

A MODIFIED RSCA METHOD FOR LOW-RESOLUTION HLA-C TYPING

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Table 1. HLA-C test panel.

Sample	HLA-C type	Sample	HLA-C type	Sample	HLA-C type
1	Cw*0102, 1602	13	Cw*0702, 0803	24	Cw*0501, 0401
2	Cw*0102, 0704	14	Cw*0702, 1502	25	Cw*1801, 0802
3	Cw*0202	15	Cw*0702, 1203	26	Cw*1203, 0401
4	Cw*0202, 0501	16	Cw*0801, 1202	27	Cw*0701, 1203
5	Cw*0302, 0804	17	Cw*1402, 0702	28	Cw*1502, 0202
6	Cw*0303, 0304	18	Cw*1505, 0602	29	Cw*1202, 0302
7	Cw*0401, 0602	19	Cw*1601	30	Cw*0302, 0603
8	Cw*0403, 1202	20	Cw*1701	31	Cw*0702, 0304
9	Cw*0501, 1203	21	Cw*0304, 0801	32	Cw*0102, 0501
10	Cw*0602	22	Cw*0802, 1402	33	Cw*0303, 0702
11	Cw*0602, 1507	23	Cw*0304, 0701	34	Cw*0501
12	Cw*0702, 1801				

Method adopted for the Li-Cor 4200

A 313 bp region of intron 1 and exon 2, selected as the polymorphism observed broadly corresponds to the 15 Cw allele families, was used to produce smaller heteroduplexes. Using Cw*0701 and Cw*0102 reference strands, the mobility of the alleles in the reference panel were re-established and 48 random donors from the Welsh Bone Marrow Donor Registry (previously HLA-C typed by PCR-SSP) were tested.

Results

Four Cw alleles, *0102, *0401, *0701 and *1402, were clearly differentiated from all other alleles tested. However, the remainder fell within four broad allele groups: (1) Cw*0202, *0302, *0303, *0304, and *0403; (2) Cw*0602, *0702, *0704, and *1801; (3) Cw*0501, *0801, *0802, *0803, *1202, *1203, *1601, *1602 and *1701; (4) Cw*1502, *1505 and *1507. Additional small mobility differences within these groups were observed for Cw*0202, *0403 and *1502. Figures 4 and 5.

Introduction

Reference strand mediated conformation analysis (RSCA) differentiates alleles on the basis of electrophoretic mobility of DNA heteroduplexes. Successful HLA-A and -B typing by RSCA has been reported, however the mobility values of HLA-C alleles are indistinct (Arguello et al. (1998) Tissue Antigens, 52, 57). We have evaluated RSCA HLA-C typing using the Li-Cor 4200 automated DNA sequencer as an alternative to low-resolution typing by PCR-SSP.

Standard HLA-C RSCA typing method

The mobility of 25 HLA-C alleles was established using the method of Arguello et al. on the Li-Cor 4200. A panel of 34 reference DNA samples covering all serological specificities, Cw*12 and Cw*14 to Cw*18 was used. However, resolution was poor as many alleles shared mobility (Figure 1).

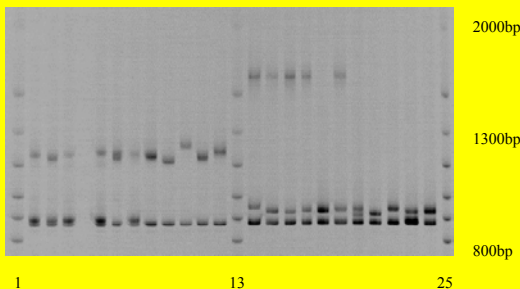


Figure 1. Samples 12-22 from the HLA-C test panel (Table 1) tested with fluorescent labelled reference strand (FLR) Cw*0701 (tracks 2-12) and FLR Cw*0303 (tracks 14-24). 100bp ladder (Microzone Ltd) tracks 1, 13 and 25. Smallest product in each lane is the FLR homoduplex.

Modifications to improve resolution

Attempts to improve resolution included changes in concentration and constituents of the gel matrix, the introduction of single-stranded elements into the heteroduplex and the use of alternative reference strands. However, resolution was not improved due, in part, to poor reproducibility possibly caused by the different conformations adopted by large (909 base pair) heteroduplexes spanning exon 2, intron 2 and exon 3 (Figures 2 and 3).

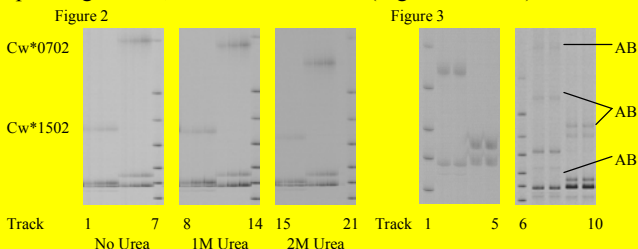


Figure 2. RSCA results demonstrating the effect of urea on mobility. Sample type is Cw*0702, *1502. Heteroduplexes with FLR Cw*0701 (tracks 1-3, 8-10, 15-17) and Cw*0303 (tracks 4-6, 11-13, 18-20). 100bp ladder tracks 7, 14, 21. Urea reduced the mobility of heteroduplexes formed between FLR Cw*0701 and Cw*1502 (tracks 8-10, 15-17) and Cw*0303 and Cw*0702 (tracks 11-13, 18-20) when used in the gel formulation. Smallest product is the FLR homoduplex.

Figure 3. Cw*0501 and Cw*0602 share mobility when combined with FLR Cw*0701 (tracks 2-3, 7-8) and Cw*0303 (tracks 4-5, 9-10). Additional bands (AB) were observed when a single-stranded element was introduced to the heteroduplex. However, these also shared mobility. 100bp ladder tracks 1 and 6. Smallest product is the FLR homoduplex.

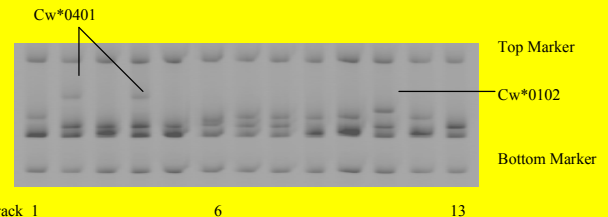


Figure 4. Test panel samples 23-34 (Table 1, no. 29 in duplicate) with FLR Cw*0701. Both sample and FLR derived from a region in exon 2. 100 bp ladder not shown. All tracks have a top (400bp) and bottom (200bp) marker. Note mobility of most alleles is shared and close to that of the FLR homoduplex. Cw*0401 (tracks 2 and 4) and Cw* 0102 (track 11) however, could be differentiated.

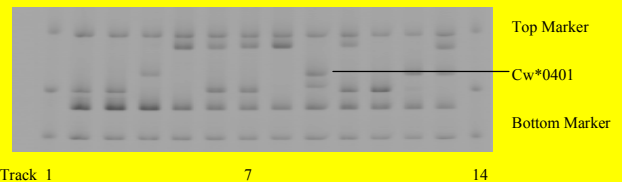


Figure 5. Test panel samples 1-11 (Table 1, no. 1 in duplicate) with FLR Cw* 0102. Both sample and FLR derived from a region in exon 2. 100bp ladder in tracks 1 and 14, all sample tracks have a top (400bp) and bottom (200bp) marker. Note the wide spread of mobility compared with Cw*0701 FLR (Figure 4). Cw*1402 displayed mobility close to the FLR homoduplex (not shown on this gel), however the mobility was unique.

Conclusion

On the basis of our evaluation and further experiments we conclude that RSCA using the Li-Cor 4200 requires significant improvements to both reproducibility and resolution before it can be adopted as a low-resolution HLA-C typing technique.

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