

Performance of Saliva DNA, stabilised in Isohelix GeneFix™ collectors, and isolated by Chemagen or Isohelix GeneFix™ Saliva DNA kits, on MLPA assays.

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 available 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).

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 of DNA extracted alternatively from 2ml saliva samples in downstream MLPA analysis using 2 different extraction methods.

Method

4 x saliva samples (2ml each) from a single adult male volunteer were taken using the GeneFix™ saliva collection device and shipped to the laboratory using the standard Royal Mail postal service. Details of the GeneFix™ saliva collection devices 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.

The DNA was extracted from the 4 saliva samples using 2 methods:

1. Chemagen Method (Perkin-Elmer)

The first 2 saliva samples were extracted using a protocol modified for use with the Chemagen MSM1 automated DNA extraction system. This involved heating the stabilised saliva samples (total volume = 4ml) for 2 hours at 55°C before extracting the samples using the standard Chemagen extraction method normally used for blood samples of between 1 and 3ml. The DNA was eluted in a volume of 260µl.

2. GeneFix™ Saliva DNA Mini Kit Method (Isohelix)

The other 2 saliva replicates were extracted using the Isohelix GeneFix™ Saliva DNA mini kit (www.isohelix.com) according to the manufacturer’s instructions and eluted in a volume of 100µl.

The 4 aliquots of eluted DNA from the above extraction methods were then diluted to a concentration of 20ng/µl before carrying out MLPA analysis of the LMX1B gene 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 the 8 exons of the LMX1B gene 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 data for the 4 samples tested showed that the DNA extracted from the saliva samples using the modified Chemagen method produced data of acceptable quality as indicated by the consistency of the amplitude of the histogram plot. However, the 2 DNA samples extracted using the Isohelix GeneFix™ Saliva DNA mini kit show improved dosage quotient data as indicated by the even amplitude of the histogram plots. This visual observation was verified in the statistical analysis of the dosage analysis data (data not shown).

Figure 1

MLPA dosage quotient data – Chemagen MSM1 extracted DNA (**replicate 1**)

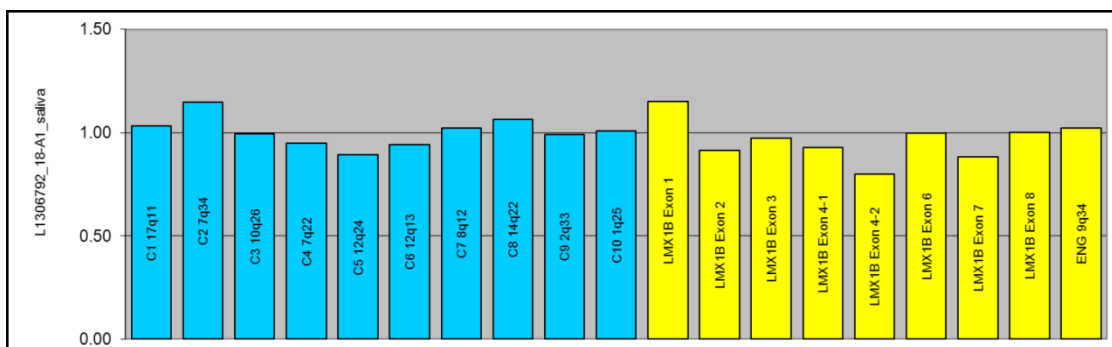


Figure 2
MLPA dosage quotient data – Chemagen MSM1 extracted DNA (**replicate 2**)

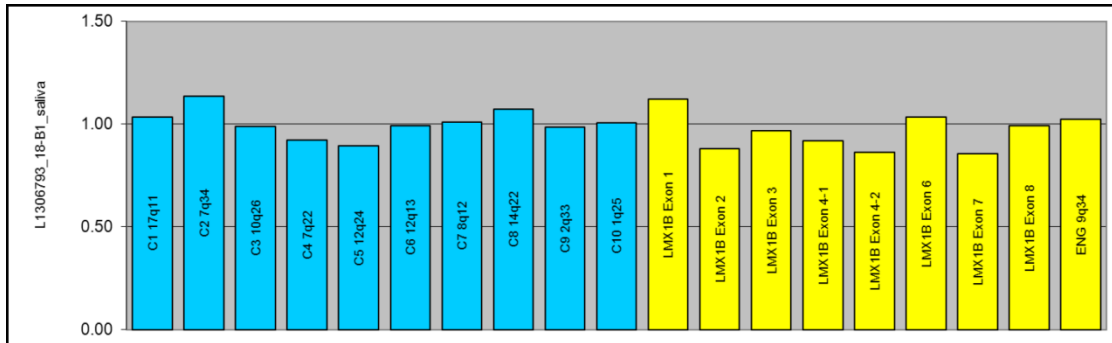


Figure 3
MLPA dosage quotient data – Isohelix GeneFix™ Saliva DNA mini kit isolated DNA (**replicate 1**)

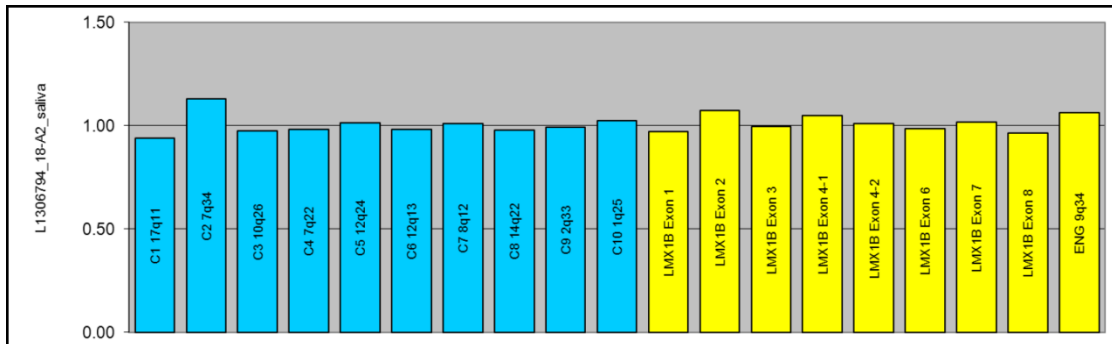
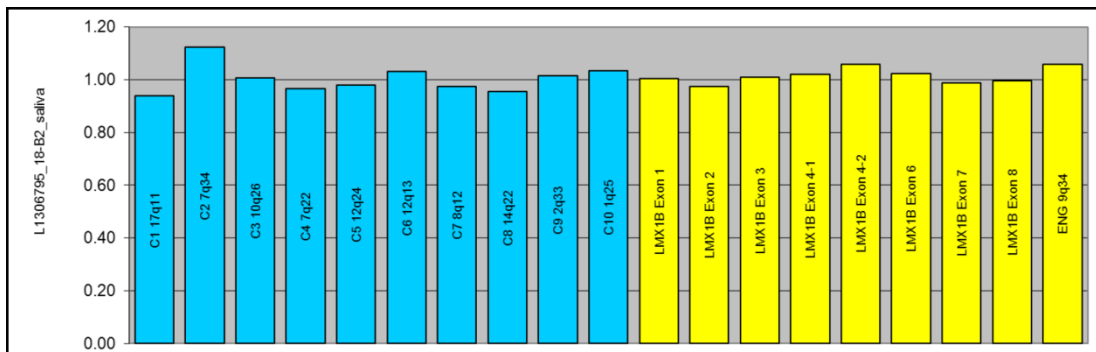


Figure 4
MLPA dosage quotient data – Isohelix GeneFix™ Saliva DNA mini kit isolated DNA (**replicate 2**)



Conclusions

The GeneFix™ collection device (www.isohelex.com) provides a simple non-invasive and robust method by which clinical staff can provide DNA testing laboratories with a suitable sample for downstream analysis.

The laboratory can subsequently recover high quality DNA from these saliva samples using existing and commonly used automated DNA extraction systems such as the Chemagic MSM1 system (Perkin-Elmer).

Saliva samples collected in the GeneFix™ device can also be processed into high-quality DNA using various manual DNA extraction methods such as the new GeneFix™ Saliva DNA mini kit from Isohelix.

DNA samples extracted using both the Chemagic MSM1 and Isohelix GeneFix™ Saliva DNA mini kit methods have been analysed using the Multiplex Ligation Probe Amplification (MLPA) assay which is very sensitive to DNA quality.

The MLPA data demonstrated the high quality of DNA extracted from both methods with the sample extracted using the Isohelix GeneFix™ Saliva DNA mini kit method showing the highest quality dosage quotient results.

References:

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

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