

## Validation of volume reduction strategies for high-throughput capillary sequencing using Genomic Solutions Cartesian MicroSys low volume liquid handling system

### Cartesian Application Note 1200

#### BACKGROUND

Within the modern genomics laboratory various large-scale genetic studies are conducted and there is a constant drive to increase the throughput and value of these platforms by automating many of the routine bench procedures and reducing reaction volumes used from suppliers' kits.

The Cartesian MicroSys liquid handling system, utilizing proprietary non-contact synQUAD technology, enables reduction of both these bottlenecks. The MicroSys can be used to automate sequencing reaction preparation in a 96 well plate format and consequently the MicroSys has become a necessary tool for large scale high-throughput sequencing.

#### INTRODUCTION

Initially the MicroSys was purchased to cut high costs incurred for performing single nucleotide polymorphism (SNP) genotyping using Applied Biosystems SNP genotyping assays. However the flexibility of the MicroSys has allowed other applications to be explored including reducing costs for performing sequencing reactions using Applied Biosystems BigDye v3.1 and 3730 DNA analyzer.

The MicroSys minimizes routine bench work and automates the initial extensive volume reduction gradient to determine if reactions can be performed at lower volumes. This is important since not all sequencing reactions are the same, with massive variations in sequencing parameters existing between different genes. Therefore it is essential to optimise the volume reduction before proceeding with factory-style sequencing.

Once an optimum reduced reaction volume has been determined large-scale sequencing can commence. The data presented in this report will show how the Cartesian MicroSys has been used to optimise sequencing volumes and obtain gradient data to perform actual sequencing projects.

#### MATERIALS AND METHODS

DNA (25 ng) for volume reduction gradient and standard reactions was amplified by PCR using HotStart Taq DNA Polymerase (Qiagen). Thermal cycling (MJ Research DNA Tetrad™ thermal cycler) parameters were 95°C activation followed by denaturing (94°C for 1 minute), annealing (65°C for 1 minute) and extension (72°C for 1 minute) with reaction ending at 60°C for 30 minutes.

PCR efficiency was established by performing agarose gel electrophoresis (Invitrogen) and UV examination. PCR products were purified by magnetic bead technology (Agencourt); 10 µl of purified PCR product was used as template for the sequencing reactions. Sequencing reaction master mixes were prepared for the volume reduction gradient as shown in Table 1.

Reaction components	Total reaction volume (µl)						
	10	6.6	5.0	4.0	3.32	2.86	2.5
Ready reaction (Applied Biosystems)	0.5	0.3	0.25	0.2	0.16	0.14	0.125
5X sequencing buffer (Applied Biosystems)	3.5	2.3	1.75	1.4	1.16	1.0	0.875
Primer (0.2 µM)	0.16	0.1	0.08	0.064	0.05	0.05	0.04
Water	5.84	3.9	2.92	2.336	1.95	1.67	1.46
Template (purified PCR product)	10	10	10	10	10	10	10

Table 1: Sequencing reaction master mix set up for volume reduction gradient-96 well format

### MATERIALS AND METHODS (Con't)

Twenty-five rounds of sequencing reactions were completed using 96°C (for 45 seconds) denaturing, annealing at 50°C for 25 seconds and extension at 60°C for 4 minutes (MJ Research DNA Tetrad™ thermal cycler). Sequencing products were purified using CleanSeq bead technology (Agencourt) following the manufacturers instructions. Sequencing was read through the 3730 DNA analyzer (Applied Biosystems) and data was collected through Applied Biosystems data collection software. Sequences were examined using SeqScape v2.1 (Applied Biosystems).

### RESULTS AND DISCUSSION

Sequencing data was preliminarily checked on the array viewer of the data collection software and assembled data was examined using SeqScape v2.1. As expected raw array data showed reducing intensity as the reaction volume was reduced (Figure 1).

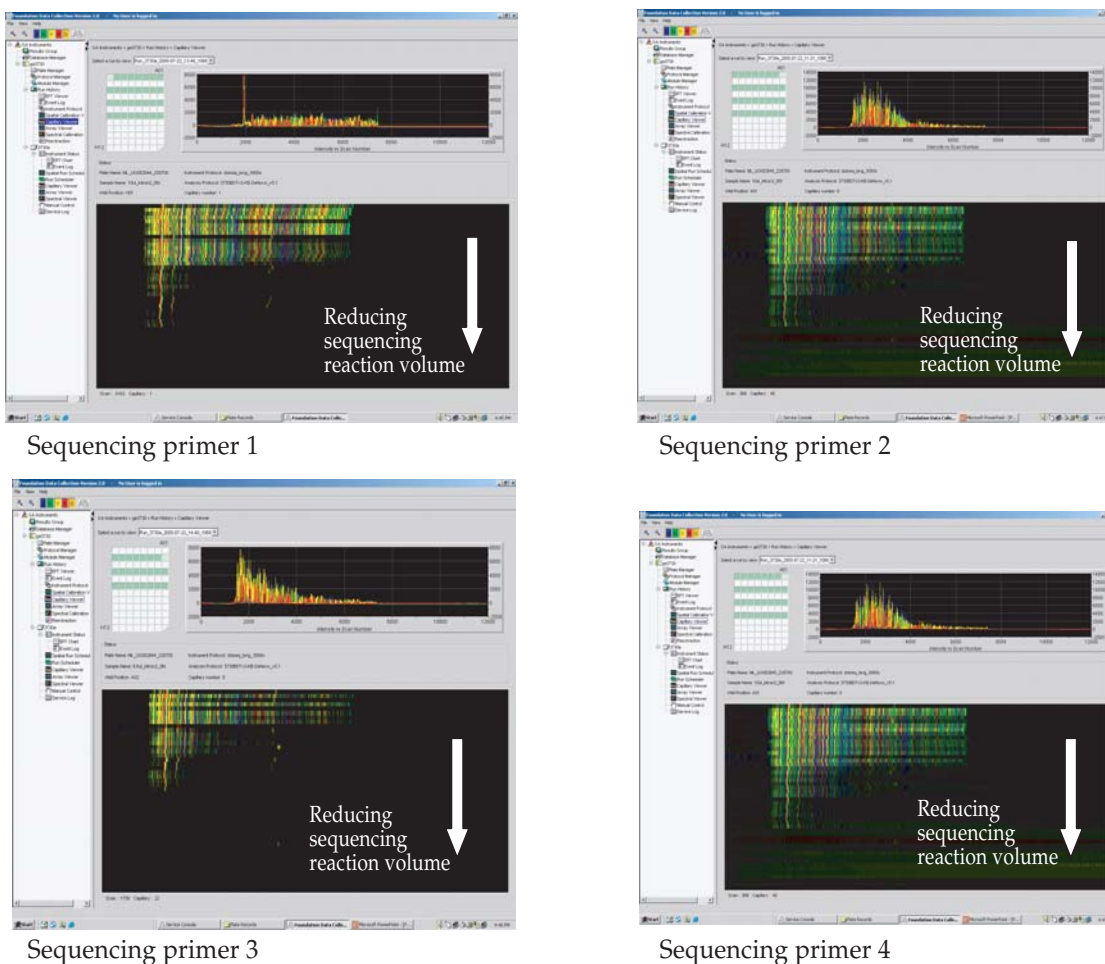
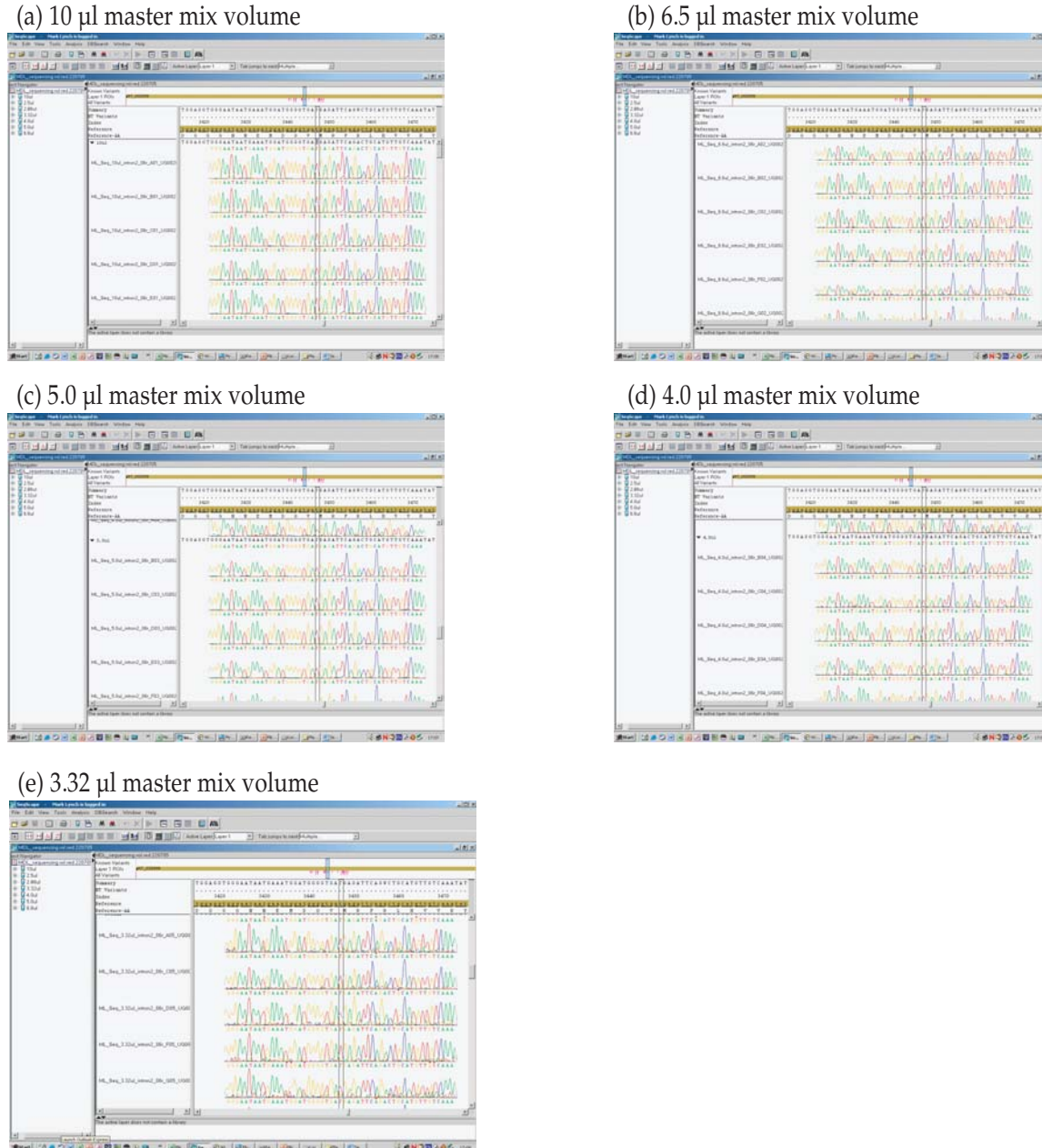


Figure 1: DNA sequencing raw sample data illustrating the effect of reducing sequencing reaction volume on data generated using various sequencing primers. Data from Applied Biosystems 3730 data collection software.

## RESULTS AND DISCUSSION (Con't)

Sequences were assembled for analysis using SeqScape v2.1 and results illustrated that quality data could be generated from master mix volumes ranging from 10  $\mu$ l - 3.32  $\mu$ l (Figure 2), however most sequencing reactions failed at master mix volumes below 3.32  $\mu$ l.



*Figure 2: Assembled sequencing data from sequence volume reduction gradient experiment showing master mix volumes from 10  $\mu$ l to 3.32  $\mu$ l.*

From these sequencing volume reductions a sequencing reaction volume of 5  $\mu$ l is now being considered and has been used for some applications. A test was conducted using this volume (Table 1) and it has been shown that even at half the supported reaction volume quality data can be achieved (Figure 3) for this gene. It is possible that, for other genes, the reaction volume could be reduced even further due to the large variations in sequencing parameters between genes.

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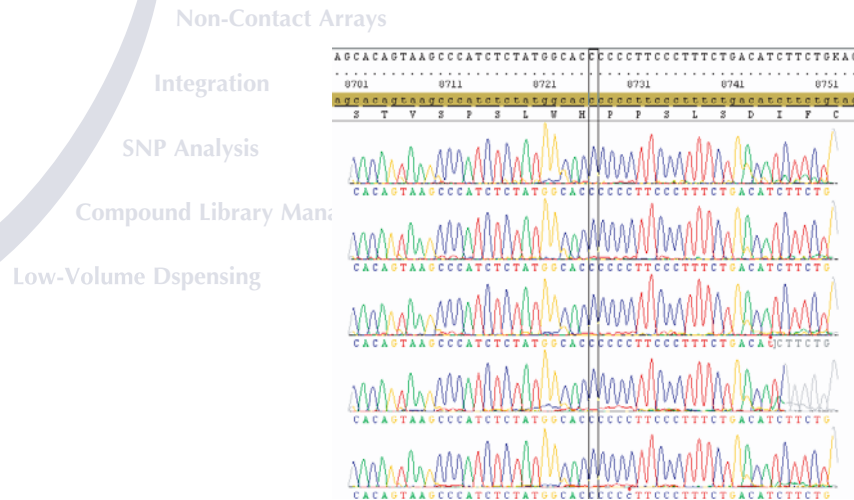


Figure 3: Assembled sequencing data from reactions set up at 5 µl reaction volume.

## CONCLUSIONS

Within 6 months of purchase the flexibility of the Cartesian MicroSys has enabled an extension of applications beyond TaqMan SNP genotyping assay preparation. Sequencing reaction volumes can be reduced and the MicroSys can be used to prepare 96 well plates for capillary sequencing.

By investing in the Cartesian MicroSys utilizing proprietary synQUAD liquid handling technology it has been possible to fully automate much of the tedious large-scale sample preparation associated with high-throughput sequencing. The automation is robust and reliable with accurate and precise non-contact dispensing into 96 well plates containing DNA. Reaction volumes are reduced and as a consequence so are costs. After initial sequencing-specific volume reduction gradient tests throughput has been demonstrated to be fast, reliable and cost effective.

For details on automating and miniaturizing high throughput genotyping please refer to Application Note No. HA1201 or contact Jo Butlin, Global Product Manager for Cartesian systems at [jo.butlin@genomicsolutions.co.uk](mailto:jo.butlin@genomicsolutions.co.uk)

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