

Use of the PHERAstar for SNP Genotyping Chemistry Development and High Throughput Production Genotyping

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- FRET based multichromatic assay
- Fastest read times
- Miniaturised format (1–2 μL) in 384- and 1536-wells
- Improved call rates and decreased failure of assays

Introduction

Single Nucleotide Polymorphisms (SNP's) have recently become an invaluable tool in the field of Genetic Research. They are employed in a wide range of scientific fields from Pharmacogenetics to animal breed identification through to disease gene mapping. They are a popular choice of genetic marker due to their ease of assay and analysis, however many standard assays remain relatively expensive to use in high throughput.

In this application note we show the use of the PHERAstar multimode plate reader for fluorescence intensity measurements in both SNP Genotyping chemistry development and high throughput production genotyping. KBiosciences's proprietary FRET based assay technology and BMG LABTECH's PHERAstar plate reader allow for cost effective, rapid and reliable analysis of sample volumes as small as 1 μL in 1536-well plates.

Materials & Methods

All materials were sourced from Sigma Aldrich, with the exception of 384- and 1536- well microplates which were produced in house (Part Numbers - Pro 384 and 1536 respectively). Oligonucleotides were also sourced from Operon, Germany.

The chemistry used was a KBiosciences proprietary system based on a homogeneous Fluorescence Resonance Energy Transfer (FRET) detection system, using competitive allele specific PCR. Briefly, two oligos are designed specific to each allele of the SNP. Each one of these oligos is tailed with an 18bp sequence distinct from each other. Also included in the reaction is Taq polymerase, dNTP's, an internal standard dye (Rhodamine X, (Rox)) and reverse primer.

Modified versions of Taq polymerase are unable to extend primers that are mismatched at their 3' terminal base. This is used to discriminate the two alleles.

The reaction is monitored by the creation of fluorescence from two novel FRET reporter Oligos that are included in the reaction. All reactions were conducted in a volume of 1 μL (1536-well plates) or 2 μL (384-well plates) and thermal cycled in a H2OBIT thermal cycler (KBiosystems, Basildon, UK).

Results

SNP based genotyping is essentially a qualitative technique with the output being a cluster plot. In the case of the PHERAstar assessment an initial evaluation was carried out to determine the optimal conditions for reading. During this phase the effect of gain, excitation and emission filter choice and number of flashes per well were determined.

The optimal wavelengths were determined to be:

Excitation wavelength 1 (Fam) – 485 nm - 10 nm

Excitation wavelength 2 (Hex) – 520 nm - 10 nm

Excitation wavelength 3 (Rox) – 575 nm - 10 nm

Emission wavelength 1 (Fam) – 520 nm - 20 nm

Emission wavelength 2 (Hex) – 560 nm - 10 nm

Emission wavelength 3 (Rox) – 610 nm - 10 nm

Following optimisation, the performance of the PHERAstar was checked with a number of plates. An example of a typical read is shown in figure 1, as a cluster plot from the KBiosciences Genotyping LIMS package.

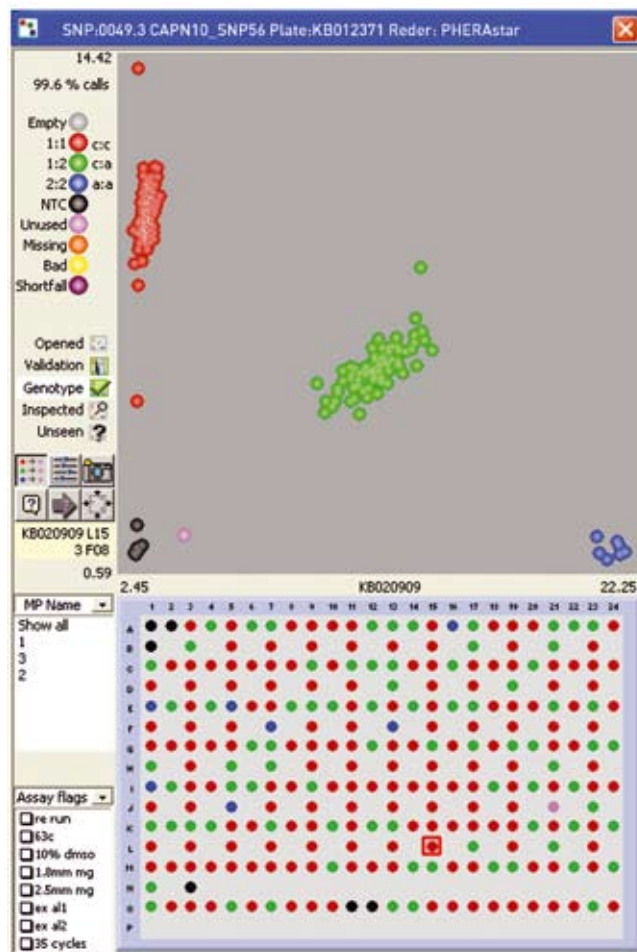


Fig. 1: Cluster plot of a typical 384-well format read using the PHERAstar in combination with the KBiosciences Genotyping LIMS package.

In all cases the quality of the data is greatly affected by the performance of the plate reader. In every case tested the PHERAstar gave greater data quality leading to improved call rates (ability to call a genotype) and decreased failure of assays. This is shown in the table below from a 100 plate study (mixture of plate types).

Table 1: Several readers were evaluated for speed and performance with KBiosciences's inhouse chemistry.

Plate reader	No of assays passed	Call rate	Time/384-plate
BMG LABTECH PHERAstar	270	98.4	2 mins 45 sec

Conclusion

The BMG LABTECH PHERAstar produces data quality surpassing other state of the art plate readers. The PHERAstar was also the fastest reader tested, giving the highest throughput in plates per hour, and is now fully integrated into the KBiosciences pipeline with a Caliper Twister where it can be left unattended for hours at a time.

Future Development

Further work has recently been completed to assess the performance of the fluorescent dyes Vic (Applied Biosystems), and Yakima Yellow

(Epoch Biosciences). To date these two dyes have been shown to give superior performance to the Hex used in this study.

In this evaluation we utilized only one of the PHERAstar's two photomultiplier tubes. Future assessments are being conducted to evaluate the ability of the PHERAstar to monitor emission in two channels simultaneously, where the internal standard and two allele specific dyes are read together. Simultaneous dual emission would reduce the number of reads per plate from three to two, decreasing read-times by a third and potentially further improving accuracy.

Background

KBiosciences based in Hoddesdon, Hertfordshire is a rapidly growing company that has been created to exploit the use of miniaturisation and its own chemistry development to drive down SNP genotyping costs. It offers access to this technology by way of a fee for service operation. However, realising that some researchers are unable to send DNA out of their own laboratories KBiosciences has been completing the development of its own in-house Genotyping Chemistry.

To aid in the throughput in the laboratory KBiosciences undertook to evaluate the BMG LABTECH PHERAstar for its SNP Genotyping pipeline.

More information can be found at:
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