High Speed FRET based SNP Genotyping Measurement on the PHERAstar

BMG LABTECH

Phil Robinson, KBiosciences, Hoddesdon, Hertfordshire, EN11 0EX, UK
Franka Ganske, BMG LABTECH, Offenburg, Germany

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- ○ FRET based SNP genotyping with new KASPar™ system
- ○ Optimized optical modules with dual emission
- ○ Improvements in speed and accuracy of measurement

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%.
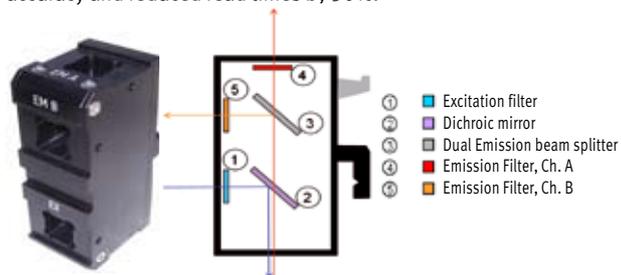

① Excitation filter
② Dichroic mirror
③ Dual Emission beam splitter
④ Emission Filter, Ch. A
⑤ Emission Filter, Ch. B

Fig 1. SNP Genotyping optical module with dual emission wavelength detection (SDE module)

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, dNTPs, 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).The 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 and 3 show the results obtained from measurements with different emission modules. The results show, sharper clusters are obtained when the dual emission module is used.
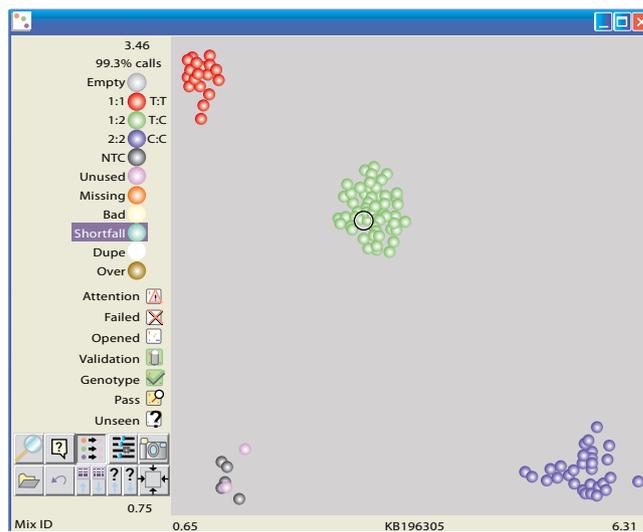

Fig 2. Cluster plot from the KBiosciences Genotyping LIMS package from a measurement using the single emission module
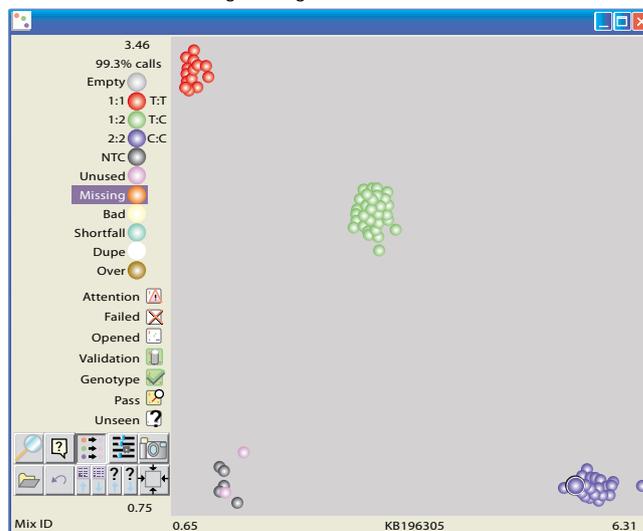

Fig 3. Cluster plot from the KBiosciences Genotyping LIMS package from a measurement using the dual emission module

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.

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 chose to use PHERAstars in their HTS pipeline.

More information can be found at: www.kbioscience.co.uk