SuperPhi DNA Amplification (RCA) Kit with Random Primers

Cat#: Phi100 or Phi1000

FOR RESEARCH USE ONLY

SuperPhi DNA Amplification Kit is intended for molecular biology use and *in vitro* use only. This product is not intended for diagnosis, prevention or treatment of a disease in human beings or animals.

Store Kit at <-20°C on Receipt

Introduction

Rolling Circle Amplification (RCA) is an isothermal enzymatic process, which amplifies circular or genomic DNA by a Phi29 DNA polymerase-based rolling circle mechanism in the presence of the short DNA or RNA primers, as shown in Fig. 1. Phi29 DNA polymerase is a monomeric protein with two distinct functional domains, C-terminal DNA polymerase domain for DNA synthesis and a spatially separated N-terminal domain with a 3'-5' exonuclease activity for proofreading activity. Co-operation as well as delicate competition between two functional domains, ensure the accurate and efficient DNA synthesis at an optimal rate. Furthermore, this enzyme has capacity to strongly bind to single stranded DNA of double stranded nucleic acid, which makes it favor for multiple displacement amplification (MDA), through debranching double stranded DNA. RCA technology is better than PCR-based methods for DNA amplification because of the high processivity and proofreading activity of Phi29 polymerase. Specially, when genomic DNA is amplified, RCA generates larger fragments (more than 10 kb) with better coverage and less amplification bias, than PCR-based approaches. The RCA technology is so simple, powerful, and versatile that it has been extensively used for DNA cloning, sequencing, SNP and STR genotyping, and genomic DNA amplification, etc. Since the amplified DNA is a concatemer containing tens to thousands of tandem repeats, RCA technology has been employed to generate complex but fine DNA nanostructures, such as DNA origami, nanotubes, and nanoribbons, through manipulating the circular tailor-designed template. These DNA nanostructures have critical potentials in biomedical research, disease diagnostics and therapeutics.

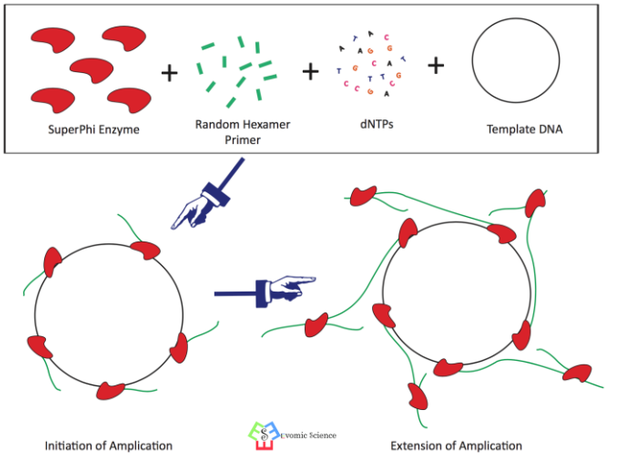

Fig.1. ***Schematic diagram of the RCA process.*** Random hexamer primers anneal to the circular single strand DNA at multiple sites. Phi29 DNA polymerase extends with highest fidelity. With the progress of reaction, Phi29 will extend on the synthesized DNA and amplify DNA at least thousand folds.

Through optimizing RCA reaction condition and engineering Phi29 enzyme, two classes of RCA DNA amplification technologies, RCA 1.0 and RCA 2.0 as described below, were developed for the various

applications. SuperPhi DNA Amplification Kit, a type of RCA 1.0 technology, was suitable for DNA cloning, direct DNA sequencing, and regular DNA amplification; while RCA 2.0 technology would be specifically desired for circle DNA/RNA detection, single cell genomic amplification, etc.

SuperPhi DNA Amplification Kit, based on RCA 1.0 Technology:

Our SuperPhi DNA Amplification Kit (RCA 1.0) was developed to rapidly amplify circular template DNA. This amplification produces microgram quantities of DNA from less than picograms of starting material within 4 hrs at 30°C. Template DNAs can be low or high copy plasmid from bacterial colony, liquid culture, glycerol stock, DNA from BAC culture, M13 plaque, M13 Phage culture supernatant, fosmids or any lambda vectors, DNA ligation/assembly reactions etc. This amplification technology also eliminates the need for overnight cell culture and conventional plasmid or M13 preparations. The amplified DNA, as high molecular weight and double-stranded concatemers of the circular template, can be used for cloning and direct DNA sequencing without further purification. Specially, the amplified RCA products as concatemers, after diluted, can be used to directly transform *Bacillus*. Compared to the traditional *bacillus* transformation methods, SuperPhi RCA products would give the highest transformation rate, which is of critical importance to *bacillus* gene cloning, expression and library construction. Our highly pure SuperPhi enzyme in SuperPhi DNA Amplification Kit ensures that the amplified DNAs are from your template DNA, but not from the host or environmental DNAs, which are usually found in other Phi29-based DNA amplification kits (Fig.2).

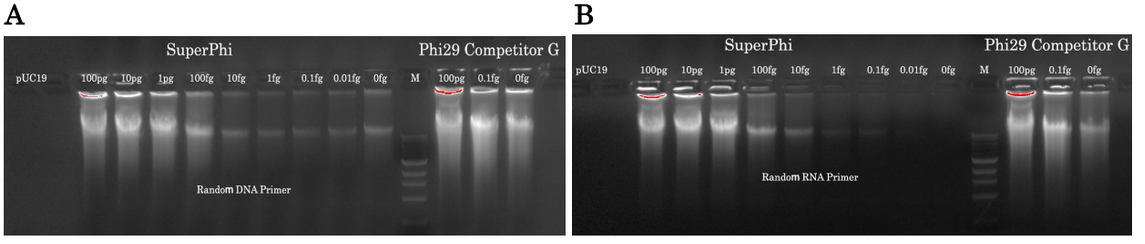

Fig.2. ***Comparison of purity and activity of our SuperPhi enzyme with Phi29 from competitor G.*** The indicated amount of denatured pUC19 were mixed with random DNA primer (**A**) and random RNA primer (**B**) in the presence of SuperPhi enzyme and Phi29 enzyme from competitor G. After 4 hrs at 30°C, the reaction was stopped and loaded on agarose gel. The intensity of 15 kb amplified product using SuperPhi did response well to the input dosage of pUC19, while that in competitor G did not (in **A** and **B**). Even on condition of no input pUC19, the reaction containing Phi29 from competitor G still produced amplified DNA when using RNA primer (in **B**). That indicated that competitor Phi29 enzyme was contaminated with foreign DNA.

Features of SuperPhi DNA Amplification Kits

- SuperPhi29 DNA polymerase for isothermal multiple displacement amplification (MDA);
- Clean manufacturing process ensures components are free of detectable DNA contamination;
- Greater accuracy than existing other PCR based MDA methods;
- Ease with minimal handing time: 15~20 min for up to 96 samples;
- Easily automated;
- Yield up to 2 μg of highly pure DNA.
- Able to detect down to 1 picogram of circular DNA or genomic DNA.
- Any circle DNA:
    - low or high copy plasmid from bacterial colony,
    - liquid culture and glycerol stock,
    - DNA from BAC culture, M13 plaque, M13 Phage culture supernatant,
    - Fosmids or any lambda vectors,
    - DNA ligation/assembly reactions.
- No further purification of amplified DNA: Direct sequencing and cloning reaction.
- Better results for sequencing than traditional sequencing.
- Compatible with other sequencing kits.
- No need for growing liquid bacterial cultures.

Handling

This kit is used to amplify picogram of DNA. Any contaminated DNA may deteriorate your result. Always wear gloves, and prepare the reaction in a laminar flow hood or similar device to avoid contaminations. Use molecular biology grade clean reagents, sterile reaction tubes and DNA-free pipette tips. Thaw Sample Buffer, Reaction Buffer and SuperPhi Enzyme on ice and keep them in ice all the time.

Quality Control

Each batch of SuperPhi DNA Amplification Kit is tested in-house to ensure consistent product quality. Enzymes used in the kit have been tested separately to ensure the highest purity with no DNA contamination.

The amplified DNA by RCA1.0 technology is suitable for:

- Direct DNA restriction enzyme digestion without further purification;
- Direct DNA sequencing (Sanger's method) without further purification;
- Essential for *Bacillus* plasmid transformation.

SuperPhi DNA Amplification Kits are available for both 100 and 1000 reactions.

Reagent and Equipment to be Supplied by the Users

- Sterile vials, pipettes and pipette tips
- Microcentrifuge
- Cold block
- Sterile, ideally DNA-free certified 0.2 ml PCR tubes
- Thermocycler
- Vortexer

Kit Components:

Components	100 Reactions (Cat#: Phi100)	1000 Reaction (Cat#: Phi1000)	Storage
Sample Buffer	800 µl	10x 800 µl	-20°C
Reaction Buffer	500 µl	10x 500 µl	-20°C
SuperPhi Enzyme	100 µl	10x 100 µl	-80°C

Detailed Protocol

SuperPhi DNA Amplification Kit has capacity to detect as low as 1 pictogram of template DNA under optimal condition. 0.2~0.5 µl of saturated overnight culture or 1/10 to 1/100 of the colony (approximately 10^2 ~10^4 cells) would be enough for SuperPhi DNA Amplification reaction. Please keep in mind that excess culture or colony will inhibit SuperPhi DNA Amplification reaction! The protocol described below is a general protocol for amplifying circular DNA from various sources. Yields and kinetics may vary if crude or un-quantified samples are amplified. We recommend to consider a starting point for adapting your specific reaction.

1. Preparation of Sample Mix:

Sample Mix could be prepared, depending on material sources, as described below:

1.1. Purified DNA or DNA ligation/assembly reactions:

Transfer 3 µl of Sample Buffer into a 0.2 ml PCR tube.
Add 1 µl of circular DNA (≥ 1 pg/µl) to the above PCR tube.
Heat to 95 ˚C for 3 minutes and then quickly cool to 4˚C.
Keep the samples on ice until use.

1.2. Bacterial colonies:

Transfer 4 µl of Sample Buffer into a 0.2 ml PCR tube.
Pick 1/10 to 1/100 of the colony (approximately 10^2 ~10^4 cells) and add to the above PCR Tub.
Heat to 95 ˚C for 3 minutes and then quickly cool to 4˚C.
Keep the samples on ice until use.

1.3. Liquid bacterial culture:

Transfer 3.5~3.8 µl of Sample Buffer into a 0.2 ml PCR tube.
Add 0.2~0.5 µl of saturated overnight culture to the above PCR tube.
Heat to 95 ˚C for 3 minutes and then quickly cool to 4˚C.
Keep the samples on ice until use.

1.4. Glycerol stock:

Transfer 3.5~3.8 µl of Sample Buffer into a 0.2 ml PCR tube.

Add 0.2~0.5 μl of glycerol stock to the above PCR tube.

Heat to 95 ˚C for 3 minutes and then quickly cool to 4˚C.

Keep the samples on ice until use.

Note: *Heating at higher temperature or longer time may increase the probability of nicking target DNA and releasing host genomic DNA into cell lysis to the reaction, where the host genomic DNA will compete with the desired template DNA during amplification.*

2. DNA Amplification:

Add 5 μl of Reaction Buffer (**Vortex before use**) and 1 μl of SuperPhi Enzyme to the above PCR tube contains 4 μl of Sample Mix as showed below:

Component	Volume/reaction
Sample Mix	4 μl
Reaction Buffer	5 μl
SuperPhi Enzyme	1 μl
Final Volume	**10 μl**

One can prepare the master mix of Reaction Buffer and SuperPhi Enzyme for multiple reactions. The final volume is 10 μl. Mix well and incubate at 30 ˚C for 3~124 hrs.

3. Inactivate SuperPhi: Incubating at 65 ˚C for 10 min, and then cool to 4 ˚C.

4. Perform Downstream Application:

- The amplified DNA can be directly used for the cycle sequencing reaction without purification;
- The amplified DNA can be directly used for DNA restriction enzyme digestion;
- An aliquot of the amplified DNA can be examined by agarose gel;
- Specially, the amplified RCA products as concatemers, maybe after diluted, can be used to directly transform *Bacillus*. Compared to the traditional *bacillus* transformation methods, the RCA1.0 products would give the highest transformation rate, which is of critical importance to *bacillus* gene cloning, expression and library construction.

5. FAQ and Troubleshooting: Please contact us at info@evomicscience.com.

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