GENDX
personalising diagnostics

NGS Early Access Program: Findings in a multicentre evaluation

Introduction

NGS technology is developing fast and has come to a point where it can be applied routinely. Many HLA typing laboratories are considering introducing NGS technologies in their diagnostics routines.
To facilitate HLA typing by NGS, we developed NGS-go®, a generic HLA gene amplification strategy, and NGSengine®, a software package to perform HLA typing based on NGS data.

An NGS Early Access Program (EAP) was set up to determine if our NGS solutions fulfill the needs and requirements of users within the HLA field. This EAP gave laboratories the chance to evaluate both NGS-go® and NGSengine® with their NGS instrument of choice, and even more important, it gave laboratories influence on the design of the software package.

Over 20 laboratories from various geographical regions participated in the EAP (Figure 1). There was a wide variety in the levels of NGS experiences amongst the participating labs. Many of the participating labs are in the early days of HLA testing with NGS and have not yet established a workflow.
All labs were given access to NGS-go® reagents and NGSengine® software and were stimulated to provide feedback on the performance. Based on their feedback, NGSengine® was adjusted and significantly improved, resulting in monthly updates of NGSengine®.

Results

Almost more then 30% of the labs had access to more than one NGS platform (Figure 2). As expected, the three most abundant platforms were Illumina MiSeq (40%), IonTorrent PGM (25%) and Roche 454 (25%) (Figure 3).

Sequencing data was generated on Illumina MiSeq and HiSeq, Roche 454, Ion Torrent PGM and PacBio RS sequencers with several different amplification strategies.

All labs used their own favorite workflow for library preparation. Sequencing data files contained a wide variety of read lengths, and number of reads. Some of the commercially available enzymatic fragmentation kits have a sequence bias, resulting in lower coverage in the exon 2/3 region (Figure 4).

All data available from different NGS platforms could be analyzed by NGSengine®. For the majority of the samples, the expected HLA genotype assignments were obtained. In some samples, clearly new alleles were identified. In addition, intron data was obtained that was not available yet at the IMGT/HLA database. If NGS-go® reagents were used to amplify the whole genes, in several examples the phasing between the alleles could be established throughout the whole gene, resulting in gene-wide unambiguously identified allele sequences.

Figure 1. Locations of EAP participants

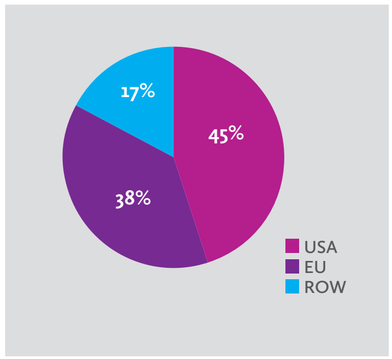


Figure 2. Number of different NGS platforms available

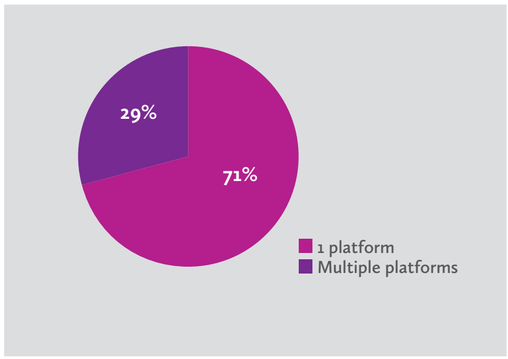


Figure 3. NGS platforms used for EAP

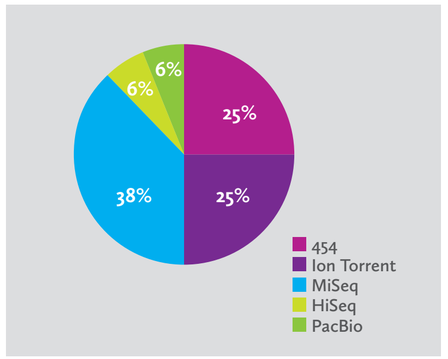
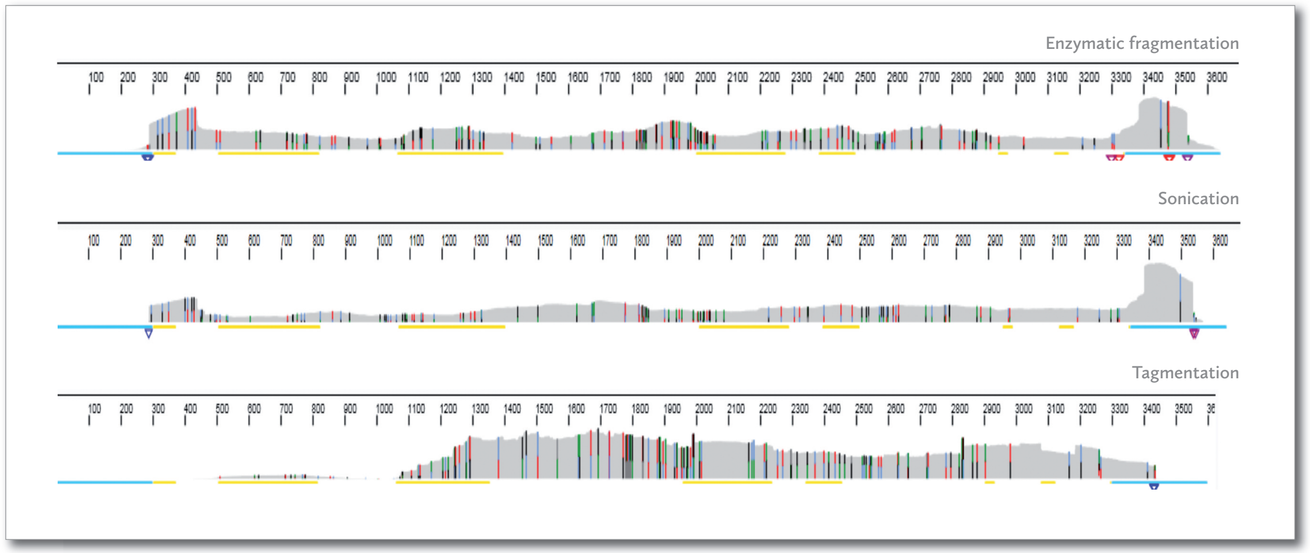


Figure 4. Comparison between fragmentation technologies



Key Features NGSengine®

- High percentage of analysed reads
- Automatic gene identification
- Clear quality statistics
- Whole gene representation
- One button analysis

Key Features NGS-go®

- Single PCR reation per locus
- 9 HLA loci available
- Complete exon readability
- Class I entire gene coverage
- Class II coverage off relevant

Conclusions

Based on the data provided by the different EAP participants, the following conclusions could be drawn:

- Reliable typing results could be obtained
  - with all sequencing platforms
  - with different amplification strategies
  - with different workflows for library preparation
  - with different read lengths
- Read lengths of 150 nt or more, result in reliable phasing
- A uniform depth of coverage of 100 can result in reliable typing results

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