

Standard DNA amplification assay

Pipette the following into a PCR tube, mix and make up to a final volume of 50 μ L. We recommend dispensing all reagents on ice, adding the enzyme last. It is important to vortex all buffers and MgCl₂ solutions before use to remove any gradients that may result from repeated freeze/thaw steps.

If you do have already your own PCR-Protocol established, please use your existing pipetting scheme and Thermocycler protocol.

fon:

+49 (0)7357 - 91 63 77

fax:

+49 (0)7357 - 91 63 78

eMail:

info@genaxxon.com

internet:

www.genaxxon.com

Pipetting scheme

Components	Quantities
Template DNA	x μ L DNA (50 ng plasmid DNA (or 300 ng - 500 ng genomic DNA)
2 mM dNTPs	5.0 μ L
10X amplification buffer	5.0 μ L
25 mM MgCl ₂	5.0 μ L
primer 1:	1 μ L of 15 μ M solution (15 pmol/ μ L)
primer 2:	1 μ L of 15 μ M solution (15 pmol/ μ L)
LongMax Polymerase mixture	1 μ L (5 units)
sterile, RNase and DNase free water	up to 50 μ L

Drops should be collected by centrifugation and 50 μ L of mineral oil (m3024.0810) should be layered upon the reaction mixture.

PCR programme

Cycling Profile - DNA Template	
94°C for 5 minutes	step 1
94°C for 35 sec.	1 - 35 cycle(s)
annealing temp. for 35 sec.	1 - 35 cycle(s)
extension at 68°C **, 1 min. per 1 kb to be amplified.	1 - 35 cycle(s)
94°C for 35 sec.	
68°C for 7 minutes	last step
cool down to 4°C	

Cycling Profile - Control DNA ***	
94°C for 5 minutes	step 1
94°C for 35 sec.	1 - 35 cycle(s)
60°C for 30 sec.	1 - 35 cycle(s)
extension at 68°C, 7 min	1 - 35 cycle(s)
94°C for 35 sec.	
68°C for 7 minutes	last step
cool down to 4°C	

* Cycling times are proposals and have to be adjusted to the special needs.

** The longer the template to be amplified, the longer the amplification time.

*** The amplified control DNA is expected to show a size of about 14 kb on a 0.7% agarose gel.

Note: For every template/primer pair the optimal reaction conditions have to be evaluated empirically, changing the primer/template ratio, the ionic strength (with MgCl₂) and the cycle parameters (time and temperatures).

Trouble shooting:

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Check if annealing temperature has been calculated correctly. The optimal annealing temperature of primers lies about 5°C below the T_M of the oligo.

TM calculation: For oligos up to 15 bases: $TM = 4x(G+C) + 2x(A+T)$
 For larger oligos: $TM = 81.5 + 16.6([\log_{10} J^*]) + 0.4(\%G+C) - (600/n) - 0.63(\%FA)$

A = Adenosine, C = Cytosine, G = Guanine, T = Thymidine, FA = Formamide, J* = concentration of monovalent cations, n = number of bases; T_M = calculated melting temperature,

Bands smear over the Gel

Increase annealing temperature

Add up to 5% DMSO to the Reaction mixture

Reduce the amount of template DNA

Check if the primers bind more than once on the template DNA. Perform control reactions with only one of the primers.

Low Yields

Increase annealing temperature

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