TAIL PCR to identify transgenes in Soybean

Modified from "High-Throughput TAIL-PCR as a Tool to Identify DNA Flanking Insertions" By Tatjana Singer, Ellen Burke

Thermal asymmetric interlaced polymerase chain reaction (TAIL-PCR) is a fast and efficient method to amplify unknown sequences adjacent to known insertion sites in *Arabidopsis*. Nested, insertion-specific primers are used together with arbitrary degenerate primers (AD primers), which are designed to differ in their annealing temperatures. Alternating cycles of high and low annealing temperature yield specific products bordered by an insertion-specific primer on one side and an AD primer on the other. Further specifity is obtained through subsequent rounds of TAIL-PCR, using nested insertion-specific primers. The increasing availability of whole genome sequences renders TAIL-PCR an attractive tool to easily identify insertion sites in large genome tagging populations through the direct sequencing of TAIL-PCR products. For large-scale functional genomics approaches, it is desirable to obtain flanking sequences for each individual in the population in a fast and cost-effective manner. In this chapter, we describe a TAIL-PCR method amenable for high-throughput production (HT-TAIL-PCR) in *Arabidopsis* Based on this protocol, HT-TAIL-PCR may be easily adapted for other organisms.

1. Prepare oligos
    a. Prepare 100µM stock solutions for LB specific primers in TE buffer
    b. Prepare 200µM stock solutions for AD primers in TE buffer
    c. Store @ -20^{o}C

Primer	Tm	Sequence
AD1	45.6	NGTCGASWGANAWGAA
AD2	49.4	TGWGNAGSANCASAGA
AD3	44.2	AGWGNAGWANCAWAGG
AD4	45.2	STTGNTASTNCTNTGC
AD5	43.7	NTCGASTWTSGWGTT
AD6	44.6	WGTGNAGWANCANAGA
LB3	58.8	GATGGGTTTTTATGATTAGAGTCCCGCAATTATAC
LB2	61.9	TTCTTAAGATTGAATCCTGTTGCCGGTCTTGC
LB1	63.5	TCACCGAAATCTGATGACCCCTAGAGTCAAGC

2. Prepare AD-pool primer concentrations and aliquot into 1.5mL Eppendorf tubes

Primer	µL	[conc]
AD1	30	12µM
AD2	30	12µM
AD3	30	12µM
AD6	40	16µM
H2O	370	
Total (µL)	500	

3. Prepare transgene specific primers

Primer	µL	H2O(µL)	[conc]
LB3	10	90	10µM
LB2	10	90	10µM
LB1	10	90	10µM

4. Prepare 10mM stocks of dNTPs
5. Set-up single reaction for primary TAIL PCR using WT and transgenic DNA template

1x Reaction vol.	Reagents/Stock	[Final]
5.0µL	dH_2O	
1.0µL	10x Qiagen PCR Buffer	1x
0.2µL	10mM dNTPs	0.2mM
0.2µL	10µM LB1 primer	0.2µM
2.5µL	4xAD-pool	1x (3-4µM)
0.1µL	5U/µL HotStart Taq	0.5 U
1.0µL	DNA template	
10.0µL	**Total vol.**	

6. Carry-out first-round PCR according to program below

Steps	Temp		Time
1	94°C	for	15 min
2	94°C	for	30 sec
3	62°C	for	1 min
4	72°C	for	2:30 min
5	**Five cycles of steps 3-5**		
6	94°C	for	30 sec
7	25°C	for	3 min (50% ramp) 0.4°C/min
8	72°C	for	2:30 min (32% ramp) 0.3°C/min
9	**Two cycles of steps 7-9**		

10	94°C	for	10 sec
11	68°C	for	1 min
12	72°C	for	2:30 min
13	94°C	for	10 sec
14	68°C	for	1 min
15	72°C	for	2:30 min
16	94°C	for	10 sec
17	44°C	for	1 min
18	72°C	for	2:30 min
19	**15 cycles of steps 11-19**		
20	72°C	for	5 min
21	10°C	soak	

7. Prior to secondary TAIL PCR dilute primary TAIL-PCR 1:100 and use 1µL of that dilution as template for secondary TAIL-PCR.
8. Set-up single reaction for secondary TAIL PCR

1x Reaction vol.	**Reagents/Stock**	**[Final]**
6.0µL	dH_2O	
1.0µL	10x Qiagen PCR Buffer	1x
0.2µL	10mM dNTPs	0.2mM
0.2µL	10µM LB2primer	0.2µM
1.5µL	4xAD-pool	0.6x (1.8-2.4µM)
0.1µL	5U/µL HotStart Taq	0.5 U
1.0µL	DNA template	1:100 diluted first TAIL
10.0µL	**Total vol.**	

9. Carry-out first-round PCR according to program below

Steps	**Temp**		**Time**
1	94°C	for	15 min
2	94°C	for	10 sec
3	64°C	for	1 min
4	72°C	for	2:30 min
5	**Five cycles of steps 2-4**		
6	94°C	for	10 sec
7	64°C	for	1 min
8	72°C	for	2:30 min
9	94°C	for	10 sec
10	64°C	for	1 min
11	72°C	for	2:30 min
12	94°C	for	10 sec

13	44°C	for	1 min
14	72°C	for	2:30 min
15	**15 cycles of steps 6-9**		
16	94°C	for	10 sec
17	44°C	for	1 min
18	72°C	for	3 min
19	**Five cycles of steps 16-18**		
20	72°C	for	5 min
21	10°C	soak	

10. Prior to tertiary TAIL PCR dilute secondary TAIL-PCR 1:50 and use 1µL of that dilution as template for tertiary TAIL-PCR.
11. Set-up single reaction for tertiary TAIL PCR

1x Reaction vol.	**Reagents/Stock**	**[Final]**
11.0µL	dH_2O	
2.0µL	10x Qiagen PCR Buffer	1x
0.4µL	10mM dNTPs	0.2mM
0.4µL	10µM LB3 primer	0.2µM
5µL	4xAD-pool	1x (3-4µM)
0.2µL	5U/µL HotStart Taq	0.5 U
1.0µL	DNA template	1:50 diluted secondary TAIL
20.0µL	**Total vol.**	

12. Carry-out first-round PCR according to program below

Steps	**Temp**		**Time**
1	94°C	for	15 min
2	94°C	for	10 sec
3	44°C	for	1 min
4	72°C	for	2 min
5	**20 cycles of steps 2-4**		
6	72°C	for	5 min
7	10°C	soak	

13. Run 20µL tertiary TAIL-PCR samples on 1% agarose gel and gel elute fragments
14. Clone into pGem T easy and sequence clones