DNA Polymerases

Taq Polymerase

Description:

Taq DNA Polymerase is a thermostable DNA polymerase of thermophillic bacterium *Thermus aquaticus*. The enzyme catalyzes 5'-3' synthesis of DNA, has no detectable 3'-5' exonuclease (proof-reading) activity, but maintains the 5'-3' exonuclease activity. In addition, Taq DNA Polymerase exhibits deoxynucleotidyl transferase activity, resulting in addition of extra adenines at 3'-end of PCR products.

Features:

- Thermostable enzyme of approximately 94 kDa from *Thermus aquaticus*
- Ultra pure recombinant protein
- Replicates DNA at 74°C and exhibits a half-life of 40 minutes at 95°C
- Generates mostly 3' dA overhang PCR products which are suitable for TA cloning
- DNA sequencing and labeling
- Incorporates modified nucleotides (E.g.: biotin, deoxygenin,fluroscently-labelled nucleotides)
- Supplied with two buffers- 10X Hibuffer A with KCl and 10X Hibuffer S with $(NH4)_2SO4$ [$(NH4)_2SO4$ allows for PCR at wide range of Mg^{+2} concentrations and decrease in unspecific priming]

E.coli cells with a pol gene from *Thermus aquaticus*

Guidelines for PCR optimization using HiMedia's Taq Polymerase:

DNA Template

1. Use high quality, purified DNA templates
2. Approximately 104 copies are required to detect the amplification in 25-30 PCR cycles
3. Use higher DNA concentration when few PCR cycles are desired

Primers

1. Generally 20-30 bp in size
2. GC content between 40-60% ideally
3. Melting temperatures should be between 42-65°C
4. Final concentration to be used 0.1-0.5µM of each primer

Magnesium Concentration

1. Ideal for Taq Polymerase is 1.5-2.0mM
2. Optimum concentration depends on template, buffer and dNTPs
3. Higher than optimal concentration yields undesired products and if concentration is too low the concentration, no amplification products are detected

dNTPs

1. Typical concentration to be used is 200µM
2. Higher than optimal concentration of dNTPs yields higher yield but low fidelity

PCR grade water

1. It is advisable to use PCR or MB grade water for any kind of PCR assay . As this water is free of nucleases and free of nucleic acid contamination that may cause false-positive signals in PCR

Taq Polymerase

1. Typical concentration to be used is 0.5 to 2 units per 50µl of reaction

PCR reaction

1. Thaw all reaction components on ice
2. To PCR reaction, add Taq Polymerase at the end
3. Once the reaction is set, immediately transfer the tubes to pre-heated thermal cycler
4. Start the reaction with desired cycling conditions with annealing temperature set to 5°C difference of melting temperature between forward and reverse primers

Representative Data

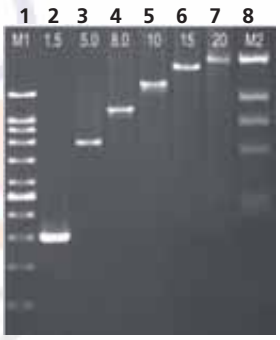

Fig. No 41 a : Figure representing amplicon sizes obtained using Taq DNA Polymerase (MBT060)

Lane	Amplicon Size	Taq DNA Polymerase Concentration	dNTP Concentration	Formamide Concentration
1	M1-1Kb Ladder			
2	1.5Kb	1.0U	0.2mM	NA
3	5.0Kb	1.25U	0.25mM	3%
4	8.0Kb	1.25U	0.25mM	3%
5	10.0Kb	1.25U	0.36mM	3%
6	15.0Kb	1.25U	0.36mM	3%
7	20.0Kb	1.25U	0.36mM	3%
8	M2-Lambda Hind III marker			