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2X PCR Master Mix

Description

Components Provided:

Product	
1	2X Master PCR mix
2	MolecularÂ BiologyÂ Grade Water

2 X Master PCR Mix:Â Comprises of proprietary Taq polymerase 2X Master PCR mix. It is ready-to-use mix for efficient amplification of DNA templates. PCR mix is made in nuclease-free water with optimal concentrations of Taq DNA Polymerase, dNTPs, MgCl2 and stabilizers.

PCR Protocol :Â Â

1. Add 25 Âµl 2X Master PCR mix into a PCR
2. Add 20 Âµl of nuclease free
3. Add 2 Âµl of each primer from a working stock of 10 picomole/Î¼l of each Final concentration of each primer should be 0.4 Î¼M.
4. Add 1 Âµl of your template DNA (10-20 nano grams)

HelpfulÂ HintsÂ aboutÂ primersÂ concentration:

a).Forward and Reverse primers are generally 20â€“40 nucleotides in length with GC content of 45 to 65%. The final concentration of each primer in a reaction varies from 0.05â€“1 Î¼M. If you are not sure of the right primers concentrations for a PCR reaction, We recommend with a final concentration for 0.4 ÂµM of each primer.

- *IfÂ youÂ haveÂ aÂ primerÂ suspendedÂ inÂ sterileÂ water at a concentration of 100 picomoles/Î¼l, dilute it 10 fold to final concentration of 10 picomoles/Î¼l (10 Î¼M) for each working Add 2 Î¼l of each primer to 50 Î¼l of PCR volume.*

- *IfÂ youÂ addÂ 2Â ÂµlÂ ofÂ aÂ 10Â Î¼MÂ stockÂ into a 50 Î¼l reaction, then the concentration of your each primer in the 50 Î¼l reaction would be 2/50th of 10 Î¼M, or 4 Î¼M (400 nM).*

- *IfÂ yourÂ PCRÂ primerÂ isÂ suppliedÂ asÂ 40Â nmolÂ per tube dried, suspend it in 400 Î¼l of sterile water, which will be equivalent to 100 picomoles/Î¼l (100 Î¼M)*

5. Vortex samples for few seconds or mix the contents by pipetting up and down with a
6. If PCR machine does not have a heated lid, add two drops of mineral oil into each
7. Use following conditions for a thermocycler (if your fragment size is about 1 KB):

DenaturingÂ Step:Â 95 Â°C for 5 minutesÂ **AmplificationÂ Steps**Â (**35Â steps**)

1. 95 Â°C for 1 minute (Denaturing step)

2. 60 Â°C for 1 minute (annealing step)
3. 72 Â°C for 1 minute (extension step)

Final extension Step:

1. 70 Â°C for 5 minutes (final extension)

Helpful Hints about extension time:Â An extension time of 1 minute per Kb is recommended. Extension time from of 20 seconds ~ (300bp) to 5 minutes ~ (5 Kb) can be used depending upon the size of your fragment.

TroubleshootingÂ Â

Problem	**Possible Cause**	**Suggest**
	Not Sufficient Template	â€¢Â Â
	Target sequence missing in the template	â€¢Â Â
		â€¢Â Â
Â	Incorrect Primers design	complen
Â		â€¢Â Â
Â	Primers Concentration too low	â€¢Â Â
Â	Primers quality	â€¢Â Â and stor
Â	Insufficient number of PCR cycles	â€¢Â Â cycles).
Â		â€¢Â Â
Â		â€¢Â Â
Â	**Â**	and time
No or low PCR products	**Â**	â€¢Â Â annealin
	Â	
	Denaturation, annealing and extension are not optimal	â€¢Â Â length. C
		â€¢Â Â of long t

	â€¢Â Â Â Â Â Purify templates by alcohol precipitation or by	
Inhibitor (s) present in the reaction mix	using clean up kit.	
	â€¢Â Â Â Â Â Optimize the sample volume.	
Incorrect thermocycler program	â€¢Â Â Â Â Â Verify program for temperatures and times.	
		â€¢Â Â
	Non-optimal template concentration	â€¢Â Â
		â€¢Â Â
Multiple band	Contaminated template	
/non-specificÂ PCR Products		â€¢Â Â
Â		â€¢Â Â
Â	Poor primer design	â€¢Â Â
Â		â€¢Â Â
Â		â€¢Â Â
Â		â€¢Â Â
Â	Too high concentration of	0.05-1 Â
Â	primers	â€¢Â Â
Â	GC content in the primers in	â€¢Â Â
Â	no appropriate	â€¢Â Â
Â		â€¢Â Â
Â	Contaminated reaction	pipette ti
Smear PCR products	mixture components	â€¢Â Â
		â€¢Â Â
	Too low or incorrect annealing	intermitte
	temperature	â€¢Â Â

Premature replication	â€¢Â Â Â Â Â Change the primers. â€¢Â Â Â Â Â Use fresh non-degraded template DNA. â€¢Â Â Â Â Â Change PCR tubes and try different thermocycler.
Template degraded, primers degraded or both template and primers are contaminated	â€¢Â Â Â Â Â Use PCR grade sterile water. â€¢Â Â Â Â Â Check the quality of DNA template by electrophoresis. â€¢Â Â Â Â Â Check 260/280 ratio of DNA template.

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