

# Cost Effective High Throughput DNA Sequencing Using Novel Automated Nanoliter Dispensing Technology

Oza, U., Khoja, H., McIntosh, B., Doneen, B  
Genomic Solutions, Inc. 17851 Sky Park Circle, Irvine, CA 92614 (USA)

## Abstract

Cost effective, high throughput DNA sequencing could accelerate further advances in Genomics. Currently the high cost of reagents are prohibitive to the acquisition of high throughput sequence data. Among the most costly reagents used for DNA sequencing are Big Dye™, preparation of template DNA, and the thermostable DNA polymerase.

The solution to reducing sequencing costs and increasing throughput is to reduce reaction volumes and to deliver these volumes using cost-efficient high speed robotic dispensing instruments. This application note investigates Hummingbird™ technology to dispense low volumes of DNA, and synQUAD™ technology to dispense reduced volumes of Big Dye. The data shows that

Hummingbird accurately dispenses DNA without carryover or cross contamination, and synQUAD dispenses reduced volumes of the Big Dye with high precision. The study also demonstrates that 1 µL of template DNA and 0.5 - 2 µL of Big Dye dispensed to PCR plates using Hummingbird and synQUAD give high resolution sequences in excess of 1000 basepairs.

Together these technologies can **increase throughput** of sequencing while **substantially lowering the costs**.

## Materials & Methods

### 1. Hummingbird Evaluation

- Low volume DNA Dispense
- Carry-over Studies

A Hummingbird system configured with a 384 capillary cassette was used to complete the low volume DNA dispense as well as the carry-over studies. PCR plates were placed directly onto the Hummingbird system.

Two source plates were made. The first plate contained 5 µL/well of DNA and water, in alternating wells to form a checkerboard pattern. The second one contained water in all wells with the exception of four positive controls (wells H10-H11 and wells I10-I11 were spiked with DNA). Two identical destination plates were manually filled with PCR master mix solution containing the PCR buffer, dNTP mix, primers and enzyme.

Materials Used:	Instrument Parameters:
Hummingbird system with a 384 capillary cassette (1000 nL)	Aspiration Time: 20 sec
384 PCR Plates	Dispense Time 0.2 sec
PCR Master Mix (PCR Kit F-551S from MJ Research)	Dispense Pressure: 12 psi
DNA Template (Lamba-monocut DNA from NE Biolabs)	Wash Time: 1 sec
MJ PTC225 Thermocycler	Waste Time: 1 sec
Owl Separation System- Model A6	Number of Pre-run wash: 1
Lambda DNA Hind - III markers	Post-run wash time: 5 sec
1% Agarose gels Ethidium Bromide 10 mg/mL stock	Number of Washes between Dispenses: 5

1 µL of DNA/water was aspirated from source plate 1 and dispensed into PCR plate 1 under conditions described above. After the DNA dispense, the capillary cassette was washed 5 times with water. 1 µL of water was then aspirated from source plate 2 and dispensed into PCR plate 2.

The PCR plates were placed in the 384 block of the thermocycler and run according to instructions in the kit:

Denaturation time	30 sec at 94 °C
Annealing time	30 sec at 62 °C
Extension time	30 sec at 72 °C
No. of PCR cycles	= 36

After the PCR, the plates were centrifuged, and 1 µL/well of gel loading dye- bromophenol blue was added. The samples were then loaded onto a 1% agarose gel containing 0.5 µg/ml of Ethidium bromide and electrophoresed. 200 samples were loaded per gel and Lambda-DNA Hind-III markers were used to identify the DNA size. The gel was run for 1 hour at 120 volts.

### 2. synQUAD Evaluation:

- Big Dye Dispense

A Cartesian dispensing system configured with 5 mL syringes, and 190 µm (pore size) ceramic tips was used to aspirate and dispense the Big Dye reaction mix. Big Dye was aspirated from well A1 of a 96 well plate and dispensed into 24 wells of a 384 black destination plate. An air gap was used during aspiration to eliminate any loss of reagent. In addition, pre-dispenses and post-dispenses of the residual Big Dye were performed at the source plate.

Materials Used:	Instrument Parameters:
PreSys System with 5 ml Syringes	Aspiration Syringe Speed
190 µm tips	Drop volume 2 µL
Microtiter Plates: 96 well clear plates 384 well black plates	Dispense Syringe Speed
Sodium-fluorescein	Air Gap 10 µL

Sodium-fluorescein was added to the Big Dye stock solution to a final concentration of 2.5 µM. Percent coefficient of variation (%CV) was calculated by dispensing Big Dye stock solution into standard 384 well black plates. Average dispense volume was determined by measuring weight gain in the target plate, and converting this value to microliters. Wells were next topped-off with 50 µL 0.01N NaOH by manual pipetting. The plates were then centrifuged, and fluorescence was measured in a Victor-V2 Spectro-photometer. CV (Standard Deviation/ Mean \* 100) was computed.

### 3. Sequencing:

Template DNA (1 µL) and Big Dye (0.5-2 µL) were dispensed into 96 well PCR plates (ABI) manually and using Hummingbird-synQUAD technology. Sequencing was then performed on an ABI-3730 DNA Sequence Analyzer.

## Introduction

Sequencing is the process of determining the exact order of bases in a segment of DNA. In a typical sequencing reaction, DNA from a 96 or 384 well plate is added to a master mix buffer containing Big Dye Terminator reagent, primer, and the enzyme. The typical reagent volumes used are 8 µL for Big Dye and 2-5 µL for template DNA. Sequencing reactions are then cleaned and processed on DNA sequence analyzers.

Advances in automation and technology are being explored to obtain and compare DNA sequences quickly and cheaply. Big Dye, DNA Template and enzyme are the three most expensive components in a sequencing reaction and with reduction in volumes of these reagents, sequencing costs can be minimized. This application note investigated if 1) the Hummingbird system can be used for high speed dispensing of reduced volumes of DNA with no risk of carry-over and 2) the synQUAD technology can be used to dispense reduced volumes of Big Dye with high precision and accuracy and with minimal waste of the reagent during dispensing.

The Hummingbird system can dispense simultaneously nanoliter volumes of samples per transfer operation using glass capillaries in a cassette. Transfer occurs by

aspirating a sample, using capillary action, followed by dispensing with a pulse of air. The cassettes hold 96 or 384 capillaries in a format to fit standard microtiter plates. Cross contamination is always an issue in setting up high throughput DNA sequencing. Since thermo cycle sequencing reactions are less sensitive to cross contamination than PCR reactions, a PCR experiment was set up to test for carryover. This study was undertaken to establish that 1 µL DNA could be reliably dispensed using Hummingbird into PCR plates containing 5 µL master mix. The plate was run in a thermo-cycler and the amplified DNA was identified using standard electrophoresis techniques. In addition, the minimum number of washes to detect no DNA carry-over was also determined.

synQUAD technology is an extremely fast non-contact dispensing system. It has a dynamic dispensing volume range from 20 nL to 20 µL. synQUAD can operate in either an aspirate/dispense configuration or bulk dispense mode, making it possible to support the various liquid dispensing techniques used in sequencing assays. We investigated if reduced volumes of Big Dye could be dispensed into 384 plates with accuracy and precision using the synQUAD technology.

## Results

### 1. Hummingbird Evaluation

The results of the low volume DNA dispense and carry-over are given in Figures 1 - 4 below.

Figure 1: Top half of PCR plate 1  
(source plate had DNA and water in alternating wells)

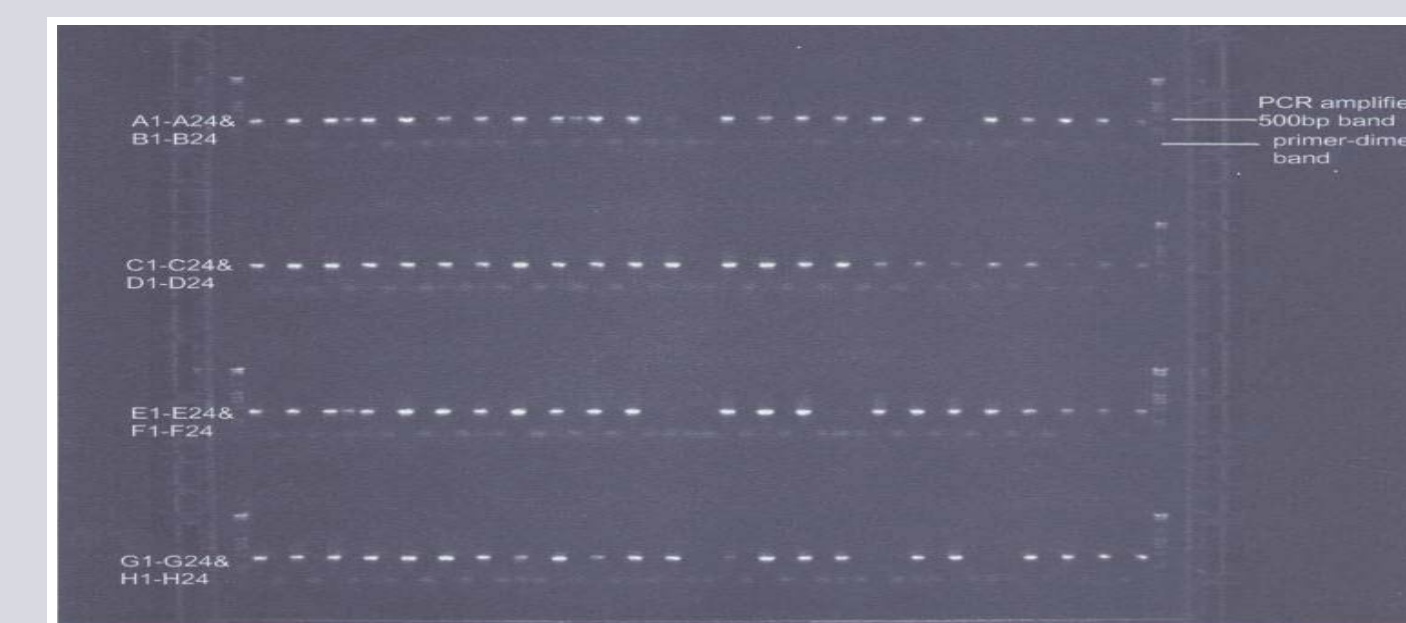


Figure 2: Bottom half of PCR plate 1  
(source plate had DNA and water in alternating wells)



As seen in Figures 1 & 2, PCR samples from plate 1 gave PCR amplified DNA bands of 500 bp in wells containing DNA. In wells where water was added, faint bands corresponding to the primer-dimer only were seen.

Figure 3: Top half of PCR plate 2  
(source plate had water in all wells except positive DNA in H10 & H11)



Figure 4: Bottom half of PCR plate 2  
(source plate had water in all wells except positive DNA in I10 & I11)



Figures 3 & 4 show no DNA band in all wells indicating there was no carry-over of DNA. In all wells the primer-dimer bands were seen. Exceptions were wells H10-H11 and I10 & I11 where DNA was manually added.

### 2. synQUAD Evaluation

Results of Big Dye Dispensing using synQUAD are shown in Figure 5 and Table 1.

Figure 5: Dispensing 0.5 µL Big Dye into row 1 of two 384-well plates (N = 24).

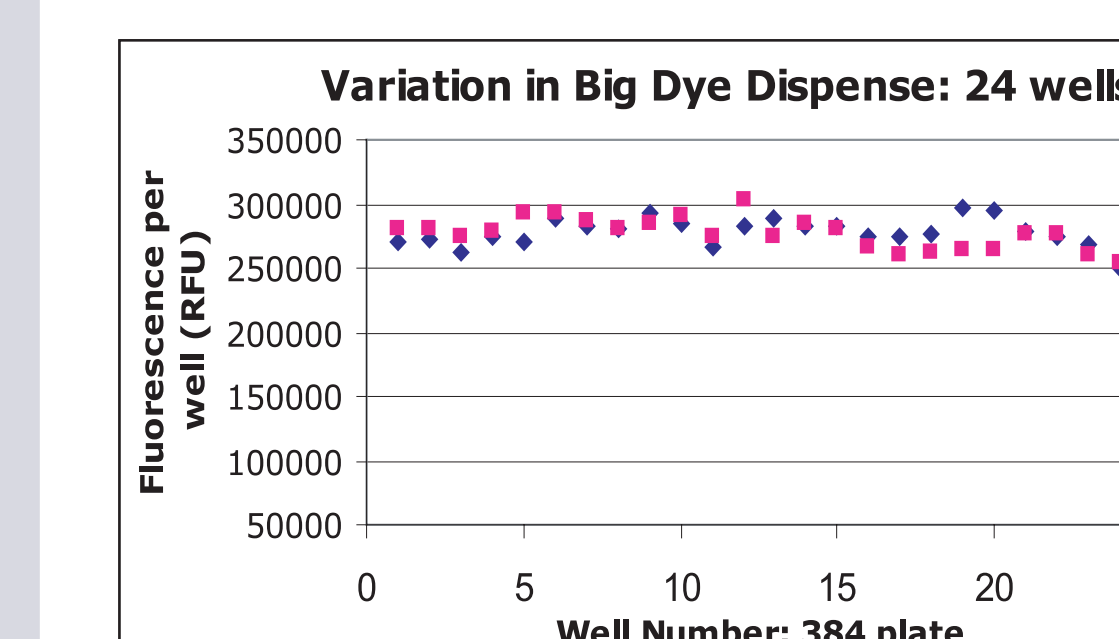
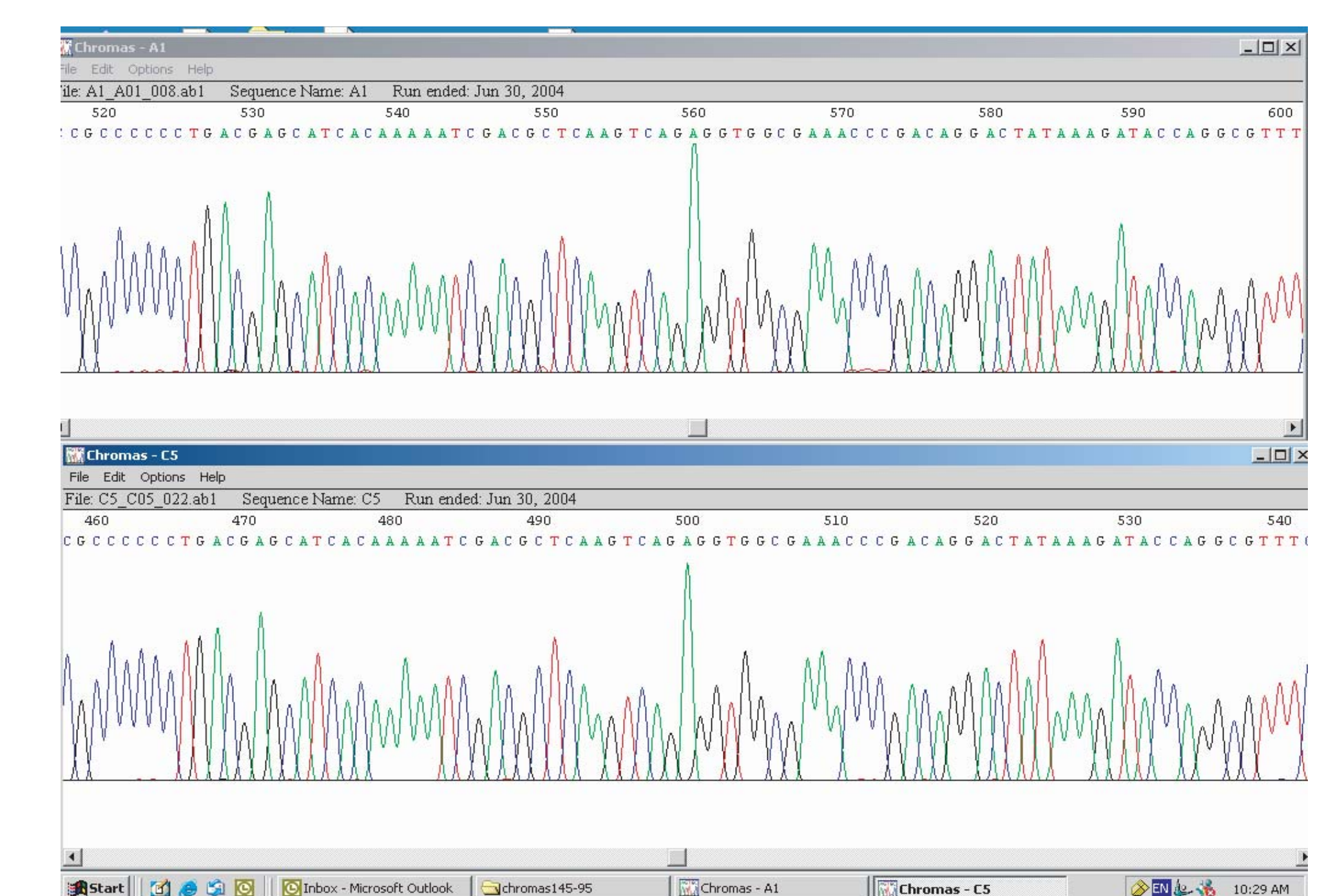


Table 1: Plate CV for synQUAD Dispensing

Plate No.	% CV	Mean Volume (µL)
1	3.8	0.49
2	4.4	0.49

### Results (cont)

Figure 6. Sequence chromatograms: Hummingbird-synQUAD dispensed reagents (upper figure) and manual dispensed reagents (lower figure). Only a segment of the 1100 bp is shown



## Conclusions

- Hummingbird can be used to efficiently and accurately dispense DNA for sequencing reactions with no carry over.
- synQUAD can dispense low volumes of Big Dye quickly into 384 plates with a CV of less than 5.0 %.
- PCR sequencing reactions in which DNA and Big Dye were rapidly dispensed using Hummingbird and synQUAD produced identical data (sequence length and resolution) to that of the much slower manually dispensed reagents.
- By Reducing Big Dye volumes from 8 µL to 0.5 µL, reagent costs are reduced 16 fold. After approximately 45 x 384 sequencing reactions, one can expect 100% return of the investment from Hummingbird and synQUAD systems.