# Minimal Requirements for Histocompatibility & Immunogenetics Laboratory

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**Medical Director, Histocompatibility & Immunogenetics laboratory**
**Medical Director, Blood & Marrow laboratory**




KING HUSSEIN CANCER FOUNDATION
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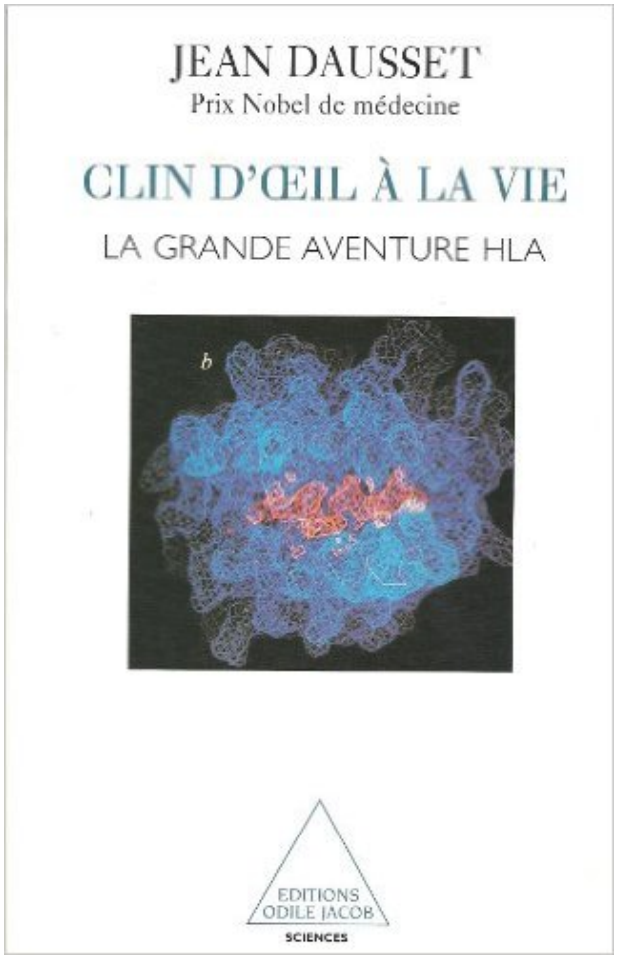
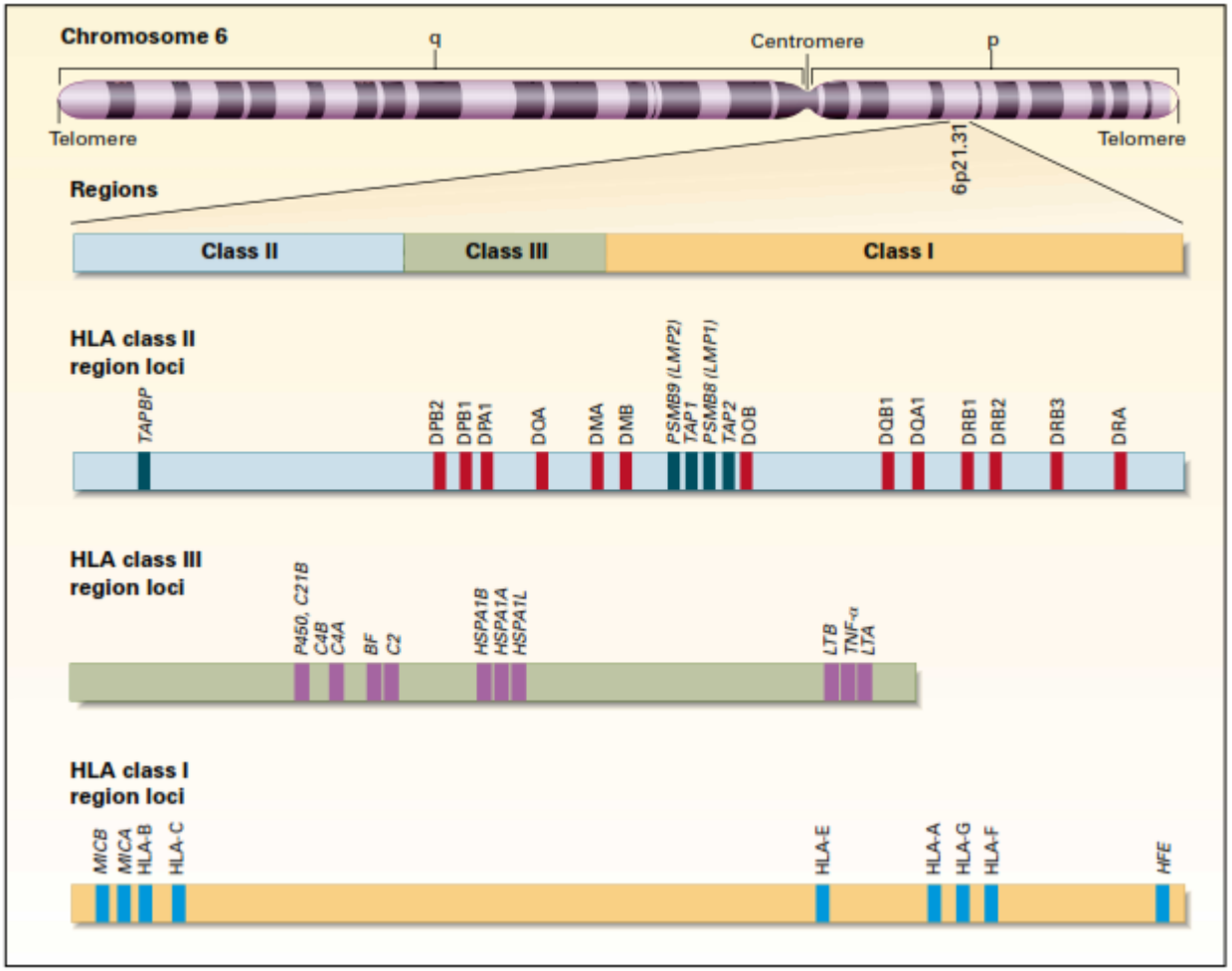
**The 4th WBMT Congress and Workshop – Riyadh, KSA - January 15-17, 2017**

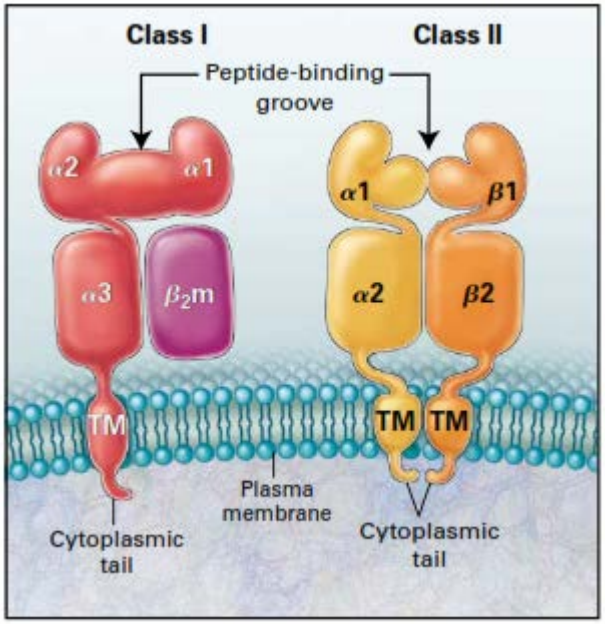
# HLA Discovery, 1958




**The Nobel Prize in Physiology or Medicine 1980**
Baruj Benacerraf, Jean Dausset, George D. Snell

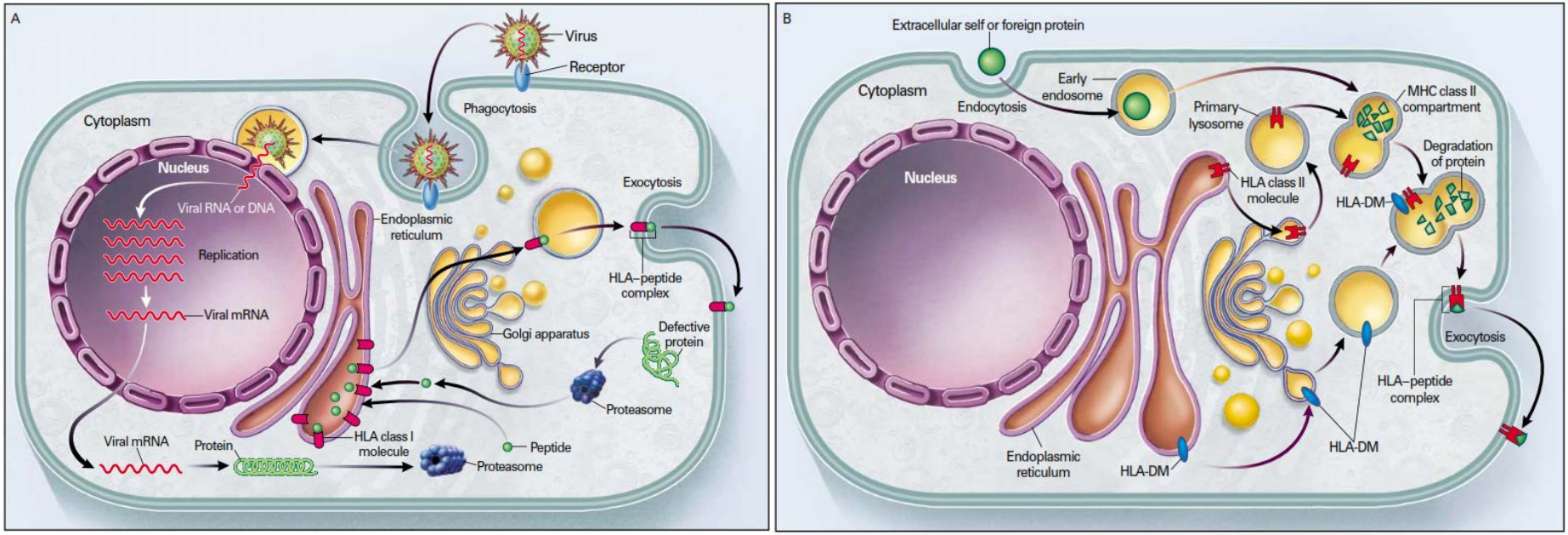
# HLA Complex, Chromosome 6




Klein and Sato. NEJM, 2000

# Antigen Processing

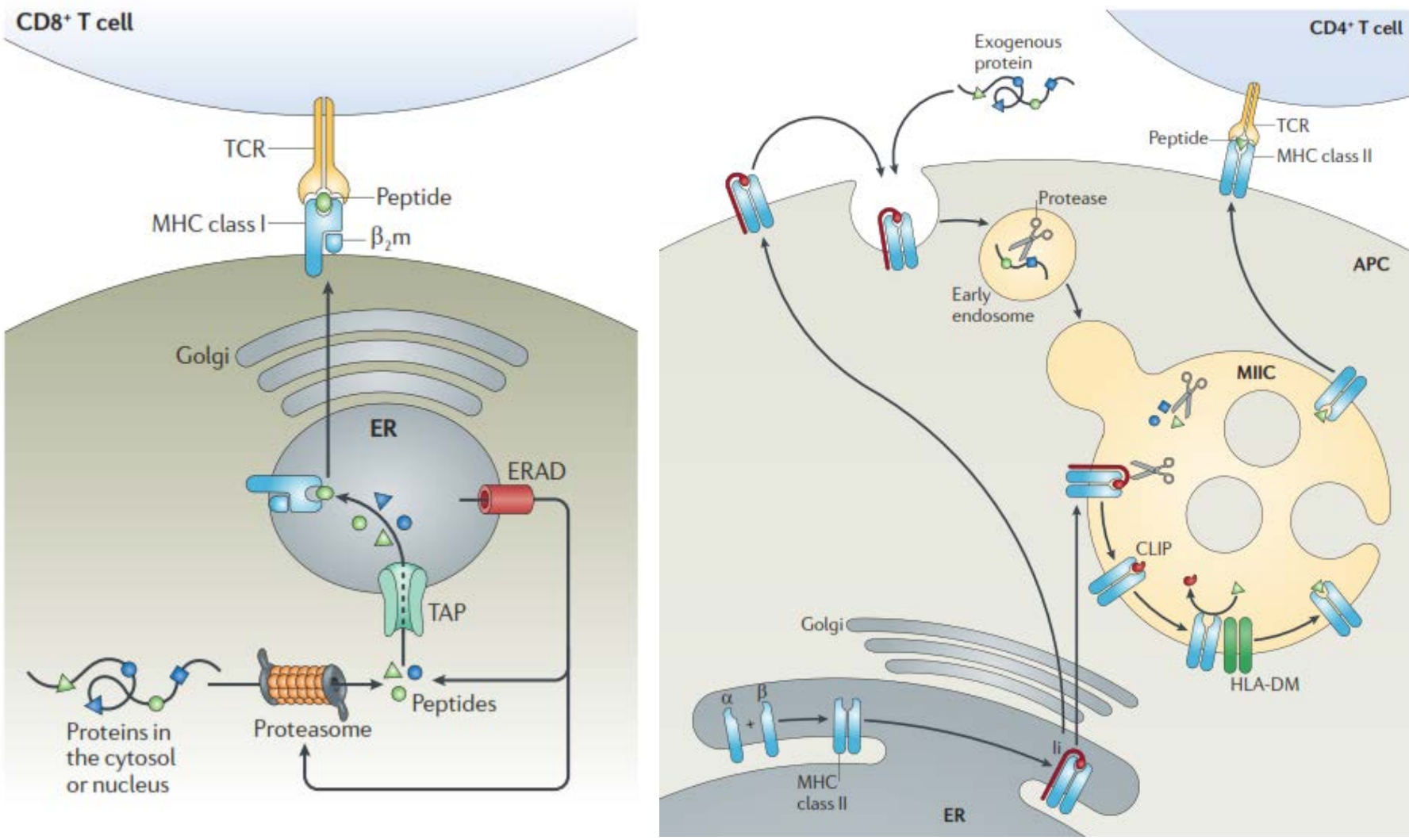



**Panel A shows the principal pathways of generating peptides for loading onto HLA class I molecules**

**Panel B shows the processing of extracellular proteins.**

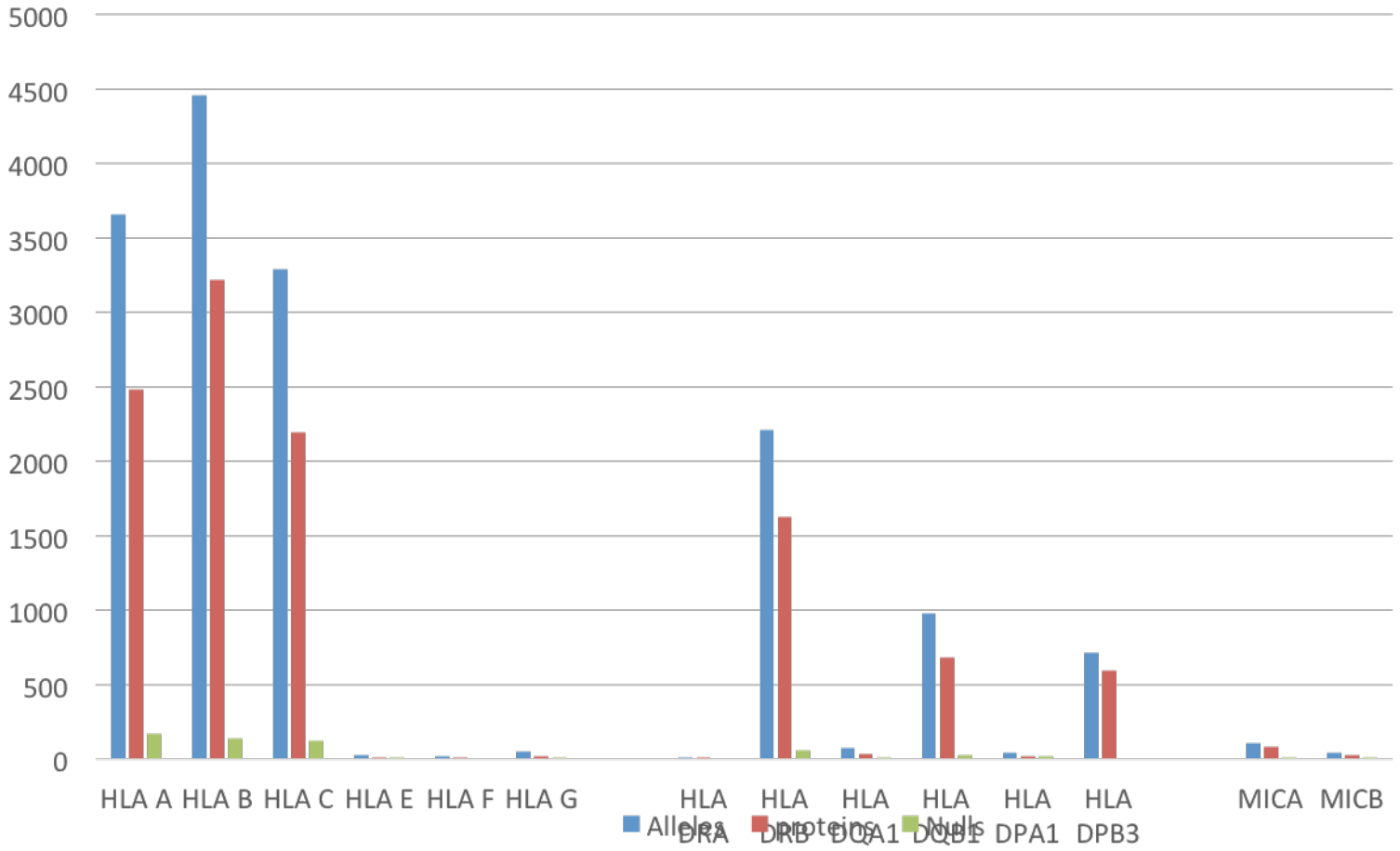
**Klein and Sato. NEJM, 2000**

# Antigen Presentation



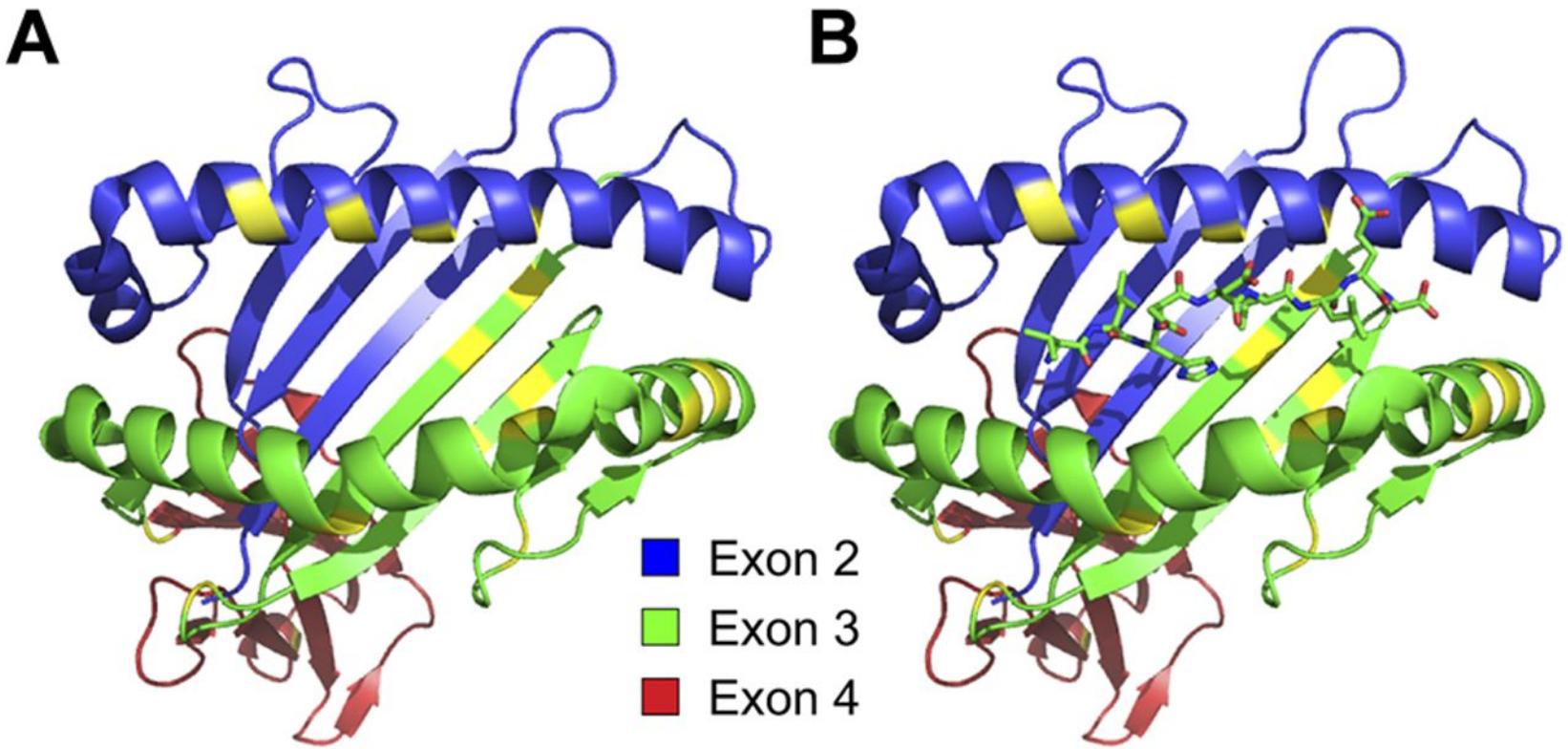

Neefjes et al. Nature Reviews, 2011

# The *HLA-A*, *-B*, *-C*, and *-DRB1* loci are the most polymorphic genes in the entire human genome



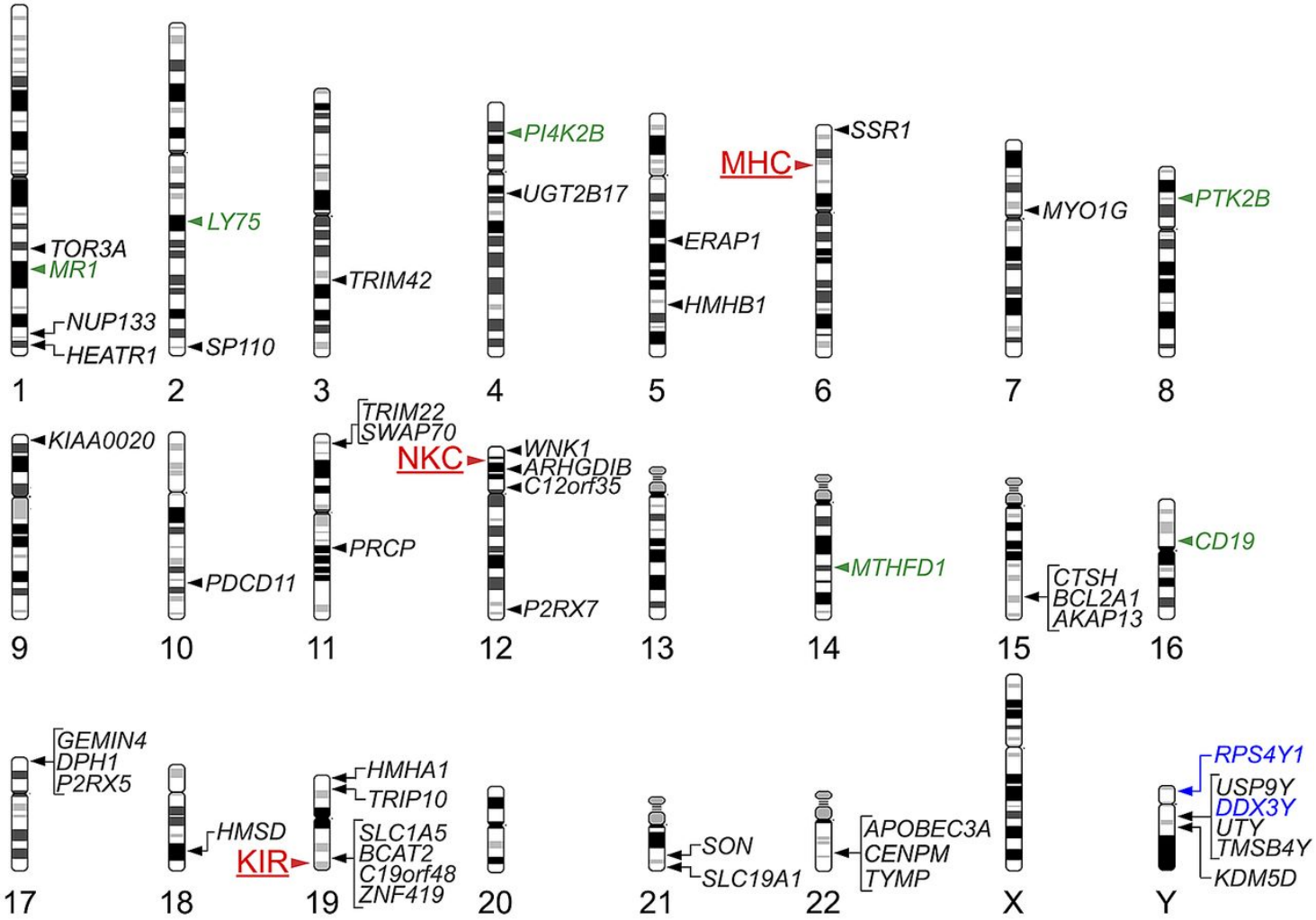

Data were taken from http://www.ebi.ac.uk/imgt/hla/stats.html; accessed January 12, 2017.

# The majority of polymorphic residues that distinguish different alleles of class I and class II molecules are located in positions that influence Peptide binding or interaction with T-cell receptors.




Edus H. Warren et al. Blood 2012;120:2796-2806

# Map of genetic loci that can influence histocompatibility in the allogeneic HCT setting




Edus H. Warren et al. Blood 2012;120:2796-2806

**Table 1. Diseases Commonly Treated with Hematopoietic Stem-Cell Transplantation.**

**Autologous transplantation***

Cancers
- Multiple myeloma
- Non-Hodgkin's lymphoma
- Hodgkin's disease
- Acute myeloid leukemia
- Neuroblastoma
- Ovarian cancer
- Germ-cell tumors

Other diseases
- Autoimmune disorders
- Amyloidosis

**Allogeneic transplantation†**

Cancers
- Acute myeloid leukemia
- Acute lymphoblastic leukemia
- Chronic myeloid leukemia
- Myelodysplastic syndromes
- Myeloproliferative disorders
- Non-Hodgkin's lymphoma
- Hodgkin's disease
- Chronic lymphocytic leukemia
- Multiple myeloma
- Juvenile chronic myeloid leukemia

Other diseases
- Aplastic anemia
- Paroxysmal nocturnal hemoglobinuria
- Fanconi's anemia
- Blackfan–Diamond anemia
- Thalassemia major
- Sickle cell anemia
- Severe combined immunodeficiency
- Wiskott–Aldrich syndrome
- Inborn errors of metabolism

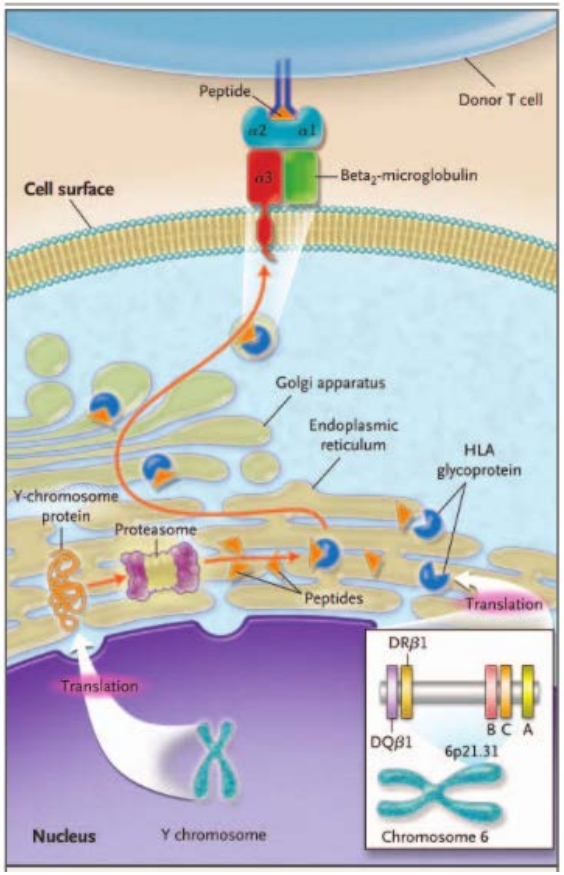
The NEW ENGLAND JOURNAL of MEDICINE

REVIEW ARTICLE

MEDICAL PROGRESS

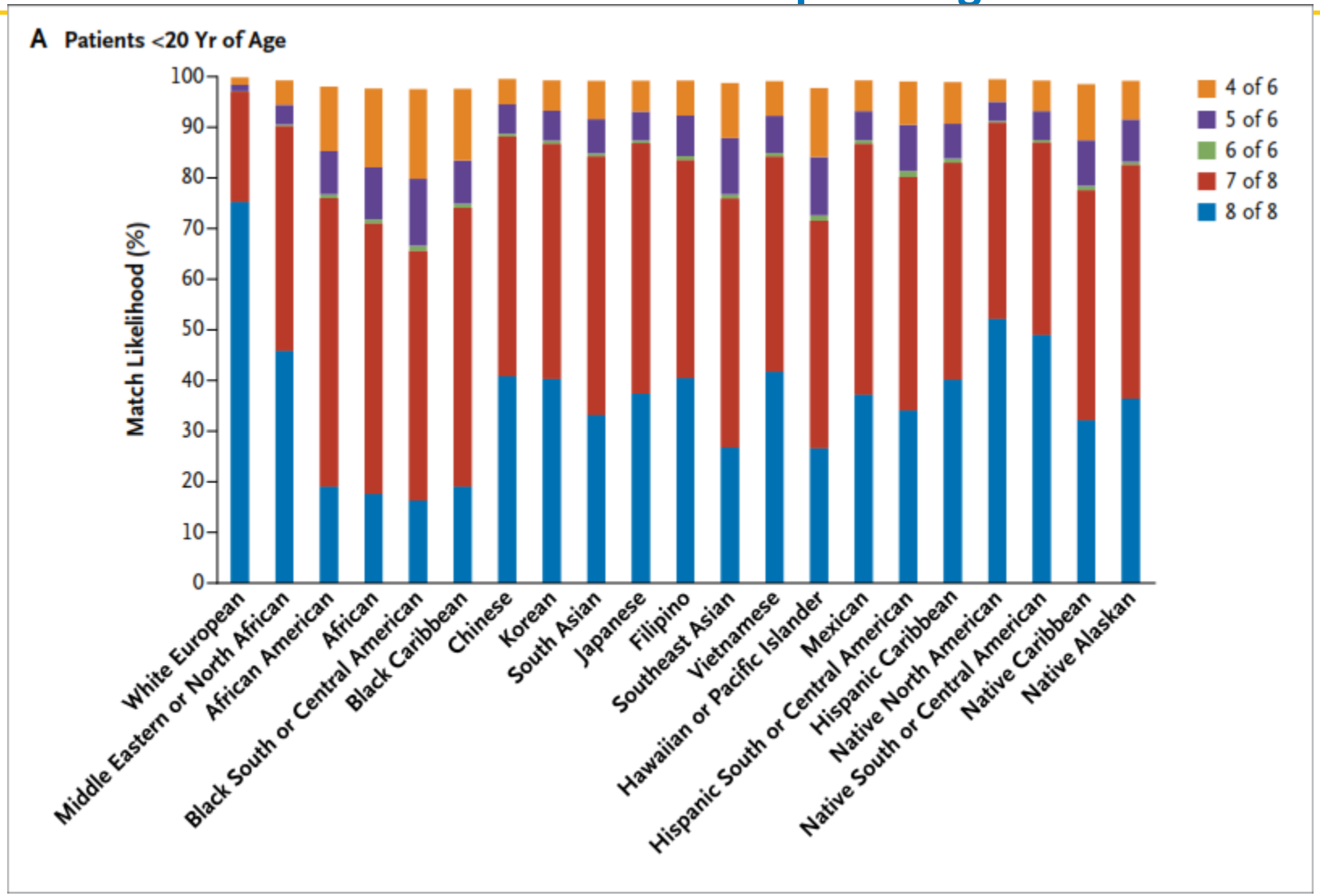
# Hematopoietic Stem-Cell Transplantation

Edward A. Copelan, M.D.



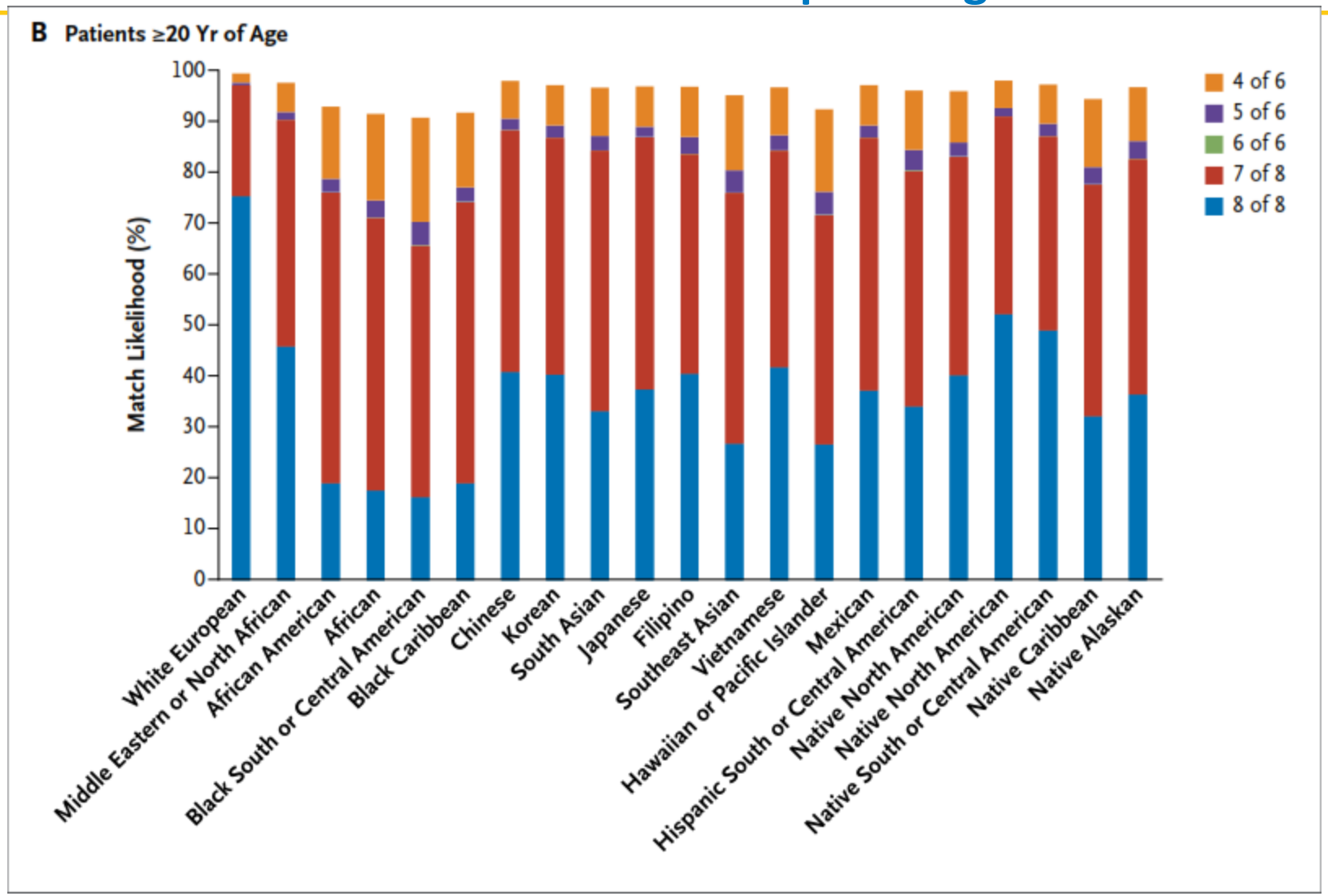

**Copelan. NEJM, 2006**

# Match Likelihoods According to Racial and Ethnic Group and Age




Gragert et al. NEJM, 2014

# Match Likelihoods According to Racial and Ethnic Group and Age




Gragert et al. NEJM, 2014

# Minimal Requirements for Histocompatibility & Immunogenetics laboratory

- Personnel Qualifications
- Quality Assurance
- Testing Methods
- Laboratory Accreditation

# Minimal Requirements for Histocompatibility & Immunogenetics laboratory

## Personnel Qualifications

- The laboratory must employ one or more individuals who meet the qualifications and fulfill the responsibilities of the **Director**, and **Technical Supervisor**
- The **number of staff** must be large enough to carry out the volume and variety of tests required

# Minimal Requirements for Histocompatibility & Immunogenetics laboratory

## Quality Assurance

- Facilities
- Specimen submission and requisition
- Laboratory Procedure Manual
- Proficiency Testing and Competency Evaluation
- Records and test reports
- Testing referred to other laboratories

# Minimal Requirements for Histocompatibility & Immunogenetics laboratory

## Quality assurance

### Facilities

- **Sufficient space** to all procedures carried out
- Proper **maintenance** of equipments, instruments and test systems (with ample records)
- Laboratories performing amplification of nucleic acids must use physical and/or biochemical barriers to **prevent DNA contamination**
- Adequate **lighting and ventilations**

# Minimal Requirements for Histocompatibility & Immunogenetics laboratory

## Quality assurance

### Specimens issues

- The laboratory must have available and follow written **policies and procedures regarding specimen collection**
- The laboratory must assure adequate information contained in the **requisition form**
- The laboratory must maintain a system to ensure reliable **specimen identification**

# Minimal Requirements for Histocompatibility & Immunogenetics laboratory

## Quality assurance

### Laboratory Procedure Manual

- All procedures in use in the laboratory must be detailed in a **procedure manual**, and **immediately available**
- Each procedure must be **reviewed periodically** (according to standards) by the Director and written evidence of this review must be in the manual

# Minimal Requirements for Histocompatibility & Immunogenetics laboratory

## Quality assurance

### Proficiency Testing

- The laboratory must participate in **External proficiency Testing (EPT) program (s)** to cover all the accredited laboratory applications (HLA typing, antibody screening and identification, crosshatching, etc.)

# Minimal Requirements for Histocompatibility & Immunogenetics laboratory

## Quality assurance

### Competency Evaluation

- The laboratory Director and the technical staff must participate in pertinent continuing education

# Minimal Requirements for Histocompatibility & Immunogenetics laboratory

## Quality assurance

### Records and test reports

- The laboratory must maintain records of subjects tested for a period dictated by national laws and accreditation standards

# Minimal Requirements for Histocompatibility & Immunogenetics laboratory

## Quality assurance

### Records and test reports

- The report must contain:
  - a. Name of the individual tested or unique identifier(s) and relationship to the patient if applicable.
  - b. Date(s) of collection of sample(s)
  - c. Date of the report

# Minimal Requirements for Histocompatibility & Immunogenetics laboratory

## Quality assurance

### Records and test reports

- The report must contain:

  d. Test results.

  e. Techniques used.

  f. Appropriate interpretations and the signature of the Laboratory Director, or, in his/her absence, by a designee

# Minimal Requirements for Histocompatibility & Immunogenetics laboratory

## Quality assurance

### Testing referral

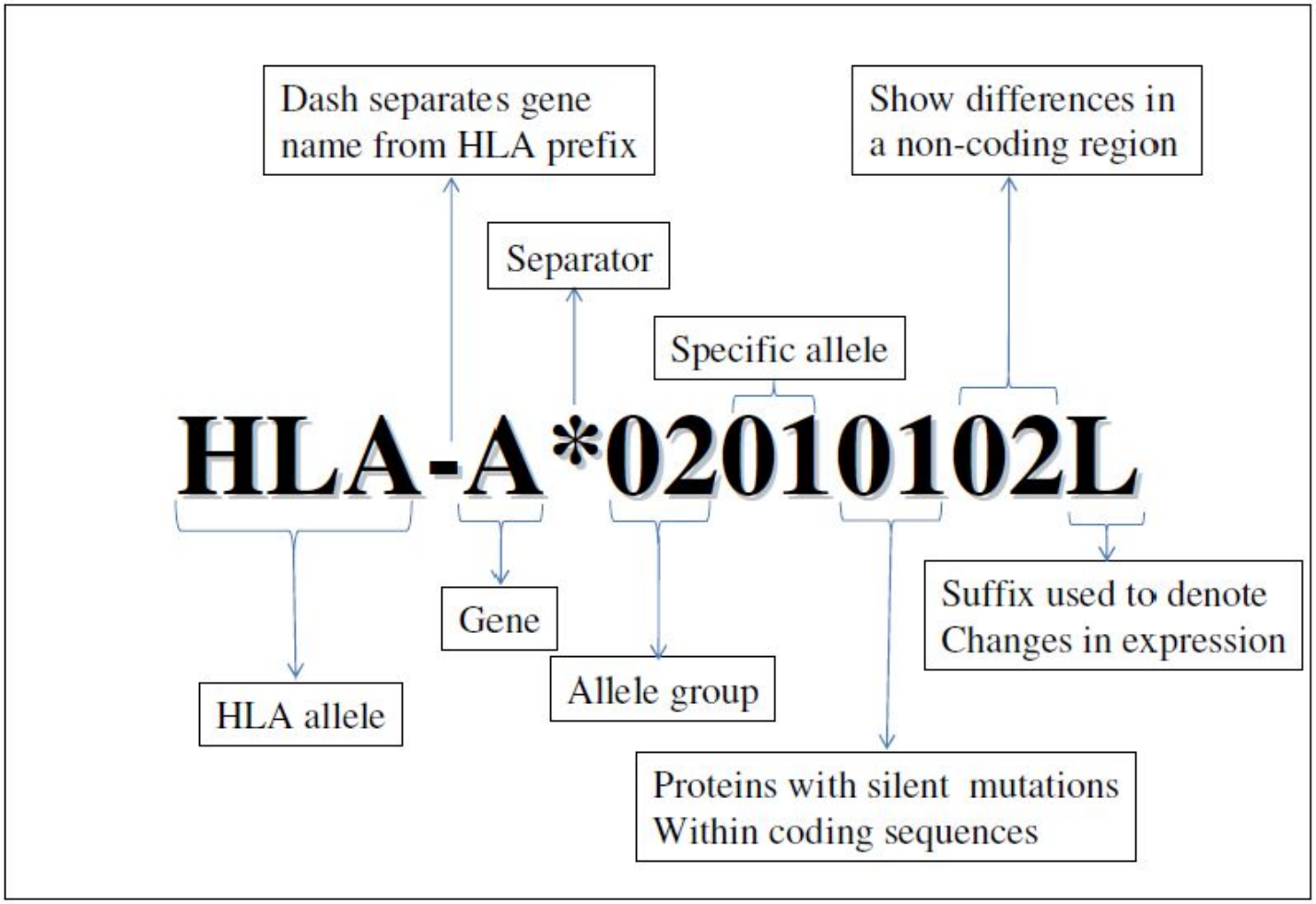
- An accredited laboratory may engage another laboratory to perform testing not done by the primary laboratory

# Minimal Requirements for Histocompatibility & Immunogenetics laboratory

## Testing Methods

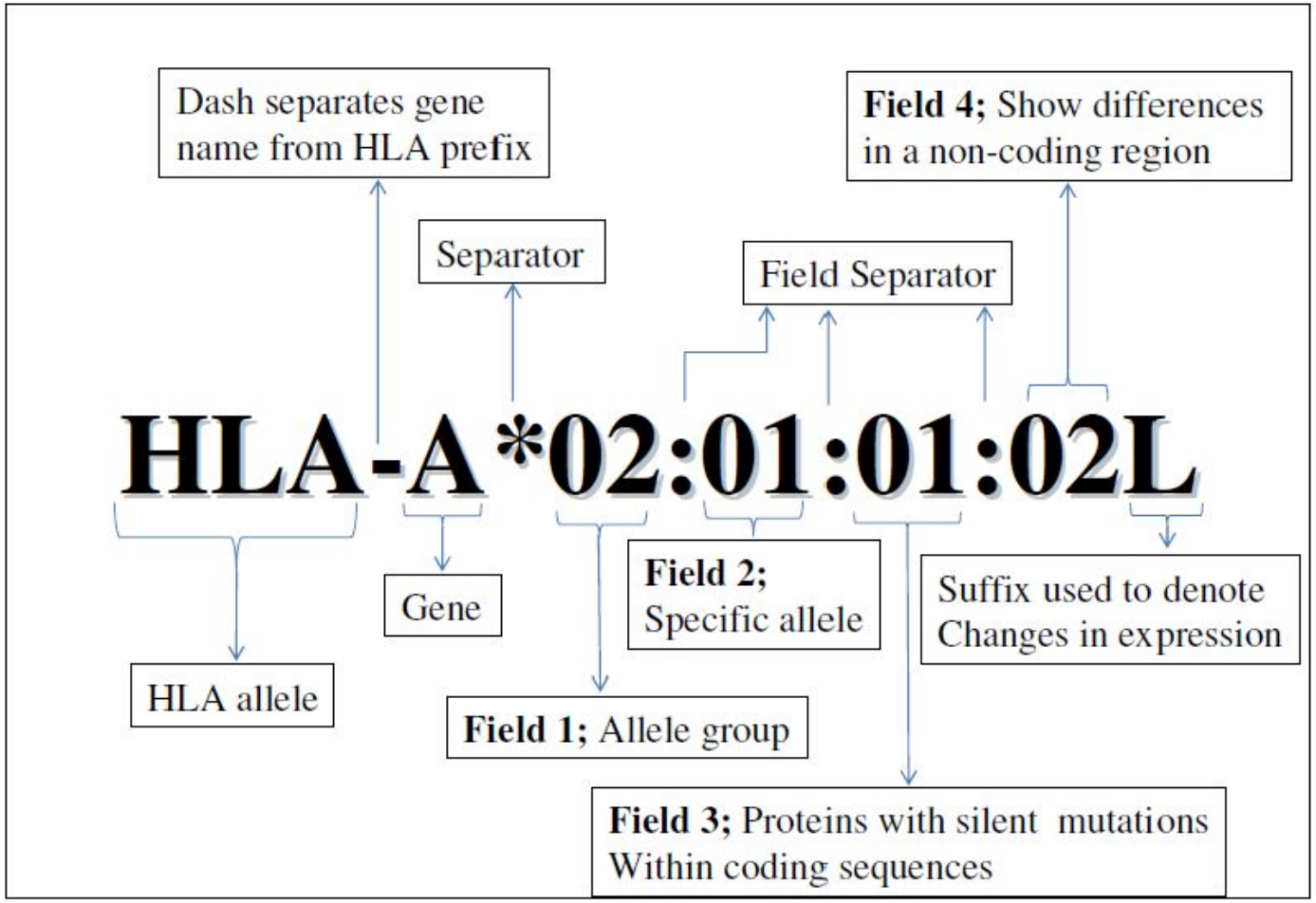
- Serological vs. Molecular-based HLA typing
  - HLA Nomenclature
  - Resolution level
  - Haplotype assignment
- Chimerism studies

# HLA Nomenclature, old




Bhadran Bose et al Transplantation Antigens and Histocompatibility Matching, 2013

# HLA Nomenclature, new




Bhadran Bose et al Transplantation Antigens and Histocompatibility Matching, 2013

# HLA Typing Methods

| | |
|---|---|
| 1950s | • Discovery of HLA system |
| 1960s | • Serological typing |
| 1980s | • First HLA genes cloned, sequenced |
| 1990s | • DNA/PCR based HLA typing |
| 1999 | • Sequence entire MHC (HGP) |
| 2000 | • Database of all HLA alleles |
| 2000s | • SBT, Luminex SSO |
| 2013 | • Next Generation Sequencing |

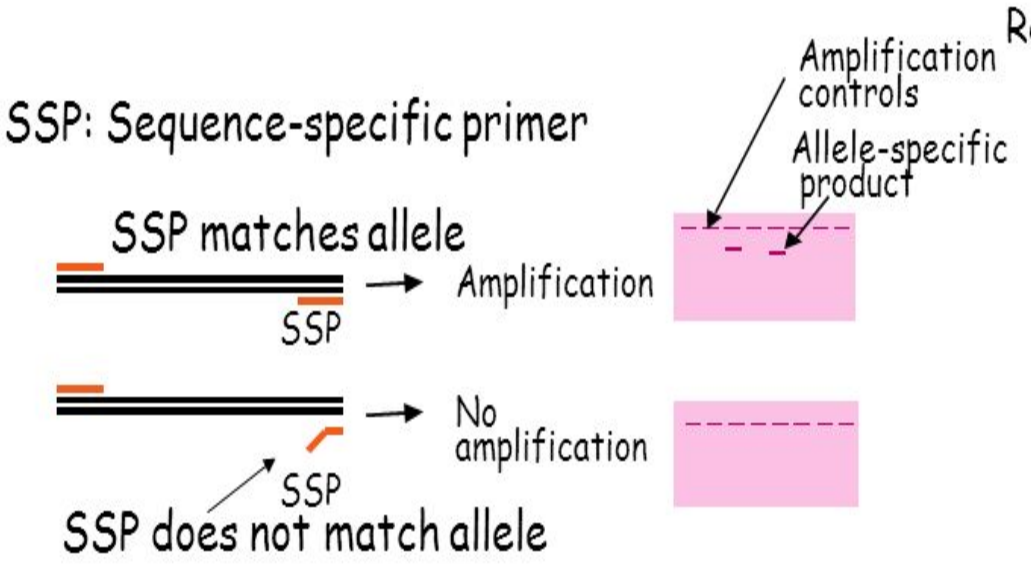
# Levels of Resolution

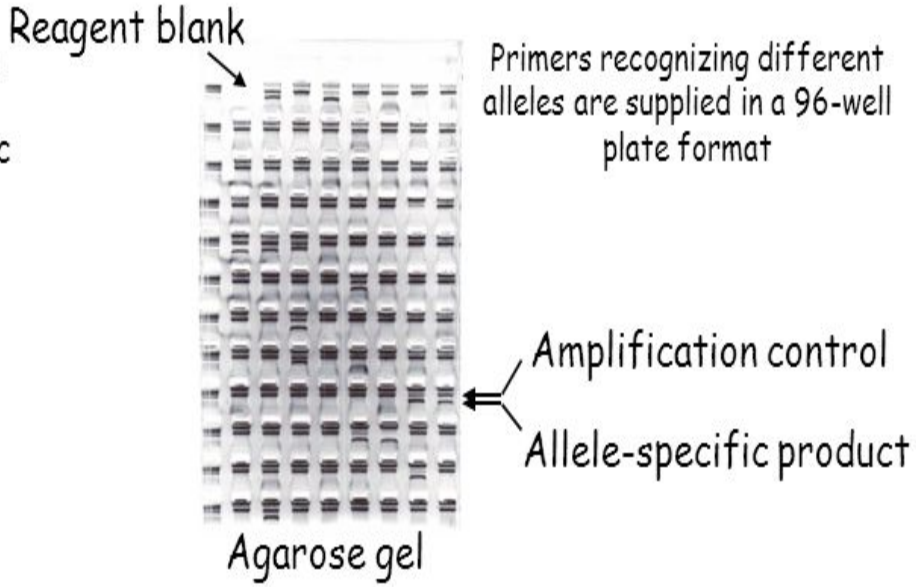
| | |
|---|---|
| **Low resolution (2 digit)** | • same serotype group (e.g. A*02) |
| **Intermediate resolution (allele string)** | • identifies alleles that have common sequence (e.g. A*02:05/08/22) |
| **High resolution (minimum 4 digit)** | • identifies single allele e.g. A*02:05 |

# HLA TYPING

## DNA-BASED TYPING METHODS

### SSP-PCR: Sequence-specific PCR (allele-specific primers)




Very rapid test that can be performed in 3-4 hours from the time a sample is received.

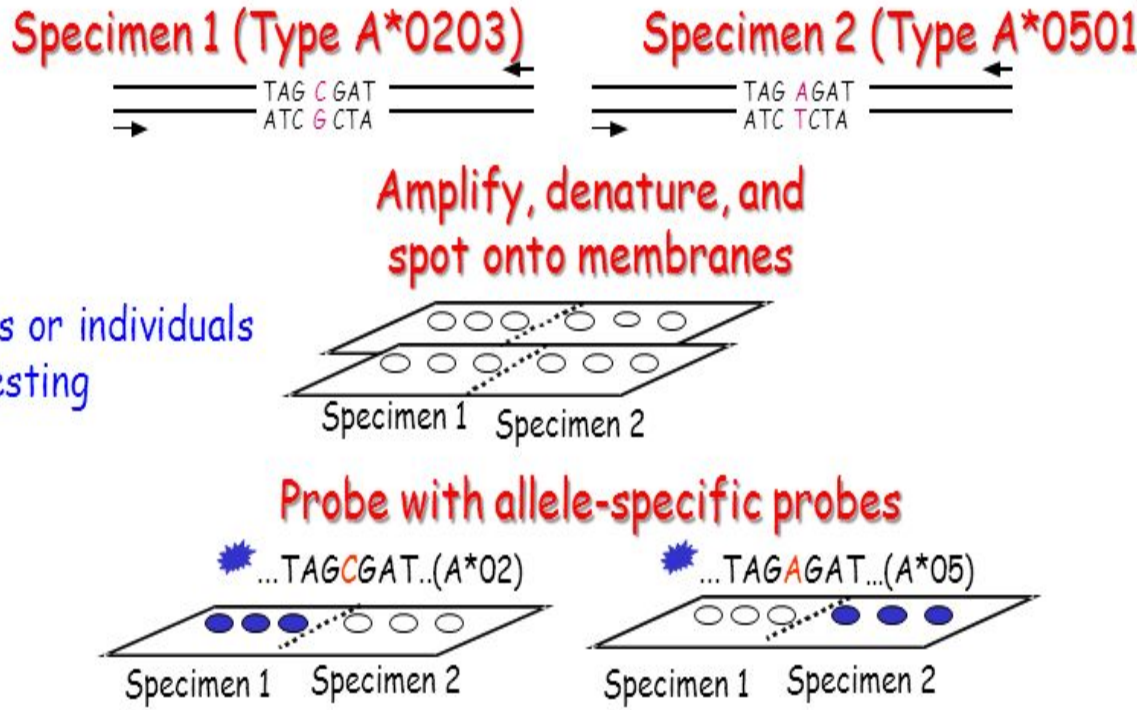
PCR-SSP is used for typing deceased organ donors when speed is an important consideration.

# HLA TYPING

## DNA-BASED TYPING METHODS

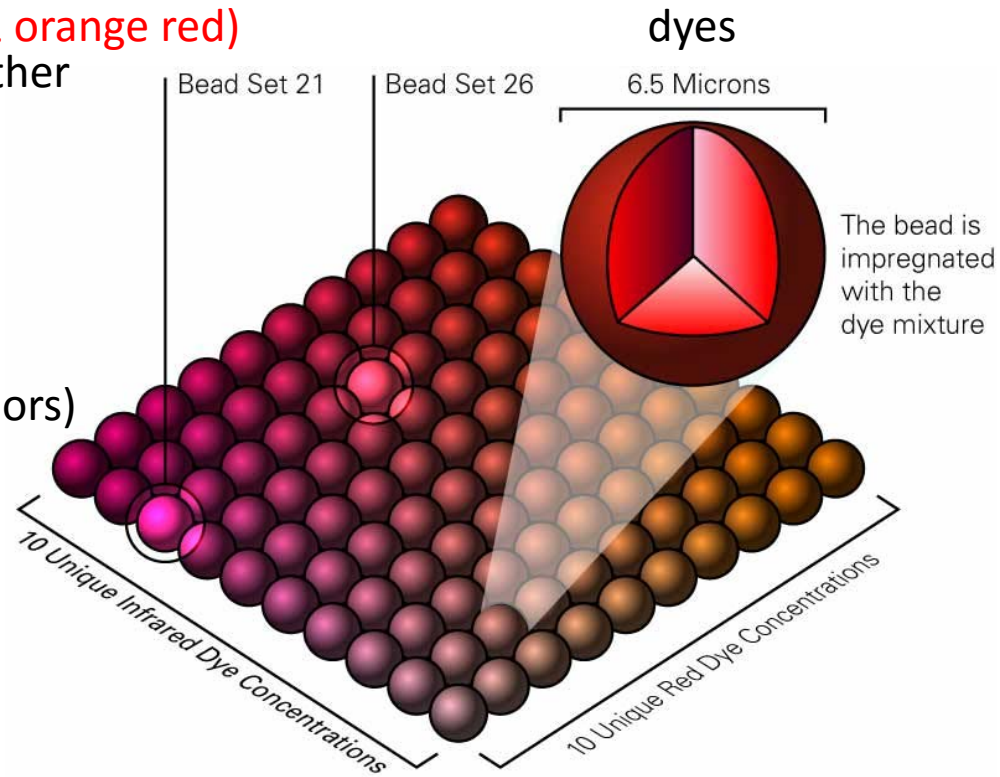
**PCR-SSOPH:** Sequence-specific oligonucleotide probe hybridization

- Intermediate resolution
- Sreening test to identify potential donors or individuals who may later require higher resolution testing
- High volume, relatively low cost

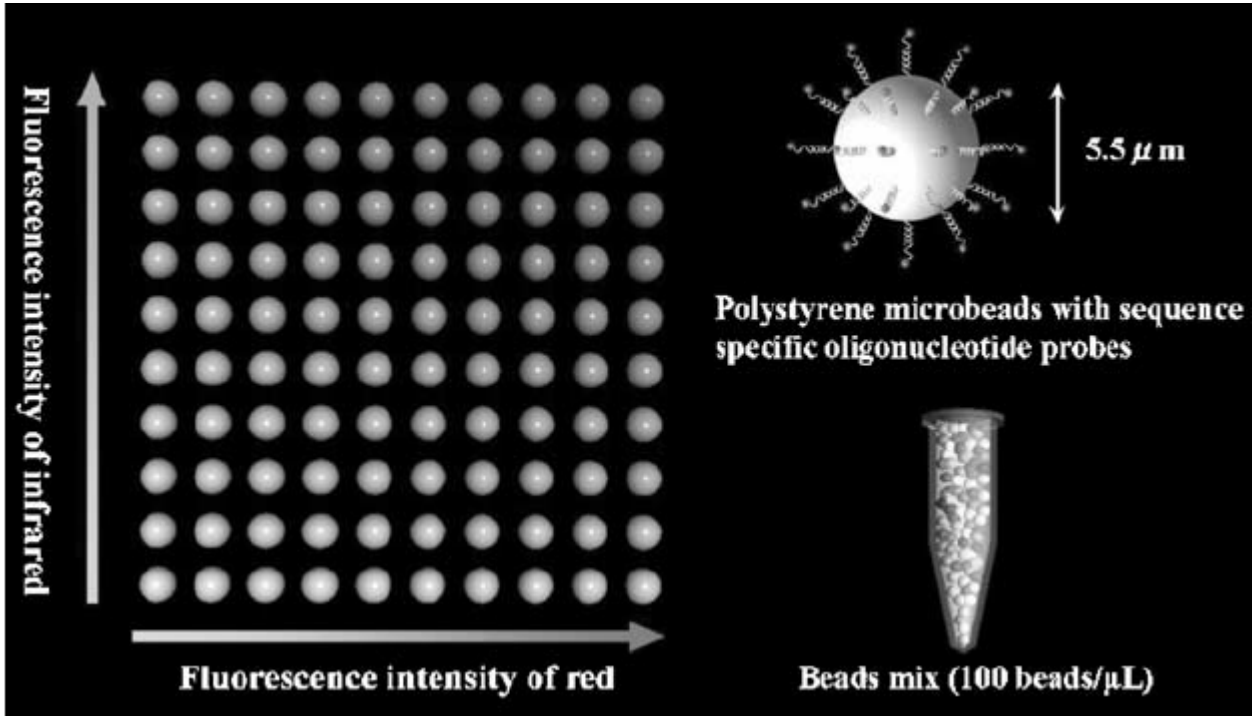


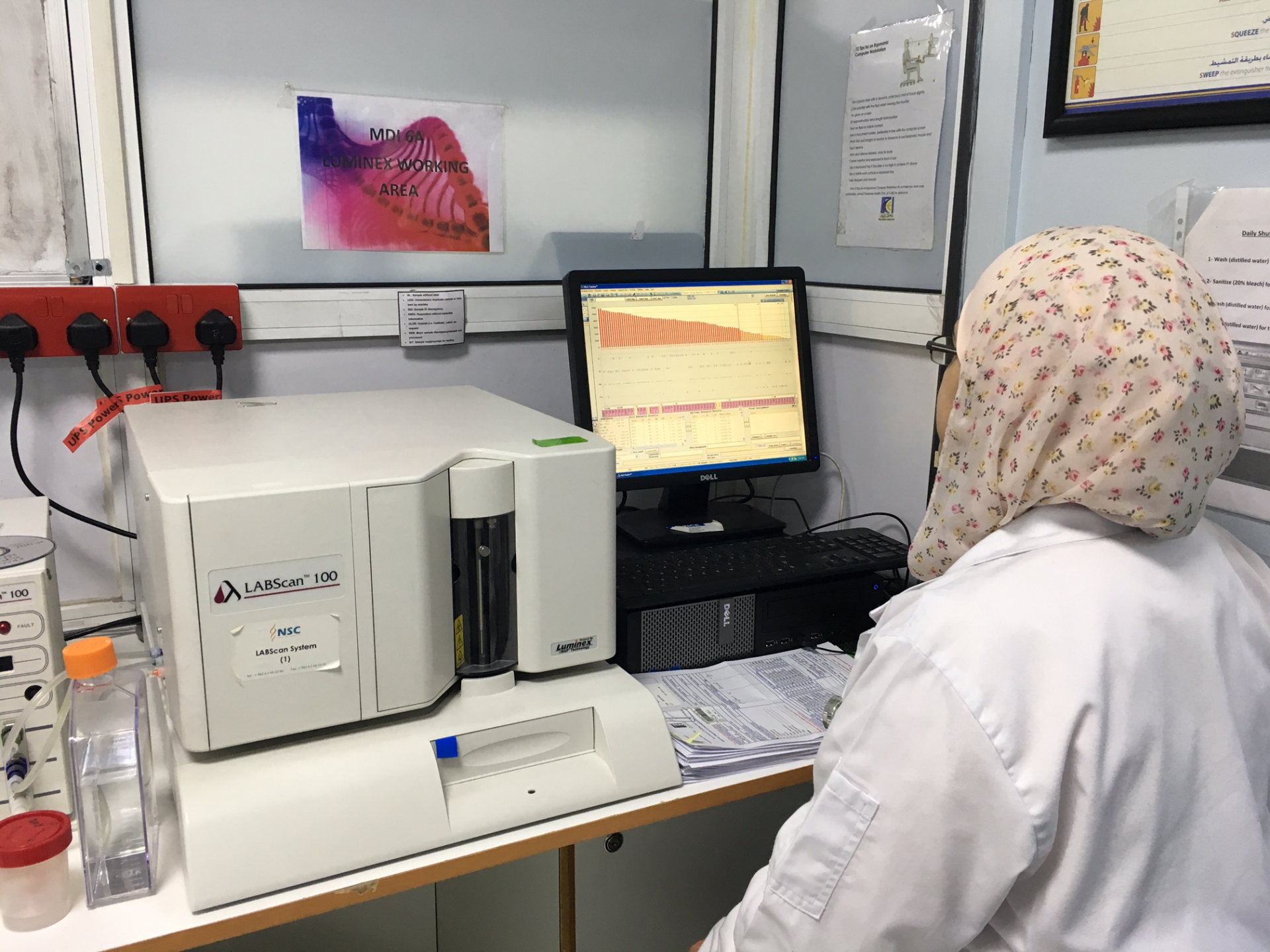
# xMAP® Technology, Multi - Analyte Profiling

- **5.6 micron (non magnetic) or 6.5 micron (magnetic) beads**
- **Up to 100 (500) different beads/well**
  - Bead color = spectral address
  - A combination of red and infrared (& orange red) dyes distinguishes one bead set from another
- **Small bead size allows:**
  - **Liquid suspension assay with**
    - a high surface-to-volume-ratio
    - fast kinetics (liquid-phase behaviors)



# xMAP® Technology, Multi - Analyte Profiling




MDI 6A
LUMINEX WORKING
AREA
LABScan™ 100
NSC
LABScan System
(1)
Luminex
DELL
DELL
UPS Power
UPS Power

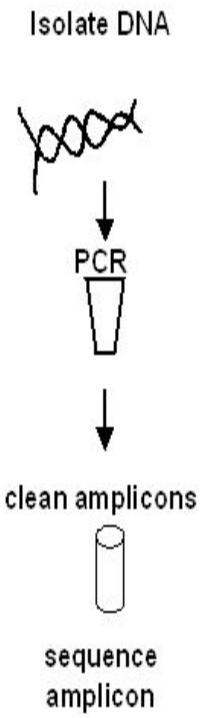
# HLA TYPING

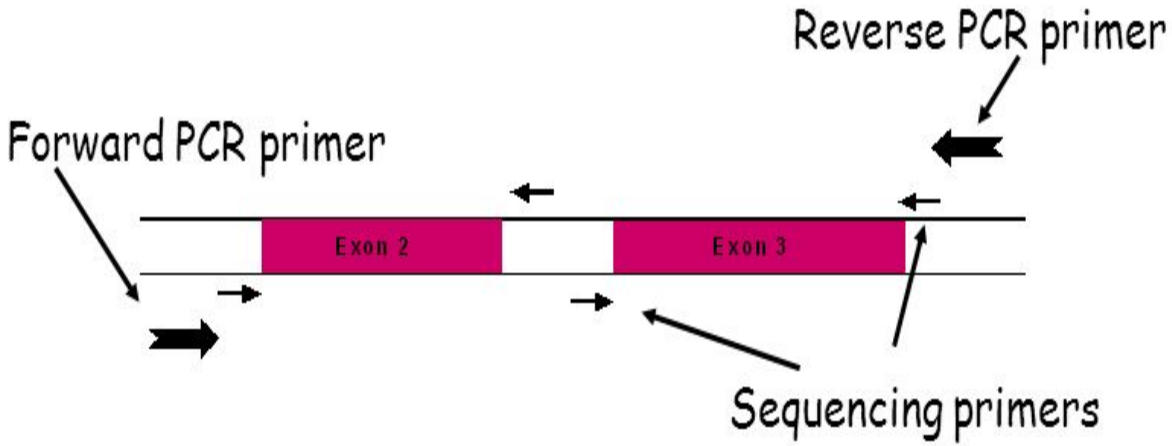
## DNA-BASED TYPING METHODS

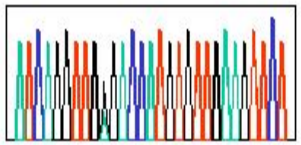
### SBT: Sequence-based typing

Polymorphic regions are amplified by PCR and then sequenced

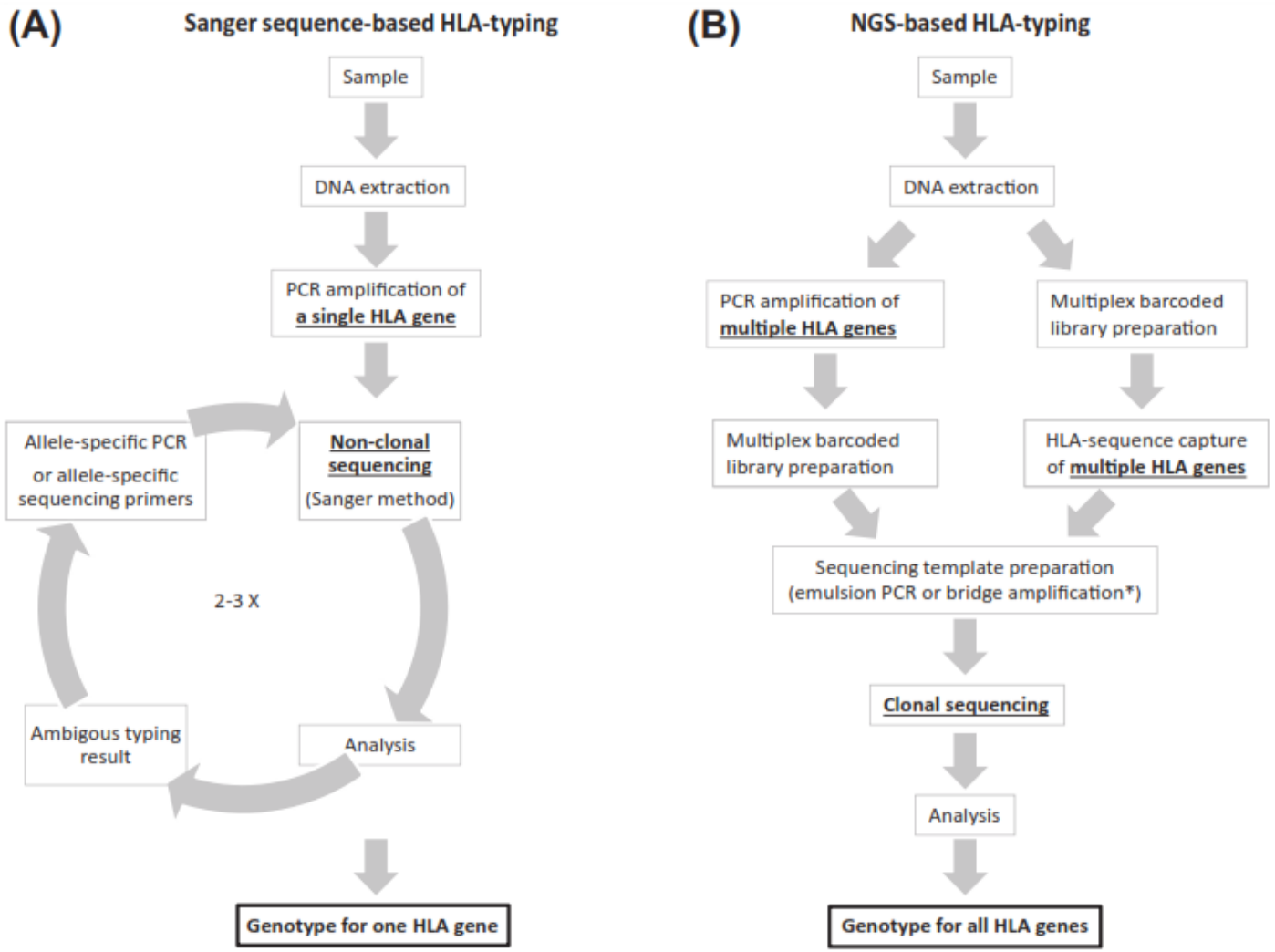
The highest resolution HLA typing



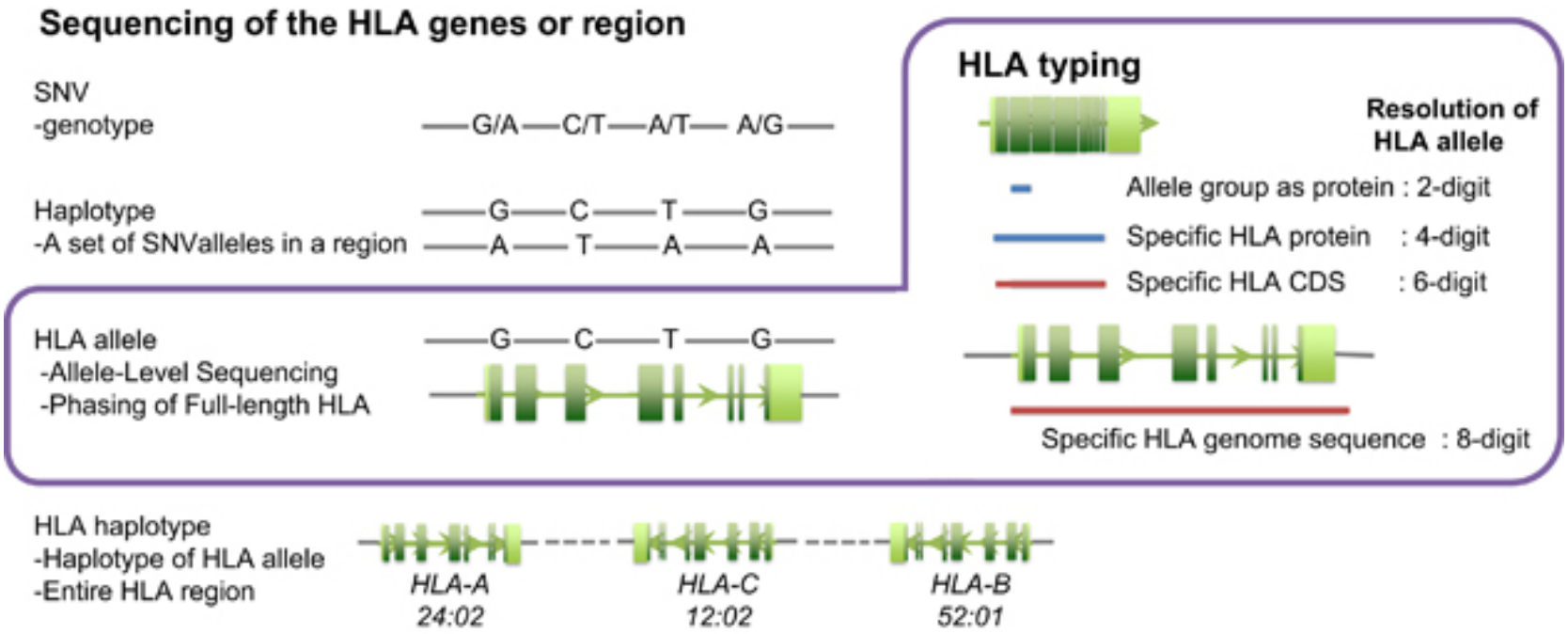



Sequences are compared to reference sequences for previously assigned alleles



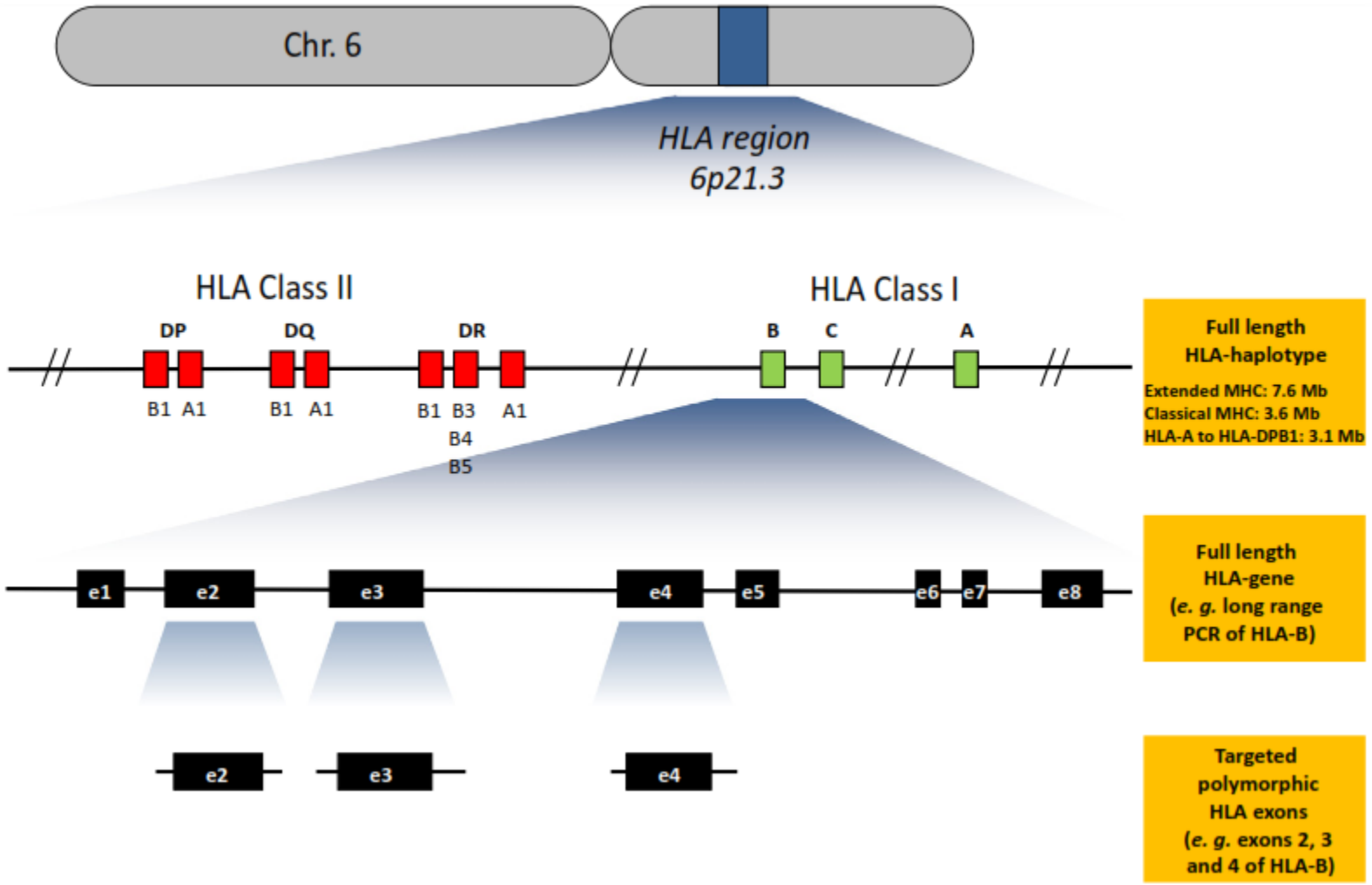

R. Carapito et al. / Human Immunology 77 (2016) 1016–1023

# Sequence-based Typing




*Journal of Human Genetics* (2015) **60,** 665–673

# Targeted regions of the HLA by NGS approaches




R. Carapito et al. / Human Immunology 77 (2016) 1016–1023

Contents lists available at ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

Invited Review

# Clinical validation of NGS technology for HLA: An early adopter's perspective

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*University of North Carolina at Chapel Hill School of Medicine, Department of Pathology and Laboratory Medicine, Chapel Hill, NC 27514, United States*

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ABSTRACT

Clinical validation of NGS for HLA typing has been a topic of interest with many laboratories investigating the merits. NGS has proven effective at reducing ambiguities and costs while providing more detailed information on HLA genes not previously sequenced. The ability of NGS to multiplex many patients within a single run presents unique challenges and sequencing new regions of HLA genes requires application of our knowledge of genetics to accurately determine HLA typing. This review represents my lab-

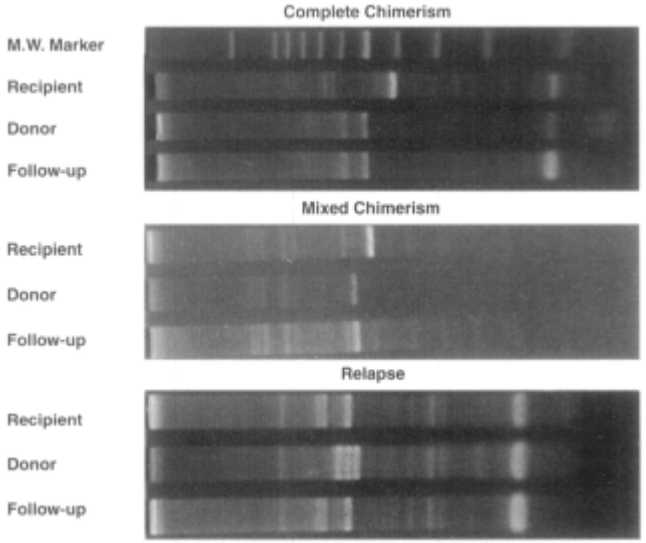
- **NGS can reduce the ambiguity rate, cost, and TAT for HLA typing.**
- **Validation of NGS for clinical HLA typing is challenging due to the numerous issues, including: sample types, complexity of the HLA genes, reliance on software for accurate HLA typing, and many more.**

HEMATOPATHOLOGY
*Original Article*

# The Use of Amplified Variable Number of Tandem Repeats (VNTR) in the Detection of Chimerism Following Bone Marrow Transplantation

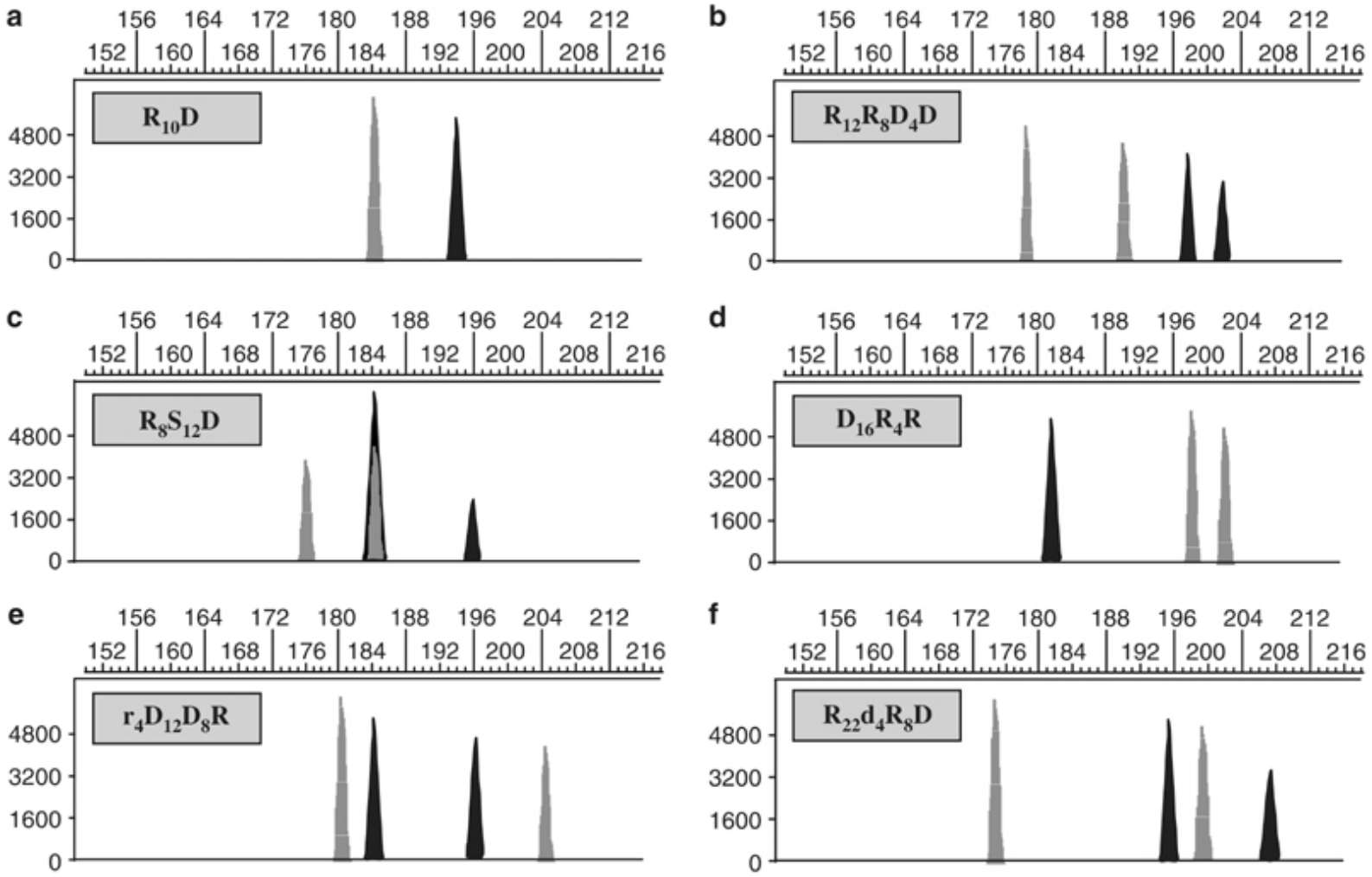
## *A Comparison With Restriction Fragment Length Polymorphism (RFLP) by Southern Blotting*

JOSEPH J. SREENAN, MD, JAMES D. PETTAY, MT(ASCP), ABDELGHANI TBAKHI, MD, GRIGORIOS TOTOS, MD, LINDA M. SANDHAUS, MD, MICHAEL L. MILLER, DO, BRIAN BOLWELL MD, AND RAYMOND R. TUBBS, DO




**Am J Clin Pathol 1997;107:292-298.**

# STR-PCR-based chimerism testing



Biology of Blood and Marrow Transplantation 7:473-485 (2001)


## REVIEW

# Establishment of Complete and Mixed Donor Chimerism After Allogeneic Lymphohematopoietic Transplantation: Recommendations From a Workshop at the 2001 Tandem Meetings

*Joseph H. Antin,[1] Richard Childs,[2] Alexandra H. Filipovich,[3] Sergio Giralt,[4] Stephen Mackinnon,[5] Thomas Spitzer,[6] Daniel Weisdorf[7]**

[1]Dana-Farber Cancer Institute, Boston, Massachusetts; [2]National Heart, Lung, and Blood Institute, Bethesda, Maryland; [3]Children's Hospital Medical Center, Cincinnati, Ohio; [4]M. D. Anderson Cancer Center, Houston, Texas; [5]University College London, London, United Kingdom; [6]Massachusetts General Hospital, Boston, Massachusetts; [7]University of Minnesota Medical School, Minneapolis, Minnesota

Correspondence and reprint requests: Joseph H. Antin, MD, Dana-Farber Cancer Institute, 44 Binney St, Boston, MA

Leukemia (2012) **26**, 1821 – 1828

**www.nature.com/leu**

**ORIGINAL ARTICLE**

# The EuroChimerism concept for a standardized approach to chimerism analysis after allogeneic stem cell transplantation

T Lion[1], F Watzinger[1], S Preuner[1], H Kreyenberg[2], M Tilanus[3], R de Weger[4], J van Loon[4], L de Vries[4], H Cavé[5], C Acquaviva[5], M Lawler[6], M Crampe[6], A Serra[7], B Saglio[7], F Colnaghi[8], A Biondi[8], JJM van Dongen[9], M van der Burg[9], M Gonzalez[10], M Alcoceba[10], G Barbany[11], M Hermanson[11], E Roosnek[12], C Steward[13], J Harvey[14], F Frommlet[15] and P Bader[2] on behalf of the EuroChimerism Consortium (EU-Project number: QLG1-CT-2002-01485)

Hematopoietic stem cell transplantation is becoming an increasingly important approach to treatment of different malignant and non-malignant disorders. There is thus growing demand for diagnostic assays permitting the surveillance of donor/recipient chimerism posttransplant. Current techniques are heterogeneous, rendering uniform evaluation and comparison of diagnostic results between centers difficult. Leading laboratories from 10 European countries have therefore performed a collaborative study supported by a European grant, the EuroChimerism Concerted Action, with the aim to develop a standardized diagnostic methodology for the detection and monitoring of chimerism in patients undergoing allogeneic stem cell transplantation. Following extensive analysis of a large set of microsatellite/short tandem repeat (STR) loci, the EuroChimerism (EUC) panel comprising 13 STR markers was established with the aim to optimally meet the specific requirements of quantitative chimerism analysis. Based on highly stringent selection criteria, the EUC panel provides multiple informative markers in any transplant setting. The standardized STR-PCR tests permit detection of donor- or recipient-derived cells at a sensitivity ranging between 0.8 and 1.6%. Moreover, the EUC assay facilitates accurate and reproducible quantification

Hindawi Publishing Corporation
Biotechnology Research International
Volume 2016, Article ID 8589270, 6 pages
http://dx.doi.org/10.1155/2016/8589270

Hindawi

*Research Article*

# Chimerism Analysis of Cell-Free DNA in Patients Treated with Hematopoietic Stem Cell Transplantation May Predict Early Relapse in Patients with Hematologic Malignancies

**Mahmoud Aljurf,[1] Hala Abalkhail,[2] Amal Alseraihy,[3] Said Y. Mohamed,[1] Mouhab Ayas,[3] Fahad Alsharif,[1] Hazza Alzahrani,[1] Abdullah Al-Jefri,[3] Ghuzayel Aldawsari,[1] Ali Al-Ahmari,[3] Asim F. Belgaumi,[3] Claudia Ulrike Walter,[2] Hassan El-Solh,[3] Walid Rasheed,[1] and Maher Albitar[4]**

[1] *Oncology Centre, King Faisal Specialist Hospital and Research Centre, Riyadh 11211, Saudi Arabia*
[2] *Pathology and Laboratory Medicine, King Faisal Specialist Hospital and Research Centre, Riyadh 11211, Saudi Arabia*

# Minimal Requirements for Histocompatibility & Immunogenetics laboratory

## Laboratory Accreditation

- To certify that the laboratory is meeting the minimum requirements as per given sets of standards
  - ASHI
  - EFI
  - CAP
  - ISO 15189


Thank you