

Ultra-Low Volume SNP Genotyping: PCR Preparation with mosquito®

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Abstract

The polymerase chain reaction (PCR) is the basis for many different technologies such as single nucleotide polymorphism (SNP) genotyping. It is desirable to perform the reaction in as small a volume as possible since some reagents are subject to royalty payments and the primers/probes and sample DNA may be scarce or expensive.

Introduction

MRC Geneservice, a major UK research service provider, currently uses a 5 μ L total volume setup for SNP genotyping assays, which is recognised as a small volume for this type of assay.

Using mosquito, a comparison was made between the assay performance at the standard 5 μ L and the smaller volume of 1.7 μ L. With its nanolitre capability, mosquito can perform smaller volume assays and 1.7 μ L was chosen to ensure that the fluorescent signal arising from the assay could be detected on the ABI 7900HT (Applied Biosystems)

The 5 μ L assay was performed in columns 1-12 of a 384-well microtitre plate. The same proportions and reagents were used for the 1.7 μ L assay but in columns 13-24 of the same plate. The prepared plate was heat sealed, cycled and read. The results were plotted as the intensities of the two allele-specific fluorophores ('VIC' and 'FAM').

Conclusion

- Preparation and analysis of PCR SNP assays down to a reaction volume of 1.7 μ L is possible using mosquito.
- The quality of data for 1.7 μ L is comparable with – and possibly higher than – the quality using 5 μ L.
- Mosquito can more than halve reagent costs in SNP analysis through the reduction of required reagents.

Authors

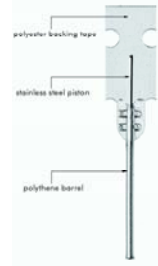
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1 mosquito Instrument



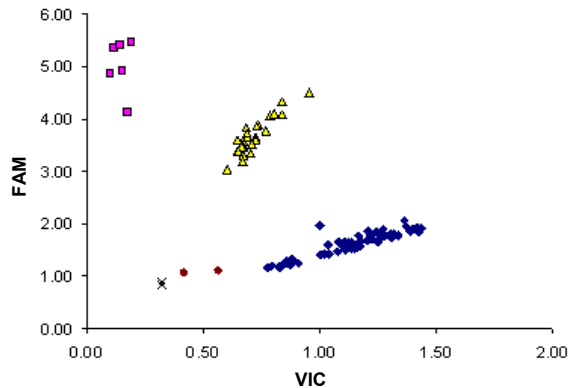
mosquito is a low volume liquid handling instrument combining a disposable tip system with a positive displacement pipette. mosquito is capable of pipetting volumes from 1.2 mL down to 50 nL with no washing required.

2 Disposable Positive Displacement Tip

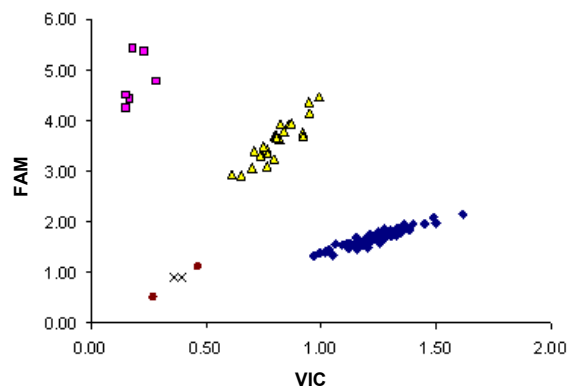


Tips are manufactured from high-grade polyethylene co-extruded with stainless steel. Tips have both positional accuracy and an ability to pierce many sealing films.

3 5 μ L Reaction on Control DNA



4 1.7 μ L Reaction on Control DNA



Figures three and four show data clusters as described below:

Bottom right: DNA samples which are homozygous for the first SNP allele (◆ VIC).

Top left: DNA samples which are homozygous for the second SNP allele (■ FAM).

Middle: DNA samples that are heterozygous for SNP alleles (▲ Both).

Near origin: No template controls (●) and 'outliers' (X) which failed to amplify significantly.

