DNA from Oragene®/saliva samples† and REPLI-g® whole genome amplification

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DNA collected with the Oragene® self-collection kti is successfully amplified using the Qiagen REPLI-g® kit. 10 ng of starting template generates about 40 µg of amplified DNA for downstream experiments.

Introduction

The Oragene self-collection kit facilitates the collection and preservation of large amounts of DNA – a median of 110 µg of DNA from 2 mL of saliva[1]. Yet a renewable source of DNA may be desirable, given the rare and valuable nature of many specimens. Towards this end, Whole Genome Amplification (WGA) allows in vitro production of virtually unlimited copies of genomic DNA.

Multiple Displacement Amplification (MDA) is a WGA method based on rolling circle amplification using Phi29 DNA polymerase and random primers. MDA generates high-fidelity genomic copies with minimal bias[2]. MDA-amplified DNA has been validated with diverse applications such as SNP genotyping, STR analysis, comparative genomic hybridization, RFLP analysis, subcloning, and DNA sequencing[3, 4, 5].

The REPLI-g kit from Qiagen utilizes MDA to exponentially amplify genomic DNA. The purpose of this study was to determine if DNA collected with Oragene could be amplified with REPLI-g, 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 500 µL aliquots of the Oragene/saliva samples using the prepIT™•L2P protocol[6]. Purified DNA was redissolved in 75 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 assay[7].

Whole genome amplification

Whole genome amplification of the DNA purified from Oragene/saliva samples was performed according to instructions provided with the REPLI-g kit. In brief, 2.5 µL of DNA was added to 2.5 µL of Buffer D1 (containing KOH & EDTA) to denature the DNA. The sample was incubated for 3 minutes at room temperature, before adding 5 µL of Buffer N1 (neutralizing buffer). Then, 40 µL of Master Mix containing the REPLI-g DNA polymerase were added to 10 µL of the denatured DNA solution. The mixture was incubated overnight at 30°C. After amplification, the REPLI-g DNA polymerase was heat-inactivated during a 10 minute incubation step at 65°C. Table 1 summarizes the steps in the protocol.

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

Step	Description
1	Mix 2.5 µL of DNA with 2.5 µL of Buffer D1
2	Incubate at room temperature for 3 minutes
3	Add 5 µL of Buffer N1
4	Mix with 40 µL of Master 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 REPLI-g protocol.*

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

Polymerase chain reaction

WGA and 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 unamplified 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[8, 9].

Results

Whole genome amplification

For the WGA reactions, the amount of starting DNA template was 10 ng, and the average amount of DNA after amplification was 50 ± 1.6 µg, representing about a 5,000-fold increase. Similarly, 10 ng of the supplied control DNA was amplified to 58.5 µg. 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 genoytping

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).

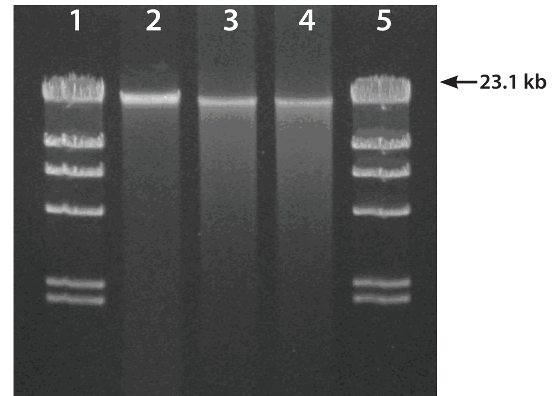

Figure 1: *Agarose gel electrophoresis of DNA samples. Lane 2 contains unamplified DNA sample. Lanes 3 and 4 show WGA reactions with sample and control DNA, respectively. A Lambda-Hind III digest was used as the marker in Lanes 1 and 5.*

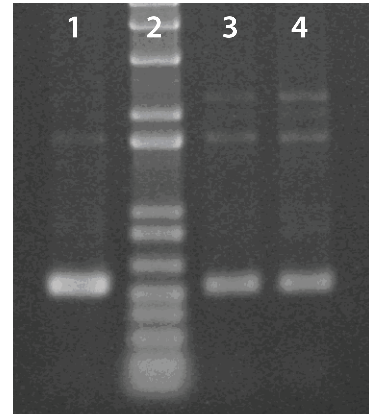

Figure 2: *PCR results. Lane 1 shows PCR with unamplified control DNA and Lane 2 contains a 100 bp ladder. Lanes 3 and 4 show PCR with WGA and unamplified DNA, respectively.*

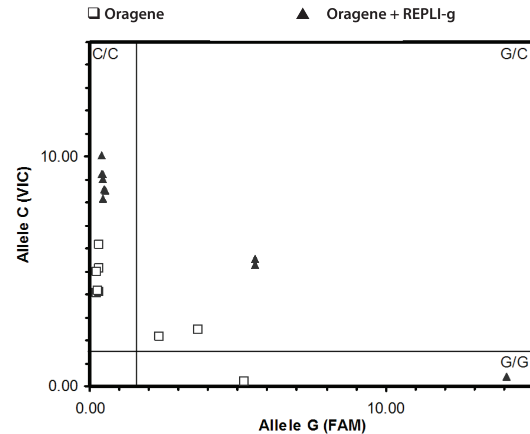

Figure 3: *Allelic discrimination plot for the thymidylate synthetase assay.*

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Discussion and conclusions

According to the manufacturer, 10 ng of starting DNA typically generates 40 µg of high molecular weight amplified DNA[10]. DNA from Oragene/saliva samples was successfully amplified with the REPLI-g kit and gave an average yield of 50 µg, 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 µg from 2 mL of saliva1. REPLI-g allows DNA from Oragene/saliva samples to be amplified around 5,000-fold. Additionally, Oragene/saliva samples have been shown to be stable for extended periods of time at room temperature[11], thereby supplying a long-term source of genomic DNA template for WGA reactions.
In summary, DNA collected with Oragene kits works well with the REPLI-g WGA kit and has the potential to generate renewable quantities of DNA for downstream experiments.

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