

GENDX

# EXPERIENCE NGS HLA TYPING



personalizing diagnostics

**NGSgo<sup>®</sup> reagents**

**NGSEngine<sup>®</sup> software**

Platform independent

HLA-A, B, C, DRB1, DQB1, DPB1, DQA1, DPA1, DRB3/4/5, G

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# NGS-Based HLA Typing by the HLA Sequencing Experts

With sequencing technology developing rapidly, we continue to offer more comprehensible and accessible high-resolution Sequencing-Based HLA Typing solutions and strive to develop platform-independent reagents and software. This allows you to choose your preferred instrument relying on our robust workflows and highly evolved software.

## Start Next-Generation Sequencing-Based HLA Typing today

We have been successfully solving HLA typing cases for years, applying our extensive knowledge of the complex HLA system and its ever growing database of alleles. With an efficient lab workflow and one-button data analysis, Next-Generation Sequencing-Based HLA Typing brings new opportunities in sample throughput, ambiguity resolving power and workflow efficiency.



## NGSgo<sup>®</sup> & NGSengine<sup>®</sup>

- Fast workflow
- Multiplex and single loci
- 12 loci: HLA-A, B, C, DRB1, DQB1, DPB1, DPA1, DQA1, DRB3/4/5, G
- MICA, MICB, and KIR
- Up to 384 samples per single run
- Overview of data quality
- Easy new allele identification

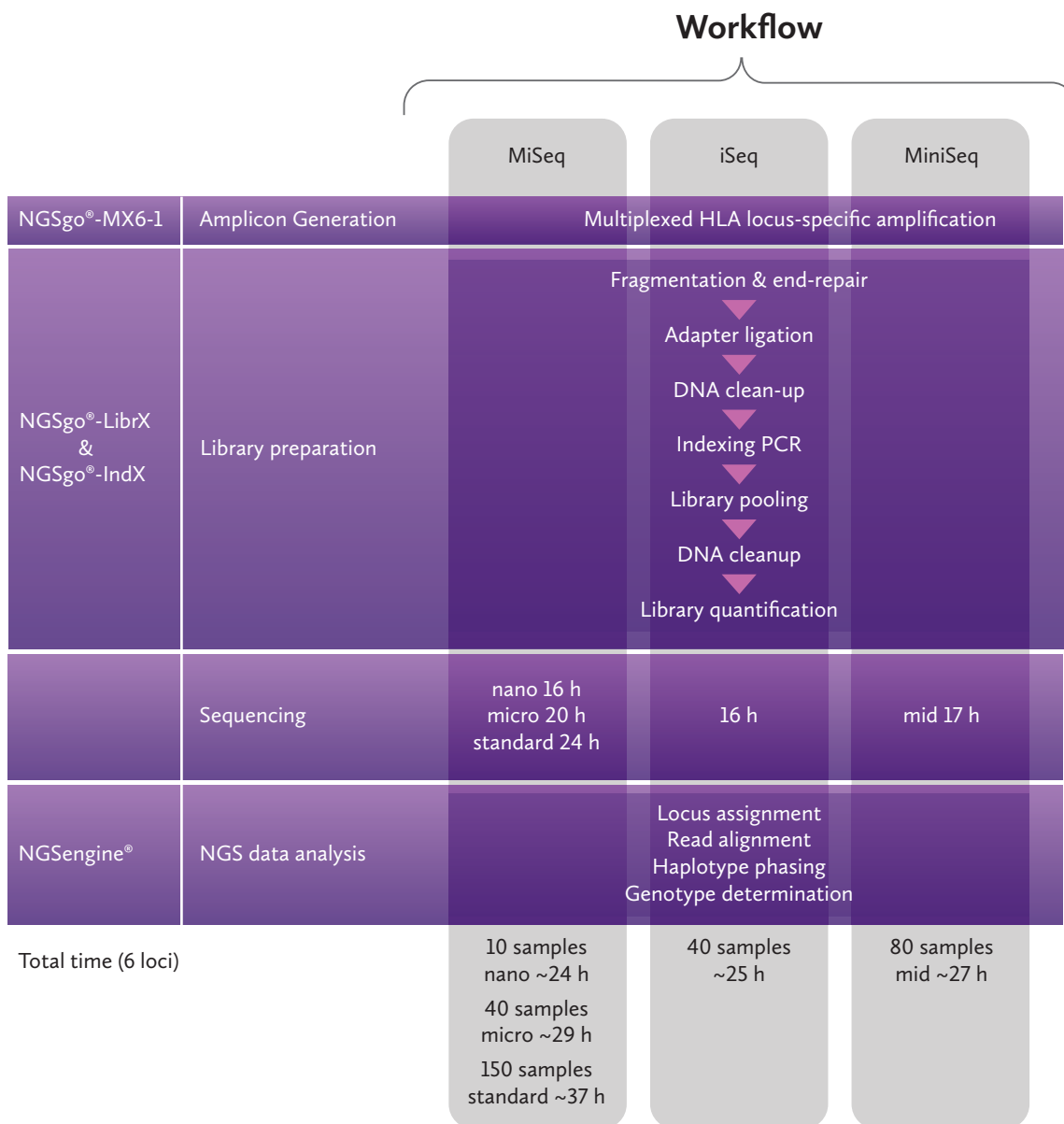
## Compatible systems

ILLUMINA	ION TORRENT	PACBIO	OXFORD NANOPORE
MiSeq*	Ion PGM*	RSII	MinION
HiSeq	Ion S5		
MiniSeq	Ion Proton		
NextSeq			
iSeq			

\* NGSgo and NGSengine are compatible with these instruments, full validation has been completed by GenDx on Illumina MiSeq, Ion Torrent PGM and PacBio RSII

# Easy multiplex amplification of six loci (HLA-A, B, C, DRB1, DQB1, and DPB1)

Workflow for Illumina MiSeq, iSeq, MiniSeq, HiSeq, and NextSeq



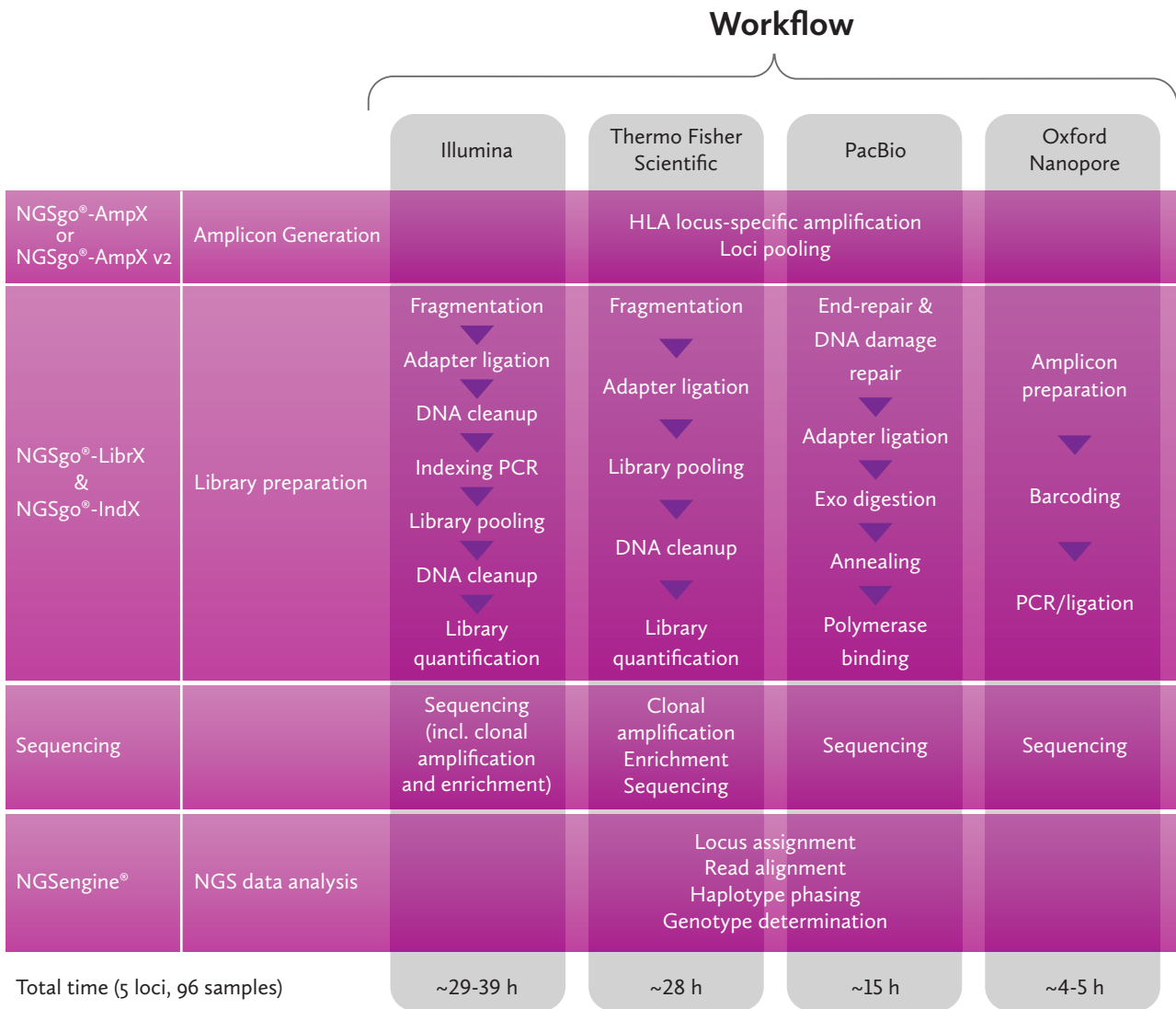
## One-Day-Workflow

HLA typing by NGS in just 24 hours!

# Single-Locus Amplifications

Combine single loci and multiplex for full flexibility

## Workflow for 4 different sequencing platforms



Optimized Workflows  
For Optimal Results

# Intelligent Workflow Design

## Robust amplification primers with proven reliability

Reliable HLA typing starts with a robust amplification strategy, and ours is based on more than a decade experience. Moreover, our amplification strategy has been tested and validated on > 100.000 samples.

## Fast workflow for multiplex and singleplex

Whether you prefer your amplification multiplexed, single locus, or a combination of both, we offer a fast workflow for any HLA locus combination. Amplicons of a single DNA sample can be pooled prior to indexing, providing full flexibility while saving time.

## Minimal number of required wash and cleanup steps

Optimal results demand several cleanup steps. In order to simplify the workflow and increase cost and time effectiveness, we have optimized the number of wash and cleanup steps without compromising quality.

## Easy enzymatic fragmentation, no additional equipment

The GenDx workflow uses enzymatic fragmentation, as this is most reliable and requires no additional equipment. The enzymatic fragmentation and fragment end-preparation required for adapter ligation are combined in one easy step, saving you time and reagents.

## Easy indexing with NGSgo-IndX plates

NGSgo-IndX for Illumina is a ready-to-use 96-well plate for indexing. Each well contains a unique dual index combination, making your workflow easier, faster, and more reliable.



# NGS Data Analysis in 4 Steps

After sequencing, the NGS instrument will produce fasta or fastq files, which can be imported and analyzed in NGSengine.



## 1 Locus assignment

Which loci are present in the sample?

Through alignment of the reads to the HLA sequencing reference database (IMGT/HLA), NGSengine is able to determine which loci are represented in a sample. This enables loci pooling in the workflow, saving time and reagents.



## 2 Alignment

Where do all the reads align?

Which read matches where with the reference database? Each read is aligned, base-by-base, to the HLA sequencing reference database (IMGT/HLA).



## 3 Haplotype phasing

Which reads are part of the same allele?

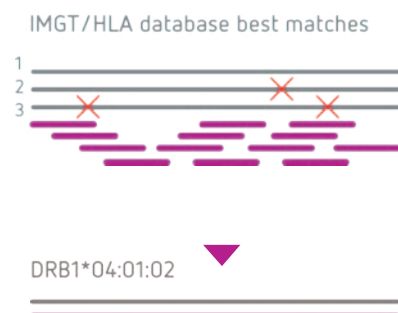
As each read can only be generated by allele 1 or allele 2 of the sample, heterozygous positions of both alleles can be used to determine which read belongs to which allele. Overlapping reads at heterozygous positions enable phasing and assignment of the reads to either allele 1 or allele 2.



## 4 Genotype determination

Which IMGT/HLA genotype matches the data?

The phased sequences are compared to the database to find the best matching genotype. Possible mismatches between the sequences and the best matching genotype are clearly indicated to allow efficient evaluation.



# Accuracy Determines Success

In the field of HLA typing a single wrong base call can affect the outcome of transplantation. This demands accurate base calling and careful consideration before calling a result.

NGSEngine makes large data sets comprehensible and easy to evaluate by providing an intuitive overview and extensive options to evaluate data statistics. Best matching genotypes are presented clearly and you can easily switch the typing resolution to view the number of fields of your choice.

NGSEngine is incredibly fast and runs on a regular laptop or desktop PC.

The screenshot displays the NGSEngine software interface. The top window shows the 'Overview' for 'Sample 2: HLA-A'. It features a sequence alignment viewer with 'gDNA | cDNA | Codons' and a detailed view of the HLA-A gene structure. Below this, there are data folders and analysis controls.

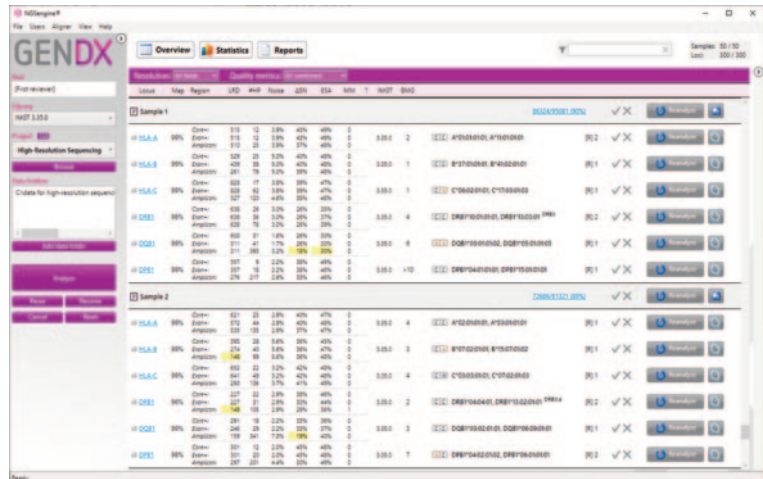
The bottom window shows a 'Statistics' view with a table of results for three samples. The table includes columns for Resolution, Quality metrics, Name, and various fields. The results are summarized as follows:

Sample	Resolution	Quality metrics	Name	Fields	Fields	Fields	Fields	
Sample 1	HLA-A	15474/15559 (99%)	136 [1-151]	1405 [310-1703]	2	[C][X] A*01010101, A*11010101	[E] 0 [H] 0	[R] 2
	HLA-B	11762/11824 (99%)	134 [1-151]	940 [261-1397]	1	[C][X] B*37010101, B*41020101	[E] 0 [H] 0	[R] 1
	HLA-C	14686/14764 (99%)	138 [1-151]	1268 [327-1605]	1	[C][X] C*06020101, C*17030103	[E] 0 [H] 0	[R] 1
	DRB1	16759/17011 (98%)	138 [1-151]	878 [830-2447]	4	[C][X] DRB1*10010101, DRB1*13030101 DRB3	[E] 0 [H] 0	[R] 2
	DRB1	14765/14856 (99%)	139 [1-151]	1106 [311-1395]	6	[C][X] DRB1*03010102, DRB1*05010105	[E] 0 [H] 0	[R] 1
Sample 2	HLA-A	12976/13062 (98%)	130 [0-151]	1054 [535-1352]	4	[C][X] A*02010101, A*03010101	[E] 0 [H] 0	[R] 1
	HLA-B	7434/7490 (99%)	126 [1-151]	558 [148-700]	3	[C][X] B*07020101, B*15070102	[E] 0 [H] 0	[R] 1
	HLA-C	12211/12299 (99%)	128 [1-151]	939 [293-1148]	4	[C][X] C*03030101, C*07020103	[E] 0 [H] 0	[R] 1
	DRB1	12414/12894 (96%)	133 [1-151]	830 [148-1013]	2	[C][X] DRB1*040401, DRB1*13020101 DRB3A	[E] 0 [H] 1	[R] 2
	DRB1	12673/12761 (99%)	132 [1-151]	939 [159-1162]	3	[C][X] DRB1*03020101, DRB1*06090101	[E] 0 [H] 0	[R] 1
Sample 3	HLA-A	15602/15780 (98%)	138 [1-151]	1338 [321-1643]	2	[C][X] A*24020101, A*68010202	[E] 0 [H] 0	[R] 1
	HLA-B	8906/9013 (98%)	135 [1-151]	659 [253-897]	4	[C][X] B*27050201, B*55010101	[E] 0 [H] 0	[R] 1
	HLA-C	14252/14415 (98%)	138 [1-151]	1123 [397-1469]	2	[C][X] C*02020101, C*03030101	[E] 0 [H] 0	[R] 1
	DRB1	10551/10953 (96%)	139 [1-151]	560 [296-1125]	2	[C][X] DRB1*13010101, DRB1*13020102 DRB3	[E] 0 [H] 0	[R] 2
	DRB1	19704/19895 (99%)	138 [1-151]	1372 [146-1799]	6	[C][X] DRB1*06030101, DRB1*06040101	[E] 0 [H] 0	[R] 1



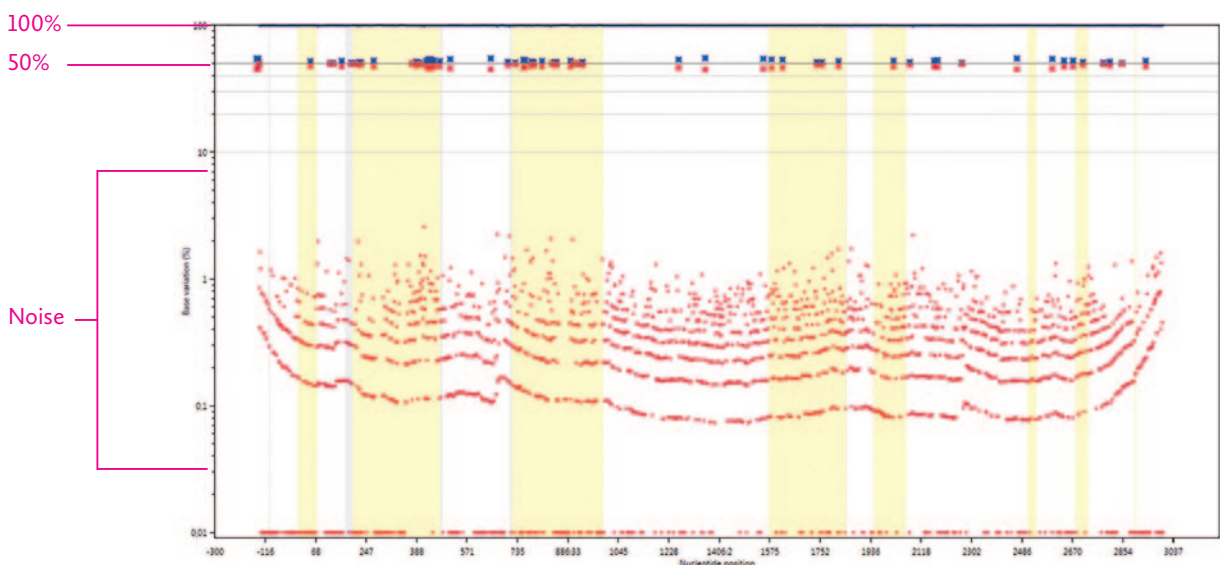
# NGSengine Quality Control

Better data quality means a higher reliability of the typing result. Therefore, NGSengine provides many tools to take a closer look at data quality. In the overview screen you can view many useful quality metrics which are color-coded for easy visualization of low, intermediate, or high quality. The intuitive software makes data inspection a pleasant experience, whether you want to quickly glance at the overall quality, or go as deep as the statistics of a single base call.



A quick and clear data impression is given by the Base Variation plot. The plot shows two dots for every position: blue for the major base and red for the percentage of other bases. A blue dot around 100% indicates a homozygous position. In case of a balanced heterozygous position, both blue and red are positioned at the 50% line.

A clear separation of the heterozygous positions around the 50% line from the noise is a strong indication of good quality data.



## Available products

NGS HLA workflow reagents (RUO and CE)		RXN
<b>NGSgo Workflow</b>		
Full workflow compatible with Illumina. Includes NGSgo-AmpX, NGSgo-LibrX, NGSgo-IndX	HLA-A, B, C, DRB1, DQB1	4 x 24
		2 x 96
	HLA GeneSuite (11 loci)	4 x 24
		2 x 96
<b>NGSgo®-AmpX</b>		
Amplification primers compatible with Illumina and Ion Torrent	Individual HLA loci	24
		96
		384
<b>NGSgo®-LibrX</b>		
Library preparation kit compatible with Illumina and Ion Torrent	Compatible with Illumina	48
		96
	Compatible with Ion Torrent	48
<b>NGSgo®-IndX</b>		
Indices & Adapters compatible with Illumina and Ion Torrent	Compatible with Illumina	4 x 24
		2 x 96
		1 x 384
	Compatible with Ion Torrent	4 x 24

NGS HLA workflow reagents (RUO)		RXN
<b>NGSgo®-AmpX v2</b>		
Amplification primers, optimized protocol (v2). Kit includes GenDx-LongMix.	Individual HLA loci	96
<b>NGSgo®-MX6-1</b>		
Multiplex amplification primers, kit includes GenDx-LongMix.	Multiplex HLA-A, B, C, DRB1, DQB1, DPB1	96

NGS workflow reagents (RUO)		RXN
<b>NGSgo®-AmpX KIR</b>		
Amplification primers, kit includes GenDx-LongMix.	KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR3DL1, KIR3DS1, KIR3DL2, KIR3DL3	24
<b>NGSgo®-AmpX MICA, MICB</b>		
Amplification primers, kit includes GenDx-LongMix.	MICA, MICB	96

NGSEngine® (RUO and CE)	
NGS HLA analysis software compatible with Illumina, Ion Torrent, PacBio, MinION	One year Entry
	One year Extension
	Five year entry
	Five year Extension

Full details available on [www.GenDx.com](http://www.GenDx.com)



HLA Training School

## Join & discover

Sharing information and knowledge on sequencing-based HLA typing and chimerism monitoring is our priority. We empower you to get the best results.

Join one of our Training Courses, Teaching Sessions, User Meetings, Webinars or hands-on laboratory experiences.

Check for dates on our website and register via [www.GenDx.com/education](http://www.GenDx.com/education)



# Contact our Technical Support team Get involved

Our Support team can assist you with practical matters and data interpretation. For all our Sanger, NGS and Chimerism monitoring solutions, reagents and software.

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