

Use of Fluidigm BioMark™ for Genotyping, Gene Expression and 454 Libraries Quantification

Olivier Bouchez^{1,2}, Katia Feve², Juliette Riquet², Jean-José Maoret³, Gérald Salin^{1,2}, Cécile Donnadiou^{1,2}, Frédéric Martins¹, Jérôme Lluch¹, Eugénie Robe¹, Diane Esquerré^{1,2}, Julien Fremez¹ & Denis Milan^{1,2}

¹ Plateforme Génomique de la Génopole Toulouse Midi-Pyrénées, INRA, Chemin de Borderouge, 31326 Castanet-Tolosan Cedex, France.

² Laboratoire de Génétique Cellulaire, INRA, Chemin de Borderouge, 31326 Castanet-Tolosan Cedex, France.

³ Plateforme Biopuces de la Génopole Toulouse Midi-Pyrénées, IFR150, Hôpital Rangueil, Bat L4, BP84225, 31432 Toulouse Cedex 4, France.

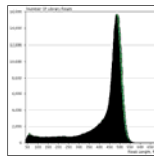
Recently, we acquired a Fluidigm BioMark™. Different applications have been developed on this technological platform. Genotyping experiments were performed, allowing us to simultaneously genotype 48 or 96 animals on 48 or 96 SNP markers (2304 or 9216 genotypes). Furthermore, it has been used to determine gene expression in 96 samples on 96 genes (9216 points) using Sybrgreen or AOD. Results obtained demonstrate that this technique is very robust and results are comparable to those obtained with classical techniques.

This allows the researchers to produce a lot of data in a reduced time. One other application available on BioMark™ is the quantification of DNA libraries (SlingShot) before sequencing on Roche 454. We developed this application for our sequencing experiments on different kind of DNA (gDNA, cDNA, Amplicons). This application is able to more precisely quantify DNA molecule number and to save time in comparison with titration recommended by Roche, and should allow us to start from a smaller quantity of DNA.

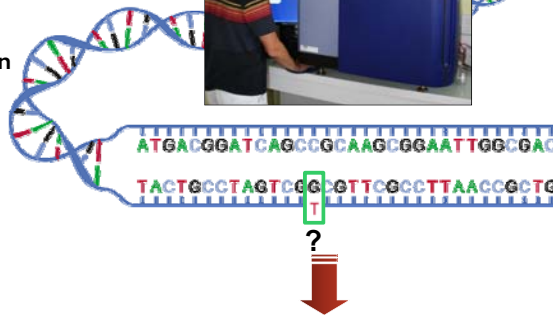
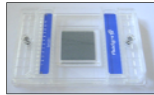
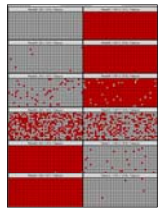
SlingShot

DNA libraries quantification for Roche 454 GS FLX sequencing

Sequencing run

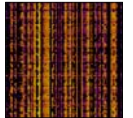
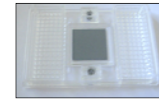
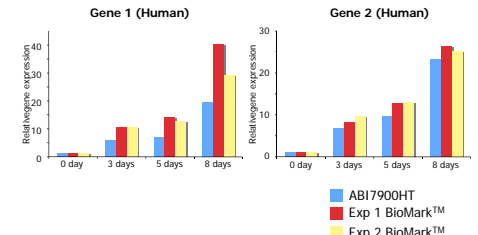


Serial dilutions of the libraries and count of red cells : exact quantification of DNA molecule number



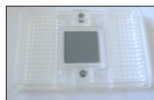
Quantitative PCR

Comparison of experiments performed on ABI7900HT and BioMark™ using AOD on 96x96 Chips



Conclusions :

- > Similar results obtained in comparison to Roche recommended titration
- > Cost gain
- > Hand-on time gain



Genotyping of 96 Pig SNPs (Taqman® probes) for association studies with quantitative traits

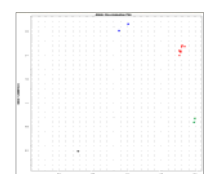
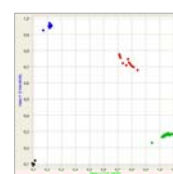
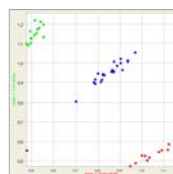
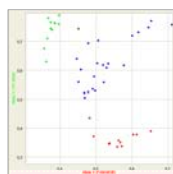
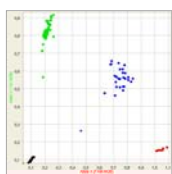
Genotyping

Good quality DNA

Bad quality DNA

Pre-amplification on bad quality DNA

Comparison of BioMark™ genotyping vs ABI7900HT genotyping



BioMark™

ABI7900HT

Conclusions :

- > Good and reproducible results obtained on SNPs genotyping
- > Similar results obtained between ABI7900HT genotyping and BioMark™ genotyping