DNA genotek

SNP genotyping of DNA from Oragene®/saliva samples† with SNPstream®

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2006-06-29

Genomic DNA collected from saliva with the Oragene® self-collection kit is successfully genotyped with the SNPstream® genotyping system, and gives the same results as DNA isolated from whole blood.

Introduction

The GenomeLab™ SNPstream genotyping system (Beckman Coulter) combines the features of solid-phase chip array, primer extension assay, and universal tags for the genotyping of single-nucleotide polymorphisms (SNPs). The instrument allows the genotyping of up to 1,000,000 SNP genotypes per day, with 96% sample call rates, and >99% concordance with reference genotypes[1]. Sources of genomic DNA such as whole blood[2] and highly degraded forensic samples[3] have been validated with the SNPstream system. The purpose of this study was to compare the SNP genotyping results between paired blood and saliva samples collected with the Oragene self-collection kit.

Materials and methods

DNA collection

Paired saliva and whole blood samples were collected from 25 donors. The saliva samples were collected using the Oragene self-collection kit, and purified according to the prepIT™•L2P purification protocol[4]. Genomic DNA was purified from the whole blood samples using the QIAamp DNA blood mini kit (Qiagen). Purified DNA was quantified by fluorescence with SYBR® Green I dye (Molecular Probes)[5].

SNP genotyping

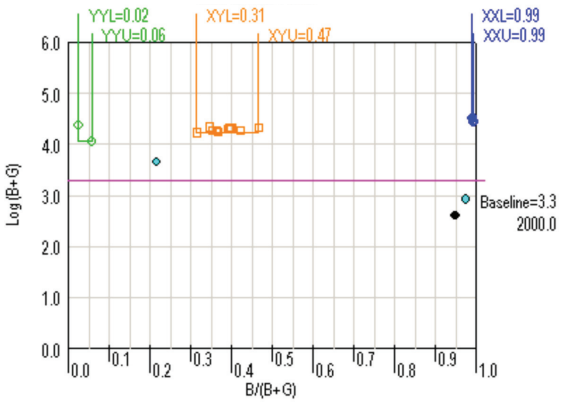
The SNPstream genotyping assay was performed according to methods previously described by Bell et al.[1]. In brief, 4 ng of each DNA sample was used for 12-plex PCR amplification. The PCR-amplified fragments were treated with a cocktail of exonuclease I and shrimp alkaline phosphatase to degrade unincorporated PCR primers and dNTPs. Tagged extension primers were extended using single TAMRA- or BODIPY-Fluorescein-labeled nucleotide terminator reactions, and spatially resolved by hybridization to the complementary oligonucleotide tag on the SNPware® Tag Array (384-well microplate format). The individual SNPs within the multiplex were identified according to the position of the arrayed oligonucleotides within each well. Based on the relative fluorescent intensities for each spot, individual sample genotype data was generated and computer-processed for graphical review.

Results

There was 100% concordance for the genotyping calls between the paired blood and saliva samples, with comparable signal intensity and overall data quality. Initially, one of the saliva samples had weak signal intensity, however successful genotyping was achieved on repeat analysis. Figure 1 shows representative graphical output of the TAMRA/BODIPY fluorescence signals obtained for SNP #5 of 12.

† Saliva samples were collected with Oragene®•DNA or Oragene®•DISCOVER.

(A) Saliva



(B) Whole Blood

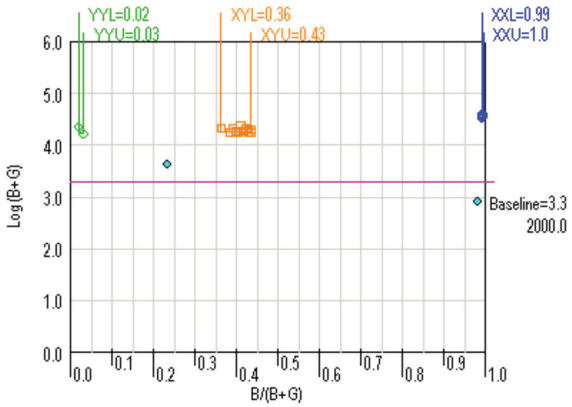


***Figure 1**: SNPstream graphical output of the TAMRA/BODIPY fluorescence signals obtained for SNP #5 of 12. Each saliva (A) or whole blood (B) sample is depicted by green, orange or blue open circles within the associated genotype clusters.*

Discussion and conclusions

DNA from the Oragene/saliva samples gave clear genotyping calls with the SNPstream genotyping system and had 100% concordance with the paired blood samples. These results are in agreement with other studies showing that genomic DNA from oral sources gives the same genotyping results as DNA from whole blood[6, 7]. In summary, genomic DNA from saliva may be collected in a non-invasive manner with the Oragene self-collection kit, and accurately genotyped with the SNPstream genotyping system.

About Ellipsis Biotherapeutics

Ellipsis Biotherapeutics Corporation provides custom SNP genotyping services using state-of-the-art technologies such as the GenomeLab SNPstream genotyping system. For more information, please visit *www.ellipsisbio.com*, or contact *genotyping@ellipsisbio.com*.

References

1 Bell, P., et al. (2002). SNPstream® UHT: ultra-high throughput SNP genotyping for pharmacogenomics and drug discovery. *BioTechniques*. 32: S70-S77.

2 Denomme, G. and Van Oene, M. (2005). High-throughput multiplex single-nucleotide polymorphism analysis for red cell and platelet antigen genotypes. *Transfusion*. 45: 660-666.

3 Budowle, B. (2004). SNP typing strategies. *Forensic Science International*. 146S: S139–S142.

4 Laboratory protocol for manual purification of DNA from 0.5 mL of sample. DNA Genotek. PD-PR-006.

5 DNA quantification using the Fluorescence/DNase (F/D) assay. Replaced with DNA quantification using SYBR Green I dye and a micro-plate reader. DNA Genotek. PD-PR-075.

6 Zheng, S., Ma, X., Buffler, P., Smith, M. and Wiencke, J. (2001). Whole genome amplification increases the efficiency and validity of buccal cell genotyping in pediatric populations. *Cancer Epidemiology, Biomarkers & Prevention*. 10: 697–700.

7 de Vries, H., et al. (1996). Validation of the determination of ΔF508 mutations of the cystic fibrosis gene in over 11000 mouthwashes. *Human Genetics*. 97: 334–336.