LA PCR *in vitro* Cloning Kit (Cat.# RR015)

Frequently Asked Questions: LA PCR *in vitro* Cloning Kit

The LA PCR *in vitro* Cloning Kit is an improved cloning system designed specifically to amplify a long region of DNA. This system takes advantage of LA (long and accurate) PCR technology by including *TaKaRa LA Taq*® DNA Polymerase. This allows for longer amplification and improved fidelity than is possible with conventional polymerases. Amplification is accomplished by cassette-mediated PCR whereby long DNA fragments, such as those found in genomic DNA, can be obtained without the need for time-consuming library construction and screening.

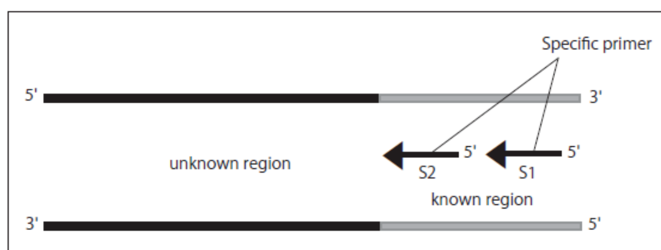
Answers to frequently asked questions about the LA PCR i*n vitro* Cloning Kit are presented here. For additional information, refer to the product User Manual and web page.

Q1: What are the guidelines for designing the specific primers S1 and S2?

A1: Design primers for directional amplification of the unknown region. Primer S2 should be designed to anneal to a location upstream (relative to the template) of the annealing sequence of Primer S1 (see figure). The distance between primer S1 and S2 does not substantially influence results.

S1 and S2 primer design considerations:

- Primer length should be between 20 - 35 mers for products up to 4 kb. We recommend 30 - 35 mers for amplification of long DNA fragments, i.e., 4 kb or larger.
- Primers should have a balanced G/C and A/T content throughout and avoid subregions with high G/C or A/T content. In particular, avoid high A/T % at the 3'-end of primers.
- Avoid primers likely to form stable secondary structures (e.g., hairpins, loops).
- Since both S1 and S2 primers are used in combination with Cassette Primer (C1, C2), avoid S1 and S2 primers likely to form primer dimers with C1 or C2. Check complementarity (3 - 4 mers from primer 3' ends.)



Q2: No band is observed after the first round of PCR. What should I do?

A2: A band may not be detected after the 1st PCR. In such cases, continue on to the 2nd PCR.

Q3: What restriction enzyme should be used for genome walking experiments?

A3: For genome walking, we recommend using *Sau*3AI for digestion of genomic DNA.

Q4: For genome walking with *Sau*3AI, why is more cassette necessary than for other types of digestions?

A4: *Sau*3AI has a four-base recognition sequence. When genomic DNA is digested with *Sau*3AI, more fragments are generated than with restriction enzymes having longer recognition sequences. Therefore, a larger amount of cassette is needed.

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