Evomic Science

FemtoPhi™ Single Cell Whole Genome Amplification Kit

Cat#: WGA25

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Instruction for
FemtoPhi® Single Cell Whole Genome Amplification Kit

Basic Principles

The DNA polymerase Phi29 based Rolling Circle Amplification (RCA) technology is one of the most powerful tools for whole genome DNA amplification. While using current commercial RCA kits, ~20% of total DNA products can't be mapped to any organism in the sequence database (**Fig. 1, Upper Panel**). Particularly, when single cell genomic DNA or very low amount of the template DNA is recruited, the non-specific DNA would represent >50% of the total product. The presence of these non-specific DNA could deteriorate the data interpretation, and severely interfere with the downstream application. It is assumed that the non-specific products are most likely derived from the false priming from primer dimmer at the C-terminal domain of Phi29 polymerase. To avoid this type of non-specific amplification using random primers in RCA reaction, we thus seek for protein engineering approach to modify the C-terminal domain of Phi29 polymerase. The effort eventually brought us the improved Phi29 polymerase, named FemtoPhi™ (**Patent pending**). The modified enzyme can prevent the extension from the short false primer dimers, thus significantly decreases the non-specific DNA amplification, as well as remarkably improves the DNA amplification efficiency (**Fig. 1**, **Lower Panel**). Since FemtoPhi™ still keeps the proofreading of 3'→5' exonuclease activity, the DNA replication is extremely accurate.

Our FemtoPhi™ Single Cell Whole Genome Amplification(WGA) Kit is optimized for whole genome DNA amplification from single cells, including bacteria, yeast, fungi, mammalian cells, and plant cells (without cell wall). Briefly, single cell is directly lysed and the released genomic DNA is denatured, and then neutralized. Whole genome released from the single cell is then amplified by isothermal strand displacement using FemtoPhi™. Unlike other WGA kits, no detectable DNA was produced in the negative control during the 10 hour reactions in our FemtoPhi™ Kit, indicating all DNA products were truly from the template DNA but not from the non-specific amplification. Purified intact genomic DNA can be amplified using this kit, too. DNA from other samples, including dried blood cards, buccal cells, tissue, serum, plasma, laser-micro-dissected cells and plant cells can also be used for FemtoPhi™ amplification, although in some cases, may need pre-treatment or repairing for efficient amplification.

Kit Specifications

In general, microgram quantities of DNA would be generated from less than picogram amounts of starting material within four hours in FemtoPhi™ reaction. Typical DNA yields are 10~30 µg per 40 µl reaction when starting with a single mammalian cell. Kinetics may vary if crude or un-quantified samples are amplified, while increasing the reaction time may be helpful for certain type of samples such as bacterial cells.

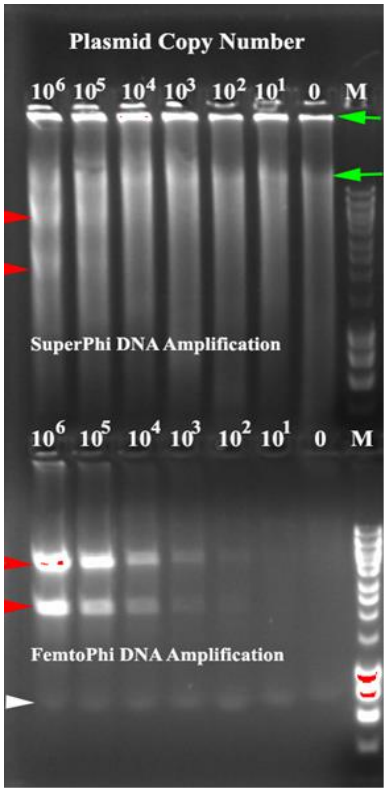

Fig.1. Comparison of Efficiency and Specificity of wildtype SuperPhi29 and the engineered FemtoPhi™ for DNA Amplification.

The indicated amount (copy number) of denatured pCT-eGFP plasmid were mixed with the corresponsive kits: SuperPhi (upper panel) and FemtoPhi™ (lower panel), respectively. The final reaction products were directly digested with *Eco*RI and then loaded on agarose gel. The **Red Arrows** indicated the expected products, while the **Green Arrows** pointed the non-specific products. The non-specific and leftover primers (**White Arrowhead**) were found in FemtoPhi™ reactions (lower panel), but not in SuperPhi reactions (upper panel). It was assumed that the non-specific primers were used to amplify DNA in SuperPhi reaction, resulting in the smear bands (upper panel). The least plasmid copy number can be detected in FemtoPhi™ reaction was 10 copies of plasmid DNA (~0.1 fg), while 10^5 copies (~1 pg) in SuperPhi reaction. The 10^6 copies of plasmid DNA are equivalent to 10pg.

Kit Components

FemtoPhi™ Single Cell Whole Genome Amplification Kit

Cat#: WGA25	Volume	Cap Color	Storage
Denaturation Buffer*	800 µl	Clear	-20ºC
Neutralization Buffer	800 µl	Yellow	-20ºC
Reaction Buffer W**	400 µl	Green	-20ºC
FemtoPhi™ Enzyme Mix	100 µl	Red	-80ºC

* Denaturation Buffer is corrosive and harmful with potassium hydroxide (KOH). Risk and safety phrases: R22–35. S26–36/37/39–45.

** Vortex Reaction Buffer W before use.

Storage Condition and Expiration Date

FemtoPhi™ enzyme mix must be stored at -80ºC, thaw the tube on ice and maintain at 0°C to 4°C during handling; store other components at -20°C. This product has been designed to deliver high quality results for up to 24 months (-80ºC) from the manufacturing date. Please refer to the expiration date on the product label.

Quality and Caution

All ingredients in this kit have been UV exposed to breakdown the contaminated DNA and avoid non-specific product during DNA amplification.

Since this kit has capacity to amplify single copy of genomic DNA or a few copies of circular DNA, please handle all components and your samples carefully to prevent any cross contamination. The DNA-free environment or hood would be preferred to work.

Reagents/ Equipment Supplied by User

- Liquid-handling supplies: Sterile vials and pipette tips; pipettes, microcentrifuge.
- Water: Use molecular biology grade water (DNases-free & nucleic acid free).
- Ice bucket: Maintaining FemtoPhi™ reagents at 4ºC during the experiment setup.
- Perform all reactions in sterile 0.2 ml PCR tubes or 96-well PCR plates.
- Thermocycler or Real-time qPCR instrument: for incubation at 30ºC and 65ºC.
- DNA/ microbiome-free hood or work environment is preferred.

Protocol

This kit is suitable for whole genome amplification of single cell from all species, including bacteria, yeast, fungi, plant (without cell wall) and mammalian cells. Purified genomic DNA or single cell (without genomic DNA extract or isolation), can be directly applied to this kit. The intact genomic DNA is essential for successful amplification using this kit. Apoptotic cells and single cell with formalin or other cross-linking agents fixed is not recommended. DNA from these damaged cells need to further repair for amplification (See our RCA product list for more information).

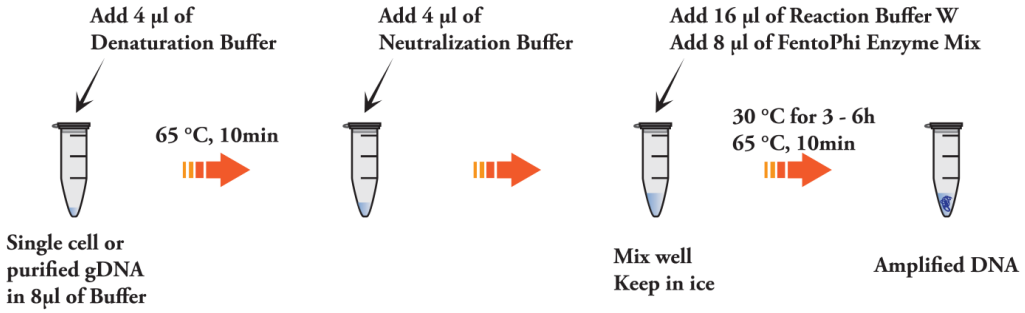

Fig. 2. Schematic Representation of FemtoPhi™ Single Cell WGA Kit Protocol.

The above steps describe a general protocol for amplifying DNA with this kit (**Fig. 2**). This protocol should be considered a starting point for optimizing the reaction in your laboratory, based on your application. Specially, for bacteria with thick cell wall, pretreatment with appropriate enzyme to break down the cell wall, may help to release the genomic DNA.

Procedures:

1. Sample Dilution:

1) **Purified genomic DNA:** Dilute genomic DNA to ~10 pg (from single mammalian cell) in 8 µl of Tris-HCl Buffer (pH8.0) in 0.2 ml PCR tube.
2) **Cells isolated using dilution method:** Count and dilute cells with 1x PBS buffer to the required final concentration (1~1000 cells/8 µl of 1x PBS buffer) in 0.2 ml PCR tube.
3) **Cells isolated using FACS:** Sort single cell to 96-well plate and bring total volume to 8 µl with 1x PBS buffer.

2. Sample Mixture:

Always keep volume ratio of Sample: Denaturation Buffer: Neutralization Buffer as **2:1:1.**

1) Add 4 µl of Denaturation Buffer to 8 µl of sample in PCR tube;
2) Briefly centrifuge the tube or plate and then incubate at 65°C for 10 minutes;
   Note: Ensure that the cell material does not stick to the tube or well wall above the buffer line; Exceeding the incubation time or temperature may nick the template DNA.
3) Add 4 µl of Neutralization Buffer and keep this Sample Mixture (total 16 µl) on ice.
   Note: For gram-positive bacteria with thick cell wall, pretreatment with appropriate enzymes to break down the cell wall, may help to release the bacterial genomic DNA.

3. DNA Amplification:

- Add 16 µl of Reaction Buffer W and 8 µl of FemtoPhi™ Enzyme Mix as showed below:
- Mix well and incubate at 30˚C for 3~18 h.

Component	Volume/reaction
Sample Mixture	16 µl
Reaction Buffer W	16 µl
FemtoPhi™ Enzyme Mix	8 µl
Final Volume	40 µl

Note: One can scale up the master mixture of Reaction Buffer W and FemtoPhi™ Enzyme Mix for multiple reactions with extra 10% of total volume. The final volume for each reaction is 40 µl.

4. Inactivate FemtoPhi™:

- Incubate at 65 ˚C for 10 min, and then cool to 4 ˚C.

5. Perform Downstream Applications:

- Keep the amplified DNA at 4°C for short-term storage or –20°C for long-term storage;
- The amplified DNA can be directly used for NGS, array CGH, and qPCR etc.;
- The amplified DNA can be directly used for the Sanger's cycle sequencing reaction;
- The amplified DNA can be directly used for DNA restriction enzyme digestion;
- An aliquot of the amplified DNA can be examined by agarose gel.

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