# FemtoPhi DNA Amplification Kit (RCA2.0)

Cat#: FP100 or FP1000

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Evomic Science

# User Instruction

# FemtoPhi DNA Amplification Kit (RCA2.0)

Cat#: FP100 or FP1000

**FOR RESEARCH USE ONLY**

FemtoPhi 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 -80°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.

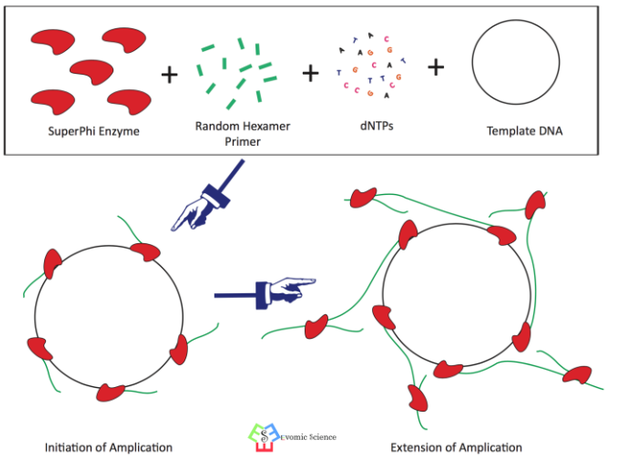



**Fig.1. Schematic diagram of the RCA process.** Random hexamer primers anneal to the circular single strand DNA at multiple sites. Phi29 DNA polymerase extends with highest fidelity. With the progress of reaction, Phi29 will extend on the synthesized DNA and amplify DNA at least thousand folds.

However, there were always around 20% of the amplified DNA product that can't be mapped to any organism in sequence database (**Fig. 2, the upper panel**). Particularly, when single cell

genomic DNA or very low amount of the template DNA, such as less than 10 picogram, was recruited to amplify, this non-specific amplified DNA would represent more than 50% of total amplified DNA. The presence of the non-specific amplified DNA could deteriorate the correct data interpretation, and severely interference the downstream application. It was assumed that the nonspecific RCA products were most likely derived from false priming at sites where primer dimers are formed. To avoid this non-specific DNA amplification due to the false priming in RCA reaction when using random primers, we thus applied protein engineering approach to engineer the C-terminal domain of Phi29 polymerase. This resulting Phi29 polymerase (**Patent pending**) would prevent the extension from the short false primer dimer and significantly decreased the non-specific DNA amplification in RCA reaction, as well as had the better DNA amplification efficiency than wild type Phi29 (**Fig. 2, the lower panel**). Furthermore, the engineered Phi29 had capacity to detect 10 plasmid DNA molecules in 10 microliter of reaction, which is almost 1000 fold sensitive over wild type Phi29. Remarkably, the sensitivity of DNA detection using engineered Phi29 is comparable to PCR or qPCR (**Fig. 3.**). Therefore, this engineered Phi29 would be an ideal enzyme to specifically amplify single cell genomic DNA and low concentration DNA from clinical or environmental samples.

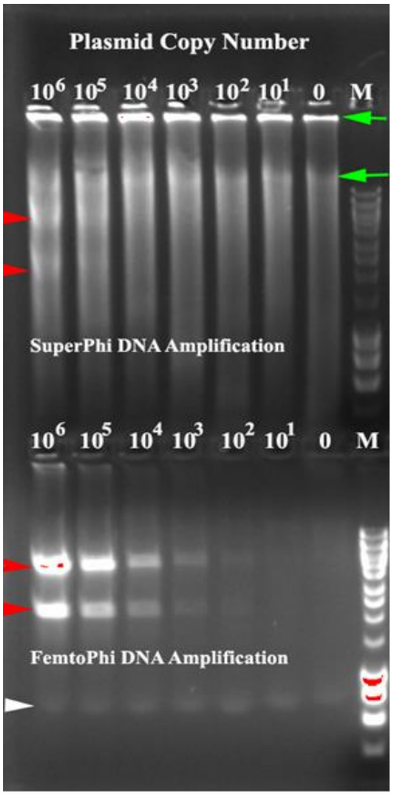



**Fig.2. Comparison of Efficiency and Specificity of SuperPhi DNA Amplification Kit and FemtoPhi DNA Amplification Kit.** The indicated amount of denatured pCT-eGFP (Copy Number) were mixed with the corresponsive reaction buffers and enzymes SuperPhi (upper panel) and FemtoPhi (lower panel), respectively. After 4 hrs at 30°C, the reactions were stopped, digested with *Eco*R1 and then loaded on agarose gel. The **Red Arrowhead** indicated the expected products, while the **Green Arrowhead** pointed the non-specific amplification products. The non-specific primers and left primers (**White Arrowhead**) were found in FemtoPhi DNA amplification reactions (lower panel), but could not be done in SuperPhi DNA Amplification reactions (upper panel). It was assumed that the non-specific primers were used to amplify DNA in SuperPhi Kit, resulting in the smear bands (upper panel). The lowest copy number of plasmid can be detected in FemtoPhi Kit was 10 copies (~0.1 femtogram), while $10^5$ copies (~1 picogram) in SuperPhi Kit. The $10^6$ copies of plasmid are equivalent to 10 picogram.

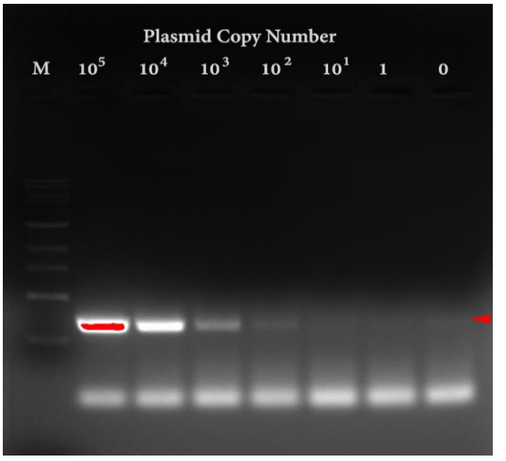



**Fig.3. The lowest copies can be detected by PCR**. The indicated amount of plasmid were input in PCR reactions. The PCR products were loaded on agarose gel. The expected products were pointed with the Red Arrowhead. The lowest copies of plasmid detected by PCR were around 100.

## Features of FemtoPhi DNA Amplification Kits

- FemtoPhi29 DNA polymerase for isothermal multiple displacement amplification (MDA);
- Greater accuracy than existing PCR and other MDA methods;
- Ease with minimal handing time: 15~20 min for up to 96 samples;
- Easily automated;
- Yield up to 4 μg of highly pure DNA.
- Able to detect down to 1 femtogram 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,
    - Gemonic DNA.
- 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;
- Clean manufacturing process ensures components are free of detectable DNA contamination.

## Handling

This kit is used to amplify femtogram 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 FemtoPhi Enzyme on ice and keep them in ice all the time.

## Quality Control

Each batch of FemtoPhi 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.

## Reagent and Equipment to be Supplied by the Users

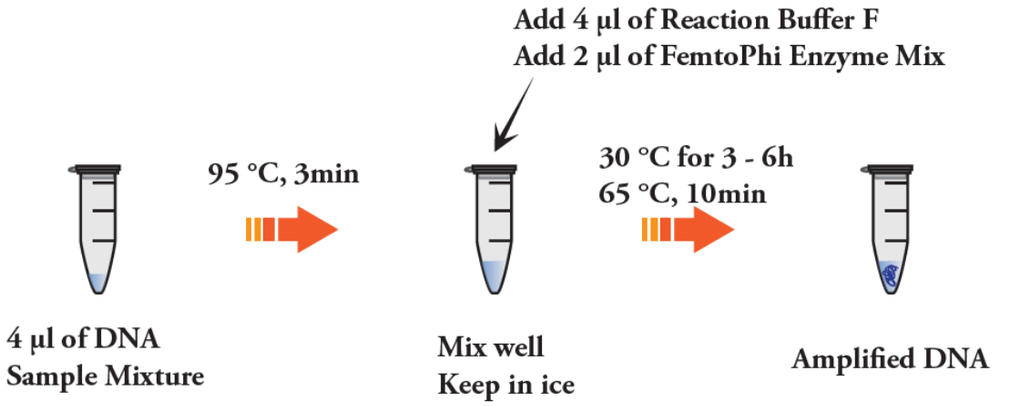
- Sterile vials, pipettes and pipette tips
- Microcentrifuge
- Cold block
- Sterile, ideally DNA-free certified 0.2 ml PCR tubes
- Thermocycler
- Vortexer

## Kit Components:

| Components | 100 Reactions (Cat#: FP100) | 1000 Reaction (Cat#: FP1000) | Storage |
|---|---|---|---|
| **Sample Buffer** | 600 µl | 10x 600 µl | -20°C |
| **Reaction Buffer** | 400 µl | 10x 400 µl | -20°C |
| **FemtoPhi Enzyme** | 200 µl | 10x 200 µl | -80°C |

## Detailed Protocol

FemtoPhi DNA Amplification Kit has capacity to detect as low as 1 femtogram of circular DNA under optimal condition. 0.1~0.5 µl of saturated overnight bacterial culture or 1/100 to 1/1000 of the bacterial colony (approximately 10 ~$10^4$ cells) would be enough for FemtoPhi DNA amplification reaction. Please keep in mind that excess culture or colony will inhibit FemtoPhi 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. The following chart outlined the procedures of using this kit.



# 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 4 µl of Reaction Buffer and 2 µl of FemtoPhi Enzyme to the above PCR tube contains 4 µl of Sample Mix as showed below:

| Component | Volume/Reaction |
|---|---|
| Sample Mix | 4 µl |
| Reaction Buffer | 4 µl |
| FemtoPhi Enzyme | 2 µl |
| Final Volume | 10 µl |

The final volume is 10 µl. Mix well and incubate at 30 ˚C for 3~18 h. One can prepare the master mix of Reaction Buffer and FemtoPhi Enzyme if multiple reactions will be run.

## 3. Inactivate FemtoPhi: 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 can be used to transform *Bacillus* after diluted or purified. Compared to the traditional *Bacillus* transformation methods, the FemtoPhi product would give the highest transformation rate, which is of critical importance to *Bacillus* gene cloning, expression and library construction. Please note that, the FemtoPhi product cannot mix with *Bacillus* without further dilution or purification due to its toxicity to the cell. If you do need large amount of RCA product for *Bacillus* transformation, please follow the protocol of "Purification of RCA product" described below. Alternatively, we recommend our SuperPhi DNA Amplification Kit (Cat#: Phi100) which does not contain the toxic components, and the SuperPhi product can be directly transformed to *Bacillus*, without additional handling.

## 5. Option: Purification of RCA product

1. Please use the following protocol, in case you need further purify RCA product prior to usage.
2. Mix equal volume of FemtoPhi product with PEG-NaCl solution (20% PEG8000 in 2.5M NaCl) in a 1.5ml tube; tapping the tube until the white cloudy precipitate comes out from the solution. Incubation at 37 ˚C for 15min if you didn't see the precipitate.
3. Spin for 1 min to pellet the DNA and remove the supernatant completely but not disturb the pellet; wash the pellet by 500 µl PEG-NaCl once.
4. Dissolve the pellet by at least one volume of TE buffer or water; tap and sit at room temperature or 37 ˚C overnight to dissolve. No pipette, no vertex.

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

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