cytiva

illustra Ready-To-Go **GenomiPhi** V3 DNA Amplification Kit

Product booklet

Table of Contents

1 Introduction

Product codes

25660124

25660196

25660197

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.

It is the responsibility of the user to verify the use of the Ready-To-Go™ GenomiPhi™ V3 DNA Amplification Kit for a specific application range as the performance characteristic of this kit has not been verified to a specific organism.

Safety

NOTICE
This kit is sensitive to small amounts of DNA. Wear gloves and safety glasses at all times during the preparation to avoid contamination.

All chemicals should be considered as potentially hazardous. For use and handling of the products in a safe way, refer to the Safety Data Sheets.

Note: *Ready-To-Go GenomiPhi V3 DNA Amplification Kit is optimized for whole genome amplification from at least 10 ng of high quality genomic DNA template.*

Use of less DNA or low quality DNA (such as degraded DNA, or DNA from formalin fixed paraffin embedded samples) can result in amplification bias. Negligible amplification product is produced in the absence of template DNA up to 1.5 hours amplification.

Storage

Store the kit at ambient temperature. Once opened, completely reseal the pouch containing the Ready-To-Go GenomiPhi V3 DNA Amplification Kitcakes. To ensure maximum shelf life and optimal performance, storage in a desiccator is recommended. Open product may be stored by resealing the pouch and folding the sealed edge over several times and sealing with a clip.

Store reconstituted Control DNA (Lambda) at -20°C. For longer-term stability, once opened, store 2× denaturation buffer at 2–8°C.

Expiry

This product has been designed to deliver high quality results for up to 12 months from the date of manufacture. Please refer to the expiration date on the product label.

2 Components

Kit Contents

Table 1. Ready-To-Go GenomiPhi V3 DNA Amplification Kit

Product code	24 Reactions 25660124	96 Reactions 25660196	5 × 96 Reactions 25660197
2× Denaturation buffer	1 × 1.2 mL	1 × 1.2 mL	5 × 1.2 mL
Ready-To-Go GenomiPhi V3 cakes	3 × 8-well strips	1 × 96-well plates	5 × 96-well plates
Control DNA (Lambda), 10ng/uL when reconstituted with 50 µL of sterile water	1 × vial	1 × vial	1 × vial
Strips of 8 domed caps	12 strips	12 strips	5 × 12 strips

Reagents to be supplied by the user

- **Liquid-handling supplies** – Sterile vials and pipette tips; pipettes, micro centrifuge
- **Water** – Use PCR-grade water that is free of contaminating DNases or nucleic acid.
- **Ice bucket or cold block** - for maintaining Ready-To-Go GenomiPhi V3 DNA Amplification Kit cakes at 4°C during reconstitution and prior to DNA amplification.
- **Amplification reactions** - should be performed in the wells provided. Individual strips can be split from the rest of the plate by bending the strip upwards, carefully cutting the seal between adjacent columns of wells, then snapping-off the required strip of 8 wells.

  For fewer reactions, carefully tap out cake into suitable PCR tube, taking precautions to avoid introducing contaminating DNA (as described in *Safety, on page 3*)

- **Thermocycler or water baths** - for incubations at 30°C, 65ºC and 95ºC.

3 Product description

The Basic Principle

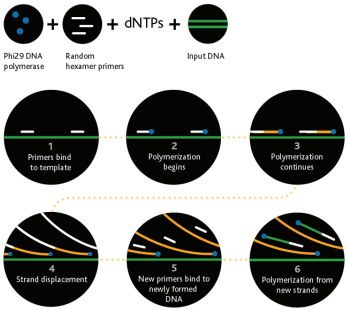


Fig 1. Overview of the Ready-To-Go GenomiPhi V3 DNA Amplification Kit procedure.

Figure 1 above shows an overview of whole genome amplification by isothermal stand displacement using the Ready-To-Go GenomiPhi V3 DNA Amplification Kit. DNA is briefly heat-denatured in denaturation buffer then cooled. This is added to the freezedried cake which contains DNA polymerase, random hexamers, nucleotides, salts and buffers. Isothermal amplification proceeds at 30°C for 1.5 hours. After amplification the enzyme is heat inactivated during a 10 minute incubation at 65°C.

Kit specifications

Typical amplification kinetics with Ready-To-Go GenomiPhi V3 DNA Amplification Kit is shown in Figure 2 below. Microgram quantities of DNA are generated from nanogram amounts of starting material in 1.5 hours. Typical DNA yields from a Ready-To-Go GenomiPhi V3 DNA Amplification Kit reaction are >12–20 µg per 20 µL reaction when starting with 10 ng of purified DNA. Kinetics will vary if crude or un-quantified samples are amplified. Increased reaction times (2 hours) may be helpful for samples such as crude blood or buccal swabs. Control reactions without added template DNA do not produce any product during 1.5 h reactions. The average product length is greater than 10 kb. DNA replication is extremely accurate due to the proofreading 3′–5′ exonuclease activity of the DNA polymerase (3, 4).

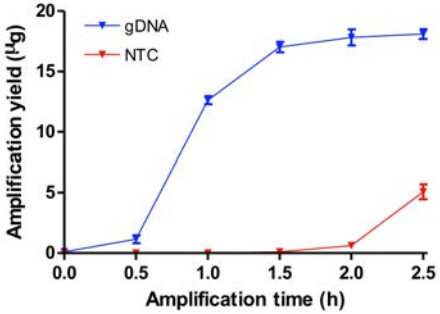


Fig 2. Amplification kinetics of a Ready-To-Go GenomiPhi V3 DNA Amplification Kit reaction.

Fig. 2, on page 7 shows the comparison of amplification of 10 ng purified gDNA with no template control (NTC). Most commercial DNA isolation kits and homemade purification procedures produce suitable DNA for the amplification.

Table 2.

Sample	See section	Typical DNA yield (µg)
10 ng Lambda DNA	*General Protocol, on page 10*	18
10 ng Human genomic DNA - heat	*General Protocol, on page 10*	16
10 ng Human genomic DNA - chemical	*Chemical Denaturation and Amplification of Template DNA, on page 12*	16
Blood lysate	*DNA Amplification from Blood Cells, on page 15*	15
Dried blood spots	*DNA Amplification from Blood-blotted Paper (e.g. Whatman's FTA Paper or Guthrie card), on page 18*	18

Table 2 above showing typical amplification yields for Ready-To-Go GenomiPhi V3 DNA Amplification Kit using either purified DNA (10 ng) or DNA amplified from blood or blood-spotted paper using protocols described later in the booklet.

4 Protocols

Short Protocol

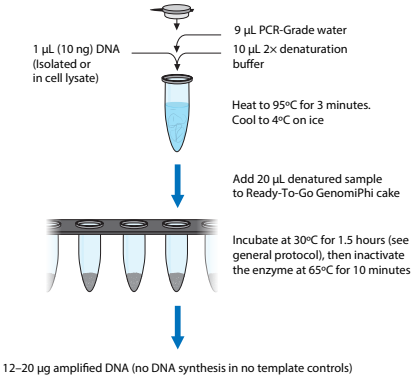


Fig 3. Schematic representation of Ready-To-Go GenomiPhi V3 DNA Amplification Kit protocol

General Protocol

The following outlined steps describe a general protocol for amplifying template DNA.

This protocol should be considered a starting point for optimizing the reaction in your laboratory.

DNA should be resuspended in PCR-grade water or TE^{-1} buffer (10 mM Tris-HCl pH 8.0; 0.1 mM EDTA). The Denaturation buffer is supplied as a 2× solution to allow some flexibility in volume of DNA that can be used.

Note: *The total volume of template DNA and water should equal 10 µL.*

When required, the Control DNA should be reconstituted with 50 µL PCR-grade water. A screw-cap has been provided to re-seal the tube. Store at -20°C after reconstitution (in aliquots if so desired).

Step	Action
1	Mix 2× denaturation buffer with template DNA

a. Add 10 µL 2× Denaturation Buffer to 1 µL of 10 ng template DNA.

b. Add 9 µL PCR-grade water.

Note:
Template DNA should be resuspended in PCR-grade water or TE^{-1} buffer. If sample DNA concentration is less than 10 ng/µL more volume can be used, the total volume of DNA plus water should equal 10 µL

Note:
10 µL denaturation buffer

9 µL PCR-grade water

Step	Action
	1 µL DNA template (10 ng)
2	Denature template DNA Heat the samples to 95°C for 3 minutes then cool to 4°C on ice.

CAUTION
Heating the DNA for longer than 3 minutes or at higher temperatures can cause damage to the DNA.

3 Reconstitute the Ready-To-Go GenomiPhi V3 cake with the denatured template DNA.

- **a.** Add the 20 µL of denatured DNA template from Step 2 to each cake.
- **b.** Seal wells with domed caps provided.
- **c.** Keep each amplification reaction on ice prior to incubation at 30°C.

Note:
The reconstituted Ready-To-Go GenomiPhi V3 cake contains all the components required for DNA amplification and will generate amplification products if exposed to temperatures > 4°C for sufficient time.

4 Incubate for DNA amplification.
Incubate the samples at 30°C for 1.5 hours.

Step	Action
5	Inactivate the Phi29 DNA polymerase enzyme. Heat the samples to 65ºC for 10 minutes then cool to 4ºC. ***Note:*** *Heating is required to inactivate the exonuclease activity of the DNA polymerase which may otherwise begin to degrade the amplification product.*
6	Storage of amplified material Store amplification reactions at -20°C. ***Note:*** *Ready-To-Go GenomiPhi DNA V3 amplification products should be stored and treated as genomic DNA. Minimize freeze-thaw cycles.*

Chemical Denaturation and Amplification of Template DNA

The steps outlined below describe a general protocol for amplifying template DNA.

Prepare chemical denaturation solution, neutralization buffer and potassium chloride solution.

Chemical Denaturation Solution

400 mM KOH

10 mM EDTA

Neutralization Buffer

400 mM HCl

600 mM Tris-HCl, pH 7.5 (prepared by mixing 4 mL of 1 M HCl and 6 mL of 1 M Tris-HCl pH 7.5)

Potassium Chloride

150 mM KCl

Step	Action
1	Denature template DNA. **a.** Mix 1 µL of template (10 ng) with 1 µL of Chemical Denaturation Solution. **b.** Incubate at room temperature for 3 minutes ***Note:*** *Mix by pipetting up and down. Do not vortex.* *1 µL of DNA + 1 µL Denaturation solution* *RT for 3 minutes*
2	Neutralize DNA solution. Add 1 µL of Neutralization Buffer and store on ice. ***Note:*** *Mix by pipetting up and down. Do not vortex.*
3	Add PCR-grade water and potassium chloride **a.** Add 7 µL of PCR-grade water and 10 µL 150 mM KCl. **b.** Keep on ice ***Note:*** *7 µL of PCR-grade water + 10 µL of potassium chloride*

Step	Action
4	Reconstitute the Ready-To-Go GenomiPhi V3 cake with the denatured template DNA. **a.** Add the 20 µl of denatured DNA template from Step 4 to each cake. **b.** Seal wells with domed caps provided. **c.** Keep each amplification reaction on ice prior to incubation at 30°C. ***Note:*** *The reconstituted Ready-To-Go GenomiPhi V3 cake contains all the components required for DNA amplification and will generate amplification products if exposed to temperatures > 4°C for sufficient time.*
5	Incubate for DNA amplification Incubate the samples at 30°C for 1.5 hours.
6	Inactivate the Phi29 DNA polymerase enzyme. Heat the samples to 65°C for 10 minutes then cool to 4°C. ***Note:*** *Heating is required to inactivate the exonuclease activity of the DNA polymerase which would otherwise degrade the amplification product.*

Step	Action
7	Storage of the amplified material. Store amplification reactions at -20°C. ***Note:*** *Ready-To-Go GenomiPhi V3 amplification products should be stored and treated as genomic DNA. Minimize freeze-thaw cycles.*

DNA Amplification from Blood Cells

Note: *Components of blood (e.g. heme) can be inhibitory to the GenomiPhi reaction.*

Amplification is faster and more reproducible when the blood sample is diluted before amplification.

A reaction time of 2 hours is suggested

Prepare cell lysis solution, neutralization buffer and potassium chloride solution

Cell lysis solution

10 mM EDTA

400 mM KOH

100 mM DTT

Neutralization Buffer

400 mM HCl

600 mM Tris-HCl, pH 7.5 (prepared by mixing 4 mL of 1 M HCl and 6 mL of 1 M Tris-HCl pH 7.5)

Potassium Chloride

150 mM KCl

Step	Action
1	Cell lysis **a.** Dilute blood 3 times with physiological buffer, e.g., PBS (137 mM NaCl; 2.7 mM KCl; 10 mM phosphate buffer pH 7.4). **b.** Add one part of this mixture with one part of cell lysis solution. **c.** Mix well with gentle tapping and incubate on ice for 10 minutes ***Note:*** *Mix by pipetting up and down. Do not vortex.* ***Note:*** *1 µL of diluted blood cells + 1 µL cell lysis solution. 10 minutes on ice.*
2	Neutralize cell lysate. Add one part of neutralization buffer to the cell lysate, mix well and store on ice. ***Note:*** *Mix by pipetting up and down. Do not vortex.*
3	Add PCR-grade water and potassium chloride. **a.** To 3 µL from Step 3, add 7 µL of nuclease-free water and 10 µL 150 mM KCl. **b.** Keep on ice. ***Note:*** *7 µL of PCR-grade water + 10 µL of potassium chloride*

Step	Action
4	Reconstitute the Ready-To-Go GenomiPhi V3 cake with the denatured template DNA. **a.** Add the 20 µL of denatured DNA template from Step 4 to each cake. **b.** Keep each amplification reaction on ice prior to incubation at 30°C. ***Note:*** *The reconstituted Ready-To-Go GenomiPhi V3 cake contains all the components required for DNA amplification and will generate amplification products if exposed to temperatures > 4°C for sufficient time.*
5	Incubate for DNA amplification Incubate the samples at 30ºC for 2.0 hours.
6	Inactivate the Phi29 DNA polymerase enzyme. Heat the samples to 65ºC for 10 minutes then cool to 4ºC. ***Note:*** *Heating is required to inactivate the exonuclease activity of the DNA polymerase which would otherwise degrade the amplification product.*
7	Storage of the amplified material. Store amplification reactions at -20°C. ***Note:*** *Ready-To-Go GenomiPhi V3 amplification products should be stored and treated as genomic DNA. Minimize freeze-thaw cycles.*

DNA Amplification from Blood-blotted Paper (e.g. Whatman's FTA Paper or Guthrie card)

Prepare cell lysis solution, neutralization buffer, 2× denaturation buffer and TE^{-1} buffer.

Cell lysis / denaturation solution

400 mM KOH

10 mM EDTA

100 mM DTT

Neutralization Buffer

400 mM HCl

600 mM Tris-HCl, pH 7.5

2× Denaturation buffer

20 mM Hepes, pH8.25

1.0 mM ETDA

0.02% Tween-20

150 mM KCl

TE^{-1} buffer

10 mM Tris-HCl pH 8.0

0.1 mM EDTA

Step	Action
1	Punch processing **a.** From the blood-blotted and dried FTA paper, punch out 1.2 or 3 mm diameter disks. **b.** Wash the disks three times for 5 minutes with FTA purification reagent (Cytiva catalog number WB120204) in a micro centrifuge tube (using 200 µL for 1.2 mm, and 500 µL for 3 mm disks) **c.** Wash each disk twice for 5 minutes with TE^{-1} buffer. Remove as much fluid as possible at the end of the second TE^{-1} buffer wash. ***Note:*** *Mix by pipetting up and down. Do not vortex.*
2	Denaturation **a.** Add 20 µL cell lysis / denaturation solution to the damp disks from Step 2. **b.** Mix well with gentle tapping and incubate on ice for 10 minutes. ***Note:*** *Mix by pipetting up and down. Do not vortex.*
3	Neutralization. **a.** Add 20 µL neutralization buffer to the cell lysate. **b.** Add 20 µL PCR-grade water, mix well and store on ice. ***Note:*** *Mix by pipetting up and down. Do not vortex.*

Step	Action
4	Add 2× denaturation buffer. **a.** To 10 µL from Step 4, add 10 µL 2× denaturation buffer **b.** Keep on ice.
5	Reconstitute the Ready-To-Go GenomiPhi V3 cake with the denatured cell lysate DNA. **a.** Add the 20 µL of denatured DNA template from Step 5 to each cake. **b.** Keep each amplification reaction on ice prior to incubation at 30°C. ***Note:*** *The reconstituted Ready-To-Go GenomiPhi V3 cake contains all the components required for DNA amplification and will generate amplification products if exposed to temperatures > 4°C for sufficient time.*
6	Incubate for DNA amplification. Incubate the samples at 30ºC for 2 hours.
7	Inactivate the Phi29 DNA polymerase enzyme. Heat the samples to 65ºC for 10 minutes then cool to 4ºC ***Note:*** *Heating is required to inactivate the exonuclease activity of the DNA polymerase which would otherwise degrade the amplification product.*

Step	Action
8	Storage of the amplified material. Store amplification reactions at -20°C. ***Note:*** *Ready-To-Go GenomiPhi V3 amplification products should be stored and treated as genomic DNA. Minimize freeze-thaw cycles.*

Quantification of Amplification Products

Quantification is generally not required as every reaction will yield approximately the same amount of DNA. Quant-iT™ PicoGreen® dsDNA quantification reagent (Life Technologies, P7581) is recommended if accurate quantitation is required.

Note: *Quantification of non-purified amplification products by UV absorption will generate inaccurate results due to the presence of unused hexamers in the completed reaction.*

Step	Action
1	Prepare TE buffer. Dilute the concentrated 20 × TE buffer included in the kit to 1 × concentration using water.

CAUTION
Use only sterile, DNase free water when preparing the dilution to ensure accurate quantification.

Step	Action
2	Prepare 1:25 dilution of PicoGreen reagent.

a. Determine the required volume of a 1:25 dilution of PicoGreen reagent.

- Volume = 100 µL/sample × # of samples

b. Determine the volume of stock PicoGreen reagent necessary to produce the required volume of a 1:25 dilution

- Volume = volume of required dilution/25

CAUTION
Reagent adsorbs to glass surfaces. Use plastic ware only. Protect the solution from light at all times.

Step	Action

3 Prepare the λ DNA standard curve.

a. Dilute the λ DNA standard supplied in the Quant-iT PicoGreen kit to a 10 ng/µL working solution. Use this working stock to prepare a standard curve (see table).

b. Add 100 µL of each dilution to each well of the assay plate.

Standard Number	λ DNA (ng)	λ DNA (10 ng/µL)	1 x TE
1	600	60 µL	40 µL
2	500	50 µL	50 µL
3	400	40 µL	60 µL
4	200	20 µL	80 µL
5	100	10 µL	90 µL
6	50	5µL	95µL
7	25	2.5µL	97.5µL
8	0	0 µL	100 µL

4 Dilute the Ready-To-Go GenomiPhi V3 amplification products.
Dilute the Ready-To-Go GenomiPhi V3 amplification products 1:10 by adding 180 µL of 1 × TE to each amplification reaction.

Note:
Due to the viscosity of the amplification product, mix amplification products thoroughly by vortexing heavily.

Step	Action
5	Add diluted Ready-To-Go GenomiPhi amplification products to the assay plate.
	a. Aliquot 95 µL of 1 × TE into each sample well.
	b. Add 5 µL of diluted sample for a final volume of 100 µL.
	Note: *Because the amplification product is diluted before the assay, the dilution factor must be taken into consideration when calculating total yields.*
6	Add diluted PicoGreen to sample wells.
	a. Add 100 µL of the 1:25 dilution of PicoGreen to all wells containing standards and samples.
	b. Mix contents well by pipetting up and down.
	c. Seal the plate with foil and spin in micro plate centrifuge for 1 minute to eliminate bubbles.

CAUTION
Protect plate from light at all times. The plate must be read 5–10 minutes after addition of PicoGreen reagent to ensure accurate quantification.

Step	Action
7	Measure the sample fluorescence. **a.** Place the sample assay plate into a fluorescence micro plate reader. **b.** Set the fluorescence reader at the following parameters: • Excitation wavelength: 480 nm • Emission wavelength: 520 nm • Gain: Optimal ***Note:*** *If it is not possible to set the instrument gain to optimal, find a way to have the instrument read the sample where the highest DNA concentration generates readings that fall within the linear dynamic range of the instrument.*
8	Calculate the concentration of the amplification product. Generate a standard curve of fluorescence versus DNA concentration. Determine the concentration of Ready-To-Go GenomiPhi V3 Kit amplified products from the equation of the line derived from the standard curve.

5 Related Products

GenomiPhi Products[1]	
illustra™ Ready-To-Go GenomiPhi HY (high yield)	25660324
	25660396
	25660397
illustra GenomiPhi V2 (liquid format)	25660031
illustraGenomiPhi HY (high yield, liquid format)	25660022
DNA Purification Products[1]	
illustra tissue and cells genomicPrep Mini Spin Kit	28904276
illustra tissue and cells genomicPrep Midi Flow Kit	28904273
illustra blood genomicPrep Mini Spin Kit	28904264
illustra blood genomicPrep Midi Flow Kit	28904261
illustra triplePrep	28942544
illustra bacteria genomicPrep Mini Spin Kit	28904258
PCR Products[1]	
illustra PureTaq Ready-To-Go PCR Beads	27955901
illustra Hot Start Mix Ready-To-Go	28900653
illustra GFX™ PCR DNA and Gel Band Purification Kit	28903470
illustra ExoProStar™ - PCR and Sequence Reaction Clean-Up	US78210
illustra ExoProStar 1-Step - PCR and Sequence Reaction Clean-Up	US77702
DNA Polymerase (cloned)	27079804
illustraSolution dNTPs (multiple formats available)	28406552

[1] please see cytiva.com for an overview of available pack sizes.

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).
4. Nelson, J.R. et al; BioTechniques 32, S44-S47 (2002).

Appendix A

Troubleshooting

Problems	Possible cause and suggestions
Reduced yield/ no amplification product.	**Contamination of template DNA** • Excessive contaminants carried over from the starting material can inhibit the DNA polymerase. Dilute or clean-up the DNA and re-amplify. • Extending the amplification time will help when inhibitory material is causing reduced yields. **Inactive Enzyme** • It is critical that the Ready-To-Go GenomiPhi V3 DNA Amplification Kit freeze-dried cakes are stored properly. Once opened, completely reseal the pouch, fold the sealed edge over several times and seal with a clip. In high humidity environments, store unopened and resealed pouches in a desiccator to maximise product life. • Each cake should be a bright white solid having the shape of the base of the well. Cakes that have been exposed to moisture will become increasingly more translucent in appearance and this will impact both stability and performance. • Perform a control (Lambda DNA) reaction to confirm performance of the Ready-To-Go GenomiPhi V3 DNA Amplification Kit.
Reduced yield/ no amplification product.	**Low quality DNA** • Amplification kinetics strongly favor intact templates. Avoid template preparation steps that can damage DNA. **Prolonged denaturation** • Heating at 95°C for 3 minutes is sufficient to denature template DNA. Longer denaturation times can nick the template and decrease the amplification efficiency.

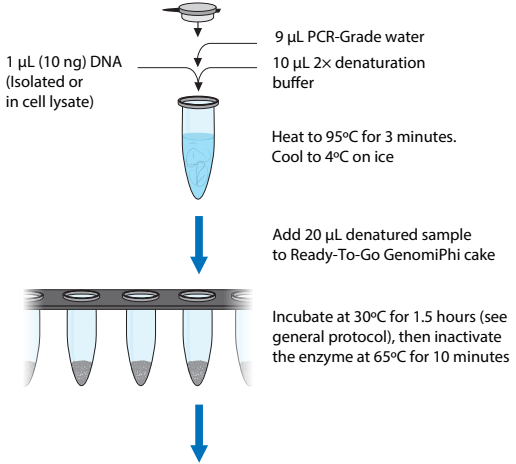
Problems	Possible cause and suggestions
Poor performance in downstream applications.	**Degraded/low amounts of template DNA** • In the absence of input DNA or poor quality of input DNA, there will be no or minimal DNA synthesis in the amplification reactions within 1.5 hours. • Degraded or low amounts of starting DNA template may not amplify consistently or representatively. Increase the amount of starting DNA. • Use high quality genomic DNA for amplification **Inhibition of optimized downstream conditions** • Components of the Ready-To-Go GenomiPhi V3 reaction may affect previously optimized conditions for some downstream applications. Purify the amplification products after amplification.

Appendix B

Quick reference protocol

Ready-To-Go GenomiPhi V3 DNA Amplification Kit

25660124, 25660196, 25660197



12–20 µg amplified DNA (no DNA synthesis in no template controls)

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