Robust Amplification Strategy for Full-Length Characterization of HLA-DRB1 Alleles

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Introduction

The full potential of NGS-based HLA genotyping is currently affected by the lack of sequence information outside the ARD for most named alleles. As of January 2019, full-length HLA-DRB1 alleles are still strongly underrepresented in the IPD-IMGT/HLA database. In particular, only 15% of the CWD alleles are completely covered. While for other HLA genes all alleles are of similar length, the size of HLA-DRB1 alleles varies between 11 and 16 kb, mainly due to the length of intron 1. Large allele size differences present a particular challenge for long-range PCR because amplification of the longer allele is commonly suppressed in the presence of a shorter allele.

Grouping and Long-Range PCR

To avoid allele bias in full length HLA-DRB1 sequencing, we aimed for separating alleles with big length differences in our long-range PCR approach. Therefore, we created group-specific amplification primers by sorting all known alleles into one of three groups based on their length (S/M/L). Approximately two thirds of the samples contain alleles of different length and end up in two different groups. Consequently, they are amplified in two separate PCR reactions and create artificially homozygous results which will be joined with their partner allele after analysis.

Long-Range PCR Group	Alleles	Allele Length	Primer
S	DRB1*01/15/16	11 – 12 kB	F2 + R2/R3
M	DRB1*03/08/10/11/12/13/14	13 – 14 kB	F1 + R2/R3
L	DRB1*04/07/09	15 – 16 kB	F1 + R1

Forward Primer:
F1: CAGATGCTGATTCGTTCTCCAACACT
F2: TTCTCCAACACGAGATTACCCAACC

Reverse Primer:
R1: AGCACAAAACTTGGAAGATGAGGGG
R2: GAGCACAAAAGTTGAAGATGAGGCG
R3: GAGCACAAAAGTTGAAGATGAGGCAC

Table 1: Grouping of known HLA-DRB1 alleles based on their length. Pretyped samples are assigned to their respective group(s) using first field information.

10 – 400 ng	Genomic DNA
1x	Polymerase Reaction Buffer
400 µM each	dNTPs
200 nM each	Primer (S, M or L)
0,25 µl	Advantage Genomic LA Polymerase (TaKaRa)

Step	Time	Temperature	Cycles
Initial Denaturation	1 min	94°C	1
Denaturation	10 s	98°C	35
Annealing / Elongation	12 min	68°C	
Final Elongation	10 min	72°C	1

Table 2: Long -range PCR conditions for a 25 µl PCR reaction (S, M or L)

Workflow

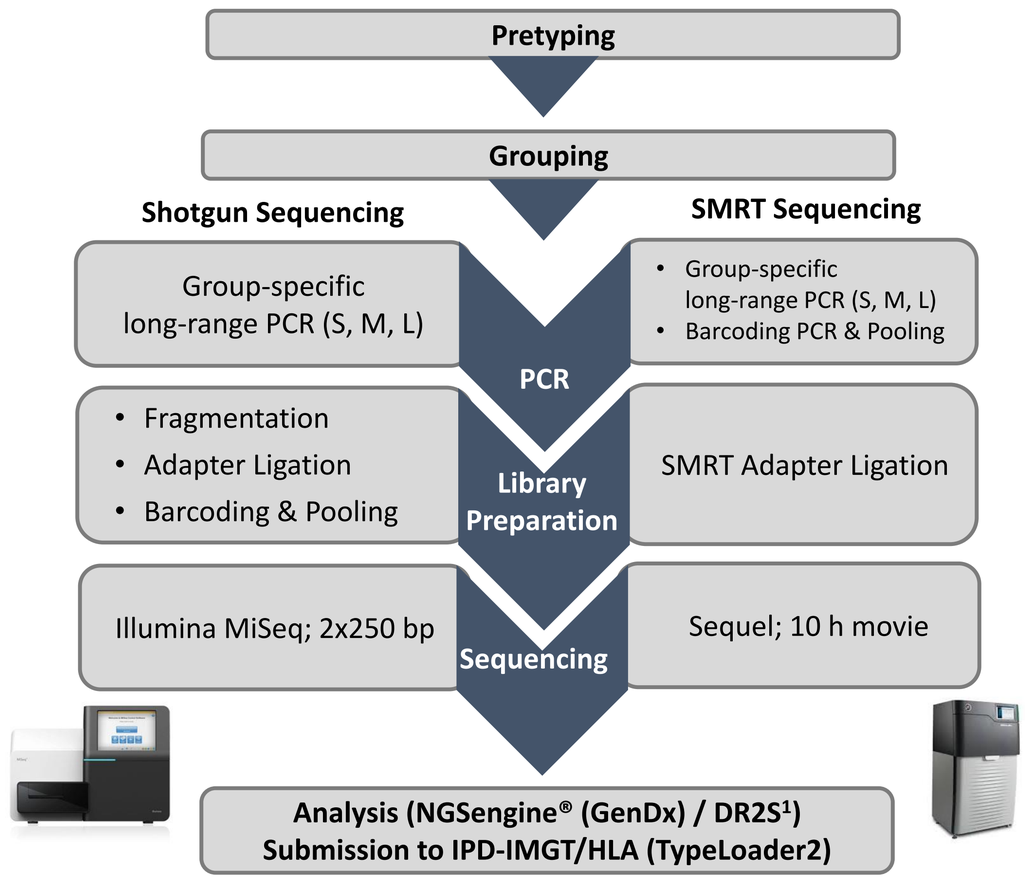

Figure 1: Workflow for the characterization of novel HLA-DRB1 alleles
Novel HLA-DRB1 alleles are identified during our high-throughput workflow. This pretyping information is used for selecting the appropriate long-range PCR group and appropriate primers (Table 1). If pretyping information is not available, all three PCR reactions may be performed for each sample, knowing that one or two of them will not generate a result.
The chosen PCR reaction(s) are performed twice to generate both short-read shotgun sequencing and long-read SMRT sequencing data. These are combined during analysis to obtain optimal phasing information and error correction. Finally, the novel allele is submitted to IPD-IMGT/HLA using TypeLoader2[2].

Results and Outlook

The workflow was established using 92 buccal-swab samples with available pretyping information. Both alleles were amplified by group-specific long-range PCR(s) and sequenced on both MiSeq and Sequel. The PCR success rate was 92% with PCR failures distributed over all three groups. After sequencing on both platforms, 85% of HLA-DRB1 alleles could be analyzed, all of them with the correct typing result. Overall, complete and correct full-length HLA-DRB1 results for both alleles could be obtained for 66% of the samples.

After successful evaluation, this workflow will now be used for full-length characterization of novel HLA-DRB1 alleles to complement our well-established workflow for HLA-A/-B/-C/-DQB1/-DPB1[1]. Furthermore, we plan to generate full-length sequences for common, but only partially described, HLA-DRB1 alleles. All sequences will be submitted to IPD-IMGT/HLA.

References

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