

Oragene®•DNA and GenomePlex® Whole Genome Amplification

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DNA collected with Oragene•DNA is successfully amplified using the GenomePlex® Kit, thus creating a renewable source of DNA for downstream experiments.

Introduction

The ability to replenish valuable DNA specimens may be useful in a variety of situations. For example, biobanks may receive requests for DNA samples from specific diseases. Often, the patient may be deceased or otherwise unable to provide additional DNA. For situations like this, Whole Genome Amplification (WGA) is a method by which small amounts of starting DNA material can be multiplied time and again to generate a renewable source of genomic DNA.

The GenomePlex WGA Kit (Sigma-Aldrich) randomly cuts genomic DNA into a library of fragments, ranging from 200 to 1,500 base pairs in size, with an average of 400 bp. Universal priming sites are added to the DNA fragments, which are then amplified using a high-fidelity DNA polymerase, without degradation of representation (ref. 1).

The purpose of this study was to determine if DNA collected with Oragene•DNA could be amplified with the GenomePlex Kit, and to test the performance of the amplified DNA for PCR and SNP genotyping applications.

Materials and Methods

DNA collection

Saliva was collected from 10 donors using Oragene•DNA Self-Collection Kits. DNA was purified from 500 µL aliquots of the Oragene•DNA/saliva samples using the ethanol precipitation protocol supplied with the kits. 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 (Molecular Probes) according to the F/D assay (ref. 2).

Whole Genome Amplification

The WGA reactions were performed according to instructions provided with the GenomePlex Kit. The first stage of the protocol involved random fragmentation of the genomic DNA and conversion of the resulting small fragments to PCR-amplifiable OmniPlex® Library molecules flanked by universal priming sites. The second stage involved amplification of the OmniPlex Library using universal oligonucleotide primers and a limited number of temperature cycles. 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 comparison with a Lambda-Hind III digest ladder.

Step	Description
1	Heat 10 µL of DNA template to 95°C for 4 min
2	Cool on ice and add Library Preparation and Stabilization buffers
3	Heat to 95°C for 2 min
4	Cool on ice and add Library Preparation enzyme
5	Perform single cycles at 16°C, 24°C, and 37°C for 20 min each, then 75°C for 5 min and hold at 4°C
6	Library amplification is performed by adding 60 µL Master Mix containing JumpStart Taq
7	Heat to 95°C in a thermal cycler followed by 14 cycles of 95°C for 15 sec and 65°C for 5 min
8	Store at 4°C or -20°C

Table 1. Summary of the GenomePlex protocol.

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 (ref. 3, 4).

Results

Whole Genome Amplification

For the WGA reactions, the amount of starting DNA template was 10 ng. The average amount of DNA after amplification was 6.52 ± 0.20 µg, representing about a 650-fold increase. This compares to about an 883-fold increase generated with the control DNA. 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.

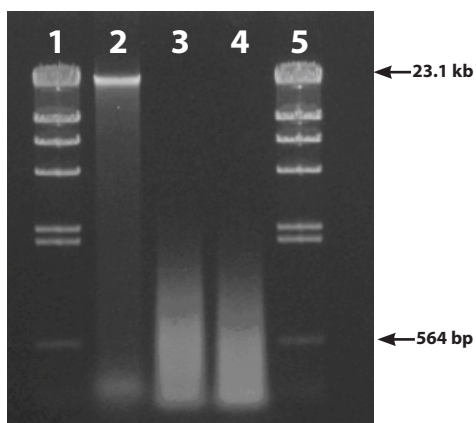


Figure 1. Agarose gel electrophoresis of DNA samples. Lane 2 contains unamplified DNA sample. Lanes 3 and 4 show WGA reactions with sample and control DNA, respectively. A Lambda-Hind III digest was used as the marker in Lanes 1 and 5.

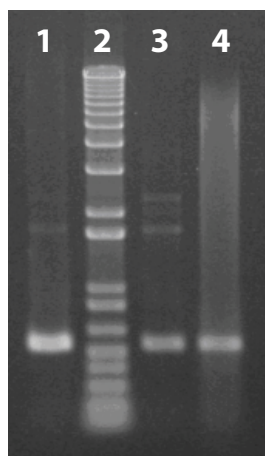


Figure 2. PCR results. Lane 1 shows PCR with unamplified control DNA and Lane 2 contains a 100-bp ladder. Lanes 3 and 4 show PCR with WGA and unamplified DNA template, respectively.

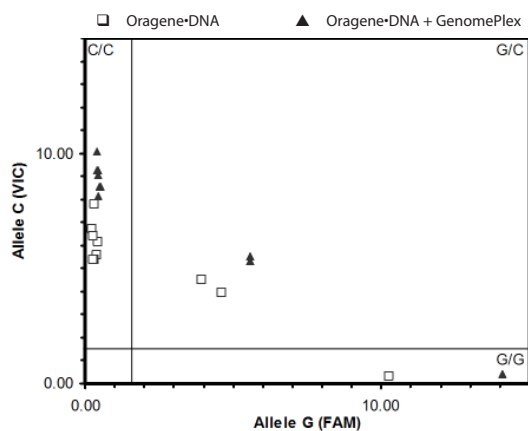


Figure 3. Allelic discrimination plot for the thymidylate synthetase assay.

SNP genotyping

Figure 3 shows an allelic discrimination plot for the thymidylate synthetase real-time PCR 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).

Discussion and Conclusions

According to the manufacturer, 10 ng of starting DNA should generate 5 to 10 μg of amplified DNA (ref. 5). DNA from Oragene•DNA gave an average yield of 6.52 μg , which is within the expected range. The amplified DNA worked well for both PCR and TaqMan reactions—the PCR amplicons were of similar intensity and the SNP genotyping calls were identical.

With Oragene•DNA, the median DNA yield is 110 μg from 2 mL of saliva (ref. 6). The GenomePlex Kit allows DNA from Oragene•DNA to be amplified around 650-fold, thus resulting in a renewable source of DNA. In summary, DNA collected with Oragene•DNA is successfully amplified with the GenomePlex Kit, and the amplified DNA works well with downstream applications such as PCR and SNP genotyping.

References

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