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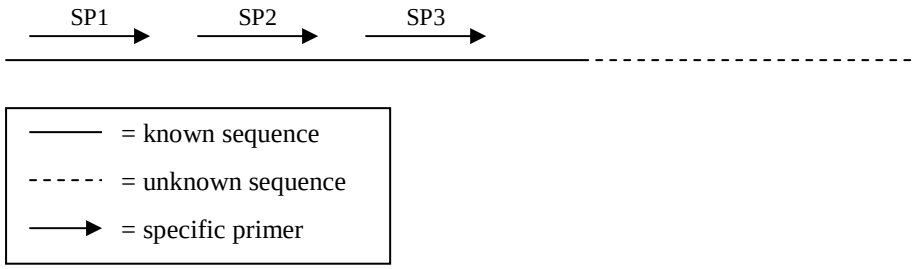
TAIL (THERMAL ASYMMETRIC INTERLACED) PCR

for obtaining DNA sequence from flanking regions

Adapted from *Liu, Y.-G. & Whittier, R. F.* (1995)
Thermal Asymmetric Interlaced PCR: Automatable amplification and sequencing of insert end fragments from P1 and YAC clones for chromosome walking. Genomics 25: 674-681.

1. Primer Design

Design three adjacent primers from your sequence (priming outwards from the sequence). These become the specific (SP) primers. SP1 is the innermost primer, SP2 is the middle primer and SP3 is nearest the end of the known sequence, and are used in the 1°, 2° and 3° PCR reactions respectively.



The SP primers need to be ~20-mers and have a T_m of 60-62°C.

Use the following arbitrary degenerate (AD) primers.

Name	Sequence (5'-3')	Length	T_m	Degeneracy
TAIL-AD1	NGTCGASWGANAWGAA	16-mer	46°C	128×
TAIL-AD2	GTNCGASWCANAWGTT	16-mer	46°C	128×
TAIL-AD3	WGTGNAGWANCANAGA	16-mer	45°C	256×

S = C or G
W = A or T

Choose one AD primer (e.g. AD1) and use it for the 1°, 2° and 3° reactions. If this primer fails try AD2, and so on. More bands can be expected when using AD3 (because of the higher degeneracy).

2. Primary (1°) PCR Reaction

Set up the 1° PCR reaction (20µL volume).

	×1	
Milli-Q H_2O	0.3	
Betaine (5M)	4.0	(1M)
dNTPs (2mM)	2.5	(0.25mM)
10× PCR Buffer	2.0	(1×)
$MgCl_2$ (25mM)	1.0	(1.25mM)
AD Primer* (10pmol/µL)	8.0	(4µM)
SP1 Primer (10pmol/µL)	1.0	(0.5µM)
Genomic DNA	1.0	
Taq Polymerase (5U/µL)	0.2	(1U)
TOTAL	**20µL**	

* Use AD primer AD1, AD2 or AD3.

Use the following 1° PCR program:

Step			
1	94°C	2 min	×1
2	94°C	30 sec	
3	60°C	1 min	×5
4	72°C	2 min	
5	94°C	30 sec	
6	25°C	1 sec	×1
7*	72°C	2 min	
8	94°C	30 sec	
9	44°C	1 min	×10
10	72°C	2 min	
11	94°C	30 sec	
12	60°C	1 min	
13	72°C	2 min	
14	94°C	30 sec	
15	60°C	1 min	×15 supercycles
16	72°C	2 min	
17	94°C	30 sec	
18	44°C	1 min	
19	72°C	2 min	
20	72°C	5 min	×1
21	4°C	HOLD	

* Ramp from 25°C to 72°C over 3 min (Δ47°C = +0.25°C/s). All other ramping speeds are limited to 1°C/s.

3. Secondary (2°) PCR Reaction

Dilute the 1° PCR reaction 100× with MilliQ-H_2O. Set up the 2° PCR reaction (20µL volume).

	×1	
Milli-Q H_2O	2.3	
Betaine (5M)	4.0	(1M)
dNTPs (2mM)	2.5	(0.25mM)
10× PCR Buffer	2.0	(1×)
$MgCl_2$ (25mM)	1.0	(1.25mM)
AD Primer* (10pmol/µL)	6.0	(3µM)
SP2 Primer (10pmol/µL)	1.0	(0.5µM)
1° PCR Product (diluted 100×)	1.0	
Taq Polymerase (5U/µL)	0.2	(1U)
TOTAL	**20µL**	

* Use the same AD primer as in the 1° reaction.

Use the following 2° PCR program:

Step			
1	94°C	2 min	×1
2	94°C	30 sec	×15 supercycles
3	60°C	1 min	
4	72°C	2 min	
5	94°C	30 sec	
6	60°C	1 min	
7	72°C	2 min	
8	94°C	30 sec	
9	44°C	1 min	
10	72°C	2 min	
11	72°C	5 min	×1
12	4°C	HOLD	

Ramping speed should be limited to 1°C/s.

4. Tertiary (3°) PCR Reaction

Dilute the 2° PCR reaction 100× with MilliQ-H_2O. Set up the 3° PCR reaction (20µL volume).

	×1	
Milli-Q H_2O	4.3	
Betaine (5M)	4.0	(1M)
dNTPs (2mM)	2.5	(0.25mM)
10× PCR Buffer	2.0	(1×)
$MgCl_2$ (25mM)	1.0	(1.25mM)
AD Primer* (10pmol/µL)	4.0	(2µM)
SP3 Primer (10pmol/µL)	1.0	(0.5µM)
2° PCR Product (diluted 100×)	1.0	
Taq Polymerase (5U/µL)	0.2	(1U)
TOTAL	**20µL**	

* Use the same AD primer as in the 1° and 2° reaction.

Use the same PCR program as for the 2° PCR reaction.

5. Agarose Electrophoresis of Products

Run the 1°, 2° and 3° products in adjacent lanes on a 1.5% agarose gel (5µL each of the 1° and 2° reactions and all 20µL of the 3° reaction). Look for the characteristic shift in size of the desired product. The change in product size should correspond to the length between SP primers.

If the 3° reaction contains just one product a PCR cleanup can be performed and the product sequenced directly (using the SP3 primer and/or the AD primer). More often there are multiple 3° products, in which case the desired band can be excised from agarose and gel extracted, before being sequenced directly.

If direct sequencing is problematic the product can be cloned and sequenced.