

GenPlates® for the storage of Oragene®•DNA samples

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Saliva samples collected with Oragene•DNA may be conveniently stored in a dry form on GenPlates®. From the GenPlate, the DNA may be recovered and purified with GenSolve™ kits. The recovered DNA is high quality and suitable for PCR, real-time PCR, and SNP genotyping applications.

Introduction

GenPlates from GenVault are the core of an integrated biosample management system. GenPlates eliminate the need for freezers and liquid nitrogen tanks while maintaining biosamples in a stable format suitable for long term archiving, automated retrieval and simplified distribution. The 384-well GenPlates contain 6 mm disc-shaped elements of Whatman FTA® paper in each well. Once a sample is applied to a GenPlate, a chain of custody is established because each GenPlate is labeled with a unique physical barcode and each element within the GenPlate is labeled with GenCode®, a biological barcode which permanently identifies the actual sample regardless of its physical state (ref. 1). The purpose of this study was to determine the compatibility of GenPlates for the storage and recovery of DNA from saliva samples collected with Oragene•DNA.

Materials and Methods

DNA collection

Saliva samples were collected from six donors using the Oragene•DNA Self-Collection Kit. An aliquot was removed and purified using the Oragene•DNA Purification Protocol (ref. 2). Purified DNA was quantified by fluorescence with SYBR® Green (Molecular Probes) (ref. 3).

GenPlate storage

Unpurified Oragene•DNA/saliva samples were spotted onto GenPlates according to the manufacturer's instructions. After storage for two weeks at room temperature, DNA was recovered and purified using the GenSolve kit (GenVault) (ref. 4). Purified DNA was quantified by fluorescence with SYBR Green.

DNA analysis

The DNA recovered from the GenPlates was analyzed by three different methods. First, the amount of human and bacterial DNA in each DNA sample was quantified by real-time PCR with SYBR Green. The reactions amplified a 143-bp fragment of the human Thymidylate Synthetase (TYMS) gene and a highly-conserved fragment of the bacterial 16S rRNA gene. The human primers were Forward 5'-gcc ctc tgc cag ttc ta-3' and Reverse 5'-ttc agg ccc gtc atg t-3', and the bacterial primers were Forward 5'-cct acg gga ggc agc ag-3' and Reverse 5'-att acc gcg gct gct gg-3'. All of the real-time PCR reactions were performed using a Rotor-Gene™ RG-3000A (Corbett Research).

Next, a 2,568-bp fragment of the human mitochondrial genome was amplified by PCR using a PTC-100 thermal cycler (MJ Research). The primer sequences were Forward 5'-ctg tgg ggg gtc tct ttg ggg-3' and Reverse 5'-cgc acg gac tac aac cac gac-3'.

Finally, SNP genotyping was performed using two LightCycler® Mutation Detection Kits (Roche Diagnostics). The CYP2C9 kit detects two major SNPs of the human cytochrome P450 2C9 gene: C to T at position 430 (CYP2C9*2), and A to C at position 1075 (CYP2C9*3). The Apo E Kit detects two point mutations in codons 112 and 158 of the human Apolipoprotein E gene: C to T at position 3932 and C to T at position 4070, respectively. All reactions were performed using the LightCycler 2.0 System (Roche Diagnostics).

Results

With the Oragene•DNA purification protocol, the six donors had an average DNA yield of 94.5 µg for the 4-mL Oragene•DNA/saliva sample. With the GenSolve kits from the GenPlates, the DNA recovery efficiency was 72% of the Oragene•DNA yield. Based on the real-time PCR results, the average percentage of human DNA was 92% and the average percentage of bacterial DNA was 6.7%.

All of the PCR and SNP genotyping reactions were successful. Figure 1 shows representative PCR results and Figure 2 shows SNP genotyping results for the Apo E gene.

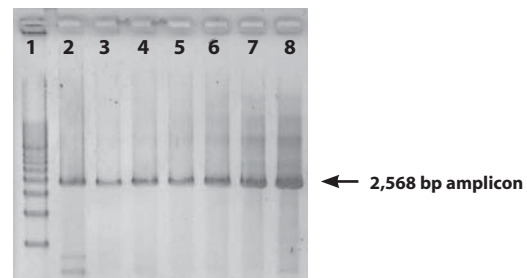


Figure 1. Agarose gel of PCR reactions, with SYBR® Gold staining. Lane 1 is a 500 bp ladder and Lane 2 is a reaction with human control DNA. Lanes 3 to 8 contain Samples 1 to 6, respectively.

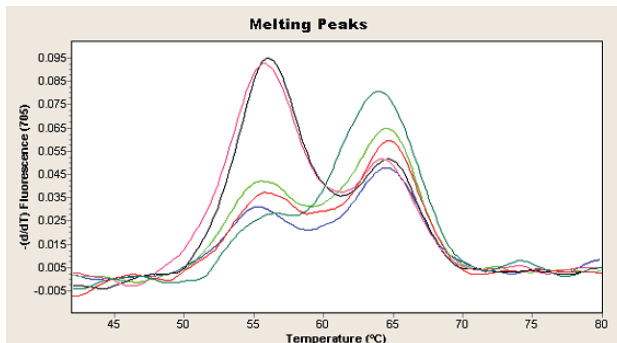


Figure 2. SNP genotyping results for codon 158 of the Apo E gene. Samples 1 and 6 were homozygous (CGC), and Samples 2, 3, 4, and 5 were heterozygous (TGC).

Discussion and Conclusions

Oragene•DNA/saliva samples were successfully stored in a dry form on the GenPlates. Using the GenSolve kit, the recovered DNA was high quality and suitable for PCR, real-time PCR and SNP genotyping applications. The majority of DNA in the Oragene•DNA/saliva samples was human rather than bacterial in origin. In contrast, mouthwash or buccal swab samples may have a median bacterial DNA content as high as 88.5% (ref. 5, 6). In conclusion, Oragene•DNA was compatible with the GenPlate and GenSolve kits, and this system may be particularly useful for archiving large numbers of samples because of savings in labor, storage, and specimen retrieval costs.

References

1. Dutton, G. (2005). Thinking outside the icebox on DNA storage. *The Scientist*. July 18.
2. Laboratory Protocol for Manual Purification of DNA from 0.5 mL of Oragene•DNA/saliva. PD-PR-006
3. DNA quantification using the Fluorescence/DNase (F/D) assay. (2004). *DNA Genotek*. Nov.
4. GenSolve™ DNA recovery kit instruction manual: manual and automated protocols. Version 2.1. (2005). *GenVault*. Revision #URM0007.
5. Chartier, J. and Birnboim, H.C. (2005). Bacterial DNA content with Oragene•DNA, *DNA Genotek*. PD-WP-003
6. Garcia-Closas, M., Egan, K., Abruzzo, J., Newcomb, P., Titus-Ernstoff, L., Franklin, T. et al. (2001) Collection of Genomic DNA from Adults in Epidemiological Studies by Buccal Cytobrush and Mouthwash. *Cancer Epidemiology, Biomarkers & Prevention*. 10, 687-696.