



## DNA from Oragene®/saliva samples† and whole genome amplification with GenomiPhi™

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*DNA collected with the Oragene® self-collection kit is successfully amplified using the GenomiPhi™ DNA amplification kit and has the potential to generate near-unlimited quantities of DNA for downstream experiments.*

### Introduction

As the genotyping of single-nucleotide polymorphisms (SNPs) becomes more automated and less costly, the limiting factor for genome-wide genetic analysis is the amount of DNA that can be collected from study subjects. A renewable source of genomic DNA is desirable. The traditional method of transforming lymphoblastic cell lines is usually too expensive for population studies with large sample sizes. A less expensive alternative is whole genome amplification (WGA), which allows in vitro production of numerous copies of the entire genome.

Multiple displacement amplification (MDA) is a WGA method that uses Phi29 DNA polymerase and random priming. MDA generates high-fidelity genomic copies with minimal bias<sup>1</sup>.

The GenomiPhi DNA amplification kit from Amersham Biosciences utilizes MDA to exponentially amplify genomic DNA. The purpose of this study was to determine if DNA collected with Oragene could be amplified with GenomiPhi and to test the quality of the amplified DNA for PCR and SNP genotyping.

### Materials and methods

#### DNA collection

Saliva was collected from 10 donors using Oragene self-collection kits. DNA was purified from 200 µL aliquots of Oragene/saliva samples using the prepIT™•L2P protocol<sup>2</sup>. Purified DNA was

redissolved in 200 µL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). DNA was quantified using a fluorimeter and SYBR® Green I dye (Molecular Probes) according to the F/D protocol<sup>3</sup>.

#### Whole genome amplification

Following instructions provided with the GenomiPhi kit, 1 µL of DNA was added to 9 µL of sample buffer containing random hexamers and heated to 95°C to denature the DNA. The sample was cooled and mixed with 9 µL of reaction buffer containing salts and deoxynucleotides and 1 µL of enzyme mix. The mixture was incubated overnight at 30°C. After amplification, the Phi29 DNA polymerase was heat-inactivated during a 10 minute incubation at 65°C. Table 1 summarizes the steps in the protocol.

The amplified DNA was quantified using the F/D protocol. The molecular weight was analyzed by agarose gel electrophoresis with ethidium bromide staining and comparison with a Lambda-Hind III digest ladder.

Step	Description
1	Mix 1 µL of DNA with 9 µL of sample buffer
2	Heat to 95°C for 3 minutes
3	Cool to 4°C on ice
4	Mix cooled sample with 9 µL of reaction buffer and 1 µL of enzyme mix
5	Incubate sample overnight at 30°C (16-18 h)
6	Heat-inactivate sample at 65°C for 10 minutes
7	Store at 4°C or -20°C

**Table 1:** Summary of the GenomiPhi protocol.

† Saliva samples were collected with Oragene®•DNA or Oragene®•DISCOVER.

### Polymerase chain reaction

WGA and original unamplified DNA samples were analyzed by PCR and agarose gel electrophoresis for a 560 bp fragment of the human thymidylate synthetase gene.

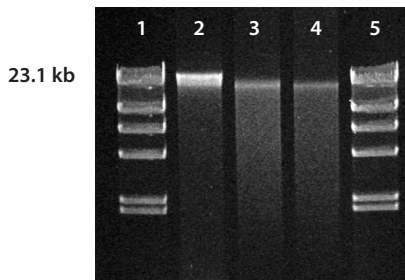
### SNP genotyping

A TaqMan SNP Genotyping Assay for the thymidylate synthetase (TYMS) gene was obtained from Applied Biosystems (TaqMan Assay ID: C\_\_1637541\_1\_). WGA and original DNA samples were genotyped using a Rotor-Gene 3000™ real-time quantitative thermal cycler (Corbett Research). Reactions were set up according to the manufacturer's instructions<sup>4,5</sup>.

## Results

### Whole genome amplification

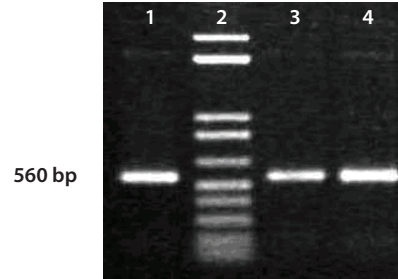
For the WGA reactions, the average amount of starting DNA template was  $13 \pm 2$  ng (mean  $\pm$  SEM). The control DNA supplied with the kit had a concentration of 10 ng/ $\mu$ L. The average amount of DNA after amplification was  $7.60 \pm 0.13$   $\mu$ g, representing about a 600-fold increase. Figure 1 compares the molecular weight of WGA and unamplified DNA samples.



**Figure 1:** Agarose gel electrophoresis of DNA samples. Lane 2 contains unamplified DNA sample. Lanes 3 and 4 show WGA reactions with sample and control DNA respectively. A Lambda-Hind III digest was used as the marker in Lanes 1 and 5.

### Polymerase chain reaction

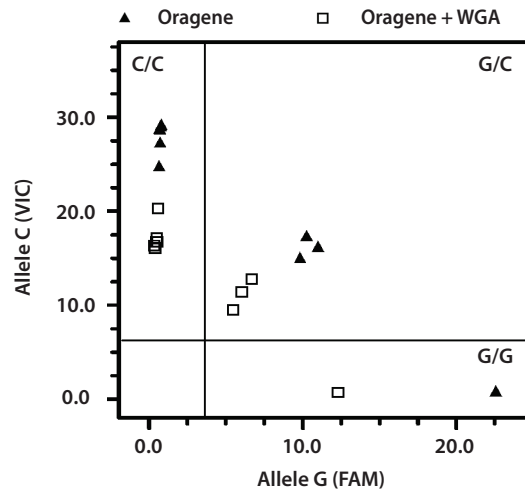
Figure 2 shows a typical PCR result for WGA and unamplified DNA samples.



**Figure 2:** PCR results. Lane 1 shows PCR with unamplified control DNA and Lane 2 contains a 100 bp ladder. Lanes 3 and 4 show PCR with amplified and unamplified DNA respectively.

### SNP genotyping

Figure 3 shows an allelic discrimination plot for the thymidylate synthetase assay. The plot is presented as the signal (average fluorescence between cycles 41 and 45) minus the background (average fluorescence between cycles 21 and 25).



**Figure 3:** Allelic discrimination plot for the thymidylate synthetase assay.

