TempliPhi

DNA Amplification Kit

Amersham Biosciences

Convenient protocol

for Template Preparation

63-0046-15 / Rev AB / 1-03

Rapid and simple

TempliPhi™ DNA Sequencing Template Amplification Kits are designed to prepare circular DNA templates for direct use in cycle sequencing with no need for further purification. Kits are available for 100, 500 and 10 000 reactions and utilize bacteriophage Phi29 DNA polymerase and rolling circle amplification (RCA) technology for rapid amplification of circular template DNA. This isothermal amplification produces microgram quantities of DNA from picograms of starting material in 4-6 h. *In vitro* amplification of very small amounts of template DNA eliminates the need for overnight cell culture and conventional plasmid or M13 preparations.

TempliPhi DNA Amplification Kits offer:

- **Convenience and ease of use**
  One protocol for DNA amplification of most samples eliminates the need for multiple protocols and kits. Starting materials that can be used include 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.

- **Quality, reproducibility and time savings**
  Yields up to 1.0–1.5 µg of highly pure DNA in 4–6 h from 1 to 100 pg DNA starting material.

- **Minimal hands on time**
  Total hands on time of 15–20 min for sample size of 1–96. Amplified DNA can be used directly in any cycle sequencing reaction without further purification.

- **Improved results**
  Can improve sequencing pass rates and readlengths when compared to samples prepared by traditional methods.

- **Increased efficiency**
  Increases throughput by allowing DNA template preparation and sequencing to be completed in one day.

- **Cost savings**
  Eliminates the need for growing liquid bacterial cultures, for special equipment and is easily automated. DNA amplifications are performed at 30 °C and DNA can be used directly without any processing.

- **Compatibility**
  DNA amplified with TempliPhi Kits can be used with both DYEnamic™ ET Terminator and ABI PRISM™ BigDye™ sequencing chemistries on all MegaBACE™ and ABI PRISM DNA analysis platforms.

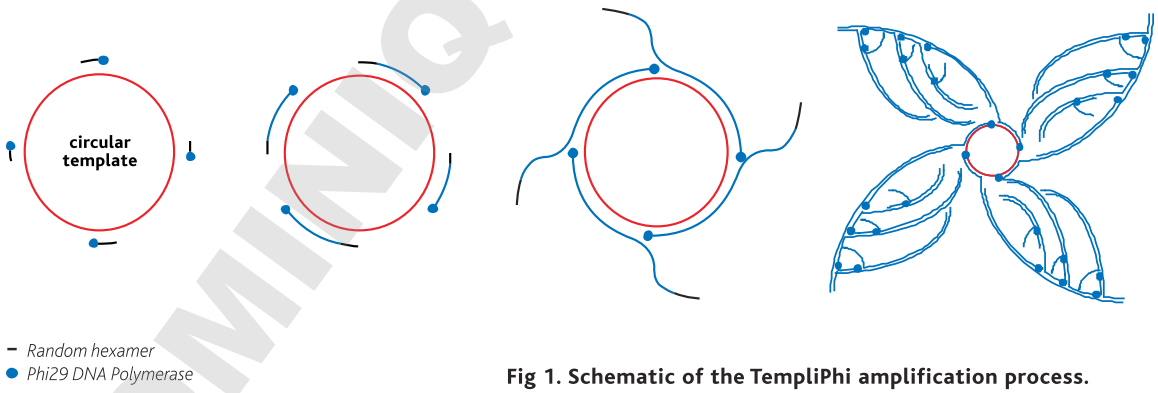

Fig 1. Schematic of the TempliPhi amplification process.
Random hexamer primers anneal to the circular template DNA at multiple sites. Phi29 DNA polymerase extends each of these primers. When the DNA polymerase reaches a downstream-extended primer, strand displacement synthesis occurs. The displaced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

protocol for preparing DNA templates

Rolling Circle Amplification Templates are effectively prepared by rolling circle amplification, or RCA (Fig 1). The starting material for amplification can be a small amount of bacterial cells containing a plasmid, an isolated plasmid, an M13 phage, or any circular DNA sample. A small portion of bacterial colonies can be picked from agar plates and added directly to the TempliPhi amplification reaction. Alternately, microliter quantities of a saturated culture can serve as starting material. With TempliPhi 100/500 Kits, amplification is completed in 4-6 h at 30 °C without the need for thermal cycling (Fig 2). The TempliPhi amplification reaction produces high molecular weight, double-stranded concatemers of circular template. Note that when starting with M13 clones, the TempliPhi amplification product is double-stranded DNA and can be directly sequenced with forward and reverse primers. Amplified DNA can be used in a cycle sequencing reaction without any purification.

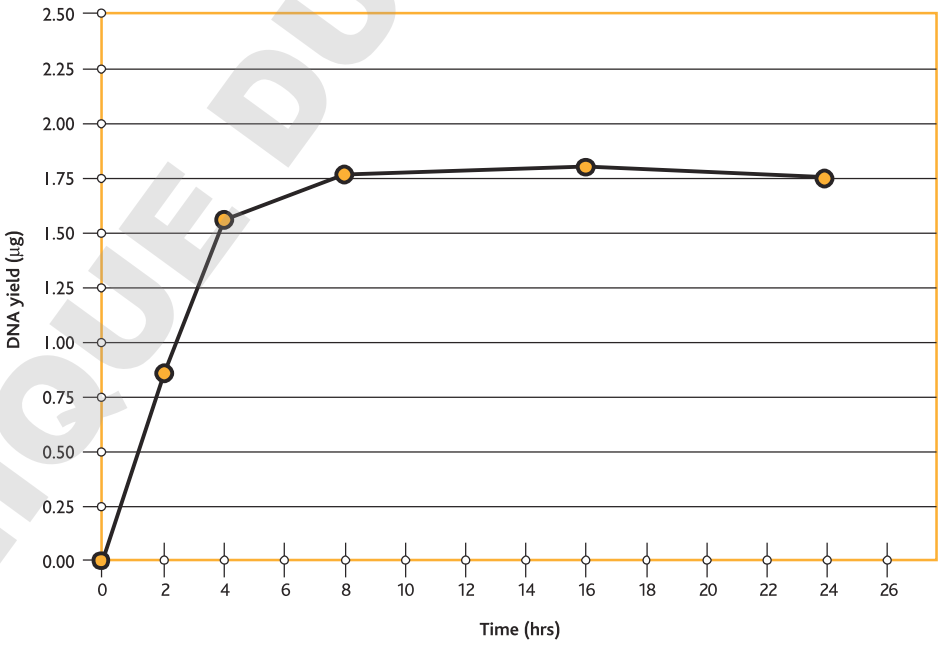

Fig 2. Kinetics of DNA amplification using TempliPhi 100 Amplification Kit.
Amplification of 1ng pUC19 DNA over 24 hrs. The amount of DNA was quantitated using PicoGreen™ dsDNA Quantitation Reagents at the given times. Data is representative of triplicate experiment.

Product use and kit contents Two different types of TempliPhi DNA Amplification Kits are available to meet different throughput requirements (Fig 3). TempliPhi DNA Sequencing Template Amplification Kit for 10 000 reactions contains premixed components and generates templates after overnight incubation. The 100 and 500 reaction size kits contain separate, unmixed components for enhanced stability. All TempliPhi Kits streamline the template preparation process (Fig 4).

flexibility...

TempliPhi Amplification Kits were designed to efficiently prepare DNA sequencing templates, but the isothermal process has been found useful for other applications. For instance, plasmid DNA amplified with TempliPhi Kits can be conveniently transformed into bacteria–without an additional plasmid preparation step. And a modified protocol for bacterial artificial chromosome (BAC) DNA preparation using TempliPhi 100 and 500 Amplification Kits is efficient, streamlined, and generates up to 15 µg of DNA.

	Sample Buffer		Reaction Buffer		Enzyme Mix		Positive Control pUC19 DNA	
	Quantity	*Storage*	*Quantity*	*Storage*	*Quantity*	*Storage*	*Quantity*	*Storage*
100 Reaction Kit	1 x 0.5 ml	-20 °C (1 month) or -70 °C (12 months)	1 x 0.5 ml	-20 °C (1 month) or -70 °C (6 months)	1 x 20 µl	-20 °C (1 month) or -70 °C (6 months)	1 x 50 µl, 2 ng/µl	-20 °C (12 month) or -70 °C (12 months)
500 Reaction Kit	5 x 0.5 ml	-20 °C (1 month) or -70 °C (12 months)	5 x 0.5 ml	-20 °C (1 month) or -70 °C (6 months)	5 x 20 µl	-20 °C (1 month) or -70 °C (6 months)	1 x 50 µl, 2 ng/µl	-20 °C (1 month) or -70 °C (12 months)

	TempliPhi Premix		Denature Buffer		Positive Control pUC19 DNA	
	Quantity	*Storage*	*Quantity*	*Storage*	*Quantity*	*Storage*
10 000 Reaction Kit	5 x 20 ml	-70 °C (6 months)	5 x 20 ml	-20 °C or -70 °C (12 months)	1 x 50 µl 2 ng/ul	-20 °C or -70 °C (12 months)

Fig 3. Components of each kit.

Alkaline lysis method

- Pick colony into medium, grow 16 h
- Centrifuge to pellet cells
- Remove supernatant
- Add TE
- Add NaOH/SDS
- Add potassium acetate
- Centrifuge
- Remove supernatant to new tube
- Precipitate DNA
- Resuspend in TE
- Remove aliquot to sequencing reaction

TempliPhi 10 000 Reaction Kit method

- Transfer <1µl of sample into 10 µl of Denature Buffer
- Denature at 95 ˚C for 3 min. Cool samples to room temperature or 4 ˚C
- Add 10 µl of TempliPhi Premix
- Incubate at 30 ˚C for at least 16-18 h
- Heat inactivate at 65 ˚C for 10 min Cool to 4 ˚C
- Transfer 1–2 µl (150–300 ng amplified product) directly to a 20 µl cycle sequencing reaction

TempliPhi 100/500 Reaction Kit method

- Transfer ≤ 1 µl of sample into 5 µl of sample buffer
- Denature at 95 ˚C for 3 min. Cool samples to 4 ˚C
- Mix 5 µl of Reaction Buffer with 2 µl Enzyme Mix, and add 5 µl of this mixture to sample
- Incubate at 30 ˚C for at least 4 h
- Heat inactivate at 65 ˚C for 10 min and cool to 4 ˚C
- Transfer 1-2 µl (150–300 ng amplified product) directly to a 20 µl standard cycle sequencing reaction

Fig 4. Comparison of TempliPhi protocols with traditional alkaline lysis method for plasmid miniprep. TempliPhi DNA Sequencing Template Amplification Kit uses a simple protocol that requires less than 20 min of hands-on time for amplifying 96 samples from bacterial colonies.

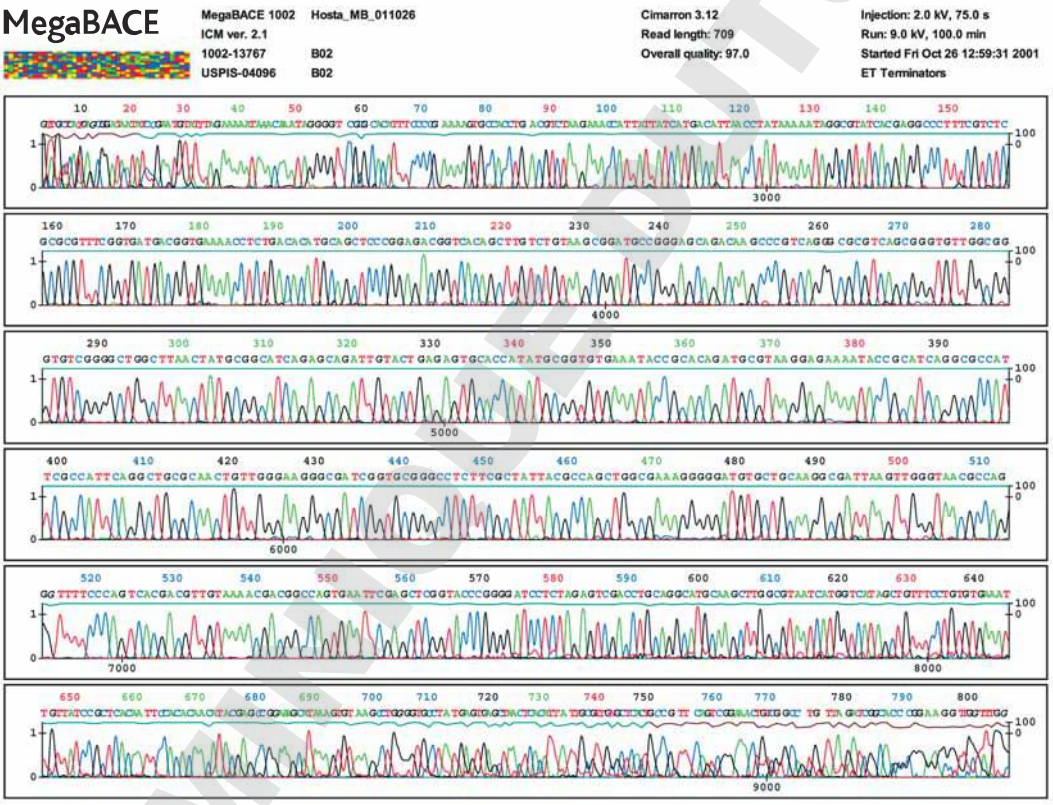

Fig 5. Sequence Performance of TempliPhi amplified DNA on MegaBACE 1000 DNA Analysis System. Template preparation was carried out directly from a single bacterial (DH5α) colony containing plasmid. Using recommended protocols and TempliPhi 100 Amplification Kit, 1 µl of the post-amplification product was used directly in a DYEnamic ET Terminator Cycle Sequencing reaction and analyzed on MegaBACE 1000 DNA Analysis System.

TempliPhi amplified DNA

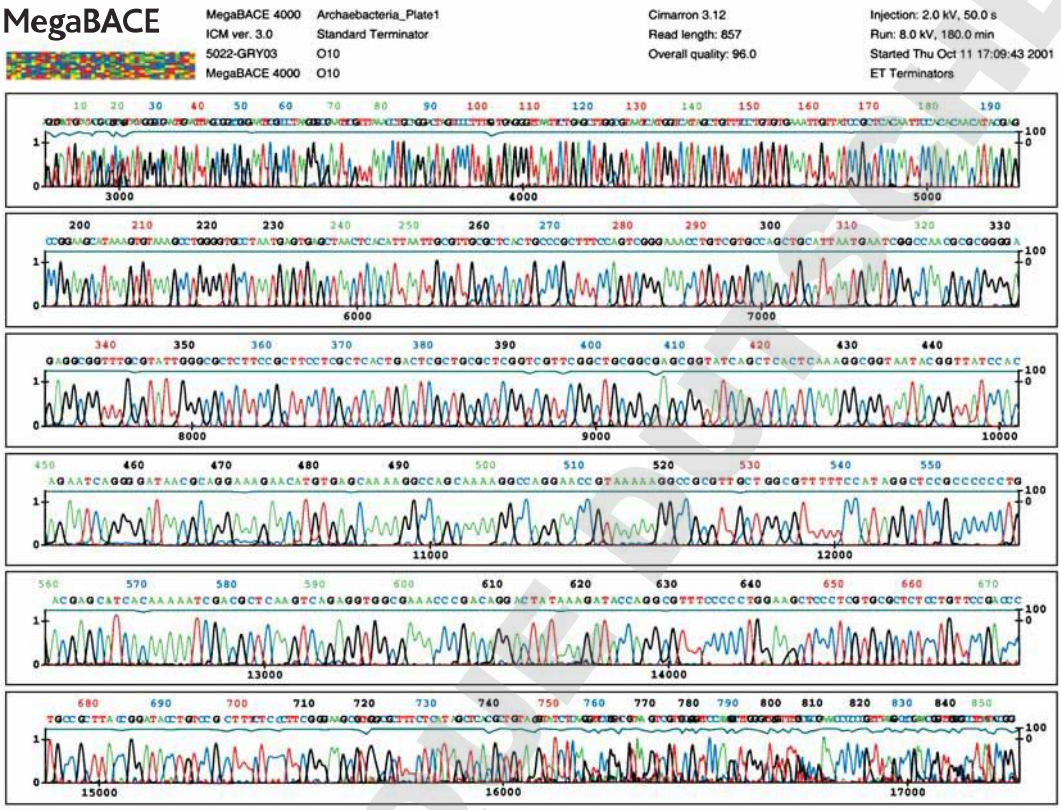

Fig 6. Sequence Performance of TempliPhi amplified DNA on MegaBACE 4000 DNA Analysis System. Template preparation was carried out directly from a 10% glycerol stock containing double stranded plasmid. Using recommended protocols and TempliPhi 10 000 Amplification Kit, 3 µl of the post-amplification product was used directly in a DYEnamic ET Terminator Cycle Sequencing reaction and analyzed on MegaBACE 4000 DNA Analysis System.

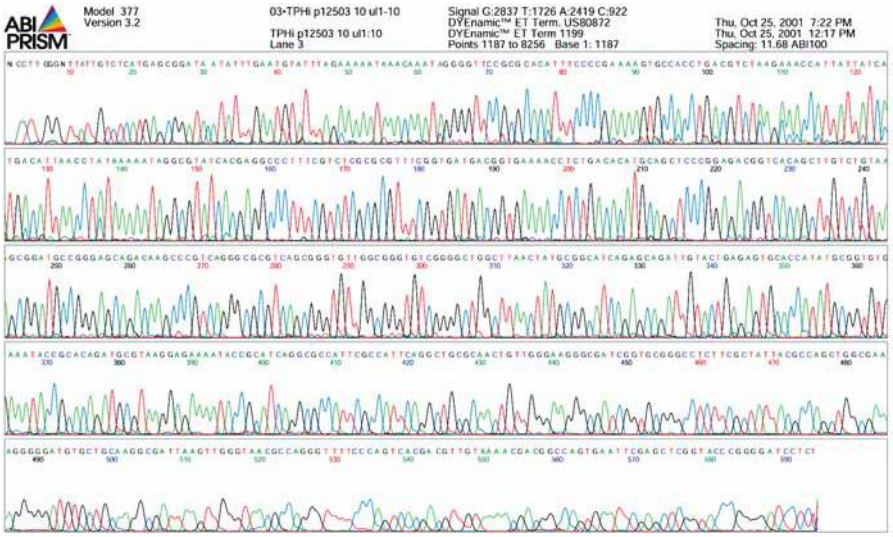

Fig 7. Sequence Performance of TempliPhi amplified DNA on ABI PRISM 377 DNA Sequencer. Template preparation was carried out directly from a single bacterial (DH5α) colony containing plasmid. Using recommended protocols and TempliPhi 100 Amplification Kit, 1 µl of the post-amplification product was used directly in a DYEnamic ET Terminator Cycle Sequencing reaction and analyzed on ABI PRISM 377 DNA Sequencer.

Fig 8. Sequence Performance of TempliPhi amplified DNA on ABI PRISM 3100 Genetic Analyzer. Template preparation was carried out directly from a single bacterial (DH5α) colony containing plasmid. Using recommended protocols and TempliPhi 100 Amplification Kit, 1µl of the post-amplification product was used directly in a DYEnamic ET Terminator Cycle Sequencing reaction and analyzed on ABI PRISM 3100 Genetic Analyzer.

References

1. Dean, F.B., Nelson, J.R., Giesler, T.L., and Lasken, R.S. 2001. Rapid Amplification of plasmid and phage DNA using Phi29 DNA polymerase and multiply-primed rolling circle amplification. Genome Research **11**:1095-1099.

2. Lizardi, P.M., Huang, X., Zhu, Z., Bray-ward, P., Thomas, D.C., and Ward, D.C. 1998. Mutation detection and single-molecule counting using isothermal rolling-circle amplification. Nature Genetics **19**:225-232.

order information

TempliPhi 100 Amplification Kit / 100 reactions	25-6400-10
TempliPhi 500 Amplification Kit / 500 reactions	25-6400-50
TempliPhi DNA Sequencing Template Amplification Kit / 10 000 reactions	25-6400-01

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