PO 09

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

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Introduction

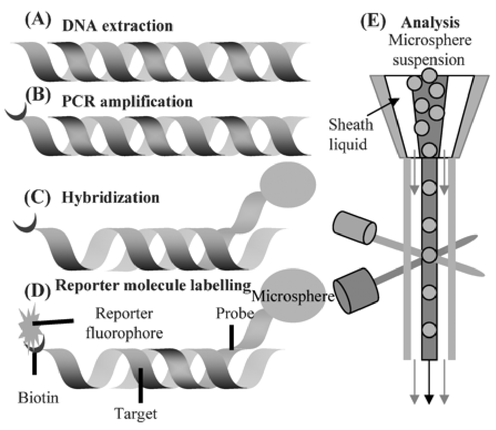
Over the last decade or so, blood group genotyping has become firmly established in the field of Transfusion Medicine. This is especially the case with regard to the clinical management of transfusion-dependent patients (e.g. patients with Sickle Cell Disease and Auto-immune Haemolytic Anaemia). Ante-natal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

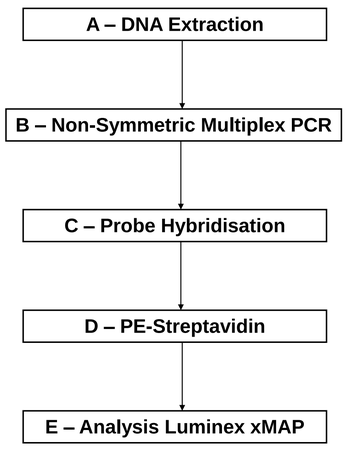
The integration of molecular typing has not been as successful in the typing of donors, despite the availibilty of micro-array platforms such as BioArray Solutions BeadChip and ProGenika's BLOODChip [Denomme *et al*. 2011]. Although the benefits of extensively typing donors is obvious, there must always 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 biotinylated amplification products. Hybridisation occurs with specific probes. R-phycoerythrin-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 annealling. 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).

LIFECODES RBC Kit (Beta)	
System	**Antigen**
Kell	K, k, Kp^a, Kp^b, Kp^c, Js^a, Js^b
Kidd	Jk^a, Jk^b, Jk^{WT}, Jk_{null}
Duffy	Fy^a, Fy^b, Fy^x, Fy_{GATA}
MNS	M, N, S, s, S-s-U^{var}
Rh	C, c, E, e
Dombrock	Do^a, Do^b

LIFECODES RBC-R Kit (Beta)	
System	**Antigen**
Colton	Co^a, Co^b
Dombrock	Do^a, Do^b, Hy, Jo^a
Scianna	Sc1, Sc2
Lutheran	Lu^a, Lu^b
Diego	Di^a, Di^b, Wr^a, Wr^b
Landsteiner-Weiner	LW^a, LW^b
Cartwright	Yt^a, Yt^b
Knops	Kn^a, Kn^b, McC^a, McC^b, Sl1, Sl2
Cromer	Cr^a+, Cr^a-

Methods

Sample Selection

Donor samples were selected from repeat Irish donors. Donors were selected to be as fully phenotyped as possible.

DNA was extracted using Genevision Geno M-6 semi-automated DNA extraction robot. Final DNA concentration range was 39-130 ng/µl.

80 donor DNA samples were then selected from previously genotyped donors. The samples were previously genotyped using BioArray Solutions BeadChip [O'Connor *et al*. 2009] and Inno-Train's Ready-Gene SSP-PCR CE-approved kits (in-house validation).

Non-symmetric PCR

PCR was performed using an AB GeneAmp 9700 thermal cycler. 1.5U *Taq* polymerase (Gen-Probe) was used per multiplex reaction. DNA was not diluted and 2µl was used per PCR. The PCR program was a follows:

Temperature	Duration	Cycles
95˚ C	240 sec	1
95˚ C	30 sec	45
51˚ C	45 sec	
65˚ C	150 sec	
65˚ C	300 sec	1

Methods

Hybridisation

Following probe-microsphere activation (5-10 minutes at 55-60˚ C), 15µl of well-mixed probe was aliquoted 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:

Temperature	Duration	Cycles
56˚ C	22 min	1
56˚ C	Hold	1

Acquisition and Analysis

Fluorescence and microsphere analysis was performed using a Luminex 100 Fluoroanalyzer, with xMAP Technology. Raw data was analsyed using GenProbe's RBC MatchIT Software.

Results

GenProbe and BeadChip

A total of 80 BeadChip-genotyped samples were tested by the Gen-Probe Beta kits for a total of 2400 SNPs. 5 discordant results were obtained (*MNS4, DI1, LU1* x 2*, 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 (*FY_{GATA}, K3/K4, DI3/DI4, YT1/YT2, KN1/KN2*) were compared. For 208 SNPs analysed, concordance was **100%** (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' (*LU2*) 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 typed included C/c, E/e, K/k, Fy^a/Fy^b, Jk^a/Jk^b, M/N and S/s (Lu^a and Co^b 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 LIFECODE 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 cytometry, 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 technolgies 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 'teething' 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 realtively labour intensive, however the Luminex platform lends itself well to automation. GenProbe are developing a automated platform with the Hamilton Starlet, which provides an easy-to-use medium-to-high throughput platform for donor red cell genotyping.

References

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