# A Next Generation Sequencing based genotyping strategy for MICA and MICB

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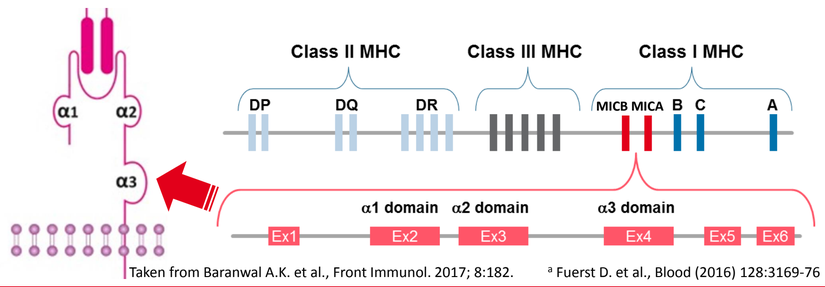
## Introduction

The standard typing profile of our in-house developed high-throughput typing software neXtype included up to now HLA genes A, B, C, E, DRB1, DQB1, and DPB1 as well as genes for ABO, RhD, CCR5, and KIR. Given recent indications[a] that the Major Histocompatibility Complex (MHC) class I chain-related molecules MICA play an important role in hematopoietic stem cell transplantations, we decided to extend our typing profile by including results for MICA and MICB. These genes are located on the chromosome 6 close to the HLA-B locus within the MHC class I region. The binding sites for natural killer cells ($\alpha$1- and $\alpha$2 domain) are encoded by exon 2 and 3, respectively. The $\alpha$3 domain and the transmembrane region are encoded by exon 4 and 5, respectively.




Taken from Baranwal A.K. et al., Front Immunol. 2017; 8:182. [a] Fuerst D. et al., Blood (2016) 128:3169-76

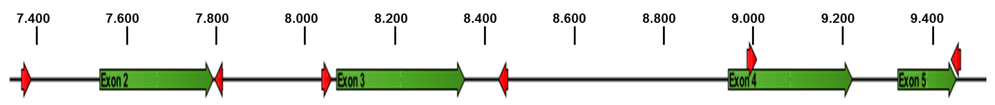
## Methods

- Next Generation Sequencing on Illumina MiSeq or HiSeq 2500 instruments.
- Short amplicon based approach.
- Making use of only one primer pair for exon 2 and 3. And two pairs for exons 4 and 5 together to establish phasing information.
- Assign each read to one of the already known exon allele sequences (EAG) given in the database (IMGT/HLA: https://www.ebi.ac.uk/ipd/imgt/hla/).
- To handle the vast number of unknown exon 5 sequences the exon 4 is in addition evaluated independently.
- Differentiation between MICA and MICB alleles by possible combinations of exons. All primers are exon specific only.

## The Algorithm

Primer recognition → Allele matching → Clean up → Exon combination → Result rating → User interaction → Final result

Primer position[b]:




[b] Example for MICA*001, [green arrow: exon; red arrow: primer]

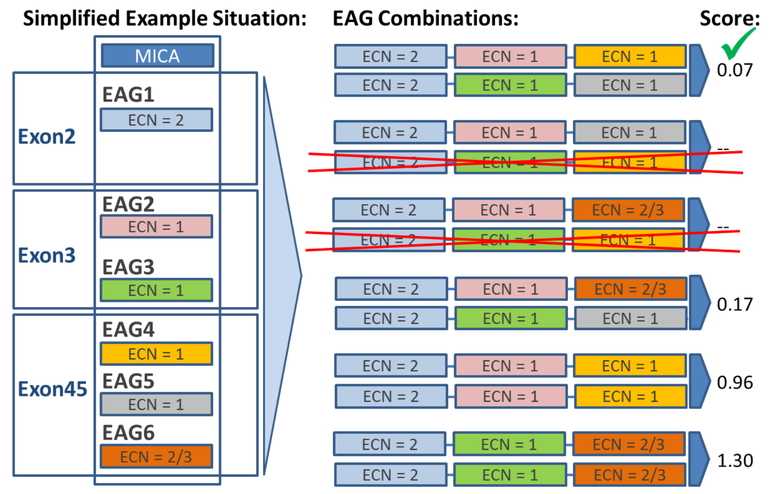
- Copy number assignment:

| Case 1 | | Case 2 | | Case 3 | |
|---|---|---|---|---|---|
| EAG | Copy Number | EAG | Copy Number | EAG | Copy Number |
| EAG1 | 2 | EAG1 | 1 | EAG1 | 1 |
| | | EAG2 | 1 | EAG2 | 1 |
| | | | | EAG3 | 2/3 |
| | | | | ... | |
| | | | | EAGn | 2/n |

- Scoring:
  To evaluate which of the EAG combinations is the most likely one, we take the minimum of the scoring values. The score is defined by:

$$Score = \frac{1}{\sum ECN_{calc}} \sum_{i=EAG1}^{EAGn} \left(ECN^{i}_{used} - ECN^{i}_{calc}\right)^2$$

- Combination of EAGs:
  Generation of possible combinations of EAG




- GL-String:
  The final result is expressed in the genotype list (GL) string syntax for MICA and MICB.

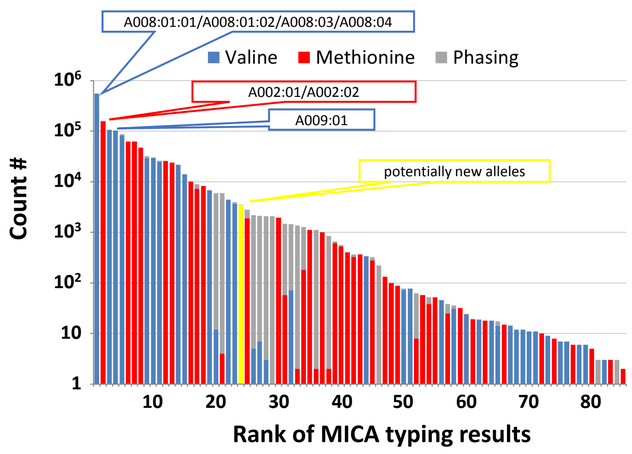
Example: **MICA*008:01:01/MICA*008:01:02/MICA*008:03/MICA*008:04 + MICA*002:01/MICA*002:02**

## First Results

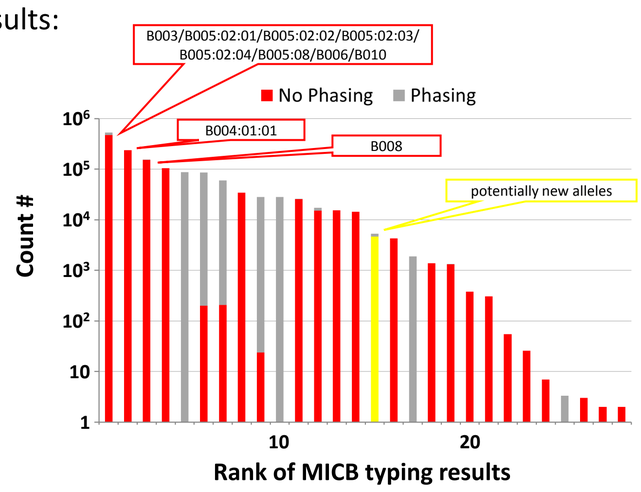
- We typed over 720.000 potential donors.
- In 96% (75%) of the MICA (MICB) samples, we achieved unambiguous results with respect to exon 2 and 3.
- In 0,4% (0,5%) of the samples potentially new alleles were detected for MICA (MICB).
- The remaining results were of lower resolution due to phasing ambiguities.
- The MICA-129 dimorphism can be resolved in all cases. The assignment leads to 71% for “MICA val” and 29% for “MICA met”.
- The three most frequent typing results are:

| Rank # | MICA | % | MICB | % |
|---|---|---|---|---|
| 1 | A008:01:01/A008:01:02/A008:03/A008:04 | 38% | B003/B005:02:01/B005:02:02/B005:02:03/B005:02:04/B005:08/B006/B010 | 37% |
| 2 | A002:01/A002:02 | 11% | B004:01:01 | 16% |
| 3 | A009:01 | 7% | B008 | 11% |
| ... | ... | ... | ... | ... |

- Distribution of MICA and MICB typing results:




MICA distribution with MICA-129 categorization in case of no phasing




See also poster P173 in Poster Group No. 6

## Validation & Conclusion

### Validation:

For validation, sets of 118 MICA and 94 MICB samples were processed and compared to pre-typed results. For 3 MICA and 1 MICB samples no result could be assigned due to a PCR failure. All other results were in full concordance with the pre-typings.

### Conclusion:

We established an efficient high-throughput workflow for MICA and MICB genotyping. The results are valuable contributions to the standard stem cell donor typing profile, already accessible with the DKMS Donor Navigator® Software[c].

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