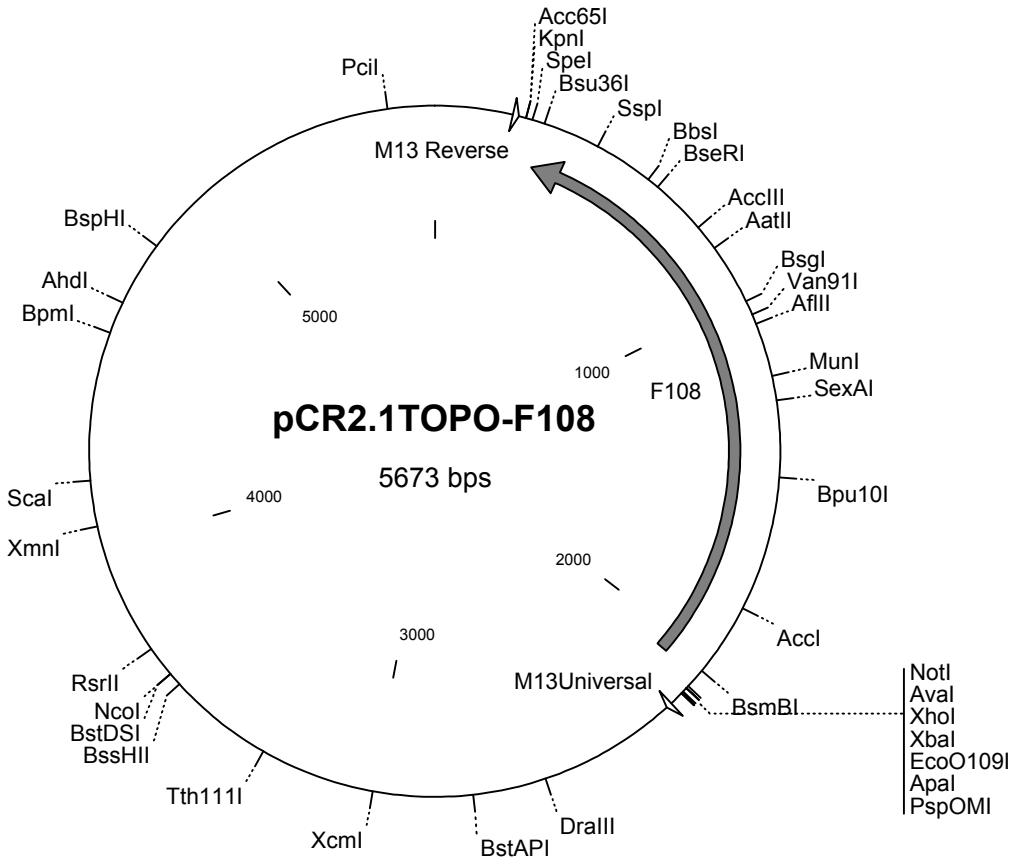
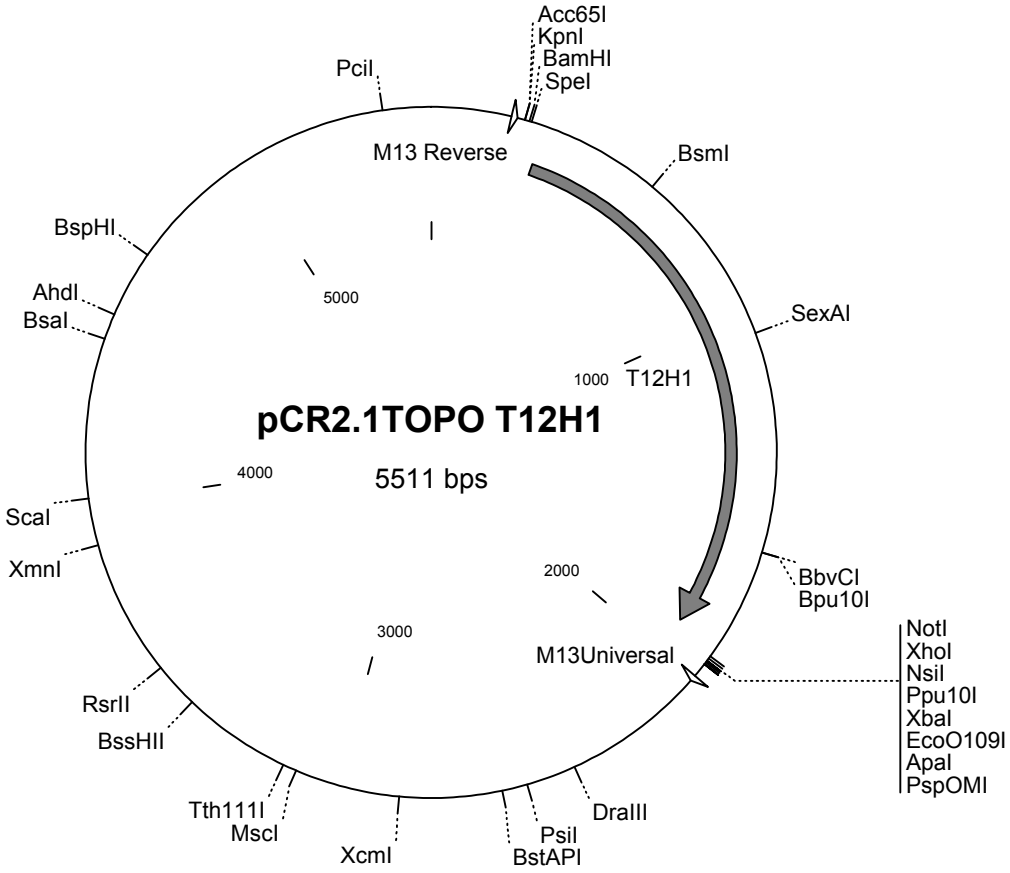
Appendix B

Plasmids and primers constructed

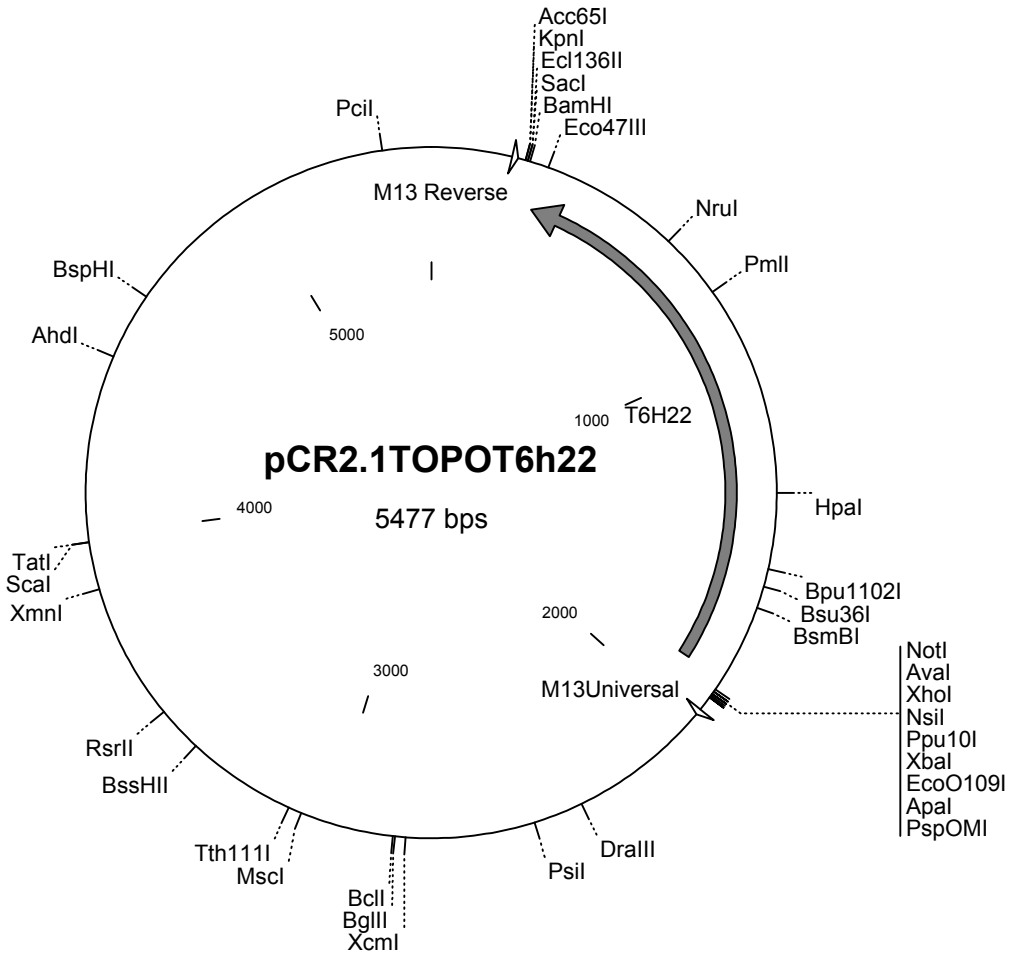
Plasmids in this section were constructed for use in this dissertation research. Maps were produced using CloneManager 5.0 for Windows. Sequences for the three Arabidopsis cDNAs have been published on Genbank, as described in Chapter 4 of this work. A list of primers used in this work, as well as an alignment of key Arabidopsis primers to the cloned sequences is also included for reference (Alignment produced from MacVector 2.1).



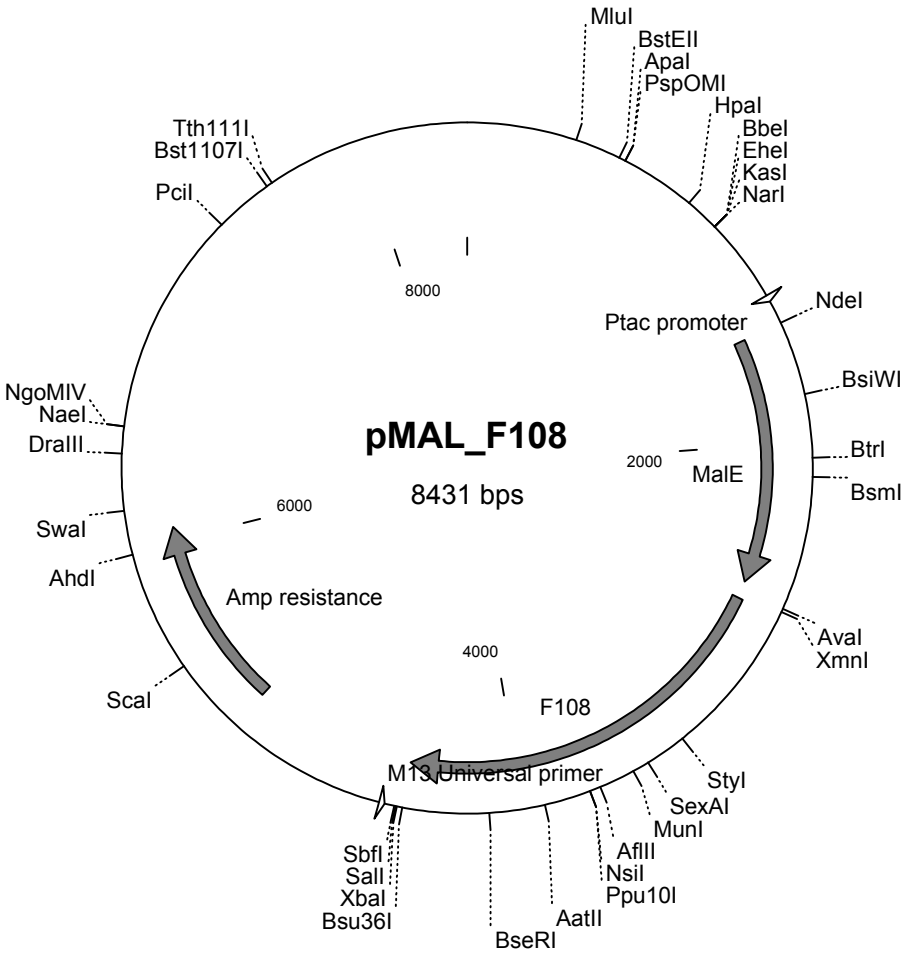
Vector:	pCR2.1TOPO (Invitrogen)
Insert:	F108 PCR product
Resistance marker(s):	kanamycin, ampicillin
Host strain:	Top10 (Invitrogen)



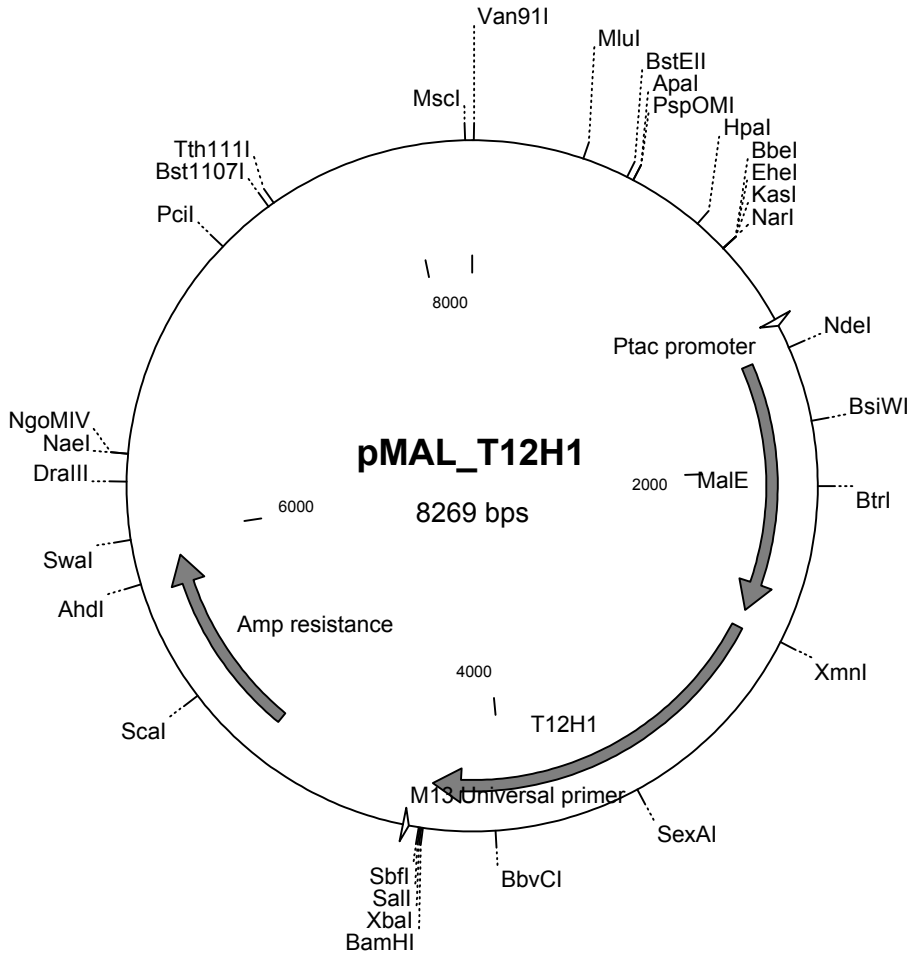
Vector:	pCR2.1TOPO (Invitrogen)
Insert:	T12H1 PCR product
Resistance marker(s):	kanamycin, ampicillin
Host strain:	Top10 (Invitrogen)



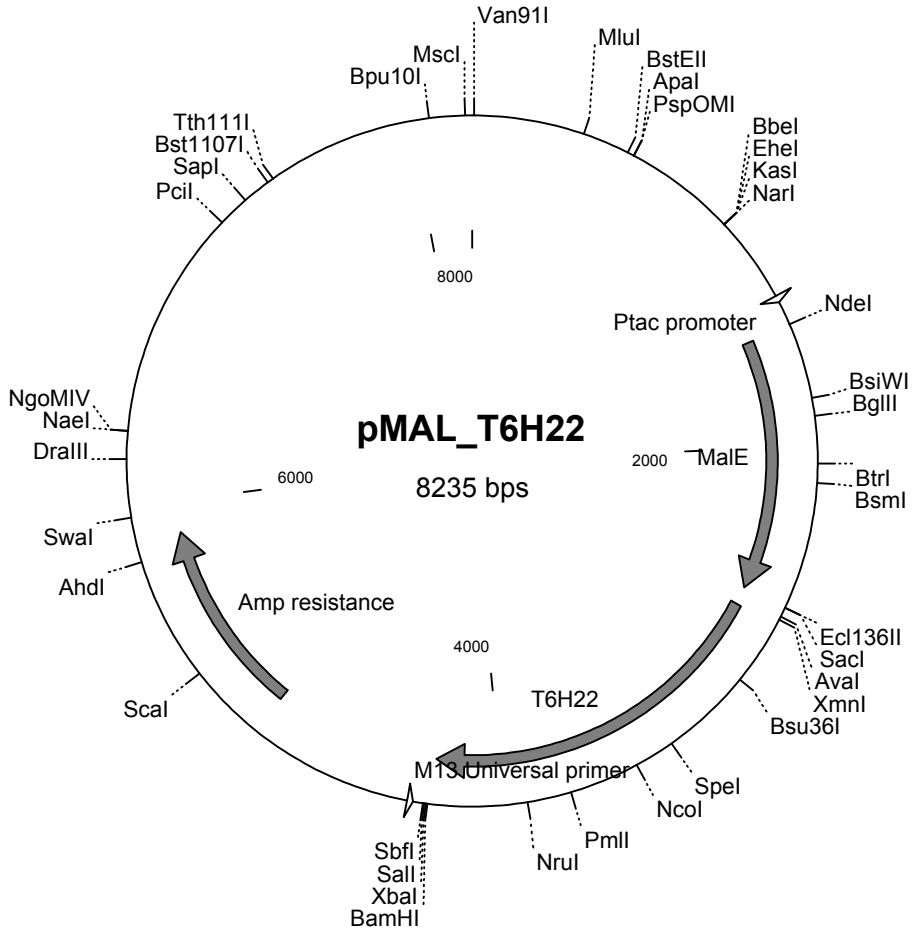
Vector:	pCR2.1TOPO (Invitrogen)
Insert:	T6H22 PCR product
Resistance marker(s):	kanamycin, ampicillin
Host strain:	Top10 (Invitrogen)



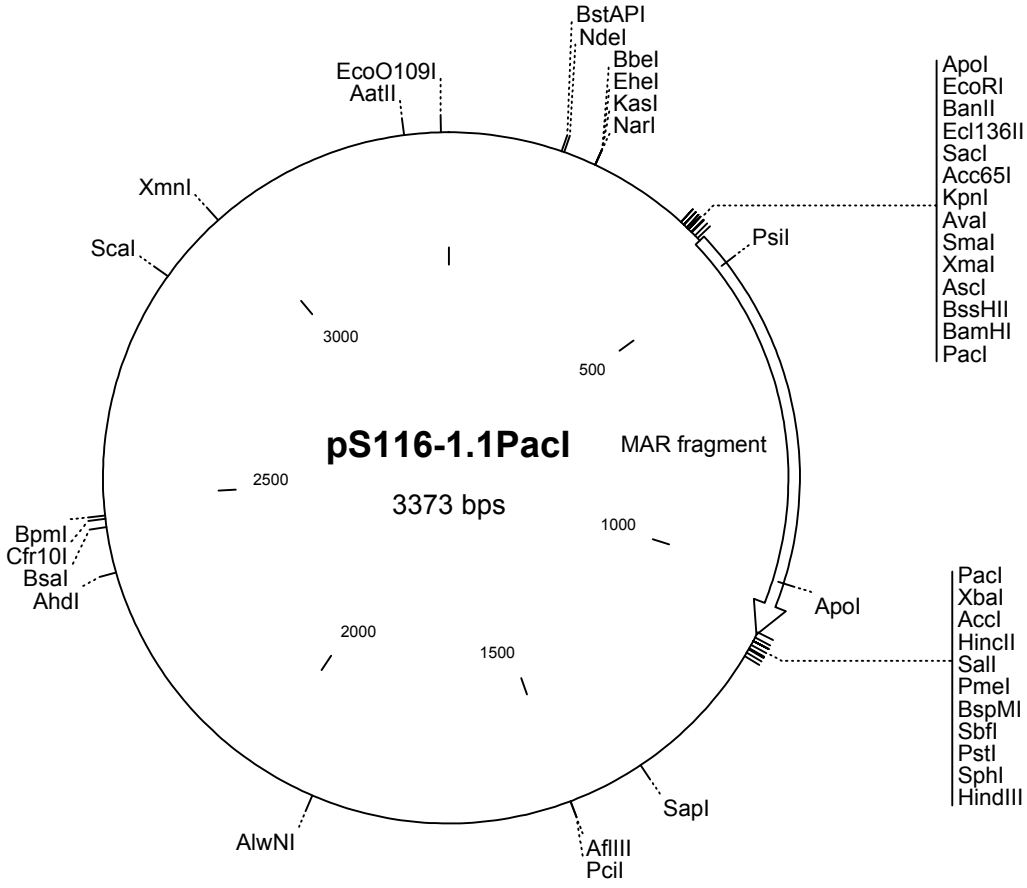
Vector:	pMAL c2x (New England Biolabs)
Insert:	EcoRI fragment from pCR2.1TOPO-F108
Resistance marker(s):	ampicillin
Host strain:	TB1



Vector:	pMAL c2x (New England Biolabs)
Insert:	EcoRI fragment from pCR2.1TOPO-T12H1
Resistance marker(s):	ampicillin
Host strain:	TB1



Vector:	pMAL c2x (New England Biolabs)
Insert:	EcoRI fragment from pCR2.1TOPO-T6H22
Resistance marker(s):	ampicillin
Host strain:	TB1

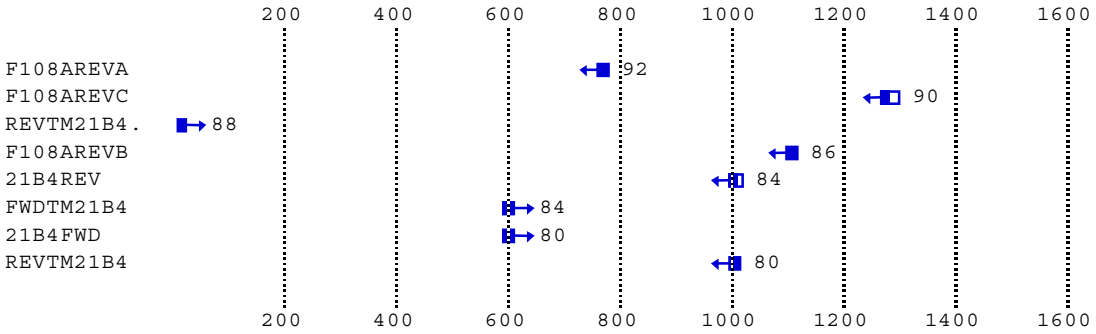


Vector:	pNEB193 (New England Biolabs)
Insert:	S116-1.1B PacI restriction fragment
Resistance marker(s):	ampicillin
Host strain:	XL-1 Blue (Stratagene)

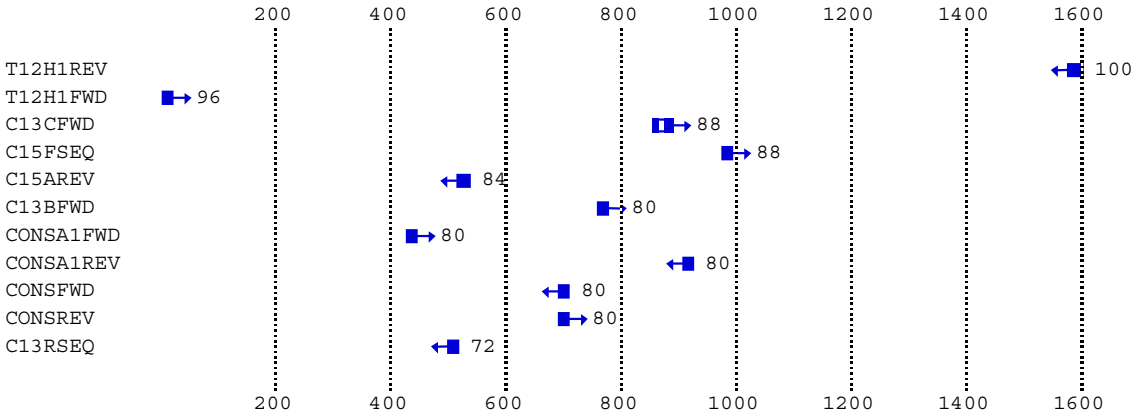
Primer Name	date rec'd	sequence 12345678901234567890123456789012345678	length	degeneracy	strand	Nearest Neighbor Tm
TOBACCO PRIMERS						
BND3FWD	4/30/98	ATHGTNAAYGAYAAYTAYYTNTAYGC	26	2048	coding(IVNDNYLYA)	
FWDBND3A	5/20/98	GAYAAYTAYYTNTAYGCNAAR	21	256	coding(DNYXYAK)	
BND3REV	4/30/98	CKNCCDATRAANSWNSWRTGRAADATNARNCCRTA	35	98304	complement(YGXIFHXXFIG)	
REVBND3B	5/20/98	ATNARNCCRTAYTTNGGNGT	20	2048	complement(TPKYGX)	
REVBND3C	5/20/98	RTGRAADATNAANCCRTA	18	384	complement(YGXIFH)	
REVBND3A	10/5/98	YTTNGCRTANARRTARTTRTC	21	256	complement(DNYXYAK)	
FWDBND3C	10/5/98	TAYGGNTTNATHTTYCAY	18	384	coding(YGXIFH)	
HATPKYGFWD	10/12/98	CAHGCYACTCCWAARTATGG	20	24	coding(HATPKYG)	
HATPKYG2	9/23/99	GCGGCCGCACTAGTCAHGCYACTCCWAARTATGG	34	24	coding(HATPKYG)	
HATPKYGREV	10/12/98	CCATAYTTWGGAGTRGCATG	20	8	complement(HATPKYG)	60
ARABIDOPSIS PRIMERS						
FWDFULL2.12	8/18/98	ATGCTAATACTGTTTGAG	18	NONE	coding	43
FWDFULL.12	8/10/98	ATGCTAATACTGTTTG*G*AG	19	NONE	Error in sequence, don't use	48
REVFULL.12	8/10/98	CTAGCAGAGGAATAATC	17	NONE	complement	42
FWDFULL.13	8/10/98	ATGGTTAAGCTAAAAGC	17	NONE	coding	45
REVFULL.13	8/10/98	TTACCATAGCAACTGG	16	NONE	complement	45
FWDTM21B4.12	4/7/98	CAATCTTTGGTTTGCCTTTAGC	22	NONE	complement	60
REVTM21B4.12	4/7/98	AATACTGTTTGAGACGCCAGGT	22	NONE	coding	60
FWDTM21B4.13	4/7/98	CAAGGGAAACCGTTTTTGAA	20	NONE	complement	60
REVTM21B4.13	4/7/98	TCTGCAGAGTCAGCTCGAAG	20	NONE	coding	60
FWDTM21B4		GACAATATCTTATATGCCAAG	21	NONE	coding(DNILYAK)	49
REVTM21B4		ATTAAACCATACTTTGGAGT	20	NONE	complement(TPKYGL)	48
RIFULL12FWD		*GAATTC*ATGCTAATACTGTTTGAG	24	NONE	coding	55
HIFULL12REV		*GGATCC*TAGCAGAGGAATAATC	22	NONE	complement	56
RIFULL13FWD		*GAATTC*ATGGTTAAGCTAAAAGC	23	NONE	coding	56
HIFULL13REV		*GGATCC*TTACCATAGCAACTGG	24	NONE	complement	60
CONSFWD	1/25/99	CTGCGTCCTTAAGATCTGCC	20	NONE	complement(ADLKDA)	60
CONSREV	1/25/99	GGCAGATCTTAAGGACGCAG	20	NONE	coding(ADLKDA)	60
CON2FWD	2/18/99	GAATTTTATGACAAAGGTGTTGCC	24	NONE	coding(EFYDKGVA)	61
CON2REV	2/18/99	TTCTTCTTCTTGCTTTTCTTCACC	24	NONE	complement(VKKSKKKK)	60
CONSA1FWD	3/10/99	CAGTCTCGCCAGATATAAGC	20	NONE	coding(SLARYK)	55
CONSA1REV	3/10/99	GCTTGGAGAGGTTCAATAAA	20	NONE	complement(LLNLSK)	55
CONSA2FWD	3/10/99	AAGTCTTAGGGGATGAAGATAAG	23	NONE	coding(VLGDEDK)	55
CONSA2REV	3/10/99	TTCTCTATCAACTCCTTCATTACA	24	NONE	complement(VMKELIEN)	55
NTCONBREV	5/12/99	GGAAGATGGGCATTTAGCAA	20	NONE	complement()	60
21B4FWD	9/23/99	*GCGGCCGCACTAGT*GACAATATCTTATATGCCAAG	35	NONE	coding(DNILYAK)	*75* 50
21B4REV	9/23/99	*GTCGACTCGAG*ATTAAACCATACTTTGGAGT	31	NONE	complement(TPKYGL)	*67* 48
C23AFWD	10/3/99	*GCGGCCGCACTAGT*CCTCGGTGAAGAAAAGCAAG	34	NONE	coding	*81* 60
C23CFWD	10/1/99	*GCGGCCGCACTAGT*AAGGGAAGGAGCCAGTTGAT	34	NONE	coding	*80* 60
C13BFWD	10/1/99	*GCGGCCGCACTAGT*CCGTGAACTCTGTGACCAG	33	NONE	coding	*82* 58
C13CFWD	10/1/99	*GCGGCCGCACTAGT*AGCTTGTTGGTGCTCGTCTT	34	NONE	coding	*82* 57
F108AREVB	10/1/99	*GTCGACTCGA*GAGCATCACAACGGATAGCA	30	NONE	complement	*75* 63
F108AREVC	10/1/99	*GTCGACTCGAG*ATCAGACCTCCGGATCCTTT	31	NONE	complement	*75* 60
C25CREV	10/15/99	*GTCGACTCGAG*CGATGACCTCCTTTGCCTTA	31	NONE	complement(KAKEVI)	*74* 60
C15AREV	1/6/00	*GTCGACTCGAG*TATGTGTTCAGCTCTTTGT	30	NONE	complement	*63* 50
C15FSEQ	3/9/00	TAGAGCTCTTAAGACAAAACAC	22	NONE		52
C13RSEQ	3/9/00	TTGTCAAGATCATCAAGC	18	NONE		48
C25RSEQ	3/9/00	TCACCAGGGTTTTAAGTC	18	NONE		50
C23FSEQ	3/9/00	AAGAACGTGGATGTAATG	18	NONE		48
F108RSEQ	3/9/00	ACAGAGTTCTCGGATGTG	18	NONE		52
F108AREVA	3/9/00	AGAAAGAACCTGGTCACAGAGTT	23	NONE		58
F108FWD	4/12/00	ATGCTAATACTGTTTGAGACGC	22	NONE	coding	49
F108REV	4/12/00	TTTTTAAGGACTAAACCAAACATATT	26	NONE	complement	50
T6H22FWD	4/12/00	ATGGCGATGTATGTTATCTACGA	23	NONE	coding	51
T6H22REV	4/12/00	CTATTCAGCGCTCTTAGACTTCTT	24	NONE	complement	51
T12H1FWD	4/12/00	ATGGTCTTAGTGCTATACGAGACA	24	NONE	coding	50
T12H1REV	4/12/00	TCAGTCCTTACTCTTCTTTTTCTTC	25	NONE	complement	50
S.CEREVISAE PRIMERS						
FWDSIK1	4/7/98	ACTAGTGAAAGATGGCTCCTATTG	24	NONE	coding	58
REVSIK1	4/7/98	CTCGAGAAGGGAATAAAAGATAATA	25	NONE	complement	57

Figure B1. Listing of primers used in this work.

F108



T12H1



T6H22

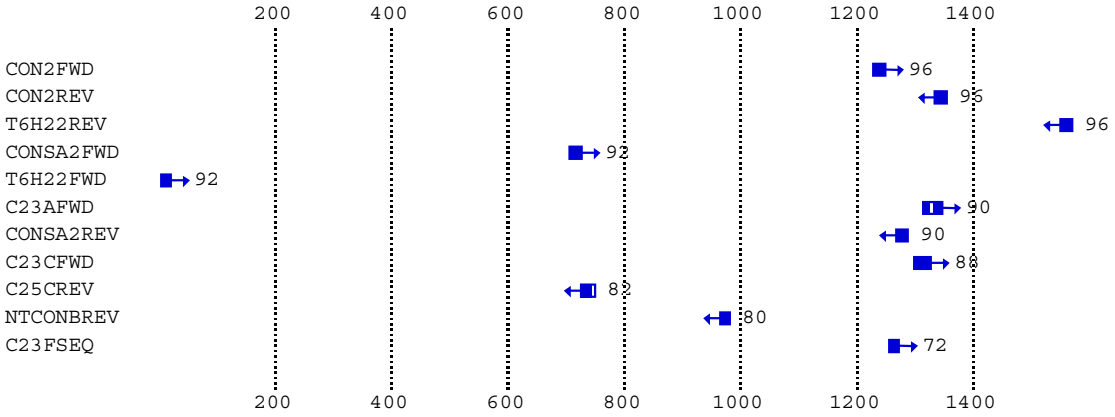


Figure B2. Alignment of PCR primers with cloned Arabidopsis genes. PCR primers were synthesized for cloning and sequencing of cDNA sequences from Arabidopsis. The primers are shown above aligned with the final sequences. Only primers specific for a single target are shown. Scores reflect the homology of primer to target sequence. Primers contain approximately twenty base pairs of perfect 3' homology to the target sequence, though some primers contain 5' restriction sites to facilitate cloning. Specific sequence of each primer is in Figure B1