# Amplification of DNA from diverse sources with the GenomiPhi HY DNA Amplification Kit

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# Amplification of DNA from diverse sources with the illustra GenomiPhi HY DNA Amplification Kit

***Key words:*** *DNA amplification • Phi29 DNA polymerase • GenomiPhi • Simplex PCR • Multiplex PCR • SNP genotyping*

## Abstract

**The illustra™ GenomiPhi™ HY DNA Amplification Kit simplifies the process of genomic DNA preparation and stock replenishment and makes it easier to prepare multiple genomic DNA samples simultaneously. The Phi29 DNA polymerase-based kit is designed to produce large amounts (40 to 50 µg) of DNA in just 4 h. The amplified DNA can be used directly in most downstream applications such as simplex or multiplex PCR, cloning, and SNP genotyping.**

## Introduction

Genomic DNA isolation is a fundamental step in genetic analysis, and obtaining high-quality DNA is vital for success. Representative amplification of genomic DNA with high fidelity from a small input quantity is useful in applications involving genetic analysis, such as cancer research, genetic testing, DNA archiving, and forensics (1-5).

The GenomiPhi method of whole-genome amplification uses Phi29 DNA polymerase, a highly processive enzyme with excellent strand-displacement activity, in combination with random-sequence hexamer primers to amplify DNA in an isothermal process. Random-sequence hexamer primers anneal to the template DNA at multiple sites on denatured linear template. Phi29 DNA polymerase initiates replication at these sites simultaneously. As synthesis proceeds, strand displacement of upstream replicated DNA generates new single-stranded DNA. The subsequent priming and strand-displacement replication of this DNA results in the formation of large quantities of several-kilobase-long, double-stranded DNA, that is suitable for subsequent genetic analysis applications requiring high molecular weight DNA. Microgram quantities of high molecular weight DNA can be produced from as little as 10 ng of genomic DNA using this simple and robust procedure.

Here we describe the amplification of human genomic DNA from whole blood and buccal swabs using the GenomiPhi HY DNA Amplification Kit. The amplified DNA was then used directly in simplex and multiplex PCR reactions to amplify regions of mitochondrial DNA.

## Materials

### *Products Used*

| | |
|---|---|
| illustra GenomiPhi HY DNA Amplification Kit | 25-6600-20 |
| GFX™ Genomic Blood DNA Purification Kit | 27-9603-01 |
| illustra Hot Start Master Mix | 25-1500-01 |

### *Other Materials*

| | |
|---|---|
| CEPH human 10859 DNA (Coriell Cell Repositories) | NA10859 |
| Whole human blood, ACD preservative (BioReclamation) | HMWBACD |
| Cotton or polyester swabs (Puritan) | 25-806 1WC |
| Quant-IT™ PicoGreen™ dsDNA reagent (Invitrogen) | P-7581 |

Lysis solution: 0.4 M KOH, 0.1 M DTT, 10 mM EDTA

Neutralization Solution: 0.6 M Tris-HCl, pH 7.5, 0.4 M HCl

Primer set 1: 5'-CCCTAACACCAGCCTAACCAGATT-3';
5'-CGTGCTTGATGCTTGTCCCTTTTG-3'

Primer set 2: 5'-CCCCATCCTTACCACCCTCGTTA-3';
5'-GGGTTGTGGCTCAGTGTCAGTTCG-3'

Primer set 3: 5'-CAGGGTTTGTTAAGATGGCAGAGC-3';
5'-TAAGGTCGGGGCGGTGATGTAGA-3'

## Methods

### 1. Preparation of lysate from blood

a. Aliquot 20 µl of blood into a 1.5 ml centrifuge tube and add 40 µl of 1x PBS. Mix gently by vortexing briefly.

b. Add 60 µl of lysis solution, mix the solution by vortexing briefly and leave on ice for 10 min.

c. Add 60 µl of neutralization solution and mix well by tapping the tube.

d. Use 2.5 µl of lysate per amplification reaction.

### 2. Preparation of lysate from cheek cells

a. Cut the shaft off the applicator using a clean pair of scissors and place the brush containing the cells in a 1.5 ml centrifuge tube and add 400 µl of 1x PBS. Mix gently.

b. Add 400 µl of lysis solution, mix the solution by vortexing briefly and leave on ice for 10 min.

c. Add 400 µl of neutralization solution and mix well by tapping the tube.

d. Use 2.5 µl of lysate per amplification reaction.

### 3. Optional purification of DNA from blood and buccal swabs

You can avoid inhibition of the amplification reaction by purifying the template DNA from blood with the illustra blood genomicPrep Mini Spin Kit (GE Healthcare; 28-9042-64). The GFX Genomic Blood DNA Purification Kit can be used to purify template DNA from both blood and buccal swabs.

### 4. DNA amplification

a. Aliquot 22.5 µl of sample buffer into each reaction tube, and add 10 ng of purified genomic DNA or 2.5 µl of lysate.

b. Incubate at 95°C for 1 min and then cool to 4°C on ice to denature the DNA and anneal hexamers.

c. To each cooled reaction, add 22.5 µl of reaction buffer and 2.5 µl of enzyme mix. Mix by vortexing gently and centrifuge briefly.

d. Incubate at 30°C for 4 h.

e. Incubate at 65°C for 10 min to heat inactivate the enzyme.

### 5. Quantitation of amplification products

a. Prepare 100 µl of DNA standards of 0, 25, 50, 100, 200, 400, and 500 ng using the lambda DNA supplied with the GenomiPhi HY DNA Amplification Kit in a 96-well plate suitable for fluorescent assays (e.g., a black, clear-bottomed plate).

b. Add 150 µl 1x TE buffer to each 50 µl amplification reaction. Mix thoroughly by pipetting sample up and down at least 10 times or by vortexing gently. The amplification product is highly viscous and concentrated (1 µg/µl), and thorough mixing at this stage is essential for accurate quantitation.

c. Using a fresh tip, transfer 10 µl of diluted amplification product to the black plate containing lambda DNA standards. A change of tip is required as the DNA can stick to the outside of the tip and lead to inaccurate quantitation.

d. Add 90 µl of 1x TE buffer to each amplification sample in the black plate. Thoroughly mix sample by pipetting up and down at least 10 times or by vortexing gently.

e. Prepare 1:25 dilution of the PicoGreen dsDNA quantitation reagent.

f. Add 100 µl of diluted PicoGreen reagent to lambda DNA standards and amplification sample in the black plate. Seal plate with foil and spin at 2000 *g* for 1 min. Read plate within 10 min.

g. Quantitate amplification products from the standard curve.

### 6. PCR

a. For each PCR, add 25 µl of illustra Hot Start Master Mix, 100 ng of amplified DNA, 1.5 µl of each 10 µM primer, and water to a total volume of 50 µl.

b. Heat to 95°C for 2 min, followed by 35 cycles of 95°C for 30 s, 56°C for 30 s and 72°C for 52 s, then 72°C for 5 min.

## Results and discussion

### *Components in blood and buccal samples can inhibit Phi29 DNA polymerase*

A 50 µl reaction should produce approximately 45 µg of product in 4 h with the GenomiPhi HY DNA Amplification Kit. The presence of inhibitory compounds like heme (Fig 1) in crude lysates (such as those prepared from buccal and blood cells) can slow down the amplification reaction kinetics. To overcome this inhibition, you can either extend the duration of amplification by up to 2 h or purify the DNA template with the illustra blood genomicPrep Mini Spin Kit prior to the amplification reaction.

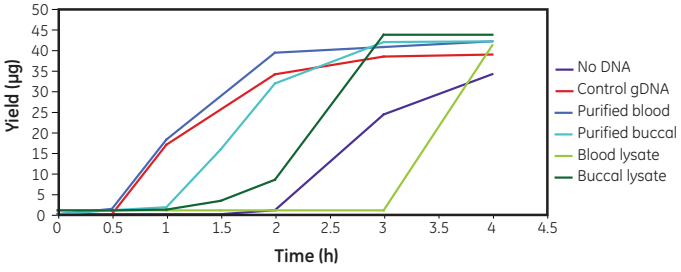


**Fig 1.** GenomiPhi HY DNA Amplification Kit reaction kinetics from different templates. Purified CEPH human genomic DNA was used for the control reaction. The Quant-IT PicoGreen dsDNA Kit was used to determine DNA yields.

### *Amplification product in samples without DNA*

The GenomiPhi HY DNA Amplification Kit usually produces an amplification product in non-DNA containing samples. However, the amplification reaction is comparatively inefficient, resulting in a negligible amount of amplification product after 2 h (Fig 2). In contrast, the control genomic DNA reaction generated about 35 µg of amplification product in 2 h (Fig 2).

Hexamers tend to be amplified in the absence of a DNA substrate resulting in a nonspecific amplification product. The product of a no-DNA reaction can be neither cut with restriction endonucleases nor amplified by PCR.

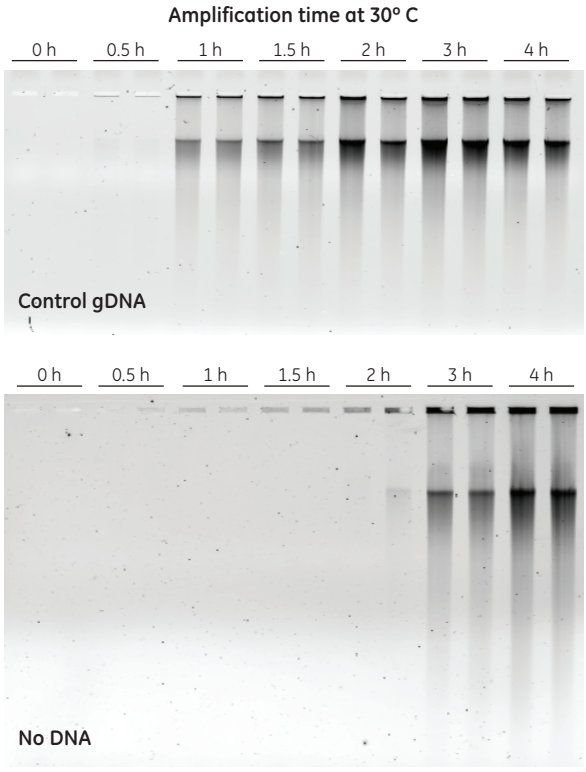


**Fig 2.** Comparison of DNA and hexamer amplification kinetics. Amplification of target DNA occurs efficiently with the GenomiPhi HY DNA Amplification Kit resulting in a detectable amplification product after incubating for 1 h at 30°C. Amplification of the hexamers, which occurs in the absence of DNA, proceeds slowly generating detectable DNA only after a 2 h incubation at 30°C. The products of both DNA and hexamer reactions are indistinguishable by agarose gel electrophoresis. Two microliters of amplification reaction was run on 1% agarose gel.

## *Quantitation*

In general, quantitation is not necessary as every reaction yields the same amount of DNA (approximately 50 µg) if the reaction is not inhibited. If quantitation is required, then a dye appropriate for quantitating double-stranded DNA, such as PicoGreen ds DNA quantitation reagent can be used.

Spectrophotometic measurement ($A_{260}$) is not recommended because some of the components remaining in a completed amplification reaction—such as unused hexamers—can contribute to inaccurate readings. The amplification product is highly viscous due to the high concentration of the amplified DNA (approximately 1 µg/µl), making accurate quantitation difficult. Therefore the amplification product should be diluted 10-100 fold in Tris-EDTA (TE) buffer and thoroughly mixed by gentle vortexing or pipetting. Fresh tips should be used for sample transfer as the DNA can stick to the outside of the pipette tips and lead to inaccurate DNA quantitation. Any yields greater than 55 µg (1.1 µg/µl) indicate inaccurate quantitation.

## *Use of amplification products in downstream applications*

For most applications, the amplified product can be used directly in downstream applications (Figs 3 and 4). If the amplified DNA does not perform equivalently to genomic DNA in downstream applications, then it may be necessary to purify the amplification product using a MicroSpin™ G-50 column (GE Healthcare; 27-5330-01). Poor performance in downstream applications may also be indicative of too little or poor quality DNA added to the amplification reaction. Please see www.genomiphi.com for more troubleshooting information.

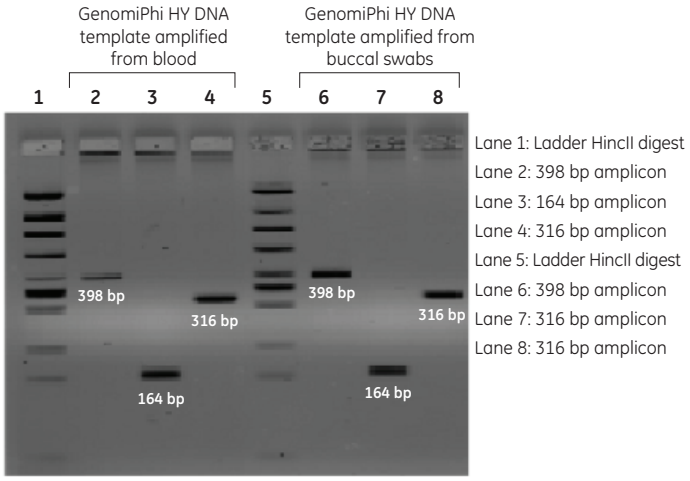


**Fig 3.** Simplex PCR using template DNA purified with the GenomiPhi HY DNA Amplification Kit from blood and buccal samples.

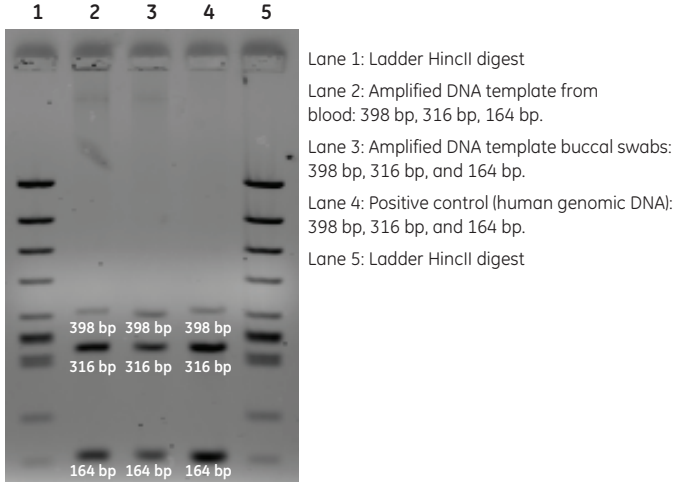


**Fig 4.** Multiplex PCR using template DNA purified with the GenomiPhi HY DNA Amplification Kit from blood and buccal samples.

## Conclusion

The GenomiPhi HY DNA Amplification Kit generates about 50 µg of amplified DNA in 4 h. Components of crude blood and buccal cell lysates can inhibit the amplification reaction but purifying the DNA sample prior to amplification or extending the duration of amplification to 5 or 6 h can overcome this inhibition.

Hexamer amplification occurs in the absence of DNA template but the reaction is is inefficient and no significant amplification product is produced within the first 2 h of incubation at 30°C. If you use purified DNA as template, stopping the reaction after 2 h allows for an easy distinction between plus-DNA and minus-DNA amplification reactions while still yielding large quantities of amplified product.

The amplification product is highly viscous and difficult to pipette accurately. It is recommended that you dilute the amplification product 10- 100-fold in 1x TE buffer with thorough mixing either by pipetting up and down or gently vortexing before using in downstream applications. The amplified DNA is ready to be used directly in most applications.

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