

LA PCR Kit, Version 2.1 (Cat.# RR013A)

## Frequently Asked Questions: LA PCR Kit, Version 2.1

The LA PCR Kit, Ver. 2.1 includes all the reagents necessary for amplification of long DNA templates with routine extension to 30 kb (and up to 48 kb possible with some templates.) This kit combines *TaKaRa LA Taq*<sup>®</sup> DNA Polymerase with an optimized buffer system resulting in longer and more accurate PCR products than conventional PCR reagents. High fidelity (6.5-fold better than *Taq*) is facilitated by an efficient 3'→5' exonuclease activity. Control template and primers are included to ensure premium PCR performance.

Answers to frequently asked questions about LA PCR Kit, Version 2.1 are presented here. For additional information, refer to the product User Manual and web page.

### Q1: Why is a two-step PCR protocol recommended when using the LA PCR Kit Ver. 2.1?

A1: For successful long PCR, long primers (~30 bases) are often used to increase specificity. Because of the high  $T_m$  value of these long primers, the temperature difference between the annealing and extension temperatures is often small. Therefore, when the annealing temperature exceeds 60°C, good amplification can be obtained by using the same temperature for annealing and extension. We recommend using a two-step PCR protocol with LA PCR Kit Ver. 2.1 because this kit is designed for long PCR. When using shorter primers, a three-step PCR protocol can be used. However, successful amplification using two-step PCR may be possible when using ~20mer primers.

### Q2: Do PCR products amplified with the LA PCR Kit Ver. 2.1 have an A overhang?

A2: Yes, PCR products have an A overhang and can be used for TA cloning. Alternatively, products can be used for blunt end cloning after blunting and phosphorylation by T4 Polynucleotide Kinase.