SBTengine®, The Ultimate Solution For High Resolution HLA Typing

Introduction

Sequencing based typing (SBT) has become the gold standard in HLA-typing. In recent years various strategies have been developed. However the most time consuming and error prone part of SBT is still data-analysis including reporting and archiving of the generated data. In addition, the ever vast increasing number of HLA alleles, increase the number of ambiguous results which require a special approach to resolve. In the most recent IMGT/HLA database release the total number has succeeded even 6000 different HLA-alleles.

Results

We have improved our software package SBTengine® significantly; e.g. allowing now a reduced number of crucial positions to check manually, as the software is using a quality scoring per nucleotide. Moreover also the CWD list1 and NMDP allele frequency2 list are exploited elegantly such that the attention of the reviewer is directed to most important crucial positions (Figure 2). SBTengine® combines all sequences of a single sample, either homozygous or heterozygous to a conclusive allele assignment. The software guides the user easily through the typing process. In case of an ambiguity, the DART system of SBTengine® suggests a consecutive action, either to sequence an additional region or to apply a Group Specific Sequencing Primer to separate the HLA alleles (Figure 1). Furthermore, almost all known null-alleles are excluded or confirmed automatically. SBTengine® is an open software package and accepts any reagents used.

Reducing number of crucial positions that have to be checked manually

1. When the analysis results in multiple correct matches, indicating genotype or allele ambiguities, SBTengine® will suggest the resolving strategy, hence use GSSP Bg808 and use also the selective nucleotide.
2. The homozygous sequence is shown, including the automatic recognition of the Bg808 GSSP.
3. Usage of the GSSP can easily be toggled on/off to see the effect on the final analysis result.
4. Usage of the selective nucleotide of the GSSP in the analyses can easily be toggled on/off to review the effect on the final analysis result.
5. The allele frequency table that has been chosen.
6. The use of the Common and Well Documented (CWD) allele list will drastically reduce the number of crucial positions that have to be checked, as only the CDW alleles are taken into account.
7. Homozygous sequencing results derived from a Group Specific Sequencing Primer (GSSP). The percentage indicates the presence of the selected allele versus the other allele. A high number (>80%) indicates a good separation.
8. If required the selective nucleotide of the GSSP maybe used for the final data analyses. If this is the case the used nucleotide is shown i.e. position 355 C.
9. Heterozygous sequencing results derived from a Core sequencing primer, i.e. exon 3-forward
10. The relative presence of both alleles is shown. In daily practice a ratio between 30/70 or 70/30 is considered as a trustful amplification. The number of heterozygous positions in this region is shown as well.

Use of CWD list and quality score

1. When the analysis results in multiple correct matches, indicating genotype or allele ambiguities, SBTengine® will suggest the resolving strategy, hence use GSSP Bg808 and use also the selective nucleotide.
2. The homozygous sequence is shown, including the automatic recognition of the Bg808 GSSP.
3. Usage of the GSSP can easily be toggled on/off to see the effect on the final analysis result.
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Conclusion

We have implemented new and unique features of the latest version of SBTengine®. The versatility of SBTengine® will enable laboratories to easily implement SBT Typing into their daily routine laboratory activities and to obtain high resolution and accurate HLA typing in a straightforward manner. End-users may use within SBTengine any commercial SBT reagent line, home brew reagents or even a combination of multiple strategies.