

Validation of volume reduction strategies for TaqMan SNP genotyping assays using Digilab MicroSys low volume liquid handling system

Digilab Application Note 1201

BACKGROUND

State of the art genomics laboratories perform many large-scale genetic studies. There is a constant drive to increase the throughput of these studies by automating many of the routine bench procedures and reducing reaction volumes used from suppliers' kits.

The Digilab MicroSys liquid handling system, utilizing proprietary non-contact SynQuad technology, can remove these inconvenient bottlenecks. The MicroSys can be used to automate TaqMan SNP genotyping reaction preparation in 384 well plate formats and consequently has become an essential tool for large scale genotyping projects.

INTRODUCTION

The MicroSys helps to minimize routine bench work and automate the initial extensive volume reduction gradient to determine if reactions can be performed at lower volumes. This is important since not all TaqMan SNP genotyping assays are the same, with massive variations in sensitivity existing between different assays. Therefore it is essential to optimise the volume reduction before proceeding with factory-style genotyping.

Once an optimum reduced reaction volume has been determined large-scale genotyping can commence. The data presented in this report will show how the Digilab MicroSys has been used to optimise assay volumes and to perform a TaqMan SNP genotyping project.

MATERIALS AND METHODS

For initial optimization experiments the same DNA sample was aliquoted from a stock tube to a 384 well plate using liquid handling robotics (Tecan Genesis RSP 150). For standard genotyping assays multiple DNA samples (384) were aliquoted from 96 well plates to a 384 well plate using liquid handling robotics (Tecan Genesis RSP 150).

DNA was dried down within the 384 well plate by heating at 95°C on a PCR block (MJ Research DNA Tetrad™ thermal cycler) until 5 µl (25 ng) had evaporated. Optimization volume reduction gradient experiments were conducted and reactions prepared as shown in Table 1.

Reaction components	Well reaction volumes (µl)							
	5.0	4.0	3.0	2.5	2.0	1.5	1.0	0.5
TaqMan universal master mix (Applied Biosystems)	2.5	2.0	1.5	1.25	1.0	0.75	0.5	0.25
Primer-Probe mix (Applied Biosystems)	0.25	0.2	0.15	0.125	0.1	0.075	0.05	0.025
Water	2.25	1.8	1.35	1.125	0.9	0.675	0.45	0.225
DNA (25 ng)	DD	DD	DD	DD	DD	DD	DD	DD

DD – dried down, total amount 25 ng

Table 1: TaqMan reaction master mix set up for volume reduction gradient-384 well format

MATERIALS AND METHODS (Con't)

Reaction mix containing buffer, primer-probes and water was aliquoted to dried down DNA using the Digilab MicroSys at the reaction volumes indicated in Table 1.

PCR amplification was performed using Applied Biosystems' TaqMan 7900HT sequence detection software (SDS). PCR was performed by an initial activation of AmpliTaq gold (95°C for 10 minutes) followed by 40 cycles of denaturing (95°C for 15 seconds) and annealing/extension (60°C for 1 minute). In all instances a reaction volume of 5 µl was inserted into the SDS file. Amplification data was evaluated using SDS software with automatic crossing threshold (Ct) analysis enabled.

For standard (non-gradient) assays allelic discrimination plate read assays were performed on the TaqMan 7900HT using SDS (Applied Biosystems) with autocaller function enabled.

RESULTS AND DISCUSSION

Volume reduction gradient

Amplification occurred in all 44 wells containing sample at 5.0 µl - 1.5 µl reaction volumes while amplification failed to occur in the two non-template control wells (NTC). The green lines shown in Figure 1g-h illustrate the failure rate for samples at volumes below 1.5 µl. It was not possible to perform an allelic discrimination assay for the volume reduction gradient since the entire DNA used for optimisation was the same sample. Based on the amplification plots for this particular SNP genotyping assay a reaction volume of 2.0 µl was considered satisfactory and used for large-scale genotyping.

Amplification profiles generated with Applied Biosystems sequence detection software with automatic Ct function enabled are shown in Figure 1.

Large scale genotyping

A large-scale genotyping application was set up to illustrate that this TaqMan SNP genotyping assay worked at 2 µl, less than half the supported reaction volume. Amplification plots were examined (Figure 2a), generated by automatic Ct, followed by allelic discrimination plate read (Figure 2b), created with genotyping autocaller enabled.

As Figure 2 illustrates, amplification was highly successful at a 2 µl reaction volume and the data was able to be genotyped in the same way as at 5 µl reaction volume. Using the Digilab MicroSys to set up TaqMan SNP genotyping assays not only enabled significant cost reductions but also eliminated much of the routine aliquoting associated with large-scale, high-throughput genotyping without compromising on speed, accuracy or contamination control.

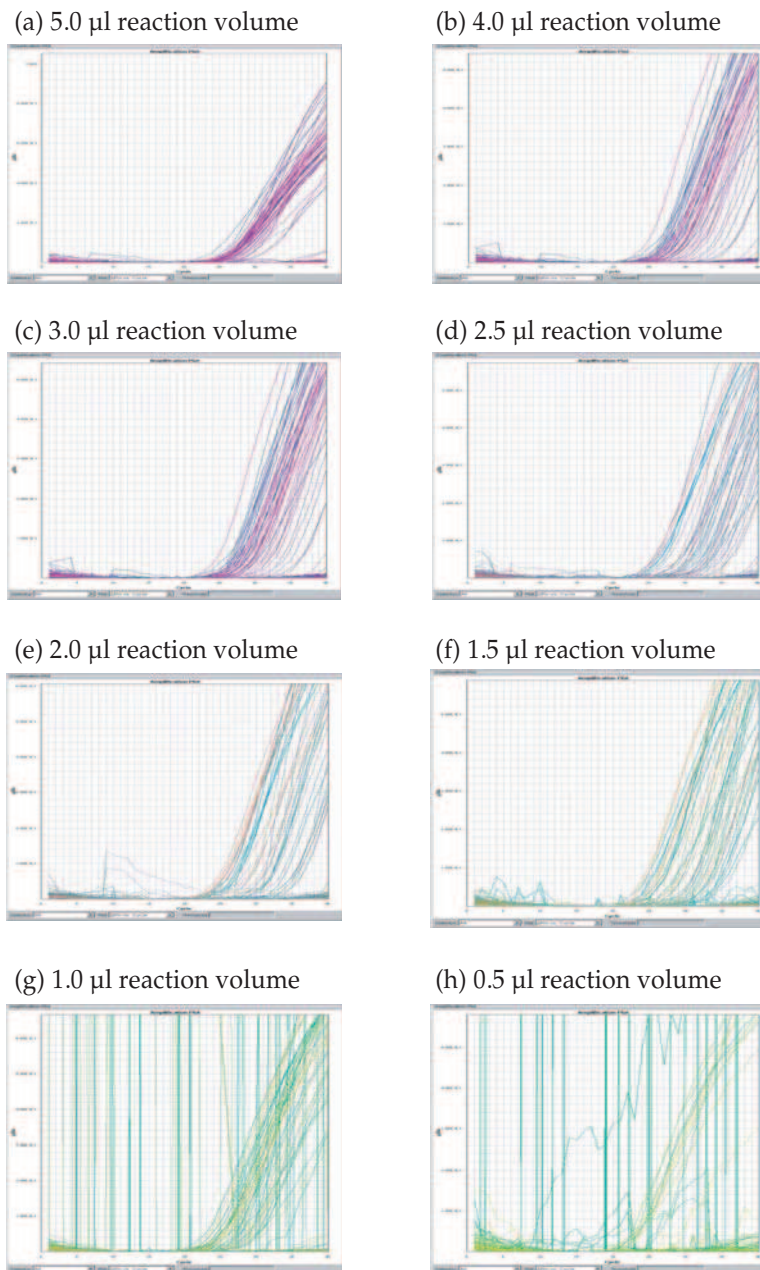


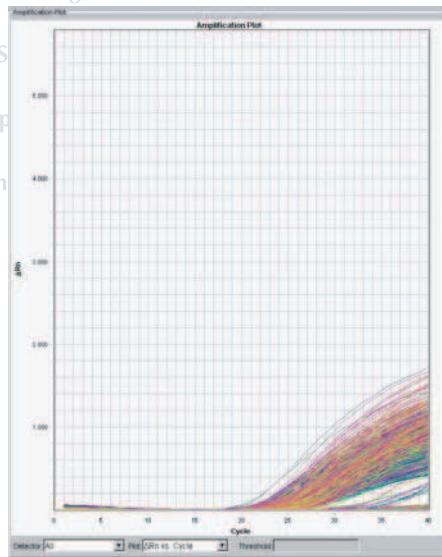
Figure 1: Amplification plots for TaqMan SNP genotyping volume reduction gradient

TaqMan SNP genotyping assays using the MicroSys

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Non-Contact Arrays

(a) Amplification plot



(b) Allelic discrimination plot

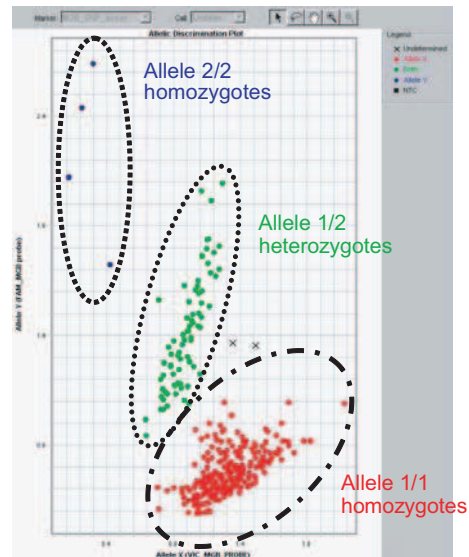


Figure 2: Amplification and allelic discrimination profiles from large-scale, 384 subject TaqMan SNP genotyping. NTCs and failed samples were removed from analysis.

CONCLUSIONS

Investing in the Digilab MicroSys has allowed full automation of much of the tedious large-scale sample preparation usually associated with high-throughput genotyping. The automation is robust and reliable with accurate and precise non-contact dispensing into 384 well plates containing dried DNA. Reaction volumes are reduced and as a consequence so are costs. After initial assay-specific volume reduction gradient tests throughput has been greatly improved.

The flexibility of the Digilab MicroSys opens up the possibility of automation and miniaturization of multiple applications. Consequently an investigation into high throughput sequencing using the MicroSys was performed.

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