

TAIL PCR (THERMAL ASYMMETRIC INTERLACED PCR)

TAIL-PCR is a powerful tool for the recovery of DNA fragments adjacent to known sequences. Basically, TAIL-PCR utilises three nested primers in consecutive reactions together with an arbitrary degenerate (AD) primer having a lower T_m (melting temperature) so that the relative amplification frequencies of specific and non-specific products can be thermally controlled.

Primary reaction.

In the primary reaction, one low stringency PCR cycle is conducted to create one or more annealing sites for the AD primer in the targeted sequence. Specific products are then amplified over non-specific ones by interspersions of two high-stringency PCR cycles with one reduced-stringency PCR cycle.

1. Set up 4 reactions as follows (one with each AD primer):

2 μ l	10 X PCR buffer
1.2 μ l	25 mM $MgCl_2$
0.2 μ l	10 mM dNTP's
0.2 μ l	100 $ng\mu l^{-1}$ specific primer 1 (furthest away from AD) (0.15 μ M final)
2 μ l	20 μ M AD primer (2 μ M final)
0.2 μ l	Taq DNA polymerase
0.4 μ l	DMSO
1 μ l	DNA (1-20 $ng\mu l^{-1}$)
12.8 μ l	H_2O

2. Cycle as follows: (if doesn't work, try dropping annealing step to 60°C)

92°C (3'), 95°C (1')	X 1
94°C (30s), 65°C (1'), 72°C (2')	X 5
94°C (30s), 25°C (2'), ramping to 72°C over 2', 72°C (2')	X 1
94°C (30s), 65°C (1'), 72°C (2')	
94°C (30s), 65°C (1'), 72°C (2')	
94°C (30s), 44°C (1'), 72°C (2')	X 15
72°C (5')	X1
cool to 4°C	

There is no need to run out this primary reaction. It should contain a medium yield of specific products, a high yield of non-targeted products, and a low yield of non-specific products. The nested primers used in the secondary and tertiary reactions result in very low yields of non-specific products, very high yields of specific products and no amplification of non-targeted products.

Secondary reaction

For the secondary reaction, a 1/40 dilution of the primary PCR product is used as template, and the specific primer is the middle one of the three specific primers.

1. Set up reaction as follows:

2.5 μ l	10 X PCR buffer
1.5 μ l	25 mM $MgCl_2$

0.25 μ l	10 mM dNTP's
0.3 μ l	100 ng μ l ⁻¹ specific primer 2 (middle nested) (0.2 μ M final)
2.5 μ l	20 μ M AD primer (2 μ M final)
0.2 μ l	Taq DNA polymerase
0.5 μ l	DMSO
1 μ l	DNA (1/40 dilution of primary PCR products)
16.25 μ l	H ₂ O

2. Cycle as follows: (if doesn't work, try dropping annealing step to 60°C)

94°C (30s), 65°C (1'), 72°C (2')	
94°C (30s), 65°C (1'), 72°C (2')	
94°C (30s), 45°C (1'), 72°C (2')	X 12
72°C (5')	X 1
cool to 4°C	

Tertiary reaction

For the tertiary reaction, the SAME template (i.e primary PCR product) is used but this time in a 1/10 dilution. I usually simply add 4 X of the 1/40 dilution used for the secondary reaction. This removes the possibility of getting false positives. The specific primer used is the primer nearest the unknown sequence.

1. Set up reaction as follows:

5 μ l	10 X PCR buffer
3 μ l	25 mM MgCl ₂
0.5 μ l	10 mM dNTP's
0.6 μ l	100ng μ l ⁻¹ specific primer 3 (closest to AD) (0.2 μ M final)
5 μ l	20 μ M any one AD primer (2 μ M final)
0.4 μ l	Taq DNA polymerase
1 μ l	DMSO
4 μ l	DNA (1/40 dilution of primary PCR products)
31 μ l	H ₂ O

2. Cycle as follows:

94°C (30s), 45°C (1'), 72°C (2')	X 20
72°C (5')	X 1
cool to 4°C	

Agarose gel analysis

The secondary and tertiary products are run in adjacent lanes on a 1.2% agarose gel. The specificity of the products is confirmed by the expected size change between the secondary and tertiary products.