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Pwo-DNA Polymerase

Deoxynucleoside-triphosphate: DNA deoxynucleotidyl-transferase (DNA-directed); EC 2.7.7.7

Suggested PCR Protocol

Pipette the following into a PCR tube, mix and make up to a final volume of 50µL. 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.

Components	Quantities
Template DNA	1ng - 10ng plasmid DNA or 5ng - 500ng genomic DNA
Nucleotides	1µL (10mM) each dNTP
10X amplification buffer	5µL
primer 1:	4-7µL of 3 µM solution (10 - 20 pmol absolute)
primer 2:	4-7µL of 3 µM solution (10 - 20 pmol absolute)
sterile, bidestilled water	up to 50µL
Pwo-Polymerase	0.3 - 0.8µL (0.5 - 2.0 units)

The following cycling protocol has to be seen as suggestion. Cycle numbers and cycle times have to be adjusted.

Cycling Profile *	
94°C for 2-4 minutes	step 1(denaturing of DNA)
	(cycling part)
94°C for 30-60 sec.	1 - 30 cycle(s)
annealing temp. for 30-60 sec.	1 - 30 cycle(s)
extension (72°C) for 60-240 sec. **	1 - 30 cycle(s)
94°C for 30 sec.	final step (filling up step)
72°C for 5-10 minutes	
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

After running the amplification reaction, 5µL of each sample should be analyzed in a 1% agarose gel containing 0.5µg/mL of ethidium bromide.

Note: For every template/primer pair the optimal reaction conditions have to be evaluated empirically, chancing the primer/template ratio, the ionic strength (with MgSO4) and the cycle parameters (time and temperatures).

Using PCR-product for T/A-cloning

As ReproFast (M3003), ReproHot (M3012), Pfu (M3004) and Pwo (M3002) are proof-reading DNA-polymerases, they will generate mainly blund-end products that are not applicable for T/A-cloning. To use PCR-products produced by either of the above mentioned enzymes the following additional step has to be performed.

Addition of "A" to a blunt-end DNA fragment

1 - 2µL of DNA fragment (PCR product isolated, purified and resuspended in water or TE buffer)
1µL of 10X Taq buffer
1µL of 25mM $MgCl_2$
x µL of ATP solution (to a final conc. of 0.2mM)
1µL of *Taq* Polymerase (5 Units /µL)

add nuclease free water to a final volume of 10µL
Incubate at 70°C for 10 to 30 minutes

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Trouble shooting:

No PCR® Product

Check if annealing temperature has been calculated correctly. The optimal annealing temperature of primers lies about 5°C below the TM 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([log10[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; TM = calculated melting temperature,

Remark: If primers with mismatches are used the *Pwo* Polymerase **must** be added immediately before the start of the PCR®. Do **not** let the reaction mix stand at room temperature since primers will be degraded by the *Pwo* Polymerase.

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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