illustra TempliPhi 2000 Reaction Kit

Product Booklet

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1 Introduction

Product code

28964286

Important

Read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

Storage

Store Denature buffer and control DNA at -70°C or -15°C to -30°C. Store TempliPhi™ premix at -70°C. Do not store TempliPhi premix at -15°C to -30°C. Premix that is not frozen solid will lose activity within 5 days. Thaw the premix on ice and maintain at 0°C to 4°C during handling.

Note: *Do not allow premix to warm above 4°C prior to amplification.*

Stability

The TempliPhi premix is stable at -70°C for 6 months from the date of manufacture. The denature buffer and positive control DNA are stable at -70°C or -15°C to -30°C for 12 months from the date of manufacture.

Components

Sufficient for 2000 sequencing template amplification reactions.

TempliPhi premix: 1 × 20 mL

Denature buffer: 10 mM Tris-HCl, pH 8.2, 0.5 mM EDTA, 1 × 20 mL

Positive control pUC19 DNA: 1 × 10 µL, 2 µg/mL

2 Background

illustra™ TempliPhi Kits have been developed specifically to prepare templates for DNA sequencing. The TempliPhi method utilizes bacteriophage φ29 DNA polymerase to exponentially amplify single- or double-stranded circular DNA templates by rolling circle amplification (RCA)(1, 2). This isothermal amplification method can produce microgram quantities of DNA from picograms amounts of starting material in a few hours. Amplification in vitro of very small amounts of template DNA eliminates the need for overnight cell culture and conventional plasmid or M13 DNA purification. The proofreading activity of φ29 DNA polymerase ensures high fidelity DNA replication (3).

The starting material for amplification can be a small amount of bacterial cells containing a plasmid, a purified plasmid or intact M13 phage. Portions of bacterial colonies can be picked from agar plates and added directly to the TempliPhi reaction. Alternately, microliter quantities of saturated bacterial culture or a glycerol stock can serve as starting material. Depending on the quality of the starting material, amplification is completed in 16–18 hours at 30°C, with no

need for thermal cycling. The product of the TempliPhi reaction is high molecular weight, double-stranded concatamers of the input circular template (see the figure below). Note that when working with M13 clones, the TempliPhi product is double-stranded DNA and can be sequenced directly with forward and reverse primers. Amplified DNA can be used directly in a cycle sequencing reaction without any purification.

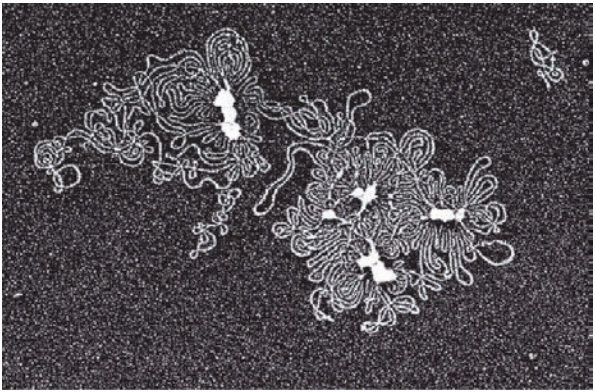


Fig 1. Electron micrograph of nondenatured TempliPhi amplified product. pUC19 DNA (1 ng) was amplified with the TempliPhi 2000 Reaction Kit for 18 hours. For size comparison, two copies of pUC 19 DNA appear in the top right section of the image.

3 Protocols

Overview

The illustra TempliPhi 2000 Reaction Kit consists primarily of two components, denature buffer and TempliPhi premix. The TempliPhi premix contains all of the components necessary for amplification, and is adjusted to a pH that supports DNA synthesis. Random hexamer primers anneal to the circular template DNA at multiple sites, and φ29 DNA polymerase extends each of these primers. When the DNA polymerase reaches a downstream extended primer, strand displacement occurs. This displaced strand is rendered singlestranded and available for priming by more hexamers. The process

continues, resulting in exponential, isothermal amplification, until the deoxynucleotides in the TempliPhi premix are consumed. When amplification is complete, approximately 3.5 µg of DNA is produced that can be used directly in a sequencing reaction (see the figure below).

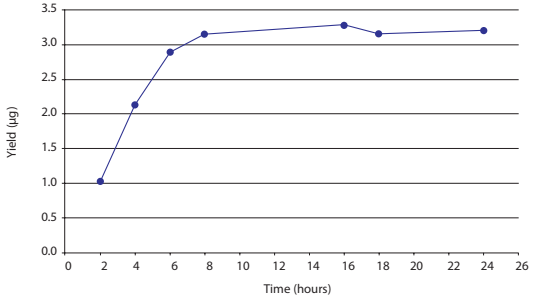


Fig 2. Typical amplification kinetics with illustra TempliPhi 2000 Reaction Kit. Amplification of pUC19 DNA (1 ng) for 24 hours. DNA yield was determined with PicoGreen™ double-stranded DNA quantitation reagent (Molecular Probes, Inc.). Amplification is typically complete after 16 hours and the DNA yield is approximately 3.5 µg.

TempliPhi DNA amplification

The steps outlined below describe a general protocol to amplify sequencing templates. This protocol is a starting point for optimizing the reaction in your laboratory. Where appropriate, notes within the protocol indicate parameters that can be further investigated for your particular templates and workflow. These notes also provide warnings about limitations of the method.

Step	Action
1	Thaw the TempliPhi premix and denature buffer on ice. The TempliPhi premix can require up to 6 hours to thaw. Thaw the premix overnight on ice in a refrigerator; do not thaw the premix at temperatures above 0°C. Do not allow the premix to warm above 4°C prior to amplification. Gently vortex the TempliPhi premix and denature buffer prior to dispensing to ensure thorough mixing.
2	Dispense 10 µL aliquots of denature buffer into an appropriate reaction tube or plate. The vessel must be capable of being capped or sealed to avoid evaporation during subsequent steps and for storage of the amplified product.

Step	Action
3	Transfer samples to the dispensed denature buffer. Follow the recommendations below based on the starting material.

Material	Recommendations
Liquid bacterial cultures	Transfer 0.2–0.5 µL of saturated bacterial culture directly to the dispensed denature buffer. Transfer only a small amount of the culture — components of saturated culture media can inhibit the TempliPhi reaction. Cap the tube or seal the plate. Proceed to step 4. ***Note:*** *The TempliPhi method is very sensitive to inhibitors — it is crucial to add the smallest possible volume of culture or supernatant to the denature buffer to prevent inhibition of the TempliPhi reaction from growth by-products. Amplification is faster and more reproducible when template DNA is prepared from cultures not grown in extremely rich growth media. LB media supplemented with the appropriate antibiotic is recommended. If the source culture was grown to high cell density in a rich medium, dilute the culture 10- to 100-fold in water or TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) and transfer 1 µL of this diluted material to the denature buffer.*

Material	Recommendations
Bacterial colonies	Transfer a small portion of a colony directly into the dispensed denature buffer. A gentle touch with a sterile straight needle or toothpick on the colony surface provides sufficient starting material. Avoid transferring excess cell material or agar from the plate as they can inhibit the TempliPhi reaction. Cap the tube or seal the plate. Proceed to step 4. ***Note:*** *Attempt to transfer one-tenth to one-one-hundreth of a colony (approximately 10^2–10^4 cells). It is crucial not to transfer too much material to the TempliPhi reaction. Use of more than 10^5 cells will decrease the TempliPhi reaction rate and produce incomplete reactions. Alternately, transfer cell material to a tube containing 50 µL of TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) or water, vortex, and transfer 0.2–0.5 µL of the suspension to the dispensed denature buffer.*
M13 phage in liquid cultures	Transfer 0.2–0.5 µL of phage supernatant directly into the denature buffer. Cap the tube or seal the plate. Proceed to step 4.
M13 plaques	Transfer a minute portion of the plaque using a sterile needle or toothpick into the dispensed denature buffer. A gentle touch of the plaque with a needle provides sufficient starting material. Transferring the entire plaque into the denature buffer might inhibit the amplification reaction due to carryover of excess cellular debris and/or agar. Cap the tube or seal the plate. Proceed to step 4.

Material	Recommendations
Bacterial glycerol stocks	Dilute 1 µL of the glycerol stock into 50 µL of TE (10 mMTris-HCl, pH 8.0, 1 mM EDTA) or water. Transfer 0.2–0.5 µL of diluted stock to the dispensed denature buffer. Cap the tube or seal the plate. Proceed to step 4.
Purified plasmid or M13 samples	Add between 1 pg–10 ng of plasmid or M13 DNA suspended in sterile water or TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) into the dispensed denature buffer. Cap the tube or seal the plate. Proceed to step 4.

4 Denature the sample. Heat the sealed plate or tube at 95°C for 3 minutes and then cool to room temperature or 4°C. The rate of cooling is not crucial.

Note:
This step partially lyses bacterial cells or phage particles sufficiently to release the circular template into the denature buffer. Avoid heating at higher temperatures or for longer times, which can release bacterial chromosomal DNA that competes with the desired template during amplification. Overheating can also nick DNA, which can decrease the efficiency of rolling circle amplification.

Step	Action
5	Add 10 µL of TempliPhi premix to the cooled sample. Mix briefly by vortexing. ***Note:*** *Store the TempliPhi premix at -70°C until ready for use. Thaw the premix on ice; do not thaw at temperatures above 0°C. The premix might require 6 hours to thaw; premix can be thawed overnight on ice in a refrigerator. Do not allow the premix to warm above 4°C prior to the incubation described in step 6 because φ29 DNA polymerase is active at 5°C. Avoid multiple freezingthawing cycles. The TempliPhi premix is stable at 4°C for up to 24 hours.* ***Note:*** *The TempliPhi premix contains φ29 DNA polymerase and all the components sufficient to generate large quantities of DNA. Incubation of premix alone will generate nonspecific DNA. (Please refer to "No sequencing result" discussion within Chapter 5 Troubleshooting guide, on page 21).* ***Note:*** *The concentrations of the premix components are designed to provide optimal performance when used as specified in this protocol. Dilution or volume reduction of the TempliPhi premix are not supported and will adversely impact results.*

Step	Action
6	Seal the tube or plate and incubate at 30°C for 16–18 hours. ***Note:*** *If minimal amounts of inhibitory material were transferred along with the template, the reaction will typically be complete after a 16–18 hour incubation and produce approximately 3.5 µg of DNA. The reaction will cease to generate more product when the deoxynucleotides are consumed. After a 4 hour incubation, > 1.5 µg of DNA should be produced. Use a double-stranded DNA quantitation reagent such as PicoGreen to quantify product yield. Do not quantify DNA yield by UV absorption at 260 nm - unused hexamers and nucleotides present in the reaction will contribute to the absorption measurement.*
7	Heat-inactivate the enzyme by incubating at 65°C for 10 minutes. Cool to 4°C. ***Note:*** *Heating prevents degradation of template DNA during storage by inactivating the proofreading exonuclease activity of φ29 DNA polymerase. It also prevents potential interference of the polymerase with subsequent cycle sequencing reactions. The heat inactivation step can be eliminated if the amplified DNA samples will not be stored prior to sequencing.*

Step	Action
8	Perform cycle sequencing. The amplified DNA does not require further purification. Due to the viscous nature of the amplified product, a dilution step is recommended prior to transfer. Add 40 µL of water or TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) to the amplified product and mix well by pipetting up and down, or vortexing if required. After mixing, transfer 2–5 µL (100–250 ng) of diluted sample per 20 µL sequencing reaction. If the incubation period was less than 12 hours, add 4–8 µL of diluted product. ***Note:*** *The amount of amplified product to use in a sequencing reaction should be optimized and may not be the same as for purified DNA. The protocol described here typically generates approximately 3.5 µg of DNA in a volume of 20 µL. The buffer system employed in the TempliPhi product is compatible with DYEnamic ET Terminator Cycle Sequencing Kits and other sequencing methods. Following heat inactivation of the enzyme, the unused amplified template can be stored at -15°C to -30°C or 4°C for at least one month with no loss of performance in cycle sequencing. TempliPhiamplified DNA does not alter or adversely affect most popular postsequencing reaction cleanup methods.*

TempliPhi DNA amplification: short protocol

If you have not previously used TempliPhi 2000 Reaction Kits, please read the full length protocol prior to using the kit.

Step	Action
1	Thaw the TempliPhi premix and denature buffer on ice. Vortex gently to ensure thorough mixing.
2	Dispense 10 µL aliquots of denature buffer into an appropriate reaction tube or plate.
3	Transfer samples to the dispensed denature buffer. Refer to Protocol 1 for procedures specific to your starting material. Seal the tubes or plate.
4	Heat the sealed plate or tubes at 95°C for 3 minutes and then cool to room temperature or 4°C.
5	Add 10 µL of TempliPhi premix to the cooled sample. Vortex briefly to mix.
6	Seal the tube or plate and incubate at 30°C for 16–18 hours.
7	Heat-inactivate the enzyme by incubating at 65°C for 10 minutes. Cool to 4°C.
8	Store the amplified DNA at -15°C to -30°C or 4°C.

Control DNA amplification

The control DNA in the kit consists of 10 µl of pUC19 DNA (2 ng/µL).

Step	Action
1	Transfer 0.5 µL of control DNA to a reaction tube containing 10 µL of denature buffer. Perform protocol *TempliPhi DNA amplification: short protocol, on page 14*., steps 4–7.

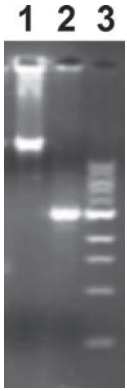


Fig 3. Agarose gel electrophoresis of 1 µL of an amplified bacterial colony (DH5α) transformed with pUC19. Lane 1, undigested product. Lane 2, product digested with EcoR I. Lane 3, kilobase ladder molecular weight marker (major band is 3 kb). The gel consists of 0.6% agarose electrophoresed in 0.5x TBE at 20 V/cm, treated with GelStar nucleic acid stain and visualized under near UV light.

Step	Action
	Note: *The pattern in Lane 1 is common when input DNA is not added to the TempliPhi reaction. Please refer to comments in Chapter 5 Troubleshooting guide, on page 21 regarding non-specific amplification.*

4 Additional information

Frequently asked questions

- **What is the fidelity of φ29 DNA polymerase?**

  φ29 DNA polymerase has an error rate of 1 in 106–107 nucleotides (3).

- **Can I amplify bacterial artificial chromosomes (BACs) with the TempliPhi 2000 Reaction Kit?**

  For BACs we recommend the TempliPhi Large Construct Kit 25-6400-80.

- **Can I amplify linear DNA with the TempliPhi 2000 Reaction Kit?**

  No. TempliPhi Kits were specifically developed to prepare circular templates for DNA sequencing. illustra GenomiPhi™ V2 DNA Amplification Kit (25-6600-31/32) should be used to amplify linear, genomic DNA.

- **Can I obtain φ29 DNA polymerase without hexamers?**

  No. φ29 DNA polymerase is not available as a stand-alone product.

- **Can the random hexamers used in TempliPhi interfere with sequencing reactions?**

  No. The melting temperature of the hexamers used in the TempliPhi premix is far below the typical cycle sequencing temperature ranges.

- **Can TempliPhi DNA overload the MegaBACE sample injection?**

  Yes. Too much of any DNA can potentially overload MegaBACE capillaries. Allowing TempliPhi amplification to proceed to completion (16–18 hour amplification) can reduce variability of DNA yield between different samples. This extended amplification period ensures that all of the nucleotides in the reaction are incorporated into DNA.

- **What is in the TempliPhi premix and denature buffer in the TempliPhi 2000 Reaction Kit?**

  The composition of the TempliPhi premix is proprietary. The formulation of denature buffer is 10 mM Tris-HCl, pH 8.2, 0.5 mM EDTA.

- **What is the stability of TempliPhi 2000 Reaction Kit?**

  The TempliPhi premix is stable at -70°C for 6 months from the date of manufacture. The kit components must freeze solidly during storage. Do not store the TempliPhi premix at -15°C to -30°C and avoid repeated freezing and thawing. The denature buffer and positive control DNA are stable at -70°C or -20°C for 12 months from the date of manufacture.

- **How can I refreeze TempliPhi 2000 Reaction kits?**

Place the kit at -70°C and look at the TempliPhi premix and denature buffer to confirm that both are frozen solidly. Repeated freezing and thawing is not recommended because it leads to enzyme inactivation.

- **Can TempliPhi amplification be carried out at room temperature?**

  Yes, but the amplification rate will be slower. Incubate for 16–18 hours to ensure that the reaction is complete.

- **Can I use colonies from an old plate, or glycerol stocks as starting material?**

  Yes. DNA from colonies on plates up to 14 days old (stored at 4°C) have successfully been amplified using the TempliPhi kit. However, fresh colonies are recommended whenever possible. For glycerol stocks, dilute 1 µl of glycerol stock in 50 µL of TE or water, and use 0.2–0.5 µL of diluted stock in the amplification reaction.

- **Does the starting material effect the amplification rate?**

  Yes. DNA amplification is quicker using colonies as the starting material rather than culture. Used culture media (found in saturated, overnight cultures) contains components that inhibit the TempliPhi reaction.

- **Do I have to precipitate TempliPhi-amplified DNA after amplification?**

  No. TempliPhi-amplified DNA can be used directly in sequencing reactions. A precipitation step may be helpful when sequencing a template that requires more than 500 ng of DNA. It is recommended that no more than 3 µL of unpurified TempliPhi-amplified DNA is added to a 20 µL sequencing reaction.

- **Can I amplify GC-rich DNA?**

  For GC-rich DNA we recommend the TempliPhi Sequence Resolver Kit 28-9035-30/31.

- **Why do I get amplification in a negative control DNA tube?**

  In the absence of input DNA, an amplification product will probably still be produced. Like any other highly-sensitive, exponential amplification method, minute quantities of contaminating DNA (or primers) can serve as an efficient amplification substrate.

- **Can I clone the TempliPhi-amplified DNA products?**

  TempliPhi-amplified DNA can be used for cloning after simple processing. DNA can be digested with a single-cutter restriction enzyme, ligated and then used for transformations. Please refer to *cytiva.com* for an application note regarding transformation with TempliPhi-amplified DNA.

- **Can I quantitate TempliPhi-amplified DNA by UV absorbance?**

  No. Unused hexamers and nucleotides in the reaction will contribute to the absorbance measurement. Quantitate the amplified DNA using a double-stranded quantitation reagent such as PicoGreen.

5 Troubleshooting guide

Problem: 1. No amplification or insufficient amplification

It is unusual to achieve no amplification. Confirm the success of the amplification reaction by analyzing a small amount of the sample (1 µL) on a low-percentage agarose gel (0.6%). The final yield of DNA from an 16–18 hour amplification reaction as described in the protocol is approximately 3.5 µg DNA.

Possible causes	Remedies
Too much culture or starting material was added to the amplification reaction.	The best troubleshooting advice is to use less input template material. The amount of input template material will vary depending on the source of the template. Follow the recommendations in the protocol. Components of saturated culture media, agar and large amounts of input cellular material inhibit the TempliPhi reaction. It is recommended that the volume of culture or supernatant added to the TempliPhi reaction be less than 10% of the final TempliPhi reaction volume. If it is difficult to transfer small volumes, dilute a portion of the liquid culture in sterile TE buffer or water and transfer a larger volume. Avoid adding more than 2 µL of media. An excellent optimization step is to prepare a dilution series of starting material. Add 1 µL of two-fold serially diluted material and monitor amplification by agarose gel electrophoresis at the most convenient incubation time for your workflow. Perform the control reaction.

Possible causes	Remedies
Inefficient denaturation.	Although full lysis of the cells in step 4 of this TempliPhi protocol (*TempliPhi DNA amplification, on page 7*) is not necessary, denaturation must be sufficient to allow release of plasmid as well as efficient primer annealing. Denature at 95°C for 3 minutes. Longer denature times are not recommended because template DNA will be nicked, which decreases the efficiency of rolling circle amplification.
Enzyme inactivated	It is critical that the premix is completely frozen during storage. Storage of premix in a -70°C freezer is strongly recommended. If a -20°C freezer is used, it is essential to confirm that the premix is completely frozen. A frost-free freezer must not be used. If the premix is stored in a liquid state, it rapidly inactivates after 5 days.Thaw only as much premix as is necessary for the experiment. Perform the control reaction to confirm performance of the premix. The premix is stable at -70°C for 6 months from the date of manufacture.
Insufficient DNA.	Research at Cytiva demonstrates robust amplification with as little as 1 pg of starting material. Perform a broad titration of your starting material to determine the range that produces optimal amplification
DNA not circular.	The kinetics of the TempliPhi method strongly favor circular templates. Templates that have been digested with restriction enzymes or that are severely nicked will be poor substrates for the TempliPhi reaction. Start with circular templates. Do not denature longer than 5 minutes at 95°C.

Problem: 2. No sequencing result

In the absence of input DNA, an amplification product will probably still be produced. (*Fig. 4, on page 24*). Like any other highly sensitive, exponential amplification strategy, minute quantities of contaminating DNA (or primers) can serve as an efficient amplification substrate, but will not perform in the sequencing reaction. These nonspecific amplicons do not affect the sequencing reaction in the presence of correct amplicons. Negative control reactions (no input DNA) are expected to produce an amplification product.

Possible causes	Remedies
No amplification	Confirm that a product has been generated by analyzing a small sample (1 µL) on a low percentage agarose gel (0.6%). If no product is observed after staining with a double-stranded DNAsensitive dye such as ethidium bromide or GelStar, refer to the troubleshooting discussion above under "Problem: No amplification."

Possible causes	Remedies
Nonspecific amplification, no input DNA.	In the absence of input DNA, there will still most likely be an amplification product but this product will not produce a sequencing result. The expected product of the TempliPhi reaction is double-stranded. It can be digested with a restriction enzyme appropriate to your sample to yield a characteristic banding pattern on an agarose gel. If you do not observe the expected pattern, nonspecific amplification due to insufficient amounts of template DNA or insufficient denaturation could be the problem. Titrate your sample to determine the range that produces optimal amplification of your template. Confirm that denaturation was performed at 95°C for 3 minutes.

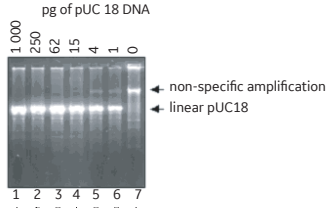
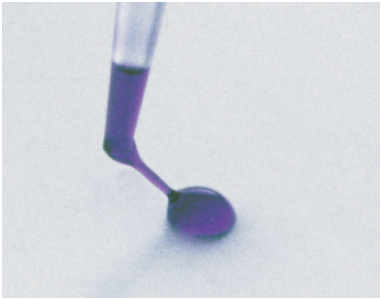


Fig 4. Agarose gel electrophoresis of amplified purified pUC18 DNA. Lane 1–6, 1000-1 pg of pUC18 DNA amplified by TempliPhi 2000 Reaction Kit for 18 hours. Product digested with EcoRI. Lane 7, no DNA control showing non-specific amplification product. The gel consists of a 0.6% agarose electrophoresed in 0.5 × TBE at 20 V/cm, treated with GelStar nucleic acid stain and visualized under near UV light.

Possible causes	Remedies
Non-specific amplification, contaminating input DNA.	The TempliPhi reaction is very sensitive; minute amounts of any input circular DNA will be efficiently amplified. It is therefore important to use clean implements and containers. Transfer colonies or plaques with the same care used when inoculating sterile media.
Nonspecific amplification, incorrect storage of TempliPhi premix.	The TempliPhi premix contains all the components necessary to generate a nonspecific amplification product. The premix must be thawed and kept on ice prior to use. Do not allow the premix to warm above 4°C prior to amplification. Once thawed, the premix must be used promptly and not stored because φ29 DNA polymerase is active, even at 5°C.
Poor sequencing results, yield of amplified product is not consistent from sample to sample.	Low sequencing success rates can be caused by inconsistent amplification between samples. Quantify the amplified products using a double-stranded DNA quantitation reagent such as PicoGreen. Do not quantify DNA yield by UV absorption. Approximately 3.5 µg of amplified product should be produced in a TempliPhi reaction after 16–18 hours. Variable yield from sample-to-sample is usually caused by inhibition of the reaction by contaminants in the input material. Components of saturated culture media, agar and large amounts of input cellular material are inhibitory to the TempliPhi reaction. Reduce the amount of starting material added to the amplification reaction, or increase the incubation period (do not exceed 24 hours).

Possible causes	Remedies
Poor sequencing results, incorrect amount of TempliPhi product used in sequencing reaction.	The amplified product can be viscous and difficult to pipette accurately. A dilution step is recommended prior to transfer. Add 40 µL of water or TE to the amplified product, and mix well by pipetting up and down three times or vortexing. After mixing, use 2–5 µL (100–250 ng) of diluted sample per 20 µL sequencing reaction. (Add 4–8 µL of diluted product if incubation time was less than 12 hours). The optimal amount of amplified product to use in a sequencing reaction may not be the same as for purified DNA. Titrate your amplified product to determine the range that produces optimal sequencing results for your samples.  **Fig 5.** The viscous product of TempliPhi amplification can be difficult to pipette accurately. Dye was added to the TempliPhiamplified product to increase DNA visibility in the photograph.

Possible causes	Remedies
Poor sequencing results, volume reduction and sequencing premix dilution.	A common reason for failed sequencing reactions is excessive reduction of the reaction volume and dilution of the premix. TempliPhi-amplified products have been tested under a wide range of conditions and found to be compatible with Cytiva and other sequencing chemistry, without the need for postamplification purification/cleanup. However, excessive dilution of the sequencing premix could produce an imbalance of essential components in the sequencing reaction. Avoid dilution of the sequencing premix. Poor performance attributed to sequencing premix dilution will not be supported by Cytiva.
Poor sequencing results, enzyme not inactivated prior to storage	Following amplification, the enzyme should be inactivated by heating at 65°C for 10 minutes. Heating prevents degradation of template DNA during storage by inactivating the proofreading exonuclease activity of φ29 DNA polymerase.

6 References

1. Dean, F. *et al., Genome Research* 11, 1095–1099 (2001).
2. Lizardi, P. *et al., Nat. Genet*. 19, 225–232 (1998).
3. Estaban, J.A. *et al., J. Biol. Chem*. 268, 2719–2726 (1993).

7 Related products

illustra TempliPhi 100 Amplification Kit, 100 reactions	25640010
illustra TempliPhi500 Amplification Kit, 500 reactions	25640050
illustra TempliPhi Sequence Resolver Kit, 50 reactions	28903530
illustra TempliPhi Sequence Resolver Kit, 200 reactions	28903531

illustra TempliPhi Large Construct Kit, 1000 reactions	25640080
illustra GenomiPhi V2 DNA Amplification Kit, 100 reactions	25660031
illustra GenomiPhi V2 DNA Amplification Kit, 500 reactions	25660032
ExoSAP-IT, 500 reactions	US78201
ExoSAP-IT, 2000 reactions	US78202
Shrimp Alkaline Phosphatase, 1000 units	E70092Z
Exonuclease I, 2500 units	E70073Z

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