGATCATCGTATCCATTCT

DXL18	TTAAGCCAAAGCGTGAAACACCCATACTCCTTGGTGAATGGTGGGATACGAACCCTATTG
DXL18S	CTAAGCCAAAGCGTGAAACACCCATACTCCTTGGTGAATGGTGGGATACGAACCCTATTG

DXL18	ATGTGGTGAGGGAGGCAACTAGAACCGGAGCGGCTCCTAATATATCTGATGCTTATACCA
DXL18S	ATGTGGTGAGGGAAGCAACTAGAACCGGAGCGGCTCCTAATATATCTGATGCTTATACCA

DXL18	TTAACGGTCAACCTGGTGATCTTAT-AACTGCTCGAGCAAAGATACTACCATCGT
DXL18S	TTAACGGTCAACCTGGTGATCTTTTAACTGCTCCAGCAAAGATACTACCATCGT

clustalw.dnd

(DXL18:0.012274,DXL18S:0.012274);

C.5 Sequencing data comparison of plasmid clones with full length CDS region of LAC18_MF

CLUSTAL 2.1 Multiple Sequence Alignments

Sequence type explicitly set to DNA
Sequence format is Pearson
Sequence 1: DX15L18 434 bp
Sequence 2: DX15L18Mf 434 bp
Start of Pairwise alignments
Aligning...

Sequences (1:2) Aligned. Score: 93.0876
Guide tree file created: [\[clustalw.dnd\]](#)

There are 1 groups
Start of Multiple Alignment

Aligning...
Group 1: Sequences: 2 Score:7916
Alignment Score 2908

CLUSTAL-Alignment file created [\[clustalw.aln\]](#)

[clustalw.aln](#)

CLUSTAL 2.1 multiple sequence alignment

DX15L18	CGACCGATATTGATGAGAACCTCTTCTTCACAATCGGCCTAGGACTCAACAATTGTCCTA
DX15L18Mf	CGACCGATATTGATGAGAACCTCTTCTTCACAATCGGCCTAGGACTCAACAATTGTCCCA

DX15L18	AAACTTTAGAGCTAGGCGGTGTCAAGGACCCAACGGCACTCGTTTCACTGCTAGTATGA
DX15L18Mf	AAACTTTAGAGCTAGGCGGTGTCAAGGACCCAACGGCACTCGTTTCACTGCTAGTATGA

DX15L18	ACAATGTGTCCTTCGTGTTTCCTTCAAAAAGCCTCGTTACTGCAAGCATATAAGCAAAACA
DX15L18Mf	ACAATGTGTCCTTTGTGTTTCCTTCAAAAAGCCTCGCTATTGCAAGCATATAAGCAAAAAA
	***** ** ***** *
DX15L18	TTCCTGGAGTTTTACCACCGATTTTCCAGCAAAACCACCAGTAAAATTTGACTTACACTG
DX15L18Mf	TTCCTGGAGTCTTACCACCGATTTTCCAGCAAAACCACAAGTAAAATTTGACTTACACTG
	***** ***** *****
DX15L18	GCAATGTTAGTCGTTCCCTCTTTCAACCTGCTCGTGGAACCTAAGCTGTACAAGTTGAAGT
DX15L18Mf	GCAATGTTAGTCGTTCCCTCTTTCAACCTGCTCGTGGAACCTAAGCTGTACAAGTTGAAGT

DX15L18	ACGGATCAAGGGTGCAGGTTGTGCTACAGGACACGAGCATTGTACACCAGAAAACCATC
DX15L18Mf	ACGGATCAAGGGTGCAGATTGTGCTACAGGACACGAGCATCGTACACCAGAAAACCATC
	***** ***** *****
DX15L18	CGATCCATCTCCATGGGATACGACTTCTACATCATCGCTGAAGGATTTGGGGATTTTCAA
DX15L18Mf	CGATCCATCTCCATGG-ATACGACTTCTACATCATCGCTGAAGGATTTGGGAATTT-CAA
	***** ***** *****
DX15L18	TTCCCAAAAACTG--
DX15L18Mf	TCCCAAACTGATAAA
	* * * * *

clustalw.dnd

(DX15L18:0.034562,DX15L18Mf:0.034562);

CLUSTAL 2.1 Multiple Sequence Alignments

CLUSTAL-Alignment file created [clustalw.aln]

CLUSTAL 2.1 multiple sequence alignment

Ptr_LAC27_CDS	ATGGAGTACTATCAGGCTCGGCAAGTCTGCTAGTCT
Ptr_LAC18_CDS	ATGGAGGTTATCAACCGTATCTTTGCTAATCGCCATTGTTCTTTCTTCTTGCTCCTGCTT
	* * * * *
Ptr_LAC27_CDS	ATTTTCATTTTTCCGCGCATTGGTCGAGTGCAAGGTCCGTCCTTTATAATTTTCAGGGTAGTC
Ptr_LAC18_CDS	CTGGCCTCAGCAATGTCATTAGCTATTGCAAAAACCCACCACCATGATTTTTACTGTTCAA
	* * * * *
Ptr_LAC27_CDS	CTGACGAACACACAACAAAGCTTTGTTCAACTAAGTCCATCCCCACCATCAATGGAAAGTTT
Ptr_LAC18_CDS	GCAACAAAAGTGAAAAGGCTATGCAAAAACCCACAACAGCATCACAGTAAACGGAATGTTT
	** * * * * * * * * * * * * * * * *
Ptr_LAC27_CDS	CCGGGACCCACTATTTTATGCAAGGGAAGGTGATAATGTCAATATAAGGCTCACTAACCAG
Ptr_LAC18_CDS	CCGGGGCCAACCTTGGAAAGTGAAGAACGGAGACACTCTAGTTGTAAAAGTTGTGAACAGA
	***** * * * * * * * * * * * * * * *
Ptr_LAC27_CDS	GTCCAATACAATGTTACCGTCCATTGGCATGGAGTGAGGCAGTTGCGTACGGGCTGGGGCC
Ptr_LAC18_CDS	GCCCGATATAATGTTACCATTCACTGGCACGGTATTAGGCAAATGAGAACGGGCTGGGCA
	* *
Ptr_LAC27_CDS	GATGGGCCAGCATACATTACACAGTGTCCAATACAGCCTGGGCAAAGTTATCTCTACAAT
Ptr_LAC18_CDS	GATGGGCCAGAATTTGTGACACAATGCCAATTAGACCAGGTGGAAGTTACACCTACAGG
	***** * * * * * * * * * * * * * * *
Ptr_LAC27_CDS	TTCACTCTTACAGGCCAGAGAGGCACACTTCTCTGGCATGCACATATTTTCATGGTTAAGG
Ptr_LAC18_CDS	TTTACTATTGAAGGACAGAAGAAGGAACACTTTGGTGGCATGCTCATAGGTCCTGGCTTAGA
	* *

Ptr_LAC27_CDS	GCAACGATACATGGTGCCATTGTCATCTTTCCTAAGAAAGGGGTTCTTACCCATTTCCA
Ptr_LAC18_CDS	GCCACTGTTTATGGTGCTCTAATCATCCATCCAAGAGAAGGATCATCGTATCCATTCTCT
	** ** *
Ptr_LAC27_CDS	AAACCTGACAAGGAAAAGATCATCATATTGAGTGAATGGTGAAGGCTGATGTTGAAGCT
Ptr_LAC18_CDS	AAGCCAAAGCGTGAACACCCATACTCCTTGGTGAATGGTGGGATACGAACCCATTGAT
	** ** *
Ptr_LAC27_CDS	GTGGTCAACCAAGCAACAATGACTGGCTTGCCTCCAAATATATCAGATGCACACACCGTT
Ptr_LAC18_CDS	GTGGTGAGGGAAGCAACTAGAACCGGAGCGGCTCTAATATATCTGATGCTTATACCATT
	***** *
Ptr_LAC27_CDS	AATGGCCATACGGGGGAGTTCCCTGGCTGCACTTCTCCGGGTTTCAC---GTTACATGT
Ptr_LAC18_CDS	ACGGTCAACCTGGTGATCTTTTAACTGCTCCAGCAAAGATACTACCATCGTTCCG-AT
	** ** *
Ptr_LAC27_CDS	TGAAAGTGGCAAGACCTATTTGCTACGCATTATCAATGCAGCATTAAATGATGAACTCTT
Ptr_LAC18_CDS	CGACTCTGGTGAGACAAACCTCCTCCGAGTCATCAATGCTGCACTCAATCAACCACTTTT
	* *
Ptr_LAC27_CDS	CTTCAAGATTGCAGGGCATAATATTACCGTTGTTGAAGTGGATGCTACTTTCCTAAGCC
Ptr_LAC18_CDS	CTTCAACATAGCCAACCAAGTTTACAGTTGTCGGTGCCGATGCATCCTATCTTAAGCC
	***** ** *
Ptr_LAC27_CDS	CTTAGCACCGACACGATATTTATCGGTCCAGGCCAAACCACAAATGCCCTGCTTACTGC
Ptr_LAC18_CDS	CTTTACACCTCGGTGATCATGCTAGGACCTGGCCAAACCACAGATGTTTGTCTCTGG
	***** *
Ptr_LAC27_CDS	AGATAAAAGCATTGGCAAGTATTTAATAGCTGTCTCTCCCTTCATGGATACTGTAGTTGC
Ptr_LAC18_CDS	CGACCAACTACCTGGCCGATATTACATGGCAGCCGAGCCTATCAAAGTGCAGCAAAATGC
	* *
Ptr_LAC27_CDS	TG---TTGATAATGTGACTGCAATCGCTTCTTGCCTATAAGGGAACCCCTTGCACTTCTC
Ptr_LAC18_CDS	ACCATTTGACAATACTACCACCACTGCCATTCTTGAATACAAATCTGTTCTT-TGCCCTG
	*** ** *
Ptr_LAC27_CDS	CCCACCTGTCCTAACCACCACTCTGCTATAAACGCAACTCCGGCAACTTCA-ACTTTCA
Ptr_LAC18_CDS	CGAAGTGTACAAAGAAACCATTTATGCCACCACTTCCAG-CTTACAATGACACAGCTACA
	* *
Ptr_LAC27_CDS	TGGACAAACTTCGAAGCCTGAATTCAAAGAAGTACCC---AGCCAATGTCCCATTAACA
Ptr_LAC18_CDS	GTACAGCCTTCAGTA---GAAGCTTTAGAAGTCCTCGAAAAGTTGAGGTCCCGACCGAT
	* *
Ptr_LAC27_CDS	GTAGACCATGATCTATACTTCACGATCGGTGTCGGGATTGACCCTTGTGCAACATGTACT
Ptr_LAC18_CDS	ATTGATGAGAACCTCTTCTTCACAATCGGCCTAGGACTCAACAATTGTCCCAAAAACCTT
	* *
Ptr_LAC27_CDS	AATGGTAG-----CAAGG---CTGTGGCA-----GATATAAATAATGTT
Ptr_LAC18_CDS	AGAGCTAGGCGGTGTCAAGGACCAACGGCACTCGTTTCACTGCTAGTATGAACAATGTG
	* *
Ptr_LAC27_CDS	TCTTTTATAATGCCAACTACAGCTT---TACTTCAAGCACACTACTATAACATAAGTGGG
Ptr_LAC18_CDS	TCCTTTGTGTTTCTTCAAAGCCTCGCTATTGCAAGCATATAAGCAAAAAATTCCTGGA
	** ** *
Ptr_LAC27_CDS	GTCTTCACAGATGATTTCCAGCAAAACCACCGATTCTTTTAATTATACGGGAAATAAT
Ptr_LAC18_CDS	GTCTTCACCACCGATTTTCCAGCAAAACCACAAGTAAATTTGACTACACTGGCAATGTT
	***** *
Ptr_LAC27_CDS	ACGGCAATGAATCTAAAGACC-ACAAATGGGACTAGAGCTTATAGGCTGGCTTTTAATTC
Ptr_LAC18_CDS	A-GTCGTTCCCTCTTTCACCTGCTCGTGGAACCTAAGCTGTACAAGTTGAAGTACGGATC
	* *
Ptr_LAC27_CDS	AGCAGTTCAGTGGTGCTGCAAGGAACCACTATAATAGCACCTGAGAGTCATCCATTCCA
Ptr_LAC18_CDS	AAGGGTGCAAGTTGTGCTACAGGACACGAGCATCGTCACACCAGAAAACCATCCGATCCA
	* *
Ptr_LAC27_CDS	TCTTCATGGATTTAATTTCTTTGTTGTTGGCAAGGGAATAGGAAATTTTGATCCAGATAA
Ptr_LAC18_CDS	TCTCATGATACGACTTCTACATCATCGCTGAAGGATTTGGGAATTTCAATCCCAAAAC


```

*** ***** * **** * * * * *** * * * ***** **** * *
Ptr_LAC27_CDS      TGATCCAAAGAAATTTAATCTGGCTGATCCTGTTGAAAGGAACACAGTCAGTGACCTAC
Ptr_LAC18_CDS      TGATAAATCCAAATTC AACCTTGTTGATCCACCAATGAGGAATACAGTTGCAGTACCCGT
*** * ***** ** ** * ***** ***** *****
Ptr_LAC27_CDS      TGCCGGATGGATTGCAATCAGATTCAAGGCAGATAATCCAGGTGTTTGGTTTTGCATTG
Ptr_LAC18_CDS      GAATGGATGGGCGGTTATTAGATTGTTGCTGACAACCCAGGTGTGTGGCTAATGCACTG
***** * ** ***** ** ** * ***** ** * **** **
Ptr_LAC27_CDS      CCATTTGGAAGTACACACGACATGGGGACTAAAGATGGCATTGTGGTGGACAATGGTA-
Ptr_LAC18_CDS      TCACTTGGATGTTACATCACATGGGGTCTGGCCATGGCGTTTTTGGTAGAAGAGGGGAT
** ***** ** ***** ***** ** ***** ** * ** *
Ptr_LAC27_CDS      -AGGGCCCAATGAGTCAATACTACCACCTCCTAGTGACCTTCCAACGTGCTAG
Ptr_LAC18_CDS      TGGGATTTTACAGAGT--GTCGAGCCTCCTCCAGCCGATCTGCCAATATGTAA
** * **** * ** ***** ** ** ***** ** **

```

[clustalw.dnd](#)

(Ptr_LAC27_CDS:0.27368,Ptr_LAC18_CDS:0.27368);

C.7 Sequence similarity of the CDS region for thirteen SDX LACs which are targets of miR397a

CLUSTAL 2.1 Multiple Sequence Alignments

```
Sequence type explicitly set to DNA
Sequence format is Pearson
Sequence 1: Ptr_LAC2_Potri.001G184300.1    1731 bp
Sequence 2: Ptr_LAC15_Potri.006G097000.1    1680 bp
Sequence 3: Ptr_LAC24_Potri.009G156800.1    1731 bp
Sequence 4: Ptr_LAC40_Potri.016G112000.1    1674 bp
Sequence 5: Ptr_LAC49_Potri.006G097100.1    1680 bp
Sequence 6: Ptr_LAC18_Potri.008G073700.1    1725 bp
Sequence 7: Ptr_LAC26_Potri.010G183600.1    1728 bp
Sequence 8: Ptr_LAC7_Potri.001G401300.1     1746 bp
Sequence 9: Ptr_LAC11_Potri.006G087100.1    1743 bp
Sequence 10: Ptr_LAC12_Potri.006G087500.1    1740 bp
Sequence 11: Ptr_LAC14_Potri.006G096900.1    1677 bp
Sequence 12: Ptr_LAC23_Potri.009G156600.1    1731 bp
Sequence 13: Ptr_LAC30_Potri.011G120300.1    1746 bp
Start of Pairwise alignments
Aligning...
```

```
Sequences (1:2) Aligned. Score: 50.6548
Sequences (1:3) Aligned. Score: 98.3247
Sequences (1:4) Aligned. Score: 51.9116
Sequences (1:5) Aligned. Score: 50.8929
Sequences (1:6) Aligned. Score: 45.6232
Sequences (1:7) Aligned. Score: 44.8495
Sequences (1:8) Aligned. Score: 66.7244
Sequences (1:9) Aligned. Score: 63.8359
Sequences (1:10) Aligned. Score: 64.8758
Sequences (1:11) Aligned. Score: 50.328
Sequences (1:12) Aligned. Score: 97.9203
Sequences (1:13) Aligned. Score: 64.8758
Sequences (2:3) Aligned. Score: 50.9524
Sequences (2:4) Aligned. Score: 90.5615
Sequences (2:5) Aligned. Score: 99.2857
Sequences (2:6) Aligned. Score: 48.5119
Sequences (2:7) Aligned. Score: 48.2738
Sequences (2:8) Aligned. Score: 53.9286
Sequences (2:9) Aligned. Score: 51.4881
Sequences (2:10) Aligned. Score: 53.5119
Sequences (2:11) Aligned. Score: 98.0322
Sequences (2:12) Aligned. Score: 50.6548
Sequences (2:13) Aligned. Score: 52.2619
Sequences (3:4) Aligned. Score: 51.6726
Sequences (3:5) Aligned. Score: 50.9524
Sequences (3:6) Aligned. Score: 45.5652
Sequences (3:7) Aligned. Score: 44.9074
Sequences (3:8) Aligned. Score: 67.1288
Sequences (3:9) Aligned. Score: 64.067
Sequences (3:10) Aligned. Score: 65.2802
Sequences (3:11) Aligned. Score: 50.3876
Sequences (3:12) Aligned. Score: 99.3645
Sequences (3:13) Aligned. Score: 64.7603
Sequences (4:5) Aligned. Score: 91.0394
Sequences (4:6) Aligned. Score: 48.0884
```

Sequences (4:7) Aligned. Score: 49.1637
Sequences (4:8) Aligned. Score: 54.6595
Sequences (4:9) Aligned. Score: 50.5376
Sequences (4:10) Aligned. Score: 54.3011
Sequences (4:11) Aligned. Score: 90.9797
Sequences (4:12) Aligned. Score: 51.4337
Sequences (4:13) Aligned. Score: 53.5842
Sequences (5:6) Aligned. Score: 48.75
Sequences (5:7) Aligned. Score: 48.5119
Sequences (5:8) Aligned. Score: 53.8095
Sequences (5:9) Aligned. Score: 52.2619
Sequences (5:10) Aligned. Score: 53.75
Sequences (5:11) Aligned. Score: 98.0918
Sequences (5:12) Aligned. Score: 50.8929
Sequences (5:13) Aligned. Score: 53.0357
Sequences (6:7) Aligned. Score: 89.5072
Sequences (6:8) Aligned. Score: 45.2754
Sequences (6:9) Aligned. Score: 43.1884
Sequences (6:10) Aligned. Score: 45.1594
Sequences (6:11) Aligned. Score: 48.2409
Sequences (6:12) Aligned. Score: 45.5072
Sequences (6:13) Aligned. Score: 44.8116
Sequences (7:8) Aligned. Score: 46.5278
Sequences (7:9) Aligned. Score: 42.5347
Sequences (7:10) Aligned. Score: 45.6597
Sequences (7:11) Aligned. Score: 47.6446
Sequences (7:12) Aligned. Score: 44.9653
Sequences (7:13) Aligned. Score: 45.1968
Sequences (8:9) Aligned. Score: 65.8635
Sequences (8:10) Aligned. Score: 66.3218
Sequences (8:11) Aligned. Score: 54.3232
Sequences (8:12) Aligned. Score: 66.84
Sequences (8:13) Aligned. Score: 90.5498
Sequences (9:10) Aligned. Score: 64.0805
Sequences (9:11) Aligned. Score: 51.8187
Sequences (9:12) Aligned. Score: 63.7204
Sequences (9:13) Aligned. Score: 65.8061
Sequences (10:11) Aligned. Score: 53.8462
Sequences (10:12) Aligned. Score: 64.7025
Sequences (10:13) Aligned. Score: 66.6092
Sequences (11:12) Aligned. Score: 50.328
Sequences (11:13) Aligned. Score: 52.7132
Sequences (12:13) Aligned. Score: 65.1646
Guide tree file created: [\[clustalw.dnd\]](#)

There are 12 groups
Start of Multiple Alignment

Aligning...
Group 1: Sequences: 2 Score:31768
Group 2: Sequences: 3 Score:31461
Group 3: Sequences: 4 Score:29906
Group 4: Sequences: 2 Score:32765
Group 5: Sequences: 3 Score:32513
Group 6: Sequences: 2 Score:31179
Group 7: Sequences: 3 Score:25683
Group 8: Sequences: 6 Score:24631
Group 9: Sequences: 7 Score:25168
Group 10: Sequences: 11 Score:21423
Group 11: Sequences: 2 Score:30711
Group 12: Sequences: 13 Score:20400
Alignment Score 578831

CLUSTAL-Alignment file created [\[clustalw.aln\]](#)

[clustalw.aln](#)

CLUSTAL 2.1 multiple sequence alignment

Ptr_LAC15_Potri.006G097000.1	-----ATGGAGTACTCCAATT
Ptr_LAC49_Potri.006G097100.1	-----ATGGAGTACTCCAATT
Ptr_LAC14_Potri.006G096900.1	-----ATGGAGTACGCTTGCT
Ptr_LAC40_Potri.016G112000.1	-----ATGGAGTACTCTTGCT
Ptr_LAC24_Potri.009G156800.1	ATGGGTGCTTCAGTCCCTGCATCGCCAGAAATTCCTTGACAATTTCTACT
Ptr_LAC23_Potri.009G156600.1	ATGGGTGCTTCAGTCCCTGCATCGCCAGAAATTCCTTGACAATTTCTACT
Ptr_LAC2_Potri.001G184300.1	ATGGGTGCTTCAGTCCCTGCATCGCCAGAAATTCCTTGACAATTTCTACT
Ptr_LAC7_Potri.001G401300.1	ATGGGTGCTTCATTTCTTCCATCACCAGCATTTTC---TTGCAGTTTTTC-
Ptr_LAC30_Potri.011G120300.1	ATGGGCGCCTCTCTTCTTCTCCACCAGCATTTTC---TGGCAGTGTTTC-
Ptr_LAC11_Potri.006G087100.1	ATGGGCAACTCTCCGCGTTCCACAGTATTGCCTTCAATGGCAGCTCTGCA
Ptr_LAC12_Potri.006G087500.1	-----ATGGGTTCTGCCTTTT-TGAGGATTTTAGT
Ptr_LAC18_Potri.008G073700.1	-----ATGGAGGTTATCAACCGTATCTTTGCTAATCGCCAT
Ptr_LAC26_Potri.010G183600.1	-----ATGGAGGTTATCAAGTCTATCTTTGCTGATCGTCAT

Ptr_LAC15_Potri.006G097000.1	TGCTCATCCGTTTTATGCTTCTTGCTGTC--TGCCTCTCCCCG-CTTTG
Ptr_LAC49_Potri.006G097100.1	GGCTCATCCGTTTTATGCTTCTTGCTGTC--TGCCTCTCCCTG-CTTTG
Ptr_LAC14_Potri.006G096900.1	GGC---TCCGATTTATGCTTCTTGCTGTC--TGCCTCTCCCTG-CTTTG
Ptr_LAC40_Potri.016G112000.1	-----TCCGTTTTATGCTCCTTGCTGTC--TGCCTCTCCCTG-CTGTG
Ptr_LAC24_Potri.009G156800.1	CTTTGCTATGAGCTGCCTCTGGGCTTTCTTGAGGTGCGCCGGTG-CAAA
Ptr_LAC23_Potri.009G156600.1	CTTTGCTATGAGCTGCCTCTGGGCTTTCTTGAGGTGCGCCGGTG-CAAA
Ptr_LAC2_Potri.001G184300.1	ATTTGCTATGAGCTGCCTCTGGGCTTTCTTGAGGTGCGCCGGTG-CAAA
Ptr_LAC7_Potri.001G401300.1	---TCATCTCATTTGTCACTTTGTCTATC--CATCCAGAGCTG-CA---
Ptr_LAC30_Potri.011G120300.1	---TCTTCTCATTTGTCACTTTGTCTGTC--AATCCTGAGCCTG-CA---
Ptr_LAC11_Potri.006G087100.1	GC-TCTTGTTTTTTCTTTTCTCTTTA--GTGCCGGAATTCG-CA---
Ptr_LAC12_Potri.006G087500.1	CACTGCGTGTGTGCTCTTTGGATTTTCTCTGAGCTGGTAGTTG-CAAA
Ptr_LAC18_Potri.008G073700.1	TGTTCTTTCTTCTTGCTC--CTGCTTCT--GGCCTCAGCAATGTCATTA
Ptr_LAC26_Potri.010G183600.1	TGTTCTTTCTTCTTGCTGCTGCTTCT--AGCCTCAACAATGTCATTA

* * *

Ptr_LAC15_Potri.006G097000.1	GTCGAGTGCAGGATTCGGCATTACAAGTTCAATGTGGTGATGAAAAATAC
Ptr_LAC49_Potri.006G097100.1	GTCGAGTGCAGGATTCGGCATTACAAGTTCAATGTGGTGATGAAAAATAC
Ptr_LAC14_Potri.006G096900.1	GTCAGTGCAGGATTCGGCATTACAAGTTCAATGTGGTGATGAAAAATAC
Ptr_LAC40_Potri.016G112000.1	GTCGAGTGCAGGATTCGGCATTACAAGTTCAATGTGGTGATGAAAAATAC
Ptr_LAC24_Potri.009G156800.1	CATGCAGGCATTACGAGGCACTACAAGTTCAACATAAACTGACAAATGT
Ptr_LAC23_Potri.009G156600.1	CATGCAGGCATTACGAGGCACTACAAGTTCAACATAAACTGACAAATGT
Ptr_LAC2_Potri.001G184300.1	CATGCAGGCATTACGAGGCACTACAAGTTCAACATAAACTGAAAAATGT
Ptr_LAC7_Potri.001G401300.1	---CTTGCCATTACGAGGCACTACAAGTTGATGTTATGTTGACAAAAATGT
Ptr_LAC30_Potri.011G120300.1	---CTTGCCATTACTAGGCACTACAAGTTGATGTTATGTTGACAAAAATGT
Ptr_LAC11_Potri.006G087100.1	---GCGGCCATAACAGGCACTACACGTTCAATATCACACACAAGAAATTT
Ptr_LAC12_Potri.006G087500.1	CATGCAGGCATTACTAGACATTACAAGTTTGACATCAAGTTGCAAAATGT
Ptr_LAC18_Potri.008G073700.1	GCTATTGCAAAACCCACCACTGATTTTGTGTTCAAGCAACAAAGT
Ptr_LAC26_Potri.010G183600.1	GCGATTGCAGAGATCCACCACCATGATTTTGTGTTCAAGCAACGAAAGT

** * ** *

Ptr_LAC15_Potri.006G097000.1	TACCAGACTATGCTCTAGGAAGCCGATTGTTACCGTCAATGGCCGCTTCC
Ptr_LAC49_Potri.006G097100.1	TACCAGACTATGCTCTAGGAAGCCGATTGTTACCGTCAATGGCCGCTTCC
Ptr_LAC14_Potri.006G096900.1	TACCAGACTATGCTCTAGGAAGCCGATTGTTACCGTCAATGGCCGCTTCC
Ptr_LAC40_Potri.016G112000.1	TACCAGACTATGCTCAAGCAAGCCGATTGTTACCGTCAATGGCCGCTTCC
Ptr_LAC24_Potri.009G156800.1	CACCCGCTTGTCACACTAAGAGCATGGTGACTGTTAATGGCAAGTTCC
Ptr_LAC23_Potri.009G156600.1	CACCCGCTTGTCACACTAAGAGCATGGTGACTGTTAATGGCAAGTTCC
Ptr_LAC2_Potri.001G184300.1	CACCCGCTTGTCACACTAAGAGCATGGTGACTGTTAATGGCAAGTTCC
Ptr_LAC7_Potri.001G401300.1	GACCCGCTTTGCCACACCAAGAGCATGTAACAGTCAACGGGAAGTTCC
Ptr_LAC30_Potri.011G120300.1	GACACGCTTTGCCATACCAAGAGCATGGTTACAGTCAATGGGAAGTTCC
Ptr_LAC11_Potri.006G087100.1	TACGAGATTATGCCACACAAGGAGCTAGTGACAGTAAATGGACAGTTCC
Ptr_LAC12_Potri.006G087500.1	GACGAGGTTGTGCCGACAAAGAGCATCGTTACCGTCAATGGTCAGATCC
Ptr_LAC18_Potri.008G073700.1	GAAAAGGCTATGCAAAACCCACAACAGCATCACAGTAAACGGAATGTTCC
Ptr_LAC26_Potri.010G183600.1	GAAGAGGCTGTGCAAAACCCACAATAGCATCACAGTCAATGGGATGTTCC

* * * * *

Ptr_LAC15_Potri.006G097000.1	CAGGACCCACTCTGTATGCCAGAGAACACGATACAGTTCTTGTAAGTT
Ptr_LAC49_Potri.006G097100.1	CAGGACCCACTCTGTATGCCAGAGAACACGATACAGTTCTTGTAAGTT
Ptr_LAC14_Potri.006G096900.1	CAGGACCCACTCTGTATGCCAGAGAACACGATACAGTTCTTGTAAGTT
Ptr_LAC40_Potri.016G112000.1	CAGGACCAACTCTGTATGCCAGGGAAGACGATACAGTTCTTGTAAGTT
Ptr_LAC24_Potri.009G156800.1	CGGGGCTCGCGTAGTTGCCAGAGAAGGTGACCGTTTAGTGGTGAAGGTG
Ptr_LAC23_Potri.009G156600.1	CGGGGCTCGCGTAGTTGCCAGAGAAGGTGACCGTTTAGTGGTGAAGGTG
Ptr_LAC2_Potri.001G184300.1	CGGGGCTCGCGTAGTTGCCAGAGAAGGTGACCGTTTAGTGGTGAAGGTG
Ptr_LAC7_Potri.001G401300.1	CGGGGCTCGCATGTTGCTAGGAGGGTGATCGTCTTCTTATTAAGTT

Ptr_LAC30_Potri.011G120300.1	CGGGGCCCTCGCATTGTAGCTAGAGAGGGTGATCGTCTTGTTATTAGAATG
Ptr_LAC11_Potri.006G087100.1	CAGGTCCTCGCCTGGTAGCAAGGGAAGGTGATCAAGTTCTAGTTAAGGTG
Ptr_LAC12_Potri.006G087500.1	CTGGGCCCTCGAATCATAGCAAGGGAAGGGGATCGGCTCTTGATCAAGGTC
Ptr_LAC18_Potri.008G073700.1	CGGGGCCAACCTTGGAAAGTGAAGAACGGAGACACTCTAGTTGTAAAAGTT
Ptr_LAC26_Potri.010G183600.1	CAGGGCCAACCCTGGAAGTGAAAAATGGAGACACTCTAGTTGTAAAAGTT
	* * * * *
Ptr_LAC15_Potri.006G097000.1	GTTAACCATGTCAAATATAATGTTTCTATTCACTGGCATGGCATTAGGCA
Ptr_LAC49_Potri.006G097100.1	GTTAACCATGTCAAATATAATGTTTCTATTCACTGGCATGGCATTAGGCA
Ptr_LAC14_Potri.006G096900.1	GTTAACCATGTCAAATATAATGTTTCTATTCACTGGCATGGCATTAGGCA
Ptr_LAC40_Potri.016G112000.1	GTTAACCGTGTCAAATATAATTTGTCTATCCACTGGCATGGCATTAGGCA
Ptr_LAC24_Potri.009G156800.1	GTCAATCATGTTCCAAATAATATCAGCATCCATTGGCATGGAATTCGACA
Ptr_LAC23_Potri.009G156600.1	GTCAATCATGTTCCAAATAATATCAGCATCCATTGGCATGGAATTCGACA
Ptr_LAC2_Potri.001G184300.1	GTCAATCATGTTCCAAATAATATCAGCATCCATTGGCATGGAATTCGACA
Ptr_LAC7_Potri.001G401300.1	GTTAACCATGTCCAAAACAATATCTCTATTCACTGGCATGGAATTCGGCA
Ptr_LAC30_Potri.011G120300.1	GTCAACCATGTCCAAAACAATATCTCTATTCACTGGCATGGGATTGACGA
Ptr_LAC11_Potri.006G087100.1	GTCAATCATGTTGCTGAAAATATTACCATTCACTGGCATGGAGTAAGGCA
Ptr_LAC12_Potri.006G087500.1	GTTAACCATGTCCAAACAATGTGACACTCCATTGGCATGGAATCAGGCA
Ptr_LAC18_Potri.008G073700.1	GTGAACAGAGCCGATATAATGTTACCATTCACTGGCAGCGTATTAGGCA
Ptr_LAC26_Potri.010G183600.1	GTCAACAAAGCCGATACACGTTACCATTCACTGGCATGGTATTAGGCA
	** * * * *
Ptr_LAC15_Potri.006G097000.1	ACTAAGAACGGGCTGGGCTGATGGACCAGCATACATTACACAATGCCCCA
Ptr_LAC49_Potri.006G097100.1	ACTAAGAACGGGCTGGGCTGATGGACCAGCATACATTACACAATGCCCCA
Ptr_LAC14_Potri.006G096900.1	ACTAAGAACGGGCTGGGCTGATGGACCAGCATACATTACACAATGCCCCA
Ptr_LAC40_Potri.016G112000.1	ATTAAGAACGGGTTGGGCTGATGGACCAGCATACATTACACAATGCCCTA
Ptr_LAC24_Potri.009G156800.1	ACTTCAAAGTGGATGGGCAGATGGACCAGCATACATCACGCAATGCCCTA
Ptr_LAC23_Potri.009G156600.1	ACTTCAAAGTGGATGGGCAGATGGACCAGCATACATCACGCAATGCCCTA
Ptr_LAC2_Potri.001G184300.1	ACTTCAAAGTGGATGGGCAGATGGACCAGCATACATCACGCAATGCCCTA
Ptr_LAC7_Potri.001G401300.1	GCTTCGAAGTGGGTTGGGCTGATGGACCGGCATACATAACTCAGTGCCCTA
Ptr_LAC30_Potri.011G120300.1	GCTTCGCAGCGGGTGGGCGACGGACAGCATACGTAACCTCAGTGCCCTA
Ptr_LAC11_Potri.006G087100.1	GCTAACAAAGCGGATGGGCAGATGGTCCAGCTTACGTAACACAATGTCCCA
Ptr_LAC12_Potri.006G087500.1	ACTAAGGAGCGGATGGGCAGATGGACCTGCCTACGTAACACAGTGCCCAA
Ptr_LAC18_Potri.008G073700.1	AATGAGAACGGGCTGGGCAGATGGGCCAGAATTTGTGACACAATGCCCAA
Ptr_LAC26_Potri.010G183600.1	AATGAGAACGGGCTGGGCAGATGGGCCAGAATTTGTGACACAATGCCCAA
	* * * * *
Ptr_LAC15_Potri.006G097000.1	TTAGCCAGGGCAAAGCTATGTCTACAATTTACAATCACTGGTCAGAGG
Ptr_LAC49_Potri.006G097100.1	TTAGCCAGGGCAAAGCTATGTCTACAATTTACAATCACTGGTCAGAGG
Ptr_LAC14_Potri.006G096900.1	TTAGCCAGGGCAAAGCTATGTCTACAATTTACAATCACTGGTCAGAGG
Ptr_LAC40_Potri.016G112000.1	TTAGCCAGGGCAAAGCTATGTCTACAATTTACTACTACAGGGCAGAGA
Ptr_LAC24_Potri.009G156800.1	TTCAAACAACACAGACTTATGTTTACAACCTTCACTATTACAGGACAAGA
Ptr_LAC23_Potri.009G156600.1	TTCAAACAACACAGACTTATGTTTACAACCTTCACTATTACAGGACAAGA
Ptr_LAC2_Potri.001G184300.1	TTCAAACAACACAGACTTATGTTTACAACCTTCACTGTTACAGGACAAGA
Ptr_LAC7_Potri.001G401300.1	TACAAACTGGACAGAGCTATGTGTACAACCTACACCATCGTAGGACAAGA
Ptr_LAC30_Potri.011G120300.1	TACAAACTGGACAAGCTATGTGTACAACCTACACCATGTAGGACAGAGA
Ptr_LAC11_Potri.006G087100.1	TACAAACTGGCCAGGCATATACGTACAATTTACACCTCACTGGGCAGAGA
Ptr_LAC12_Potri.006G087500.1	TTCAAACGGGACAGAGCTATGTATACAATTTACAGTTACTGGACAAGA
Ptr_LAC18_Potri.008G073700.1	TTAGACCAGGTGGAAGTTACACCTACAGGTTTACTATTGAAGGACAAGAA
Ptr_LAC26_Potri.010G183600.1	TCAGACCAGGTGGGAGTTATACCTACAGGTTTAAATATTGAAGGACAAGAA
	* * * * *
Ptr_LAC15_Potri.006G097000.1	GGCACACTTCTTTGGCAGCAGACATATTCTCTGGCTAAGGGCCACAGTTCA
Ptr_LAC49_Potri.006G097100.1	GGCACACTTCTTTGGCAGCAGACATATTCTCTGGCTAAGGGCCACAGTTCA
Ptr_LAC14_Potri.006G096900.1	GGCACACTTCTTTGGCAGCAGACATATTCTCTGGCTAAGGGCCACAGTTCA
Ptr_LAC40_Potri.016G112000.1	GGCACACTTCTTTGGCATGCACATATTCTCTGGCTAAGAGCCACTGTCCA
Ptr_LAC24_Potri.009G156800.1	GGAACTCTCTTCTGGCATGCTCACCTCTCATGGCTTAGAGCTTCTGTCTA
Ptr_LAC23_Potri.009G156600.1	GGAACTCTCTTCTGGCATGCTCACCTCTCATGGCTTAGAGCTTCTGTCTA
Ptr_LAC2_Potri.001G184300.1	GGAACTCTCTTCTGGCATGCTCACCTCTCATGGCTTAGAGCTTCTGTCTA
Ptr_LAC7_Potri.001G401300.1	GGCACTCTTTGGTGGCATGCTCATATATCATGGCTAAGATCAACTCTCTA
Ptr_LAC30_Potri.011G120300.1	GGCACTCTTTGGTGGCATGCCCATATATCATGGTTAAGATCAACTCTCCA
Ptr_LAC11_Potri.006G087100.1	GGAACTCTTGTGGCATGCGCACATTTTATGGCTAAGATCATCACTCTA
Ptr_LAC12_Potri.006G087500.1	GGGACATTGTTCTGGCATGCTCATTTTATGGTTAAGAGCTACCCTTTA
Ptr_LAC18_Potri.008G073700.1	GGAACTCTTTGGTGGCATGCTCATAGCTCTTGGCTTAGAGCCACTGTTTA
Ptr_LAC26_Potri.010G183600.1	GGGACACTTTGGTGGCATGCTCATAGCTCTTGGCTTAGAGCCACTGTTTA
	* * * * *
Ptr_LAC15_Potri.006G097000.1	TGGTGCCCTGGTTGTCTTGCTTAAACGCGGCATCCCTACCCATTCCCAG
Ptr_LAC49_Potri.006G097100.1	TGGTGCCATGGTTGTCTTGCTTAAACGCGGCATCCCTACCCATTCCCAG
Ptr_LAC14_Potri.006G096900.1	TGGTGCCATGGTTGTCTTGCTTAAACGCGGCATCCCTACCCATTCCCAG

Ptr_LAC40_Potri.016G112000.1	CGGTGCATTAGTTGTCTTGCCTAAGCTCGGTGTCCCTACCCATTCCCAG
Ptr_LAC24_Potri.009G156800.1	TGGACCCCTTATCATCTTACCTAAGCGCAACGTTTCTTACCCATTTGCCA
Ptr_LAC23_Potri.009G156600.1	TGGACCCCTTATCATCTTACCTAAGCGCAACGTTTCTTACCCATTTGCCA
Ptr_LAC2_Potri.001G184300.1	TGGACCCCTTATCATCTTACCTAAGCGCAACGTTTCTTACCCATTTGCCA
Ptr_LAC7_Potri.001G401300.1	CGGTCCACTTATCATTCTTCCCAAACCTCGGCACTACTTATCCATTGTGTA
Ptr_LAC30_Potri.011G120300.1	CGGTCCAATAATCCTTCTTCCCAAACCTCGGCACACCTTACCCATTTGCTA
Ptr_LAC11_Potri.006G087100.1	TGGACCCATTATTATTCTACCAAAGCTCAACGAGTCTTACCCATTGAAGA
Ptr_LAC12_Potri.006G087500.1	TGGACCCATTGTATCCTCCCTAAGAAAGGCGTTTCTTACCCATTTCAC
Ptr_LAC18_Potri.008G073700.1	TGGTGTCTAATCATCCATCCAAGAGAAGGATCATCGTATCCATTCTCTA
Ptr_LAC26_Potri.010G183600.1	TGGTGTCTAATTATCCATCCAAGAGAAGGATCTCGTATCCGTTGCTA
	** * * * * * * * * * * *
Ptr_LAC15_Potri.006G097000.1	CTCCTCATAAAGAAGTGCTTGTGTATTAGCTGAATGGTGGAAATCAGAT
Ptr_LAC49_Potri.006G097100.1	CTCCTCATAAAGAAGTGCTTGTGTATTAGCTGAATGGTGGAAATCAGAT
Ptr_LAC14_Potri.006G096900.1	GTCTCATAAAGAAGTGCTTGTGTATTAGCTGAATGGTGGAAATCAGAT
Ptr_LAC40_Potri.016G112000.1	CTCCCCACAAGGAAGTAGTTGTTGTTTATAGCGGAATGGTGGAAATCAGAT
Ptr_LAC24_Potri.009G156800.1	AACCCCAACAAGGAAGTGACTATCATGTTGGGAGAGTGGTTCAATGCTGAT
Ptr_LAC23_Potri.009G156600.1	AACCCCAACAAGGAAGTGACTATCATGTTGGGAGAGTGGTTCAATGCTGAT
Ptr_LAC2_Potri.001G184300.1	AACCCCAACAAGGAAGTGACTATCATGTTGGGAGAGTGGTTCAATGCTGAT
Ptr_LAC7_Potri.001G401300.1	AACCTCACAAGGAAGTCCCAATCATCTTCGGAGAGTGGTTCAATGTCAGAT
Ptr_LAC30_Potri.011G120300.1	AACCTTACAAGGAGGTTCCCAATCATCTTTGGGAGTGGTTTAAATGTCAGAT
Ptr_LAC11_Potri.006G087100.1	AACCTCACAAGGAATCCCATCTTTTGGGAGATGGTTTAAATGTAGAC
Ptr_LAC12_Potri.006G087500.1	TACCTCACAAGAAGTCCCTATCATTTTGGGAGATGGTGGAAAGCAGAC
Ptr_LAC18_Potri.008G073700.1	AGCCAAAGCGTGAACACCCATACTCCTTGGTGAATGGTGGGATACGAAC
Ptr_LAC26_Potri.010G183600.1	AGCCAAAGCGTGAACACCCATACTTCTTGGCGAATGGTGGGATGCGAAT
	** * * * * * * * * * * *
Ptr_LAC15_Potri.006G097000.1	ACTGAAGCTGTGATCAACGAGGCTCTTAAATCTGGATTAGCTCCAAATGT
Ptr_LAC49_Potri.006G097100.1	ACTGAAGCTGTGATCAACGAGGCTCTTAAATCTGGATTAGCTCCAAATGT
Ptr_LAC14_Potri.006G096900.1	ACTGAAGCTGTGATCAACGAGGCTCTTAAATCTGGATTAGCTCCAAATGT
Ptr_LAC40_Potri.016G112000.1	ACCGAAGCTGTGATCAATGAAGCTCTTAAATCCGATTAGCACCAATGT
Ptr_LAC24_Potri.009G156800.1	ACTGAGGCAGTGATTAGCCAGGCTTTACAGACAGGAGGGGGGCCAAATGT
Ptr_LAC23_Potri.009G156600.1	ACTGAGGCAGTGATTAGCCAGGCTTTACAGACAGGAGGGGGGCCAAATGT
Ptr_LAC2_Potri.001G184300.1	ACTGAGGCAGTGATTAGCCAGGCTTTACAGACAGGAGGGGGGCCAAATGT
Ptr_LAC7_Potri.001G401300.1	CCAGAGGCAATCATTAAACAGGCAATGCAACCGGTTGGAGGCCAAATGT
Ptr_LAC30_Potri.011G120300.1	CCAGAGGCGATCATTAGTCAGGCAATGCAAACTGGTGGAGGCCAAATGT
Ptr_LAC11_Potri.006G087100.1	CCAGAAGCTGTAATTGCCAGGCTCTTACAGACGGGGCAGGTCCAAATGT
Ptr_LAC12_Potri.006G087500.1	ACGGAGAAAAATTATCAGCCAGGCTTGAAAAACAGGAGGAGCGCCAAATAT
Ptr_LAC18_Potri.008G073700.1	CCTATTGATGTGGTGAGGGAAGCAACTAGAACCAGGAGCGGCTCCTAATAT
Ptr_LAC26_Potri.010G183600.1	CCCGTTGATGTGGTGAGGAGGCAACTAGAACTGGAGCAGTCCAAATAT
	* * * * * * * * * * *
Ptr_LAC15_Potri.006G097000.1	CTCTGATGCTCACACAATTAACGGCCATCCAGGAGCTGTCTCAGCTTGTT
Ptr_LAC49_Potri.006G097100.1	CTCTGATGCTCACACAATTAACGGCCATCCAGGAGCTGTCTCAGCTTGTT
Ptr_LAC14_Potri.006G096900.1	CTCTGATGCTCACACAATTAATGGCCATCCAGGAGCTGTCTCAACTTGTT
Ptr_LAC40_Potri.016G112000.1	CTCTGATGCTCACACAATTAATGGGCATCCAGGAGCTGTCTCAACTTGTT
Ptr_LAC24_Potri.009G156800.1	CTCTGAAGCCTACACCTTTAATGGACTTCCAGGTCCATTGTACAATTGCT
Ptr_LAC23_Potri.009G156600.1	CTCTGAAGCCTACACCTTTAATGGACTTCCAGGTCCATTGTACAATTGCT
Ptr_LAC2_Potri.001G184300.1	CTCTGAAGCCTACACCTTTAATGGACTTCCAGGTCCACTGTACAATTGCT
Ptr_LAC7_Potri.001G401300.1	CTCTGATGCCTACACTATCAATGGATTTCAGGGCCGTTGTATAACTGCT
Ptr_LAC30_Potri.011G120300.1	CTCTGATGCTTATACTATCAATGGACTTCCAGGGCCATTGTATAACTGCT
Ptr_LAC11_Potri.006G087100.1	TTGAGATGCGTACACCATCAATGGCTTCCAGGGCCTTTATACAATTGTT
Ptr_LAC12_Potri.006G087500.1	TTCTGATGCCTACACCATTAATGGTCATCTGGACTTTGTATAACTGTT
Ptr_LAC18_Potri.008G073700.1	ATCTGATGCTTATACCATTAACGGTCAACCTGGTGATCTTTTAACTGCT
Ptr_LAC26_Potri.010G183600.1	ATCTGATGCATATACCATTAAATGGTCAACCTGGTGATCTTTATAATTGTT
	** * * * * * * * * * * *
Ptr_LAC15_Potri.006G097000.1	CTTCACAGGGCGGTTTCACATTGCCAGTCAAAAGTGGCGAGACCTACATG
Ptr_LAC49_Potri.006G097100.1	CTTCACAGGGCGGTTTCACATTGCCAGTCAAAAGTGGCGAGACCTACATG
Ptr_LAC14_Potri.006G096900.1	CTTCACAGGGCGGTTTCACATTGCCAGTCAAAAGTGGCGAGACCTACATG
Ptr_LAC40_Potri.016G112000.1	CTTCACAAGGCGGTTTCACTTTGCCAGTCCAAAGTGGCAAGACCTACATG
Ptr_LAC24_Potri.009G156800.1	CAGAAAAATAATACATAACAAGCTAAAGGTGAAGCCAGGAAAGACGTATCTT
Ptr_LAC23_Potri.009G156600.1	CAGAAAAATAATACATAACAAGTAAAGGTGAAGCCAGGAAAGACGTATCTT
Ptr_LAC2_Potri.001G184300.1	CAGAAAAATAATACATAACAAGCTAAAGGTGAAGCCAGGAAAGACATATCTT
Ptr_LAC7_Potri.001G401300.1	CTGCCAAAGATACTTTCAAGCTGAAGGTAAAGCCAGGAAAGACTTACCTT
Ptr_LAC30_Potri.011G120300.1	CTGCCAAAGATACTTTCAAGCTGAAGGTAAAGCCAGGAAAGACTTACCTT
Ptr_LAC11_Potri.006G087100.1	CTGCTAAAGATACATACAAACGAAGGTAAAGCCGGGAAAAACCTACCTT
Ptr_LAC12_Potri.006G087500.1	CAGCCAAAGATACATTCAAGCTCAAGGTGAAGCCAGGAAAAACCTATCTC
Ptr_LAC18_Potri.008G073700.1	CCAGCAAAGATACTACCATCGTTCGATCGACTCTGGTGAGACAAACCTC
Ptr_LAC26_Potri.010G183600.1	CCAGCGAAGATACTACCATCGTTCGATCGCTCTGGTGAGACAAACCTC

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*      *      **      *      *      **      *      *      *
Ptr_LAC15_Potri.006G097000.1      CTACGGTTGATCAATGCTGCACTCAATGAAGAGCTCTTCTTCAAAATTGC
Ptr_LAC49_Potri.006G097100.1      CTACGGTTGATCAATGCTGCACTCAATGAAGAGCTCTTCTTCAAAATTGC
Ptr_LAC14_Potri.006G096900.1      CTACGGTTGATCAATGCTGCACTCAATGAAGAGCTCTTCTTCAAAATTGC
Ptr_LAC40_Potri.016G112000.1      CTACGGTGATCAATGCTGCACTCAATGAGGAGCTCTTCTTCAAAATTGC
Ptr_LAC24_Potri.009G156800.1      CTCCGATTGATCAATGCTGCACTCAATGATGACCTCTTTTTCAGCATTGC
Ptr_LAC23_Potri.009G156600.1      CTCCGATTGATCAATGTTGCACTCAATGATGACCTATTTTTCAGCATTGC
Ptr_LAC2_Potri.001G184300.1      CTCCGATTGATCAATGCTGCACTCAATGATGACCTCTTTTTAGCATTGC
Ptr_LAC7_Potri.001G401300.1      CTTGCGATGATCAACGCGCACTCAATGACGAGCTCTTCTTCAGCATAGC
Ptr_LAC30_Potri.011G120300.1      CTTGCGATGATCAATGCTGCCCTCAATGACGAGCTCTTCTTCAGCATAGC
Ptr_LAC11_Potri.006G087100.1      CTCCGACTAATCAACGCTGCACTCAACGACGAGCTCTTTTTCAGCATTGC
Ptr_LAC12_Potri.006G087500.1      CTTGTTTAATCAATGCTGCACTTAACGACGAGCTCTTCTTCAGCATTGC
Ptr_LAC18_Potri.008G073700.1      CTCCGAGTCATCAATGCTGCACTCAATCAACCATTCTTCCACCATAGC
Ptr_LAC26_Potri.010G183600.1      CTTGAGTCATCAATGCTGCACTCAATCAACGCTTTTCTTACAATAGC
** * * * * * * * * * * * * * * * * * * * * * * * * * * * *

Ptr_LAC15_Potri.006G097000.1      AGGGCATAAGCTTACAGTCGTGAAAGTGGATGCCACCTATGTTAAACCAT
Ptr_LAC49_Potri.006G097100.1      AGGGCACAAGCTTACAGTCGTGAAAGTGGATGCCACCTATGTTAAACCAT
Ptr_LAC14_Potri.006G096900.1      AGGGCATAAGCTTACAGTCGTGAAAGTGGATGCCACCTATGTTAAACCAT
Ptr_LAC40_Potri.016G112000.1      AGGGCACAAGCTTACTGTCGTGGAAGTTGATGCCACCTACGTTAAACCTT
Ptr_LAC24_Potri.009G156800.1      AAACCACACCTTACCCTGTTGTTGAAGTGGATGCTACTTACGCGAAGCCTT
Ptr_LAC23_Potri.009G156600.1      AAACCACACCTTACCCTGTTGTTGAAGTGGATGCTACTTACGCGAAGCCTT
Ptr_LAC2_Potri.001G184300.1      AAACCACACCTTACCCTGTTGTTGAAGTGGATGCTACTTACGCGAAGCCTT
Ptr_LAC7_Potri.001G401300.1      AAACCATACACTCACAGTTGTTGATGTTGATGCTATTTATGTGAACCAT
Ptr_LAC30_Potri.011G120300.1      AAACCACACAGTCACAGTTGTTGATGTTGATGCTGTTTATGTCAAGCCAT
Ptr_LAC11_Potri.006G087100.1      GAACCACACTCTCACCCTGTTGAAAGTGGATGCTGTTTATGTCAAGCCTT
Ptr_LAC12_Potri.006G087500.1      AAACCACAGCCTCACTGTGGTTGAAAGTGGATGCTGTTTATGTCAAGCCTT
Ptr_LAC18_Potri.008G073700.1      CAACCACAAGTTTACAGTTGTCGGTGCCGATGCATCCTATCTTAAAGCCTT
Ptr_LAC26_Potri.010G183600.1      CAATCACAAGTTCACAGTTATTGGTGCTGATGCCTCTTATCTTAAACCTT
* * * * * * * * * * * * * * * * * * * * * * * * * * * *

Ptr_LAC15_Potri.006G097000.1      TCAAAACCGATACAGTCCTAATTGCCCCAGGCCAGACCACCAATGTCCTT
Ptr_LAC49_Potri.006G097100.1      TCAAAACCGATACAGTCCTAATTGCCCCAGGCCAGACCACCAATGTCCTT
Ptr_LAC14_Potri.006G096900.1      TCAAAACCGATACAGTCCTAATTGCCCCAGGCCAGACCACCAATGTCCTT
Ptr_LAC40_Potri.016G112000.1      TCAAAACTGATACAGTCCTAATTGCCCCAGGCCAGACCACCAATGTCCTC
Ptr_LAC24_Potri.009G156800.1      TTGAGACTAACCTCTTGGTTATCACAGCAGGACAGACCACCAATGTTCTT
Ptr_LAC23_Potri.009G156600.1      TTGAGACCAACCTCTTGGTTATCACAGCAGGACAGACCACCAATGTTCTT
Ptr_LAC2_Potri.001G184300.1      TTGAGACCAACCTCTTGGTTATAACAGCAGGACAGACCACCAATGTTCTT
Ptr_LAC7_Potri.001G401300.1      TTGATACCGAGACACTTCTCATTGCTCCTGGACAAACCACCAATGTTCTT
Ptr_LAC30_Potri.011G120300.1      TTGATGCCGAGACACTTCTCATTACCCTTGGACAAACCACCAATGTTCTT
Ptr_LAC11_Potri.006G087100.1      TTGAAGCTGACACGCTCCTCATCAGTCCAGGCCAAACTACCAATGTTCTA
Ptr_LAC12_Potri.006G087500.1      TTAAACCCACATTTGACTCATCACCCGGGACAAACCACCAATGTTCTA
Ptr_LAC18_Potri.008G073700.1      TTACCACCTCGGTATCATGCTAGGACCTGGCCAAACCACAGATGTTTTG
Ptr_LAC26_Potri.010G183600.1      TTACTACCTCGGTATAATGCTAGGACCCAGGCCAAACCACCTGATGTTTTG
*      *      *      *      *      *      *      *      *

Ptr_LAC15_Potri.006G097000.1      GTCACAGCTAACAAAAATACAGGCAA-----GTACTTGTTGCTGCCTC
Ptr_LAC49_Potri.006G097100.1      GTCACAACCTAACAAAAATACAGGCAA-----GTACTTGTTGCTGCCTC
Ptr_LAC14_Potri.006G096900.1      GTCACAACCTAACAAAAATACAGGCAA-----GTACTTGTTGCTGCCTC
Ptr_LAC40_Potri.016G112000.1      GTCACAACCTAACAAAAATACAGGCAA-----GTACTTGTTGCTGCATC
Ptr_LAC24_Potri.009G156800.1      CTGAAGGCCAAACCCATTGCCCCCAATGCATCATTTACATGTTAGCAAG
Ptr_LAC23_Potri.009G156600.1      CTGAAGGCCAAACCCATTGCCCCCAATGCATCATTTACATGTTAGCAAG
Ptr_LAC2_Potri.001G184300.1      CTAAAGACCAAGCCCCACCAACCAACGCCAGTTTTTTCATGCTGCTAG
Ptr_LAC7_Potri.001G401300.1      CTGAAAACCAAAACCCGACTACCCAAATGCCCAATTTTTCATGCTGCTAG
Ptr_LAC30_Potri.011G120300.1      CTCAAAACCAAGCCACATCTCCCTAATGCAACCTTTTACATGTTTGGCGG
Ptr_LAC11_Potri.006G087100.1      CTCATGGCCAAAGCTAAGGCCCTTAATTCCACCTTTCTTATGGCCGCTAG
Ptr_LAC12_Potri.006G087500.1      ATCTCTGGCGACCAACTACCTGGCCG-----ATATTACATGGCAGCCG
Ptr_LAC18_Potri.008G073700.1      ATCTCAGGCGACCAACTACCTGGCCG-----ATATTACATGGCAGCTCG
*      *      *      *      *      *      *      *      *

Ptr_LAC15_Potri.006G097000.1      CCCGTTTCATGGATGCTCCAATTGCT--GTGGACAACATGACAGCAACAG
Ptr_LAC49_Potri.006G097100.1      CCCGTTTCATGGATGCTCCAATTGCT--GTGGACAACATGACAGCAACAG
Ptr_LAC14_Potri.006G096900.1      CCCGTTTCATGGATTCTCCAATTGCT--GTGGACAACATGACAGCAACAG
Ptr_LAC40_Potri.016G112000.1      CCCATTTCATGGATGCTCCAATTGCT--GTAGACAACATGACTGCAACAG
Ptr_LAC24_Potri.009G156800.1      ACCATACTTTACTGGCCAAGGCACA--TTTGACAACACAACCTGTTGCAG
Ptr_LAC23_Potri.009G156600.1      ACCATACTTTACTGGCCAGGGCACA--TTTGACAACACAACCTGTTGCAG
Ptr_LAC2_Potri.001G184300.1      ACCATACTTTACTGGCCAAGGCACA--TTTGACAACACAACCTGTTGCAG
Ptr_LAC7_Potri.001G401300.1      ACCTTACGTGACTGGTCAAGGAAC--TTTGATAATTCAACTGTTGCTG

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Ptr_LAC30_Potri.011G120300.1	ACCTTACGCGACTGGTCAAGGAAGCT---TTCGATAATTCAACTGTTGCTG
Ptr_LAC11_Potri.006G087100.1	GCCTTATTTCTCAGGCATGGGAAGC---TTTGACAACTCTACCACTGCTG
Ptr_LAC12_Potri.006G087500.1	GCCATATGCCACCGGCCAGGCTCC---TTTGACAATAACAACCACTGCTG
Ptr_LAC18_Potri.008G073700.1	AGCCTATCAAAGTGCACAAATGCACCAATTTGACAATACTACCACCACTG
Ptr_LAC26_Potri.010G183600.1	AGCTTATCAGAGTGCACAAATGCACCATTGACAATACTACAACCACTG
	* * * * *
Ptr_LAC15_Potri.006G097000.1	CCACTTTGCAGTATTTCAGGAGCACTTGCTA-----ACTCCC-----
Ptr_LAC49_Potri.006G097100.1	CCACTTTGCAGTATTTCAGGAGCACTTGCTA-----ACTCCC-----
Ptr_LAC14_Potri.006G096900.1	CCACTTTGCAGTATTTCAGGAGCACTTGCTA-----ACTCCC-----
Ptr_LAC40_Potri.016G112000.1	CCACTTTACACTATTTCAGGAGCACTTTCTA-----ACTCCC-----
Ptr_LAC24_Potri.009G156800.1	GGATACTCGAGTATGAAACTTCTTCAA-----ATTCAA-----
Ptr_LAC23_Potri.009G156600.1	GGATACTCGAGTATGAAACTTCTTCAA-----ATTCAA-----
Ptr_LAC2_Potri.001G184300.1	GGATACTCGAGTATGAAACTTCTTCAA-----ATTCAA-----
Ptr_LAC7_Potri.001G401300.1	GAATCTTAGAATATGAAGAAATCAAAACAAACCAATTAATCAAGCCACTCC
Ptr_LAC30_Potri.011G120300.1	GCATTTTAGAATATGAAGTGCACAAACAAACCAAGTCAATCAAAACCACTCC
Ptr_LAC11_Potri.006G087100.1	GTGTCTCTGGTTTATAAACATCCATCTAGTAATAATCACTTGA-----
Ptr_LAC12_Potri.006G087500.1	GAATACTCGAATATGACCAGAACCTTCTGCAACAACTCCAAAGCAAG
Ptr_LAC18_Potri.008G073700.1	CCATTCTTGAATACAAATCTGTTCTTTGCCCTGCGAAGTGATC-----
Ptr_LAC26_Potri.010G183600.1	CCATTCTTGAATACAAATCTGCCCTTTGCCCTGCTAAGTGATC-----
	* ** * *
Ptr_LAC15_Potri.006G097000.1	-----CTACAACTCTCACCA-CCCCACCTCCAAAGAATGCCAC
Ptr_LAC49_Potri.006G097100.1	-----CTACAACTCTCACCA-CCCCACCTCCAAAGAATGCCAC
Ptr_LAC14_Potri.006G096900.1	-----CTACAACTCTCACCA-CCCCACCTCCAAAGAATGCCAC
Ptr_LAC40_Potri.016G112000.1	-----CCACAACTCTCACCA-TCCCACCTCCCAAGAATGCCAC
Ptr_LAC24_Potri.009G156800.1	-----CAGCTTTTAAACCAACCTTCTCCTCCGATTAATGCTAC
Ptr_LAC23_Potri.009G156600.1	-----CAGCTTTTAAACCAACCTTCTCCTCGATTAATGCTAC
Ptr_LAC2_Potri.001G184300.1	-----CAGCTTTTAAATCAACCTTCTCCTCCGATTAATGCTAC
Ptr_LAC7_Potri.001G401300.1	CCTAAGAAACTGCCGTTCTATAAACCAAATCTACCACCTCTAAATGACAC
Ptr_LAC30_Potri.011G120300.1	ACCAAGAAACTCCCACTCTATAAACCAAATCTACCACCACTAAACGACAC
Ptr_LAC11_Potri.006G087100.1	---AAAAGCTTCTACACT-CAAGCCAACTCTGCCACCAATCAATGCTAC
Ptr_LAC12_Potri.006G087500.1	AACAAGAAACTCCCTCTCTTAAACCGTCTCTTCCAGTGTTAATGACAC
Ptr_LAC18_Potri.008G073700.1	-----AAAGAAACCATTTATGCCACCACTTCCAGCTTACAATGACAC
Ptr_LAC26_Potri.010G183600.1	-----AACAAAACCAAGTTATGCCCTCGACTTCCAGCTTATAACGACAC
	* * * * *
Ptr_LAC15_Potri.006G097000.1	TGCAGTTGCCAACCAATTTACCAACTCTCTACGCAGCCTTAACCTAAGAA
Ptr_LAC49_Potri.006G097100.1	TGCAGTTGCCAACCAATTTACCAACTCTCTACGCAGCCTTAACCTAAGAA
Ptr_LAC14_Potri.006G096900.1	TGCAGTTGCCAACCAATTTACCAACTCTCTACGCAGCCTTAACCTAAGAA
Ptr_LAC40_Potri.016G112000.1	TGCAGTTGCCAACCAATTTACTAATTCTCTACGCAGCTTAAACTCGAAAA
Ptr_LAC24_Potri.009G156800.1	AAATGTCGTCGCGAATTTTACAAGAAGACTTCGCAGTCTGGCTAATTCTC
Ptr_LAC23_Potri.009G156600.1	AAATGTCGTCGCGAATTTTACAAGAAGACTTCGCAGTCTGGCTAATTCTC
Ptr_LAC2_Potri.001G184300.1	AAATGTCGTCGCGAATTTTACAAGAAGACTTCGCAGTCTAGCTAATTCTC
Ptr_LAC7_Potri.001G401300.1	TTCAATTTGCTACAAATTTTACAAGCAAACTCCGCAGCTAGCAAGTGAC
Ptr_LAC30_Potri.011G120300.1	TTCAATTTGCTACAAATTTTCTAGCAAGCTCCGCAGCTAGCCAGTGAC
Ptr_LAC11_Potri.006G087100.1	TGGCTTTGTTGCGAATTTTCAACAAGAAATTCCTAGCTTAGCCACAGCTA
Ptr_LAC12_Potri.006G087500.1	AACATTTGCCACAAAGTTTGTCAAGAAATCCGTAGCTTGGCCAATGCAA
Ptr_LAC18_Potri.008G073700.1	AGCTACAGTACAGCCTTCAGTAGAAGCTTTAGAAGTCC-----TCGA
Ptr_LAC26_Potri.010G183600.1	AGCTACAGTACAGCATTAGTGAAGCTTGAAGCTCC-----TCGA
	* ** * *
Ptr_LAC15_Potri.006G097000.1	GATTTCTGCCAAAGTCCCATTGAACGTTGATCACAACCTTTTCTTTACA
Ptr_LAC49_Potri.006G097100.1	GATTTCTGCCAAAGTCCCATTGAACGTTGATCACAACCTTTTCTTTACA
Ptr_LAC14_Potri.006G096900.1	GATTTCTGCCAAAGTCCCATTGAACGTTGATCACAACCTTTTCTTTACA
Ptr_LAC40_Potri.016G112000.1	CGTTCCCTGCCAAAGTCCCATTAAACCGTTGATCAGACGCTTTTCTTCACT
Ptr_LAC24_Potri.009G156800.1	GATTCCCGGTTAATGTCCCTCAACAGTAGACAAGAAATTTTCTTCACT
Ptr_LAC23_Potri.009G156600.1	GATTCCCGGTTAATGTCCCTCAACAGTAGACAAGAAATTTTCTTCACT
Ptr_LAC2_Potri.001G184300.1	GATTCCCGGTTAATGTCCCTCAACAGTAGACAAGAAATTTTCTTCACT
Ptr_LAC7_Potri.001G401300.1	AATTCCCTGCCAATGTGCCGCAAAAGGTAGACAGGCAATTTTCTTCACT
Ptr_LAC30_Potri.011G120300.1	ACTTCCCTGCCAATGTCCCTCAAAAGGTTGACAGACAATTTTCTTCACT
Ptr_LAC11_Potri.006G087100.1	AATTTCCAGCTAATGTGCCACAACAGTGACAGGAAATTTCTTCTCACT
Ptr_LAC12_Potri.006G087500.1	GATTTCCAGCTAAGGTTCCCAAGAAAGTTGACAGACGCTTCTTTTCACTA
Ptr_LAC18_Potri.008G073700.1	AA-----AGTTGAGGTCCGACCGATATTGATGAGAACCTTCTTCTTCACTA
Ptr_LAC26_Potri.010G183600.1	AA-----AGTCGAGGTCCCAACAGATATTGACGAGAACCTTCTTCTTCACTA
	* * * * *
Ptr_LAC15_Potri.006G097000.1	GTTGGTCTAGGAGTTAACCCTATGTTCAAGTTGC---AAAGCT-----GG
Ptr_LAC49_Potri.006G097100.1	GTTGGTCTAGGAGTTAACCCTATGTTCAAGTTGC---AAAGCT-----GG
Ptr_LAC14_Potri.006G096900.1	GTTAGTCTAGGAGTTAACCCTATGTTCAAGTTGC---AAAGCT-----GG

GTTGGTCTAGGAATTAACCCGTGTCCAACCTGC---AAAGCT-----GG
 GTTGGTCTGGGAAACAGCCCGTGCCCAAAAAAC---CAGACA-----TG
 GTTGGTCTGGGAAACAGCCCGTGCCCAAAAAAC---CAGACA-----TG
 GTTGGTCTGGGAAACAGCCCGTGCCCAAAAAAC---CAGACA-----TG
 GTAAGCCTTGAACAACCCATGCTCCAAAAAC---AAAACC-----TG
 GTAGGCTTGGAAACAACCCATGCTCCAAAAAC---CAAACC-----TG
 GTGGGACTTGGGACTAACCCGTGCCCTAAAAAT---ACAACC-----TG
 ATAGGCTAGGACTACTCCCTTGCTCACAACAC---AAGACC-----TG
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 * * * * * * * * * *

T-----AATGGCA---GCAGGGTTGTTGCTAGTATTAACAATGTCA
T-----AATGGCA---GCAGGGTTGTTGCTAGTATTAACAATGTCA
T-----AATGGCA---GCAGGGTTGTTGCTAGTATTAACAATGTCA
T-----AATGGTA---GCAGAGTTGTTGCTAGTATCAACAATGTCA
TCAAGGGCCTAATGGCA---CGAAATTTTCAGCTTCTGTTAACAACATCT
TCAAGGGCCTAATGGCA---CGAAATTTTCAGCTTCTGTTAACAACATCT
TCAAGGACCTAATGGCA---CGAAATTTTCAGCTTCTGTTAACAACATCT
CCAGGGACCAATGTGAA---CAATGTTTGCAGCTTCAGTAAATATGTAT
CCAGGGACCAACGGAA---CGAGGTTTGTGCTTTCAGTCAATATGTAT
TCAGGGACCAATAATACGAAATTTGCTGCTTCGTTATTAACAATGTGT
CCAAGGACCTAATAACA---CCATGTTTGCAGCTTCAGTCAACAATGTGT
TCAAGGACCAACGGCA---CTCGTTTCACTGCTAGTATGAACAATGTGT
TCAAGGACCAACGGCA---CTCGTTTCAACGCTAGCATGAACAATGTTT
* * * * * * * * * *

CATTTGTGATGCCAACCACTGCC---CTGCTCCAAGCACATTTCTTCAAC
CATTTGTGATGCCAACCACTGCC---CTGCTCCAAGCACATTTCTTCAAC
CATTTGTGATGCCAACCACTGCC---CTGCTCCAAGCACATTTCTTCAAC
CATTTGTGATGCCAACCACTGCC---CTGCTCCAAGCACATTTCTTCAAC
CCATGGCTCTCCTTTCATCAGCA---CTCCTTCAATCTATTTTTTTCAA
CCATGGCTCTCCTTTCATCAGCA---CTCCTTCAATCTACTTTTTTCAA
CCATGGCTCTCCTTTCATCAGCA---CTCCTTCAATCTATTTTTTTCAA
CATTTGTGATGCCAACCAAGCT---TACTCTCAAGCCCATTTTGGT
CATTTGTGAATGCCAACCAAGCT---TACTCTCAAGCCACCATTTTGGT
CATTTGTATTACCTCTGTAGCT---CTCTGCAATCTACTTCTTGGGA
CATTTGTGCAGCAAAATATGCC---CTCTGCAATCTACTTCTTGA
CCTTTGTGTTTCCCTTCAAAGCCTCGCTATTGCAAGCATATAAGCAAAA
CTTTTGTGTTTCCCTTCAAACATTGCGTTACTGCAAGCGTATCAGCAAAA
* * * * * * * * * *

--ATCA- GCGGTGTGTTTACCACCTGATTTTCTGCAAAAGCCACCGCATGT
 --ATCA- GCGGTGTGTTTACCACCTGATTTTCTGCAAAAGCCACCGCATGT
 --ATCA- GCGGTGTGTTTACCACCTGATTTTCTGCAAAAGCCACCGCATGT
 --ATCA- GTGGTGTTTACCACCTGATTTTCTGCAAAAGCCACCATCATGT
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 AAATCAATGGGGTTTACACCTCTGATTTCCCAAGCTTTCTCTCCATCC
 CAATCAATGGGGTTTACACCTCTGATTTCCCAAGCTTTCTCTCCATCC
 CAATCAAAAGGGGTTTACAGCCTTAACCTTCCAATCAATCCATAATCCC
 CAATCAAGGGGTGTTTACAGTCTTATTTTCCAATCAGTCCACTGATCCC
 CAGTCAATGGTGTCTTACCTTCGATTTTCTCAGAAACCAACATAACCC
 CGGTCAAAGGGGTGATCAACCACTGATTTCTCCACCAACCCACTTTCAA
 --ATTCT- CTGGAGTCTTACCACCGATTTTCCAGCAAAACCAACAGTAAA
 --GTTC- CTGGAATTTTACACCACTGACTTTCCAGCAAAACCAACAGTAAA

TTTCAATTACACTGGCACTCC --ACCTACAAATTTACAGACC -AAAAGTG
TTTCAATTACACTGGCACTCC --ACCTACAAATTTACAGACC -AAAAGTG
TTTCAATTACACTGGCACTCC --ACCTACAAATTTACAGACC -AAAAGTG
TTTTAATTACACAGGCATCC --ACCCACAATTTACAGACC -ACAAGTG
ATTCAACTACACAGGGACCCC --ACCAAAATAACTCTGTGTG -GCCAATG
ATTCAACTATACAGGGACCCC --ACCAAAATAACTCTGTGTG -GCCAATG
ATTCAACTACACAGGGACCCC --ACCAAAATAACTCTGTGTG -GCCAATG
TTTTAATTATACCGGCACCCC --ACCAAAATAACTATGGTG -AGCAATG
TTTTAACTATACTGGCACACC --ACCCAAATAACTATGGTG -AGCAATG
ATTTAATTATACGGGTATCCC --ACCAAAATAACTATGGTG -AGCAATG
ATTCAACTATACAGGGACACC --GCCTAGCAATACCATGACT -GCCAAG
ATTGATACACTGGGAATGTGATGCTTCCTCTTTACAGCTGCTGCTG
GTTTGATTACACTGGTAATGTGATGCTTCCTCTTTACAGCTGCTGCTG


```

** * ** ** ** * * *
Ptr_LAC15_Potri.006G097000.1 GAACTAAAGTTTATAGGCTGAGCTACAACCTCGACAGTCCAACCTGTTATG
Ptr_LAC49_Potri.006G097100.1 GAACTAAAGTTTATAGGCTGAGCTACAACCTCGACAGTCCAACCTGTGCATG
Ptr_LAC14_Potri.006G096900.1 GAACTAAAGTTTATAGGCTGAGCTACAACCTCGACAGTCCAACCTGTTATG
Ptr_LAC40_Potri.016G112000.1 GAACAAAAGCATATAGGTTGCCCTACAACCTCGACAGTCCAACCTGTTATG
Ptr_LAC24_Potri.009G156800.1 GTACCAAGCTTTGTAGTGGTACCATTAAACACAAGCGTGGAGGTAGTGATG
Ptr_LAC23_Potri.009G156600.1 GTACCAAGCTTTGTAGTGGTACCATTAAACACAAGCGTGGAGGTAGTGATG
Ptr_LAC2_Potri.001G184300.1 GTAACAAGCTTTGTAGTGGTACCATTAAACACAAGTGTGGAGGTAGTGATG
Ptr_LAC7_Potri.001G401300.1 GAACAAAGCTAGTTGTCTTCTCTTTAAACACCAAGCGTGGAGCTCATTATG
Ptr_LAC30_Potri.011G120300.1 GAACAAAGCTAGTTGTCTTCTCTTTAAACACAAGCGTGGAGCTCATCATG
Ptr_LAC11_Potri.006G087100.1 GCACAAAAGCAGTGGTGTAAACATTCAACACGAGCGTGGAGTTGGTAATG
Ptr_LAC12_Potri.006G087500.1 GAACCAAGGTGGTGGTCTTCTTTCAATACTAGCGTGGAGTTGGTGATG
Ptr_LAC18_Potri.008G073700.1 GAACTAAGCTGTACAAGTTGAAGTACGGATCAAGGGTGCAGATTGTGCTA
Ptr_LAC26_Potri.010G183600.1 GAACTAAGCTGTACAAGTTGAAGTACGGATCAAGGGTGCAGATTGTGTTG
* * ** * * * * * *
Ptr_LAC15_Potri.006G097000.1 CAAGATACTGGGATCATATCCCTTGAGAACCATCCGATCCATTTACATGG
Ptr_LAC49_Potri.006G097100.1 CAAGATACTGGGATCATATCCCTTGAGAACCATCCGATCCATTTACATGG
Ptr_LAC14_Potri.006G096900.1 CAAGATACTGGTATCATATCCCTTGAGAACCATCCGATCCATTTACATGG
Ptr_LAC40_Potri.016G112000.1 CAAGATACTGGGATCATATCCCTTGAGAACCATCCAATCCATCTCATATGG
Ptr_LAC24_Potri.009G156800.1 CAGGGCACTCGCATTTTCGGTGCTGAGAGCCACCCTCTCCATCTTCATGG
Ptr_LAC23_Potri.009G156600.1 CAGGGCACTCGCATTTTCGGTGCTGAGAGCCACCCTCTCCATCTTCATGG
Ptr_LAC2_Potri.001G184300.1 CAGGGCACTCGAATTTTCGGTGCTGAGAGCCACCCTCTCCATCTTCATGG
Ptr_LAC7_Potri.001G401300.1 CAGGACACCAGCATACTTGGTGCTGAGAGCCACCCTCTTCATTTGCATGG
Ptr_LAC30_Potri.011G120300.1 CAGGGCACTAGCATACTTGGTGAGAGAGCCACCCTCTTCATTTGCATGG
Ptr_LAC11_Potri.006G087100.1 CAGGGCACAAGCATAGTCGTCGCCAAAGTCACCACTCCATCTGCATGG
Ptr_LAC12_Potri.006G087500.1 CAGGACACCAGCATTATTGGTGACAGAGTCAACCCTCTTCACCTCCATGG
Ptr_LAC18_Potri.008G073700.1 CAGGACACGAGCATCGTCACACCAGAAAACCATCCGATCCATCTCCATGG
Ptr_LAC26_Potri.010G183600.1 CAGGACACGAGCATTGTGCACACCAGAAAACCAACCAATCCATCTTCATGG
** * ** ** ** * * ** * ** * ** * ** *
Ptr_LAC15_Potri.006G097000.1 ATTCATTTCTTTGCTGTCGGTAGGGGAGTAGGGAATTACAATCCGAAGA
Ptr_LAC49_Potri.006G097100.1 ATTCATTTCTTTGCTGTCGGTAGGGGAGTAGGGAATTACAATCCGAAGA
Ptr_LAC14_Potri.006G096900.1 ATTCATTTCTTTGCTGTCGGTAGGGGAGTAGGGAATTACAATCCGAAGA
Ptr_LAC40_Potri.016G112000.1 ATTCATTTCTTTGCTGTCGGTAGGGGAGTAGGGAATTACAATCCGAAGA
Ptr_LAC24_Potri.009G156800.1 CTTCATTTCTATGTTGTTGGAGAAGGGTTTGGAAATTTTGATCCAAATA
Ptr_LAC23_Potri.009G156600.1 CTTCATTTCTATGTTGTTGGAGAAGGGTTTGGAAATTTTGATCCAAATA
Ptr_LAC2_Potri.001G184300.1 CTTCATTTCTATGTTGTTGGAGAAGGGTTTGGAAATTTTGATCCAAATA
Ptr_LAC7_Potri.001G401300.1 TTTCAATTTCTTTGTTGTTGGTGAAGGATTTGGGAACTTTGATCCAAAAA
Ptr_LAC30_Potri.011G120300.1 TTTCAATTTCTTTGTTGTCGGTCAAGGTTTGGGAACTTTGATCCAAAGCA
Ptr_LAC11_Potri.006G087100.1 TTTTAATTTCTTTGTTGTTGGTGAAGGTTTGGCAACTACGATCCCAACA
Ptr_LAC12_Potri.006G087500.1 CTTCACCTCTTTGTGGTTGGTCAGGGTTTGGCAACTTTGACCCCAAGA
Ptr_LAC18_Potri.008G073700.1 ATACGACTTCTACATCATCGCTGAAGGATTTGGGAATTTCAATCCCAAAA
Ptr_LAC26_Potri.010G183600.1 ATACGACTTCTACATCATTGCAGAAGGATTTGGAAATTTCAATCCCAAAA
* * **** * * ** * ** * * * * *
Ptr_LAC15_Potri.006G097000.1 CTGATACTAAGAAATTTAACCTCGTTGATCCTGTTGAACGGAACACAATT
Ptr_LAC49_Potri.006G097100.1 CTGATACTAAGAAATTTAACCTCGTTGATCCTGTTGAACGGAACACAATT
Ptr_LAC14_Potri.006G096900.1 CTGATACTAAGAAATTTAACCTCGTTGATCCTGTTGAACGGAACACAATT
Ptr_LAC40_Potri.016G112000.1 CTGATCCTAAGAAATTTAACCTTGTGACCCTGTTGAACGGAACACAATT
Ptr_LAC24_Potri.009G156800.1 ATGACCCCAAGAACTTTAACCTTGTGATCCTGTTGAAAGGAACACTGTT
Ptr_LAC23_Potri.009G156600.1 ATGACCCCAAGAACTTTAACCTTGTGATCCTGTTGAAAGGAACACTGTT
Ptr_LAC2_Potri.001G184300.1 ATGACCCCAAGAACTTTAACCTTGTGATCCTGTTGAAAGGAACACTGTT
Ptr_LAC7_Potri.001G401300.1 AGGATCCGGCAAATTTCAATCTTGTGACCCGTTGAAAGGAATACCGTG
Ptr_LAC30_Potri.011G120300.1 AGGATCCCGCAAATTTCAATCTTGTGACCCGTTGAAAGGAATACCTGTG
Ptr_LAC11_Potri.006G087100.1 AAGATCCTTCCAATTTAATCTGGTCGACCCTATGGAAAGGAACACGGCG
Ptr_LAC12_Potri.006G087500.1 AGGACCTGTCAAGTTCAACCTTGTGACCCTGCCGAGAGGAACACTGTT
Ptr_LAC18_Potri.008G073700.1 CTGATAAATCCAAATTTCAACCTTGTGATCCACCAATGAGGAATACAGTT
Ptr_LAC26_Potri.010G183600.1 CTCATAAATCTAAATTTAATCTTGTGGATCCACCAATGAGGAATACAGTT
* ** ** ** ** ** * * * * *
Ptr_LAC15_Potri.006G097000.1 GGAGTACCTTCTGGTGGATGGGTGGCGATAAGATTTGCGCGCGATAATCC
Ptr_LAC49_Potri.006G097100.1 GGAGTACCTTCTGGTGGATGGGTGGCGATAAGATTTGCGCGCGATAATCC
Ptr_LAC14_Potri.006G096900.1 GGAGTACCTTCTGGTGGATGGGTGGCGATAAGATTTGCGCGCGATAATCC
Ptr_LAC40_Potri.016G112000.1 GGAGTACCTTCTGGTGGATGGGTGGCGATAAGATTTGCGCGCGATAATCC
Ptr_LAC24_Potri.009G156800.1 GGTGTGCCTACTGCTGGTGGGTGGCAATTCTGTTTTCATGCAGACAATCC
Ptr_LAC23_Potri.009G156600.1 GGTGTGCCTACTGCTGGTGGGTGGCAATTCTGTTTTCATGCAGACAATCC
Ptr_LAC2_Potri.001G184300.1 GGTGTGCCTACTGCTGGTGGGTGGCAATTCTGTTTTCATGCAGACAATCC
Ptr_LAC7_Potri.001G401300.1 GGAGTGCCCTCTGGAGGCTGGGTGCTATACGTTTCTAGCCGACAAACC

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Ptr_LAC30_Potri.011G120300.1      GGAGTGCCCTCTGGAGGCTGGGTTGCTATACGGTTTCTAGCAGACAATCC
Ptr_LAC11_Potri.006G087100.1      GGAGTTCCAGCTGGTGGCTGGATCGCTATTGCTTCCAGCGGACAATCC
Ptr_LAC12_Potri.006G087500.1      GGTGTGCCATCTGGCGGGTGGGTCGCCATACGGTTCCCTTGCTGACAATCC
Ptr_LAC18_Potri.008G073700.1      GCAGTACCCGTGAATGGATGGGCGGTTATTAGATTGTTGCTGACAACCC
Ptr_LAC26_Potri.010G183600.1      GCAGTGCCCTTCAATGGATGGGCGAGTCATTAGATTGTTGCTGACAATCC
*   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *

Ptr_LAC15_Potri.006G097000.1      AGGAGTTTGGTTCATGCATTGCCATCTAGAGGTGCACACTACATGGGGAC
Ptr_LAC49_Potri.006G097100.1      AGGAGTTTGGTTCATGCATTGCCATCTGGAGGTGCACACTACATGGGGAC
Ptr_LAC14_Potri.006G096900.1      AGGAGTTTGGTTCATGCATTGCCATCTAGAGGTGCACACTACATGGGGAC
Ptr_LAC40_Potri.016G112000.1      TGGAGTTTGGTTCATGCATTGCCATCTCGAGGTGCACACTACATGGGGAC
Ptr_LAC24_Potri.009G156800.1      AGGAGTATGGTTTATGCATGCCACTTTGATGTCCATTTGAGCTGGGGGT
Ptr_LAC23_Potri.009G156600.1      AGGAGTATGGTTTATGCATGCCACTTTGATGTCCATTTGAGCTGGGGGT
Ptr_LAC2_Potri.001G184300.1      AGGAGTATGGTTTATGCATGCCACTTTGATGTCCATTTGAGCTGGGGGT
Ptr_LAC7_Potri.001G401300.1      AGGTGTATGGTTCATGCATTGCCATCTAGAAGTGCACACAAGCTGGGGCT
Ptr_LAC30_Potri.011G120300.1      AGGCGTATGGTTCATGCATTGCCATCTAGAAGTGCACACAAGCTGGGGCT
Ptr_LAC11_Potri.006G087100.1      AGGGGTGTGGTTCATGCATGCCATCTTGATGTGCACACTAGCTGGGGGC
Ptr_LAC12_Potri.006G087500.1      AGGAGTTTGGTTCATGCATGTCTTTGGAAGTACACACCAGCTGGGGCC
Ptr_LAC18_Potri.008G073700.1      AGGTGTGTGGCTAATGCATGTCACTTGGATGTTTACATCACATGGGGTC
Ptr_LAC26_Potri.010G183600.1      AGGTGTGTGGCTAATGCATGTCACTTGGATGTTTACATCACATGGGGTC
*   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *

Ptr_LAC15_Potri.006G097000.1      TTAAGATGGCGTTCTTGGTAGACAATGGCAAAGGCCCTAAGGAGTCTCTT
Ptr_LAC49_Potri.006G097100.1      TTAAGATGGCGTTCTTGGTAGACAATGGCAAAGGCCCTAAGGAGTCTCTT
Ptr_LAC14_Potri.006G096900.1      TTAAGATGGCGTTCTTGGTAGACAATGGCAAAGGCCCTAAGGAGTCTCTT
Ptr_LAC40_Potri.016G112000.1      TTAAGATGGCATTCTTGGTGGACAATGGCAAAGGCCCTAACGAGTCACTT
Ptr_LAC24_Potri.009G156800.1      TAAGGATGGCATGGATTGTCTTGATGGAACACTTCCAGTCAGAAGCTC
Ptr_LAC23_Potri.009G156600.1      TAAGGATGGCATGGATTGTCTTGATGGAACACTTCCAGTCAGAAGCTC
Ptr_LAC2_Potri.001G184300.1      TAAGGATGGCATGGATTGTCTTGATGGAACACTTCCAGTCAGAAGCTC
Ptr_LAC7_Potri.001G401300.1      TGAAGATGGCTTGGGTTGTCTGGATGGAAAGCTTCCCAATCAGAAGCTG
Ptr_LAC30_Potri.011G120300.1      TGAAGATGGCTTGGGTTGTCTTGATGGAAGCTTCCCAATCAGAAGCTG
Ptr_LAC11_Potri.006G087100.1      TCCGAATGGCATGGATAGTCTTGATGGACCCCAACCGAATCAAAAAATA
Ptr_LAC12_Potri.006G087500.1      TGAAGATGGCTTGGGTGGTCAATGACGGAAAACGGCCGAGCCAAAAGCTG
Ptr_LAC18_Potri.008G073700.1      TGGCCATGGCGTTTCTTGATAGAAGAGGGGATTGGGATTTTACAGAGTGTC
Ptr_LAC26_Potri.010G183600.1      TGGCCATGGCTTCTTGGTAGAGGATGGGATTGGGGAATTACAGAGTGTA
*   *   *   *   *   *   *   *   *   *   *   *   *   *   *

Ptr_LAC15_Potri.006G097000.1      CTACCGCCGCCAAGCGACCTTCCAAAAATGTTGA
Ptr_LAC49_Potri.006G097100.1      CTACCGCCGCCAAGCGATCTTCCAAAAATGTTGA
Ptr_LAC14_Potri.006G096900.1      CTACCGCCGCCAAGCGACCTTCCAAAAATGTTGA
Ptr_LAC40_Potri.016G112000.1      CTGCCGCCTCCAAGCGATCTTCCAAAAATGTTAA
Ptr_LAC24_Potri.009G156800.1      CCTCCTCCACCATCTGATCTTCCCAAGTGTGTA
Ptr_LAC23_Potri.009G156600.1      CCTCCTCCACCATCTGATCTTCCCAAGTGTGTA
Ptr_LAC2_Potri.001G184300.1      CCTCCTCCACCATCTGATCTTCCCAAGTGTGTA
Ptr_LAC7_Potri.001G401300.1      CTTCTCCACCAAGCTGATCTTCCCAAGTGTGTA
Ptr_LAC30_Potri.011G120300.1      CTTCTCCACCAAGCTGATCTTCCCAAGTGTGTA
Ptr_LAC11_Potri.006G087100.1      CCACCAACCAAGCTGATCTTCCCAAGTGTGTA
Ptr_LAC12_Potri.006G087500.1      CCACCTCCACCTTCTGATCTTCCCAAGTGTGTA
Ptr_LAC18_Potri.008G073700.1      GAGCCTCCTCCAGCGATCTGCCAATATGTTAA
Ptr_LAC26_Potri.010G183600.1      GAGCCTCCTCCAGCAGATCTGCCGATATGTTAA
*   *   *   *   *   *   *   *   *   *   *   *   *   *   *

```

clustalw.dnd

```

(
(
(
Ptr_LAC2_Potri.001G184300.1:0.00958,
(
Ptr_LAC24_Potri.009G156800.1:0.00197,
Ptr_LAC23_Potri.009G156600.1:0.00439)
:0.00601)
:0.16738,
(
(
(
(
(
Ptr_LAC15_Potri.006G097000.1:0.00503,

```

```
Ptr_LAC49_Potri.006G097100.1:0.00211)
:0.00547,
Ptr_LAC14_Potri.006G096900.1:0.01034)
:0.03859,
Ptr_LAC40_Potri.016G112000.1:0.04282)
:0.17336,
(
Ptr_LAC18_Potri.008G073700.1:0.05224,
Ptr_LAC26_Potri.010G183600.1:0.05268)
:0.24436)
:0.07798,
Ptr_LAC12_Potri.006G087500.1:0.16701)
:0.00616)
:0.00365,
(
Ptr_LAC7_Potri.001G401300.1:0.04168,
Ptr_LAC30_Potri.011G120300.1:0.05283)
:0.11232,
Ptr_LAC11_Potri.006G087100.1:0.18208);
```

C.8 Sequence similarity of the CDS region for all seventeen SDX LACs

CLUSTAL 2.1 Multiple Sequence Alignments

```
Sequence type explicitly set to DNA
Sequence format is Pearson
Sequence 1: Ptr_LAC2_Potri.001G184300.1 1731 bp
Sequence 2: Ptr_LAC15_Potri.006G097000.1 1680 bp
Sequence 3: Ptr_LAC24_Potri.009G156800.1 1731 bp
Sequence 4: Ptr_LAC40_Potri.016G112000.1 1674 bp
Sequence 5: Ptr_LAC49_Potri.006G097100.1 1680 bp
Sequence 6: Ptr_LAC18_Potri.008G073700.1 1725 bp
Sequence 7: Ptr_LAC26_Potri.010G183600.1 1728 bp
Sequence 8: Ptr_LAC7_Potri.001G401300.1 1746 bp
Sequence 9: Ptr_LAC11_Potri.006G087100.1 1743 bp
Sequence 10: Ptr_LAC12_Potri.006G087500.1 1740 bp
Sequence 11: Ptr_LAC14_Potri.006G096900.1 1677 bp
Sequence 12: Ptr_LAC23_Potri.009G156600.1 1731 bp
Sequence 13: Ptr_LAC30_Potri.011G120300.1 1746 bp
Sequence 14: Ptr_LAC17_Potri.008G064000.1 1671 bp
Sequence 15: Ptr_LAC19_Potri.008G073800.1 1749 bp
Sequence 16: Ptr_LAC25_Potri.010G183500.1 1749 bp
Sequence 17: Ptr_LAC27_Potri.010G193100.1 1668 bp
Start of Pairwise alignments
Aligning...
```

```
Sequences (1:2) Aligned. Score: 50.6548
Sequences (1:3) Aligned. Score: 98.3247
Sequences (1:4) Aligned. Score: 51.9116
Sequences (1:5) Aligned. Score: 50.8929
Sequences (1:6) Aligned. Score: 45.6232
Sequences (1:7) Aligned. Score: 44.8495
Sequences (1:8) Aligned. Score: 66.7244
Sequences (1:9) Aligned. Score: 63.8359
Sequences (1:10) Aligned. Score: 64.8758
Sequences (1:11) Aligned. Score: 50.328
Sequences (1:12) Aligned. Score: 97.9203
Sequences (1:13) Aligned. Score: 64.8758
Sequences (1:14) Aligned. Score: 49.2519
Sequences (1:15) Aligned. Score: 43.963
Sequences (1:16) Aligned. Score: 43.7319
Sequences (1:17) Aligned. Score: 49.4604
Sequences (2:3) Aligned. Score: 50.9524
Sequences (2:4) Aligned. Score: 90.5615
Sequences (2:5) Aligned. Score: 99.2857
Sequences (2:6) Aligned. Score: 48.5119
Sequences (2:7) Aligned. Score: 48.2738
Sequences (2:8) Aligned. Score: 53.9286
Sequences (2:9) Aligned. Score: 51.4881
Sequences (2:10) Aligned. Score: 53.5119
Sequences (2:11) Aligned. Score: 98.0322
Sequences (2:12) Aligned. Score: 50.6548
Sequences (2:13) Aligned. Score: 52.2619
Sequences (2:14) Aligned. Score: 62.8965
Sequences (2:15) Aligned. Score: 46.25
Sequences (2:16) Aligned. Score: 45.5357
Sequences (2:17) Aligned. Score: 62.9496
```

Sequences (3:4) Aligned. Score: 51.6726
 Sequences (3:5) Aligned. Score: 50.9524
 Sequences (3:6) Aligned. Score: 45.5652
 Sequences (3:7) Aligned. Score: 44.9074
 Sequences (3:8) Aligned. Score: 67.1288
 Sequences (3:9) Aligned. Score: 64.067
 Sequences (3:10) Aligned. Score: 65.2802
 Sequences (3:11) Aligned. Score: 50.3876
 Sequences (3:12) Aligned. Score: 99.3645
 Sequences (3:13) Aligned. Score: 64.7603
 Sequences (3:14) Aligned. Score: 49.1323
 Sequences (3:15) Aligned. Score: 44.483
 Sequences (3:16) Aligned. Score: 44.0208
 Sequences (3:17) Aligned. Score: 49.4005
 Sequences (4:5) Aligned. Score: 91.0394
 Sequences (4:6) Aligned. Score: 48.0884
 Sequences (4:7) Aligned. Score: 49.1637
 Sequences (4:8) Aligned. Score: 54.6595
 Sequences (4:9) Aligned. Score: 50.5376
 Sequences (4:10) Aligned. Score: 54.3011
 Sequences (4:11) Aligned. Score: 90.9797
 Sequences (4:12) Aligned. Score: 51.4337
 Sequences (4:13) Aligned. Score: 53.5842
 Sequences (4:14) Aligned. Score: 62.8965
 Sequences (4:15) Aligned. Score: 47.1924
 Sequences (4:16) Aligned. Score: 46.2963
 Sequences (4:17) Aligned. Score: 63.6691
 Sequences (5:6) Aligned. Score: 48.75
 Sequences (5:7) Aligned. Score: 48.5119
 Sequences (5:8) Aligned. Score: 53.8095
 Sequences (5:9) Aligned. Score: 52.2619
 Sequences (5:10) Aligned. Score: 53.75
 Sequences (5:11) Aligned. Score: 98.0918
 Sequences (5:12) Aligned. Score: 50.8929
 Sequences (5:13) Aligned. Score: 53.0357
 Sequences (5:14) Aligned. Score: 63.3154
 Sequences (5:15) Aligned. Score: 46.369
 Sequences (5:16) Aligned. Score: 45.4167
 Sequences (5:17) Aligned. Score: 63.1295
 Sequences (6:7) Aligned. Score: 89.5072
 Sequences (6:8) Aligned. Score: 45.2754
 Sequences (6:9) Aligned. Score: 43.1884
 Sequences (6:10) Aligned. Score: 45.1594
 Sequences (6:11) Aligned. Score: 48.2409
 Sequences (6:12) Aligned. Score: 45.5072
 Sequences (6:13) Aligned. Score: 44.8116
 Sequences (6:14) Aligned. Score: 44.1652
 Sequences (6:15) Aligned. Score: 74.8406
 Sequences (6:16) Aligned. Score: 75.1304
 Sequences (6:17) Aligned. Score: 45.2638
 Sequences (7:8) Aligned. Score: 46.5278
 Sequences (7:9) Aligned. Score: 42.5347
 Sequences (7:10) Aligned. Score: 45.6597
 Sequences (7:11) Aligned. Score: 47.6446
 Sequences (7:12) Aligned. Score: 44.9653
 Sequences (7:13) Aligned. Score: 45.1968
 Sequences (7:14) Aligned. Score: 44.4644
 Sequences (7:15) Aligned. Score: 75.1157
 Sequences (7:16) Aligned. Score: 75.463
 Sequences (7:17) Aligned. Score: 44.9041
 Sequences (8:9) Aligned. Score: 65.8635
 Sequences (8:10) Aligned. Score: 66.3218
 Sequences (8:11) Aligned. Score: 54.3232
 Sequences (8:12) Aligned. Score: 66.84
 Sequences (8:13) Aligned. Score: 90.5498
 Sequences (8:14) Aligned. Score: 48.474
 Sequences (8:15) Aligned. Score: 44.6163
 Sequences (8:16) Aligned. Score: 44.1581
 Sequences (8:17) Aligned. Score: 49.4005
 Sequences (9:10) Aligned. Score: 64.0805


```

Sequences (9:11) Aligned. Score: 51.8187
Sequences (9:12) Aligned. Score: 63.7204
Sequences (9:13) Aligned. Score: 65.8061
Sequences (9:14) Aligned. Score: 48.2944
Sequences (9:15) Aligned. Score: 44.8078
Sequences (9:16) Aligned. Score: 44.3488
Sequences (9:17) Aligned. Score: 48.8609
Sequences (10:11) Aligned. Score: 53.8462
Sequences (10:12) Aligned. Score: 64.7025
Sequences (10:13) Aligned. Score: 66.6092
Sequences (10:14) Aligned. Score: 47.5763
Sequences (10:15) Aligned. Score: 43.8506
Sequences (10:16) Aligned. Score: 45.8046
Sequences (10:17) Aligned. Score: 48.741
Sequences (11:12) Aligned. Score: 50.328
Sequences (11:13) Aligned. Score: 52.7132
Sequences (11:14) Aligned. Score: 63.1957
Sequences (11:15) Aligned. Score: 45.975
Sequences (11:16) Aligned. Score: 45.4979
Sequences (11:17) Aligned. Score: 63.4293
Sequences (12:13) Aligned. Score: 65.1646
Sequences (12:14) Aligned. Score: 49.3118
Sequences (12:15) Aligned. Score: 44.3096
Sequences (12:16) Aligned. Score: 43.9053
Sequences (12:17) Aligned. Score: 49.5803
Sequences (13:14) Aligned. Score: 48.8929
Sequences (13:15) Aligned. Score: 44.0435
Sequences (13:16) Aligned. Score: 42.6117
Sequences (13:17) Aligned. Score: 48.8609
Sequences (14:15) Aligned. Score: 43.447
Sequences (14:16) Aligned. Score: 42.7887
Sequences (14:17) Aligned. Score: 89.6882
Sequences (15:16) Aligned. Score: 91.6524
Sequences (15:17) Aligned. Score: 43.8249
Sequences (16:17) Aligned. Score: 43.4652
Guide tree file created: [clustalw.dnd]

```

There are 16 groups
Start of Multiple Alignment

```

Aligning...
Group 1: Sequences: 2      Score:31768
Group 2: Sequences: 3      Score:31459
Group 3: Sequences: 4      Score:29901
Group 4: Sequences: 2      Score:29656
Group 5: Sequences: 6      Score:24201
Group 6: Sequences: 2      Score:32765
Group 7: Sequences: 3      Score:32513
Group 8: Sequences: 2      Score:31179
Group 9: Sequences: 3      Score:25684
Group 10: Sequences: 6     Score:24631
Group 11: Sequences: 7     Score:25168
Group 12: Sequences: 13    Score:20603
Group 13: Sequences: 2     Score:30711
Group 14: Sequences: 2     Score:31454
Group 15: Sequences: 4     Score:27637
Group 16: Sequences: 17    Score:19997
Alignment Score 956795

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CLUSTAL-Alignment file created [clustalw.aln]

clustalw.aln

CLUSTAL 2.1 multiple sequence alignment

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Ptr_LAC15_Potri.006G097000.1  -----ATGGAGTACTCCAATTGCTCATCCGTT
Ptr_LAC49_Potri.006G097100.1  -----ATGGAGTACTCCAATTGGCTCATCCGTT

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Ptr_LAC14_Potri.006G096900.1
 Ptr_LAC40_Potri.016G112000.1
 Ptr_LAC17_Potri.008G064000.1
 Ptr_LAC27_Potri.010G193100.1
 Ptr_LAC24_Potri.009G156800.1
 Ptr_LAC23_Potri.009G156600.1
 Ptr_LAC2_Potri.001G184300.1
 Ptr_LAC7_Potri.001G401300.1
 Ptr_LAC30_Potri.011G120300.1
 Ptr_LAC11_Potri.006G087100.1
 Ptr_LAC12_Potri.006G087500.1
 Ptr_LAC18_Potri.008G073700.1
 Ptr_LAC26_Potri.010G183600.1
 Ptr_LAC19_Potri.008G073800.1
 Ptr_LAC25_Potri.010G183500.1

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 Ptr_LAC26_Potri.010G183600.1

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 -----ATGGAGTACTCTTGCT-----TCCGTT
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 -----ATGGAGTACTATCAGG-----CTCGGA
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 -----ATGGGTCTGCCTTTT-TGAGGATTTTAGT
 -----ATGGAGGTTATCAACCGTATCTTTGCTA-----ATCGC
 -----ATGGAGGTTATCAAGTCTATCTTTGCTG-----ATCGT
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 -----ATGGAGGTTTCAACAACATTTTGGCATCAATCATCGT

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 TTAT---GCTTCTTGCT----GTCTGCCTCCTC----CCTGCTTTGGT
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 TTAT---GCTCCTTGCT----GTCTGCCTCCTC----CCTGCTGGT
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 CAAT---GCTGCTAGTG----ATTTTCATTTTT---CCGCATTGGT
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 CTTTGCTATGAGCTGCC----TCTGGGCTTTCTGAGGTGCGCGGTGC
 ATTTGCTATGAGCTGCC----TCTGGGCTTTCTGAGGTGCGCGGTGC
 ---TCATCTCATTTGTC----ACTTTGTCTATC--CATCCAGAGCTGTC
 ---TCTTCTCATTTGTC----ACTTTGTCTGTC--AATCCTGAGCTGTC
 GC-TCTTGTTTTTTTTC----TTTTTCTCTTA--GTGCCGACTTCGC
 CACTGCGTTGTGTGCTC----TTTGGATTTCTCTGAGCTGGTAGTTGC
 CATTGTTCTTTCTTCTGTC---TC---CTGCTTGGCCTCAGCAATGTC
 CATTGTTCTTTCTTCTGTC---TCGTGCTGCTTCTAGCCTCAACAATGTC
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 TGCTCTTCGTTCTTCTTAGGCTTCTGTTGCTCCTAGCTTCTGCATTGTC
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GCCAAAC---CCCACAAGGAAGTGACTATCATGTTGGGAGAGTGGTTCAA
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GCTAAAC---CTTACAAGGAGGTTCCAATCATCTTTGGGAGTGGTTTAA
AAGA AAC---CCTACAAGGAATCCCCATCTTTTGGGAGATGGTTTAA
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Ptr_LAC12_Potri.006G087500.1
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Ptr_LAC30_Potri.011G120300.1      CTGCTAGACCTTACGCGACTGGTCAAGGAACT---TTCGATAATTCAACT
Ptr_LAC11_Potri.006G087100.1      TTGCCGGGCCTTATTTCTCAGGCATGGGAAGC---TTTGACAACCTTACC
Ptr_LAC12_Potri.006G087500.1      CCGCTAGGCCATATGCCACCGGCCAGGCTCC---TTTGACAATACAACC
Ptr_LAC18_Potri.008G073700.1      CAGCCCGAGCCTATCAAAGTGCACAAAATGCACCATTTGACAATACTACC
Ptr_LAC26_Potri.010G183600.1      CAGCTCGAGCTTATCAGAGTGCACAAAATGCACCATTTGACAATACTACA
Ptr_LAC19_Potri.008G073800.1      CAGCACGTGCCTATCAAAGCGCGCAAAAATGCACCATTCGACAATACTACC
Ptr_LAC25_Potri.010G183500.1      CAGCACGTGCCTACCAAAGCGCACAAAATGCACCATTCGACAATACTACC
                                *      *      *      *      *      *      *

Ptr_LAC15_Potri.006G097000.1      GCAACAGCCACTTTGCAGTATTAGGAGCACTTG-----CTAACTCCC-
Ptr_LAC49_Potri.006G097100.1      GCAACAGCCACTTTGCAGTATTAGGAGCACTTG-----CTAACTCCC-
Ptr_LAC14_Potri.006G096900.1      GCAACAGCCACTTTGCAGTATTAGGAGCACTTG-----CTAACTCCC-
Ptr_LAC40_Potri.016G112000.1      GCAACAGCCACTTTACACTATTAGGAGCACTTT-----CTAACTCCC-
Ptr_LAC17_Potri.008G064000.1      GCAATCGCTTTCTTGCCTATAAGGGAACCATTTG-----CATTTTCCC-
Ptr_LAC27_Potri.010G193100.1      GCAATCGCTTTCTTGCCTATAAGGGAACCATTTG-----CATTTTCCC-
Ptr_LAC24_Potri.009G156800.1      GTTGCAGGGATACTCGAGTATGAAACTTCTTCAA-----ATTCAA-
Ptr_LAC23_Potri.009G156600.1      GTTGCAGGGATACTCGAGTATGAAACTTCTTCAA-----ATTCAA-
Ptr_LAC11_Potri.006G087100.1      GTTGCAGGGATACTCGAGTATGAAACTTCTTCAA-----ATTCAA-
Ptr_LAC7_Potri.001G401300.1      GTTGTGGAATCTTAGAATATGAAGAATCAAACAAAACCATTAATCAAG
Ptr_LAC30_Potri.011G120300.1      GTTGTGGAATCTTAGAATATGAAGTGCACAAAACCAACAGTCAATCAAA
Ptr_LAC11_Potri.006G087100.1      ACTGCTGGTGTCTTGGTTTATAAACATCCATCTAGTAATAATCACTTGA-
Ptr_LAC12_Potri.006G087500.1      ACTGCTGGAATACTCGAATATGACCAGAACCCCTTCTGCAACAACTCCAA
Ptr_LAC18_Potri.008G073700.1      ACCACTGCCATTCTTGAATACAAATCTGTTCTTGGCCTGCGAAGTGTA-
Ptr_LAC26_Potri.010G183600.1      ACCACTGCCATTCTTGAATACAAATCTGCCCTTGGCCTGCTAAGTGTA-
Ptr_LAC19_Potri.008G073800.1      ACTACCGCTATTCTTGAATACAAATCTCCGCTGTGCTGCCAAGAATTG
Ptr_LAC25_Potri.010G183500.1      ACTACTGCCATTCTTGAATACAAATCATCCCTTGTGCTGCCAAAATTG
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Ptr_LAC15_Potri.006G097000.1      -----CTACAACTCTCACCA-CCCCACCTCCAAAGA
Ptr_LAC49_Potri.006G097100.1      -----CTACAACTCTCACCA-CCCCACCTCCAAAGA
Ptr_LAC14_Potri.006G096900.1      -----CTACAACTCTCACCA-CCCCACCTCCAAAGA
Ptr_LAC40_Potri.016G112000.1      -----CCACAACTCTCACCA-TCCCACCTCCCAAGA
Ptr_LAC17_Potri.008G064000.1      -----CACCAGTCCTGACCA-CTACTCCTGCTATAA
Ptr_LAC27_Potri.010G193100.1      -----CACCTGTCTAACCA-CCACTCCTGCTATAA
Ptr_LAC24_Potri.009G156800.1      -----CAGCTTTTAAACCAACCCTTCTCCGATTA
Ptr_LAC23_Potri.009G156600.1      -----CAGCTTTTAAACCAACCCTTCTCCGATTA
Ptr_LAC2_Potri.001G184300.1      -----CAGCTTTTAAATCAACCCTTCTCCGATTA
Ptr_LAC7_Potri.001G401300.1      CCACTCCCCTAAGAAACTGCCGTCTATAAAACCAAACTACCACTCTAA
Ptr_LAC30_Potri.011G120300.1      CCACTCCACCAAGAAACTCCCCTCTATAAAACCAAACTACCACTCTAA
Ptr_LAC11_Potri.006G087100.1      -----AAAAGCTTCTTACACT-CAAGCCAACTCTGCCACCAATCA
Ptr_LAC12_Potri.006G087500.1      AAGCAAGAACAAAGAACTCCCTCTTCTTAAACCGTCTCTTCCAGTGTTTA
Ptr_LAC18_Potri.008G073700.1      -----CAAAGAAACCATTTATGCCACCACTTCCAGCTTACA
Ptr_LAC26_Potri.010G183600.1      -----CAACAAAACCAAGTTATGCCTCGACTTCCAGCTTATA
Ptr_LAC19_Potri.008G073800.1      -----TTCATCAAAACAAACCAATCATGCCACCGTGTGCCGGCTACA
Ptr_LAC25_Potri.010G183500.1      -----TTCATCAAAACAAACCAATCATGCCACCATACCGACCTTCA
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Ptr_LAC49_Potri.006G097100.1      ATGCCACTGCAGTTGCCAACCAATTTACCAACTCTCTACGCAGCCTTAAC
Ptr_LAC14_Potri.006G096900.1      ATGCCACTGCAGTTGCCAACCAATTTACCAACTCTCTACGCAGCCTTAAC
Ptr_LAC40_Potri.016G112000.1      ATGCCACTGCAGTTGCCAACCAATTTACTAATTTCTCTACGCAGCTTAAAC
Ptr_LAC17_Potri.008G064000.1      ACGCAACTCCAGTAACATCAACTTTTCATGGACAATCTTGAAGCCTGAAT
Ptr_LAC27_Potri.010G193100.1      ACGCAACTCCGGCAACTTCAACTTTTCATGGACAATCTTGAAGCCTGAAT
Ptr_LAC24_Potri.009G156800.1      ATGCTACAAATGTCGTCGCGAATTTTACAAGAAGACTTCGCAAGTCTGGCT
Ptr_LAC23_Potri.009G156600.1      ATGCTACAAATGTCGTCGCGAATTTTACAAGAAGACTTCGCAAGTCTGGCT
Ptr_LAC11_Potri.006G087100.1      ATGCTACAAATGTCGTCGCGAATTTTACAAGAAAATTCGCAAGTTAGCT
Ptr_LAC7_Potri.001G401300.1      ATGACACTTCATTTGCTACAAATTTTACAAGCAAACTCCGAGCCTAGCA
Ptr_LAC30_Potri.011G120300.1      ACGACACTTCATTTGCTACAAATTTTCTAGCAAGCTCCGAGCTTAGCC
Ptr_LAC11_Potri.006G087100.1      ATGCTACTGGCTTTGTTGCGAATTTTACCAAGAAAATTCGTAAGCTTAGCC
Ptr_LAC12_Potri.006G087500.1      ATGACACAACATTTGCCACAAAGTTTGTCAAGAAAATCCGTAGCTTGGCC
Ptr_LAC18_Potri.008G073700.1      ATGACACAGCTACAGTCACAGCCTTCAGTAGAAGCTTTAGAAGTCTCTGA
Ptr_LAC26_Potri.010G183600.1      ACGACACAGCTACAGTTACAGCATTAGTGGAAGCCTTAGAAGCCTCTGA
Ptr_LAC19_Potri.008G073800.1      ATGACACAGCCACTGTCACTACTTTTCACTACTAGCTTCAAAAGTGTGAT
Ptr_LAC25_Potri.010G183500.1      ATGACACAGCCACTGTCACTGCTTTCACTAGTAGCTTCAAAAGTACTGAT
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Ptr_LAC15_Potri.006G097000.1      TCAAGAAGATTTCTGCCAAAAGTCCCATGAACGTTGATCACAACCTTTT
Ptr_LAC49_Potri.006G097100.1      TCAAGAAGATTTCTGCCAAAAGTCCCATGAACGTTGATCACAACCTTTT
Ptr_LAC14_Potri.006G096900.1      TCAAGAAGATTTCTGCCAAAAGTCCCATGAACGTTGATCACAACCTTTT
Ptr_LAC40_Potri.016G112000.1      TCGAAAACGTTCCCTGCCAAAAGTCCCATTAACCGTTGATCAGCCTTTT

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Ptr_LAC17_Potri.008G064000.1      TCAAAGAAGTTCCAGCCAATGTTCCATTAACAGTGGACCATTCCTATA
Ptr_LAC27_Potri.010G193100.1      TCAAAGAAGTACCAGCCAATGTCCATTAAACAGTAGACCATGATCTATA
Ptr_LAC24_Potri.009G156800.1      AATTCTCGATTCCCGTTAATGTCCCTCAAACAGTAGACAAGAAATTTT
Ptr_LAC23_Potri.009G156600.1      AATTCTCGATTCCCGTTAATGTCCCTCAAACAGTAGACAAGAAATTTT
Ptr_LAC2_Potri.001G184300.1       AGTGCAGAATTCCCTGCCAATGTGCCGCAAAAGGTAGACAGGCAATTTT
Ptr_LAC7_Potri.001G401300.1       AGTGCAGACTTCCCTGCCAATGTTCTCAAAGGTTGACAGACAATTTT
Ptr_LAC30_Potri.011G120300.1      AACGCTAAATTTCCAGCTAATGTGCCACAAACAGTGGACAGGAAATCTT
Ptr_LAC11_Potri.006G087100.1      AATGCAAGATTCCAGCTAAGGTCCCAAAGAAAGTTGACAGACGCTTCTT
Ptr_LAC12_Potri.006G087500.1      AAAGTTGAGGTCCCGACCGATATT-----GATGAGAACCTCTT
Ptr_LAC18_Potri.008G073700.1      AAAGTCGAGGTCCCAACAGATATT-----GACGAGAACCTCTT
Ptr_LAC26_Potri.010G183600.1      AAAACCTTAGTCCCAACTGACATT-----GATGAGAGTTTATT
Ptr_LAC19_Potri.008G073800.1      AAAACCTTTGTCCGACTGACATC-----GATGAGAGTTTATT
Ptr_LAC25_Potri.010G183500.1      **      *      *      **      *      *

Ptr_LAC15_Potri.006G097000.1      CTTTACAGTTGGTCTAGGAGTTAACCCATGTCC-----AAGT----
Ptr_LAC49_Potri.006G097100.1      CTTTACAGTTGGTCTAGGAGTTAACCCATGTCC-----AAGT----
Ptr_LAC14_Potri.006G096900.1      CTTTACAGTTAGTCTAGGAGTTAACCCATGTCC-----AAGT----
Ptr_LAC40_Potri.016G112000.1      CTTCACTGTTGGTCTAGGAATTAACCCGTGTCC-----AACT----
Ptr_LAC17_Potri.008G064000.1      CTTCACTATCGGTGTGGGATTGACCCCTTGCGC-----AACA----
Ptr_LAC27_Potri.010G193100.1      CTTACAGATCGGTGTGGGATTGACCCCTTGTC-----AACA----
Ptr_LAC24_Potri.009G156800.1      CTTACAGTTGGTCTGGGAAACAGCCCGTGCCCAAAAAACAGACA----
Ptr_LAC23_Potri.009G156600.1      CTTACAGTTGGTCTGGGAAACAGCCCGTGCCCAAAAAACAGACA----
Ptr_LAC2_Potri.001G184300.1      CTTACAGTTGGTCTAGGAAACAGCCCGTGCCCAAAAAACAGACA----
Ptr_LAC7_Potri.001G401300.1      CTTCAAGTGAAGCCTTGGAACAAACCCATGTCTCAAAAAACAAACC---
Ptr_LAC30_Potri.011G120300.1      CTTCACTGTAGGCCTTGGAACAAACCCATGTCTCAAAAAACAAACC---
Ptr_LAC11_Potri.006G087100.1      CTTCACTGTGGGACTTGGGACTAACCCGTGCCCTAAAAATACAACC---
Ptr_LAC12_Potri.006G087500.1      TTTCACAATAGGTCTAGGATCACTCCCTTGCTCACAAAAACAAGACC---
Ptr_LAC18_Potri.008G073700.1      CTTCACAATCGGCCTAGGACTCAACAATTGTCCCAAAAACTTTAGAG---
Ptr_LAC26_Potri.010G183600.1      CTTCACAATTGGTCTAGGGCTTAACAACATGTCTAAAAATTCAGAG---
Ptr_LAC19_Potri.008G073800.1      CTTCACAATTGGTTTAGGTCTCAATCCTTGCCCGTCTAATTTTAAACAAGT
Ptr_LAC25_Potri.010G183500.1      CTTACAGTTGGTTTAGGTCTCAATCCATGCCACCAAAATTTTAAACAAGT
** *      *      *      *      **      *      *

Ptr_LAC15_Potri.006G097000.1      -----TGCAAAGCTGGTAATGGCA---GCAGGGTTGTTGCTAGTATT
Ptr_LAC49_Potri.006G097100.1      -----TGCAAAGCTGGTAATGGCA---GCAGGGTTGTTGCTAGTATT
Ptr_LAC14_Potri.006G096900.1      -----TGCAAAGCTGGTAATGGCA---GCAGGGTTGTTGCTAGTATT
Ptr_LAC40_Potri.016G112000.1      -----TGCAAAGCTGGTAATGGTA---GCAGAGTTGTTGCTAGTATC
Ptr_LAC17_Potri.008G064000.1      -----TG-----TGTCATGGTA---GCAAGGCTGTGGGAGCTATT
Ptr_LAC27_Potri.010G193100.1      -----TG-----TACTAATGGTA---GCAAGGCTGTGGCAGATATA
Ptr_LAC24_Potri.009G156800.1      -----TGTCAAGGGCCTAATGGCA---CGAAATTTTCAGTCTCTGTT
Ptr_LAC23_Potri.009G156600.1      -----TGTCAAGGGCCTAATGGCA---CGAAATTTTCAGTCTCTGTT
Ptr_LAC2_Potri.001G184300.1      -----TGTCAAGGACCTAATGGCA---CGAAATTTTCAGTCTCTGTT
Ptr_LAC7_Potri.001G401300.1      -----TGCCAGGGACCAATGGAA---CAATGTTTGCAGCTTCAGTT
Ptr_LAC30_Potri.011G120300.1      -----TGCCAGGGACCAACCGGAA---CGAGGTTTGTGCTTCAGTC
Ptr_LAC11_Potri.006G087100.1      -----TGTCAAGGACCAATAATAACGAAATTTGCTGCTTCGATT
Ptr_LAC12_Potri.006G087500.1      -----TGCCAGGACCTAATAACA---CCATGTTTGCAGTCTCCGCT
Ptr_LAC18_Potri.008G073700.1      CTAGGCGGTGTCAAGGACCAACGGCA---CTCGTTTCACTGCTAGTATG
Ptr_LAC26_Potri.010G183600.1      CTAGGCGGTGTCAAGGACCAACGGCA---CTCGTTTCACTGCTAGTATG
Ptr_LAC19_Potri.008G073800.1      CTAGCCAGTGTCAAGGGCTTAACGGAA---CCCGCTTCACTGCAAGCATG
Ptr_LAC25_Potri.010G183500.1      CTAGCCAGTGTCAAGGGCTAATGGAA---CCCGCTTCACTGCAAGCATG
**      **      *      *      *

Ptr_LAC15_Potri.006G097000.1      AACAAATGTCACATTTGTGATGCCAACCA---CTGCCCTGCTCCAAGCACA
Ptr_LAC49_Potri.006G097100.1      AACAAATGTCACATTTGTGATGCCAACCA---CTGCCCTGCTCCAAGCACA
Ptr_LAC14_Potri.006G096900.1      AACAAATGTCACATTTGTGATGCCAACCA---CTGCCCTGCTCCAAGCACA
Ptr_LAC40_Potri.016G112000.1      AACAAATGTCACATTTGTGATGCCAACCA---CTGCCCTGCTCCAAGCACA
Ptr_LAC17_Potri.008G064000.1      AATAATATTTCTTTTATAATGCCAACTA---CAGCTCTACTTCAAGCACA
Ptr_LAC27_Potri.010G193100.1      AATAATATTTCTTTTATAATGCCAACTA---CAGCTTACTTCAAGCACA
Ptr_LAC24_Potri.009G156800.1      AACAAACATCTCCATGGCTCTCCCTTCAT---CAGCACTCCTTCAATCCTA
Ptr_LAC23_Potri.009G156600.1      AACAAACATCTCCATGGCTCTCCCTTCAT---CAGCACTCCTTCAATCCTA
Ptr_LAC7_Potri.001G401300.1      AACAAACATCTCCATGGCTCTCCCTTCAT---CAGCACTCCTTCAATCCTA
Ptr_LAC30_Potri.011G120300.1      AATAATGTATCATTTGTGATGCCAACCA---AAGCTCTACTCCAAGCCCA
Ptr_LAC11_Potri.006G087100.1      AATAATGTATCATTTGTGATGCCAACCA---CAGCTCTACTCCAAGCCCA
Ptr_LAC12_Potri.006G087500.1      AACAAATGTGCATTTGTATTACCTCTGT---TAGCTCTTCTGCAATCCTA
Ptr_LAC18_Potri.008G073700.1      AACAAATGTGCATTTGTGCAGCCAAATA---TAGCCCTCTGCAATCCTA
Ptr_LAC26_Potri.010G183600.1      AACAAATGTGCCTTTGTGTTTCCCTTCAAACATTGCGTTACTGCAGGCGTA
Ptr_LAC19_Potri.008G073800.1      AACAAACGTATCTTTTGTGCTCCCATCCAACCTCTCTCTGTTGCAAGCTCA
Ptr_LAC25_Potri.010G183500.1      AACAAATGTATCTTTTGTGCTCCCATCCAACCTCTCTCTATTGCAAGCTCA

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Ptr_LAC15_Potri.006G097000.1 TTTCTTCAACA---TCAGCGGTGTGTTACCACTGATTTTCTGCAAAGC
Ptr_LAC49_Potri.006G097100.1 TTTCTTCAACA---TCAGCGGTGTGTTACCACTGATTTTCTGCAAAGC
Ptr_LAC14_Potri.006G096900.1 TTTCTTCAACA---TCAGCGGTGTGTTACCACTGATTTTCTGCAAAGC
Ptr_LAC40_Potri.016G112000.1 TTTCTTCAACA---TCAGTGGTGTGTTACCACTGATTTTCTGCAAAGC
Ptr_LAC17_Potri.008G064000.1 CTACTATAGCA---TAAGTGGGGTTTTACAGACGATTTTCCAGCAATGC
Ptr_LAC27_Potri.010G193100.1 CTACTATAACA---TAAGTGGGGTCTTCACAGATGATTTCCAGCAAAAC
Ptr_LAC24_Potri.009G156800.1 TTTTTTCAAAAAATCAAATGGGGTTTACACCTCTGATTTCCCAAGCTTTT
Ptr_LAC23_Potri.009G156600.1 CTTTTTCAAAAAATCAAATGGGGTTTACACCTCTGATTTCCCAAGCTTTT
Ptr_LAC2_Potri.001G184300.1 TTTTTTCAAAAAATCAAATGGGGTTTACACCTCTGATTTCCCAAGCTTTT
Ptr_LAC7_Potri.001G401300.1 TCATTTTGGTCAATCAAAGGGCGTTTACAGCCCTAACTTTCCAATCAATC
Ptr_LAC30_Potri.011G120300.1 CCATTTTGGTCAATCAAGGGGTGTTTACAGTCTCTATTTTCCAATCAGTC
Ptr_LAC11_Potri.006G087100.1 CTTCTTTGGACAGTCAAATGGTGTCTTCACCTCCGATTTTCTCAGAACCC
Ptr_LAC12_Potri.006G087500.1 TTTCTTAAACCGGTCAAAGGGGTGTGTACACAACCTGATTTCCCTACCAACC
Ptr_LAC18_Potri.008G073700.1 TAAGCAAAAA---TTCTGGAGTCTTCACCACCGATTTTCCAGCAAAAC
Ptr_LAC26_Potri.010G183600.1 TCAGCAAAAAAG---TTCTGGAATTTACACCAGTACTTTCCAGCAAAAC
Ptr_LAC19_Potri.008G073800.1 TCACCAACGCA---TACAAGGAGTTTTACCACTGATTTTCCAGCAAAATC
Ptr_LAC25_Potri.010G183500.1 TCACCAACGCA---TACAAGGAGTTTTACCACTGATTTCCAGCAAAATC
** * * * * * * * * *

Ptr_LAC15_Potri.006G097000.1 CACCGCATGTTTTCAATTACACTGG---CACTCCACCTACAAATTTACAG
Ptr_LAC49_Potri.006G097100.1 CACCGCATGTTTTCAATTACACTGG---CACTCCACCTACAAATTTACAG
Ptr_LAC14_Potri.006G096900.1 CACCGCATGTTTTCAATTACACTGG---CACTCCACCTACAAATTTACAG
Ptr_LAC40_Potri.016G112000.1 CACCACATGTTTTTAATTACACAGG---CACTCCACCCACAAATTTACAG
Ptr_LAC17_Potri.008G064000.1 CGCCAAATTCITTTAATTATACGGGAAATAATACGGCATTAAATCTGCAG
Ptr_LAC27_Potri.010G193100.1 CACCGATTTCTTTTAAATTATACGGGAAATAATACGGCAATGAATCTAAAG
Ptr_LAC24_Potri.009G156800.1 CTCTCCATCCATTCAACTACACAGG---GACCCCAACCAATAAATACTCTT
Ptr_LAC23_Potri.009G156600.1 CTCTCCATCCATTCAACTACACAGG---GACCCCAACCAATAAATACTCTT
Ptr_LAC2_Potri.001G184300.1 CTCTCCATCCATTCAACTACACAGG---GACCCCAACCAATAAATACTCTT
Ptr_LAC7_Potri.001G401300.1 CACTAATCCCTTTTAAATTATACCGG---CACCCCAACCAACAATACTATG
Ptr_LAC30_Potri.011G120300.1 CACTGATCCCTTTTAACTATACTGG---CACACCACCGAACAATACTATG
Ptr_LAC11_Potri.006G087100.1 CAACAATACCAATTCATTTATACGGG---TACTCCACCAATAAATACTATG
Ptr_LAC12_Potri.006G087500.1 CACCTTTCAAATTCAACTATACAGG---GACACCGCTAGCAATACCATG
Ptr_LAC18_Potri.008G073700.1 CACAAGTAAATTTGACTTACACTGGCAATGTTAGTCGTTCCCTCTTTTCAA
Ptr_LAC26_Potri.010G183600.1 CACCAGTAAAGTTTGATTACACTGGTAATGTGAGTCGTTCCCTCTTTTCAA
Ptr_LAC19_Potri.008G073800.1 CACCACGAAATTTGATTACACTGGTAATGTGAGCCGGTCCCTCTTTTCAA
Ptr_LAC25_Potri.010G183500.1 CACCACGAAATTTGATTACACTGGTAACGTGAGCAGGTCCTCTTTTCAA
* ** * * * *

Ptr_LAC15_Potri.006G097000.1 ACCAAAAAGTGGAACATAAGTTTATAGGCTGAGCTACAACCTCGACAGTCCA
Ptr_LAC49_Potri.006G097100.1 ACCAAAAAGTGGAACATAAGTTTATAGGCTGAGCTACAACCTCGACAGTCCA
Ptr_LAC14_Potri.006G096900.1 ACCAAAAAGTGGAACATAAGTTTATAGGCTGAGCTACAACCTCGACAGTCCA
Ptr_LAC40_Potri.016G112000.1 ACCACAAGTGGAACAAAAGCATATAGGTTGCCCTACAACCTCGACAGTCCA
Ptr_LAC17_Potri.008G064000.1 ACCATCAATGGGACTAGAACTTATAGGCTGGCTTTTAAATTCGACAGTTCA
Ptr_LAC27_Potri.010G193100.1 ACCACAATGGGACTAGAGCTTATAGGCTGGCTTTTAAATTCAGCAGTTCA
Ptr_LAC24_Potri.009G156800.1 GTGGCCAATGGTACCAAGCTTGTAGTGGTACCATTTAACACAAGCGTGGA
Ptr_LAC23_Potri.009G156600.1 GTGGCCAATGGTACCAAGCTTGTAGTGGTACCATTTAACACAAGCGTGGA
Ptr_LAC2_Potri.001G184300.1 GTGACCAATGGTAACAAGCTTGTAGTGGTACCATTTAACACAAGTGTTGA
Ptr_LAC7_Potri.001G401300.1 GTGAGCAATGGAACAAAGCTAGTTGTGCTTCCCTTTAACCACGAGCGTGGA
Ptr_LAC30_Potri.011G120300.1 GTGAGCAATGGAACAAAGCTAGTTGTGCTTCCCTTTAACCACGAGCGTGGA
Ptr_LAC11_Potri.006G087100.1 GTGAGCAATGGAACAAAGCTAGTTGTGCTTCCCTTTAACCACGAGCGTGGA
Ptr_LAC12_Potri.006G087500.1 ACTGCCAAAGGAACCAAGGTGGTGGTCTTCCCTTCAATACTAGCGTGGA
Ptr_LAC18_Potri.008G073700.1 CCTGCTCGTGGAACTAAGCTGTACAAGTTGAAGTACGGATCAAGGGTGCA
Ptr_LAC26_Potri.010G183600.1 CCTGTTCTGTGGAACATAAGCTGTACAAGTTGAAGTACGGATCAAGGGTGCA
Ptr_LAC19_Potri.008G073800.1 CCTGTTCTGTGGGACTAAGCTGTACAATTTGAAATATGGTTCAAGGGTTCA
Ptr_LAC25_Potri.010G183500.1 CCTGTTCCAGGGAATAAGCTGTACAGATTGAAATATGGGTCAAGAGTGCA
** * * * * * * * * *

Ptr_LAC15_Potri.006G097000.1 ACTTGTTATGCAAGATACTGGGATCATATCCCCTGAGAACCATCCGATCC
Ptr_LAC49_Potri.006G097100.1 ACTTGTTATGCAAGATACTGGGATCATATCCCCTGAGAACCATCCGATCC
Ptr_LAC14_Potri.006G096900.1 ACTTGTTATGCAAGATACTGGTATCATATCCCCTGAGAACCATCCGATCC
Ptr_LAC40_Potri.016G112000.1 ACTTGTTATGCAAGATACTGGGATCATATCCCCTGAGAACCATCCCAATCC
Ptr_LAC17_Potri.008G064000.1 ACTTGTTGTTGCAAGGAACCACTATAATAGCACCTGAGAGTCATCCGTTCC
Ptr_LAC27_Potri.010G193100.1 AGTGGTGTCTGCAAGGAACCACTATAATAGCACCTGAGAGTCATCCATTCC
Ptr_LAC24_Potri.009G156800.1 GGTAGTGATGCAGGGCACTCGCATTTTCGGTGTGAGAGCCACCCCTCTCC
Ptr_LAC23_Potri.009G156600.1 GGTAGTGATGCAGGGCACTCGCATTTTCGGTGTGAGAGCCACCCCTCTCC
Ptr_LAC2_Potri.001G184300.1 GGTAGTGATGCAGGGCACTCGAATTTTCGGTGTGAGAGCCACCCCTCTCC
Ptr_LAC7_Potri.001G401300.1 GCTCATTTATGCAGGACACCACTACTTGGTGTGAGAGCCACCCCTCTTC
Ptr_LAC30_Potri.011G120300.1 GCTCATCATGCAGGGCACTAGCATACTTGGTGTGAGAGCCACCCCTCTTC

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Ptr_LAC11_Potri.006G087100.1
Ptr_LAC12_Potri.006G087500.1
Ptr_LAC18_Potri.008G073700.1
Ptr_LAC26_Potri.010G183600.1
Ptr_LAC19_Potri.008G073800.1
Ptr_LAC25_Potri.010G183500.1

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GTTGGTGATGCAGGACACCAGCATTATTGGTGACAGAGTCACCCCTCTTC
GATTGTGCTACAGGACACGAGCATCGTCACACCAGAAAACCATCCGATCC
GATTGTGTTGCAGGACACGAGCATTGTCACACCAGAAAACCCCAATCC
GATTGTATTGCAAGACACGAGCATTGTCACACCAGAAAACCCCAATCC
GATTGTGCTACAAGACACGAGCATTGTCACATCAGAAAACCCCAATTC
* *

Ptr_LAC15_Potri.006G097000.1
Ptr_LAC49_Potri.006G097100.1
Ptr_LAC14_Potri.006G096900.1
Ptr_LAC40_Potri.016G112000.1
Ptr_LAC17_Potri.008G064000.1
Ptr_LAC27_Potri.010G193100.1
Ptr_LAC24_Potri.009G156800.1
Ptr_LAC23_Potri.009G156600.1
Ptr_LAC2_Potri.001G184300.1
Ptr_LAC7_Potri.001G401300.1
Ptr_LAC30_Potri.011G120300.1
Ptr_LAC11_Potri.006G087100.1
Ptr_LAC12_Potri.006G087500.1
Ptr_LAC18_Potri.008G073700.1
Ptr_LAC26_Potri.010G183600.1
Ptr_LAC19_Potri.008G073800.1
Ptr_LAC25_Potri.010G183500.1

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ATTTACATGGATTCAATTTCTTTGCTGTCGGTAGGGGAGTAGGGAATTAC
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ATCTTCATGGATTAAATTTCTTTGTTGTTGGCAAGGGATTGGGAATTTT
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ATCTTCATGGCTTCAATTTCTATGTTGTTGGAGAAGGGTTTGGAAATTTT
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ATCTGCATGGTTTAAATTTCTTTGTTGGTGGACAAGGTTTGGCAACTAC
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ATCTTCATGGATATGACTTTTACATTATCGCACAAAGGATTGGAAATTT
* *

Ptr_LAC15_Potri.006G097000.1
Ptr_LAC49_Potri.006G097100.1
Ptr_LAC14_Potri.006G096900.1
Ptr_LAC40_Potri.016G112000.1
Ptr_LAC17_Potri.008G064000.1
Ptr_LAC27_Potri.010G193100.1
Ptr_LAC24_Potri.009G156800.1
Ptr_LAC23_Potri.009G156600.1
Ptr_LAC2_Potri.001G184300.1
Ptr_LAC7_Potri.001G401300.1
Ptr_LAC30_Potri.011G120300.1
Ptr_LAC11_Potri.006G087100.1
Ptr_LAC12_Potri.006G087500.1
Ptr_LAC18_Potri.008G073700.1
Ptr_LAC26_Potri.010G183600.1
Ptr_LAC19_Potri.008G073800.1
Ptr_LAC25_Potri.010G183500.1

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AATCCGAAGACTGATACTAAGAAATTTAACCTCGTTGATCCTGTTGAACG
AATCCGAAGACTGATACTAAGAAATTTAACCTCGTTGATCCTGTTGAACG
AATCCGAAGACTGATCCTAAGAAATTTCAACCTGTTGACCTGTTGAACG
GATGCAGATAATGATCCAAAAAATTTAATCTGGCTGATCCTGTTGAAAG
GATCCAGATAATGATCCAAAGAAATTTAATCTGGCTGATCCTGTTGAAAG
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GATCCAAAAAAGGATCCGGCAAATTTCAATCTGTTGACCCCGTTGAAAG
GATCCAAAGCAAGGATCCCGCAAATTTCAATCTGTTGACCCCGTTGAAAG
GATCCCAACAAAGATCCTTCAATTTTAAATCTGGTCGACCTATGGAAAG
GACCCCAAGAAAGACCTGTCAAGTTCAACCTTGTGACCTGCCGAGAG
AATCCCAAACTGATAAATCATAATTTCAACCTGTTGATCCACCAATGAG
AATCCCAAACTCATAAATCATAATTTAATCTTGGATCCACCAATGAG
AATCCCCGAGCAGATCCATCAAAATTTAACCTGTTGATCCACCTCTCAG
AATCCCCGAACAGACCATCAAAATTTAACCTCGTTGATCCACCTCTCAG
* *

Ptr_LAC15_Potri.006G097000.1
Ptr_LAC49_Potri.006G097100.1
Ptr_LAC14_Potri.006G096900.1
Ptr_LAC40_Potri.016G112000.1
Ptr_LAC17_Potri.008G064000.1
Ptr_LAC27_Potri.010G193100.1
Ptr_LAC24_Potri.009G156800.1
Ptr_LAC23_Potri.009G156600.1
Ptr_LAC2_Potri.001G184300.1
Ptr_LAC7_Potri.001G401300.1
Ptr_LAC30_Potri.011G120300.1
Ptr_LAC11_Potri.006G087100.1
Ptr_LAC12_Potri.006G087500.1
Ptr_LAC18_Potri.008G073700.1
Ptr_LAC26_Potri.010G183600.1
Ptr_LAC19_Potri.008G073800.1
Ptr_LAC25_Potri.010G183500.1

GAACACAATTGGAGTACCTTCTGGTGGATGGGTGGCGATAAGATTTGCGG
GAACACAATTGGAGTACCTTCTGGTGGATGGGTGGCGATAAGATTTGCGG
GAACACAATTGGAGTACCTTCTGGTGGATGGGTGGCGATAAGATTTGCGG
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AAACACAATCAGTGACCTACTGCTGGATGGGCTGCGATCAGATTGAGGG
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GAATACGTGGGAGTGCCCTCTGGAGGCTGGGTGCTATACGTTTCTAG
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GAATACAGTTGCAGTACCCGTGAATGGATGGGCGGTTATTAGATTTGTTG
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AAACACAGTTGCAGTCCCTGTAACGGATGGGCGGTCATTGATTGCTCG
AAACACAGTTGCAGTGCCTGTAATGGATGGGCGGTCATTAGATTCGATAG
* *

Ptr_LAC15_Potri.006G097000.1
Ptr_LAC49_Potri.006G097100.1
Ptr_LAC14_Potri.006G096900.1
Ptr_LAC40_Potri.016G112000.1
Ptr_LAC17_Potri.008G064000.1

CCGATAATCCAGGAGTTTGGTTTCATGCATTGCCATCTAGAGGTGCACACT
CCGATAATCCAGGAGTTTGGTTTCATGCATTGCCATCTAGAGGTGCACACT
CCGATAATCCAGGAGTTTGGTTTCATGCATTGCCATCTAGAGGTGCACACT
CCGATAATCCAGGAGTTTGGTTTCATGCATTGCCATCTAGAGGTGCACACT
CAGATAATCCAGGAGTTTGGTTTTCATGCATTGCCATTTGGAAGTACATACG

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Ptr_LAC27_Potri.010G193100.1 CAGATAATCCAGGTGTTTGGTTTTTGATTGCCATTTGGAAGTACACACG
Ptr_LAC24_Potri.009G156800.1 CAGACAATCCAGGAGTATGGTTTATGCACTGCCACTTTGATGTCCATTTG
Ptr_LAC23_Potri.009G156600.1 CAGACAATCCAGGAGTATGGTTTATGCACTGCCACTTTGATGTCCATTTG
Ptr_LAC2_Potri.001G184300.1 CAGACAATCCAGGAGTATGGTTTATGCACTGCCACTTTGATGTCCATTTG
Ptr_LAC7_Potri.001G401300.1 CCGACAACCCAGGTGTATGGTTCATGCATTGCCATCTAGAAGTGCACACA
Ptr_LAC30_Potri.011G120300.1 CAGACAATCCAGGCGTATGGTTCATGCATTGCCATCTAGAAGTGCACACA
Ptr_LAC11_Potri.006G087100.1 CGGACAATCCAGGGGTGTGGTTCATGCATTGCCATCTTGATGTGCACACT
Ptr_LAC12_Potri.006G087500.1 CTGACAATCCAGGAGTTTGGTTCATGCATTGCTATTTGGAAGTACACACC
Ptr_LAC18_Potri.008G073700.1 CTGACAACCCAGGTGTGTGGCTAATGCACTGTCACTTGGATGTTCCACATC
Ptr_LAC26_Potri.010G183600.1 CTGACAATCCAGGTGTGTGGCTAATGCACTGTCACTTGGATGTTCCACATC
Ptr_LAC19_Potri.008G073800.1 CAGACAATCCAGGTGTATGGCTAATGCATTGCCACCTGGATGTCCACATC
Ptr_LAC25_Potri.010G183500.1 CAGATAATCCAGGTGTTTGGCTAATGCATTGCCACTTGGATGTTCCACATC
* * * * *

Ptr_LAC15_Potri.006G097000.1 ACATGGGGACTTAAGATGGCGTTCTTGGTAGACAATGGCAAAGGCCCTAA
Ptr_LAC49_Potri.006G097100.1 ACATGGGGACTTAAGATGGCGTTCTTGGTAGACAATGGCAAAGGCCCTAA
Ptr_LAC14_Potri.006G096900.1 ACATGGGGACTTAAGATGGCGTTCTTGGTAGACAATGGCAAAGGCCCTAA
Ptr_LAC40_Potri.016G112000.1 ACATGGGGACTTAAGATGGCATTTCTTGGTGGACAATGGCAAAGGCCCTAA
Ptr_LAC17_Potri.008G064000.1 ACATGGGGACTTAAGATGGTATTTGTGGTTGACAATGGTGAAGGTCCAGA
Ptr_LAC27_Potri.010G193100.1 ACATGGGGACTTAAGATGGCATTTTGTGGTGGACAATGGTGAAGGCCCAAG
Ptr_LAC24_Potri.009G156800.1 AGCTGGGGGTTAAGGATGGCATGGATTGTCTTGGATGGAACACTTCCCAG
Ptr_LAC23_Potri.009G156600.1 AGCTGGGGGTTAAGGATGGCATGGATTGTCTTGGATGGAACACTTCCCAG
Ptr_LAC2_Potri.001G184300.1 AGCTGGGGGTTAAGGATGGCATGGATTGTCTTGGATGGAACACTTCCCAG
Ptr_LAC7_Potri.001G401300.1 AGCTGGGGGTTAAGGATGGCTTGGGTTGTCTTGGATGGAAGCTTCCCAA
Ptr_LAC30_Potri.011G120300.1 AGCTGGGGGTTAAGGATGGCTTGGGTTGTCTTGGATGGAAGCTTCCCAA
Ptr_LAC11_Potri.006G087100.1 AGCTGGGGGCTCCGAATGGCATGGATAGTCTTGGATGGACCCCAACCGAA
Ptr_LAC12_Potri.006G087500.1 AGCTGGGGGCTGAAGATGGCTTGGGTTGTCAATGACGGAAACGCGCCGAG
Ptr_LAC18_Potri.008G073700.1 ACATGGGGGCTTGCCCATGGCGTTTTTGGTAGAAGAGGGGATTGGGATTTT
Ptr_LAC26_Potri.010G183600.1 ACATGGGGTTTGGCCATGGCTTTCTTGGTAGAGGATGGGATTGGGGAATT
Ptr_LAC19_Potri.008G073800.1 ACCTGGGGTCTTGCCACAGCTTTCTTGGTAGAGAATGGAGTTGGACAATT
Ptr_LAC25_Potri.010G183500.1 ACCTGGGGTCTTGCCACGCGTTTCTTGGTCGAGAATGGAGTCGGAGAATT
* * * * *

Ptr_LAC15_Potri.006G097000.1 GGAGTCTCTTCTACCGCCGCCAAGCGACCTTCCAAAATGTTGA
Ptr_LAC49_Potri.006G097100.1 GGAGTCTCTTCTACCGCCGCCAAGCGATCTTCCAAAATGTTGA
Ptr_LAC14_Potri.006G096900.1 GGAGTCTCTTCTACCGCCGCCAAGCGACCTTCCAAAATGTTGA
Ptr_LAC40_Potri.016G112000.1 CGAGTCACTTCTGCCGCTCCAAGCGATCTTCCAAAATGTTAA
Ptr_LAC17_Potri.008G064000.1 CGAGTCACTACTACCACCTCCAGTGACCTCCCAACTGCTAG
Ptr_LAC27_Potri.010G193100.1 TGAGTCAATACTACCACCTCCTAGTGACCTTCCAACTGCTAG
Ptr_LAC24_Potri.009G156800.1 TCAGAAGCTCCCTCCTCCACCATCTGATCTTCCCAAGTGTTGA
Ptr_LAC23_Potri.009G156600.1 TCAGAAGCTCCCTCCTCCACCATCTGATCTTCCCAAGTGTTGA
Ptr_LAC2_Potri.001G184300.1 TCAGAAGCTCCCTCCTCCACCATCTGATCTTCCCAAGTGTTGA
Ptr_LAC7_Potri.001G401300.1 TCAGAAGCTGCTTCTCCACGCGATCTTCCCAAGTGTTGA
Ptr_LAC30_Potri.011G120300.1 TCAGAAGCTGCTTCTCCACGCTGATCTTCCCAAGTGTTAA
Ptr_LAC11_Potri.006G087100.1 TCAAAAATAACCAACCAACCGTCTGATCTTCCAAAGTGCTGA
Ptr_LAC12_Potri.006G087500.1 CCAAAAGCTGCCACCTCCACCTTCTGATCTCCCAAGTGTTAA
Ptr_LAC18_Potri.008G073700.1 ACAGAGTGTCGAGCCTCCTCCAGCGATCTGCCAATATGTTAA
Ptr_LAC26_Potri.010G183600.1 ACAGAGTGTAAGAGCCTCCTCCAGCAGATCTGCCGATATGTTAA
Ptr_LAC19_Potri.008G073800.1 GCAATCCATTGAGTCGCCTCCAGAAGATCTGCCTCTATGCTGA
Ptr_LAC25_Potri.010G183500.1 GCAATCCATTGAGTCCCTCCAGAAGATCTGCCTCTATGCTGA
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clustalw.dnd

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Ptr_LAC2_Potri.001G184300.1:0.00966,
(
Ptr_LAC24_Potri.009G156800.1:0.00238,
Ptr_LAC23_Potri.009G156600.1:0.00398)
:0.00594)
:0.16697,
(
(
Ptr_LAC7_Potri.001G401300.1:0.04288,
Ptr_LAC30_Potri.011G120300.1:0.05162)
:0.11425,
Ptr_LAC11_Potri.006G087100.1:0.18016)
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)
)

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:0.00405)
:0.00229,
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Ptr_LAC15_Potri.006G097000.1:0.00512,
Ptr_LAC49_Potri.006G097100.1:0.00203)
:0.00612,
Ptr_LAC14_Potri.006G096900.1:0.00969)
:0.03744,
Ptr_LAC40_Potri.016G112000.1:0.04397)
:0.11983,
(
Ptr_LAC17_Potri.008G064000.1:0.05360,
Ptr_LAC27_Potri.010G193100.1:0.04952)
:0.15069)
:0.05715,
(
(
Ptr_LAC18_Potri.008G073700.1:0.05238,
Ptr_LAC26_Potri.010G183600.1:0.05255)
:0.06511,
(
Ptr_LAC19_Potri.008G073800.1:0.04010,
Ptr_LAC25_Potri.010G183500.1:0.04338)
:0.08931)
:0.17776)
:0.07638,
Ptr_LAC12_Potri.006G087500.1:0.17106);

```

C.9 Phylogenetic tree of thirteen SDX LACs which are targets of miR397a

C.10 Phylogenetic tree of all seventeen SDX LACs

D Appendix D

D.1 Sequence similarity for miRNAs

121STTM166-C-2-R

CTACGGGGGAGTCACGTAACCAACCCTGCCTTAGCATCTTTTCCCTAACGGCCTCCTGCCTAA
GCGGTACTTCTAGCTTCGAACGGCGTCTGGGCTCCAGGTTTAGTCGTCTCGTGTCTGGTTTATA
TTCACGACAAAGATCTATAGGGACTTTAGGAGATCTGGATTTTAGTACTGGATTTTGGTTTATAG
GAATTAGAAATTTTATTGATAGAAGTATTTTACAAATACAAATACATACTAAGGGTTTCTTAT
ATGCTCAACACATGAGCGAAACCCTATAAGAACCCTAATTTCCCTTATCGGGAAACTACTCAC
ACATTATTTATGGAGAAAATAGAGAGAGATAGATTTGTAGAGAGAGA**CTGGTGATTTCAGCG**
TACCGAATTC**TCGGACCAGGTAGCTTCATTCCTTCTTTAGACCATATTTAAATTA**
GACCATAACAACAACAAC**TCGGACCAGGTAGCTTCATCCCC**AAGCTTGGGCTGTCCTCTCCA
AATGAAATGAACTTCCTTATATAGAGGAAGGGTCTTGCGAAGGATAGTGGGATTGTGCGTCAT
CCCTTACGTCAGTG

Reverse complement(pBI121 STTM165/166)

5'GGGG**G**ATGAAG**CTA**CCTGGTCCGA3'**GTTGTTGTTGTTATGGTCTAATTTAAATATGGTCTAA**
AGAAGAAGAAT5'GGGG**A**TGAAG**CTA**CCTGGTCCGA3'GAATTCGGTACGCTGAAATCACCA
G

121STTM397-C-2-R

GTCAACGTAACCAACCCTGCCTTAGCATCTTTTCCCTAACGGCCTCCTGCCTAAGCGGTACTTC
TAGCTTCGAACGGCGTCTGGGCTCCAGGTCTAGTCGTCTCGTGTCTGGTTTATATTCACGACAA
AGATCTATAGGGACTTTAGGAGATCTGGATTTTAGTACTGGATTTTGGTTTATAGGAATTAGAA
ATTTTATTGATAGAAGTATTTTACAAATACAAATACATACTAAGGGTTTCTTATATGCTCAACA
CATGAGCGAAACCCTATAAGAACCCTAATTTCCCTTATCGGGAAACTACTCACACATTATTTA
TGGAGAAAATAGAGAGAGATAGATTTGTAGAGAGAGACTGGTGATTTCAGCGTACCGAATTC
TTATTGAGTGTAGCAGCGTTGATG**ATTCTTCTTCTTTAGACCATATTTAAATTAGACCATAACA**
ACAACAACTCATTGAGTGTAGCAGCGTTGATGAAGCTTGGGCTGTCCTCTCCAAATGAAATGA
ACTTCCTTATATAGAGGAAGGGTCTTGCGAAGGATAGTGGGGTTGTGCGTCATCCCTTATGTC
AGTGGAGATGTCACATCAATCCACTTGCTTTGAAGACGTGGTTGGAACGTCTTCTTTTCCACG
ATGCTCCTCGTGGGTGGGGTCCATCTTTGGGACCACTGTCGGCAGAGGCATCTTGAATGATA
GCCTTTCCTTTATCGCAATGATGGCATTGTAGGAGCCACCTTCCTTTTCTACTGTCCTTTTCGAT
GAGTGACAGATAGCTGGGCAATGG

D.2 Sequence similarity of all eight HB genes that are targets of miR166

CLUSTAL 2.1 Multiple Sequence Alignments

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Sequence type explicitly set to DNA
Sequence format is Pearson
Sequence 1: Ptr_HB1_Potri.009G014500.1_CDS      2568 bp
Sequence 2: Ptr_HB2_Potri.004G211300.1_POPREV_CDS 2541 bp
Sequence 3: Ptr_HB3_Potri.011G098300.1_PHABULOSA_CDS 2532 bp
Sequence 4: Ptr_HB4_Potri.001G372300.1_CDS      2535 bp
Sequence 5: Ptr_HB5_Potri.001G188800.1_PCN_CDS   2559 bp
Sequence 6: Ptr_HB6_Potri.003G050100.1_CDS      2514 bp
Sequence 7: Ptr_HB7_Potri.018G045100.1_CDS      2532 bp
Sequence 8: Ptr_HB8_Potri.006G237500.1_CDS      2535 bp
Start of Pairwise alignments
Aligning...
```

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Sequences (1:2) Aligned. Score: 86.344
Sequences (1:3) Aligned. Score: 56.4376
Sequences (1:4) Aligned. Score: 58.2643
Sequences (1:5) Aligned. Score: 53.4193
Sequences (1:6) Aligned. Score: 54.7335
Sequences (1:7) Aligned. Score: 51.7378
Sequences (1:8) Aligned. Score: 53.4911
Sequences (2:3) Aligned. Score: 51.6588
Sequences (2:4) Aligned. Score: 53.215
Sequences (2:5) Aligned. Score: 46.7532
Sequences (2:6) Aligned. Score: 50.2387
Sequences (2:7) Aligned. Score: 52.4882
Sequences (2:8) Aligned. Score: 49.1913
Sequences (3:4) Aligned. Score: 93.3649
Sequences (3:5) Aligned. Score: 57.109
Sequences (3:6) Aligned. Score: 58.5123
Sequences (3:7) Aligned. Score: 54.3049
Sequences (3:8) Aligned. Score: 58.4518
Sequences (4:5) Aligned. Score: 57.5148
Sequences (4:6) Aligned. Score: 60.0636
Sequences (4:7) Aligned. Score: 57.4645
Sequences (4:8) Aligned. Score: 58.8166
Sequences (5:6) Aligned. Score: 92.8401
Sequences (5:7) Aligned. Score: 71.6035
Sequences (5:8) Aligned. Score: 73.0178
Sequences (6:7) Aligned. Score: 75.895
Sequences (6:8) Aligned. Score: 77.6452
Sequences (7:8) Aligned. Score: 93.0095
Guide tree file created: [clustalw.dnd]
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There are 7 groups
Start of Multiple Alignment
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Aligning...
Group 1: Sequences: 2      Score:45745
Group 2: Sequences: 2      Score:45284
Group 3: Sequences: 4      Score:40248
Group 4: Sequences: 2      Score:46082
Group 5: Sequences: 6      Score:34697
Group 6: Sequences: 2      Score:45259
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Group 7: Sequences: 8 Score:33968
Alignment Score 334663

CLUSTAL-Alignment file created [clustalw.aln]

clustalw.aln

CLUSTAL 2.1 multiple sequence alignment

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Ptr_HB7_Potri.018G045100.1_CDS      -----ATGGTAGAAGGGATGATGGGTGTAAGCTCGACGTGTAAGGATGG
Ptr_HB8_Potri.006G237500.1_CDS      -----ATGATGGCTGTAAGCTCGACGTGTAAGGATGG
Ptr_HB5_Potri.001G188800.1_PCN      -----ATGATGGCAAT-----GTCCTGCAAGGATGG
Ptr_HB6_Potri.003G050100.1_CDS      -----ATGGAAT-----GTCCTGCAAGGATGG
Ptr_HB3_Potri.011G098300.1_PHA      -----ATGGCGCT-----TTCTATGCACAG
Ptr_HB4_Potri.001G372300.1_CDS      -----ATGGCGCT-----TTCTATACACAG
Ptr_HB1_Potri.009G014500.1_CDS      ATGGCGATGGCGGTGGCCCCCTCAGCACAGAGAGAGCAGTAGTAGTGGGAG
Ptr_HB2_Potri.004G211300.1_POP      ATGGCCATGGAAGTGGCCCCCTCTGCACCGAGAGAGCAGCAGTAGTGGGAG
                                   *               *               *

Ptr_HB7_Potri.018G045100.1_CDS      AAGTACTAGGATAACAATGGACAACGGAAGTACGTAAGGTACACACCAG
Ptr_HB8_Potri.006G237500.1_CDS      GAGTACTAAGATAACAATGGACAATGGAAAGTACGTAAGGTACACACCAG
Ptr_HB5_Potri.001G188800.1_PCN      TAAGAATCCTATTAAACATGGACAATGGCAAGTATGTCCGGTACACGCCTG
Ptr_HB6_Potri.003G050100.1_CDS      TAAGCAGCCTAT---CATGGACAATGGCAAGTATGTCCGGTACACGCCTG
Ptr_HB3_Potri.011G098300.1_PHA      TAAGGACAAGCA---GATGGATTCAAGTAAGTATGTCCGGTATACACCTG
Ptr_HB4_Potri.001G372300.1_CDS      TAAGGACAAGCA---CATGGATTCAAGTAAGTATGTGAGGTATACACCTG
Ptr_HB1_Potri.009G014500.1_CDS      CTAAACAAGCACCTCACTGATAATGGAAGTATGTTCCGGTACACATCAG
Ptr_HB2_Potri.004G211300.1_POP      CATAACAAGCACCTTACTGATGGGAAGTATGTTCCGGTACACAGCAG
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Ptr_HB7_Potri.018G045100.1_CDS      AGCAAGTGAAGCACTAGAAAGGCTTTACCATGAATGTCTTAAACCAACT
Ptr_HB8_Potri.006G237500.1_CDS      AACAAAGTGAAGCACTAGAAAGGCTTTACCATGAATGTCTTAAAGCAAGC
Ptr_HB5_Potri.001G188800.1_PCN      AGCAGGTGCGAAGCCTTGGAAAGGCTCTATCATGATTGCCCAAACCCAGC
Ptr_HB6_Potri.003G050100.1_CDS      AGCAGGTGCGAAGCCTTGGAAAGGCTCTATCATGATTGTCCCAAACCCAGC
Ptr_HB3_Potri.011G098300.1_PHA      AGCAAGTTGAGGCTTTGGAGAGGGTGTATACAGAATGTCCAAGCCTAGT
Ptr_HB4_Potri.001G372300.1_CDS      AGCAAGTTGAGGCATTGGAGAGGGTGTATACAGAATGTCTTAAGCCTAGT
Ptr_HB1_Potri.009G014500.1_CDS      AGCAAGTGGAGGCTCTTGAAGAGTTTACGCCGAGTGCCCTAAGCCAAGC
Ptr_HB2_Potri.004G211300.1_POP      AGCAAGTGGAGGCTCTTGAAGAGTTTATGCGGAGTGCCCTAAGCCCAAGC
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Ptr_HB7_Potri.018G045100.1_CDS      TCTACGCGTCGCCAACAGTTGATCAGGGAATGCCCTATTCTTTCTCACAT
Ptr_HB8_Potri.006G237500.1_CDS      TCTATGCGTCGCCAACAGTTGATTAGGGAATGCCCTATTCTATCTCACAT
Ptr_HB5_Potri.001G188800.1_PCN      TCCATTTCGCCGCCAGCAGCTCATTAGAGAGTGCCCAATTCTCTCCAACAT
Ptr_HB6_Potri.003G050100.1_CDS      TCCATTTCGCCGCCAGCAGCTCATTAGGAGTGTTCAATTCTCTCCAATAT
Ptr_HB3_Potri.011G098300.1_PHA      TCCTTGAGAAGACAACAACCTATTAGAGAGTGTCCTATTCTCTCAACAT
Ptr_HB4_Potri.001G372300.1_CDS      TCCTTGAGAAGACAACAACCTATTAGAGAGTGTCCTATTCTCTCAACAT
Ptr_HB1_Potri.009G014500.1_CDS      TCCTTGCGCAGGCAACAGTTGATCAGAGAGTGTCCTATTTTGGCTAATAT
Ptr_HB2_Potri.004G211300.1_POP      TCCTTGCGTAGGCAACAATTGATTAGAGAGTGCCCATTTTAGCTAATAT
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Ptr_HB7_Potri.018G045100.1_CDS      CGAGCCTAAGCAAAATCAAAGTCTGGTTCCAAAATCGAAGATGTAGAGAGA
Ptr_HB8_Potri.006G237500.1_CDS      CGAGCCTAAGCAAAATCAAAGTCTGGTTCCAAAATCGAAGATGTAGAGAGA
Ptr_HB5_Potri.001G188800.1_PCN      TGAGCCCAAACAAATCAAAGTCTGGTTCCAAAATAGAAGGTGCCGAGAGA
Ptr_HB6_Potri.003G050100.1_CDS      TGAGCCCAAACAAATCAAAGTCTGGTTCCAAAATAGAAGATGTCGAGAGA
Ptr_HB3_Potri.011G098300.1_PHA      TGAGCCTAAACAGATCAAAGTCTGGTTTCAAATCGCAGATGTCGTGAAA
Ptr_HB4_Potri.001G372300.1_CDS      TGAGCCCAAACAGATCAAAGTCTGGTTTCAAACCGCAGATGTCGTGAAA
Ptr_HB1_Potri.009G014500.1_CDS      TGAGCCCAAAGCAGATCAAAGTCTGGTTTCAAATCGCAGGTGTCGAGAGA
Ptr_HB2_Potri.004G211300.1_POP      TGAGCCGAAGCAGATCAAAGTCTGGTTTCAAATCGCAGGTGTAGAGAGA
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Ptr_HB7_Potri.018G045100.1_CDS      AGCAGAGGAAAGAGGCATCGCGCTGCAAGCTGTGAATAGGAAATTGACG
Ptr_HB8_Potri.006G237500.1_CDS      AGCAGAGAAAAGAGGCATCGCGCTTCAAGCTGTGAATAGGAAATTAAACA
Ptr_HB5_Potri.001G188800.1_PCN      AGCAAAGGAAAGAGGCGTCGCGCTCCAAGCGGTGAATAGGAAGCTGACT
Ptr_HB6_Potri.003G050100.1_CDS      AACAGAGGAAAGAGAGCGTCTCGCTCCAGCGGTGAATAGGAAGCTGAGT
Ptr_HB3_Potri.011G098300.1_PHA      AACAGAGAAAGGAATCTTCTCGTCTCCAGACAGTGAATAGAAAGCTGACT
Ptr_HB4_Potri.001G372300.1_CDS      AACAGAGAAAGGAAGCTTCTCGTCTCCAGACAGTGAATAGAAAGCTGACT
Ptr_HB1_Potri.009G014500.1_CDS      AGCAGAGAAAAGAGTCTCAAGACTCCAAACTGTGAACAGGAAATTGACT
Ptr_HB2_Potri.004G211300.1_POP      AGCAGAGAAAAGAGTCTCAAGACTCCAGACTGTGAACAGGAAATTGACA
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Ptr_HB7_Potri.018G045100.1_CDS      TTGCACTGGTGTGGCAGCACGTGCATGCGGCCTTGTGGGTCTAGAACCGA
Ptr_HB8_Potri.006G237500.1_CDS      ATGCACTGGTGTGGCAGCACGTGCATGTGGCCTTGTGGGTCTAGAACCTA
Ptr_HB5_Potri.001G188800.1_PCN      TTGTTCTGGTGTGGGAGCACGAGCCTGCGGCCTGGTGGGTCTTGAACCTA
Ptr_HB6_Potri.003G050100.1_CDS      TTGTGCTGGTGTGGGAGCACGAGCTTGTGGCCTAGTGGGTCTTGAACCTA
Ptr_HB3_Potri.011G098300.1_PHA      CTGCAGTGGGGTAGCAGCGCAGCCTGTGGCCTTGTGAGCCTGGAGCCCA
Ptr_HB4_Potri.001G372300.1_CDS      CTGTAGTGGGGTAGCAGCACGAGCCTGCGGCCTTGTGAGCCTGGAGCCCA
Ptr_HB1_Potri.009G014500.1_CDS      GTGCAGTGGAGTGGCAGCTAGAGCCTGCGGTCTTGTAAAGTTTAGAACCTA
Ptr_HB2_Potri.004G211300.1_POP      GTGCGGTGGAGTGGCAGCAAGAGCCTGCGGTCTTGTAAAGTTTAGAGCCTA
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Ptr_HB7_Potri.018G045100.1_CDS      CAAGAGTTGCTGAAATCCTTAAGGATCGGCCATCATGGTTTCGTGATTGC
Ptr_HB8_Potri.006G237500.1_CDS      CGAGAGTTGCCGAAATCCTTAAAGATCGGCCATCATGGTTTCGTGATTGC
Ptr_HB5_Potri.001G188800.1_PCN      CAAGGGTTGCAGAAATCCTCAAGGATCGGCCATCATGGTTTCGTGATTGC
Ptr_HB6_Potri.003G050100.1_CDS      CAAGGGTTGCTGAAATCCTCAAGGATCGGCCATCATGGTTTCGTGATTGC
Ptr_HB3_Potri.011G098300.1_PHA      TTAAGGTTGCTGAAATCCTCAAAGATCGTCCTTCTTGGTTTCGTGACTGC
Ptr_HB4_Potri.001G372300.1_CDS      CAAAGGTTGCTGAAATCCTCAAAGATCGTCATCTTGGTTTCGCGACTGC
Ptr_HB1_Potri.009G014500.1_CDS      CAAAGCTTGACAGATCCTTAAAGATCGTCAATCTTGGTTCCGGGATTGT
Ptr_HB2_Potri.004G211300.1_POP      AAAAGATTGCAGAGATCCTTAAAGATCGTTCATCTTGGTTCCGTGATTGT
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Ptr_HB7_Potri.018G045100.1_CDS      CGAGCTGTGGATGTTATCAATGCGATGTCCACTGCAATGGTGGAACAT
Ptr_HB8_Potri.006G237500.1_CDS      CGAGCTGTGGATGTTGTCAATGCATTGTCCACCGGAAGCGTGGAACAT
Ptr_HB5_Potri.001G188800.1_PCN      CGAGCTGTGGATGTGCTGAATGTGCTGCCACTGCTAATGGGGGAACGAT
Ptr_HB6_Potri.003G050100.1_CDS      CGAGCTGTGGATGTGCTTAATGTGCTGCCACCGCAATGGTGGAACCAT
Ptr_HB3_Potri.011G098300.1_PHA      CGATGCCTAGACATAATGAGTGTGATTCTACAGGAAGTGAGGGACAAT
Ptr_HB4_Potri.001G372300.1_CDS      CGTTGCCTTGACATATTGAGTGTGATTCTACAGGAAGTGAGGGACAAT
Ptr_HB1_Potri.009G014500.1_CDS      CGGAACCTTGAAGTTTCTACTGTGTTCTGCTGGAACCGTGGAACAAT
Ptr_HB2_Potri.004G211300.1_POP      CGGAACCTTGAAGTTTCTACTATGTTCTGCTGGAATGGTGGAACAAT
** * * * * * * * * * * * * * * * *

Ptr_HB7_Potri.018G045100.1_CDS      TGAAGTGCCTTATATGCAGCTCTATGCGCCTACAACTTTGGCACCAGCTC
Ptr_HB8_Potri.006G237500.1_CDS      TGAATTGCTTTATATGCAGCTCTATGCGCCTACAACTTTGGCACCAGCTC
Ptr_HB5_Potri.001G188800.1_PCN      TGAGCTGCTTTACATGCAGCTCTATGCGCCAACAACTTTGGCACCAGGCTC
Ptr_HB6_Potri.003G050100.1_CDS      TGAGCTGCTTTATATGCAGCTCTATGCGCCAACACTACGTGGCACCTGGTC
Ptr_HB3_Potri.011G098300.1_PHA      TGAGCTGATATACATGCAGACTTATGCACCTACAACACTGGCCGACGAC
Ptr_HB4_Potri.001G372300.1_CDS      TGAGCTGATATACATGCAGACTTACGCACCAACAACATTGGCTGCAGCAC
Ptr_HB1_Potri.009G014500.1_CDS      TGAAGTATTATACAGTCAGATATATGCTCCGACAACCTAGCTCCTGCGC
Ptr_HB2_Potri.004G211300.1_POP      TGAAGTATTATACAGTCAGATATATGCTCCAACCTACTCTTGCTCCAGCAC
*** * * * * * * * * * * * * * * * *

Ptr_HB7_Potri.018G045100.1_CDS      GTGACTTCTTGTTACTGCGCTACACATCTGTTTTGGAGGATGGTAGTCTT
Ptr_HB8_Potri.006G237500.1_CDS      GTGACTTCTGGTTACTGCGCTACACATCTGTTTTGGAGGATGGTAGTCTT
Ptr_HB5_Potri.001G188800.1_PCN      GTGACTTCTGGTTGTTGCGTTATACTTCTGTTTTAGAAGATGGAAGCCTT
Ptr_HB6_Potri.003G050100.1_CDS      GTGACTTCTGGTTGTTGCGTTATACTTCTGTTTTAGAAGATGGAAGCCTT
Ptr_HB3_Potri.011G098300.1_PHA      GTGACTTTTGGACACTAAGATACACTACAACCTTGGAGAGCGGGAGTCTT
Ptr_HB4_Potri.001G372300.1_CDS      GTGACTTTTGGACACTAAGATATACTACAACCTTGGAGAGTGGAAGTCTT
Ptr_HB1_Potri.009G014500.1_CDS      GGGATTCTTGGACTTGAGATACACTATAAATTAGAGAATGGCAGTCTT
Ptr_HB2_Potri.004G211300.1_POP      GGGATATGTGGACTCTGAGATACACTACAAGTTTAGAGAATGGCAGCCTT
* * * * * * * * * * * * * * * *

Ptr_HB7_Potri.018G045100.1_CDS      GTGGTCTGTGAGAGATCACTAAACAACACTCAAATGGTCCAAGTATGCC
Ptr_HB8_Potri.006G237500.1_CDS      GTGGTCTGTGAGAGATCTTTAACAACACCCAAATGGTCCAAGCATGCC
Ptr_HB5_Potri.001G188800.1_PCN      GTGGTATGTGAGAGATCTCTGAAAAAATACTCAAATGGCCCGAGCATGCC
Ptr_HB6_Potri.003G050100.1_CDS      GTGGTTTGTGAGAGATCTCTGAAAAAATACTCAAATGGCCCGAGCATGCC
Ptr_HB3_Potri.011G098300.1_PHA      GTGATATGTGAGAGGTCATTGACTTCTTCTACTGGTGGCCCAACAGGACC
Ptr_HB4_Potri.001G372300.1_CDS      GTTATATGTGAGAGGTCATTGACTTCTTCTACTGGTGGCCCAACAGGACC
Ptr_HB1_Potri.009G014500.1_CDS      GTGGTGTGTGAGAGATCTTTCTGGTTCTGGTGTGGACCAAAATGCAGC
Ptr_HB2_Potri.004G211300.1_POP      GTGGTCTGTGAGAGATCACTTCTGGTTATGGTGTGGCCCGGATGCAGC
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Ptr_HB7_Potri.018G045100.1_CDS      ACCCACTCAGCATTTTGTGAGAGCAGAAATGTTGCCTAGCGGGTATCTTA
Ptr_HB8_Potri.006G237500.1_CDS      ACCTACACAGCATTTTGTGAGAGCAGAAATGCTGCCTAGCGGGTATCTTA
Ptr_HB5_Potri.001G188800.1_PCN      ACCAGTGCAGCATTTTGTGAGAGCAGAAATGCTACCAAGTGGGTATCTGG
Ptr_HB6_Potri.003G050100.1_CDS      ACCAGTGCAGCATTTTGTGAGAGCAGAAATGCTCCCAAGTGGGTATCTGG
Ptr_HB3_Potri.011G098300.1_PHA      TCCCTTCTCAAGCTTTGTAAGAGCTGATATGCTTCTTAGTGGCTATCTAA
Ptr_HB4_Potri.001G372300.1_CDS      TCTCCTTCAAGCTTCAAGAGCTGAAATGCTTCCCAAGTGGCTATCTAA
Ptr_HB1_Potri.009G014500.1_CDS      TGCAGCTGCTCAGTTTGTGAGGGCTGAAATGCTTCTAGTGGTTATTGGA
Ptr_HB2_Potri.004G211300.1_POP      TGCCGCTGCCAGTTTGTGAGGGCTGAAATGCTTCTAGTGGCTATTGGA

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Ptr_HB7_Potri.018G045100.1_CDS      TCAGACCATGTGAAGGAGGTGGATCAATTATTCATATAGTTGATCATATG
Ptr_HB8_Potri.006G237500.1_CDS      TTAGACCTTGTGAAGGAGGTGGATCAATTATTCATGTAGTTGATCATATG
Ptr_HB5_Potri.001G188800.1_PCN      TACGGCCTTGTGAAGGTGGTGGTTCATATACACATAGTTGACCACATG
Ptr_HB6_Potri.003G050100.1_CDS      TACGGCCTTGTGAAGGTGGTGGTTCATATACACATAGTTGACCACATG
Ptr_HB3_Potri.011G098300.1_PHA      TTCGACCATGTGAGGGTGGTGGCTCCATCATTACATTGTTGATCATGTT
Ptr_HB4_Potri.001G372300.1_CDS      TTCGACCATGTGAGGGCAGTGGCTCCATCATTACATTGTTGATCATGTT
Ptr_HB1_Potri.009G014500.1_CDS      TAAGGCCATGTGAAGGAGGAGGGTCAATTATCCACATCGTGGATCACCTG
Ptr_HB2_Potri.004G211300.1_POP      TAAGGCCATGTGAAGGAGG--GTCAATTATCCACATCGTGGATCACCTG
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Ptr_HB7_Potri.018G045100.1_CDS      GACTTGGAGCCCTGGAGTGTTCCTGAAGTGTTCGCGCCCACTTTATGAGTC
Ptr_HB8_Potri.006G237500.1_CDS      GATTTAGAGCCTTGGAGTGTACCTGAAGTGTTCGCGCCCGCTTTACGAGTC
Ptr_HB5_Potri.001G188800.1_PCN      GATTTGGAGCCTTGGAGTGTGCCTGAAGTGTTCAGTCCGCTTTATGAATC
Ptr_HB6_Potri.003G050100.1_CDS      GATTTGGAGCCTTGGAGTGTGCCTGAAGTACTACGCCCGCTGTATGAATC
Ptr_HB3_Potri.011G098300.1_PHA      GACTTAGATGTTTGGAGTGTTCCTGAAGTCTGAGGCCACTTTATGAGTC
Ptr_HB4_Potri.001G372300.1_CDS      GATTTAGATGTTTGGAGTGTTCCTGAAGTCTGAGGCCACTTTATGAATC
Ptr_HB1_Potri.009G014500.1_CDS      AATCTTCAGGCATGGAGTGTGCCTGAGGTGCTGCGACCACCTTTATGAATC
Ptr_HB2_Potri.004G211300.1_POP      AATCTTCAGGCATGGAGTGTGCCTGAGGTGCTTCGACCACCTTTATGAATC
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Ptr_HB7_Potri.018G045100.1_CDS      ATCGACGTTGCTTGCCAGAGAACAACATGGCGGCTTTACGCCATTTGA
Ptr_HB8_Potri.006G237500.1_CDS      ATCAACGTTGCTTGCTCAGAAGACAACAATGGCGGCTTTACGCCATTTGA
Ptr_HB5_Potri.001G188800.1_PCN      CTCAACTGTACTCGCTCAAAAGACAACCATGGCGGCTCTACGCCAAGTGA
Ptr_HB6_Potri.003G050100.1_CDS      CTCAACTGTACTTGCTCAAAAGACAACAATGGTGGCTCTACGCCAGCTGC
Ptr_HB3_Potri.011G098300.1_PHA      ATCCAAAATTCTTGCTCAGAAAATGACCATGGCGGCTTGCAGTACATAA
Ptr_HB4_Potri.001G372300.1_CDS      ATCCAAAATTCTTGCTCAGAAAATGACCATGGCGGCTTGCAGGACATAA
Ptr_HB1_Potri.009G014500.1_CDS      ATCAAAAGCAGTGGCACAAGAAAGTGACAATCACGGCTCTGCGCCATGTCA
Ptr_HB2_Potri.004G211300.1_POP      ATCAAAAGCAGTGGCACAAGAAATGACCATTCGCGCACTGCGTTATGTCA
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Ptr_HB7_Potri.018G045100.1_CDS      GGCAGATTTCTCAAGAAATTTCCAGCCAAATGTCACTGGTTGGGGAAGA
Ptr_HB8_Potri.006G237500.1_CDS      GGCAGGTTTCTCAAGAAGTTTCTCAGCCAAACGTCACTGGTTGGGGAAGA
Ptr_HB5_Potri.001G188800.1_PCN      GGCAAAATTGCTCAGGAAGCTTCTCAGTCTAGTGTGACCAACTGGGGCAGA
Ptr_HB6_Potri.003G050100.1_CDS      GGCAGATAGCTCAGGAAGCTTCTCAGTCCAATGTGACCAACTGGGGCAGA
Ptr_HB3_Potri.011G098300.1_PHA      GACAAATTGCACAAGAGACTAGTGGGGAAATTCAATGATGGAGGTGGTGGT
Ptr_HB4_Potri.001G372300.1_CDS      GACAAATTGCTCAAGAGACTAGCGGGGAAATTCAATGATGGAGGGGGCCGC
Ptr_HB1_Potri.009G014500.1_CDS      GGCAAAATAGCTCATGAACCAAGTGGTGAGGTGGTGTATGGTTTGGGCAGG
Ptr_HB2_Potri.004G211300.1_POP      GGCAAGTAGCTCATGAACCAAGTGGTGAGGTGGTGTATGGTTTGGGCAGG
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Ptr_HB7_Potri.018G045100.1_CDS      AGACCTGCAGCTCTACGTGCACTAAGTCAAAGACTGAGCAAGGGCTTCAA
Ptr_HB8_Potri.006G237500.1_CDS      AGACCTGCAGCTTTGCGTGCAATTAAGTCAGAGACTGAGCAAGGGCTTCAA
Ptr_HB5_Potri.001G188800.1_PCN      CGACCTGCAGCTTTACGAGCGTTGAGCCAGAGGTTGAGCAGGGGTTTTAA
Ptr_HB6_Potri.003G050100.1_CDS      CGACCTGCAGCTCTACGAGCACTGAGCCAGAGGTTGAGCAGGGGTTTTAA
Ptr_HB3_Potri.011G098300.1_PHA      CAGCCTGCTGTTTTAAGGACATTTAGTCAGAGGCTCTGAGGGGTTTTCAA
Ptr_HB4_Potri.001G372300.1_CDS      CAGCCTGCTGTTCTAAGGACATTTAGTCAGAGGCTCTGAGGGGTTCAA
Ptr_HB1_Potri.009G014500.1_CDS      CAGCCAGCTGTGCTGAGAACCCTTTAGCCAAAGATTAAGCAGAGGCTTCAA
Ptr_HB2_Potri.004G211300.1_POP      CAACCAGCTGTTCTGAGAACCCTTTAACCAGAGATTAAGCAGAGGTTTCAA
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Ptr_HB7_Potri.018G045100.1_CDS      TGAAGCTGTTAATGGATTTGCTGATGAGGGATGGTCTTTGTTGAAAGTG
Ptr_HB8_Potri.006G237500.1_CDS      TGAAGCTGTTAATGGATTTGCTGATGAGGGATGGTCTATGTTGAAAGTG
Ptr_HB5_Potri.001G188800.1_PCN      TGAGGCTCTCAATGGATTTAGTGATGAGGGGTGGTCAATGATTGGAATG
Ptr_HB6_Potri.003G050100.1_CDS      TGAGGCTCTCAATGGATTTAGTGATGAGGGATGGTCAATGATCGGAAATG
Ptr_HB3_Potri.011G098300.1_PHA      TGATGCTGTAAATGGATTCACAGATAGTGGGTGGTCACTTCTGGATGGTG
Ptr_HB4_Potri.001G372300.1_CDS      TGATGCTGTGAATGGGTTACAGATGATGGTTGGTCACTTCTGGGCAGTG
Ptr_HB1_Potri.009G014500.1_CDS      TGATGCAATCAATGGGTTAATGATGATGGTTGGTCACTGATGAATCTG
Ptr_HB2_Potri.004G211300.1_POP      TGATGCCATCAATGGGTTAATGATGATGGCTGGTCACTGATGAATGCGG
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Ptr_HB7_Potri.018G045100.1_CDS      ATGGCATTGATGATGTAACAGTCTTGTAAACTCTTCTCTACCAAAACG
Ptr_HB8_Potri.006G237500.1_CDS      ATGGCATTGATGATGTAACAGTCTTGTAAACTCATCTCTGCCAAAATG
Ptr_HB5_Potri.001G188800.1_PCN      ACGGCATGGACGATGTTACTATCTAGTGAACATCTCTCTGACAAGTTG
Ptr_HB6_Potri.003G050100.1_CDS      ATGGCATGGATGATGTTACTATCTCGTGAACATCATCTCTGACAAGTTG
Ptr_HB3_Potri.011G098300.1_PHA      ATGGTGGGGATGATGTGACCATCGTCATAAATTCATCGCCAACATAAATTT
Ptr_HB4_Potri.001G372300.1_CDS      ATGGTGGGGATGATGTGACCATTGTGATAAATTCATCTCAAATAAATTT
Ptr_HB1_Potri.009G014500.1_CDS      ATGGAGCTGAGGATGTAATAATTGCTGTTAACACAACCC--AAGAAGTTG
Ptr_HB2_Potri.004G211300.1_POP      ATGGAGCTGAGGATGTAATAATTGAGTTAACTCAACC--AAGAATTTG

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Ptr_HB7_Potri.018G045100.1_CDS      ATGGGAGTAAATTTTTCGTATGACAATGGATTCCATCCATGAACAACGC
Ptr_HB8_Potri.006G237500.1_CDS      ATGGGTGTAAATTTTCTTATGCCAATGGATTCCATCTATGAGTAATGC
Ptr_HB5_Potri.001G188800.1_PCN      ATGGGTTTAAATCTTTCTCTCCAATGGGTTTCCAGCTGTCAGCAGTGC
Ptr_HB6_Potri.003G050100.1_CDS      ATGGGTTCAAATCTTTCTTCACTAATGGGTTTCCAGCTGTCAGCAGTGC
Ptr_HB3_Potri.011G098300.1_PHA      CTAGGATC---TCAATACAACGCATCTATATCACCTACATTTGGAGGAG-
Ptr_HB4_Potri.001G372300.1_CDS      CTAGGATC---TCAATACAATGCATCTATGTTTCTACATTTGGAGGAGG
Ptr_HB1_Potri.009G014500.1_CDS      ATCAGTGC---TAATAATCTGCTCATTCACTTTCAITCTTTGGGAGGGA-
Ptr_HB2_Potri.004G211300.1_POP      ATTGGTGC---TAATAATTCTGCTCATTCTTTTCGTTCTTTGGGAGGGA-
* *      *      *      *      *      *

Ptr_HB7_Potri.018G045100.1_CDS      AGTGCCTTTGTGCAAAAGCATCCATGTTATTACAG-----
Ptr_HB8_Potri.006G237500.1_CDS      AGTGCCTTTGTGCGAAAAGCATCCATGTTGTTACAG-----
Ptr_HB5_Potri.001G188800.1_PCN      AGTCCTGTGCGCTAAAGCATCAATGCTTTTACAGGCAGGGATACAAAATT
Ptr_HB6_Potri.003G050100.1_CDS      TGTCTGTGTGCTAAAGCATCGATGCTTTTACAG-----
Ptr_HB3_Potri.011G098300.1_PHA      --TCCTGTGTGCGAAGGCATCAATGTTGCTGCAG-----
Ptr_HB4_Potri.001G372300.1_CDS      GGTGCTGTGCGCAAGGCATCGATGTTGCTGCAG-----
Ptr_HB1_Potri.009G014500.1_CDS      --TTCTTTGTGCAAAAGCTTCCATGCTACTGCAA-----
Ptr_HB2_Potri.004G211300.1_POP      --TTCTTTGTGCGAAAAGCTTCCATGCTACTGCAA-----
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Ptr_HB7_Potri.018G045100.1_CDS      -----AACGTGCCTCCAGCAATACTTCTCAGA
Ptr_HB8_Potri.006G237500.1_CDS      -----AATGTACCTCCAGCAATACTTCTCAGA
Ptr_HB5_Potri.001G188800.1_PCN      GTTTCCTTTCTTTACAACACTTGAATGTCCTCCTGCAATCCTTCTCAGG
Ptr_HB6_Potri.003G050100.1_CDS      -----AATGTCCTCCTGCAATCCTTCTCAGA
Ptr_HB3_Potri.011G098300.1_PHA      -----AATGTTCCCTCCTGCTTTGCTTGTTCGT
Ptr_HB4_Potri.001G372300.1_CDS      -----AATGTTCCCTCCTGCTTTGCTTGTTCGT
Ptr_HB1_Potri.009G014500.1_CDS      -----AATGTGCCTCCAGCTGTGCTGGTCCCG
Ptr_HB2_Potri.004G211300.1_POP      -----AATGTGCATCCTGCTGTGCTGGTCTGT
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Ptr_HB7_Potri.018G045100.1_CDS      TTCTTGCGTGAACATCGATCAGAATGGGCAGACAATGGAATTGATGCTTA
Ptr_HB8_Potri.006G237500.1_CDS      TTCTTGCGTGAACATCGATCAGAATGGGCAGACAGTGGTATTGATGCTTA
Ptr_HB5_Potri.001G188800.1_PCN      TTCTTGCGGGAGCACAGGTCAGAGTGGGCAGATAACAATATCGATGCCTA
Ptr_HB6_Potri.003G050100.1_CDS      TTCTTGCGAGAGCACAGGTCAGAATGGGCAGATAACAATATTGATGCCTA
Ptr_HB3_Potri.011G098300.1_PHA      TTTCTGAGAGAACATCGTTCGGAGTGGGCTGATTATGGGTTGATGTGTA
Ptr_HB4_Potri.001G372300.1_CDS      TTTTGTGAGAGAACATCGTTCTGAGTGGGCTGACTACGGGTTGATGCCTA
Ptr_HB1_Potri.009G014500.1_CDS      TTCTTAAGGGAGCATCGCTCTGAGTGGGCTGATTTCAAGTGTGATGCCTA
Ptr_HB2_Potri.004G211300.1_POP      TTCTTAAGGGAGCATCACGCTGAGTGGGCTGATTTCAAGTGTGATGCCTA
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Ptr_HB7_Potri.018G045100.1_CDS      TGCAGCTTCGGCCATTAAAGCTGGTCCTTGCAGCTTGCCAGTGTCTCGAG
Ptr_HB8_Potri.006G237500.1_CDS      TGCAGCTGCTGCTGTTAAAGCTGGTCCTTGTAGCTTGCCAAATGTCTCGAG
Ptr_HB5_Potri.001G188800.1_PCN      TGCAGCCGCAGCGGTTAAAGTCGGTCCTTGCAGTTTACAAGGGTCTAGAG
Ptr_HB6_Potri.003G050100.1_CDS      TGCAGCTGCAGCAGTTAAAGTTGGTCCTTTAGCCTACAAGGTTCTCGAG
Ptr_HB3_Potri.011G098300.1_PHA      CTCGTGTCATGTCTTAAAGCAAGTCCTTATGCAGTTCATGTGCAAGAC
Ptr_HB4_Potri.001G372300.1_CDS      CTCGTGTCATGTCTTAAAGCAAGTCCTTATGCAGTTCCTTGTGCCAGAC
Ptr_HB1_Potri.009G014500.1_CDS      TTCTGCTGCATCGTTGAAAGCTGGTTCATATGCATATCCAGGGATGAGAT
Ptr_HB2_Potri.004G211300.1_POP      TTCTGCTGCATTTGTGGAAGGCTGGTTCATATGCATATCCAGGAATGAGGC
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Ptr_HB7_Potri.018G045100.1_CDS      CTGGAAATTT---TGGGGGTCAAGTTATCTCCCCCTGGCTCACACAATT
Ptr_HB8_Potri.006G237500.1_CDS      CTGGAAATTT---TGGGGGTCAAGTTATCTCCCCCTTGGCTCACACAATT
Ptr_HB5_Potri.001G188800.1_PCN      TTGGAAATTT---TGGCGGTCAAGTTATACTTCCACTTGCTCACACCGTG
Ptr_HB6_Potri.003G050100.1_CDS      TTGGAAGTTT---CGGGGGTCAAGTTATACTTCCGCTGGCTCACACTATT
Ptr_HB3_Potri.011G098300.1_PHA      CTGGTGGCTTCCCTAGTAGCCAGGTCATTTTACCTCTTGACATACCGTG
Ptr_HB4_Potri.001G372300.1_CDS      CTGGTGGCTTCCCTAGTAGCCAGGTCATTTTACCTCTTGCGCATACCATG
Ptr_HB1_Potri.009G014500.1_CDS      CTATGAGGTTTACTGGGAGCCAAATCATCATGCCATTGGGCCACACAATT
Ptr_HB2_Potri.004G211300.1_POP      CCATGAGGTTTACTGGGAGCCAAATCACCATGCCACTGGGCCACACAATT
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Ptr_HB7_Potri.018G045100.1_CDS      GAGAATGAAGAGGCAA-----AGGTCATTAAAGCTTGAAAA
Ptr_HB8_Potri.006G237500.1_CDS      GAGCATGAAGAGGCAAGTACTTCATTTATGGAGGTCATTAAAGCTTGAAAA
Ptr_HB5_Potri.001G188800.1_PCN      GAGCACGAAGAGTTTCT-----TGGAGGTCATAAAACTAGAAGG
Ptr_HB6_Potri.003G050100.1_CDS      GAACATGAAGAGTTCC-----TGGAGGTCATAAAACTGGAAGG
Ptr_HB3_Potri.011G098300.1_PHA      GAGCATGAGGAGTTCT-----TGGAGGTAGTTTCGGCTGGAGGG
Ptr_HB4_Potri.001G372300.1_CDS      GAGCATGAGGAGTTCT-----TGGAGGTAGTTTCGGCTGGAGGG
Ptr_HB1_Potri.009G014500.1_CDS      GAACAAGAAGAGTTGC-----TTGAAGTTATTGCACTTGAAGG
Ptr_HB2_Potri.004G211300.1_POP      GAACAAGAAGACTTGC-----TTGAAGTTATTGCACTTGAAGG

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Ptr_HB7_Potri.018G045100.1_CDS
Ptr_HB8_Potri.006G237500.1_CDS
Ptr_HB5_Potri.001G188800.1_PCN
Ptr_HB6_Potri.003G050100.1_CDS
Ptr_HB3_Potri.011G098300.1_PHA
Ptr_HB4_Potri.001G372300.1_CDS
Ptr_HB1_Potri.009G014500.1_CDS
Ptr_HB2_Potri.004G211300.1_POP

Ptr_HB7_Potri.018G045100.1_CDS
Ptr_HB8_Potri.006G237500.1_CDS
Ptr_HB5_Potri.001G188800.1_PCN
Ptr_HB6_Potri.003G050100.1_CDS
Ptr_HB3_Potri.011G098300.1_PHA
Ptr_HB4_Potri.001G372300.1_CDS
Ptr_HB1_Potri.009G014500.1_CDS
Ptr_HB2_Potri.004G211300.1_POP

Ptr_HB7_Potri.018G045100.1_CDS
Ptr_HB8_Potri.006G237500.1_CDS
Ptr_HB5_Potri.001G188800.1_PCN
Ptr_HB6_Potri.003G050100.1_CDS
Ptr_HB3_Potri.011G098300.1_PHA
Ptr_HB4_Potri.001G372300.1_CDS
Ptr_HB1_Potri.009G014500.1_CDS
Ptr_HB2_Potri.004G211300.1_POP

Ptr_HB7_Potri.018G045100.1_CDS
Ptr_HB8_Potri.006G237500.1_CDS
Ptr_HB5_Potri.001G188800.1_PCN
Ptr_HB6_Potri.003G050100.1_CDS
Ptr_HB3_Potri.011G098300.1_PHA
Ptr_HB4_Potri.001G372300.1_CDS
Ptr_HB1_Potri.009G014500.1_CDS
Ptr_HB2_Potri.004G211300.1_POP

Ptr_HB7_Potri.018G045100.1_CDS
Ptr_HB8_Potri.006G237500.1_CDS
Ptr_HB5_Potri.001G188800.1_PCN
Ptr_HB6_Potri.003G050100.1_CDS
Ptr_HB3_Potri.011G098300.1_PHA
Ptr_HB4_Potri.001G372300.1_CDS
Ptr_HB1_Potri.009G014500.1_CDS
Ptr_HB2_Potri.004G211300.1_POP

Ptr_HB7_Potri.018G045100.1_CDS
Ptr_HB8_Potri.006G237500.1_CDS
Ptr_HB5_Potri.001G188800.1_PCN
Ptr_HB6_Potri.003G050100.1_CDS
Ptr_HB3_Potri.011G098300.1_PHA
Ptr_HB4_Potri.001G372300.1_CDS
Ptr_HB1_Potri.009G014500.1_CDS
Ptr_HB2_Potri.004G211300.1_POP

Ptr_HB7_Potri.018G045100.1_CDS
Ptr_HB8_Potri.006G237500.1_CDS
Ptr_HB5_Potri.001G188800.1_PCN
Ptr_HB6_Potri.003G050100.1_CDS
Ptr_HB3_Potri.011G098300.1_PHA
Ptr_HB4_Potri.001G372300.1_CDS
Ptr_HB1_Potri.009G014500.1_CDS
Ptr_HB2_Potri.004G211300.1_POP

Ptr_HB7_Potri.018G045100.1_CDS
Ptr_HB8_Potri.006G237500.1_CDS
Ptr_HB5_Potri.001G188800.1_PCN
Ptr_HB6_Potri.003G050100.1_CDS
Ptr_HB3_Potri.011G098300.1_PHA
Ptr_HB4_Potri.001G372300.1_CDS
Ptr_HB1_Potri.009G014500.1_CDS
Ptr_HB2_Potri.004G211300.1_POP

Ptr_HB7_Potri.018G045100.1_CDS
Ptr_HB8_Potri.006G237500.1_CDS
Ptr_HB5_Potri.001G188800.1_PCN
Ptr_HB6_Potri.003G050100.1_CDS
Ptr_HB3_Potri.011G098300.1_PHA
Ptr_HB4_Potri.001G372300.1_CDS
Ptr_HB1_Potri.009G014500.1_CDS
Ptr_HB2_Potri.004G211300.1_POP

TATGGGCTAT--CGGGAGGACATGCTCATGCCGGTGATGTTTTCTGT
TATGGGCTAT---CGGGAGGACATGCTCATGCCGGTGATGTTTTCTGT
GGTTTGCCATTCTCCTGAAGATGCAATTATGCCAGAGATGTGTTCTTT
TGTTGGCATTCTCCTGAAGATCCAATAATGCCAGAGATGTGTTCTTT
CCATGCATTCTCCCAGAAGATGTAGCCCTGGCACAGGATATGTAATTGT
TCATGCATTCTCCCAGAAGATGTAGCATTGGCTCGGGATATGTAATTGT
CCATTCTTTGCACAAGAAGATGCTTTTGTTCACGGGACATCCATCTCC
CCATTCTTTGCACAAGAAGATGCTTTTGTTCACAGGACATCCATCTCC
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TACAACCTTTGTAGTGGCGTGGATGAGAATGCTGTTGGCACTTGCGCAGAA
TACAACCTGTAGTGGAGTAGACGAGAATGCTGTTGGCACCTGTGCTGAA
TGCAACTCTGCTGTGGAATGGACGAGAATGCTGTTGGACATGTGCTGAA
TACAACCTGCTGTGGAATGGACGAGAATGCTGTTGGAACATGTGCTGAA
TGCAGCTATGCAAGCGGAGTTGATGAAAATGCTCTTGCTGCTTGTGCTCAG
TGCAGCTATGCAAGTGGAGTTGATGAAAATGCTGTTGGTCTTGTGCTCAG
TACAGATATGTAGTGGAAATTGATGAGAATGCTGTTGGAGCCTGTTCTGAA
TACAGATATGTAGTGGAAATTGATGAGAATGCTGTTGGAGCCTGTTCTGAA
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CTCATCTTTGCCCAATTGATGCGTCCTTTTCTGATGATGCTCCAATTAT
CTCATCTTTGCCCAATTGATGCTCTTTTCTGATGATGCTCCTATTAT
CTTATATTGCTCCCATTTGATGCTACTTTTGTGATGACGCACCTCTTTT
CTTATATTGCTCCCATTTGATGCTACTTTTGTGATGATGCACCGCTTTT
CTCGTCTTTGCGCCTATTGATGAATCCTTTGCTGATGATGCACCATTTGT
CTTGCTTTTGCCTATTGATGAATCCTTTGCTGATGATGCACCATTTGCT
CTAGTTTTTGTCCAATTGATGAAAATGTTCCCGGATGATGCTCCACTGCT
CTAGTTTTTGTCCAATTGATGAAAATGTTCCAGATGATGCTCCACTGCT
** * ** ** * ** ** ** * ** * ** * ** *

TCCTTCTGGTTTCCGCATAATTCTCTTGATTCTGGGAT-----
TCCTTCTGGTTTCCGATCATTCTCTTGATTCTGGGAT-----
GCCTTCTGGTTTTCGCATCATTCCCCTTGATTCTGGGAA-----
ACCTTCTGGTTTTCGCATCATTCCCCTTGATTCTGGGAA-----
GCCATCTGGATTTCGTGTCATACCATTAGATCCTAAAC-----
GTCATCTGGATTTCGTGTCATACCTTTGGATCCTAAAC-----
ACCCTCTGGTTTCCGCGTCATTCCACTGGAATCAAAAACAAAGGATGCGC
ACCTTCTGGTTTCCGCATCATTCTCTGGAATCAAAAGCAAAGGATACAC
* ** ** * ** * ** * ** *

-GGATGCGTCGAGTCCAAACCGCACACTTGACCTTGCACTGCTCTGGAG
-GGATGCCTCCAGTCCAAACCGCACACTTGACCTTGCACTGCTCTTGAG
-GGAAGCCTCCAGTCCAAATCGTACATTGGACCTCGCTTCTGCTCTTGAA
-GGAAGCCTCCAGTCCAAATCGTACCTTAGATCTTGCGGCTGCTCTTGAA
-AGATGCACCTGCTGCTACTCGAATTTAGATTTAGCCTCTACACTTGAA
-AGATGCACCTGCTACGACTCGCACTTTAGATTTAGCCTCTACACTTGAA
AGGAGGCATTGACCACAAATCGCACTTGGAATCAACATCAAGTCTTGAA
AGGAGGTATTGACTACAAATTTGACTCTGGATCTAACATCAAGTCTTGAA
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GTTGGACCTGCTGGAAACAGAGCATCAGTGATCTTTCAGGCCGAGTGG
GTTGGCCTGCTGGAAATAGAGCATCAGTGATCTTTCAGGCCGAGTGG
GTTGGA---GCTGGAACAGAGCATCCAGTGATTTTCTGCTAATTCTGG
GTCGGACCACTGGAACAGAGCATCCAGTGATCTTCTGCTAATTCTGG
GTAGGACCTGGTGGTGGCCCTCCAGCTAGTGAAGCTGACACCAACAGTTA
GTAGGACCTGGTGGCAGACGTCCAGCTAGTGAGGCTGACACCAACAGTTA
GTGGGCCCGGTAAACAAACCATGCTTCAGTGGATGGTTCATCATGTCTCAT
GCGGGGCTGGCAATAAACCCACACTGCAGTGGATGGCTCATCGTGTCTAG
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GTGCACAAATCAGTAATGACGATAGCATTCCAATTTGCATTTGAGATGC
GTGCACAAAGTCAGTGATGACAATAGCATTCCAATTTGCATTTGAGATGC
CTGCACAAGATCTGTAATGACAATTGCATTTGAATTTGCATTTGAGAGCC
TTGCACAAGATCTGTAATGACAATCGCATTTGAATTTGCGTTGAGAGCC
TAACCTTAGGTCAGTACTGACAATCGCTTTCCAATTTACGTTTGAGAAC
TAACCTTAGGTCAGTCTGACAATCGCGTTCCAATTTTCATTTGAGAAC
-T---TGCGATCAGTGTTGACTATTGCTTTCCAGTTCCTGTTGAGAGCA
TT---TGCGATCAGTGTTGACTATTGCTTTCCAGTTCCTTTTGAGAGCA

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Ptr_HB7_Potri.018G045100.1_CDS      ACCTTCAAGAGAATGTAGCATCCATGGCTCGGCAGTATGTTCTAGTATC
Ptr_HB8_Potri.006G237500.1_CDS      ACCTTCAAGAAAATGTGACATCCATGGCTCGGCAGTATGTTCTAGTATT
Ptr_HB5_Potri.001G188800.1_PCN      ACATGCAAGAGCATGTAGCATCAATGGCTCGGCAATATATCCGCAGCATT
Ptr_HB6_Potri.003G050100.1_CDS      ACATGCAAGAACATGTAGCATCAATGACTCGGCAATATATCCGCAGCATT
Ptr_HB3_Potri.011G098300.1_PHA      ACTTCCGAGACAATGTGGCTGCTATGGCTCGCCAATATGTGCTGGTGTT
Ptr_HB4_Potri.001G372300.1_CDS      ACATGCGAGACAATGTGGCTGCTATGGCTCGCCAATATGTTCTGGTGTT
Ptr_HB1_Potri.009G014500.1_CDS      ATCTACAGGATAATGTTGCCACCATGGCCCGTCAGTATGTCCGAGTGTG
Ptr_HB2_Potri.004G211300.1_POP      ATCTACAGGATAATGTTGCCACCATGGCACGTACGTACGTAGTGTG
* * * * *      * * * * *      * * * * *      * * * * *

Ptr_HB7_Potri.018G045100.1_CDS      ATAGCTTCTGTTCAGCGGGTAGCATTAGCGCTCTCCCTTCTCATTTTGG
Ptr_HB8_Potri.006G237500.1_CDS      ATAGCTTCTGTTCAGCGGGTAGCATTAGCACTCTCTCTTCCCATTTTGG
Ptr_HB5_Potri.001G188800.1_PCN      ATATCTTCAGTTCAGAGGGTGGCTTTAGCACTATCTCTTCTCATCAGGG
Ptr_HB6_Potri.003G050100.1_CDS      ATATCTTCAGTTCAGAGGGTGGCATTAGCACTATCTCC---TCATCTTGG
Ptr_HB3_Potri.011G098300.1_PHA      GTGGCGTCTGTTCAGAGGGTGGCAATGGCAATTTCCCTTCCCGGCTCAG
Ptr_HB4_Potri.001G372300.1_CDS      GTGGGGTCTGTTCAGAGGGTGGCAATGGCAATGCTCTTCCAGGGCTCAG
Ptr_HB1_Potri.009G014500.1_CDS      ATTTTCATCTGCCAGAGGGTGGCCACGCCATATCTCCATCAGGATTGAA
Ptr_HB2_Potri.004G211300.1_POP      ATTTTCATCTGCCAGAGGGTGGCCATGGCCATATCTCCATCAGGATTGAG
* * * * *      * * * * *      * * * * *      * * * * *

Ptr_HB7_Potri.018G045100.1_CDS      TTCTCATGCTGGTTTTTGGCCACCACATGGAACCCCTGAAGCGCACACAC
Ptr_HB8_Potri.006G237500.1_CDS      TTCTCATGCTGGTTTTTGGCCACCACCCTGGTACTCTGAAGCGCACACAC
Ptr_HB5_Potri.001G188800.1_PCN      TTCACAGGCTGGTCTTCGGTCACTGGTACTCTGAAGCACAGACAC
Ptr_HB6_Potri.003G050100.1_CDS      TTCACAGGCTGGTCTTCGGTCACTGGTACTCTGAAGCACAGACAC
Ptr_HB3_Potri.011G098300.1_PHA      CTCCAATATGGGGCCGAAGTCCCTTCCTGTCTCTCTGAGGCTTTAACTT
Ptr_HB4_Potri.001G372300.1_CDS      CTCCAATGTGGGGCCAAAGACCCTTCCTGGCTCTCTGAGGCTCTTACTT
Ptr_HB1_Potri.009G014500.1_CDS      TCCAGCTTTGGGACCAAAGCTATCTGCAGGCTCCCTGAAGCTCTAACAC
Ptr_HB2_Potri.004G211300.1_POP      TCCAGTTTTGGGACCAAAGCTATCTGCAGGATCTCTGAAGCTCTAACCT
* * * * *      * * * * *      * * * * *      * * * * *

Ptr_HB7_Potri.018G045100.1_CDS      TTGCTCGTTGGATATGCGAAAGCTACAGGAT-----
Ptr_HB8_Potri.006G237500.1_CDS      TAGCTCGTTGGATCTGTGAAAGCTACAGGTG-----
Ptr_HB5_Potri.001G188800.1_PCN      TTGCTCGTTGGATCTGCCAGAGCTACCGGAA-----
Ptr_HB6_Potri.003G050100.1_CDS      TTGCTCGTTGGATCTGCCAGAGCTACAGGAG-----
Ptr_HB3_Potri.011G098300.1_PHA      TAGCTCGATGGATATACCGAAGCTACAGGAT-----
Ptr_HB4_Potri.001G372300.1_CDS      TGGCACAATGGATATGCCGAAGCTACAGGAT-----
Ptr_HB1_Potri.009G014500.1_CDS      TGGCTCACTGGATCTGCCAAAGTTATTGGCAAGTCTGCCTCAAGTTTCA
Ptr_HB2_Potri.004G211300.1_POP      TGGCTCACTGGATCTGCCAAAGTCACAGGCAAGTTCTGCTTAA-----
* * * * *      * * * * *      * * * * *      * * * * *

Ptr_HB7_Potri.018G045100.1_CDS      -----CTATCTAGGTGTGAAACTGCTTAAA-----AATGAAGGAAG
Ptr_HB8_Potri.006G237500.1_CDS      -----CTATCTAGGTGTGAAACTGCTCAAA-----AATGAAGGAAG
Ptr_HB5_Potri.001G188800.1_PCN      -----CTATTTGGGTGTGGAGCTACTCAAATCCAGCAGTGAAGGAAG
Ptr_HB6_Potri.003G050100.1_CDS      -----CTATTTGGGTGTGGAGCTACTCAAATCCAGTGAAGGAAG
Ptr_HB3_Potri.011G098300.1_PHA      -----TCATACTGGAGGAGAATATTTGGGTTGACTCCCAAGCTGG
Ptr_HB4_Potri.001G372300.1_CDS      -----CCATACTGGAGGAGAATATTTGGGTTGAATCCCAAGCTGG
Ptr_HB1_Potri.009G014500.1_CDS      TCATGTTACCATTTAGGAGCAGAATTACTGAGATCTGATTCTGTGGGTGG
Ptr_HB2_Potri.004G211300.1_POP      -----TTACCACTTAGGAGCAGAATTACTGAGATCTGATTCTGTGGGTGG
* * * * *      * * * * *      * * * * *      * * * * *

Ptr_HB7_Potri.018G045100.1_CDS      TGATTCTATTCTTAAACACTCTGGCATCACTCTGATGCACTAGTGTGCT
Ptr_HB8_Potri.006G237500.1_CDS      TGAATCTATTCTTAAACACTCTGGCATCACTCAGATGCACTTATGTGCT
Ptr_HB5_Potri.001G188800.1_PCN      TGAATCTATTCTGAAAACCTTGTGGCATCATTAGATGCGATCATGTGCT
Ptr_HB6_Potri.003G050100.1_CDS      TGAATCTATTCTGAAAACCTTGTGGCATCATTAGATGCTATTATGTGCT
Ptr_HB3_Potri.011G098300.1_PHA      GGATGCTTTGCTGAAGCGACTGTGGCACCATTAGATGCAATTATGTGCT
Ptr_HB4_Potri.001G372300.1_CDS      CGATGCTTTGCTGAAGCAGCTGTGGCACCATTAGATGCAATTATGTGCT
Ptr_HB1_Potri.009G014500.1_CDS      AGATTCTGTCTGAAACATCTATGGCATCATCCGATGCAATTTTATGTT
Ptr_HB2_Potri.004G211300.1_POP      AGATTCTGTCTGAAACATCTATGGCATCATCCAGATGCAATTTTATGTT
* * * * *      * * * * *      * * * * *      * * * * *

Ptr_HB7_Potri.018G045100.1_CDS      GCTCTTTGAAGACT-----CTGCCGGTTTTACATTTTCAAATCAGGCT
Ptr_HB8_Potri.006G237500.1_CDS      GCTCTTTGAAGACT-----CTGCCGGTTTTACATTTGCAAAATCAAGCA
Ptr_HB5_Potri.001G188800.1_PCN      GCTCATTGAAGGCG-----CTGCCGGTCTTCACTTTTGCTAACCAGCA
Ptr_HB6_Potri.003G050100.1_CDS      GCTCACTGAAGGCG-----TTGCCCATCTTTACTTTTGCAAACCAAGCT
Ptr_HB3_Potri.011G098300.1_PHA      GCTCCTTGAAAACAAACGCATCTCCAGTTTTCACTTTTGCAAACCAAGGCT
Ptr_HB4_Potri.001G372300.1_CDS      GCTCCTTGAAAACAAATGCATCTCCAGTTTTCACTTTTGCAAACCAAGGCC
Ptr_HB1_Potri.009G014500.1_CDS      GTTCATTGGAGGCA-----CTACCAAGTTTCATCTTTGCTAACCAGGCA
Ptr_HB2_Potri.004G211300.1_POP      GTTCATTGAAGTCG-----CTGCCAGTTTTATCTTTGCCAATCAGGCA

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Ptr_HB7_Potri.018G045100.1_CDS      GGACTTGACATGCTTGAGACAACCTTGGTTGCTCTTCAAGACATCACTTT
Ptr_HB8_Potri.006G237500.1_CDS      GGACTTGACATGCTTGAGACAACCTTGGTTGCACTTCAAGACATCACTTT
Ptr_HB5_Potri.001G188800.1_PCN      GGACTTGACATGCTCGAGACAACCTTGGTCGCACTGCAAGACATAAAGTTT
Ptr_HB6_Potri.003G050100.1_CDS      GGACTTGACATGCTTGAGACAACCTTAGTTGCACTGCAAGACATAAAGTTT
Ptr_HB3_Potri.011G098300.1_PHA      GGACTTGATATGCTTGAAACTACTCTTGTGGCCCTTCAAGATATCATGCT
Ptr_HB4_Potri.001G372300.1_CDS      GGACTTGATATGCTTGAAACTACTCTTGTGGCCCTTCAAGATATTATGCT
Ptr_HB1_Potri.009G014500.1_CDS      GGACTTGACATGCTGGAGACAACCTAGTGGCTCTGCAAGACATCACACT
Ptr_HB2_Potri.004G211300.1_POP      GGGCTTGACATGCTGGAGACAACCTAGTGGCTCTACAAGATATTACACT
* * * * * * * * * * * * * * * *

Ptr_HB7_Potri.018G045100.1_CDS      GGAAAAGATATTTGATGATAATGGAAGGAAAACATTGTGCTCAGAGTTCC
Ptr_HB8_Potri.006G237500.1_CDS      GGAAAAGATATTTGATGATAATGGAAGGAAAACATTGTACTCAGAGTTCC
Ptr_HB5_Potri.001G188800.1_PCN      GGAAAAGATATTTGATGATCATGGAAGGAAAACCTCTGCTCAGAATTCC
Ptr_HB6_Potri.003G050100.1_CDS      GGAAAAGATATTTGATGATCATGGAAGAAAAACCTCTGCTCAGAATTCT
Ptr_HB3_Potri.011G098300.1_PHA      TGACAAGATTCTTGATGAAGCAGGTCGGAAGATTCTCTGCTCTGAGTTCT
Ptr_HB4_Potri.001G372300.1_CDS      TGACAAGATCCTTGATGAAGCAGGACGGAAGATTCTCTGCTCTGAGTTCT
Ptr_HB1_Potri.009G014500.1_CDS      GGATAAGATATTTGATGAATCTGGACGCAAGGCATTGTTTACAGACTTCG
Ptr_HB2_Potri.004G211300.1_POP      GGATAAGATATTCAATGAATCTGGACGCGAGGCATTGTATACAGAATTGG
* * * * * * * * * * * * * * * *

Ptr_HB7_Potri.018G045100.1_CDS      CACAAATAATGCAGCAGG-----GTTTTATGTGCCTTCCAGGTGGT
Ptr_HB8_Potri.006G237500.1_CDS      CACAAATAATGCAGCAGG-----GTTTTATGTGCCTTCAAGGTGGT
Ptr_HB5_Potri.001G188800.1_PCN      CTCAGATTATGCAACAGG-----GTTTTACTTGCTCTACAAGGTGGT
Ptr_HB6_Potri.003G050100.1_CDS      CACAGATTATGCAACAGG-----GTTTTACTTGCTTCAAGGTGGT
Ptr_HB3_Potri.011G098300.1_PHA      CTAATAATAATGCAACAGG-----GATATGCTTATCTGCCGCCGGA
Ptr_HB4_Potri.001G372300.1_CDS      CTAAGATAACGCAACAGG-----GATTGCTTATCTGCCAGCCGGA
Ptr_HB1_Potri.009G014500.1_CDS      CAAAGTTAATGCAACAGGCAACAATGGATTGCTGCTGCTGCTGGA
Ptr_HB2_Potri.004G211300.1_POP      CAAAGTTAATGCAACAGG-----GATTGCTGCTGCTGCTGCTGGA
* * * * * * * * * * * * * * * *

Ptr_HB7_Potri.018G045100.1_CDS      ATCTGCCTGTCAAGTATGGGAAGGCCAGTCTCCTATGAGAGAGCAGTGGC
Ptr_HB8_Potri.006G237500.1_CDS      ATCTGCCTGTCAAGTATGGGAAGGCCCGTCTCCTATGAAAGAGCTGTGGC
Ptr_HB5_Potri.001G188800.1_PCN      ATCTGTTTGTCAAGCATGGGCAGACAGTCTCATACGAGAGAGCGGTGTC
Ptr_HB6_Potri.003G050100.1_CDS      ATCTGTTTGTCAAGCATGGGCAGACAGTTTCATATGAAAGAGCTGTGGC
Ptr_HB3_Potri.011G098300.1_PHA      ATATGTGTATCCAGCATGGGGAGGCCGCTCTCCTACGAGCAGGCCATTGC
Ptr_HB4_Potri.001G372300.1_CDS      ATATGTGTATCCAGCATGGGGAGGCCAGTCTCCTACGAGCAGGCCGTTGC
Ptr_HB1_Potri.009G014500.1_CDS      ATCTGCATGTCAACAATGGGGCGCAATGTATCATATGAACAAGCTGTTTC
Ptr_HB2_Potri.004G211300.1_POP      ATCTGCATGTCAACAATGGGGCGTAATGTTTCATATGAGCAAGCTGTTGC
* * * * * * * * * * * * * * * *

Ptr_HB7_Potri.018G045100.1_CDS      ATGGAAGGTATTGAACGAAGAAGAAAGCGCT---CACTGCATTGCTTCA
Ptr_HB8_Potri.006G237500.1_CDS      ATGGAAGGTATTGAATGAAGAAGAGAGCGCT---CACTGCATTGCTTCA
Ptr_HB5_Potri.001G188800.1_PCN      CTGGAAAGTGTGAATGAAGAAGAAAATGCG---CATTGCATCTGCTTTA
Ptr_HB6_Potri.003G050100.1_CDS      CTGGAAAGTGTGAATGAAGAAGAAAATGCG---CATTGCATCTGCTTTA
Ptr_HB3_Potri.011G098300.1_PHA      ATGGAAGTTCCTTAATGATGATAATTCCAAC---CACTGTCTGGCTTTCA
Ptr_HB4_Potri.001G372300.1_CDS      ATGGAAGTTCCTAATGATGATGATTCCAAC---CACTGCCTGGCCTTCA
Ptr_HB1_Potri.009G014500.1_CDS      ATGGAAGTGTCTGCTGCTGAAGAGAACTGTCCACTGCATTGCTTTCT
Ptr_HB2_Potri.004G211300.1_POP      TTGGAAGTGTCTATCTGCAGAGAGAACGCTGTTTCATTGCTGCTTTCT
* * * * * * * * * * * * * * * *

Ptr_HB7_Potri.018G045100.1_CDS      TGTTTCATGAACGGTCTTTTGTGTGA
Ptr_HB8_Potri.006G237500.1_CDS      TGTTTCATCAACTGGTCTTTTGTGTGA
Ptr_HB5_Potri.001G188800.1_PCN      TGTTTATAAACTGGTCTTTTGTGTGA
Ptr_HB6_Potri.003G050100.1_CDS      TGTTTATAAACTGGTCTTTTGTGTGA
Ptr_HB3_Potri.011G098300.1_PHA      TGTTTCATTAACGGTCTTTTGTGTGA
Ptr_HB4_Potri.001G372300.1_CDS      TGTTTCATGAACGGTCTTTTGTGTGA
Ptr_HB1_Potri.009G014500.1_CDS      CTTTTGTAACTGGTCTTTTGTGTGA
Ptr_HB2_Potri.004G211300.1_POP      CTTTTGTAACTGGTCTTTTGTGTGA
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Ptr_HB1_Potri.009G014500.1_CDS:0.04783,
Ptr_HB2_Potri.004G211300.1_POPREV_CDS:0.08873)
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)
)

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(  
Ptr_HB3_Potri.011G098300.1_PHABULOSA_CDS:0.04035,  
Ptr_HB4_Potri.001G372300.1_CDS:0.02600)  
:0.16099)  
:0.10074,  
(  
Ptr_HB5_Potri.001G188800.1_PCN_CDS:0.05242,  
Ptr_HB6_Potri.003G050100.1_CDS:0.01918)  
:0.09000,  
(  
Ptr_HB7_Potri.018G045100.1_CDS:0.04187,  
Ptr_HB8_Potri.006G237500.1_CDS:0.02803)  
:0.09384);
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D.3 Phylogenetic tree of all eight HB genes that are targets of miR166

E Appendix E

Copyright documentation

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