GENDX
personalising diagnostics

NGSengine®: the ultimate tool for NGS HLA-typing

Aim

Currently applied technologies for HLA typing often show ambiguous results. For SBT by Sanger dideoxysequencing strategy, these are caused by sequencing PCR fragments with a mixture of two alleles. Additional reactions are required to resolve these ambiguities. This can be overcome with Next Generation Sequencing (NGS) technology. One of the unique features of NGS is that each individual read originates from a single molecule. Data obtained by NGS should be able to identify the two alleles separately. This allows for the development of an HLA genotyping system that yields no ambiguities.
In addition, NGS technology is developing fast and has come to a point where it can be applied routinely. We developed NGSengine®, a platform independent software package to perform HLA typing based on NGS data.

Methods

NGSengine® is an integrated HLA typing approach which includes determining the reference, aligning reads, phasing and typing into one method. Since the HLA genes are highly polymorphic, each of these steps contains HLA-specific features. Sequencing data generated on the instrument platforms Roche 454, Ion Torrent PGM, and Illumina MiSeq were kindly provided by Dimitri Monos' lab - Children's Hospital of Philadelphia and used to evaluate NGSengine®.

Results

All data available from different NGS platforms could be analyzed by NGSengine®.
In almost all samples, the expected HLA allele assignments were obtained. In some samples, clearly new alleles have been identified. In addition, intron data was obtained that was not available at the IMGT/HLA database. If whole gene amplification is applied (e.g. NGS-go™ reagents, GenDx), in many examples, the phasing between the alleles could be established throughout the whole gene, resulting in gene-wide unambiguously identified allele sequences.

Figure 1. Sample and result overview.

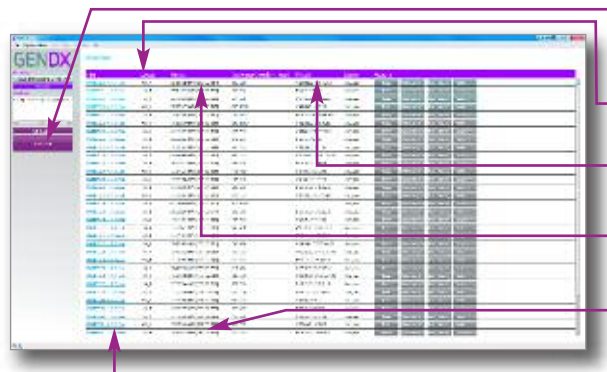

Figure 2. Alignment view.

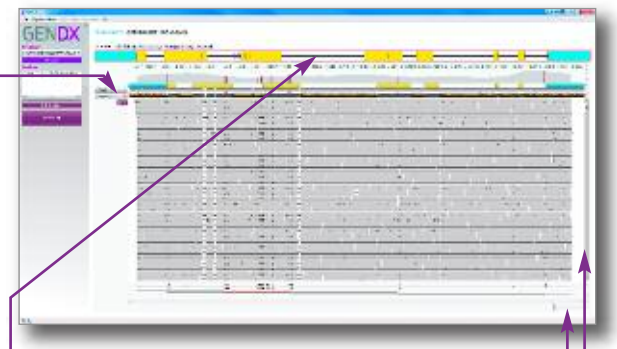

Figure 3. Alignment view zoom in.

- One button analysis
- Automatic gene identification
- Typing of heterozygous samples in one pass
- High percentage of analyzed reads
- Clear quality statistics
- Platform independent. Optimized for data from Roche 454, Ion Torrent, MiSeq
- Genotype references
- Whole gene representation
- Zoom in / Zoom out
- Read Scrolling
- Rejected read evaluation
- Well accessible overview of reads
- Phasing visualization

Key features of NGS engine® are shown in Figures 1 to 3.

Conclusion

NGSengine®:
- performs reliable unambiguous HLA allele assignment based on NGS data
- enables HLA-typing of NGS data in a single procedure
- can be applied on data from different NGS platforms
- determines gene-wide allele sequences
- identifies new alleles
- determines parts of allele sequences not yet present in the IMGT/HLA database
- is easy to use

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