TaKaRa LA Taq® DNA Polymerase (Mg^{2+} free buffer) (Cat.#s RR002A and RR002M)
TaKaRa LA Taq® DNA Polymerase (Cat.#s RR002B and RR002C)
TaKaRa LA Taq® DNA Polymerase Hot-Start Version (Cat.# RR042A)
TaKaRa LA Taq® DNA Polymerase with GC Buffer (Cat.# RR02AG) & LA PCR Kit, Version 2.1 (Cat.# RR013A)

Frequently Asked Questions: Long and Accurate PCR

Conventional *Taq* DNA polymerase suffers from lack of 3′ to 5′ proofreading capability, resulting in a relatively high error rate. *Taq* also exhibits a tendency to "fall off" or disassociate from the template and cease elongation at sites of misincorporated nucleotides. Because of this, both fidelity and yield are greatly compromised. Takara Bio is a leader in offering solutions for Long and Accurate PCR (LA PCR). LA PCR technology involves mixing *Taq* polymerase with a small amount of proofreading polymerase. One advantage of this approach is that the enzyme mix exhibits synergistically enhanced performance, with characteristics of fidelity, yield, length, and reaction reproducibility that exceed those of the individual enzymes alone.

TaKaRa LA Taq DNA Polymerase is available in the original formulation or formulated with Mg^{2+} free buffer, for hot-start PCR, or with buffer optimized for GC-rich templates. Researchers worldwide rely on *TaKaRa LA Taq* DNA Polymerase for amplification of products up to 48 kb in size with fidelity 6.5-fold higher than conventional *Taq* enzyme. *TaKaRa LA Taq* DNA Polymerase requires minimal optimization in comparison to other long PCR polymerases on the market.

Answers to frequently asked questions about LA PCR are presented here. For additional information, refer to the technical literature and web pages for *TaKaRa LA Taq* products listed at the top of this FAQ.

Q1: What reaction conditions should be used for LA PCR?

A1: The optimal reaction conditions vary according to amplicon size, reaction volume, and type of thermal cycler.

- **Cycle number:** Set the cycle number to 25 - 35 cycles depending on the quantity and complexity of template DNA and the amplicon length. Fewer cycles may not generate enough amplified product, while over-cycling may result in a diffuse smear upon electrophoresis.
- **Initial denaturation:** We recommend an initial denaturation at 94°C for 1 - 2 minutes. These conditions are sufficient even for genomic DNA templates.
- **Denaturation conditions:** When using thin-walled PCR tubes, the recommended denaturation condition is either 98°C for 10 sec. or 94°C for 20 sec. When using normal PCR tubes, the recommended denaturation condition is either 94°C for 30 sec. or 98°C for 20 sec. A denaturation time that is too short or a denaturation temperature that is too low may cause either diffuse smearing upon electrophoresis or poor amplification efficiency. A denaturation time that is too long or a denaturation temperature that is too high may generate no identifiable product.
- **Conditions for annealing and extension:** Determine the optimum annealing temperature experimentally by varying temperatures in 2°C increments over a range of 55 - 68°C. Because *TaKaRa LA Taq* DNA Polymerase maintains excellent activity from 60 to 68°C, two step PCR can be conducted by setting the annealing/extension temperature within this range. For two-step PCR with a combined annealing/extension at 68°C, the recommended extension time is 30 sec - 1 min per 1 kb. When temperature is set below 68°C, a longer extension time will be required. An annealing temperature that is too high may generate no amplification products, while a temperature that is too low may result in non-specific amplifications. An extension time that is too short may fail to produce any amplification products or may result in non-specific, short products, while overly long extension times can causes diffusely smeared electrophoresis bands.

Q2: Are there any general guidelines for designing primers for LA PCR?

A2: Primer specificity is very important for the generation of long DNA amplification products. If possible, prepare primers according to the following criteria:

1) The primer length should be 25 to 30 bases.

2) The difference between the optimum annealing temperature of paired primers should be within 2 – 3°C.

3) Choose primers with GC content around 40 - 60%.

4) Avoid primer sequences that form hairpin loops or primer-dimers, especially at the 3' end.

5) Do not use primers containing inosine.

Q3: How much primer should be used in LA PCR reactions?

A3: The optimal primer concentration will range from 0.1 µM to 1.0 µM. Using a concentration that is lower than the optimal concentration may decrease amplification yield. In contrast, the presence of excess primer may cause non-specific reactions to outperform primer-specific amplifications.

In general, primer concentrations can be determined depending on the characteristics and amounts of template DNA. Low primer concentrations are recommended either for highly complex DNA such as human genomic DNA, or for high concentrations of template DNA. High primer concentrations are recommended for low complexity templates such as plasmid DNA, or for small amounts of template DNA.

Q4: How much enzyme should be used in the reaction?

A4: Although it is recommended that 2.5 units of *TaKaRa LA Taq* DNA Polymerase be used in a 50 µl reaction, the amount can be optimized based on the reaction conditions. Both the quantity or complexity of template DNA and the length of amplified DNA fragment should be taken into consideration. If excess enzyme is used, non-specific reactions may occur and lead to a diffuse smear upon electrophoresis. Amplification efficiency may decrease when the enzyme concentration is low.

Q5: How should template DNA be prepared for LA PCR?

A5: When you perform PCR to generate DNA fragments longer than 10 kb, the preparation of intact (free of nicks) and fully purified template DNA through repeated extraction/purification is essential. Samples that are prepared by crude cell lysis with either heat treatment or protease digestion are not appropriate for LA PCR.

Q6: Can LA PCR be performed directly on λ phage particles?

A6: Lysates containing approximately 10^6 - 10^7 PFU obtained by heating at 99°C for 10 min. can generate DNA fragments of approximately 8 kb in length.

Q7: Is it possible to perform LA PCR directly on cell lysates obtained by either heat treatment (98°C for 2 min.) or protease digestion from either mammalian cells or *E. coli* cells?

A7: For *E. coli*, it is possible. Lysates* generated by heat-treatment alone could generate approximately 10 kb fragments by LA PCR.

**E. coli* cells were cultured in L-broth medium at 37°C overnight. A total of 2 µl of the cell culture was used for a 50 µl PCR reaction.

For human cells (cell cultures), the extent of purification significantly affects the length of extension achieved in PCR amplification. Heat treatment alone has a limit of several hundred bases, while samples prepared using double treatment with heat and protease may result in products exceeding 1 - 2 kb. For longer amplifications, we recommend using purified DNA.

Q8: What could cause the amplification products to appear smeared during gel electrophoresis?

A8:

Possible Causes	Comments and suggestions
Too much enzyme	Reduce the enzyme amount in 0.5 unit increments.
Denaturation time is too short	Increase the denaturation time by increments of 5 sec.
Denaturation temperature is too low	Raise the denaturation temperature by 0.5°C intervals.
dNTP concentration is too low	Increase the dNTP concentration in by increments of 50 µM.
Extension time is too long	Shorten the extension time by decrements of 1 min.
Too many PCR cycles	Reduce the number of cycles by 2 cycles per trial.
Too much template	Reduce the template amount by decrements of 20%.

Q9: What could cause multiple, nonspecific amplified products?

A9:

Possible Causes	Comments and suggestions
Primer concentration is too high	Decrease the primer concentration in steps of 0.1 µM.
Poor primer design	Enhance the specificity of primers by changing the complementary region of the template or using longer primers (up to 30-35 mers).
Too much enzyme	Reduce the enzyme amount in decrements of 0.5 units.
Too many PCR cycles	Reduce the number of cycles by 2 cycles per trial.
Annealing temperature too low	Raise the annealing temperature by intervals of 2°C per trial.
Nonspecific annealing of primers	Use the hot start method.
Extension time too short	Increase the extension time by increments of 1 min.
Poor denaturation	Raise the denaturation temperature in increments of 0.5°C per trial and extend the time by increments of 5 sec. per trial.
Too much template	Reduce the amount of template by decrements of 20%.

Q10: What is the appropriate agarose gel to use for the electrophoretic analysis of long DNA fragments generated by LA PCR?

A10: An agarose gel made with 0.4% SeaKem Gold Agarose (Lonza) is recommended.

Q11: Does *TaKaRa LA Taq* DNA Polymerase produce products with sticky ends (3'-A overhang)? Can the products be used for TA cloning?

A11: Yes, *TaKaRa LA Taq* enzyme generates DNA fragments with 3'-A overhangs that can be cloned into T-vectors. Lower cloning efficiency will occur when cloning long PCR products (>5 kb) into T-Vectors.