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

Affymetrix Clinical Services Laboratory, Affymetrix, Inc., West Sacramento, CA.

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-Coultur Biomass NXS 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 SNPs 6 array (GW6). GenChip® 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.

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 ND1100 spectrophotometer (Figure 3, Table 1) as well as on a 1% agarose gel (Figure 4).

The paired blood and saliva samples were all successfully run on the GW6 array 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.

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 GW6 array, analyzed and then compared to internal standards and to each other. Call rates and reproducibility percentages in excess of 95% 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
3) Agencourt® Genefinder™ V2 Blood and Serum Genomic DNA Isolation Kit protocol.
4) DNA and GenoteX Laboratory Protocol for Manual Purification of DNA from 0.5 mL of Oragene® DNA Kit.
5) Affymetrix Genome-wide Human SNP 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.