Application note: GFX-2

Performance of Saliva DNA collected in GeneFiX™ Saliva Collectors and isolated with GeneFix Saliva DNA kits, genotyped on the Illumina HumanOmniExpress - 12 v1.1 BeadChip.

Introduction:

The Illumina HumanOmniExpress BeadChip is a high throughput tool for genome wide association studies (GWAS) based on the bead chip microarray technology. The chip features optimised tag SNP content extrapolated from HapMap, an open record of common genetic variations occurring in humans, and is a powerful tool for associating SNPs to traits and diseases being able to assess over 700,000 SNPs per sample.

SNPs are genotyped by using a high-throughput approach and when raw data are analysed with the Illumina GenomeStudio data analysis software each scanned locus receives a 'Genotype Call' which corresponds to the detected genotype. Genotype Calls can be accurate or not, therefore they are assigned a reliability score known as GenCall Score (GC). The GenCall Score is a confidence value ranging between 0 and 1 with less reliable calls being assigned lower values, a threshold of 0.7 is generally used for accepting a Genotype Call as reliable.

One of the most significant sample quality indexes used in this assay is the Call Rate which represents the fraction of sites for which a reliable call was generated; the manufacturer specifies a rate of ≥ 0.99 as indicative of a high performing DNA sample[1]. The p10 GC, also a quality index, represents the value below which the lower 10% of all the GenCall Score is found, the higher the p10 GC value the higher is the overall expected genotyping reliability; commonly used p10 GC thresholds range between 0.3 and 0.4 [2]. A similar function is also performed by the p50 GC (the value below which the lower 50% of GenCall Scores is found).

The manufacturer does not provide defined requirements for input DNA purity (like for example an acceptable A260/280 range), however the technique is sensitive to contaminants and high purity dsDNA is required for successful SNP typing as the GenCall Score is affected by a number of parameters including sample quality and DNA fragment size.

Method:

Four 2ml saliva samples produced by different volunteers were delivered into GeneFix Saliva Collectors and stabilised with 2ml LYS4 buffer. 40 µl PK were added to each sample before incubation at 60'C for 1 hour. For genomic DNA purification 2 samples were processed through GeneFix Saliva DNA Midi Kit (GMS) according to manufacturer's instructions, the 2 other samples were processed through GeneFix Saliva Preparation Kit (GSP) also according to manufacturer's instructions.

DNA samples were sent to the Genomics & Biomarkers Theme, NIHR BRC for Mental Health, MRC SGDP Centre, Institute of Psychiatry, King's College London for SNP genotyping. This facility is illumina CSPro certified which is a collaborative service provider partnership dedicated to ensuring the delivery of the highest-quality data available for genetic analysis applications. Illumina CSPro participants undergo a rigorous two-phase certification process that includes minimum data generation, data certification, and an on-site audit of the facility and processes.

Each genomic DNA sample was interrogated for 730,525 SNP markers on the Illumina HumanOmniExpress-12 v1.1 BeadChip using the recommended input of 200ng dsDNA at a concentration of 50ng/µl as quantified by PicoGreen™ assay. Data were processed with Illumina GenomeStudio data analysis software.

Results and Discussion:

The table below demonstrates performance of the genomic DNA samples isolated with GeneFix collectors and kits utilising the Illumina HumanOmniExpress BeadChip and the Infinium ® HD Ultra assay.

Sample ID	Call Rate	p10 GC	p50 GC
GSP 1	0.996793	0.7928821	0.9088784
GSP 2	0.997817	0.7918059	0.9087574
GMS 1	0.9961593	0.7927383	0.9088505
GMS 2	0.9955646	0.7926448	0.9089208

Call Rates appear high for all the analysed samples always exceeding the expected rate of 0.99 for high performing DNAs. Achieved p10 GC and p50 GC suggest an elevated level of Genotype Calls reliability throughout the genome.

Conclusion:

Saliva collected and stabilised with the GeneFix collectors and then processed through either the column based GMS kit or the precipitation based GSP kit, yielded DNA of suitable purity, integrity and concentration for efficient use with the Illumina HumanOmniExpress BeadChip.

The data presented confirm that DNA stabilised and isolated with GeneFix collectors and kits is of high quality and suitable for demanding downstream applications. GeneFix product range offers an effective, non-invasive and convenient way of isolating pure DNA samples from human patients without sacrificing sample quality.

Acknowledgments

Isohelix gratefully acknowledges Charles Curtis and the Genomics & Biomarkers Theme, NIHR BRC for Mental Health, MRC SGDP Centre, Institute of Psychiatry and SLaM NHS Trust, King's College London for carrying out sample analysis and for providing their invaluable feedback over this application note.

[1] *http://res.illumina.com/documents/products/datasheets/datasheet_omni_whole-genome_arrays.pdf*
[2] *Butler H. and Ragoussis J. "BeadArray-based genotyping."Genomics Protocols. Humana Press, 2008. 53-74.*