

Manuscript title: Functional characterization of the late embryogenesis abundant (LEA) protein gene family from *Pinus tabuliformis* (Pinaceae) in *Escherichia coli*

Author: Jie Gao^{1*} and Ting Lan²

¹Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun, Yunnan, China

²State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Beijing 10093, China *Corresponding to Dr. Jie Gao Phone: +86-691-8713066;

E-mail: gaojie@xtbg.org.cn

Supporting information

Supplemental Figure. 1 Weblogo illustration of the conserved motif sequences in *P. tabuliformis* genes.

Supplemental Figure. 2 Growth performance of *E.coli* transformants for pTWIN-LEA and pTWIN constructs under salt treatment. BP, BL21 cells with TWIN1 vector transformed.

Supplemental Figure. 3 Growth performance of *E.coli* transformants for TWIN-LEA and TWIN constructs under heat treatment. BP, BL21 cells with TWIN1 vector transformed.

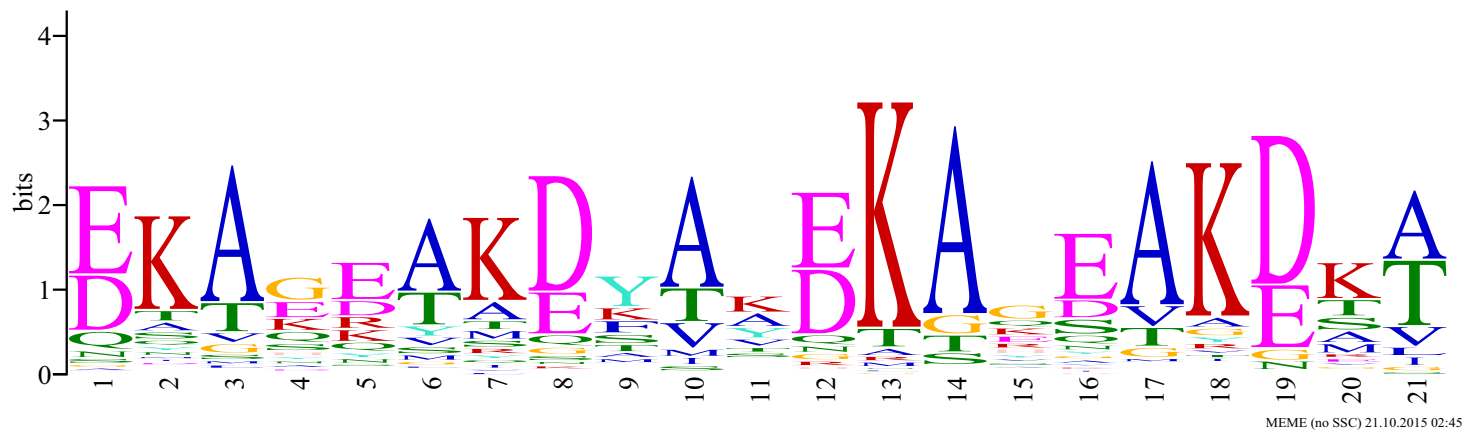
Supplemental Table 1. PCR primers used to clone and expression of *LEA* genes in *P. tabuliformis*.

Supplemental Table 2. RT-PCR primers used to detect the expression of *LEA* genes.

Supplemental Table 3. EX-PCR primers used to expression the *P. tabuliformis* *LEA* genes.

Supplemental file 4. The identified 43 protein sequences of *P. taeda*.

motif 1



motif 2



motif 3



motif 4



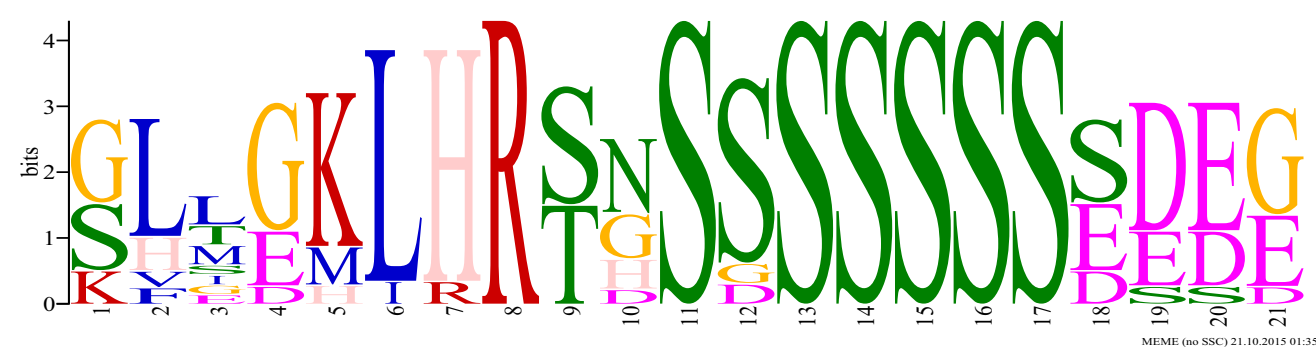
motif 5



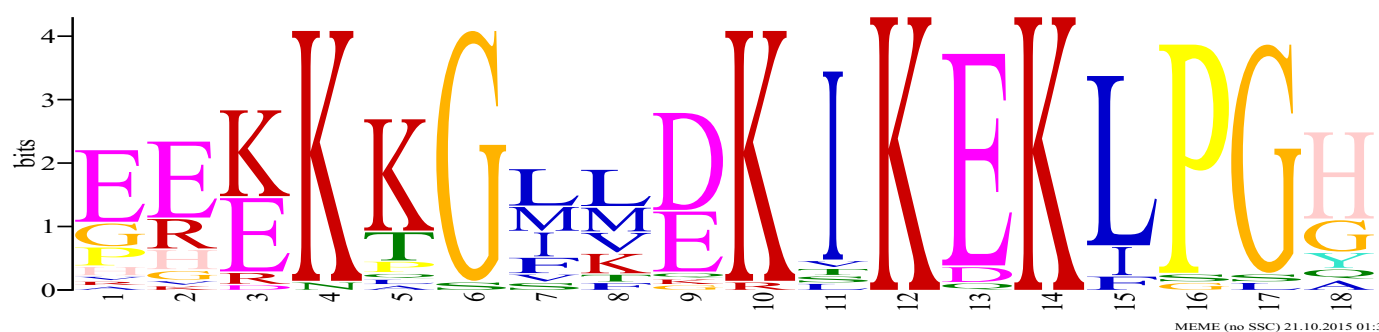
motif 6



motif S



motif K



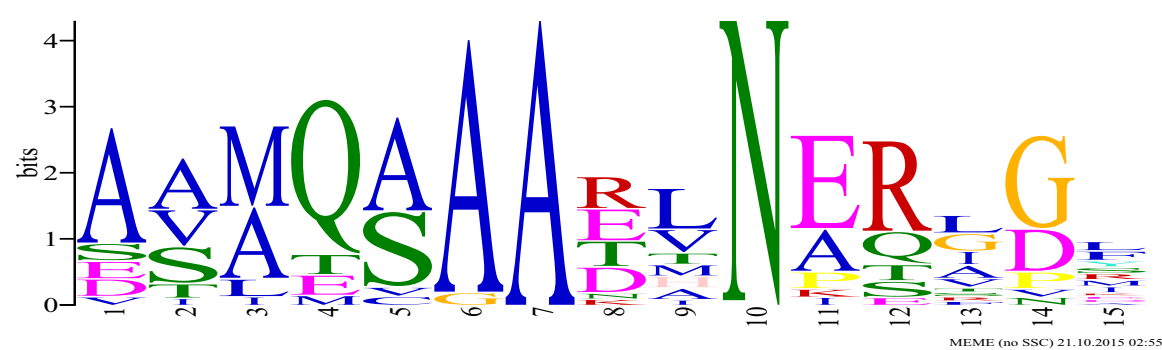
motif 9

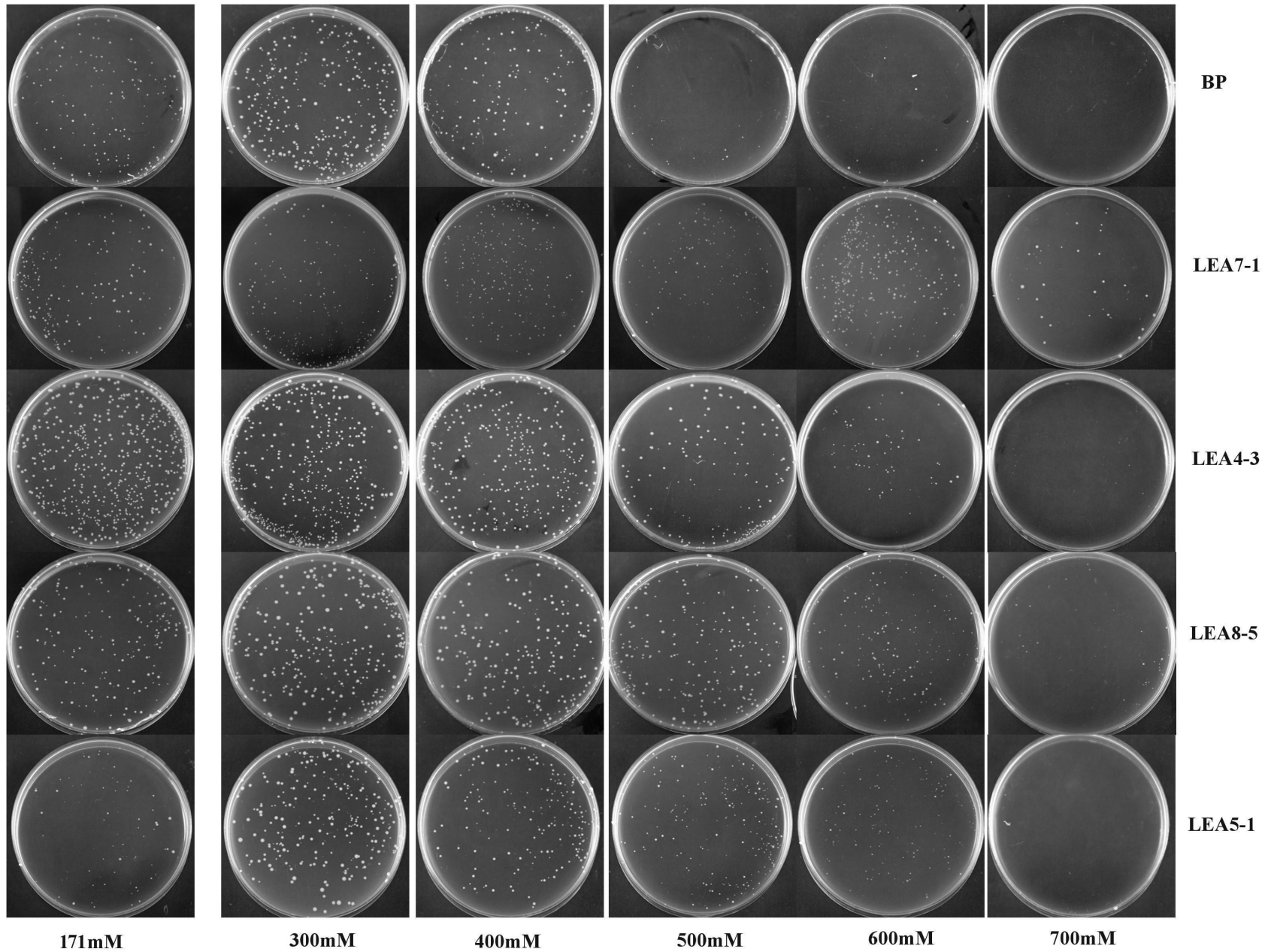


motif 10



motif 11





171mM

300mM

400mM

500mM

600mM

700mM

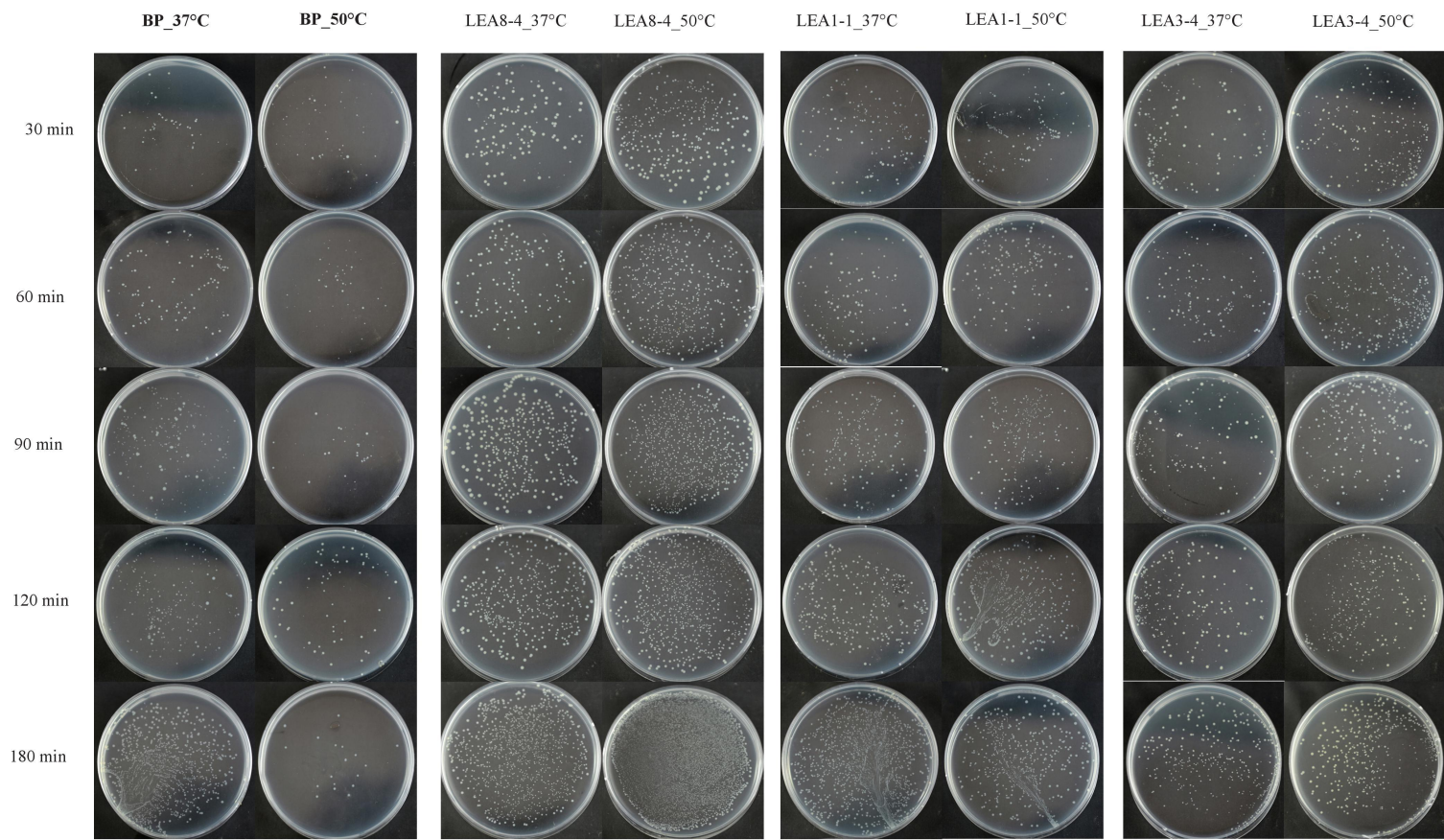
BP

LEA7-1

LEA4-3

LEA8-5

LEA5-1



Supplemental Table1. Primers used to clone the *LEA* genes.

Genes	Primer name	Sequence (5'-3')
<i>PtaLEA1-1</i>	<i>PtaRT-LEA1-1</i>	GCAGCCAAGGAGAAGATGAGC
	<i>PtaRT-LEA1-1</i>	GGGATAAGTGGTAGTGTGAGTAGGG
<i>PtaLEA1-2</i>	<i>PtaRT-LEA1-2</i>	AGCGATGTGAAGGAAGGGAT
	<i>PtaRT-LEA1-2</i>	GTAAGTCCAGGAGTACCAACATTC
<i>PtaLEA2-1</i>	<i>PtaRT-LEA2-1</i>	TATCAAGCCCGAAGCATTATC
	<i>PtaRT-LEA2-1</i>	ATGGGTCCAAATGGAGAGTCAAC
<i>PtaLEA3-1</i>	<i>PtaRT-LEA3-1</i>	ATGGCCAGATCTCTGCCC
	<i>PtaRT-LEA3-1</i>	TGCTACATCAGTTACCCCAAAA
<i>PtaLEA3-2</i>	<i>PtaRT-LEA3-2</i>	ATAATTTTCAATGGCTGCCG
	<i>PtaRT-LEA3-2</i>	AATTGAGAAATTTTACATCAATTCA
<i>PtaLEA3-3</i>	<i>PtaRT-LEA3-3</i>	TCTACATATGAAGGTTGTGGCTG
	<i>PtaRT-LEA3-3</i>	CAGAAATTTTAAATCGTGTCGTT
<i>PtaLEA3-4</i>	<i>PtaRT-LEA3-4</i>	CTCCGAATAAGTTATCTTCTCTCAG
	<i>PtaRT-LEA3-4</i>	TCCGAGTCCGCAATGTC
<i>PtaLEA3-5</i>	<i>PtaRT-LEA3-5</i>	TGGTCTTGTGTCCCGTTTTTC
	<i>PtaRT-LEA3-5</i>	TTCTGGAGGAGAGCAGCTTC
<i>PtaLEA3-6</i>	<i>PtaRT-LEA3-6</i>	ATGGCAGGACGGCTTCTAT
	<i>PtaRT-LEA3-6</i>	CTACTTTCTAGATAGGAGCTTCTGC
<i>PtaLEA4-1</i>	<i>PtaRT-LEA4-1</i>	CGGACCAGTACAATCGGG
	<i>PtaRT-LEA4-1</i>	CCCAAGGGATGCTTCAGAG
<i>PtaLEA4-2</i>	<i>PtaRT-LEA4-2</i>	CAGTGCAAGCAATATGGGC
	<i>PtaRT-LEA4-2</i>	CCAAAGGGTTTCTTGACAGC
<i>PtaLEA4-3</i>	<i>PtaRT-LEA4-3</i>	GAGGCAGAAGAGAGACGGG
	<i>PtaRT-LEA4-3</i>	GGACTCTGGGCTTTTCTTG
<i>PtaLEA5-1</i>	<i>PtaRT-LEA5-1</i>	CTAAATCGTTGGGAGTGACCA
	<i>PtaRT-LEA5-1</i>	CCGTTCTTCTTTTCTTACTTCTCC
<i>PtaLEA7-1</i>	<i>PtaRT-LEA7-1</i>	ATGAAGCGCAAGATCAATTG
	<i>PtaRT-LEA7-1</i>	AGGCAGGTCTTCGGTAGCAGTA
<i>PtaLEA7-2</i>	<i>PtaRT-LEA7-2</i>	TATTTTTCAGGGTTTCATACGAC
	<i>PtaRT-LEA7-2</i>	CCTCCTTTTTGAGTGACGG
<i>PtaLEA7-3</i>	<i>PtaRT-LEA7-3</i>	AGACTCAGAAGGGTGGTGCT
	<i>PtaRT-LEA7-3</i>	TGGTGTGGAAACCCATAGC
<i>PtaLEA7-4</i>	<i>PtaRT-LEA7-4</i>	AACGTCCTTACGGAAAGCG
	<i>PtaRT-LEA7-4</i>	CCACGAGCAGGTCTTCAGTAG
<i>PtaLEA8-1</i>	<i>PtaRT-LEA8-1</i>	CATCTGCAGAGACTGTGCCT
	<i>PtaRT-LEA8-1</i>	CCTTCATATCCATGCCCAT
<i>PtaLEA8-2</i>	<i>PtaRT-LEA8-2</i>	CAATCTGATCACATTGTCCAGAC
	<i>PtaRT-LEA8-2</i>	GGAATACTGCTTTCCATCCTC
<i>PtaLEA8-3</i>	<i>PtaRT-LEA8-3</i>	GGAGTCGAACACGGGCAT
	<i>PtaRT-LEA8-3</i>	CTTTGATTTTACCAAGCACACC
<i>PtaLEA8-4</i>	<i>PtaRT-LEA8-4</i>	ATGGCTGAACAAGCTAAGCAA
	<i>PtaRT-LEA8-4</i>	CGCCTTCTTCTCCTTTCAC

<i>PtaLEA8-5</i>	<i>PtaRT-LEA8-5</i>	ATGGCGCAACCAGCACAC
	<i>PtaRT-LEA8-5</i>	TTACTCCACGTCATTCTTATTTTGT
<i>PtaLEA8-6</i>	<i>PtaRT-LEA8-6</i>	GAAAGCATTCTGATGAGATTGG
	<i>PtaRT-LEA8-6</i>	TTATTTTATCAAGCATACCCGT

Supplemental Table 2. RT-PCR primers used to detect the expression of *LEA* genes.

Genes	Primer name	Sequence (5'-3')
<i>PtaLEA1-1</i>	<i>PtaRT-LEA1-1</i>	GCAGCCAAGGAGAAGATGAGC
	<i>PtaRT-LEA1-1</i>	GGGATAAGTGGTAGTGTGAGTAGGG
<i>PtaLEA1-2</i>	<i>PtaRT-LEA1-2</i>	AGCGATGTGAAGGAAGGGAT
	<i>PtaRT-LEA1-2</i>	GTAAGTTCCAGGAGTACCAACATTC
<i>PtaLEA2-1</i>	<i>PtaRT-LEA2-1</i>	TATCAAGCCCGGAAGCATTATC
	<i>PtaRT-LEA2-1</i>	ATGGGTCCAAATGGAGAGTCAAC
<i>PtaLEA3-1</i>	<i>PtaRT-LEA3-1</i>	ATGGCCAGATCTCTGCCC
	<i>PtaRT-LEA3-1</i>	TGCTACATCAGTTACCCCAAAA
<i>PtaLEA3-2</i>	<i>PtaRT-LEA3-2</i>	ATAATTTTCAATGGCTGCCG
	<i>PtaRT-LEA3-2</i>	AATTGAGAAATTTTACATCAATTCA
<i>PtaLEA3-3</i>	<i>PtaRT-LEA3-3</i>	TCTACATATGAAGGTTGTGGCTG
	<i>PtaRT-LEA3-3</i>	CAGAAATTTTAAATCGTGTGCGTT
<i>PtaLEA3-4</i>	<i>PtaRT-LEA3-4</i>	CTCCGAATAAGTTATCTTCTCTCAG
	<i>PtaRT-LEA3-4</i>	TCGGAGTTCGGCAATGTC
<i>PtaLEA3-5</i>	<i>PtaRT-LEA3-5</i>	TGGTCTTGTGTCCCGTTTTTC
	<i>PtaRT-LEA3-5</i>	TTCTGGAGGAGAGCAGCTTC
<i>PtaLEA3-6</i>	<i>PtaRT-LEA3-6</i>	ATGGCAGGACGGCTTCTAT
	<i>PtaRT-LEA3-6</i>	CTACTTCTAGATAGGAGCTTCTGC
<i>PtaLEA4-1</i>	<i>PtaRT-LEA4-1</i>	CGGACCAGTACAATCGGG
	<i>PtaRT-LEA4-1</i>	CCCAAGGGATGCTTCAGAG
<i>PtaLEA4-2</i>	<i>PtaRT-LEA4-2</i>	CAGTGCAAGCAATATGGGC
	<i>PtaRT-LEA4-2</i>	CCAAAGGGTTTCTTGACAGC
<i>PtaLEA4-3</i>	<i>PtaRT-LEA4-3</i>	GAGGCAGAAGAGAGACGGG
	<i>PtaRT-LEA4-3</i>	GGACTCTTGGGCTTTTTCTTG
<i>PtaLEA5-1</i>	<i>PtaRT-LEA5-1</i>	CTAAATCGTTGGGAGTGACCA
	<i>PtaRT-LEA5-1</i>	CCGTCTTCTTTTCTTACTTCTCC
<i>PtaLEA7-1</i>	<i>PtaRT-LEA7-1</i>	ATGAAGCGCAAGATCAATTG
	<i>PtaRT-LEA7-1</i>	AGGCAGGTCTTCGGTAGCAGTA
<i>PtaLEA7-2</i>	<i>PtaRT-LEA7-2</i>	TATTTTTTCAGGGTTTCATACGAC
	<i>PtaRT-LEA7-2</i>	CCTCCTTTTTGAGTGACGG
<i>PtaLEA7-3</i>	<i>PtaRT-LEA7-3</i>	AGACTCAGAAGGGTGGTGCT
	<i>PtaRT-LEA7-3</i>	TGGTGTGGAAACCCATAGC
<i>PtaLEA7-4</i>	<i>PtaRT-LEA7-4</i>	AACGTCCTTACGGAAAGCG
	<i>PtaRT-LEA7-4</i>	CCACGAGCAGGTCTTCAGTAG
<i>PtaLEA8-1</i>	<i>PtaRT-LEA8-1</i>	CATCTGCAGAGACTGTGCCT
	<i>PtaRT-LEA8-1</i>	CCTTCATATCCATGCCCAT
<i>PtaLEA8-2</i>	<i>PtaRT-LEA8-2</i>	CAATCTGATCACATTGTCCAGAC
	<i>PtaRT-LEA8-2</i>	GGAATACTGCTTTCCATCCTC
<i>PtaLEA8-3</i>	<i>PtaRT-LEA8-3</i>	GGAGTCGAACACGGGCAT
	<i>PtaRT-LEA8-3</i>	CTTTGATTTTACCAAGCACACC
<i>PtaLEA8-4</i>	<i>PtaRT-LEA8-4</i>	ATGGCTGAACAAGCTAAGCAA
	<i>PtaRT-LEA8-4</i>	CGCCTTCTTCTCTCTTCAC

<i>PtaLEA8-5</i>	<i>PtaRT-LEA8-5</i>	ATGGCGCAACCAGCACAC
	<i>PtaRT-LEA8-5</i>	TTACTCCACGTCATTCTTATTTTGT
<i>PtaLEA8-6</i>	<i>PtaRT-LEA8-6</i>	GAAAGCATTCTGATGAGATTGG
	<i>PtaRT-LEA8-6</i>	TTATTTTATCAAGCATACCCGT

Supplemental Table 3. EX-PCR primers used to expression the *P.tabliufrims* LEA genes.

Genes	Primer name	Sequence (5'-3')
<i>PtaLEA1-1</i>	<i>PtaEX-LEA1-1</i>	AGCGGCCGCATGCAACCTGTAAAGAATGCA
	<i>PtaEX-LEA1-1</i>	T TACTAGTTATCATAAGTACTTGCCGGGAG
<i>PtaLEA1-2</i>	<i>PtaEX-LEA1-2</i>	AGCGGCCGCATGCAGACGGCCAAGGAG
	<i>PtaEX-LEA1-2</i>	T TACTAGTTATCACAGGTAATCTCTACCAGTGTG
<i>PtaLEA2-1</i>	<i>PtaEX-LEA2-1</i>	AGCGGCCGCATGACAATAATCTACGATGATATC
	<i>PtaEX-LEA2-1</i>	T TACTAGTTATCAATACTCATCGTCCTCG
<i>PtaLEA3-2</i>	<i>PtaEX-LEA3-2</i>	AGCGGCCGCATGGCTGCCGGTGCTCTG
	<i>PtaEX-LEA3-2</i>	T TACTAGTTAATTTGGTCTCCGCGAGA
<i>PtaLEA3-4</i>	<i>PtaEX-LEA3-4</i>	AGCGGCCGCATGGCAAAGCTTGCTCTCC
	<i>PtaEX-LEA3-4</i>	T TACTAGTTATCAGGAGGAGGACCTTCG
<i>PtaLEA4-2</i>	<i>PtaEX-LEA4-2</i>	AGCGGCCGCATGGCGGATCAGTGCAAG
	<i>PtaEX-LEA4-2</i>	T TACTAGTTACTACTGATCGTACCACCAAAG
<i>PtaLEA4-3</i>	<i>PtaEX-LEA4-3</i>	AGCGGCCGCATGGCTTCAGAACAGAAAAC
	<i>PtaEX-LEA4-3</i>	T TACTAGTTATCACCCACGTCGTTGAAAG
<i>PtaLEA5-1</i>	<i>PtaEX-LEA5-1</i>	AGCGGCCGCATGGAGGGATTGATAATGAAAAT
	<i>PtaEX-LEA5-1</i>	T TACTAGTTAGTAGCCAAGCCTTTCCTTA
<i>PtaLEA7-1</i>	<i>PtaEX-LEA7-1</i>	AGCGGCCGCATGAATCAGGGCAGGCG
	<i>PtaEX-LEA7-1</i>	T TACTAGTTATCATGTCCGAAACCCCATG
<i>PtaLEA7-3</i>	<i>PtaEX-LEA7-3</i>	AGCGGCCGCATGCAGAGTGCGGAGGCA
	<i>PtaEX-LEA7-3</i>	T TACTAGTTACTTCTCCGAGTGGCTGTG
<i>PtaLEA8-2</i>	<i>PtaEX-LEA8-2</i>	AGCGGCCGCATGGCGGAAGAAGCACCAG
	<i>PtaEX-LEA8-2</i>	T TACTAGTTACTGTCCCATCATTCTTGTAT
<i>PtaLEA8-4</i>	<i>PtaEX-LEA8-4</i>	AGCGGCCGCATGGCTGAACAAGCTAAGCA
	<i>PtaEX-LEA8-4</i>	T TACTAGTTACTAGTGCCAGGCAGCTTCTC
<i>PtaLEA8-5</i>	<i>PtaEX-LEA8-5</i>	AGCGGCCGCATGGCGCAACCAGCACAC
	<i>PtaEX-LEA8-5</i>	T TACTAGTTACTCCACGTCATTCTATTTTGT