

Charting the Future of HLA Typing with the Next-Gen Sequencer

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Why Perform HLA Typing?

Immune System Function

The HLA System plays a crucial role in the immune system by helping it distinguish between the body's own cells and foreign cells. This is vital for the immune system to function properly.

1 Transplant Compatibility

HLA typing helps determine if a donor's tissue is compatible with the recipients. A close match reduces the risk of transplant rejection and increases the chances of a successful transplant.

2 Disease Association

Certain HLA types are associated with specific autoimmune diseases and conditions. For example, HLA-B27 is linked to various types of spondyloarthritis.

3 Immunotherapy and Research

HLA typing can help tailor immunotherapies to individual patients, making treatments more effective. It is also used in research to study genetic diversity and disease susceptibility in different populations.



HLA Typing: Resolution, Technology and Indication

HLA-DRB1*04:01:01:01



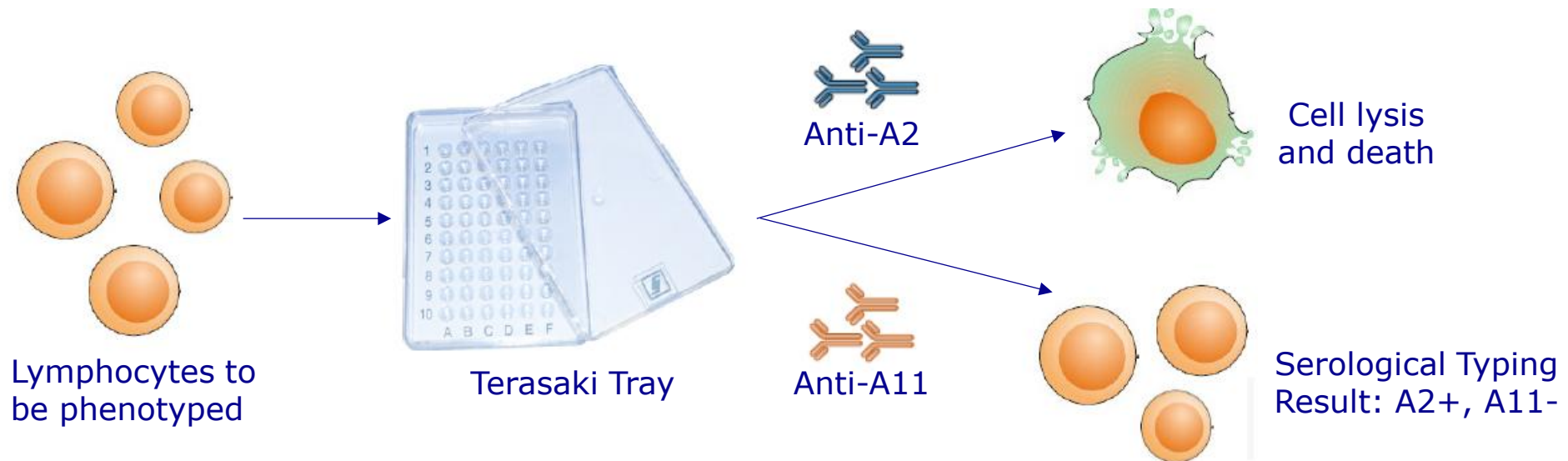
Resolution	Technology	Indication
Low Resolution	Serology	No longer routinely used
Low-intermediate Resolution	SNP-based methods (SSP, SSO, RT-PCR)	Solid organ transplantation HLA disease association Immunotherapy
High Resolution (2-fields and beyond)	SNP-based (limited to alleles of higher frequencies)	Stem cell transplantation Solid organ transplantation HLA disease association
	Sanger Sequencing	
	NGS by short-read and long-read sequencing platforms	

Different Technologies

HLA Typing Technology – Historical Evolution

1960s–1990s: Serological Typing

- **Method:** Used panels of antibodies from multi-parous women to identify HLA antigens on lymphocytes.
- **Process:** Complement-Dependent Cytotoxicity (CDC) assay used to determine the presence or absence of specific HLA.
- **Limitations:** Polyclonal antibodies had low specificity, broad reactivity and inconsistent sensitivity, leading to ambiguities in typing results.



The Advent of DNA-Based Typing

1980s–early 2000s: Molecular Typing Introduced

- **Key Innovation:** The invention of the Polymerase Chain Reaction (PCR) was pivotal, enabling DNA-level typing.
- **Methods:** Many PCR-based methods emerged:
 - Sequence-Specific Primers (SSP)
 - Sequence-Specific Oligonucleotide (SSO)
 - Real Time-PCR (RT-PCR)
 - Sanger Sequence Based Typing (SBT)



Next-Generation Sequencing: A New Era Begins

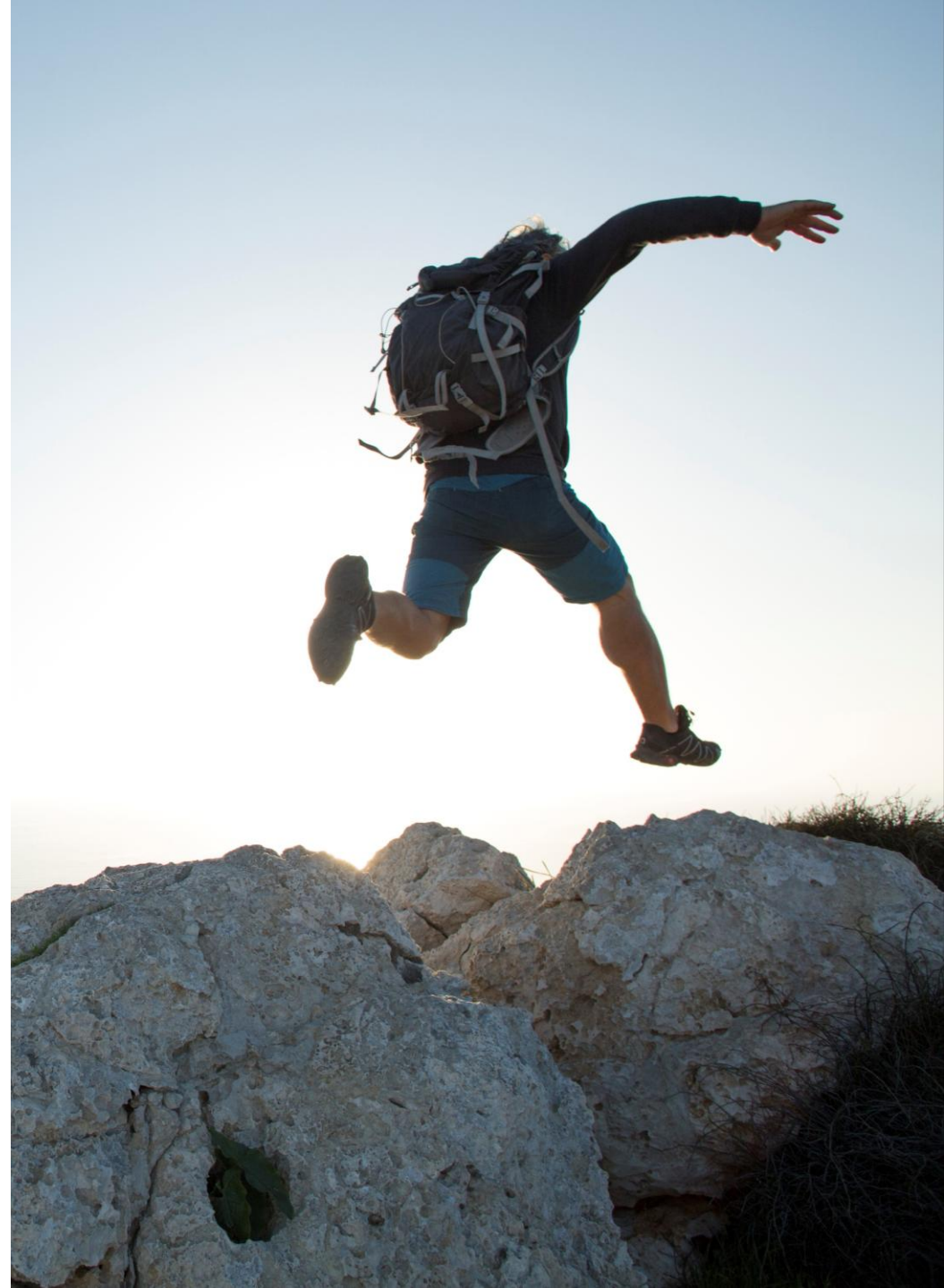
Late 2000s–Present

- **Method:** Massively parallel sequencing technologies, like those offered by Illumina, enabled high-throughput analysis of DNA.
- **Features:** DNA fragmentation, paired-end sequencing, and clonal amplification allowed for much greater depth of coverage and accuracy in resolving polymorphisms.
- **Impact:** Significantly reduced ambiguities and made high-resolution typing more accessible and affordable.
- **Limitations:**
 - **High Initial Costs and Complexity:** Initial setup costs, complexity of the workflow, and need for highly trained staff were a barrier to widespread implementation of NGS in clinical settings.
 - **Longer Turnaround Times:** Turnaround time for NGS can be longer than some established molecular typing methods.

Third-Generation Sequencing: The Next Leap in HLA Typing

Early 2010s–Present

- **Method:** Technologies like those from Pacific Biosciences and Oxford Nanopore Technologies provide long-read, single-molecule sequencing.
- **Benefits:** Long reads overcome the challenges of complex HLA regions, resolve ambiguities, and improve the phasing of polymorphisms, offering even greater accuracy for difficult alleles.
- **Importance:** Crucial for understanding complex genetic variations and improving the precision of HLA matching for transplantation.

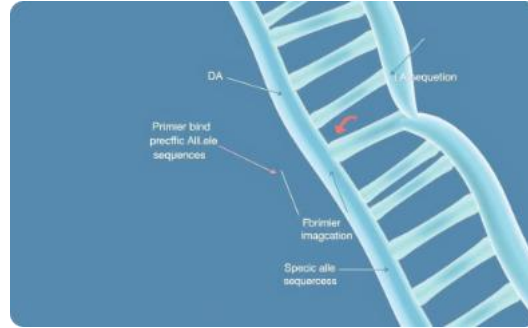


Molecular Methods



Sequence-Specific Oligonucleotide Probing (SSO)

This method uses labeled oligonucleotide probes that hybridize to specific HLA sequences. The presence of the HLA allele is detected by the binding of these probes.



Sequence-Specific Primer PCR (SSP-PCR or RT PCR)

This technique uses primers specific to HLA alleles to amplify DNA. The presence of a specific HLA allele is confirmed by the successful amplification of DNA.

Sequence-Based Typing (SBT)

This high-resolution method involves sequencing the HLA genes directly. It provides detailed information about the HLA alleles present.



Next-Generation Sequencing (NGS)

This advanced method allows for high-throughput and high-resolution HLA typing. It can sequence multiple HLA genes simultaneously, providing comprehensive results.

Transplant Market Complexity

1

1st Wave: SSO Typing

The initial wave of HLA typing technology focused on Sequence-Specific Oligonucleotide (SSO) typing methods.

2

2nd Wave: Traditional NGS Typing

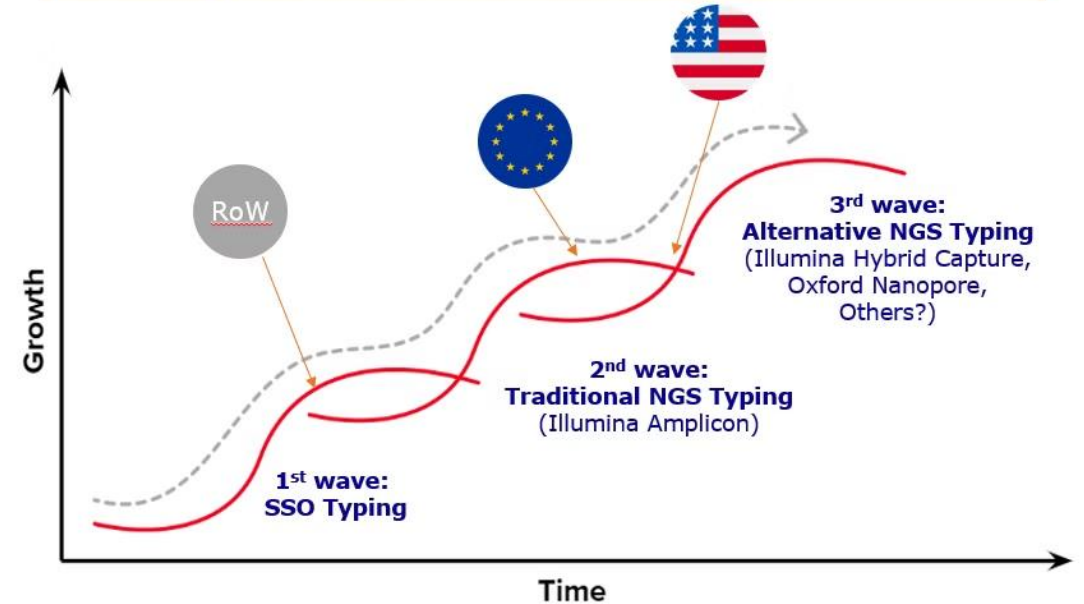
The second wave introduced Next-Generation Sequencing (NGS) techniques, particularly Illumina Amplicon-based methods.

3

3rd Wave: Alternative NGS Typing

The current wave explores alternative NGS typing methods, including Illumina Hybrid Capture, Oxford Nanopore technologies, and other emerging options.

Technology innovation waves in HLA typing



SSO Remains the Most Common Method for Organ Transplantation Internationally



Ease of Automation

SSO's popularity stems from its ease of automation, enabling batch processing without time constraints.



Typing for All Loci

While not all labs perform typing for all 11 loci, there's a growing trend toward more loci typing.



Combined Samples

SSO's versatility extends to its ability to be combined with other samples, such as those associated with disease.

Embracing Higher Resolution Typing



Higher Resolution Typing

The demand for higher resolution typing is driven by the increasing use of virtual crossmatch methods and molecular matching.



Virtual Crossmatch

Virtual crossmatch methods utilize high-resolution HLA typing as a secondary confirmation method.



Living Donor Transplants

Living donor transplants are also more likely to require higher resolution typing.

This trend is particularly pronounced in centers that also perform bone marrow transplantation in addition to solid organ transplantation..

Transplant Donor Typing



Organ Transplantation

Time-to-result requirements are crucial, making qPCR the fastest and most common method.

SSO can be used as a confirmatory test.

Oxford Nanopore is emerging as a solution, offering higher resolution.



Bone Marrow Transplantation

Time-to-result requirements are less critical.

Most labs utilize higher resolution technologies such as Illumina NGS assays, except those without an NGS system.

Oxford nanopore technologies could serve as a confirmatory test because smaller runs are faster and less expensive than small Illumina runs.



Disease Association/Immunotherapy

User Requirements

In most countries low resolution, 1st field still enough but changes are coming

Request for minimum 4th digit or Second field for specific diseases

Examples: Coeliac disease (DQB/DQA), Diabetes (DRB/DQB/DQA),
Abacavir (B*57:01), Polyarthritis (DR4/DR10), Narcolepsy
DQB*06:02

HLA labs:

Private labs or hospitals:

SSO: Patient samples run along side disease association samples

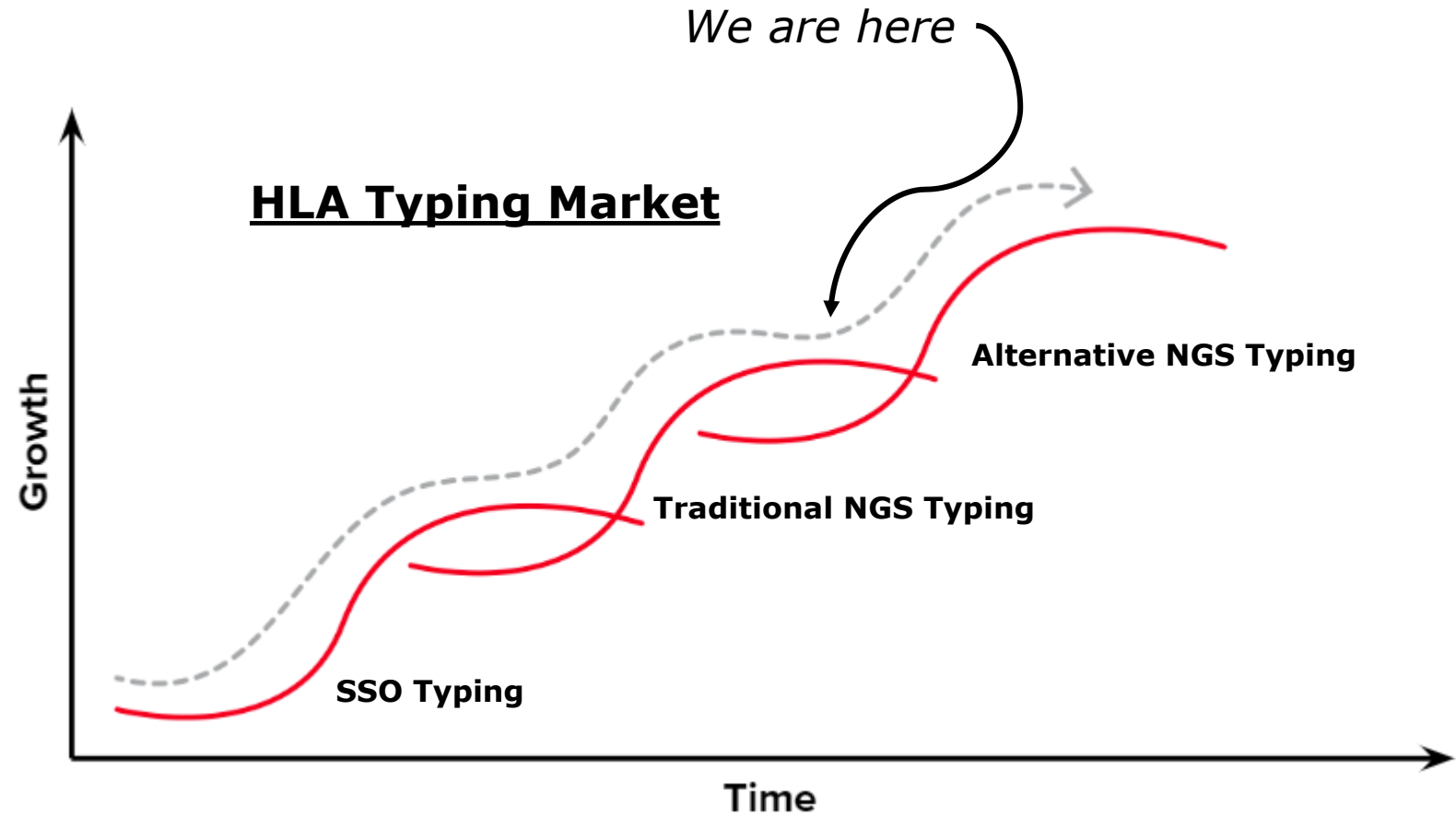
qPCR or some still SSP or SSO

NGS: single loci NGS testing mixed with full NGS testing (interest in ONT)

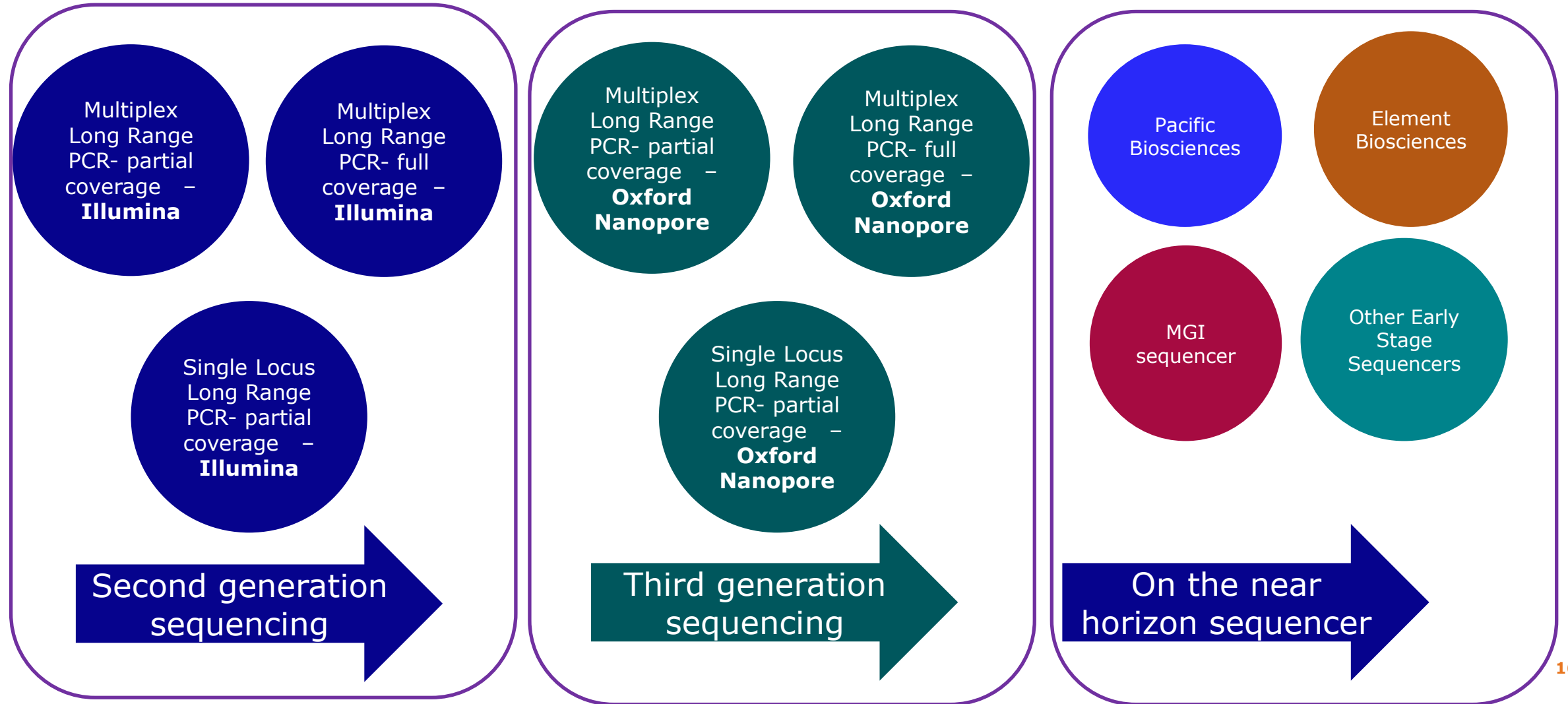
ONT can make it maybe possible



HLA Typing Evolution



HLA Typing with Long Range PCR – NGS Journey



ILLUMINA SEQUENCERS

Illumina – What is changing



MiniSeq
Low- to Mid-Throughput



MiSeq
Low- to Mid-Throughput



NextSeq
Mid- to High-Throughput



MiSeq™ i100
MiSeq™ i100 Plus

MiSeq, MiniSeq, iSeq – Obsolete in September 2025, Service until Dec, 2029
MiSeq i100 Plus – on sale now
MiSeq 100 – expected end of Q2 2025

Exploring New Illumina Sequencing Platforms

- ❖ **NextSeq 1000 and 2000 were released in Oct2020**
- ❖ **MiSeq iSeries released in late 2024 (i100 plus) and mid - 2025 (i100)**

New XLEAP-SBS chemistry and advantages:

- ❖ **Novel polymerase and improved x-block, x-linker and x-dyes -**
 - ❖ **Fewer missed incorporations or double incorporations – leads to higher accuracy**
 - ❖ **fewer imaging steps and images are sharper because of improved performance**
 - ❖ **Increased speed:** up to 2x faster incorporation speed of nucleotides, leading to quicker turnaround times.
 - ❖ **Higher accuracy:** improved fidelity and 3x greater accuracy, resulting in higher-quality sequencing data.
 - ❖ **Thermostable reagents:** enable ambient-temperature shipping and storage
 - ❖ **Significant Workflow simplification:** On board denaturation
-
- ❖ **Note:** Ambient storage for MiSeq iSeries only; reagents currently have a 3-month shelf life. NextSeq 1000/2000 reagents have a 6-month shelf life).

Illumina Platform Comparison



MiSeq™ i100
MiSeq™ i100 Plus

\$49,900

\$109,900

MiniSeq™

FC clusters	Cycles	List
Mid 8 M	300	\$717
Rapid 20 M	100	\$1,225
High 25 M	75	\$1073
	150	\$1,225
	300	\$2012

MiSeq™

FC clusters	Cycles	List
Nano 1 M	300	\$378
	500	\$454
Micro 4 M	300	\$571
	50	\$1,061
v2 15 M	300	\$1,359
	500	\$1,528
v3 25 M	150	\$1,178
	600	\$1,989

MiSeq™ i100 Series

FC clusters	Cycles	List
5M	300	\$420
	600	\$650
	100	\$550
25M	300	\$900
	600	\$1,100
	100	\$750
50M	300	\$1,100
	600	\$1,450
	100	\$1000
100M	300	\$1,450

MiSeq i100 series should be sufficient for clinical HLA typing – higher output, faster TAT

MiSeq i100										
MiSeq i100 Plus										
Flow cell ¹	5M		25M			50M			100M	
Read length (bp)	2×150	2×300	1×100	2×150	2×300	1×100	2×150	2×300	1×100	2×150
Output*	1.5 Gb	3 Gb	2.5 Gb	7.5 Gb	15 Gb	5 Gb	15 Gb	30 Gb	10 Gb	30 Gb
Run time	~7 hr	~15 hr	~4 hr	~7 hr	~15 hr	4.5 hr	~7.5 hr	~15.5 hr	~5 hr	~ 8 hr
%Q30	≥90%		≥90%			≥90%			≥90%	
Availability	Now		Now			2H 2025			2H 2025	
Value	Supports current applications, faster and more affordable					Expanded output for deeper sequencing and larger panels				

Sequencers – Oxford Nanopore

Oxford Nanopore – What is Changing?



MinION® Mk1d



GridION®



P2® Solo



P2® Integrated

	MinION Mk1d	GridION	P2 Solo	P2 integrated
Flow cells	MinION	MinION	PromethION	PromethION
Integrated Computer	NO	YES	NO	YES
Number of slots	1	5	2	2
Usable slots for HAC	1	3	2	2
Sequencing time for 1 sample	20min -1h	20min -1h	20min	20 min
Sequencing time for 48 sample	30 – 48h	30 – 48h	3-12h	3-12h

- **MinION Mk1d** – the older versions are not available after June 2025
Enhanced field and lab use with better environmental tolerance
- **P2Solo** –As customers are increasing throughput –P2 solo is a viable alternative to MinION Mk1d for High throughput customers

MGI SEQUENCERS

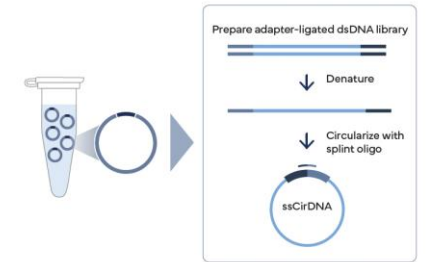
MGI sequencers



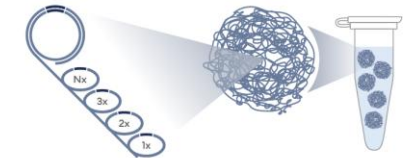
DNBSEQ-99

Feature	Illumina MiSeq (2×150 bp)	MGI DNBSEQ-G99 (2×150 bp)
Read Length	2×150 bp	2×150 bp
Throughput per Run	7.5–8.5 Gb	30–40 Gb
Run Time	24–39 hours	12 hours
Q30 Accuracy	~85–90%	~95%
Q40 Accuracy	Not typically reported	~90%
Cost per Run	Higher	Lower
Suitability for HLA Typing	Good – widely used, but lower throughput	Excellent – high throughput, high accuracy

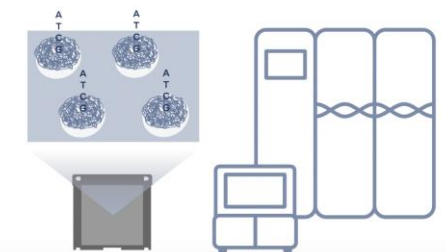
1 Prepare ssCirDNA Libraries



2 Make DNBs



3 Load DNBs and Sequence



Sequencer closest in similarity to Illumina workflow – Fragmentation, adaptor ligation, sequencing

Vision for Long Range HLA typing

- Regardless of which sequencing platform kit you choose to run our PCR kit in – we will support outputs from Clinical grade sequencers as they become available.
- Software must be able to handle data from any of the sequencing platforms

POSSIBILITIES WITH AI..

Ideas For Integration With AI

- **Personal chat bot** to help with data analysis
- **Automating data analysis** that a user would do for samples that require manual review – data analysis with more reads, checking against alternate closely related alleles
- **Prepare customized reports**
- **Integrate data** from multiple sources to better analyze sequencing and Antibody data
- **Bioinformatics** – New algorithms for variant calling and improved Phasing in heterozygous samples
- **Real Time Quality control** to monitor sequencing runs

Longer term projects that can only be done as a collaboration with community

Develop new LD tables from datamining all published data and clinical data to identify new haplotypes

Clinical Decision Projects combining data from multiple tests to provide personalized treatment plans

Our Current HLA Typing Portfolio

Our products Powering Patient Care



Typing

LIFECODES®

Luminex SSO HLA Typing Kits

Our HLA SSO typing kits identify HLA alleles present in locus-specific PCR-amplified samples. The rapid protocol, with minimal hands-on time and no centrifugation or wash steps, makes this an ideal solution for both high and low throughput testing.

MIA FORA™

Illumina NGS HLA Typing Kits

MIA FORA delivers NGS HLA typing results through advanced software using three algorithms for high accuracy. Its simplified multiplex kit reduces allele ambiguities, streamlines workflow, and shortens turnaround time.

NanoTYPE™

ONT TGS HLA Typing Kits

The NanoTYPE product family is a suite of HLA typing assays built on Oxford Nanopore Technologies (ONT) sequencing platforms.

Includes NanoTYPE CE-IVDR: Multiplex assay for 11 genes.

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Powering Patient Care