Technical Note

Use of Saliva gDNA for SNP Genotyping

Abstract

This technical note describes the results of a comparative genotyping study performed with the Genome-Wide Human SNP Array 6.0 using paired (i.e., from the same individual) genomic DNA samples from saliva and blood. The results indicate that genomic DNA samples isolated from saliva and blood perform comparably when used as a template in the Affymetrix whole-genome sampling assay (WGSA).

Introduction

The advancement of technologies to assess human genetic variation in a high-throughput, high-resolution manner has enabled scientists to perform extensive studies of the role of genetic changes in disease.

Genomic DNA derived from blood is a common source for these studies; however, blood collection has several drawbacks, including high costs, danger of infection, and sample degradation. These drawbacks have resulted in the development of novel collection, transportation, and isolation technologies for other sources of genomic DNA. In this technical note, we evaluate the use of genomic DNA (gDNA) from paired saliva (collected with Oragene™ DNA Self Collection Kits, manufactured by DNA Genotek) and blood for SNP genotyping on the Genome-Wide Human SNP Array 6.0. The results demonstrate that saliva gDNA collected with DNA Genotek's Oragene technology performs comparably to blood-extracted gDNA.

Using saliva gDNA in SNP genotyping studies

The SNP Array 6.0 enables researchers to analyze nearly 2 million markers of genetic variation. To prepare target for these arrays, sample gDNA is digested and ligated to adaptors, then amplified during a PCR reaction using a single universal primer set. Next, PCR amplicons are fragmented, labeled, and hybridized to the array (Kennedy, *et al.*). In this study, we explored the use of gDNA from both saliva and blood for genome-wide SNP genotyping.

The DNA Genotek Oragene DNA Self Collection Kit is a self-contained system for the collection, storage, and transportation of saliva. This system is available in three distinct formats (varying in shape and amount of saliva collected) as needed. Saliva collected via this system is mixed with a proprietary DNA preserving fluid which stabilizes the saliva for long-term storage at room temperature prior to DNA extraction. A DNA extraction protocol is then used to isolate the gDNA from the stabilized saliva, and the gDNA extracted in this manner has been shown to be suitable for use in various downstream applications (as described on the DNA Genotek website).

We report three conclusions from tests comparing gDNA from paired blood and saliva samples on the SNP Array 6.0:

- gDNA from both saliva and blood demonstrated similarities in concentration and purity when analyzed on the NanoDrop ND1000 spectrophotometer (see Table 1)
- gDNA, intact PCR products and fragmented PCR products from both saliva and blood have similar size distributions when electrophoresed on an agarose gel (see Figure 1)
- The paired blood and saliva samples were all successfully run on the SNP Array 6.0 and passed the performance quality thresholds. Reproducibility in genotype calls between the paired blood and saliva samples was greater than 99.6 percent (see Table 2)

In summary, it is possible to use saliva gDNA with the Affymetrix SNP genotyping WGSA assay and the SNP Array 6.0 to achieve results comparable to those achieved with blood gDNA.

Material and methods

Paired saliva and blood collection

A total of 66 paired blood and saliva samples were taken from IRB-approved volunteer donors of varying ethnic backgrounds. Saliva was collected using the DNA Genotek Oragene DNA Self Collection Kit (DNA Genotek, Canada).

gDNA extraction from blood and saliva

This experiment used the Beckman Coulter Biomek® NXP platform with Agencourt chemistry to extract gDNA from blood samples. gDNA from the paired saliva samples were extracted using the manual extraction method provided by DNA Genotek (DNA Genotek Laboratory Protocol for Manual Purification of DNA from 0.5 mL of Oragene DNA/saliva, PD-PR-006 Issue 3.2).

Determination of DNA concentration

UV spec readings (Nanodrop) were used to determine yield and concentration of the gDNA samples from both blood and saliva.

Labeling of DNA for GeneChip® analysis and hybridization

For the SNP Array 6.0, 500 ng gDNA was processed strictly according to the Genome-Wide Human SNP Nsp/Sty 6.0 User Guide.

Table 1: The average values of intact gDNA extracted from blood and saliva samples and their respective standard deviations. Also listed are the Affymetrix ranges for each metric that are considered acceptable for downstream applications. The concentration and yield recommendations are based on information in the Genome-Wide Human Nsp/Sty 6.0 User Guide.

	A260/A280 of intact gDNA +/- stdv Not established	Concentration of intact gDNA (ng/µl) +/- stdv Acceptable measurement: >50	Yield (µg) of intact gDNA +/- stdv Acceptable measurement: >0.5
Blood	1.71 +/- 0.17	117.74 +/- 63.75	4.71 +/- 2.55
Saliva	1.73 +/- 0.10	190.40 +/- 160.80*	19.00 +/- 16.10

*In rare cases the concentration of saliva gDNA was < 50 ng/µl (the concentration required for use in downstream applications). In these cases, the yield was sufficient to allow for volume adjustments to increase concentration.

Genotype analysis

Default settings were used for all data analysis. The SNP Array 6.0 data was analyzed using the Birdseed v.2 algorithm implemented in Genotyping Console™ 2.1.

Results and discussion

gDNA extraction results: average concentration and yield of gDNA in blood and saliva

Both blood and saliva yielded gDNA that met or exceeded the Affymetrix performance specifications for A260/280, concentration, and yield metrics (see Table 1).

Gel imaging results

gDNA, intact PCR products, and fragmented PCR products from saliva and blood have similar size distributions when electrophoresed on agarose gels (see Figure 1 on page 4). The appearance of saliva gDNA (gel image 2B) is more variable than the appearance of the blood gDNA (gel image 2A), but this did not affect the performance of the saliva gDNA in the WGSA assay (see Table 2).

Applying paired blood and saliva gDNA to the SNP Array 6.0

The paired blood and saliva samples were all successfully run on the SNP Array 6.0. The samples were analyzed using the Birdseed algorithm, yielding an excellent average call rate for each set of samples, as well as successful concordance between the paired blood and saliva samples. Reproducibility was greater than 99.6 percent between the paired samples (see Table 2).

Conclusion

The results presented in this technical note demonstrate that gDNA from saliva is a viable alternative to the use of blood-derived gDNA. Saliva yields DNA of sufficient purity, quantity, and concentration for use in the SNP genotyping WGSA assay. The output of this assay (labeled DNA) can be hybridized to the SNP Array 6.0, and the results are greater than 99.6 percent concordant with the results of labeled gDNA from blood that has been hybridized to the SNP Array 6.0.

References

Kennedy G. C., *et al.* Large-scale genotyping of complex DNA. *Nature Biotechnology* **21**(10):1233-7 (2003).

Matsuzaki H., *et al.* Parallel genotyping of over 10,000 SNPs using a one-primer assay on a high density oligonucleotide array. *Genome Research* **14**:414-25 (2004).

Protocol, Agencourt® Genfind™ V2 Blood and Serum Genomic DNA Isolation Kit.

DNA Genotek Laboratory Protocol for Manual Purification of DNA from 0.5 mL of Oragene® DNA/saliva (PD-PR-006 Issue 3.2).

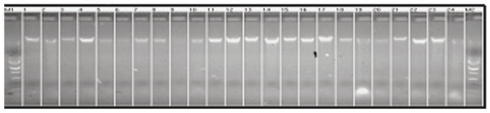
User Guide, Affymetrix® Genome-Wide Human SNP Nsp/Sty 6.0.

Table 2: Call rates for the average of all samples, average blood, and average saliva. All call rates were above the standard passing minimum rates.

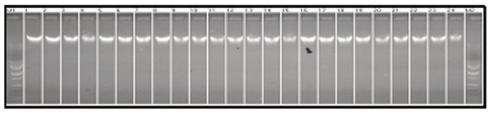
	Birdseed					Reproducibility between paired blood and saliva samples
	Call rate (%)	AB percent	AA percent	BB percent	Gender concordance	Percent (%)
Median (all)	99.65 +/- 0.03	25.82 +/- 0.13	37.26 +/- 0.07	36.49 +/- 0.06	100%	99.65 +/- 0.31
Median (blood)	99.74 +/- 0.02	25.88 +/- 0.18	37.26 +/- 0.10	36.52 +/- 0.09		
Median (saliva)	99.44 +/- 0.04	25.75 +/- 0.18	37.25 +/- 0.10	36.43 +/- 0.09		

Figure 1: Gel electrophoresis. a) 1 percent agarose gel of saliva genomic DNA; b) 1 percent agarose gel of blood genomic DNA; c) 2 percent agarose gel of PCR product from saliva samples; d) 2 percent agarose gel of PCR product from blood samples; e) 4 percent agarose gel of fragmented PCR product from saliva samples; f) 4 percent agarose gel of fragmented PCR product from blood samples.

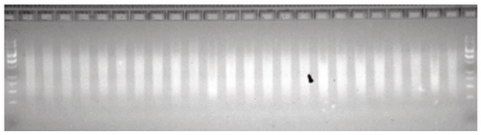
A) Saliva gDNA



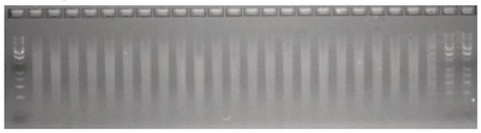
B) Blood gDNA



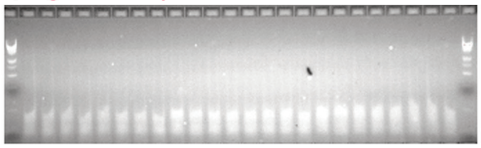
C) PCR products from saliva



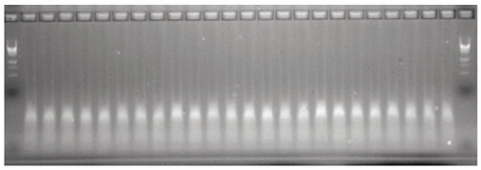
D) PCR products from blood



E) Fragmented PCR products from saliva



F) Fragmented PCR products from blood



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