Evaluation of GenProbe’s Luminex xMAP-based Blood Group Genotyping Kits using previously genotyped donor DNA

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Introduction

Over the last decade or so, blood group genotyping has become firmly established in the field of Transfusion Medicine. This is especially the case with regard to the clinical management of transfusion-dependent patients (e.g. patients with Sickle Cell Disease and Auto-immune Haemolytic Anaemia). Antenatal care has been significantly improved with the increasingly routine use of Non-Invasive Pre-natal Diagnosis of the RH D status in pregnant RHD negative females (Reid & Denomme 2011).

The integration of molecular typing has not been as successful in the typing of donors, despite the availability of micro-array platforms such as BioArray Solutions BeadChip and ProGenika’s BLOODchip (Denomme et al. 2011). Although the benefits of extensively typing donors is obvious, there must always be consideration for the cost-effectiveness of such an approach. The initial cost of using early micro-array offerings was not justifiable when compared to serological testing (this is especially the case in Europe).

Early platforms such as BeadChip and BLOODchip required dedicated hardware, with a significant capital outlay. More recently commercial offerings make use of pre-existing and established technology.

GenProbe have developed two red cell genotyping kits using, the well-established, Luminex xMAP technology. Many Histocompatibility and Immunogenetic (H&I) laboratories already use this technology, removing the need to purchase extra equipment.

Luminex uses fluoroanalysis to detect the allele of interest. In this case initial non-symmetric PCR will result in bivalent amplification products. Hybridisation occurs with specific probes. R-phycocerythrin-conjugated streptavidin then enables fluoroanalysis [microsphere are colour-coded with one of 99 available colours for each probe].

LIFECODE Kits (Beta)

GenProbe have developed two red cell genotyping kits (LIFECODES RBC and LIFECODES RBC-R). The technology uses multiplex non-symmetric polymerase chain reaction (PCR), resulting in excess ssDNA of one target strand. This eliminates the need for dsDNA de-naturation before probe annealing. Although there is a requirement for increased cycles (45), the benefits include a simplified protocol, very few post-PCR steps, with improved robustness (due to less areas for errors to occur).

Methods

Hybridisation

Following probe-microsphere activation (5-10 minutes at 55-60°C), 15µl of well-mixed probe was added into individual wells on a Costar® low profile PCR plate. 5µl of PCR product was added to relevant wells. The plate was sealed with polyethylene tape and placed in the thermal cycler (a rubber mat was also used to prevent evaporation). The Hybridisation Program was run:

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<td>95°C</td>
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Acquisition and Analysis

Fluorescence and microsphere analysis was performed using a Luminex 100 Fluorometer, with xMAP Technology. Raw data was analysed using GenProbe’s RBC MatchIT Software.

Results

GenProbe and BeadChip

A total of 80 BeadChip-genotyped samples were tested by the GenProbe Beta kits for a total of 2400 SNPs. 5 discordant results were obtained (MNS4, D11, LU1 x L2, LU2), a rate of 0.22%. The number of ‘Intermediate Calls’ (IC) with Correct Genotype for these samples was 9 (6 x LU2, 3 x RH4), a rate of 0.4%.

GenProbe and SSP-PCR

Of the 80 genotyped samples, 26 samples were also genotyped using Inno-Train SSP-PCR Kits. A further 8 SNPs (FYa, FYb, K3/K4, DX/DX4, YTT/FT2, K1/WK2) were compared. For 292 SNPs analysed, concordance was 99.6% (with no ICs).

Total SNPs Analysed

A total of 2608 SNPs were analysed with 5 discordant results, an initial concordance of 99.81%.

Repeat Testing

Following repeat testing discordant samples, only 1 ‘Intermediate Calls’ (IC) with Correct Genotype (LU3) was obtained, giving a concordance of 100%.

GenProbe and Phenotyping

A number of red cell antigen phenotypes had been determined for these donor samples. The antigen types included Cce, Kk, FyFy, JkJK, MN and S/s (Lu and Co were included for a limited number of samples).

The predictive phenotype using RBC MatchIT was accurate. Of 1075 known phenotypes, GenProbe’s LIFECODES genotyping kits predicted correct phenotypes for 1074 blood types. A sensitivity of 99.91%.

Only one sample had an incorrect predictive phenotype. This was the previously mentioned K+k+ phenotype, which was given a predictive phenotype of K+k+ (O’Connor et al. 2009). The LIFECODES kits, Inno-Train SSP-PCR kits and BeadChip gave the same genotype (and predictive phenotype) for this sample. No future investigation, such as sequencing, adsorption-elution, or flow cyrometry, has taken place on this donor. Several SNPs have been reported resulting in very weak or no expression of K (Blumenfeld & Patnaik 2004).

Discussion & Conclusion

GenProbe LIFECODES Red cell genotyping kits produced accurate genotypes and predicted accurate phenotype, when compared to other genotyping tools used for genotyping for human blood groups.

As Beta kits it was expected that there should be some discrepancies. One particular area where improvement should be expected is the determination of Lutheran genotypes/predicted phenotypes. Adjustment of cut-off MFIs, and further optimisation of PCR, will rectify this ‘hitting’ problem. Production kits will then give highly accurate and reproducible red cell genotypes.

A significant advantage of the Gen-Probe kits is the use of non-symmetric PCR. By using non-symmetric PCR both single and double-stranded DNA is produced which can directly be used in the hybridisation step. This removes the need for any denaturation and reduces the overall number of steps in the protocol. This reduces the overall chance of procedural error. The time from DNA to result for 96 (single assay) or 48 (RBC & RBC-R testing) samples is approximately 4½ hours.

GenProbe Kits offer a rapid, reduced-cost, optimal genotyping system and as Luminex hardware is already present in many immunogenetic laboratories can be used, further reducing capital needed.

For mass genotyping the GenProbe kits are relatively labour intensive, however the Luminex platform lends itself well to automation. GenProbe are developing a automated platform with the Hamiltion Statlet, which provides an easy-to-use medium-to-high throughput platform for donor red cell genotyping.

References


Acknowledgements

Special thanks to Dr. Christine Heylen (GenProbe) for providing GenProbe LIFECODES Beta Kits and for performing repeat testing on the initial 5 discordant samples.

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