Application Note: GFX-1.1

Performance of Saliva DNA, stabilised in Isohelix GeneFix™ collectors, and isolated by GeneFix™Saliva Preparation Kit on MLPA assay.

Background

MLPA (Multiplex ligation-dependent probe amplification) is a DNA-based technique developed by Schouten et al., for the detection of duplications and deletions of whole genes and individual exons. It is now widely used in both research and diagnostic genetics laboratories with a large number of commercially available kits which are targeted to specific genes (www.mrc-holland.com).

The MLPA technique uses a combination of oligonucleotide hybridisation, ligation and multiplex PCR to generate a series of amplification products whose levels can subsequently be quantified. This is most commonly achieved using fluorescent fragment analysis using instruments such as the 3130 Genetic Analyser (Life Technologies/Applied Biosystems).

By analysing the fluorescence levels for the gene of interest with reference to a series of control peaks and a reference DNA sample, it is possible to accurately quantify the copy number of the gene/exon of interest. This therefore detects events such as duplications and deletions in individual gene exons.

However, the MLPA technique requires a significant amount of high-quality DNA which has traditionally been extracted from patient blood samples.

This following application note describes the use in downstream MLPA analysis on either a fresh or a 12 months old 2 ml saliva DNA sample stabilised in Isohelix GeneFix™ collectors and purified through GeneFix™ Saliva Preparation kit.

GeneFix™ Saliva Preparation kit is a new manual/automatable DNA purification kit specifically optimised for use with GeneFix™ collectors. It features a unique, ethanol free chemistry which allows for very quick and easy DNA isolation without sacrificing on DNA yield or quality.

Method

2 x saliva samples (2ml each) from two different adult volunteers (A and B) were taken using the GeneFix™ saliva collection device. Details of the GeneFix™ collector are available separately from www.isohelix.com but the process involves quickly collecting a 2ml saliva sample into 2ml of preservation buffer which fully stabilises the sample for over 12 months at room temperature.

For both the fresh saliva sample A, and the 12 month stabilised saliva sample B*, DNA was extracted at Isohelix by using the GeneFix™ Saliva Preparation kit following the manufacturer's instructions and rehydrated into a final volume of 150µl. DNA samples were then shipped to the Liverpool Women's NHS Foundation Trust laboratory for MLPA testing.

The DNA was diluted to the required concentration of 20ng/µl before carrying out MLPA analysis using the P242 probemix "Hereditary Pancreatitis" according to the manufacturer's instructions (MRC-Holland).

The PCR amplification products were then analysed using the Applied Biosystems 3130 genetic analyser and peak areas determined using GeneMapper software (Life Technologies). Analysis of fluorescent peak areas was carried out using the spreadsheet tools designed by the National Genetics Reference Laboratory

(Manchester) resulting in the production of a composite histogram plot of dosage quotients from 14 test loci (BRAF gene, all the 5 exons of the PRSS1 gene including two probes for exon 1, the 5'UTR of the PRSS2 gene, two exons from the CASP2 gene, the 4 exons from the SPINK1 gene) as compared to 10 genomic control regions.

Results

Further details about the software tools used for the analysis of MLPA data are available from the National Genetics Reference Laboratory (Manchester). However, the spreadsheets include a statistical analysis which is highly sensitive to variations in DNA quality. The results showed that the DNA extracted from both saliva samples using the GeneFix™ Saliva Preparation kit, produced data of acceptable quality as indicated by the consistency of the amplitude of the histogram plot (Fig.1). This visual observation was further confirmed in the statistical analysis of the dosage analysis data (data not shown). Data also suggested that 12 months stabilisation on sample B had no significant effect on DNA performance at this assay (Fig.2). Note: the increased peak height of C5 10p13 for saliva sample B is due to normal genetic variation.

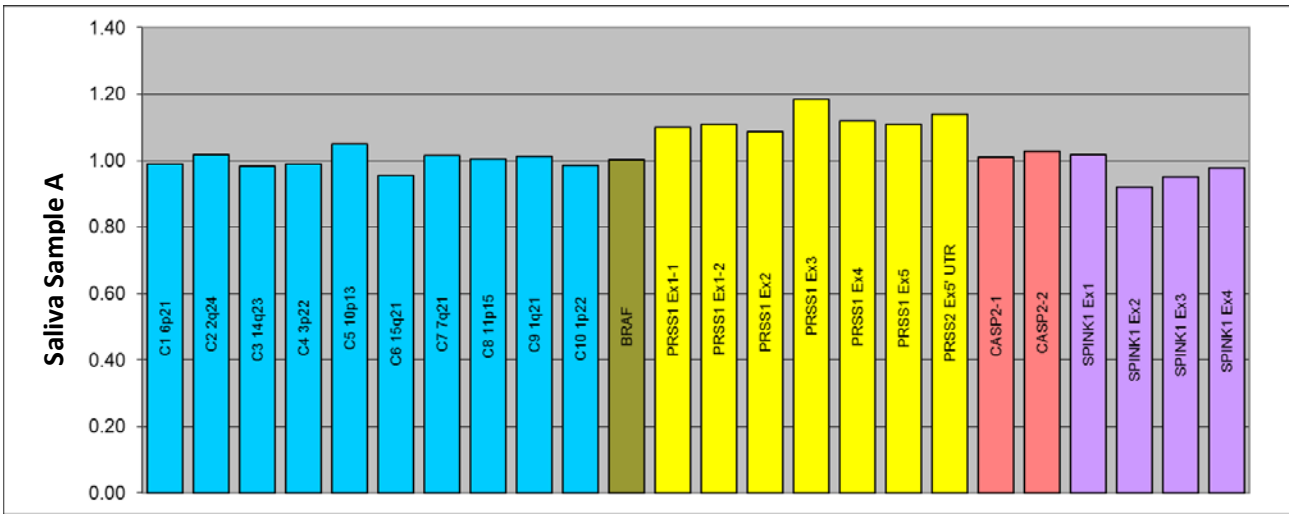

Figure 1
MLPA dosage quotient data – Isohelix Saliva Preparation Kit extracted DNA

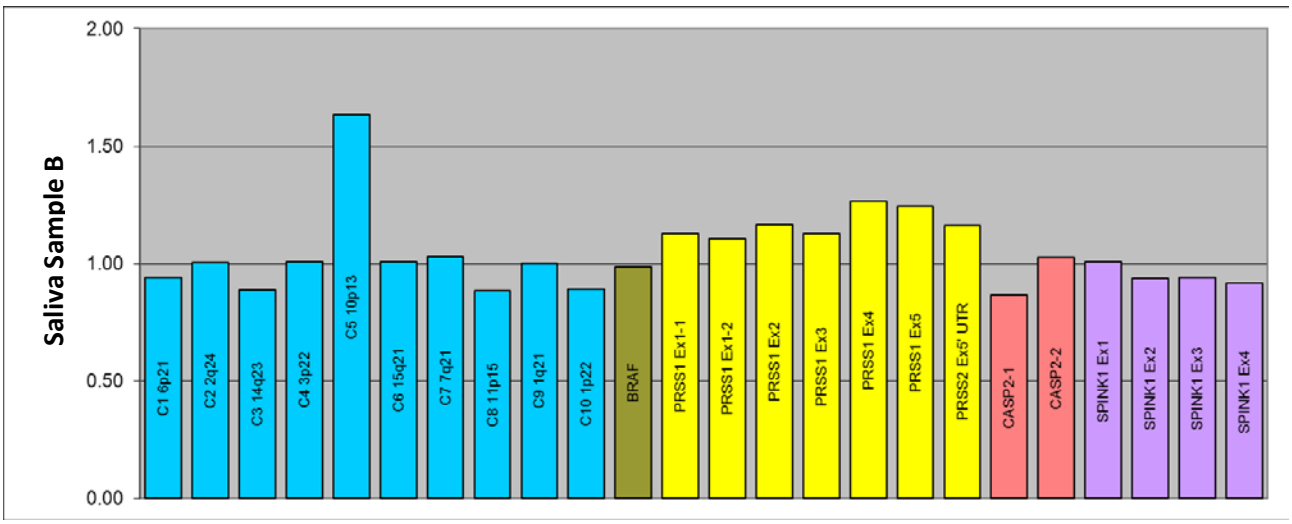

Figure 2
MLPA dosage quotient data – Isohelix Saliva Preparation Kit extracted DNA 12 month old sample

Conclusions

The GeneFix™ collection device (www.isohelix.com) offers a simple, non-invasive and robust method by which clinical staff can provide DNA testing laboratories with a suitable sample for demanding downstream analysis. The laboratory can subsequently recover high quality DNA from these saliva samples using Isohelix manual/automatable DNA extraction methods such as the new GeneFix™ Saliva Preparation kit.

DNA samples extracted using GeneFix™ Saliva Preparation kit were successfully analysed using the Multiplex Ligation Probe Amplification (MLPA) assay, which has a higher requirement for DNA quality than most other downstream processes. The MLPA data demonstrated the high quality of DNA collected and extracted using the GeneFix™collectors and Saliva-Prep DNA isolation kit. Moreover it showed that GeneFix™ system efficiently preserves saliva DNA quality for at least 12 months of storage.

References:

Schouten JP et al. (2002) Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification Nucleic Acids Res 30, e57.

*12 month sample obtained by accelerated ageing through incubation at 50°C for 60 days (Arrhenius plot)

The results were kindly presented by:
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