LApro *Taq* DNA Polymerase

Technical Manual No. TM0219 Version 01152007

I. DESCRIPTION

LApro *Taq* DNA Polymerase is a polymerase possessing 5'-3' DNA polymerase activity that is optimized to amplify longer GC-rich fragments and 3'-5' proof-reading activity. As one of the most thermostable DNA polymerase known with 3'-5' proofreading activity, the enzyme catalyze DNA synthesis at optimal temperature near 75°C with very low error rate (about ten times more accurate than standard *Taq* DNA Polymerase). The enzyme is optimized to achieve amplification of 17.5 kb products from human genomic DNA and 20 kb from λDNA. LApro *Taq* DNA Polymerase in long PCR* amplifications can reduce smearing and virtually eliminate unwanted background artifacts. LApro *Taq* DNA Polymerase generates A-ended PCR fragments that are required for TA cloning.

II. APPLICATIONS

LApro *Taq* DNA Polymerase can be used in most applications including the following:

- Up to 17.5 kb from human genomic DNA
- High fidelity amplification
- More stable than normal *Taq* DNA Polymerase
- TA cloning
- Site-directed mutagenesis
- Fragment analysis

III. CONTENTS

Components	E00013	E00014
LApro *Taq* DNA Polymerase	250 U	250 U
2× PCR Buffer	2X1.5 ml	2X1.5 ml
dNTP,10 mM (D0056)		50ul
Control template & primers		25 rnx

IV. ACTIVITY

LApro *Taq* DNA Polymerase shows very good fidelity and an average error rate around 2×10^{-6}.
LApro *Taq* DNA Polymerase has 5'-3' exonuclease activity and 3'-5' proofreading activity. It also has the extendase activity allowing TA cloning.

V. UNIT DEFINITION

One unit of enzyme incorporates 10 nmol of dNTP into acid-insoluble material in 30 minutes at 74°C.

VI. STORAGE BUFFER AND CONCENTRATION

Supplied in 5 units/µl in 50 mM Tris-HCl (pH8.1), 0.1 mM EDTA, 1 mM DTT, 0.1% (v/v) Nonidet P40, 0.1% (v/v) Tween 20, and 50% (v/v) glycerol.

VII. STORAGE

Store at -20°C. Stable for at least one year, if stored properly.

VIII. GENERAL PCR PROTOCOL USING LAPRO TAQ DNA POLYMERASE

This is a general PCR amplification protocol, optimization may be needed to get satisfactory results.

1. Set up 50 µl PCR reaction by adding the following reagents to a thin-walled PCR microcentrifuge tube or plate and mixing gently.

Reagent	Volume	Final Concentration
Water, PCR grade	20 µl	
2× Buffer	25 µl	1×
10 mM dNTP	1 µl	200 nM each
20 µM Forward primer	1 µl	400 nM
20 µM Reverse primer	1 µl	400 nM
Template DNA	1 µl	1-100 ng/reaction
5 U/µl LApro *Taq* DNA Polymerase	1 µl	0.1 U/µl
Total	50 µl	

Note: when use our positive control in complete system (E00014) just add 50ul ddH_2O to the control tube and fully dissolved (gently), then tip 2ul to the PCR microcentrifuge tube as template and primers. Pcr product length is 20kb.

2. Suggested PCR profile:
   - 3 Step PCR
     - Initial Denaturation: 94-96°C for 2 min.
     - 25-30 Cycles of Denaturation: 94-96°C for 30 sec.
     - Annealing: 50-60°C for 30 sec. (primer T_m - 5°C)
     - Extension: 72°C for 30 sec to 5 min. (1.5-2.5 kb/min)
     - Final Extension: 72°C for 7 min.
   - 2 Step PCR
     - Initial Denaturation: 94-96°C for 1 min.
     - 20-30 Cycles of Denaturation: 94-96°C for 20 sec.
     - Annealing & Extension: 68°C for Xmin. (1-2.5 kb/min)
     - Final Extension: 72°C for 7-15 min.
3. Examples of Using LApro *Taq* DNA Polymerase

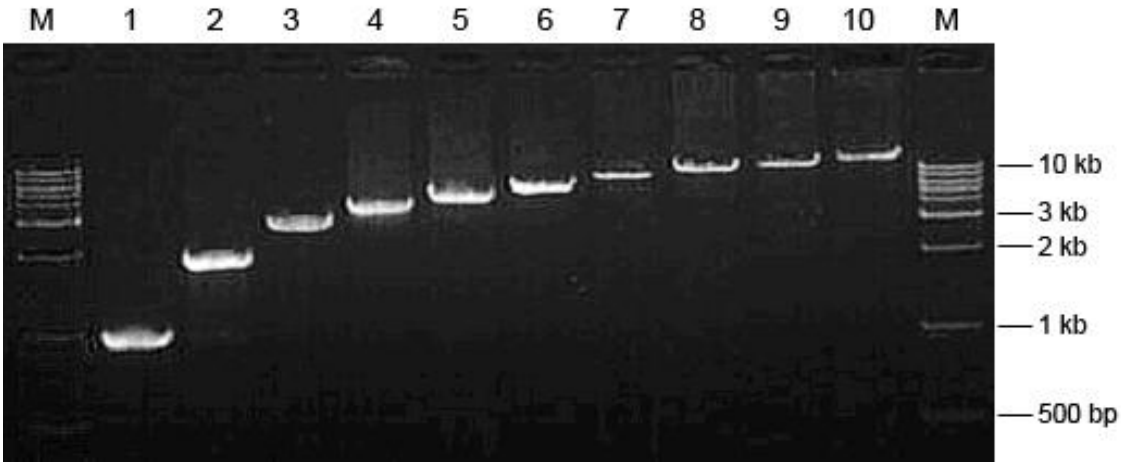

Figure 1. PCR amplification of 1 kb~20 kb fragments from λDNA using LApro *Taq* DNA Polymerase. 1U of enzyme was used in 50 µl of PCR reaction. Lane 1 - 1kb, Lane 2 - 2kb, Lane 3 - 3kb, Lane 4 - 4kb, Lane 5 - 5kb, Lane 6 - 6kb, Lane 7 - 8kb, Lane 8 - 10kb, Lane 9 - 12kb, Lane 10 - 20kb. 5µl of PCR reaction was loaded in each lane. M is DNA KB Ladder (GenScript, Cat. No. M101R).

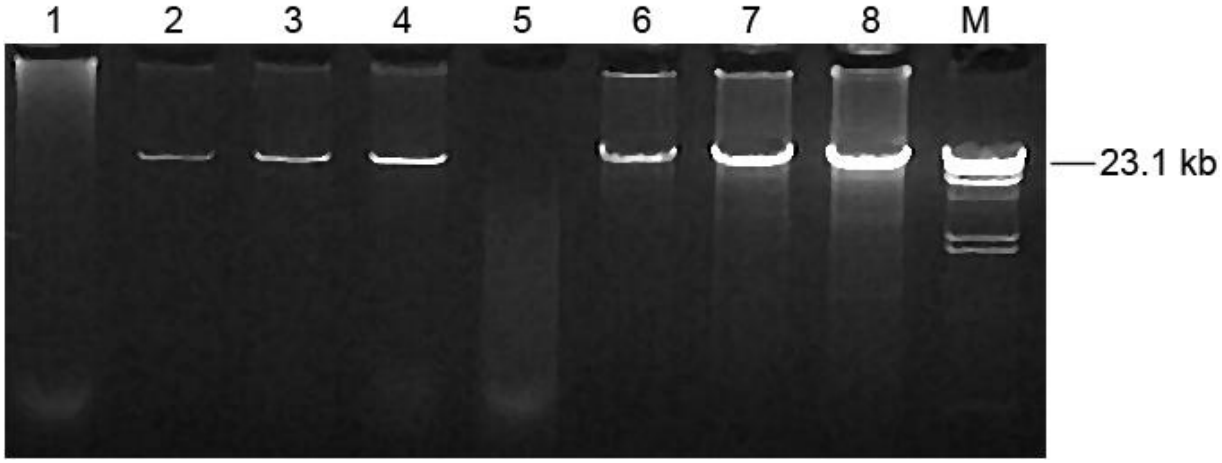

Figure 2. PCR amplification of 17.6kb fragments (lane 2, 3, 4) from human genomic DNA and 20kb (lane 6, 7, 8) from λDNA using LApro *Taq* DNA Polymerase.
Lane 1: 5 U standard *Taq* DNA Polymerase were used to amplify 17.6kb fragment from human genomic DNA
Lane 2, 3 & 4: 1 U, 2 U and 5 U of LApro *Taq* DNA Polymerase were used in 50 µl of PCR reaction to amplify 17.6 kb fragment from human genomic DNA
Lane 5: 5 U KOD DNA Polymerase were used to amplify 20 kb fragment from λDNA
Lane 6, 7 & 8: 1 U, 2 U and 5 U of LApro *Taq* DNA Polymerase were used in 50 µl of PCR reaction to amplify 20 kb fragment from λDNA. 3 µl of PCR reaction was loaded in each lane. M is a λ *Hin*dIII DNA Marker.

IX. TROUBLESHOOTING

The table below is guideline for troubleshooting.

Problem	Probable Cause	Solution
No PCR DNA	Extension time is too short;	LApro *Taq* DNA Polymerase requires a minimum extension time of 1 minute for 1~2 kb template.
	One or more PCR components	Run a positive control to make sure the PCR

	may be missing;	reaction is properly set-up.
	PCR conditions are not optimized; the annealing temperature may be too high; more cycles may be needed; the denaturation time may be too short;	Optimize the PCR conditions by decreasing annealing temperature in 2-4°C increments, or increasing the number of cycles, or increasing the denaturation time in 10 second increments. It is recommended to change only one parameter each time.
	The primers may not be designed optimally.	The primer designing is critical for high quality PCR. Longer primers of 25-30 nucleotides with a GC content of 45-60% and with a more stable 5'-end than 3'-end usually make good primers.
	Target template is highly GC-rich.	The target will be difficult to denature even with a longer denaturation step.
Non-specific DNA products	The primers may not be designed optimally.	See above.
	Annealing temperature is too low.	Optimize the PCR conditions by increasing annealing temperature in 2-4°C increments, or decreasing the number of cycles.
False positive	Reagents are contaminated.	It is recommended that a negative control without using genomic DNA be run to make sure no contamination occurs.

X. ORDER INFORMATION

LApro *Taq* DNA Polymerase, Cat. No. E00013
LApro *Taq* DNA Polymerase Complete System, Cat. No. E00014

Telephone: 732-885-9188, 732-357-3839
Fax: 732-210-0262, 732-885-5878
E-mail: info@genscript.com

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GenScript Corporation
120 Centennial Ave., Piscataway, NJ 08854
Tel: 732-885-9188, 732-357-3839
Fax: 732-210-0262, 732-885-5878
E-mail: info@genscript.com
Web: http://www.Genscript.com

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