

Assaying sperm crossover hotspots in the Class II region of the MHC

Two hotspot clusters have been discovered, one around *HLA-DNA* and the second in and 3' to *HLA-DMB*. Each hotspot is assayed in sperm DNA by first digesting DNA with an appropriate restriction enzyme that cleaves outside the test interval, then PCR amplifying aliquots of digested DNA with two allele-specific primers in repulsion phase directed to SNP sites that are heterozygous in the semen donor to selectively amplify recombinant molecules. PCR products from this 1^o PCR are then digested with S1 nuclease and reamplified using repulsion phase allele-specific primers directed to two internal heterozygous SNP sites to increase the specificity of single molecule recombinant recovery. 2^o PCR reactions showing a recombinant are then re-amplified using two internal universal primers, though in several cases the 3^o PCR included an allele-specific primer to improve the specificity of crossover recovery. Crossover breakpoints in 3^o PCR products are then mapped by dotblot hybridisation with ASOs

Details of restriction enzymes, SNPs and their locations relative to the start of the 216 kb Class II region studied, plus allele-primers (ASPs) and universal primers together with PCR cycling conditions for each hotspot region are given below. Some allele-specific primers include a synthetic 5' extension indicated in [blue lower case](#); these extension were added to improve PCR efficiency.

HLA-DNA hotspot 1

Restriction enzyme: *Bgl*II

1^o PCR:

SNPs: DD17T/C (19120 bp) and F8G/A (25717 bp)

ASPs:

DD17FAC/Dr 5' *gacgtagtcgagctggtagg*CTTTATTCAAAAAGTACTGGC 3'
or DD17FGT/Dr 5' *gacgtagtcgagctggtagg*CTTTATTCAAAAAGTGCTGGT 3'
plus Driv 5' *gacgtagtcgagctggtagg* 3'

NB these allele-specific primers also incorporate SNP site DD16G/A.

together with F8RG 5' TCCCATGAGCCTGTCC 3'
or F8RA 5' TCCCATGAGCCTGTCT 3'

PCR cycling:

1 cycle of 96° 1 min
8 cycles of 96° 20 sec, 54° 45 sec, 63° 8 min
18 cycles of 96° 20 sec, 53° 45 sec, 63° 8 min

2^o PCR:

SNPs: DD18T/C (19193 bp) and F7A/G (25530 bp)

ASPs:

DD18FGT 5' *ccccATTCCCACATCTGAAAGGTT*
or DD18FGC 5' *ccccATTCCCACATCTGAAAGGTC*

NB these allele-specific primers incorporate the G allele of SNP DD24G/T.

together with F7R2A 5' GTGGGCCCCAGGAT 3'
or F7R2G 5' GTGGGCCCCAGGAC 3'

PCR cycling:

1 cycle of 96° 1 min
5 cycles of 96° 20 sec, 64° 8 min
7 cycles of 96° 20 sec, 63° 8 min
16 cycles of 96° 20 sec, 61° 30 sec, 63° 8 min

3^o PCR:

Primers:

R-5.0F 5' CCAGTCAGAGGGAAGATGGAGAAGG 3'

together with F5RA/Pu 5' *acgaccaactcgagacagcg*TAGAGCAAGAATGTGTGTT 3'
or F5RC/Pu 5' *acgaccaactcgagacagcg*TAGAGCAAGAATGTGTGTG 3'
plus Push 5' *acgaccaactcgagacagcg* 3'

PCR cycling:

1 cycle of 96° 1 min

6 cycles of 96° 20 sec, 59° 30 sec, 63° 8 min

6 cycles of 96° 20 sec, 58° 30 sec, 63° 8 min

12 cycles of 96° 20 sec, 56° 30 sec, 63° 8 min

HLA-DNA hotspot 2

Restriction enzyme: *SapI*

1^o PCR:

SNPs: DE24C/T (24287 bp) and A12C/G (32055 bp)

ASPs:

DE24FC 5' CTCCTCTCCCTCTGTTTC 3'

or DE24FT 5' CTCCTCTCCCTCTGTTT 3'

together with A12RC/Ad 5' gtctacgtagtcagctctGGTGACTACATTTTGTTTTAC 3'

or A12RG/Ad 5' gtctacgtagtcagctctGGTGACTACATTTTGTTTTAC 3'

plus Adap 5' gtctacgtagtcagctctgg 3'

PCR cycling:

1 cycle of 96° 1 min

7 cycles of 96° 20 sec, 57° 45 sec, 63° 8 min

7 cycles of 96° 20 sec, 56° 45 sec, 63° 8 min

11 cycles of 96° 20 sec, 55° 45 sec, 63° 8 min

2^o PCR:

SNPs: F1C/T (24416 bp) and A7C/G (31882 bp)

ASPs:

F1FC 5' CCTGTGGACTCAGGGCCC 3'

or F1FT 5' CCTGTGGACTCAGGGCCT 3'

together with A7RC 5' CTAGGGAGGAGGCAGCAG 3'

or A7RG 5' CTAGGGAGGAGGCAGCAC 3'

PCR cycling:

1 cycle of 96° 1 min

7 cycles of 96° 20 sec, 65° 8 min

11 cycles of 96° 20 sec, 64° 8 min

9 cycles of 96° 20 sec, 63° 8 min

3^o PCR:

Primers:

R0.5F 5' CACCAGATGCCATGGAGACC 3'

together with A6R+ 5' CCAGTGGACGGAAGTGCC 3'

or A6R- 5' CCAGTGGACGGAAGTGCG 3'

PCR cycling:

1 cycle of 96° 1 min

4 cycles of 96° 20 sec, 65° 8 min

4 cycles of 96° 20 sec, 64° 8 min

14 cycles of 96° 20 sec, 63° 8 min

HLA-DNA hotspot 3

Restriction enzyme: *SphI*

1^o PCR:

SNPs: A6+/- (31858 bp) and BC4C/T (38149 bp)

ASPs:

A6F+ 5' AGGTGGACTCCCCGGCAC 3'

or A6F- 5' AGGTGGACTCCCCGCACT 3'

together with

BC4RC 5' GAGGAAAGCTGGGGGTAG 3'

or BC4RT 5' GAGGAAAGCTGGGGGTAA 3'

PCR cycling:

1 cycle of 96° 1 min

5 cycles of 96° 20 sec, 60° 30 sec, 61° 6 min

10 cycles of 96° 20 sec, 59° 30 sec, 61° 6 min

11 cycles of 96° 20 sec, 58° 30 sec, 61° 6 min

2^o PCR:

SNPs: A7C/G (31882 bp) and B7T/G (37366 bp)

ASPs:

A7FC 5' CGTCCACTGGAAACGTT C 3'

or A7FG 5' CGTCCACTGGAAACGTT G 3'

together with

B7RT/Ad 5' gtctacgtagtcagctctgGAAATGGTCAAATTTTATGTA 3'

or B7RG/Ad 5' gtctacgtagtcagctctgGAAATGGTCAAATTTTATGTC 3'

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plus Adap 5' gtctacgtagtcagctctgg 3'

PCR cycling:

1 cycle of 96° 1 min

5 cycles of 96° 20 sec, 60° 30 sec, 61° 6 min

10 cycles of 96° 20 sec, 59° 30 sec, 61° 6 min

12 cycles of 96° 20 sec, 58° 30 sec, 61° 6 min

3^o PCR:

Primers:

R7.6F 5' CTTGAGACCTGGTCAGGTT C 3'

together with

R13.0R 5' ggggTAATAGCCATGTAGTGAAC 3'

PCR cycling:

1 cycle of 96° 1 min

17 cycles of 96° 20 sec, 57° 30 sec, 61° 6 min

HLA-DMB hotspots 1,2

NB these are simultaneously analysed in one test interval.

Restriction enzyme: *XmnI*

1^o PCR:

SNPs: JJ6T/C (95104 bp) and J7C/T (100629 bp)

ASPs:

JJ6FT2 5' GCTCTGGTGGTGTGGT 3'

or JJ6FC 5' CTGCTCTGGTGGTGTGGC 3'

together with

J7RC 5' GCCTAAGAGCAGAGGGAG 3'

or J7RT 5' GCCTAAGAGCAGAGGGAA 3'

PCR cycling:

1 cycle of 96° 1 min

23 cycles of 96° 20 sec, 65° 8 min

2^o PCR:

SNPs: JJ7A/C (95164 bp) and J6G/A (100470 bp)

ASPs:

JJ7FA1 5' ccccCTTGCTTTGAAATGAGGA 3'

or JJ7FC2 5' ccccCTTGCTTTGAAATGAGGC 3'

together with

J6RG 5' ATAACCCCTTCTGTCCCC 3'

or J6RA 5' ATAACCCCTTCTGTCCCT 3'

PCR cycling:

1 cycle of 96° 1 min

8 cycles of 96° 20 sec, 66° 8 min

18 cycles of 96° 20 sec, 65° 8 min

3^o PCR:

Primers:

RU71.1F 5' CGGCTGGCAGACCATTGATGC 3'

together with

RU76.0R 5' CTTTATTCCCTGCCCCAGACCAGC 3'

PCR cycling:

1 cycle of 96° 1 min

24 cycles of 96° 20 sec, 60° 30 sec, 63° 8 min