

# High Speed FRET based SNP Genotyping Measurement on the PHERAstar

## Introduction

Single Nucleotide Polymorphisms (SNP's) have 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 measurements in both SNP Genotyping chemistry development and high throughput production genotyping.

The new KASPar™ system, developed by KBiosciences, was used to assess the performance enhancements of the PHERAstar, using a new SNP genotyping specific optical module with dual emission created by BMG LABTECH (Figure 1). Using two photomultipliers allows for improved accuracy and reduced read times by 30%.

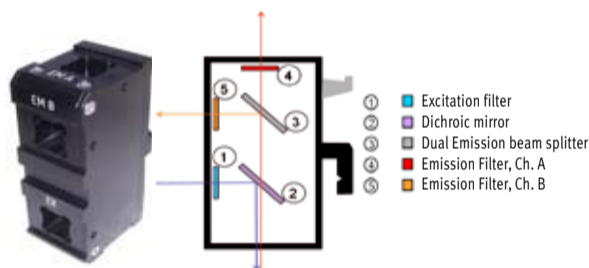


Fig. 1: SNP Genotyping optical module with dual emission wavelength detection

## Materials and 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 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 primers.

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 Hydrocycler thermal cycler (KBiosciences, Basildon, UK). Liquid handling was performed using a Deerac Equator™ GX8 (Deerac Fluidics, Dublin, Ireland).

## 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.

Dual emission modules offer the great advantage to read the internal standard (ROX) and the allele specific dyes (FAM and VIC) together. Optimal modules were determined to be:

FAM/ROX  
Excitation 485 nm  
EmissionA 610 nm  
EmissionB 520 nm

VIC/ROX  
Excitation 520 nm  
EmissionA 610 nm  
EmissionB 560 nm

Figure 2 shows the results obtained from measurements with a single emission module. Every result can be clearly assigned to a distinct cluster.

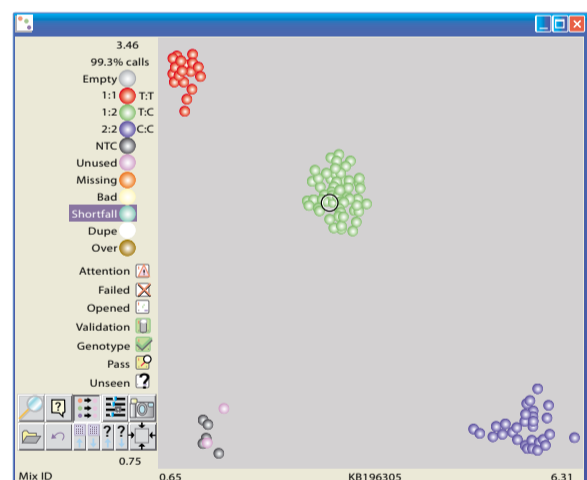


Fig. 2: Cluster plot from the KBiosciences Genotyping LIMS package from a measurement using the single emission module

The results show, sharper clusters are obtained when the dual emission module is used. (Fig. 3)

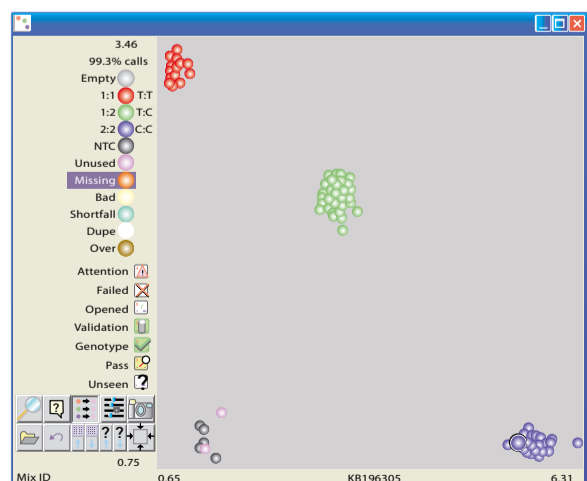


Fig. 3: Cluster plot from the KBiosciences Genotyping LIMS package from a measurement using the dual emission module

## Conclusion

The PHERAstar has always performed this application well in the past but by introducing the SDE modules a further significant improvement in performance was achieved. In addition to this, the implementation of the dual optical modules at the KBiosciences production facility has resulted in an increased throughput of 30% due to the having to read each well only twice (Fam/Rox, then Vic/Rox) instead of each Fluor separately.

Finally we have noticed that since implementation of the dual modules the mis-read rate of the PHERAstar has decreased to a level that is no longer measurable. After the initial evaluation, the whole production site of PHERAstars at the KBioscience facility has been upgraded to dual modules.



Fig. 4: BMG LABTECH's multimode plate reader PHERAstar

## Background

BMG LABTECH offers fully automated microplate measurement systems, which are designed to meet the assay needs for customers in life science research, proteomics, genomics, drug discovery and HTS. All BMG LABTECH readers can easily be integrated into robotic systems from all leading automation companies.

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.

To aid in the throughput in the laboratory KBiosciences integrated PHERAstars in their HTS pipeline. More information can be found at: [www.kbioscience.co.uk](http://www.kbioscience.co.uk)

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