TAQ DNA Polymeprase (5 units/Î¼l)

Description

Recommended PCR Condition

Template	15 â€“ 150 ng *
Forward primer	0.4 Î¼M â€“ 0.5 Î¼M
Reverse primer	0.4 Î¼M â€“ 0.5 Î¼M
10X Buffer	5 Î¼l
dNTPs	0.2 mM (each)
TAQ DNA Polymerase	0.25 Î¼l (1.25 U)
ddH2O	adjust to 50 Î¼l
Total Volume	**50 Î¼l**

2X Master Mix Prepration

10X Buffer	1 ml
dNTPâ€™s Mix (25mM) or (10mM)	100 Î¼l or 250 Î¼l
TAQ DNA Polymerase	50 Î¼l
PCR Grade Water	Adjust to 5 ml
Total Volume	**5 ml**

*** Optimal amount of DNA template depends on the source and quality of DNA. TAQ DNA Polymerase: 1.25 unit (0.25 Î¼l) per 50 Î¼l reaction is recommended. Please Use 2~2.5 unit in case target size is > 3kb.**

10X Buffer Includes : 15mM MgCl2

PCR Protocol :Â Â Â Â Â Â Â Â 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)

Helpful Hints about primers 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 recommendÂ Â Â Â Â Â Â Â Â Â Â

withÂ Â Â Â Â Â Â Â Â Â Â Â Â aÂ Â Â Â Â Â Â Â Â Â Â Â Â finalÂ Â Â Â Â Â Â Â Â Â Â Â Â concentrationÂ Â Â Â Â Â Â Â Â Â Â Â Â forÂ Â Â Â Â Â Â Â Â Â Â Â Â 0.4Â Â Â Â Â Â Â Â Â Â Â Â ÂµMÂ Â Â Â Â Â Â Â Â Â Â Â Â ofÂ Â Â Â Â Â Â Â Â Â Â Â Â eachÂ Â Â Â Â Â Â Â Â Â Â Â Â primer.Â **b).Â** If you have a primer suspended in sterile water at a concentration of 100 picomoles/Î¼l, dilute it 10 fold to final concentration of 10 picomoles/Î¼l (10 Î¼M) for each working primer. Add 2 Î¼l of each primer to 50 Î¼l of PCR volume.

- *If you add 2 Âµl of a 10 Î¼M stock into a 50 Î¼l reaction, then the concentration of your each prime in the 50 Î¼l reaction would be 2/50th of 10 Î¼M, or 4 Î¼M (400 nM).*
- *If your PCR primer is supplied as 40 nmol 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. 1. Use following conditions for a thermocycler (if your fragment size is about 1 KB):

   Denaturing Step:Â 95 Â°C for 5 minutesÂ **Amplification StepsÂ** (**35 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 3 minutes ~ (3 Kb) can be used depending upon the size of your fragment.

8. **Troubleshooting**

Problem	Possible Cause	Sugges

	Not Sufficient Template	â€¢Â Â
	Target sequence missing in the template	â€¢Â Â
	Incorrect Primers design	â€¢Â Â compler
	Primers Concentration too low	â€¢Â Â â€¢Â Â
	Primers quality	â€¢Â Â reconsti
	Insufficient number of PCR cycles	â€¢Â Â cycles). â€¢Â Â
No or low PCR products	Denaturation, annealing and extension are not optimal	â€¢Â Â â€¢Â Â and time â€¢Â Â annealir â€¢Â Â amplico â€¢Â Â amplific
	Inhibitor (s) present in the reaction mix	â€¢Â Â using cl â€¢Â Â
	Incorrect thermocycler program	â€¢Â Â

		â€¢Â Â
	Non-optimal template concentration	â€¢Â Â
	Contaminated template	â€¢Â Â
		â€¢Â Â
Multiple band		â€¢Â Â
/non-specificÂ PCR Products	Poor primer design	â€¢Â Â
		â€¢Â Â
	Too high concentration of	â€¢Â Â
	primers	â€¢Â Â ÂµM in
	GC content in the primers in	â€¢Â Â
	no appropriate	â€¢Â Â
	Contaminated reaction	â€¢Â Â
	mixture components	â€¢Â Â during s
	Too low or incorrect annealing	â€¢Â Â
	temperature	â€¢Â Â
	Premature replication	â€¢Â Â
Smear PCR products		â€¢Â Â
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	Template degraded, primers degraded or both template and primers are contaminated	â€¢Â Â
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