Miniaturisation of Liquid Handling Procedures for High Throughput Sequencing at the Broad Institute.

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Deerac Fluidics™

Broad Institute

1. ABSTRACT

Deerac Fluidics Equator™ products are designed to fit a wide range of applications requiring low volume liquid handling. The products have recently been installed as part of the high throughput sequencing production process at the Broad Institute in Boston, MA, USA (formerly the Whitehead Center for Genome Research). The Genome Sequencing and Analysis program at the Broad Institute places emphasis on the development of scalable methods employing automated laboratory procedures and informatics systems. The current rate of sequencing is over 40 million lanes per year, sequencing human, mouse, and other genomes.
This poster will describe the current process employed by Broad Institute, highlighting where the Equator™ has played a vital role in performance enhancement and cost reduction, enabling the Broad Institute to retain its position as the world's leading genome research institute.

2. INTRODUCTION TO SPOT-ON™ TECHNOLOGY

Deerac Fluidics has developed a unique proprietary technology for the dispensation of nano and micro litre volumes of a wide range of fluids. The technology is:

- Simple and robust
- Flexible to user's needs
- Clean and fast
- Cost competitive

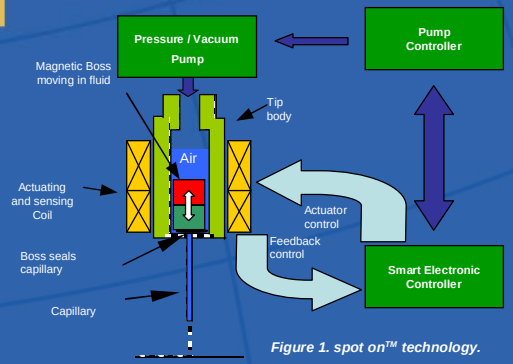

Figure 1. spot on™ technology.

Volume range	50nL-20µL
Volume increment	Freely adjustable
Speed	<15s (1536 well plate) <30s (96 well plate)
Accuracy	< 10% @ 50nL < 10% @ 1,000nL
Precision	< 10% @ 50nL < 5% @ 1,000nL
Viscosity range	0.5 - 6.0 cP
Plate formats	96, 384, 1536 & custom

Table 1. Equator™ NS 808 liquid handling product specifications

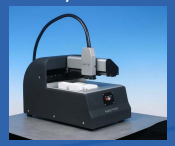

Figure 2. Equator™ NS 808

Figure 3. 1536 format dispense onto a flat surface.

3. MINIATURIZATION OF SEQUENCING AT THE BROAD INSTITUTE

3.1 Aim

To QC validate the Equator™ for aspects of the sequencing process
- To increase sequencing throughput
- To reduce reagent volumes in the sequencing reaction

3.2 Introduction

The production sequencing process employed by the Broad Institute is summarised in the schematic below.

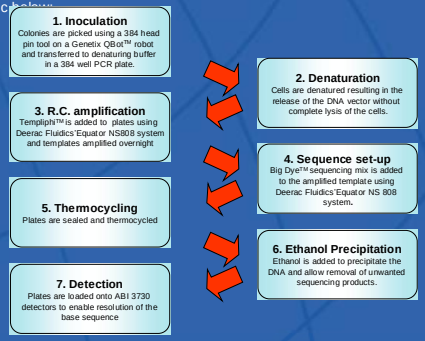

Figure 4. Summary of production sequencing process at the Broad Institute

3.3 Instrument Selection:

The Broad Institute developed a set of criteria for instruments to be considered for low volume dispensing in order to validate assay chemistry and minimise disruption.

Criteria	Requirement
Low volume dispensing	Precise dispensing below 1µL to allow the reduction of reagent costs.
Wide volume range	Range between 200nL – 10µL required to allow existing assays to be duplicated, then optimised at lower volumes.
Integration	Requirement to integrate instrument with existing production equipment.
Throughput	Maintain / improvement of throughput efficiency during sequencing process
Maintenance / Service	Instrument must be robust, stable and be easy to maintain and service.

Table 2. Criteria for selecting instruments for miniaturization

The Equator™ NS 808 pipetting system was identified as the most suitable system meeting the criteria outlined above. It was integrated into the existing high throughput sequencing line. The Equator™ NS 808 was linked with a Plate-Stack system from Perkin Elmer Life and Analytical Sciences. The integration of the Equator™ with a wide range of stackers is facilitated by the Active-X control mechanism in the spot-station™ user software.

The Equator™ NS 808 provided an improvement on previous technology used in the following manner:
- Non-contact dispensing removes cross contamination risk, reducing washing and hence transfer time.
- Fixed tips can be washed reducing day-to-day running costs.
- The Equator™ instrument could duplicate current assay volumes allowing validation of the chemistry as well as the ability to miniaturize.
- Sequencing data of the same quality is obtained with the Equator™ instrument while significantly reducing the operating costs.

3.4 Miniaturization

Two areas of the sequencing process were highlighted as being suited to immediate miniaturization: addition of Templiphi™ and sequence set-up.
Volume gradients were created across 384 or 1536 well plates using the ability of the Equator™ NS 808 to control each channel independently in order to find the optimal concentration and volume of Templiphi™ and Big Dye™ reagents .

3.5 Results:

- Miniaturisation of the addition of Templiphi™ and the sequencing set-up accomplished.
- Quality of the sequencing data not affected by assay miniaturisation.

System	Pass Rate (%)	Read Length	Bases/Read
Current	87	612	532.44
Equator™ NS 808	89	605	538.45

Table3. Sequencing results obtained before and after miniaturization

Figure 5. Sequencing chromatographs obtained before (above) and after (below) miniaturization

- Reduction of reaction size to one third its original volume.
- 10 fold reduction in dead volume. and the ratio of total assay volume to dead volume has improved from 2: 1 to 10: 1.
- Reduced amplification results in reduction of dilution steps.
- 30% increase in the throughput of these steps by employing the Equator™ product.
- Greater than 25% reduction in daily operating costs.

System	Dead Volume/ day (ml)	Time/plate (secs)
Current	239.2	66
Equator™ NS 808	29.6	45

Table 4. Comparison of assay performance before and after miniaturization

4.CONCLUSIONS:

Seamless integration with existing laboratory equipment.

- **Micro litre and sub-micro litre pipetting of expensive reagents.**
- **Maintenance of high level of data integrity.**
- **Increased throughput,**
- **Significant reduction operating costs.**