High-throughput sequencing-based HLA-typing

Introduction

Many countries have set-up donor registry HLA-typing programs to facilitate unrelated hematopoietic stem cell transplantation. The National Marrow Donor Program requires that each newly recruited donor is typed at the intermediate resolution level for HLA-A, -B and -DRB1. For the actual transplantation, typing of additional loci HLA-C and HLA-DQB1 is also required.

We initiated development of High-Throughput Sequencing-based Typing (HT-SBT) methods for identification of HLA-A, -B, -C, -DQB1 and -DRB1 alleles at the intermediate resolution level. The HT-SBT method aims to obtain the highest level of intermediate resolution possible in one single round without the need of cherry-picking.

Materials and Methods

HT-SBT method is dedicated for intermediate resolution HLA typing of large sample panels using 96-well primer plates that enable easy automation and handling. After amplification of the gene region that codes for the peptide binding groove, a specified set of sequencing reactions is performed which separate the allele groups responsible for most of the ambiguities.

These specified sequencing primer sets are composed of generic core sequencing primers and group-specific sequencing primers. For HT-SBT, 8 sequencing reactions are carried out for HLA-A, -B, and -C, 3 for HLA-DQB1 and 4 sequencing reactions for -DRB1.

Sequencing reactions are performed according to the SBTexcellerator® protocol and are subsequently run on an ABI3730xl Genetic Analyzer. The data was analyzed with SBTengine® software using a specialized feature named Batch Analysis for automated typing assignment to reduce hands-on time.

The resolving power of HT-SBT is determined based on allele frequency data available for different populations, using IMGT/HLA database version 3.9.0.

Results

The SBT-HT method developed for the different diagnostic loci shows strong specific amplification products for the vast majority of samples (n=96) examined (Figure 1).

The calculated resolving power of HT-SBT shows:

- Enhanced resolution obtained when applying group-specific sequencing (Table 1 and 2).
- The percentage of unambiguous results depends on the population and HLA locus analyzed.
- The HT-SBT is method robust, reliable and stable for different origins of DNA, including fragmented DNA like buccal swabs.

Conclusion

Here we demonstrate a new HT-SBT method that accelerates intermediate resolution by unique identification of the peptide binding groove of HLA-A, -B, -C, -DQB1, and -DRB1 loci in a robust and stable manner for large sample panels.

Enhanced ambiguity resolution is accomplished by an efficient combination of generic and group-specific sequencing primers. The combination of easy automation, enhanced sequencing strategy without need of cherry-picking and the batch analysis tool in SBTengine® allow for a high-throughput method.

The HT-SBT method will be further developed and validated, and presented to laboratories interested in performing high-throughput tissue typing.

Table 1. HLA typing resolution NOT using HT-SBT method.

<table>
<thead>
<tr>
<th></th>
<th>European_American</th>
<th>African_American</th>
<th>Hispanic</th>
<th>Asian</th>
<th>Chinese</th>
<th>Japanese</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-A</td>
<td>76%</td>
<td>75%</td>
<td>75%</td>
<td>77%</td>
<td>73%</td>
<td>67%</td>
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<tr>
<td>HLA-B</td>
<td>71%</td>
<td>73%</td>
<td>73%</td>
<td>73%</td>
<td>72%</td>
<td>68%</td>
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<tr>
<td>HLA-C</td>
<td>44%</td>
<td>44%</td>
<td>44%</td>
<td>44%</td>
<td>44%</td>
<td>44%</td>
</tr>
<tr>
<td>HLA-DQB1</td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
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<td>5%</td>
</tr>
<tr>
<td>HLA-DRB1</td>
<td>15%</td>
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<td>15%</td>
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</tbody>
</table>

Table 2. HLA typing resolution using HT-SBT method.

<table>
<thead>
<tr>
<th></th>
<th>European_American</th>
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<th>Hispanic</th>
<th>Asian</th>
<th>Chinese</th>
<th>Japanese</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-A</td>
<td>76%</td>
<td>75%</td>
<td>75%</td>
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<td>75%</td>
<td>75%</td>
</tr>
<tr>
<td>HLA-B</td>
<td>70%</td>
<td>70%</td>
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<td>70%</td>
</tr>
<tr>
<td>HLA-C</td>
<td>44%</td>
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<td>44%</td>
</tr>
<tr>
<td>HLA-DQB1</td>
<td>5%</td>
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<td>5%</td>
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<td>5%</td>
</tr>
<tr>
<td>HLA-DRB1</td>
<td>15%</td>
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</table>

Key Features

- Intermediate resolution HLA typing of large sample panels
- Enhanced resolution using dedicated group-specific sequencing primers
- One single round of sequencing without the need of cherry-picking
- 96-well skirted plate formatting for easy automation
- Pre-loaded wells with lyophilized primers containing gel loading dye for visualization and easy gel handling
- Batch analysis tool in SBTengine® for efficient HLA typing of large sample panels

Legend Table 1 & 2:
- % of unambiguous samples
- Source: http://bioinformatics.nmdp.org/HLA/Haplotype_Frequencies/index.html; National Marrow Donor Program (NMDP)
- Source: Dr. Xiangjun Liu, BFR, Beijing, China; China Marrow Donor Program (CMDP)
- Source: http://www.bmdc.jrc.or.jp/stat.html; National Marrow Donor Program (NMDP)