2x PCR mix

Cat No	Pack size	conc
PM0500	5 ml	2x
PM2500	5x5ml	2x

PCR mixes (2x) contain all components for PCR, only template DNA and primers should be added, volume adjusted by water if necessary and PCR can be started.

Description: 2x PCR mix is optimized mixture contain of Taq enzyme, reaction buffer, dNTP and enhancer as 2-fold concentration. 2x PCR mix is designed to allow the user for quick and easy preparation of reaction mixture. The PCR mix may be amplification PCR products to 3 kb and the products can be directly cloning into T-vector.

storage conditions: long time at -20˚C
short time at 4 ˚C

Template

2 x PCR mix is suitable for amplifying targets up to 3 kb from the following templates:

Genomic DNA: 10–200 ng
Plasmid DNA : 1–5 ng
cDNA : ~100 ng starting total RNA

Primers

Use 0.3 μM per primer as a general starting point. For larger amounts of template (e.g., 200 ng genomic DNA), increasing the concentration up to 0.5 μM per primer may improve yield.

Annealing Temperature

The annealing temperature is slightly higher than with typical PCR. The optimal annealing temperature should be ~2˚C lower than the Tm of the primers used. A range of 58–68˚C is recommended.

Extension Time: As little as 30 seconds per kb is suitable for most targets. Use up to 60 seconds per kb for maximum yield.

PCR Protocol:

1.haw the 2x PCR mix at room temperature. Vortex the 2x PCR mix and then spin it briefly in a micro centrifuge to collect the material in the bottom of the tube.
2. Prepare one of the following reaction mixes on ice:

Component	Volume
2x PCR mix	12.5 ul
Primer1 (20 pmol)	1-2 ul
Primer2 (20 pmol)	1-2 ul
template	1-10 ul
ddH_2O	Up to 25 ul
Total	25 ul

3. If necessary you can scale up your volume

1. Program the thermal cycler as follows:

Step	Temperature	Time	Cycle
Initial denaturation	94-96°C	0.5-2mins	1
Denaturation	94-96°C	0.2-2mins	15-30
Annealing	50-68	0.2-2mins	
Extension	68-75	1min/1kb	
Final extension	68-75	1-10mins	1

Step

After cycling, maintain the reaction at 4˚C. Samples can be stored at –20˚C until use.

Analyze products using standard agarose gel electrophoresis.

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