Incorporation of nanolitre pipetting technology into medium and high throughput SNP genotyping platforms at KBiosciences.

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1. ABSTRACT

An area of great interest in drug discovery at present is the process of genotyping in order to better understand how single nucleotide polymorphisms (SNPs) are associated with various disease states.
Companies are actively pursuing genotyping processes in medium and high throughput fashion that will enable a large number of samples to be monitored in a short time frame and with minimal labour intensity. The genotyping process can be costly to run at all levels, especially in the consumption of expensive reagents such as PCR mixes.
spot-on™ nanolitre technology from Deerac Fluidics™ (www.deerac.com) has been incorporated by UK genotyping services company KBiosciences (www.kbioscience.co.uk) into their high throughput system, which has enabled cost savings of up to 20 fold on previous techniques used.
This poster will describe the current process employed by KBiosciences. The vital role spot-on™ technology, incorporated into the Equator™ benchtop robotic liquid handling instrument, has played a in improving performance and reducing costs are highlighted. This has enabled KBiosciences to become cost competitive in the marketplace in the provision of their service.

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

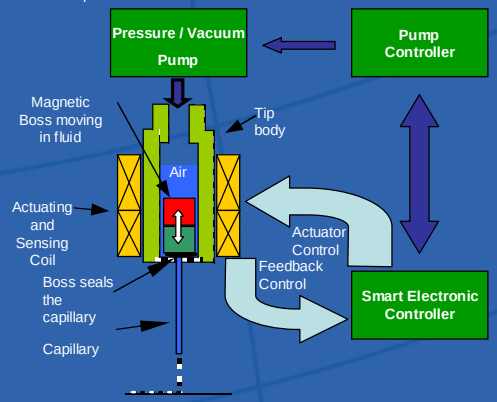

Figure 1. spot on™ technology.

Volume range	50nL-20µL
Volume Increment	Freely Adjustable
Speed	<15s (1536 wellplate) <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

3.MINIATURIZATION OF GENOTYPING AT KBIOSCIENCES

3.1 Aim

-To QC validate the Equator™ for SNP genotyping
•To increase genotyping throughput
•To reduce reagent volumes in Taqman™ PCR

3.2 Introduction to KBiosciences Genotyping Platform

KBiosciences perform high throughput SNP genotyping on 96, 384 and 1536 well plate formats. The Equator™ system is utilised in the genotyping process to dispense PCR master mix at microlitre and nanolitre volumes
Fluorophores incorporated into primers in the PCR mix are used as the basis of detecting amplified SNPs. Data is derived from a fluorescence resonance energy transfer (FRET) assay and represented in the form of a scatter allelograph. The quality of data is assessed by the compactness and separation of the discrete populations of data points.

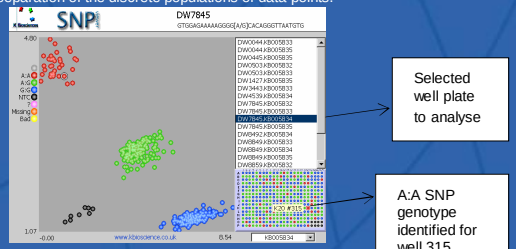

Figure 2. Scattergraph, demonstrating isolation of genotypes based on fluorescence.

3.3 Method of QC Validation of Equator™ and miniaturisation of genotyping process.

KBiosciences put the Equator™ instrument through a rigorous validation program before acceptance and integration into their genotyping platform. QC validation criteria were as follows:
• Favourable comparison of the Equator™ system to alternative low volume liquid handling technologies under the criteria outlined in Table 2 below.
• >99% call rate i.e. 99 out of 100 samples must have a called genotype. This is dependent on the quality of separation on the scattergraph (Figure 2).

Following QC validation a miniaturisation strategy was implemented to reduce volumes from the current 5ul, while maintaining the quality of the data.

3.4 Results

3.4.1 QC validation

The Equator ™ instrument was viewed to be superior to previously utilised liquid handling equipment.

Instrument Feature	Equator advantage
Ease of set up and optimisation	System is operational very quickly and can be optimised without any hardware alterations.
Reliability	The system provided a consistent excellent performance.
Accuracy and precision	Dispense performance is of consistently within specification.
Speed of operation	Dispensing speed is fast with a direct impact on increasing throughput.
Software	Graphic user interface (GUI) is simple and intuitive to operate.

Table 2. KBiosciences description of Equator™ product's competitive advantages

A high degree of separation on scattergraphs was achieved, resulting in the determination of 99% of genotypes (Figure 2).
To date the systems have performed over 5 million dispenses since installation with a limited number of failures in that period (Table 3).

No. of dispenses	No. of well plates	No. of failures (tip)
5000000	12,000	10

Table 3. Tip failure rate vs. throughput achieved with spot-on™ technology

3.4.2 Miniaturisation

Following QC validation at higher volumes, a miniaturisation programme was implemented. Initially 5ul of Taqman™ master mix was added to each well this was reduced using the nanolitre dispensing technology to 250nl – a 20 fold reduction in reagent use. The quality of the data was assessed at each volume decrease. At 250nl no loss in data quality was determined.
Due to the decrease in reagent use a reduction in assay cost was achieved from Euro 0.70 to Euro 0.05 per reaction. The transition from 96 and 384 well plates to 1536 well plates increased throughput significantly.

Figure 3. 1536 format dispense onto a flat surface.

4. CONCLUSIONS

Use of spot-on™ technology from Deerac Fluidics™ in KBiosciences genotyping process has enabled them to:

1. **Reduce reagent volume 20 fold**
2. **Bring down assay costs 14 fold.**
3. **Achieve accurate and robust liquid handling**
4. **Significantly increase throughput**
5. **Maintain the quality and integrity of their SNP data (99% call rate)**

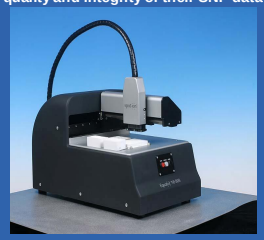

Figure 4. Equator NS 808