http://www.flychip.org.uk/spike_pcr.php

Amplification of *Arabidopsis* spike controls

Outline

The spike controls were amplified by PCR from double stranded cDNA clones and by PCR from *Arabidopsis columbia* genomic DNA. The double stranded cDNA clones were obtained from:

- Arabidopsis Biological Resource Centre (Aims) (http://aims.cps.msu.edu/)
- Incyte Genomics (Incyte) (http://www.incyte.com/)
- Functional Genomics of Plant Stress Tolerance (Arizona) (http://www.stress-genomics.org/)

PCR amplification from double stranded cDNA clones

The double stranded cDNA clone inserts were amplified using a 1 in 100 dilution of a Qiagen purified plasmid midi prep. M13 forward and reverse primers were used for all Incyte, Aims and Arizona clones.

PCR primers:

Clone	CloneID	Vector	Antibiotic	5' primer	3' primer	PCR amplicon (kb)
Arizona4	M90509	pSK+	Ampicillin	tgtaaaacgacggccagt	caggaaacagctatgac	1.0
Arizona6	U74610	pSK+	Ampicillin	tgtaaaacgacggccagt	caggaaacagctatgac	1.1
Incyte4	ATU18126	pSTBlue-1	Ampicillin	tgtaaaacgacggccagt	caggaaacagctatgac	0.6
Incyte5	L22585	pSTBlue-1	Ampicillin	tgtaaaacgacggccagt	caggaaacagctatgac	1.0
AIMS 1	AB007987	pZL1	Ampicillin	tgtaaaacgacggccagt	caggaaacagctatgac	1.3
AIMS 4	AF117335	pZL1	Ampicillin	tgtaaaacgacggccagt	caggaaacagctatgac	1.7
AIMS 5	AF168390	pZL1	Ampicillin	tgtaaaacgacggccagt	caggaaacagctatgac	1.5
AIMS 9	AF372915	pZL1	Ampicillin	tgtaaaacgacggccagt	caggaaacagctatgac	1.3
AIMS 10	Y18469	pZL1	Ampicillin	tgtaaaacgacggccagt	caggaaacagctatgac	1.6
AIMS 11	Z49777	pZL1	Ampicillin	tgtaaaacgacggccagt	caggaaacagctatgac	1.2
AIMS 19	X644464	pZL1	Ampicillin	tgtaaaacgacggccagt	caggaaacagctatgac	1.0

PCR reaction mix:

- 10 µl Sigma PCR Buffer (or ABgene Thermostart standard buffer)
- 6 µl 25 mM Mg
- 2 µl 10mM dNTPs
- 2 µl 25 pmol / µl primers
- 78 µl MilliQ water
- 1 µl Sigma Taq polymerase (or ABgene Thermostart DNA polymerase)
- 1 µl DNA template

PCR cycle:

All PCR reactions were performed in 0.2 ml microfuge tubes with a Dyad thermal cycler with the following PCR cycle.

1. 95 °C for 15 minutes
2. 94 °C for 30 seconds
3. 60 °C for 30 seconds
4. 72 °C for 4 minutes
5. Repeat steps 2 to 4, 34 times
6. 72 °C for 10 minutes
7. 4 °C cold storage before unloading

PCR products were purified using Qiagen QIA quick columns or Millipore Multiscreen-PCR plates and checked by both agarose gel electrophoresis and the Nanodrop. The DNA concentration was then adjusted to make a final stock concentration for printing.

PCR amplification from *Arabidopsis columbia* Genomic DNA

PCR primers:

Clone	CloneID	Vector	Antibiotic	5' primer	3' primer	PCR amplicon (b)
Weed 1	O82258	pCR2.1-TOPO	Ampicillin	taaagtggaacctccgatgc	gaagagctcatcgccgatac	514
Weed 3	Q9LJQ4	-	Ampicillin	ttcttcacaactcgtcaattcaa	gcaaactgatgaccaggaaga	402
Weed 4	Q9XIB8	pCR2.1-TOPO	Ampicillin	aagacgaggcgagatcttca	tgttcctttcagagtgcaaatg	396
Weed 6	O04600	pCR2.1-TOPO	Ampicillin	ttgagtaccaacggtttcagc	tatcatcggtttgcctttgc	370
Weed 7	Q9LZJ2	pCR2.1-TOPO	Ampicillin	tcatgtgaacatacaacgcaat	ggtctattgggggtggaatc	404
Weed 8	Q9LVF8	pCR2.1-TOPO	Ampicillin	tcaacctatcattcctcccatt	gcctattgaggatttgttgctt	394
Weed 9	O49366	pCR2.1-TOPO	Ampicillin	agcttgagaacataggccaca	tggcatcggttgtctctgta	343
Weed 10	O81842	pCR2.1-TOPO	Ampicillin	agcatccaaaatccaaccaa	ttcgattccgcagattatcc	361
Weed 13	Q9LU32	pCR2.1-TOPO	Ampicillin	tccaatatgatttggttgtgga	tgtatgcttgcactcgatga	330
Weed 14	O04513	pCR2.1-TOPO	Ampicillin	agggcatttggtttcatggt	atagcatgctcgatgtgcaa	306

PCR reaction mix:

- 10 µl Stratagene Yield Ace reaction buffer (or ABgene Thermostart standard buffer)
- 2 µl 10mM dNTPs
- 2 µl 25 pmol / µl primers (spike specific 5'+3')

- 84 µl MilliQ water
- 1 µl Stratagene Yield Ace polymerase (or ABgene Thermostart DNA polymerase)
- 1 µl DNA template

PCR cycle:

All PCR reactions were performed in 0.2 ml microfuge tubes with a Dyad thermal cycler with the following PCR cycle.

1. 95 °C for 15 minutes
2. 95 °C for 45 seconds
3. 60 °C for 40 seconds
4. 72 °C for 1 minute
5. Repeat steps 2 to 4, 34 times
6. 72 °C for 10 minutes
7. 4 °C cold storage before unloading

PCR products were purified using Qiagen QIA quick columns or Millipore Multiscreen-PCR plates and checked by both agarose gel electrophoresis and the Nanodrop. The DNA concentration was then adjusted to make a final stock concentration for printing.

R. Auburn (17-02-2006).