aCGH studies using GeneFix™ collected and purified saliva DNA

1. Background

1.1 Basic Principles

Array comparative genomic hybridization (aCGH) is a novel technique for the detection of copy number variations (CNVs) on a genome wide and high-resolution scale. To this end, a sample DNA is compared against a known DNA sequence (reference). Firstly, sample and reference DNAs are labelled using different fluorophores (normally green Cy3 and red Cy5) and then hybridised simultaneously and competitively on a chip seeded with specific DNA probes. After hybridization, digital imaging systems are used to capture and quantify the relative fluorescence intensities on each probe site. The fluorescence intensity ratio Cy3:Cy5 is directly proportional to the copy number ratio between sample and reference. Equal fluorescence intensity indicates equal copy numbers; conversely, altered ratios indicate either gains or losses in the sample DNA. The array technology allows scanning of thousands of probe sites for each test.

In clinical practice, aCGH is a powerful tool to provide cytogenetic information on a wide range of genetic abnormalities.

1.2 DNA Requirements

aCGH is a sensitive technique requiring sample DNA of the highest quality as protein and RNA contaminants can interfere with the assay. Array chip manufacturers such as Illumina recommend that sample DNA meets the following criteria:

- High molecular weight > 10–20 kbp
- Protein/RNA free OD 260/280 > 1.8
- Solvent free OD 260/230 > 2.0

To achieve this high level of purity, most protocols require the implementation of additional, time consuming ethanol clean up steps after the DNA extraction.

Traditionally, high quality DNA has been isolated from patient blood, but in recent years reliable DNA purification techniques were developed for saliva which can be collected without pain and without invasive procedures. GeneFix™ Saliva-Prep kit is a novel saliva DNA purification kit specifically optimised for use with GeneFix™ DNA collectors. It features a unique, ethanol-free chemistry which allows for very quick and easy DNA isolation without sacrificing on DNA yield or quality.

This application note describes the successful application of aCGH to saliva DNA collected and purified via the GeneFix™ product line (GeneFix™ DNA collector + GeneFix™ Saliva-Prep Kit) **without the need for additional ethanol clean up steps**.

2. Method

2.1 Saliva DNA purification

Saliva samples from three donors (one male, two females) were collected into GeneFix™ Saliva DNA collector tubes (Isohelix). Genomic DNA was isolated via GeneFix™ Saliva-Prep Kit (GSP) following the manufacturer's instructions:
http://www.isohelix.com/wp-content/uploads/2014/04/Instructions_GSP.pdf
The manual purification kit required less than 10 minutes hands-on time.

Additionally, a fourth saliva DNA sample was isolated via magnetic bead purification and further cleaned via ethanol purification (standard method). This sample was used for performance comparison.

2.2 aCGH Setup

The four DNA samples were prepared for aCGH testing according to the 'CytoChip Oligo reference guide" June 2015 (Illumina, available from https://support.illumina.com/downloads/cytochip-oligo-reference-guide.html). Salient steps are briefly described below.

2.3 DNA Labelling

For the incorporation of Cy3 and Cy5 fluorophores, all the saliva DNA samples and reference DNA samples were labelled via CytoChip Oligo Labeling [dUTP] kit (Illumina). Dye incorporation levels were confirmed to be within norm via spectrophotometric assay for both sample and reference DNAs.

2.4 Microarray Hybridisation and Processing

Labelled samples were hybridised for 24 hours on CytoChip ISCA v2.0 8x60K (Bluegnome) before recording fluorescence peaks through SureScan Microarray Scanner (Agilent Technologies). Data analysis was completed by using Blue Fuse Multi software (Bluegnome).

3. Results and Discussion

3.1 aCGH Plots

For each DNA sample tested an aCGH plot was produced. In the plots, each green dot represents a scanned locus. The Y axis illustrates the Cy3:Cy5 ratio while the X axis illustrates the position of the probe on the genome. Ratios between -0.3 and +0.3 are considered normal while deviation from these values may indicate copy number variations. Figures 1-4 illustrate the plots produced for, respectively, the standard method purified DNA and the three samples collected and isolated through GeneFiX™ product line.

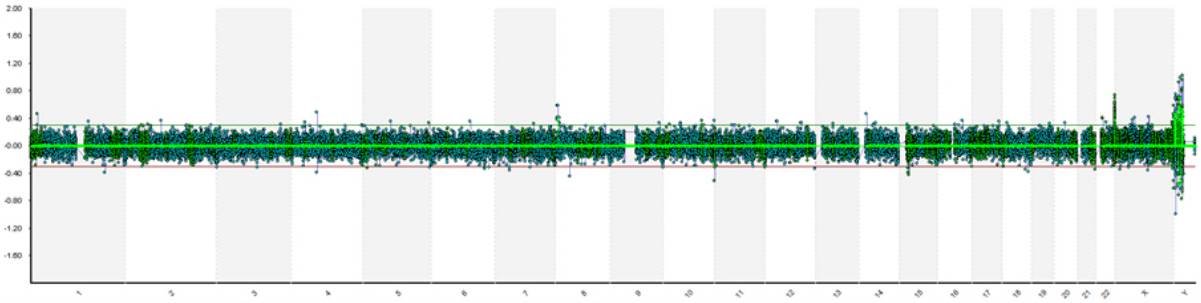

Figure 1 – Standard Saliva DNA purification (magnetic bead plus ethanol clean up)

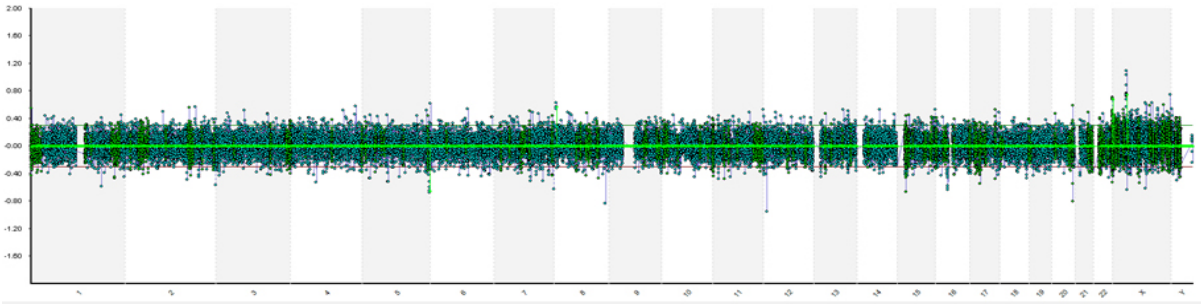

Figure 2 – GeneFiX™ purified Saliva DNA, sample 1 (female donor)

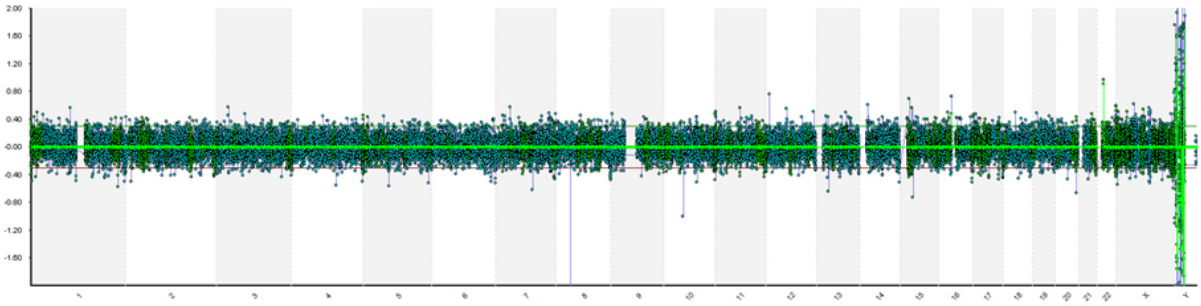

Figure 3 – GeneFiX™ purified Saliva DNA, sample 2 (male donor)

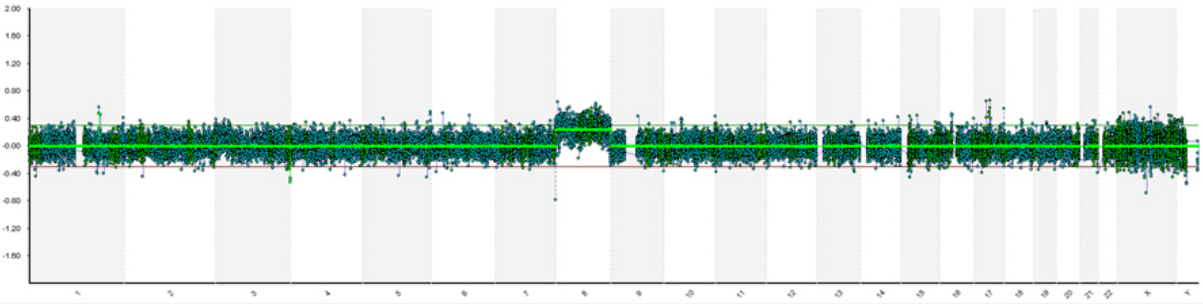

Figure 4 – GeneFiX™ purified Saliva DNA, sample 3 (female donor)

3.2 Quality Control Checks

To assess the aCGH reliability, a number of quality control (QC) metrics were recorded. The saliva DNA samples collected and isolated via the GeneFix™ product line performed equally to the standard purification DNA sample in all the QC checks and could successfully be analysed by aCGH under the experimental conditions described. Table 1 summarises the checks performed. A more detailed discussion is also presented below.

	DNA PURIFICATION METHOD							
QC metrics	**Standard**		**GeneFix™ 1**		**GeneFix™ 2**		**GeneFix™ 3**	
SD Autosome values: 0.07 – 0.15	0.12	Pass	0.14	Pass	0.14	Pass	0.14	Pass
SD Robust values: 0.07 – 0.15	0.11	Pass	0.14	Pass	0.13	Pass	0.13	Pass
Mean Spot Amplitude values: 400-2000 (Cy3 and Cy5)	533.65 775.28	Pass	452.33 621.24	Pass	432.27 749.42	Pass	457.08 797.12	Pass
Signal to background values: 5-20 (Cy3 and Cy5)	11.81 10.80	Pass	9.89 8.76	Pass	9.39 11.37	Pass	10.46 11.63	Pass

Table 1: aCGH quality control checks with reference values – The table illustrates the quality control performance of the four DNA samples analysed (one standard method purified sample, three GeneFix**™** purified samples).

3.2.1 SD Autosome and SD Robust

The SD autosome is a measure of the dispersion of the log2(Cy3:Cy5 ratio) for all sites on the array and it is a measure of the overall noise in the array. The SD Robust is constructed in the same way as the SD Autosome but incorporates only the middle 58% of the data. Because of the exclusion of the outliers, large genomic changes like trisomies will not impact on this value dramatically. Therefore SD Robust represents a reliable indicator of noise level. The results indicate low levels of noise for all samples; GeneFiX™ isolated samples performed as well as the standard purification DNA.

3.2.2 Mean Spot Amplitude (for each dye separately)

Mean spot amplitude is a record of the mean fluorescent signal intensities for the two channels Cy3 and Cy5. This metric can give an indication on how well the DNA has labelled with fluorescent dyes and high values can indicate over scanning or high background noise. Major differences between the two channels may indicate a labelling or a scanner problem, however it is normal for Cy5's signal to show a moderately higher intensity than Cy3's. Mean Spot Amplitude was normal for all samples tested indicating a reliable scan and adequate fluorophore incorporation in all cases.

3.2.3 Signal to Background Ratio (for each dye separately)

The Signal to background ratio measures the brightness of the mean signal (after the background has been subtracted) divided by the raw background signal (global signal). Again, results were satisfactory in all cases with no salient differences between GeneFiX™ and standard purification DNA samples.

4. Conclusions

GeneFix™ DNA saliva collectors (www.isohelix.com) provide a simple, non-invasive, and robust method by which clinical staff can supply DNA testing laboratories with a suitable sample for demanding downstream DNA analysis. Saliva samples collected in the GeneFix™ collector tubes can be rapidly processed into high-quality DNA using the ethanol free GeneFix™ Saliva-Prep Kit from Isohelix.

The results presented in this application note strongly suggest that saliva DNA collected and purified through the GeneFix™ product line is suitable for novel and demanding downstream applications such as aCGH where GeneFix™ purified samples performed as well as magnetic bead, ethanol cleaned samples.

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