Comparison of high density genotyping results from saliva and blood samples on Affymetrix GeneChip® GenomeWide SNP 6.0 arrays

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Introduction

Currently, EDTA-stabilized whole blood is the most common sample type used for high density genotyping. Blood has proven a very consistent and reliable source of genetic material for many avenues of testing and research, but it can also be a time consuming, expensive and invasive collection method- especially for long term or broad range studies. Finding a comparable source of genetic material, such as saliva, that is more cost effective, more stable and less invasive would be extremely beneficial to the scientific community.

This experiment used the Beckman-Coulter Biomek NX^P platform with Agencourt chemistry to extract genomic DNA from blood samples. DNA from the paired saliva samples were extracted using the manual extraction method provided by DNA Genotek. The study compared not only the DNA quality and quantity, but also the microarray call and concordance rates (CR) to indicate saliva's suitability for genetic association studies. All samples were prepared and run simultaneously on the Genome-Wide SNP6.0 arrays (GWS6.0).

GeneChip® microarrays consist of small DNA fragments (or probes), that are synthesized to specific locations on a coated array quartz surface. Millions of probes can be contained on one array. All the samples are scanned and analyzed using Affymetrix GeneChip® Genotyping Console (AGGC). AGGC performs a multiple-chip analysis fitting probe effects to increase precision on signal estimates for the two alleles of each SNP, followed by a Bayesian classification approach to make genotype calls.

Materials and Methods

Paired EDTA-stabilized whole blood samples and DNA Genotek Oragene saliva samples were collected from **66** IRB-approved volunteer donors.

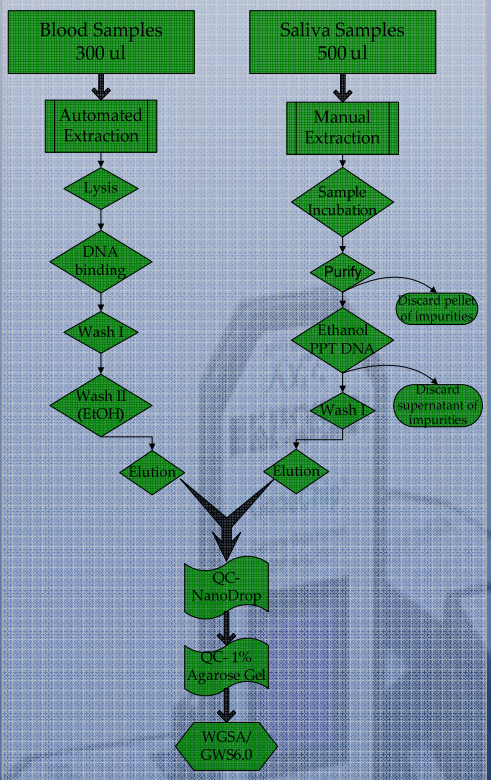

Figure 1: Overview of the extraction procedures and QC for paired blood and saliva samples. All samples were analyzed for concentration (ng/µL), purity (A260/A280 ratios) and yield (µg) on a NanoDrop ND-1000 spectrophotometer as well as for integrity on a 1% agarose gel.

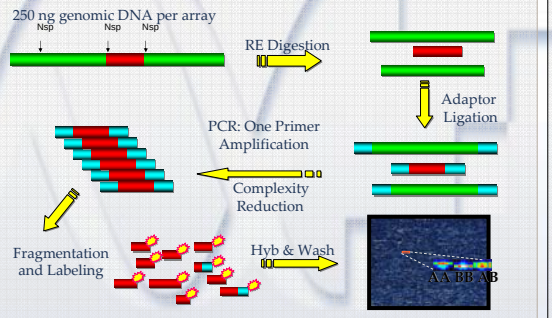

Figure 2. Overview of the Affymetrix GWS6.0 process. The WGSA protocol requires 500 ng (250ng for each Nsp and Sty reactions) of purified genomic DNA . The GWS6.0 array is used in conjunction with Affymetrix Genotyping Console to determine genotypes at each of 909,000 loci.

Results

DNA was successfully extracted from blood samples using the automated extraction procedure and also from saliva samples using a manual extraction technique. The paired samples demonstrated similarities in concentration, integrity, size and purity when analyzed on the NanoDrop ND1000 spectrophotometer **[Figure 3, Table 1]** as well as on a 1% agarose gel **[Figure 4]**.

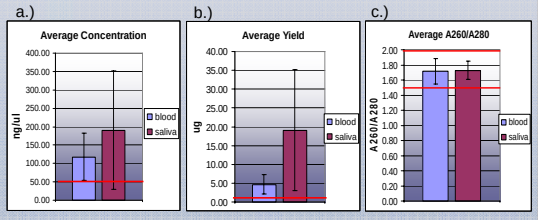

Figure 3. Average quality control metrics analyzed by the NanoDrop ND1000 spectrophotometer for blood and saliva samples. **a.)** The average concentration (ng/µl) for the blood and saliva samples. **b.)** Average total yield for the blood and saliva samples. **c.)** Average A260/A280 ratios for the blood and saliva samples. Red lines indicate minimums/maximums.

	A260/A280 +/- stdev	Concentration (ng/ul) +/- stdev	Yield (ug) +/- stdev
	Acceptable range: 1.5-2.0	Acceptable range: ≥ 50	Acceptable range: >2
Blood	1.63 +/- 0.09	149.34 +/- 59.67	5.97 +/- 2.39
Saliva	1.79 +/- 0.08	138.57 +/- 83.81	5.54 +/- 3.35

Table 1. The average metrics of DNA 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.

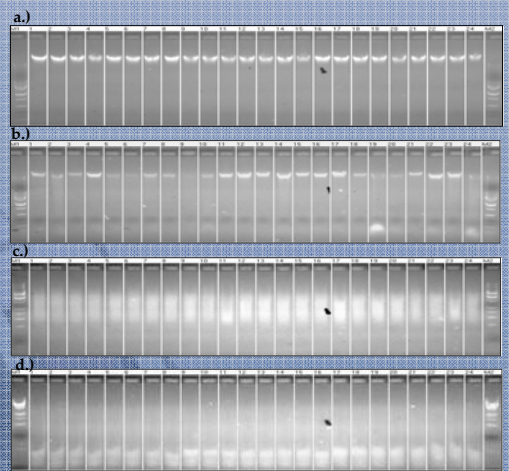

Figure 4. Gel electrophoresis. **a.)** 1% agarose gel of blood genomic DNA. **b.)** 1% agarose gel of saliva genomic DNA. **c.)** 2% agarose gel of PCR purification for both blood and saliva samples. **d.)** 4% agarose gel of fragmentation for blood and saliva samples.

The paired blood and saliva samples were all successfully run on the GWS6.0 arrays and passed the QC call rate thresholds. 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 almost 100% between the paired samples.

	QC		BIRDSEED					Concordance
	Call Rate (%)	Gender Conc.	Call Rate (%)	AB %	AA %	BB %	Gender Conc.	Percent (%)
Median (All)	96.26 +/- 0.25	100%	99.65 +/- 0.03	25.87 +/- 0.12	37.18 +/- 0.07	36.36 +/- 0.06	100%	99.65 +/- 0.31
Median (Blood)	96.45 +/- 0.39		99.74 +/- 0.02	25.91 +/- 0.17	37.25 +/- 0.09	36.51 +/- 0.08		
Median (Saliva)	95.40 +/- 0.30		99.44 +/- 0.04	25.75 +/- 0.18	37.25 +/- 0.10	36.43 +/- 0.09		

Table 2: GWS6.0 analysis QC and Birdseed call rates for the average of all samples, average blood and average saliva. All of the call rates were above the standard passing minimum rates. Reproducibility averages in excess of 99% demonstrate the similarity between the paired blood and saliva samples.

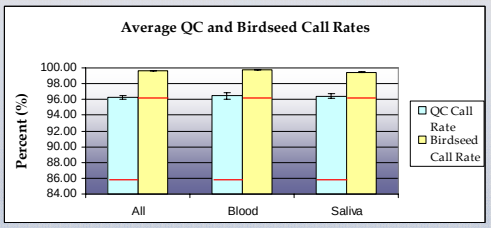

Figure 5. Graphical representation of the average QC and Birdseed call rates for the entire set of samples as well as the individual averages for the blood and saliva samples. The red lines indicate the minimum call rate percentages for QC (86%) and Birdseed (97%).

Conclusions

Concentration and purity QC metrics have demonstrated that DNA extracted from saliva is of similar quality and quantity to that extracted from the paired blood sample. The quality of the genomic DNA from saliva samples demonstrated their suitability for genotyping studies. The paired blood and saliva samples were run on the GWS6.0 arrays, analyzed and then compared to internal standards and to each other. Call rates and reproducibility percentages in excess on 99% verifies that saliva can be used successfully as an alternative source of genomic DNA for use in high density genotyping.

These results demonstrate that genomic DNA extracted from saliva may be used in a clinical and research environment as a comparable source of genetic material for high density genotyping studies. The suitability of saliva samples as a source of genomic DNA allows for the flexibility of a collection method in the clinical and research environment that is more cost effective, less invasive and more suitable for long term and/or broad range studies.

Literature cited

1.) Kennedy, G.C., et. al. Large-scale genotyping of complex DNA. *Nat Biotechnol* 21:1233-7 (2003).

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

3.) Agencourt® Genfind™ V2 Blood and Serum Genomic DNA Isolation Kit protocol.

4.) DNA Genotek Laboratory Protocol for Manual Purification of DNA from 0.5 mL of Oragene®-DNA/saliva

5.)Affymetrix Genome-Wide Human SNP Nsp/Sty 6.0 Users Guide

Acknowledgments

The blood and saliva sample donors from Affymetrix.

For further information

www.affymetrix.com

*Data is from a single lab at Affymetrix and is not a supported protocol for arrays in the field.