



Oragene™ and Whole Genome Amplification with GenomiPhi™

J. Chartier
DNA Genotek Inc., Ottawa, ON, Canada

DNA collected with Oragene 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 becoming the limited 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 (ref. 1).

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 DNA Self-Collection Kits. DNA was purified from 200 µL aliquots of Oragene/saliva samples using the ethanol precipitation protocol supplied with the kits. 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 (ref. 2).

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 min
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 min
7	Store at 4°C or -20°C

Table 1. Summary of the GenomiPhi protocol.

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 (ref. 3, 4).

Results

Whole genome amplification

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

Polymerase Chain Reaction

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

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

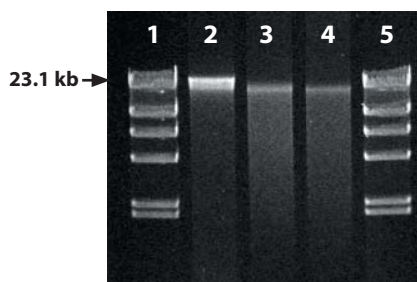


Figure 1. Agarose gel electrophoresis of DNA samples. Lane 2 contains unamplified DNA sample. Lanes 3 and 4 show WGA reactions with sample and control DNA respectively. A Lambda-Hind III digest was used as the marker in Lanes 1 and 5.

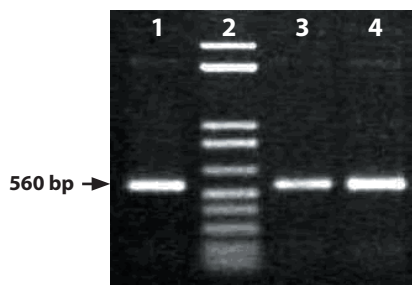


Figure 2. PCR results. Lane 1 shows PCR with unamplified control DNA and Lane 2 contains a 100-bp ladder. Lanes 3 and 4 show PCR with amplified and unamplified DNA respectively.

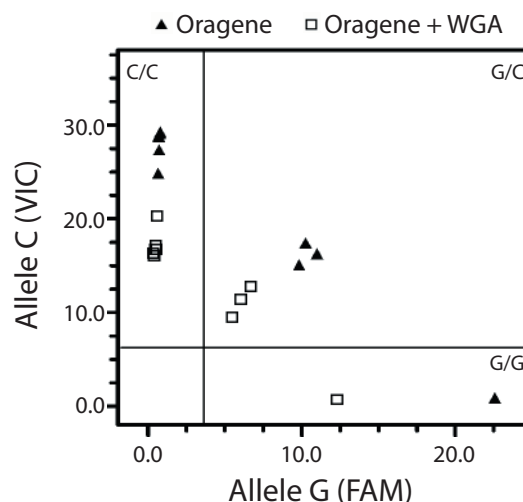


Figure 3. Allelic discrimination plot for the thymidylate synthetase assay.

between cycles 21 and 25).

Discussion and Conclusions

According to the manufacturer, 10 ng of starting DNA should generate 4 to 7 μ g of amplified DNA with a high molecular weight (ref. 5). DNA from Oragene was successfully amplified with the GenomiPhi kit and gave an average yield of 7.60 μ g, slightly higher than the expected amount. The amplified DNA worked well for both PCR and TaqMan reactions. The WGA DNA gave similar PCR bands to the original unamplified DNA and the SNP genotyping calls were identical.

With Oragene, the median DNA yield is 110 μ g from 2 mL of saliva (ref. 6). GenomiPhi allows DNA from Oragene to be amplified around 600-fold. Furthermore, this amplified DNA may be used as the template for subsequent WGA reactions. Up to 4 re-amplifications have been performed with no apparent effect on PCR of various loci across the human genome (ref. 7). In summary, DNA collected with Oragene works well with the GenomiPhi WGA kit and has the potential to generate near-unlimited quantities of DNA for downstream experiments.

References

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