MCLAB
Molecular Cloning Laboratories

Manual

Version 2.0

Product name: MCRCA DNA Amplification Kit

Cat #: PPK-100, PPK-200

Description:

MCRCA DNA amplification kit is a novel product developed specifically to prepare templates for DNA sequencing. As illustrated in Figure 1, the MCRCA method utilizes bacteriophage phi29 DNA polymerase to exponentially amplify single- or double-stranded circular DNA templates by rolling circle amplification (RCA) [1, 2]. This isothermal amplification method produces microgram quantities of DNA from picogram amounts of starting material in a few hours.

Amplification in vitro of very small amounts of template DNA eliminates the need for overnight cell culture and conventional plasmid or M13 DNA purification. The proofreading activity of phi29 DNA polymerase ensures high fidelity DNA replication.[3]

The starting material for amplification can be a small amount of bacterial cells containing plasmids, isolated plasmids, intact M13 phage, or any circular DNA samples. Bacterial colonies can be picked from agar plates and added directly to the MCRCA reaction. Alternatively, microliter quantities of a saturated bacterial culture or a glycerol stock can serve as starting material. Depending on the source of the starting material, amplification is completed in 4–18 hours at 30 ˚C with no need for thermal cycling. The product of the MCRCA reaction is high molecular weight, double-stranded concatemers of the circular template.

> Note that when starting with M13 clones, the MCRCA product is double-stranded DNA and can be sequenced with forward and reverse primers. DNA amplified by the MCRCA method can be used directly in cycle sequencing reactions without any purification.

Protocol:

Prior to template amplification, prepare 2x amplification mixture as follow:

Step 1. Reaction Mix Preparation. Add 2 µl of phi29 DNA polymerase (10 units/µl, supplied with this kit) into 100 µl MCRCA 2x Mixture (supplied with this kit), vortex and store on ice.

Step 2. Sample Preparation. For each reaction, add 9 µl Cell Lysis Buffer (supplied with this kit) into a clean tube, and add in 1 µl of one of the samples bellow, vortex for 10 sec and spin down. Heat the sample at 98° C for 5 min and cool down at room temperature for 10 min.

- Purified plasmid DNA
- Plasmid DNA containing *E. coli* culture
- *E. coli* colony resuspension in dH_2O

Step 3. Template Amplification. Add 10 µl of 2x Reaction Mix prepared in Step 1, incubate the tube at 30°C for 8 hrs. Deactivate phi29 DNA polymerase at 96 °C for 5 min.

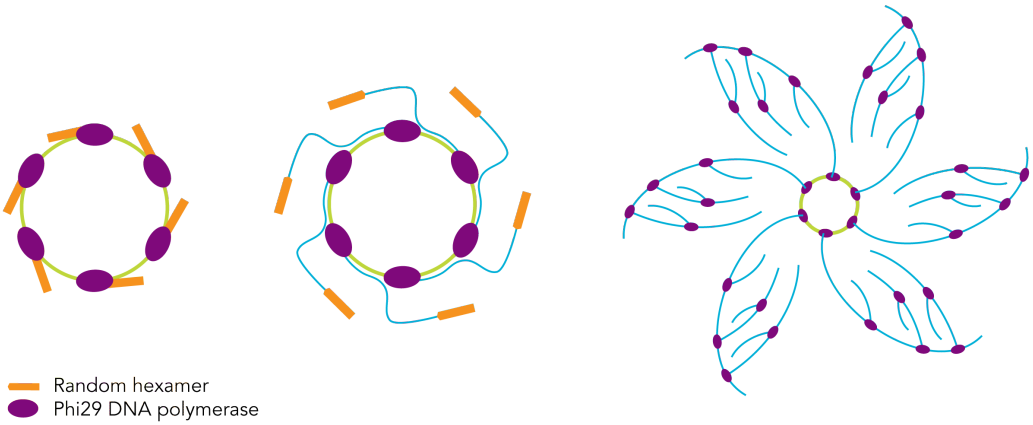


Figure 1. Schematic diagram of the MCRCA process. Random hexamer primers anneal to the circular template DNA at multiple sites. Phi29 DNA polymerase extends each of these primers. When the DNA polymerase reaches a downstream extended primer, strand displacement synthesis occurs. The displaced strand is rendered single-stranded and available to be primed by more hexamer primers. The process continues, resulting in exponential, isothermal amplification.

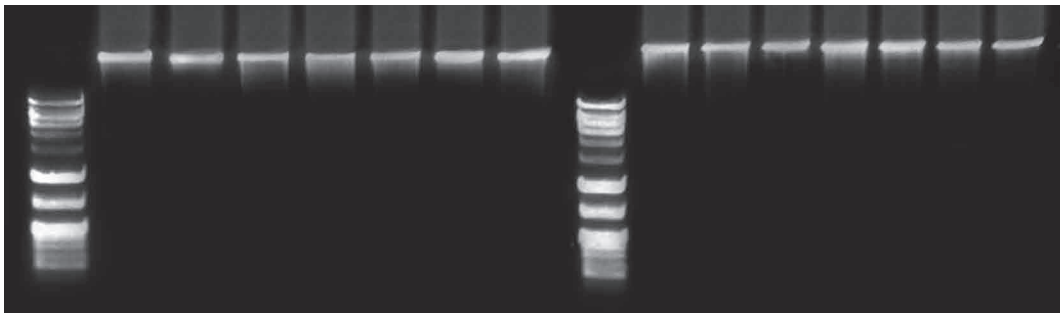


Figure 2. MCRCA 2x Mix amplified templates

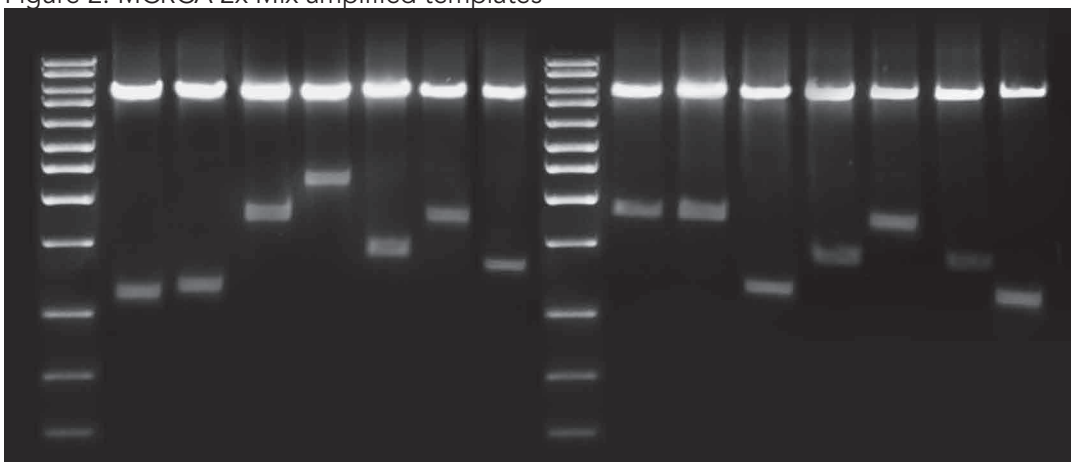
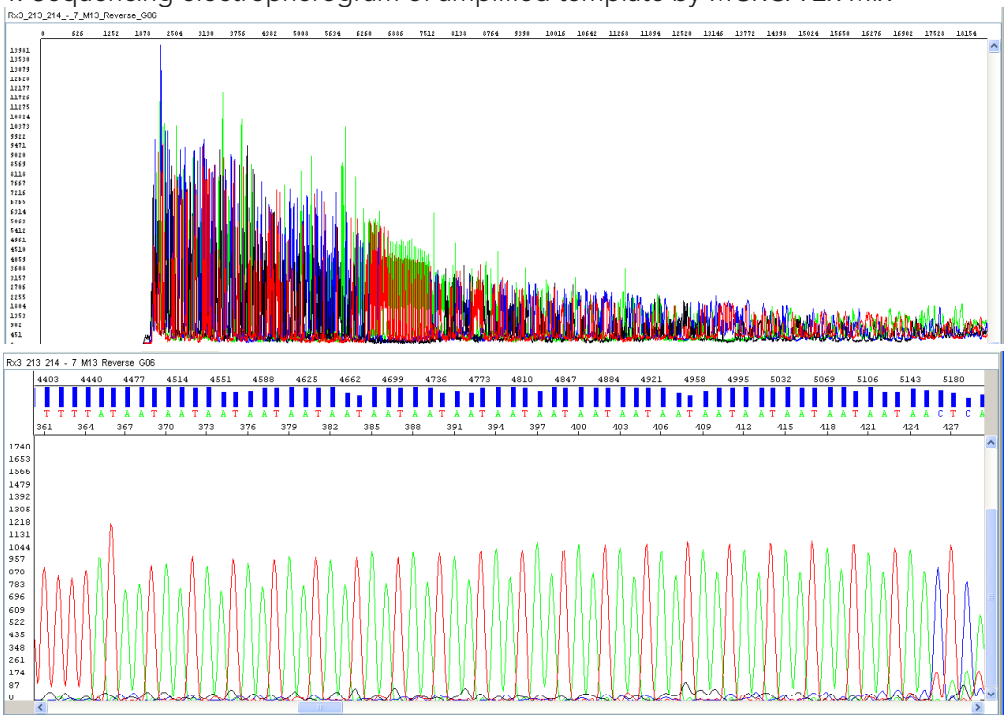


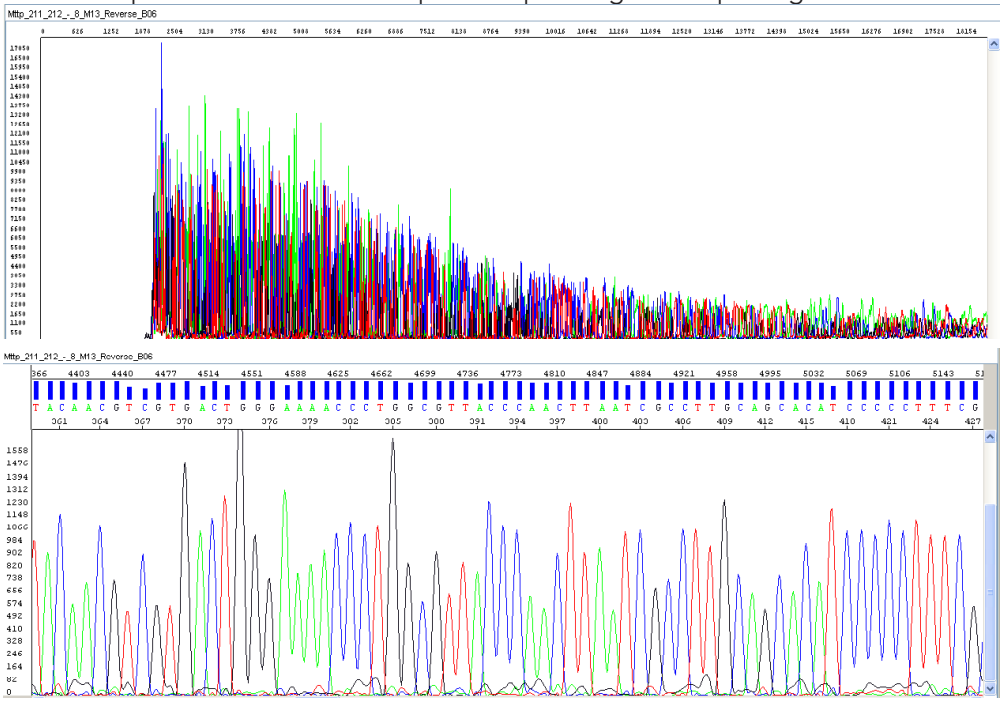
Figure 3. Restriction enzyme digested amplified templates

RCA product sequencing data

1. Sequencing electropherogram of amplified template by MCRCA 2x Mix



2. Non-amplified control DNA template sequencing electropherogram



References:

1. Dean, F. et al., Genome Research 11, 1095–1099 (2001).
2. Lizardi, P. et al., Nat. Genet. 19, 225–232 (1998).
3. Estaban, J. A. et al., J. Biol. Chem. 268, 2719–2726 (1993).