



## AuPreP Longjump Polymerase AUP-LJP- 250

A robust and very powerful Hi-fidelity polymerase meant for challenging PCR applications involving very difficult Genomic DNA templates of >3 kb to 18kb+ and Lambda DNA fragments of 28Kb+. The Product comes with a superior PCR Enhancer and Stabilizer to meet the toughest PCR situations

**(Suitable for both TA and blunt-end cloning)**

**SALIENT FEATURES AND BENEFITS--** The optimized composition of enzymatic activities enables AuPreP Longjump Polymerase to span the primer extension over long regions and demonstrate high processivity by reducing premature strand termination and template degradation. Using long primers at elevated  $Mg^{2+}$  concentrations, >28Kb or >18Kb products can be achieved from lambda templates or genomic DNA, respectively. AuPreP Longjump Polymerase provides high performance and specificity, even with 'dirty' DNA or difficult templates with an unfavorable nucleotide composition. AuPreP Longjump Polymerase possesses 5'-3' DNA polymerase activity and 3'-5' proofreading activity which reduces mis-incorporations during PCR. This combination of properties provides much higher fidelity than *Taq*. In contrast with other proofreading enzymes, AuPreP Longjump Polymerase does not cause primers degradation

**Storage conditions :** -20degrees Centigrade

**Conc:** 4U/ $\mu$ l

**Storage Buffer:** 20mM Tris-HCl, pH 7.5, 100mM NaCl, 0.1mM EDTA, 2mM DTT, 50% Glycerol & stabilizers

**Endonuclease and Exonuclease activities:** NIL Detected

**NB:** One unit is defined as the amount that incorporates 10nmoles of dNTPs into acid-precipitable form

### *Each 250 units pack contains*

AuPreP Longjump Polymerase.....	62.50 $\mu$ l
10x Buffer .....	1.2 ml
50mM MgCl <sub>2</sub> .....	1.2 ml
DMSO .....	1.0 ml
PCR Champ (enhancer) .....	1.2 ml

### IMPORTANT

Use of DMSO (supplied) in the reaction mix is recommended to increase the specificity of AuPreP Longjump Polymerase . Also supplied is a vial of PCR Champ (enhancer) , which helps to prevent the formation of false background bands and smearing, especially on difficult templates. PCR Champ (enhancer) should be used at 1.0-2.0x final concentration - the optimal amount required should be determined for each individual experiment. PCR Champ (enhancer) may also alter the ideal annealing temperature for primers - some optimization may be required. **Please note that DMSO and PCR Champ (enhancer) \ should not be used together.**



## AuPreP Longjump Polymerase Protocol & Usage Guidelines

### Reaction Mix:

AuPreP Longjump Polymerase	4u/μl	-----	1μl
Template Lambda DNA	5ng/μl	-----	1μl
DMSO or PCR Champ (optional) <sup>+</sup>		-----	2.5μl
10x Buffer		-----	5μl
100mM dNTP		-----	0.5μl
Primer mix 100μM		-----	0.3μl
50mM MgCl <sub>2</sub>		-----	1μl
Water (ddH <sub>2</sub> O)		-----	Up to 50μl

Cycling Parameters	Stage of Incubation	Incubation Temperature	Incubation Time
1x	Initial Denaturation	94°C	2 min
	Annealing	*	1 min
30x	Denaturation	94°C	30 sec
	Annealing	*	30 sec
	Extension	68°C	10 min <sup>+</sup> or 20 <sup>++</sup> min
1x	Final Elongation	68°C	10 min <sup>+</sup> or 20 <sup>++</sup> min
	Hold	4°C	

\*Annealing temperature is primer-dependent

<sup>+</sup> 10 minutes for a 10 Kb fragment.

<sup>++</sup> 20 minutes for a 20 Kb fragment

**This data is intended for use as a guide only; conditions will vary from reaction to reaction and may need optimization.**

### PCR Troubleshooting Guide

→ If **No or low PCR yield** occurs, then the following can be the reasons along with the suggested solutions.

- **Enzyme concentration too low**-----for this **Increase the amount of enzyme in 0.5U increments.**
- **Magnesium concentration not optimized**-----for this **Increase concentration in 0.25mM increments.**
- **Primer concentration is optimized**-----for this **Titrate primer concentration (0.3-1μM); ensuring that both primers have the same concentration.**

→ If **Multiple bands** occurs, then the following can be the reasons along with the suggested solutions.

- **Primer annealing temperature too low**-----for this **Increase annealing temperature. Primer annealing should be at least 5°C below the calculated T<sub>m</sub> of primers.**
- **Master mix left at room temperature**-----for this **Prepare and keep master mix on ice**
- **Low Specificity** ----- for this **Add DMSO or PCR Champ as per suitability; please refer to page 1 of AuPreP Longjump Polymerase.**

→ If **Smearing or artefacts** occur, then the following can be the reasons along with the suggested solutions.

- **Template concentration is too high** ----- for this **Prepare serial dilution of template.**
- **Too many cycles** ----- for this **Reduce the cycle number by 3-5 to remove non-specific bands.**
- **Enzyme concentration is too high** ----- for this **Decrease the amount of enzyme in 0.5U increments.**
- **Extension time too long**----- for this **Reduce extension time in 0.5-1 minute increments.**



<u>Other AuPreP™ DNA/RNA Kits</u>	<u>Other Related Products</u>
<b>AuPreP™ Plasmid Maxi Kit</b>	<b>AuPreP Oligos</b> (High Affinity Purified Oligo synthesis available in different scales, purifications & modifications)
<b>AuPreP™ Plasmid Midi Kit</b>	<b>AuPreP TaQ DNA Polymerase</b> (Ultrapure, Ultra-stable & Ultra-sensitive Taq DNA Polymerase)
<b>AuPreP™ SPIN™ SPIN Miniprep Kit</b>	<b>AuPreP Hotstart TaQ DNA Polymerase</b> (Robust Polymerase for Hotstart PCR assays)
<b>AuPreP™ Blood Genomic DNA Maxi</b>	<b>AuPreP Super Fidelity TaQ DNA Polymerase</b> (High fidelity Polymerase produces blunt ended amplicons upto 5Kb)
<b>AuPreP™ Blood Genomic DNA Extraction Midi Kit</b>	<b>PCR Doctor</b> - (PCR enhancer for AuPreP Hotstart Taq or Super Fidelity Taq especially designed for GC/AT/Dirty/Difficult Templates)
<b>AuPreP™ GEN<sup>bl</sup> DNA Extraction Kit</b>	<b>AuPreP Longjump Polymerase</b> (Robust Long Polymerase for templates > 4kb to 18kb+ for challenging PCRs )
<b>AuPreP™ DNA easy Plant Maxi kit</b>	<b>AuPreP Red PCR Master Mix</b> ( 2x Master mix with Red Dye without Enhancer)
<b>AuPreP™ DNA easy Plant Mini Kit</b>	<b>AuPreP DIAMOND MASTER-MIX</b> (2x Mastermix with PCR Enhancer & Stabilizer without tracking dyes)
<b>AuPreP™ PCR Purification Kit</b>	<b>AuPreP DIAMOND DOUBLE DYE MASTERMIX</b> (2x Mastermix with PCR Enhancer, Stabilizer & tracking dyes)
<b>AuPreP™ Plant RNA Maxi Kit</b>	<b>AuPreP DNA Extraction System</b> ( A fast Reagent for pure genomic DNA isolation for down stream applications )
<b>AuPreP™ Plasmid Maxi Kit</b>	<b>AuPreP RNA Extraction System</b> ( for Purest & High Quality RNA extraction with simple cost effective protocol )
<b>AuPreP™ RNA Easy Midi Kit</b>	<b>AuPreP Gold cDNA Synthesis Kit</b> (Highly Cost effective cDNA Synthesis Kit using RT with reduce Rnase H activity)
<b>AuPreP™ RNA™ Mini Kit</b>	<b>AuPreP Gold RT-PCR Combo Kit</b> ( 2 step RT-PCR protocol with tracking Dye )
<b>AuPreP™ RNV™ Viral RNA Extraction Miniprep Kit</b>	<b>AuPreP Extra Mile First Strand cDNA System</b> ( Premium cDNA Synthesis Kit using RT with point mutant Rnase H minus activity )
	<b>Novascript III RNase H<sup>-</sup> RT</b> (Premium Ultra-stable Rnase H minus RT for long high quality cDNA construction )
	<b>Novascript III single step RT-PCR System</b> ( Premium 1step RT-PCR system using Novascript & AuPreP Hotstart DNA Polymerase)
	<b>AuPreP Random Primer labeling Mix System</b> ( Premixed solution for the labeling of DNA with radiolabeled dCTP using random sequence oligonucleotides )