FAQs

LA Taq™ Polymerase

1) What is the principle of LA (Long and Accurate) PCR?

Generally, standard *Taq* DNA Polymerase has difficulty amplifying targets >5 kb. This is presumably (at least partially) due to its inability to correct dNTP misincorporations. The chain elongation rate from mismatched bases is greatly reduced, causing a decrease in the yield of longer products.

LA Taq™ DNA Polymerase is a thermostable polymerase which possesses a 3' to 5' exonuclease (proofreading) activity, allowing amplification of long targets. The 3' to 5' exonuclease activity detects and removes the misincorporated bases that cause slow elongation, making the reaction proceed smoothly, allowing generation of longer and more accurate PCR products.

2) What is the difference between *LA Taq*™ and *Ex Taq*™ DNA Polymerases?

Both the *LA Taq*™ and *Ex Taq*™ DNA Polymerase and buffer systems contain a proofreading activity. The *LA Taq*™ system is optimized for production of longer fragments, while *the Ex Taq*™ system is specifically optimized for high yields and superior sensitivity.

LA Taq™ DNA Polymerase is generally recommended for longer amplifications of up to 48 kb on lambda DNA and 30 kb on human genomic DNA.

In contrast, *Ex Taq*™ DNA Polymerase is generally recommended in applications where high efficiency and sensitivity are demanded in amplifications up to 20 kb of Lambda DNA and 10 kb of human genomic DNA. *Ex Taq*™ also provides consistent results with low copy number DNA.

3) What are the fidelities of Takara's DNA Polymerases?

When the mutant ratio (mutant colonies:total colonies) of *LA Taq*™ Polymerase is compared to conventional *Taq* Polymerase using the Kunkel method, *LA Taq*™ Polymerase shows 6.5-fold higher fidelity than *Taq* Polymerase.

Note: According to the Cline method, the error rate of Takara *Taq* Polymerase is 8.7×10^{-6}.

4) Does Takara's *LA Taq*™ Polymerase produce PCR products with sticky ends (3'-A overhangs)?

Yes, all of Takara's polymerases produce products with about 80% 3' A-overhangs, and these products may be cloned into T-vectors.

5) What are some general guidelines for primers to be used with Takara *LA Taq*™ Polymerase?

Primer specificity is the most important consideration. For *LA Taq*™ Polymerase, the primers should be 25-35 bases in length, with nearly 50% G+C content and balanced melting temperatures within 2-3°C of each other. With these primers, high annealing temperatures can be used to enhance reaction specificity.

6) What is the composition of Takara's *LA Taq*™ Polymerase buffers?

The buffer composition of the *LA Taq*™ buffers is proprietary, however, the magnesium concentration in the standard 10X *LA* Buffer II equals 25 mM.

Note that because the optimal Mg^{2+} concentration in a reaction may be affected by variations in the reaction mix, including concentration of dNTPs, template-primer concentrations, and chelating agents carried along with template DNA, a version of Takara's *LA Taq*™ DNA Polymerase with Mg^{2+}-free buffer is available to allow optimization.

7) What cautions should I use in handling PCR Buffers?

Repeated freeze-thawing of magnesium-containing solutions (like the 10X buffers supplied with Takara's *LA Taq*™ Polymerase) may result in the formation of a fine precipitate. This precipitate can reduce the effective concentration of Mg^{2+} in the PCR reaction, thereby impairing performance. We recommend thawing the 10X Buffer at room temperature, warming gently to 37°C for 2-3 minutes, and briefly vortexing to ensure a uniform suspension.

8) Is Takara's *LA Taq*™ Polymerase a LD (low DNA) enzyme?

Takara *LA Taq*™ Polymerase is a LD enzyme- (less than or equal to 10 fg DNA), confirmed by nested PCR of *Ori* region of *E. coli* genomic DNA.

9) Can Takara's *LA Taq*™ Polymerase be used for combinatorial or multiplex PCR?

Yes, *LA Taq*™ Polymerase can be used for both combinatorial and multiplex PCR. Combinatorial and multiplex PCR are very similar techniques, and these terms are often used interchangeably. Multiplex PCR uses one template, usually genomic DNA, and several sets of primers in the same reaction. Combinatorial PCR uses several templates and several primer sets in the same reaction.

10) What is touchdown PCR?

Touchdown PCR was originally intended to simplify the process of determining optimal primer annealing temperatures. During the initial cycles of touchdown PCR, annealing takes place at approximately 15°C above the calculated T_m. In subsequent cycles, the annealing temperature is gradually reduced by 1-2°C until it has reached approximately 5°C below the calculated T_m. Many thermal cyclers have gradient-block features, which allow touchdown PCR to be performed in a single reaction.

11) What is autosegment extension (auto-extend cycles), and when should I use it?

Autosegment extension is a technique used to increase the yield of amplification products. It is typically employed with large amplimers and is a technique used to compensate for the inactivation or depletion of reagents. At the 15th (half the total number of cycles) and subsequent cycles the extension time is extended by 15 seconds for each cycle, allowing for a significant increase in amplification efficiency in long PCR.

12) What is the composition of Takara's One Shot LA PCR Mix (i.e. premix)?

The concentration of *LA Taq*™ Polymerase in the 2X *LA Taq*™ Premix is 0.1 U/µL. The concentration of dNTPs in the 2X *LA Taq*™ Premix is 0.8 mM for each nucleotide. The final concentration of dNTPs in the 50 µL reaction is 0.4 mM for each nucleotide.

Some customers, particularly those performing high-throughput experiments, find the premix more convenient because it reduces the number of pipetting steps. This decrease in pipetting steps reduces the probability of error, decreases user-to-user variation, and minimizes the risk of contamination.

13) Can template quality affect amplification results?

Yes. Successful amplification of longer (>5 kb) products requires intact and highly purified template DNA. Performing an additional phenol/chloroform extraction and ethanol precipitation often resolves problems related to template quality.

14) What are general reaction conditions for *LA Taq*™ DNA Polymerase?

The following table represents the recommended general reaction mix for a single 50 µL reaction. To minimize the number of pipetting steps and reduce the risk of contamination, we recommend that you prepare a master mix of reagents that is sufficient for all reactions being performed.

Reagent	Volume	Final Concentration
Takara *LA Taq*™ DNA Polymerase (5 units/µl)	0.5 µL	2.5 units/50 µL
10X LA PCR Buffer II (Mg^{2+} plus)	5.0 µL	1X
dNTP Mix (2.5 mM each)	8.0 µL	400 µM
Template DNA*	1-5 µL	2.5-500 ng/50 µL
Primers	1-5 µL	0.2 µM each
Sterile ddH_20	up to 50 µL	

*DNA template amounts per 50 µL reaction:

Human genomic	0.1-1 µg
E. coli genomic or plasmid	10-100 ng
Lambda phage	0.5-2.5 ng

The following times, temperatures, and cycle numbers are starting points to use when optimizing PCR conditions for each primer pair:

Initial Denaturation (1 cycle):

94°C 1 min

Amplification (30 cycles):
98°C 10 sec
68°C 0.5-1 min/kb

Final elongation (1 cycle)
72°C 10 min

15) Can Takara's *LA Taq*™ Polymerase be used to amplify GC-rich templates or those with large amounts of secondary structure?

Takara supplies two GC-buffers for amplification of difficult templates in the LA PCR Kit, Version 2.1, and with the *LA Taq*™ Polymerase with GC-Buffers enzyme. When amplifying a 262 bp fragment (73% GC content) and a 358 bp fragment (71.5% GC content), the reaction conditions are:

Initial Denaturation (1 cycle):
94°C 1 min

Amplification (30 cycles) :
94°C 30 sec
60°C 30 sec
72°C 1 min

Final Elongation (1 cycle) :
72°C 5 min

16) What is the difference between GC Buffer I and GC Buffer II that is supplied with the *LA Taq*™ Amplification Kit, Version 2.1 and the *LA Taq*™ with GC Buffers, and how do I know which buffer to use?

Both GC Buffers I & II are buffers that have been optimized for use with DNA containing high secondary structure or GC-rich regions. GC Buffer I is best suited for amplification of longer targets, whereas GC Buffer II is useful for amplification of shorter 2-3 kb length GC-rich targets.

17) How does *LA Taq*™ Hot Start DNA Polymerase differ from the standard enzymes?

LA Taq™ Hot Start polymerase contains a monoclonal antibody to *Taq* Polymerase, which binds to the polymerase until the temperature is elevated. The binding of this antibody prevents nonspecific amplification due to mispriming and/or formation of primer dimers during PCR reaction assembly. The antibody is then denatured in the initial PCR DNA-denaturation step, releasing the polymerase and allowing DNA synthesis to proceed. Hot Start technology results in lowered background, increased specificity, and allows room-temperature reaction assembly.