.

FemtoPhi™ RCA Premix Kit with Random Primers (2x)

Cat#: FM100 or FM1000

FOR RESEARCH USE ONLY

FemtoPhi RCA Premix 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

Description

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.

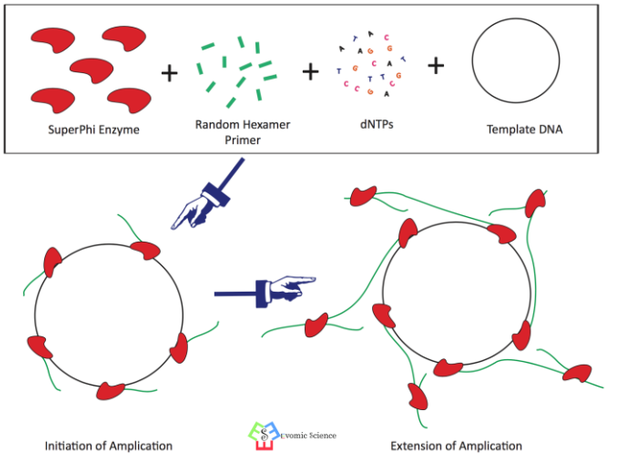


Fig.1. Schematic diagram of the RCA process. Random hexamer primers anneal to the circular single strand DNA at multiple sites. Phi29 DNA polymerase extends with highest fidelity. With the progress of reaction, Phi29 will extend on the synthesized DNA and amplify DNA at least thousand folds.

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.

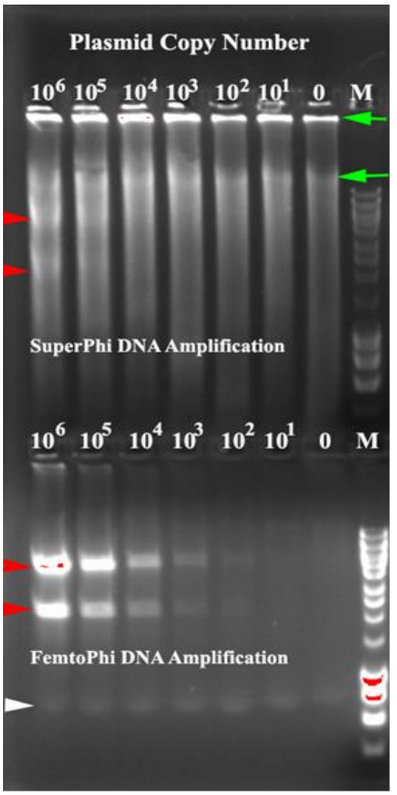
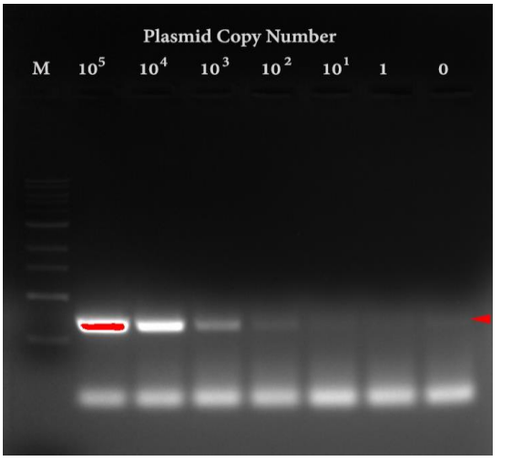


Fig.2. Comparison of Efficiency and Specificity of PicoPhi DNA Amplification Kit and FemtoPhi DNA Amplification Kit. The indicated amount of denatured pCT-eGFP (Copy Number) were mixed with the corresponsive reaction buffers and enzymes PicoPhi (upper panel) and FemtoPhi (lower panel), respectively. 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 PicoPhi DNA Amplification reactions (upper panel). It was assumed that the non-specific primers were used to amplify DNA in PicoPhi 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 PicoPhi 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.



FemtoPhi RCA Premix Kit (Cat#: FM100) is a 2x version premix of FemtoPhi RCA kit, containing all components necessary for a successful and reliable **Rolling Circle Amplification (RCA)**, such as *FemtoPhi* DNA Polymerase, dNTPs, and random primers etc, except circular DNA template. This premixed formulation saves time and reduces contamination due to a reduced number of pipetting steps required for RCA set up. *FemtoPhi* RCA Premix Ki has capacity to detect 10 femtogram of plasmid or genomic DNA molecules in 10 microliter of reaction, which is almost 1000 fold sensitive over wild type Phi29 enzyme. Remarkably, the sensitivity of DNA detection using *FemtoPhi* is comparable to PCR or qPCR. Therefore, *FemtoPhi* would be an ideal enzyme to specifically amplify single cell genomic DNA and low concentration DNA from clinical or environmental samples.

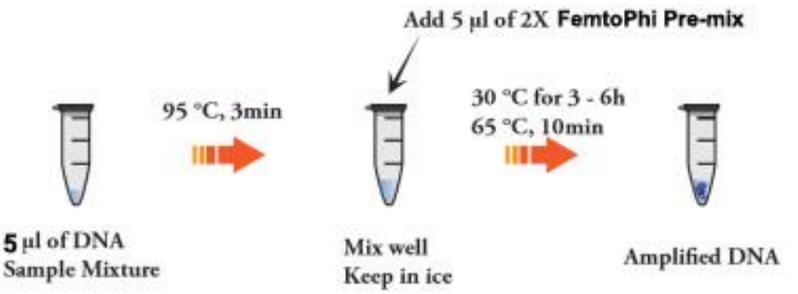
Kit Components:

Components	100 Reactions (Cat#: FM100)	1000 Reaction (Cat#: FM1000)	Storage
Sample Buffer	800 µl	10x 800 µl	-20°C
2x FemtoPhi Premix	500 µl	10x 500 µl	<-20°C

Detailed Protocol

FemtoPhi RCA Pre-mix Kit has capacity to detect as low as 10 femtogram 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 FemtoPhi RCA reaction. Please keep in mind that excess culture or colony will inhibit FemtoPhi RCA 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. Procedures of *FemtoPhi* RCA reaction were outlined below.



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 4 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 5 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 4.5~4.8 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 4.5~4.8 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 *FemtoPhi* pre-mix to the above PCR tube contains 5 μl of Sample Mix as showed below:

Component	**Volume/reaction**
Sample Mix with denatured DNA	5 μl
FemtoPhi Premix	5 μl
Final Volume	**10 μl**

Mix well and incubate at 30 ˚C for 3~24 hrs.

Note: Actually, mixing any equal volume of sample mix and FemtoPhi Pre-mix will work fine.

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, 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.**