High resolution HLA typing

Headlines:

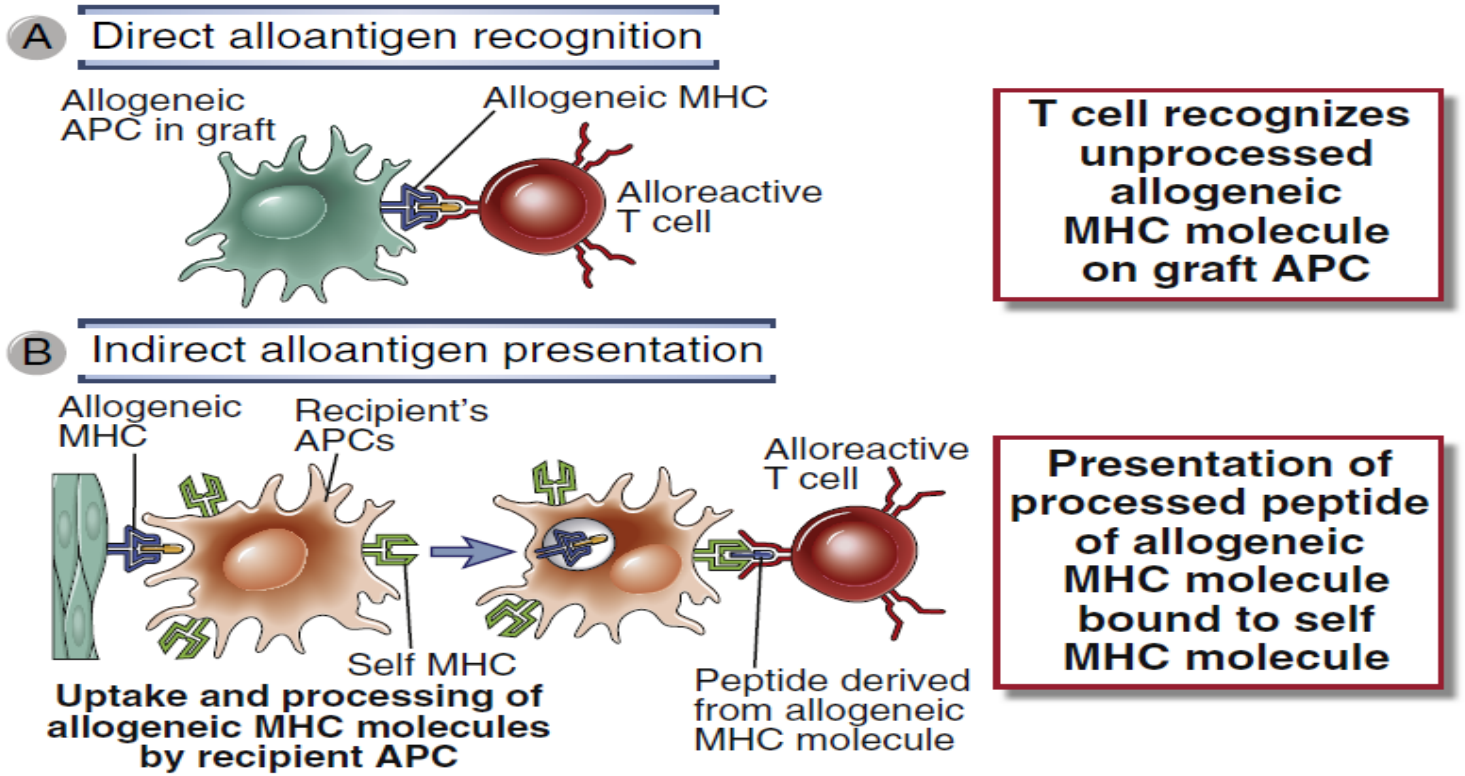
- Introduction: Allogenicity and alloreactive T cells
-The donor with compatible HLA to prevent T cell alloreactivity
-HLA typing resolution
-High resolution definitions
-High resolution HLA typing in clinical settings
-High resolution donor- recipient HLA matching
-HLA typing techniques and resulting resolutions
-NGS workflow for High resolution HLA-typing

Introduction:Allogenicity and alloreactive T cells

The immunogenetic relationship between the **donor** and **recipient** profoundly influences the outcome of **HCT**, much more so than with **solid organ transplants.**

Presentation of Allogenic MHC molecules

- **Allogenic MHC** are presented for recognition by the **alloreactive T cell of a recipient** in two fundamentally ways:



Principles of direct recognition of allogenic MHC by Alloreactive T cells

Allogenic T cells are **very frequent**

Up to 1% to 2% of all T cells in an individual, 100 to 1000 times greater than the frequency of T cells specific for any microbial peptide displayed by self MHC molecules.

Alloreactive T cells have a **high affinity TCR for** Allogenic MHC .

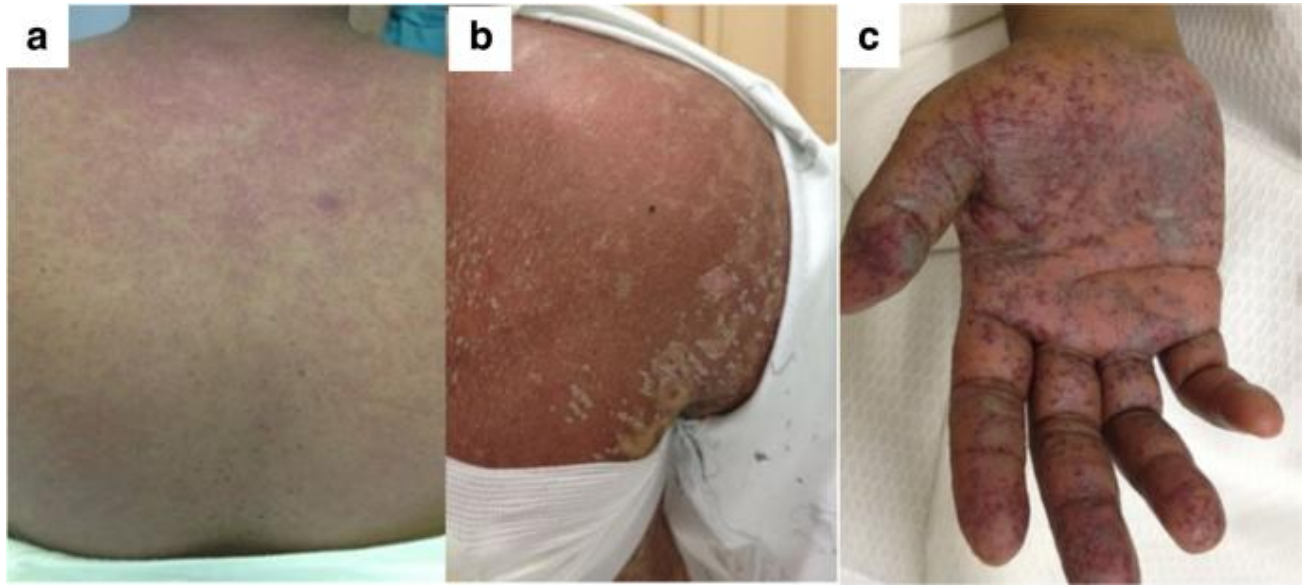
Alloreactive T cells do not rely on **Co-stimulatory CD28 signaling.**

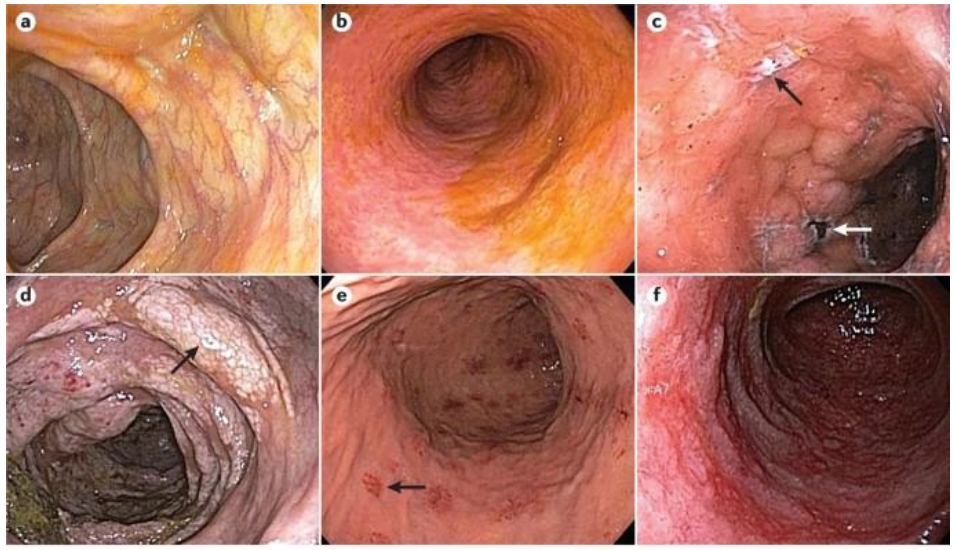
Direct recognition and activation of **alloreactive T cells** may occur **regardless of which peptide** is carried by allogenic MHC molecule.

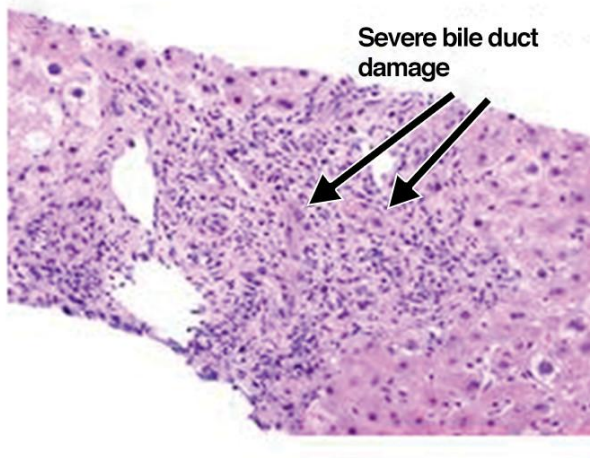
Alloreactive T cells that respond to the allogenic MHC are **cross-reactive memory T cells.**

HLA matching avoids **activation of alloreactive T cells** by major histocompatibility antigens
(MHC)

Acute graft versus host disease







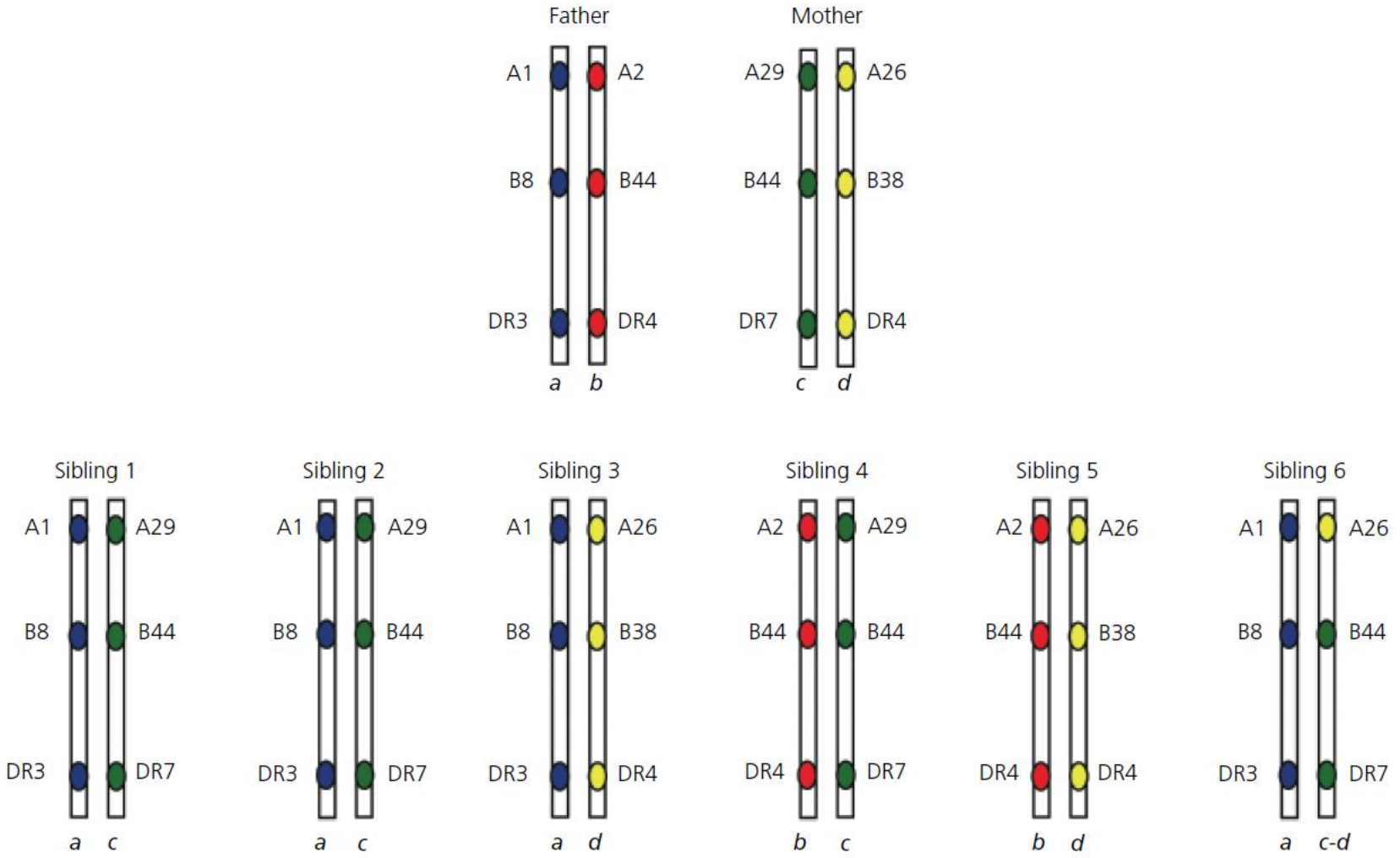
The donor with compatible HLA to prevent T cell alloreactivity

The best results are seen after transplantation from **HLA-genotypically identical sibling** donors, **matched related donor (MRD),**but only 30% of patients have such a donor.

Identical-by-descent (IBD)

Genotypically identical siblings who have inherited the same maternal and paternal chromosome 6

A family study and haplotypes for finding match related donor (MRD)



Some patients without an HLA-identical sibling have an HLA-haploidentical family member with limited mismatching between the non-shared haplotypes.

Previous studies have shown that patients receiving **an HLA-haploidentical transplant** incompatible for a single HLA-A, -B, or -DR antigen can have disease free survival (DFS) similar to that of patients with marrow from HLA-genotypically identical sibling donors, although HLA mismatching increases the risk of GVHD.

In HLA haploidentical, problems related to GVHD can be overcome by **removing T cells** from the graft or by giving **cyclophosphamide** after transplantation to eliminate T cells that are activated after they recognize recipient

HCT from **HLA-phenotypically matched unrelated donors (MUD)** or cord blood donors is an alternative for patients who lack a donor.

Unrelated HCT has been made feasible by the development **of large registries of HLA-typed individuals** and **cryopreserved cord blood units** available to serve as a source of hematopoietic cells.

With registries currently available in the United States and in other countries, it is now possible to identify an HLA-A, B-phenotypically matched DRB1 allele-matched unrelated donor for at least **70% of white persons**.

There are currently >25 million volunteer unrelated donors and UCB units listed on **Bone Marrow Donors Worldwide** (www.bmdw.org/accessed June 2015).

This **centralized database** of donors allows clinicians, and other professionals involved in donor selection, to search for donors in their own country (registry) and donors all over the world simultaneously.

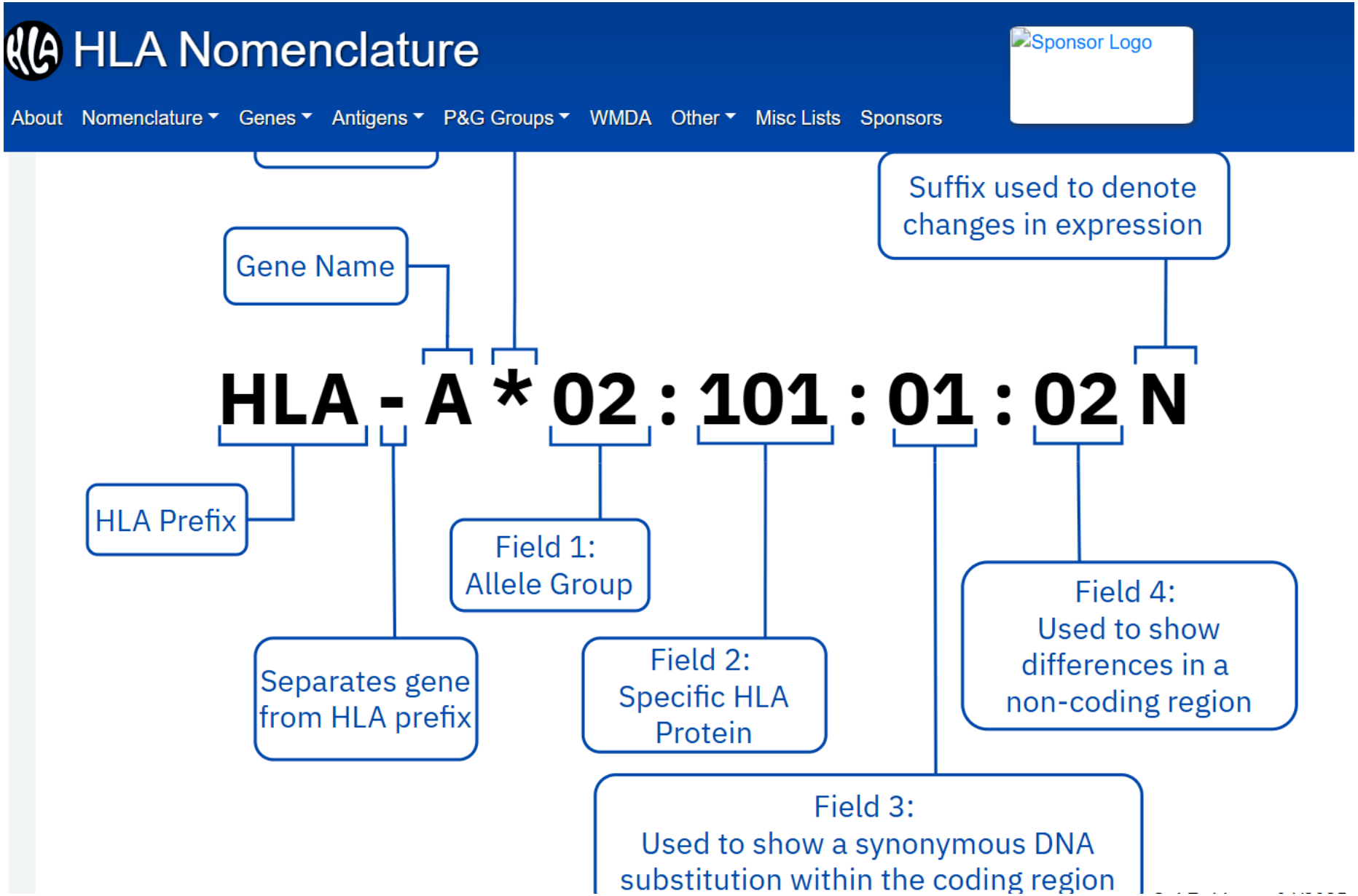
HLA alleles are more **diverse** in **Hispanic** and **black populations**, and success rates for matching unrelated donors in these populations are **lower than in white persons**.

If **a single** HLA-A, -B, or -DRB1 mismatch were allowed, then it would be possible to identify donors for many more patients.

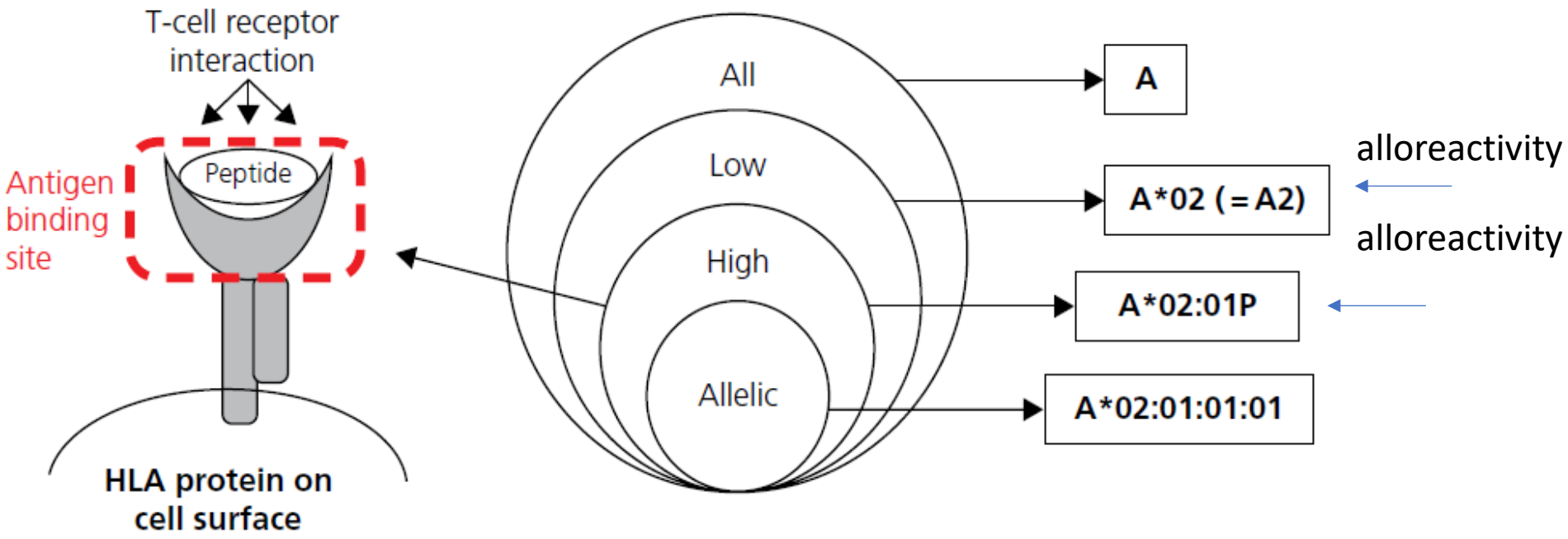
In certain circumstances, **DFS** for patients with HLA-A, -B, -C, -DRB1, and DQB1 allele-matched **unrelated donors** may be comparable to that for similar patients with HLA-genotypically identical sibling donors.

Identical-by-state (IBS)
Phenotypically matched **unrelated individuals** who fortuitously share the same HLA antigens

HLA typing resolution


HLA Nomenclature
Sponsor Logo
About
Nomenclature
Genes
Antigens
P&G Groups
WMDA
Other
Misc Lists
Sponsors
Suffix used to denote changes in expression
Gene Name
HLA - A * 02 : 101 : 01 : 02 N
HLA Prefix
Field 1: Allele Group
Separates gene from HLA prefix
Field 2: Specific HLA Protein
Field 4: Used to show differences in a non-coding region
Field 3: Used to show a synonymous DNA substitution within the coding region

HLA typing resolution. The **Venn diagram** illustrates increasing levels of HLA typing resolution



High-resolution Vs Allelic resolution

High-resolution HLA typing defines the **specific DNA sequence of the antigen binding site.**

Allelic resolution defines a single allele as defined by **a unique DNA sequence for the HLA gene**

High resolution definitions

High resolution typing: A high-resolution HLA genotype is reported in molecular HLA nomenclature to at least the second field, and may include P or G group designations.

P groups

HLA alleles having **nucleotide sequences** that encode **the same protein sequence for the peptide binding domains** (exon 2 and 3 for HLA class I and exon 2 only for HLA class II alleles).

This analysis is performed on the **protein sequence**.

The group designation will contain **a minimum of four digits**.

Designated by an upper case ‘P’ which follows the 2 field allele designation.

A*01:02P	A*01:02:01:01/A*01:02:01:02/A*01:02:01:03/A*01:02:02/A*01:412

G groups

HLA alleles that have identical nucleotide sequences across the exons encoding the peptide binding domains (exon 2 and 3 for HLA class I and exon 2 only for HLA class II alleles)

Designated by an upper case ‘G’ which follows the first 3 fields of the allele designation of the lowest numbered allele in the group.

The group designation will contain a minimum of six digits.

The algorithm used to generate the G groups does include alleles which contain unsequenced regions.

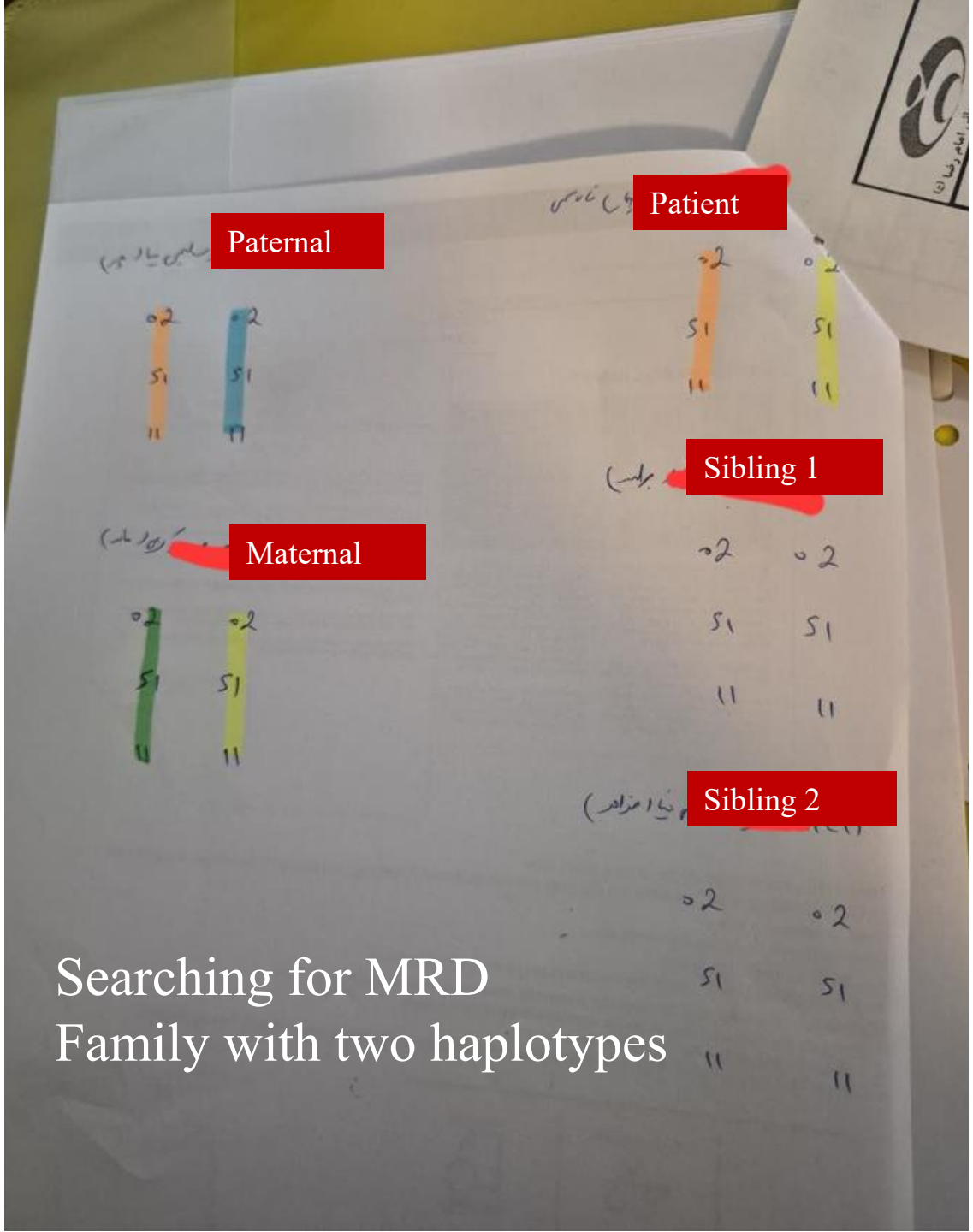
A*01:02:01G	A*01:02:01:01/A*01:02:01:02/A*01:02:01:03/A*01:412/A*01:420N

High resolution HLA typing in clinical settings

High resolution HLA typing indications

Challenges in finding definite haplotypes in family studies

Finding matched unrelated donor (MUD)



Searching for MRD
Family with two haplotypes

Finding match unrelated donor (MUD)

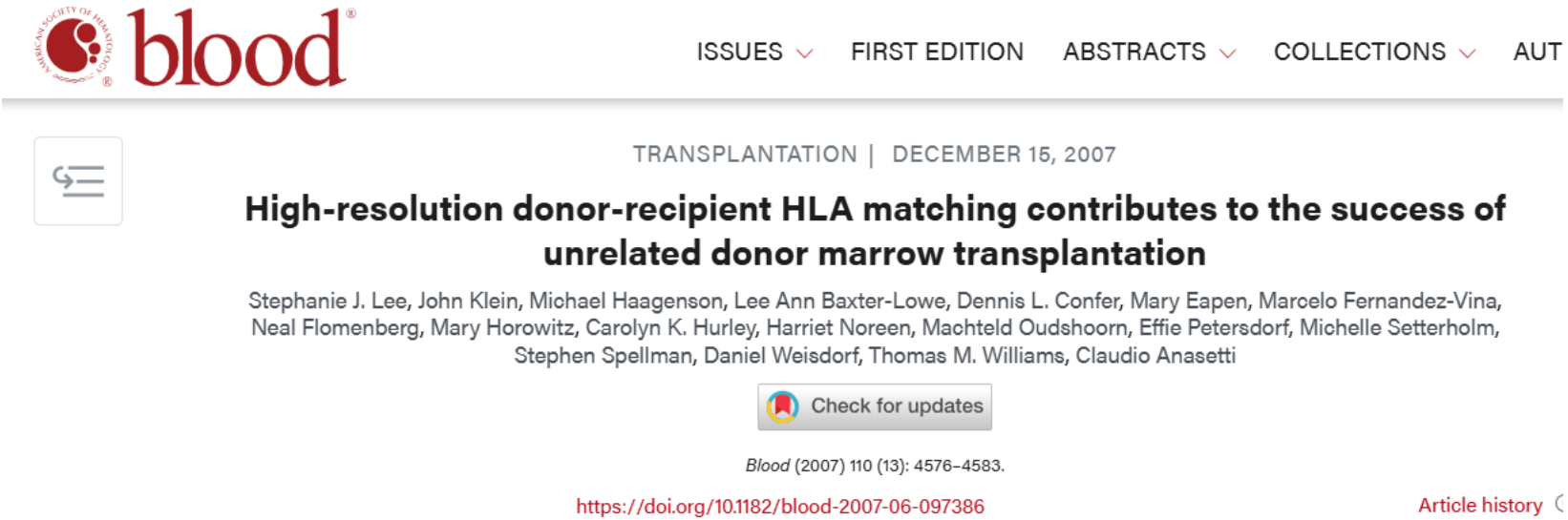
nmdp FIND CURES. SAVE LIVES.

Patient care | Get involved | What we do | Stories and impact | What are you looking for? | $ Donate | Join registry

Help people with blood cancers or disorders get a life-saving transplant

About donating blood stem cells

High resolution donor- recipient HLA matching



High-resolution DNA matching for HLA-A, -B, -C, and -DRB1 alleles is associated with higher rates of survival.

The minimal consensus on compatibility testing requires high resolution typing for HLA-A, -B, -C, and -DRB1.

Many centers in Europe also include **HLA-DQB1** compatibility in donor selection strategies.

The relevance of HLA-DPB1 matching in unrelated stem cell transplantation has long remained undefined.

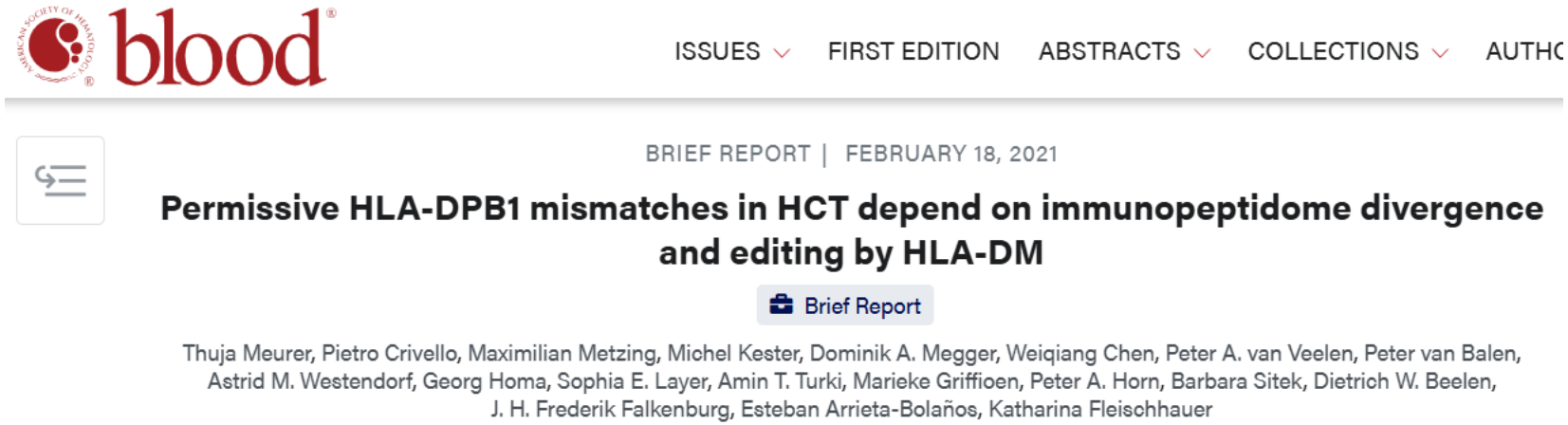
This may be due to several characteristics that distinguish HLA-DPB1 antigens from other classical HLA-molecules.

DPB1 mismatches

Classified as permissive and non-permissive.

Permissive mismatches do not elicit strong T cells.

Nonpermissive mismatches have stronger immunogenicity and elicit stronger T cell repones.

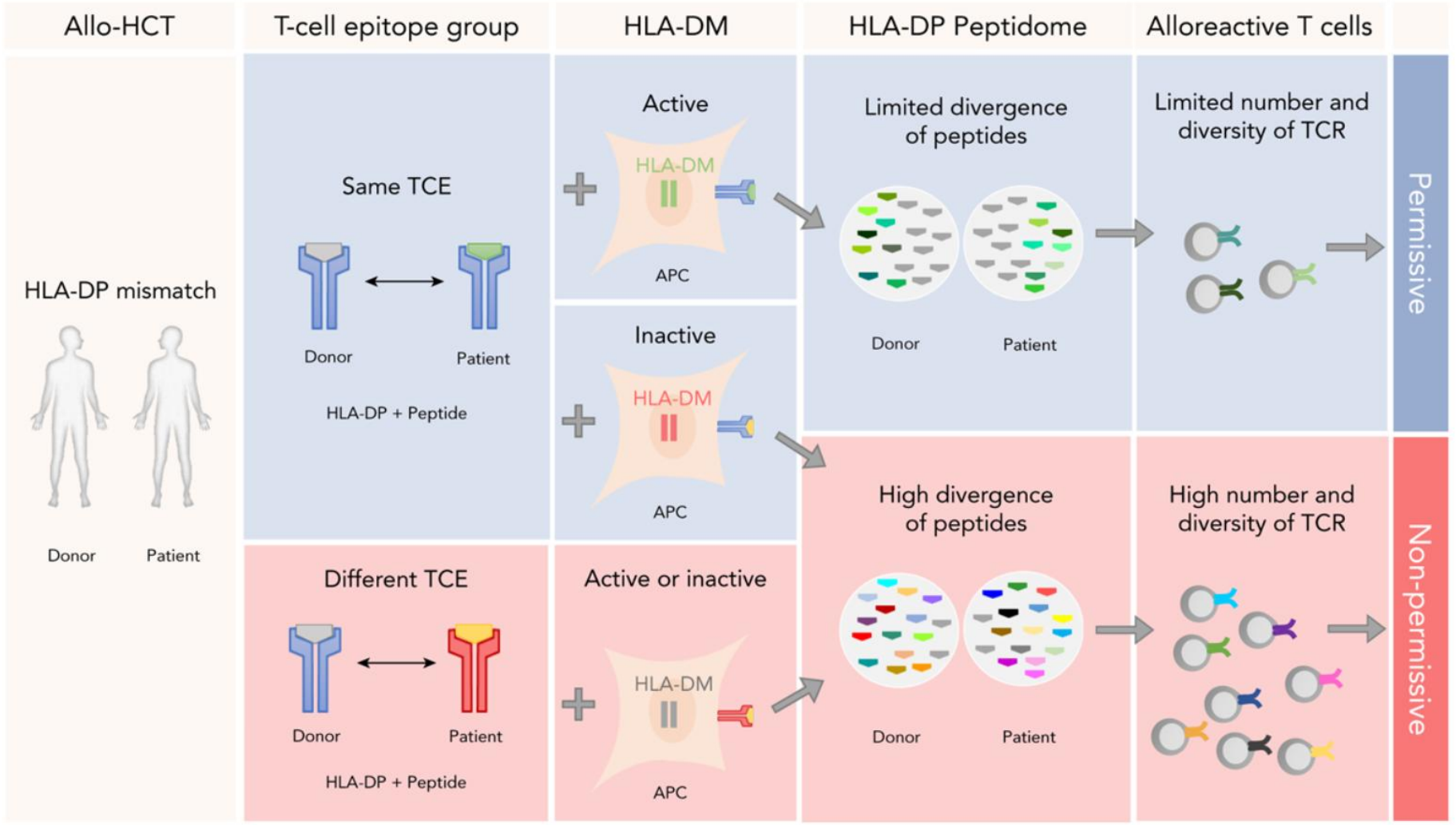


•Permissive HLA-DPB1 mismatches in HCT are characterized by **limited immunopeptidome divergence,** mirrored by **alloreactive TCR diversity**.

•The peptide editor **HLA-DM** plays a key role in harnessing T-cell alloreactivity to permissive HLA-DP by **restricting its peptide repertoire.**

HLA-DM–mediated modulation of **alloreactive T-cell responses** to **HLA-DP** in healthy individuals and transplanted

Permissive HLA-DPB1 mismatches in HCT depend on **immunopeptidome** divergence and editing by **HLA-DM**



HLA typing techniques and resulting resolutions

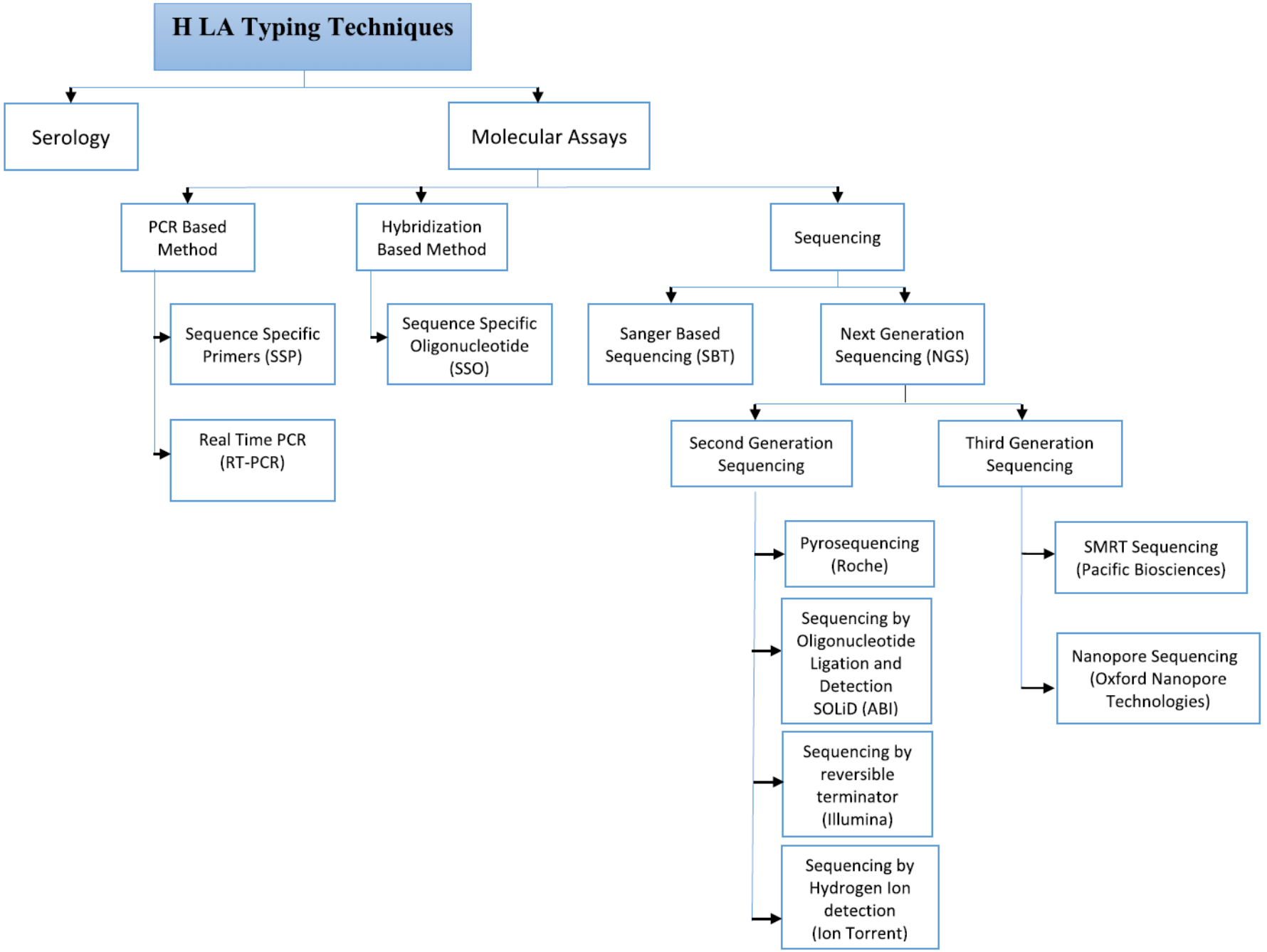

H LA Typing Techniques
Serology
Molecular Assays
PCR Based Method
Hybridization Based Method
Sequencing
Sequence Specific Primers (SSP)
Real Time PCR (RT-PCR)
Sequence Specific Oligonucleotide (SSO)
Sanger Based Sequencing (SBT)
Next Generation Sequencing (NGS)
Second Generation Sequencing
Third Generation Sequencing
Pyrosequencing (Roche)
Sequencing by Oligonucleotide Ligation and Detection SOLiD (ABI)
Sequencing by reversible terminator (Illumina)
Sequencing by Hydrogen Ion detection (Ion Torrent)
SMRT Sequencing (Pacific Biosciences)
Nanopore Sequencing (Oxford Nanopore Technologies)

Table 2.1 HLA typing techniques and resulting resolution.

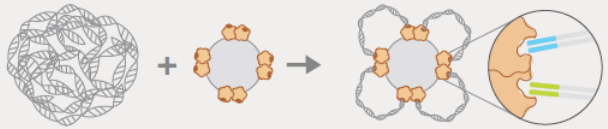
Methodology	Approach	Interpretation	Resolution	Application	Results
Serology	Cellular assay based on complement fixation by HLA specific antibodies	Cell death – yes/no	Low	Family screening Null allele confirmation	A2, A24
Sequence-specific primers (PCR-SSP)	HLA sequence-specific PCR primers	Amplification – yes/no	Low to high, dependent on DNA sequence coverage	Family screening Verification typing	Low - A*02:XX, A*24:XX or A*02AB, A*24:BC High – A*0201 g, A*24:02 g
Sequence-specific oligonucleotide probes (PCR-SSOP)	HLA sequence-specific oligonucleotide probes that bind to polymorphic sequences of amplified DNA	Probe binding – yes/no	Low to high depending on DNA sequence coverage	Family screening Verification typing	Low - A*02:XX, A*24:XX or A*02AB, A*24:BC High – A*02:01G, A*24:02G
Sanger sequence-based typing (SBT)	HLA amplicon sequencing using base termination	Base pair reads and consensus alignment	High to allele level depending on coverage	All	High – A*02:01G, A*24:02G Allele – A*02:01:01:03, A*24:02:01:01
Next-generation sequencing (NGS)	Multiple platforms, based on massive parallel sequencing reactions	Base pair calling and consensus alignment	High to allele level depending on coverage	All	High – A*02:01G, A*24:02G Allele – A*02:01:01:03, A*24:02:01:01

NGS workflow for High resolution HLA-typing

STEP

1 Whole Genome Library Generation

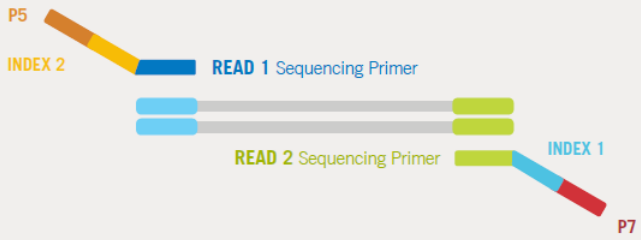
3.5 hrs / 2 hrs hands-on



Tagmentation

Genomic DNA is bound, fragmented and tagged by the bead bound transponsons - one sample per well

LESS INPUT DNA REQUIRED: >50NG OF INPUT DNA



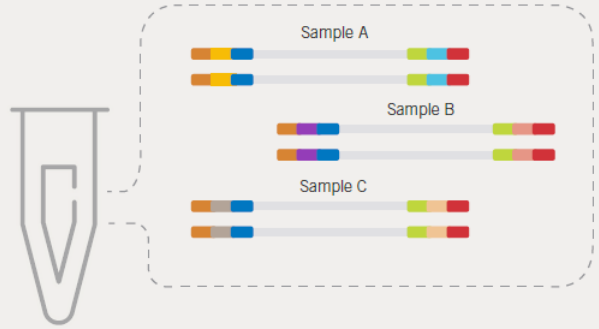
Wash beads

Removing any unbound DNA

Index PCR amplification

Clean up & size selection

Early indexing leads to **REDUCED RISK OF SAMPLE SWITCHING**



Pool all the sample Libraries into a single tube

No manual quantification of DNA is required prior to pooling

SINGLE TUBE WORKFLOW

Additional genes can be added without impacting the workflow

STEP

2 Hybrid Capture

5.5 hrs / 2 hrs hands-on

NO LONG RANGE PCR MEANS YOU GET:

- CONSISTENT ALLELE BALANCE
- REDUCED ALLELE DROP OUT RATES
- NO PCR ARTIFACTS

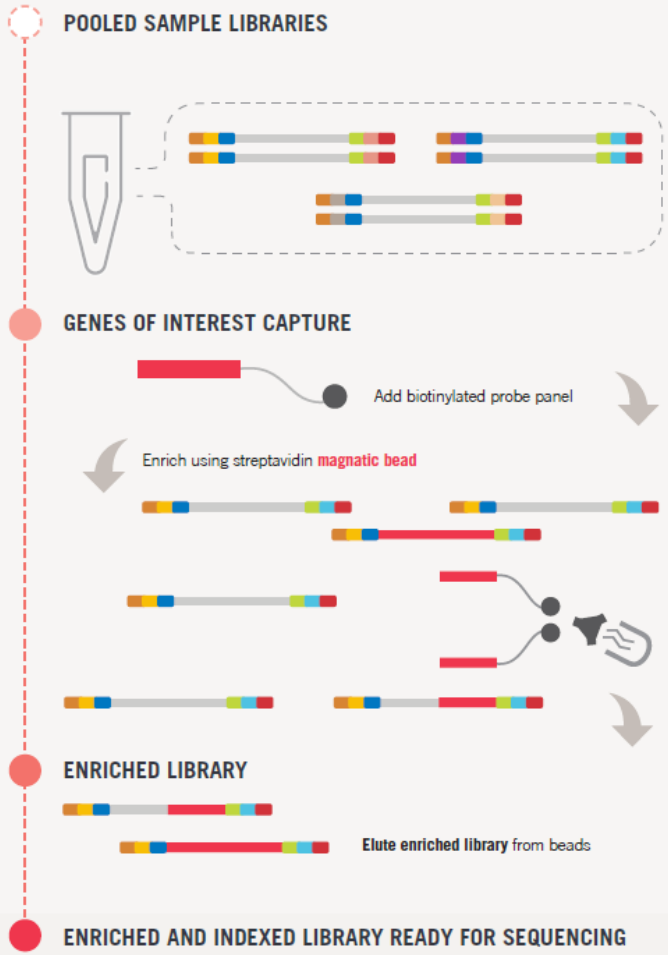
Hybridize probes

90 min or overnight at 62ºC

Capture target fragments

Amplify

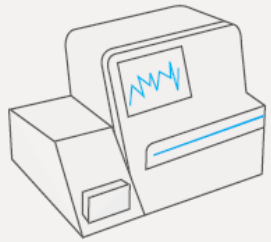
Clean up



STEP

3 Sequencing

17-24 hrs / 15 min hands-on

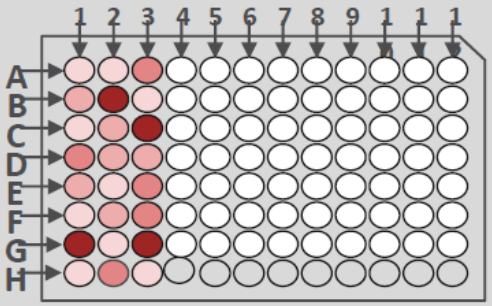


Sequencing on the Illumina platforms

MiSeq, MiniSeq, iSeq

Workflow Overview

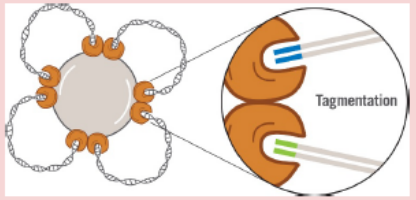
Quantification optional if >50 ng gDNA



6 to 24 samples

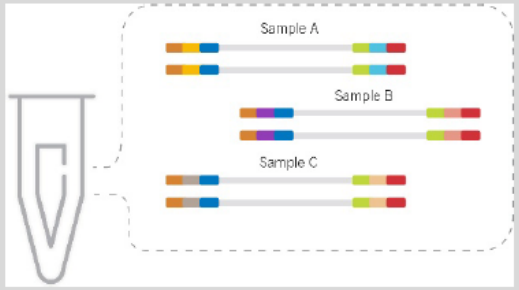
+

Add Bead-Linked Transposons (BLT) to each Sample

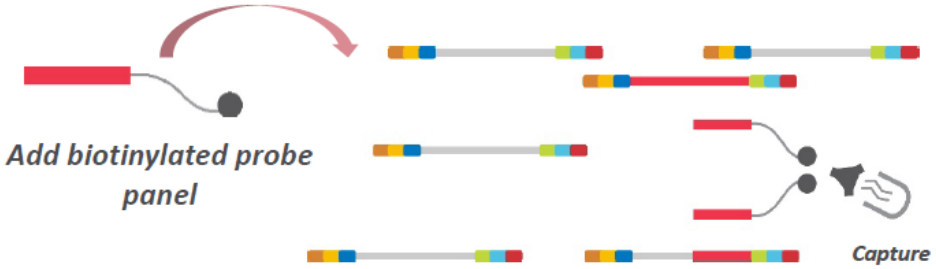


Genomic DNA binds to the Bead, is Fragmented and Tagged

Indices are Added by PCR



Libraries are Pooled into a single tube



Add biotinylated probe panel

Capture target fragments using streptavidin beads

Enrichment

Extracted and Indexed Libraries are Ready for Sequencing

NO LONG RANGE PCR MEANS YOU GET:

- **CONSISTENT ALLELE BALANCE**
- **REDUCED ALLELE DROP OUT RATES**
- **NO PCR ARTIFACTS**

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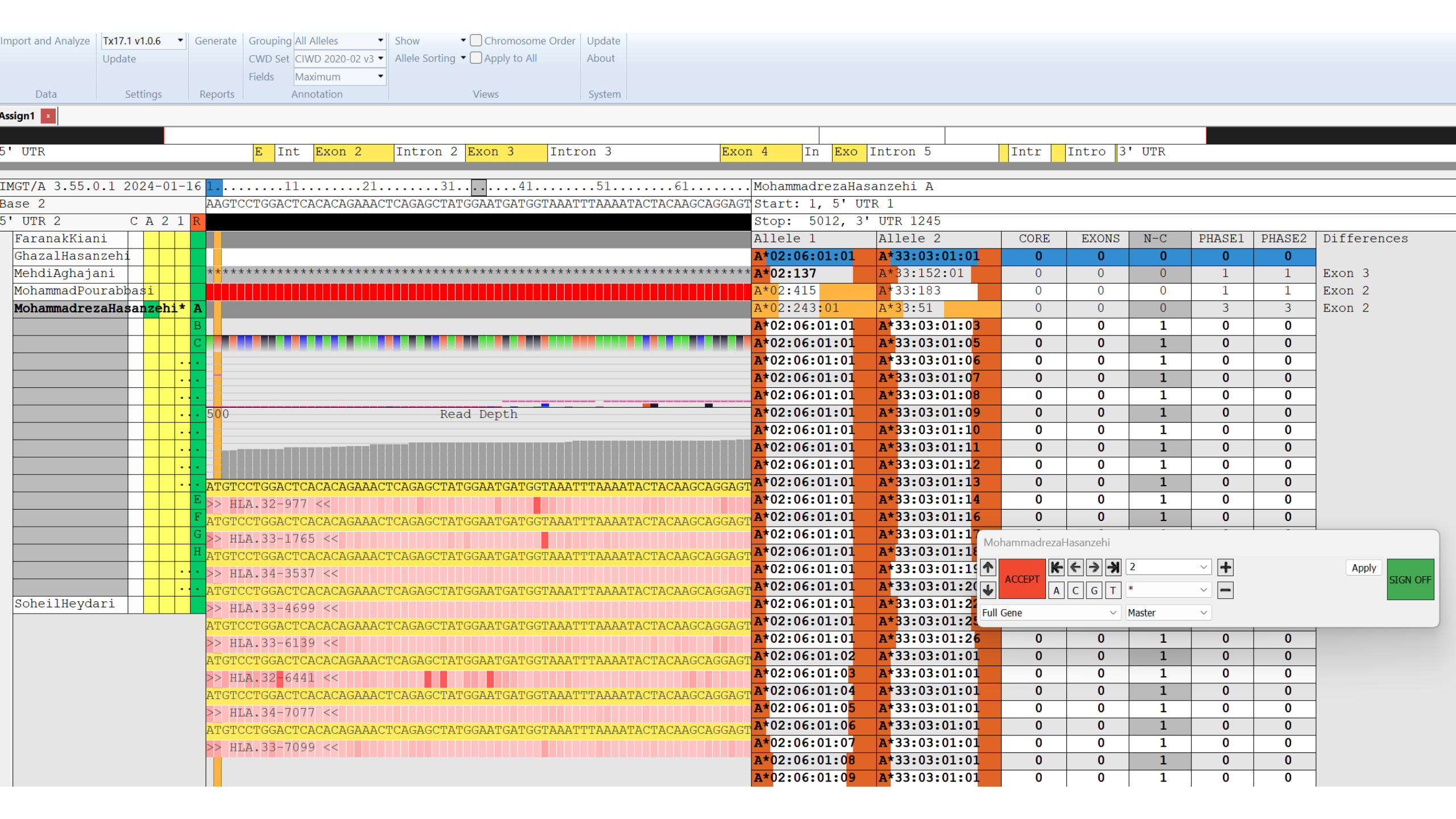
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Update
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Grouping
All Alleles
CWD Set
CIWD 2020-02 v3
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Annotation
Show
Chromosome Order
Allele Sorting
Apply to All
Views
Update
About
System
Assign1
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Intron 2
Exon 3
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Stop: 5012, 3' UTR 1245
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GhazalHasanzehi
MehdiAghajani
MohammadPourabbasi
MohammadrezaHasanzehi*
SoheilHeydari
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>> HLA.33-1765 <<
>> HLA.34-3537 <<
>> HLA.33-4699 <<
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>> HLA.32-6441 <<
>> HLA.34-7077 <<
>> HLA.33-7099 <<
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Allele 2
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EXONS
N-C
PHASE1
PHASE2
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A*02:415 A*33:183 0 0 0 1 1 Exon 2
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MohammadrezaHasanzehi
ACCEPT
A C G T
Full Gene
Master
Apply
SIGN OFF