Next Generation Sequencing based genotyping of HLA-DRB3, -DRB4, -DRB5, -DQA1, and –DPA1: the algorithmic aspect

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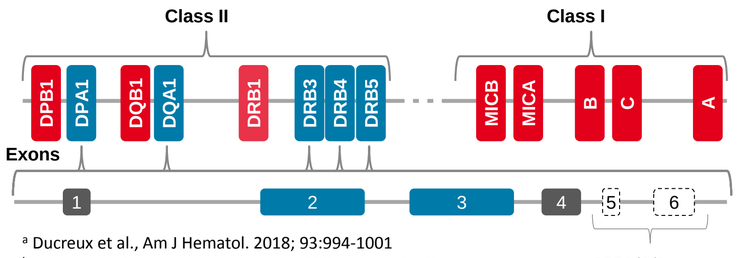
Introduction

The standard typing profile of our in-house developed high-throughput typing software neXtype included so far HLA genes A, B, C, E, DRB1, DQB1, DPB1, MICA, and MICB as well as genes for ABO, RhD, CCR5, and KIR. Given recent indications[a] that the class II genes DRB3/4/5, DQA1, and DPA1 could also play an important role in the outcome of unrelated hematopoeitic stem cell transplantation, we extended in October 2019 our typing profile accordingly. While for DQA1 and DPA1 the standard algorithm is used (as for the already established HLA loci), for DRB3/4/5 an adjustment was needed. Since the copy number (CN) for DRB3/4/5 is not fixed to two the previously established linkage disequilibrium (LD) with DRB1[b,c] is used in order to determine the actual CN for those loci.



[a] Ducreux et al., Am J Hematol. 2018; 93:994-1001
[b] Andersson et al., Immunology Today 1994, Vol.15 No.2
[c] Trowsdale, Immunogenetics 1995, 41:1-17

Methods

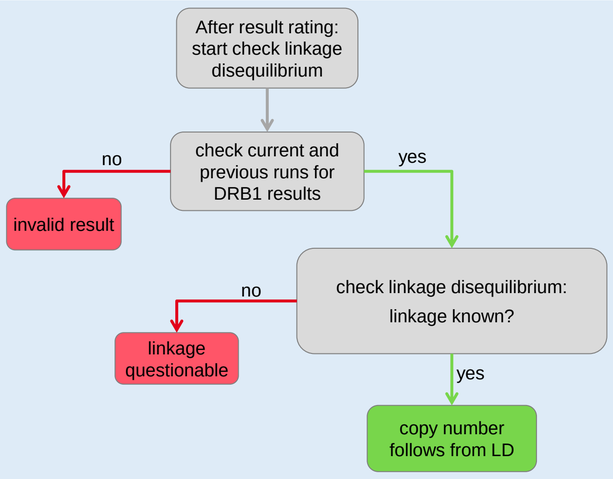
- Next Generation Sequencing on Illumina HiSeq/NovaSeq instruments with a short amplicon based approach. Making use of primer pairs for exons 2 and 3.
- Allele matching on exon level with sequences given in the database: IMGT/HLA:https://www.ebi.ac.uk/ipd/imgt/hla/.
- DQA1 and DPA1 typing results are readily provided by the existent workflow. They also serve as a positive control for the validitiy of the DRB3/4/5 result in case of absence.
- Technically the processing of DRB3/4/5 is implemented as if they were one locus using the standard algorithm up to the result rating.
- Based on previously established LD between DRB1 and DRB3/4/5 the assignment of CN of DRB3, DRB4 and DRB5 is done.

The Linkage Disequilibrium Algorithm

neXtype:

Primer recognition → Allele matching → Clean up → Exon combination → Result rating → Check linkage diseq. → User interaction → Final result

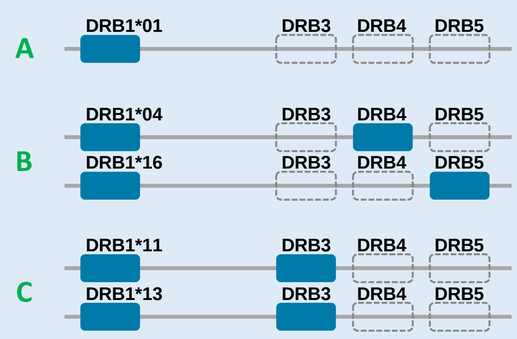
Example	DRB1	DRB3/4/5	DRB3/4/5 CN (based on typing)
A	01:01:01+01:02:01	Not present but DQA1/DPA1 positive control ok	0
B	04:04:01+16:01:01	DRB4*01:KDJV + DRB5*02:XA	1 + 1
C	11:11:01+13:01:01	DRB3*02:ERVA	1 or 2



Known Linkage[b,c]:

DRB1	DRB3/4/5
DRB1*01	-
DRB1*03	DRB3
DRB1*04	DRB4
DRB1*07	DRB4
DRB1*08	-
DRB1*09	DRB4
DRB1*10	-
DRB1*11	DRB3
DRB1*12	DRB3
DRB1*13	DRB3
DRB1*14	DRB3
DRB1*15	DRB5
DRB1*16	DRB5

Examples:



[b] Andersen et al., Immunology Today 1994, Vol.15 No.2
[c] Trowsdale, Immunogenetics 1995, 41:1-17

CN assignment (based on DRB1 – DRB3/4/5 linkage):

- default CN for DRB3/4/5 = 2 (in total)
- CN adjustment depending on linkage disequilibrium

→ A: DRB1*01 + DRB1*01 → DRB3* CN = 0, DRB4* CN = 0, DRB5* CN = 0
→ B: DRB1*04 + DRB1*16 → DRB3* CN = 0, DRB4* CN = 1, DRB5* CN = 1
→ C: DRB1*11 + DRB1*13 → DRB3* CN = 2, DRB4* CN = 0, DRB5* CN = 0

Final result (DRB1 and DRB3/4/5) expressed in genotype list (GL) string syntax:

Example	GL-String
A	DRB1*01:01:01 + DRB1*01:02:01 ^ DRB3*NNNN + DRB3*NNNN ^ DRB4*NNNN + DRB4*NNNN ^ DRB5*NNNN + DRB5*NNNN
B	DRB1*04:04:01 + DRB1*16:01:01 ^ DRB3*NNNN + DRB3*NNNN ^ DRB4*KDJV + DRB4*NNNN ^ DRB5*02:XA + DRB5*NNNN
C	DRB1*11:11:01 + DRB1*13:01:01 ^ DRB3*02:ERVA + DRB3*02:ERVA ^ DRB4*NNNN + DRB4*NNNN ^ DRB5*NNNN + DRB5*NNNN

The special code NNNN is used[d] for genes that were determined as absent.

[d] Bochtler et al., BMT (2013) 48, 1387-1388

Results

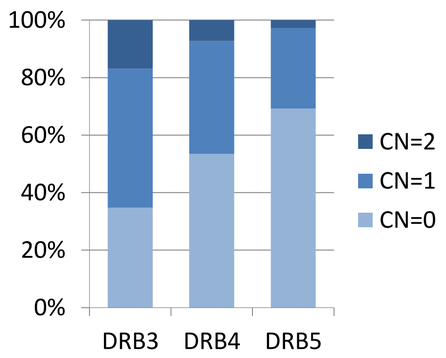
- We typed DRB3/4/5, DQA1, and DPA1 for over 1,1 million potential donors. The successful validation of the typing work-flow is presented in poster P214.
- Over 515,000 potential donors have self-assigned German ethnic background. Over 99.9% of these results are at high-resolution.
- Top five alleles within the German ethnic group for each locus, with respect to all observed alleles:

DRB3			DRB4			DRB5			DPA1			DQA1		
Allele	Count	Fraction	Allele	Count	Fraction	Allele	Count	Fraction	Allele	Count	Fraction	Allele	Count	Fraction
02:02:01G	221,330	52%	01:01:01G	239,333	86%	01:01:01G	141,288	82%	01:03:01G	855,452	83%	05:01:01G	272,406	26%
01:01:02G	154,696	37%	01:03:01:02N	27,194	10%	02:02:01G	24,733	14%	02:01:01G	87,401	8%	01:02:01G	211,465	20%
03:01:01G	44,111	10%	01:03:01N	9,484	3%	01:02:01G	7,050	4%	02:01:02G	45,769	4%	01:01:01G	146,769	14%
02:01:01G	2,286	1%	01:03:03	1,102	0%	01:05	77	0%	02:02:02G	23,228	2%	02:01:01G	127,536	12%
02:10	95	0%	01:02	396	0%	01:10N	29	0%	02:07:01G	9,987	1%	03:01:01G	113,759	11%

- We confirmed previously rarely observed haplotypic groups of DRB1 and DRB3/4/5: DRB1*01+DRB5[b] with a frequency of 0.11% and DRB1*08+DRB3[e] with a frequency of 0.02%.

[b] Andersson et al., Immunology Today 1994, Vol.15 No.2; [e] Chen et al., European Journal of Immunogenetics (1997) 24, 435-437

- CN distribution of DRB3/4/5 in the German ethnic group:



- Approx. 2% of these donors have CN = 0 in each DRB3/4/5 locus

Conclusion

We established an efficient high-throughput workflow for DRB3/4/5, DQA1, and DPA1 genotyping. The results are valuable contributions to the standard stem cell donor typing profile. Large numbers of typing results allows for confirmation of rare haplotypic groups between DRB1 and DRB3/4/5.

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