



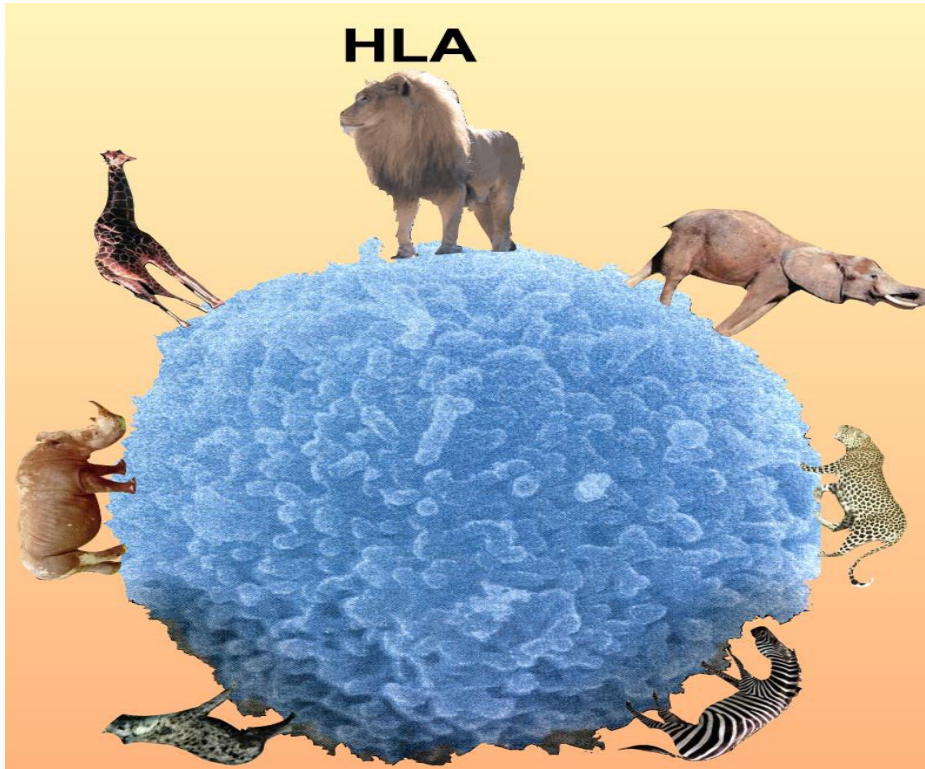
SAPIENZA  
UNIVERSITÀ DI ROMA

# HLA, Histocompatibility Testing and Transplantation

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26th April 2022

# The cell surface is a jungle !



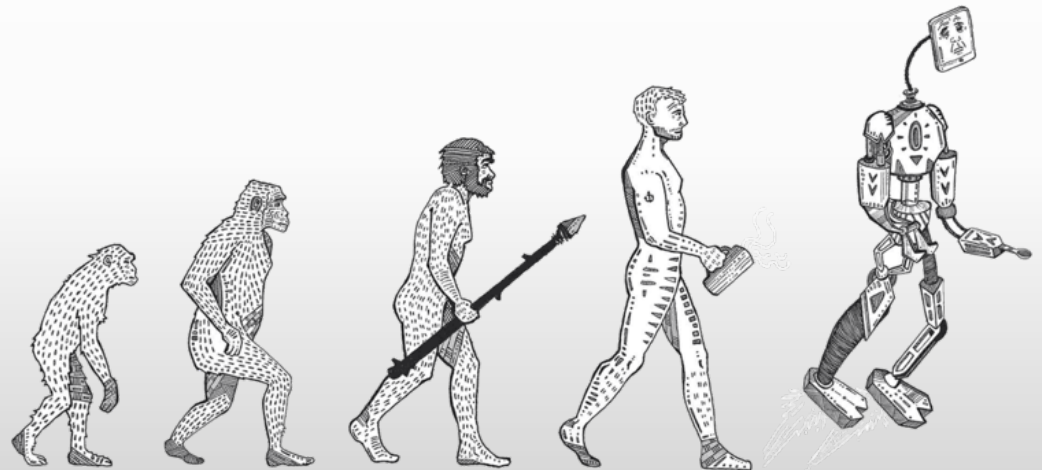
**Major histocompatibility complex (MHC),** group of genes that code for **proteins** found on the **surfaces of cells** that help the immune system to recognize foreign substances.

**In humans the complex is also called the human leukocyte antigen (HLA) system.**



# The HLA system

The human MHC is called the **HLA (Human Leukocyte Antigen)** system because these antigens were first identified and characterized using alloantibodies obtained from multiparous women and previously transfused patients, against leukocytes.



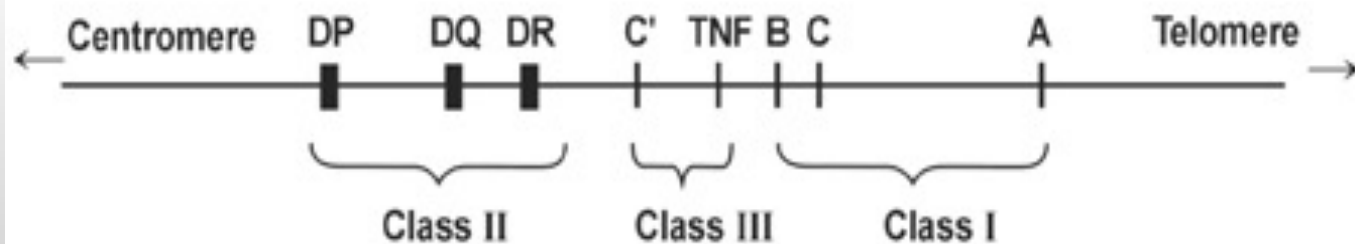
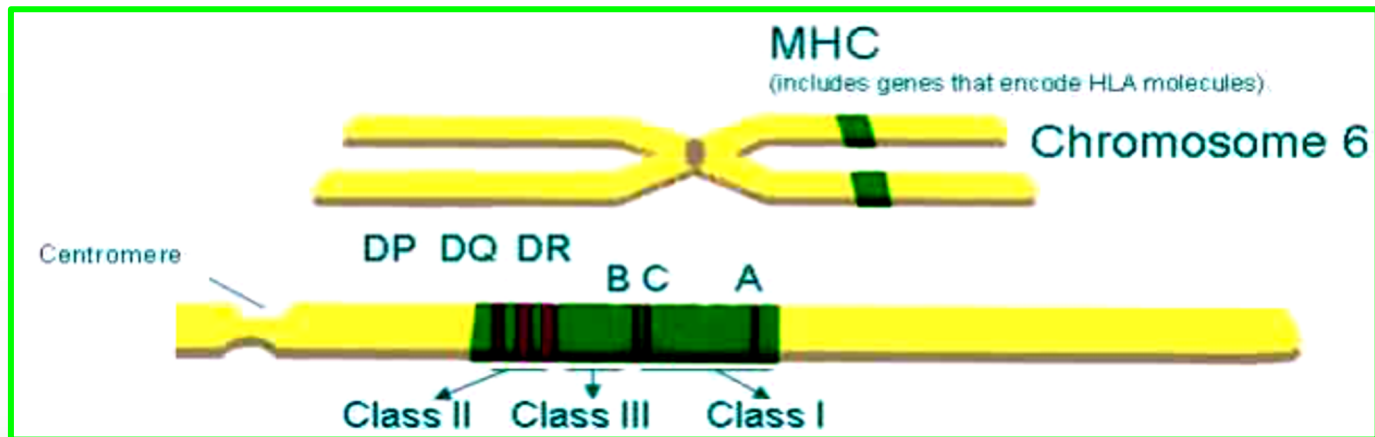


# Where?

## Genomic organization of the HLA system

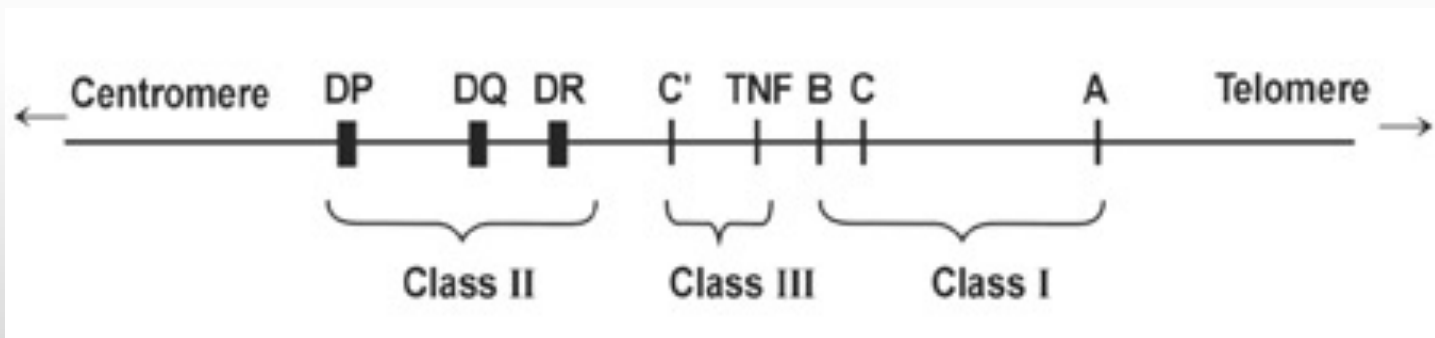
The HLA system maps to the short arm of chromosome 6 (6p21) and spans approximately 3,600 kb of DNA.

It is divided into three regions.

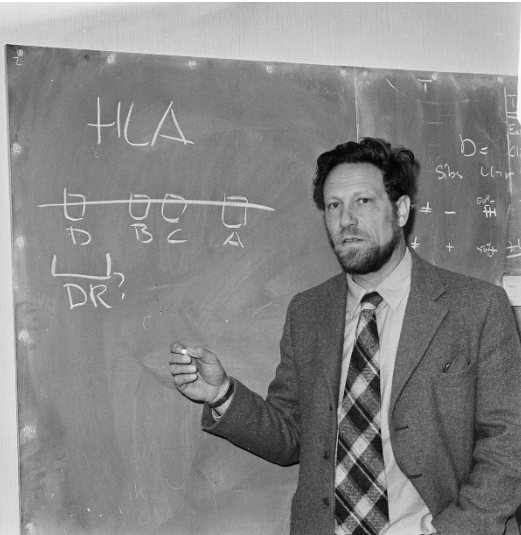


# Genomic organization of the HLA system

- ✓ The class I region contains the classical HLA-A, HLA-B, and HLA-C genes that encode the heavy chains of class I molecules.
- ✓ The class II region consists of a series of sub-regions (A and B), accounting for six main genes: HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1, HLA-DRA and HLA-DRB1.
- ✓ The class III region contains genes for complement components (C2, C4, factor B), 21-hydroxylase, TNF and others.



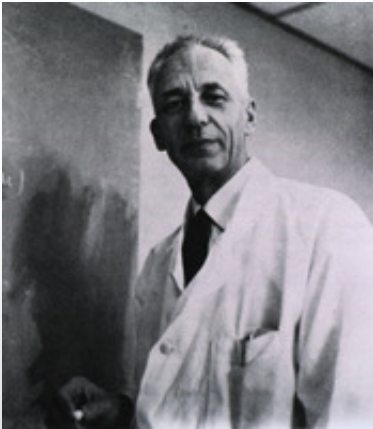
# A short history of HLA



**Jon van Rood**

Three papers appeared in **1958** by **Jean Dausset, Jon Van Rood and Rose Payne**, all describing **antibodies in human sera from multitransfused patients or multiparous women, reacting with leucocytes from many individuals who were tested.**

Antibodies in this sera detected a polymorphic system of antigens on human leucocytes.

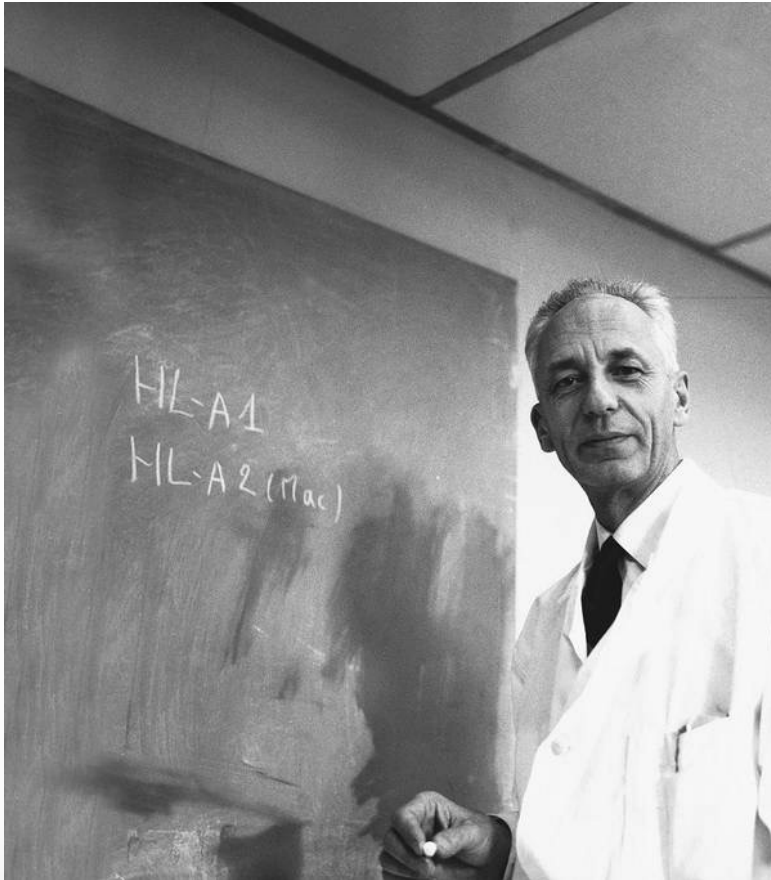


**Jean Dausset 1916-2009**  
**1980 Nobel Prize**



**Rose Payne**  
**1909- 1999**

# ***A short history of HLA***



Jean Dausset 1916-2009  
1980 Nobel Prize

**Jean Dausset**  
**described 1958**  
**the first HLA antigen named MAC**  
(an acronym made up of the initials of  
the first three donors with whom the  
serum he was testing did not react)  
For his discovery,  
**he received**  
**the Nobel Prize in 1980**

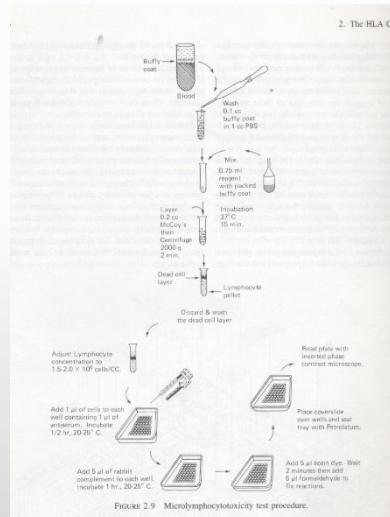
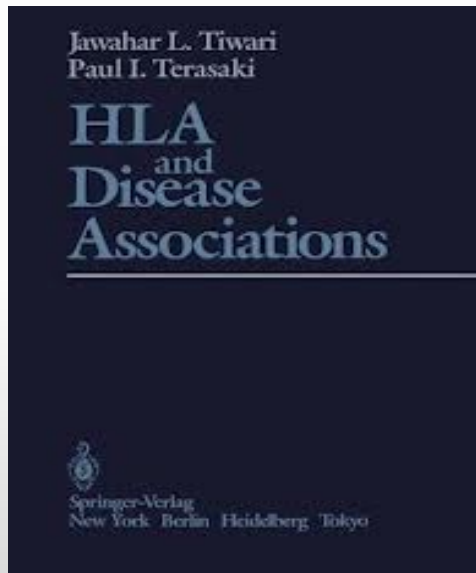
*‘Finally, in a more long-time perspective, the study of leucocyte antigens might become of great importance in tissue transplantation, in particular in bone marrow transplantation’*

# The Major Histocompatibility Complex



In 1964, Terasaki developed the microcytotoxicity test, a tissue-typing test for organ transplant donors and recipients to identify HLA

The test was adopted to detect routinely HLA antigens as the international standard for tissue typing.



This test is still used nowadays even if the overall tendency is to abandon serology

# HLA

- HLA CLASS I -> SURFACE OF ALL NUCLEATED CELLS
- HLA CLASS II -> B LYMPHOCYTES, ANTIGEN-PRESENTING CELLS (MONOCYTES, MACROPHAGES AND DENDRITIC CELLS) AND ACTIVATED T LYMPHOCYTES



JANUARY 2016 ... MORE THAN 14,000 HLA ALLELES HAVE BEEN ASSIGNED, ACCOUNTING FOR MORE THAN 10,000 DIFFERENT HLA PROTEINS

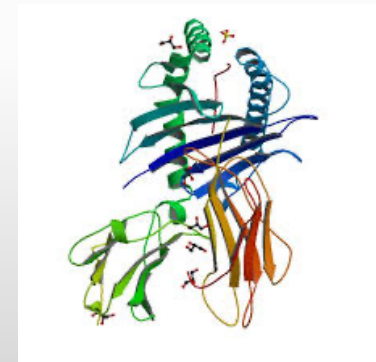
Tiercy, Haematologica , 2016

September 2018: more than 18.000 HLA class I and II

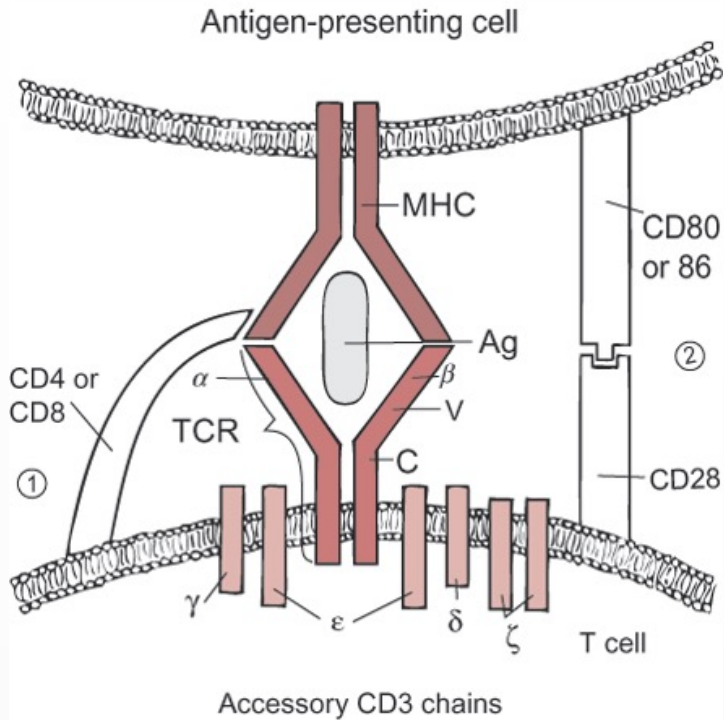


# WHAT do they do? ... HLA role

- The primary role is the regulation of the immune response, helping in the best way the immune system in recognition of foreign molecules and antigens
- HLA proteins are able to capture and present antigens of every type.
- To do it efficiently, each cell has a complement of slightly different HLA molecules, each of which is specialized in interacting with different types of antigens.



# HOW



## MHC RESTRICTION:

PEPTIDES ARE BOUND TO MHC MOLECULES AND THESE COMPLEXES ARE RECOGNIZED BY T-CELL RECEPTOR

The  $\alpha$  and  $\beta$  chains of the T-CELL RECEPTOR (TCR) bind to Antigen (AG)–MHC complex on an antigen-presenting cell (APC), and CD4 or CD8 interacts with the MHC

Both actions stimulate the T CELL (1st signal) through the accessory CD3 chains

# HLA IN DETAILS ... Why ?

## THE MHC REGION THE MOST POLYMORPHIC REGIONS OF THE HUMAN GENOME

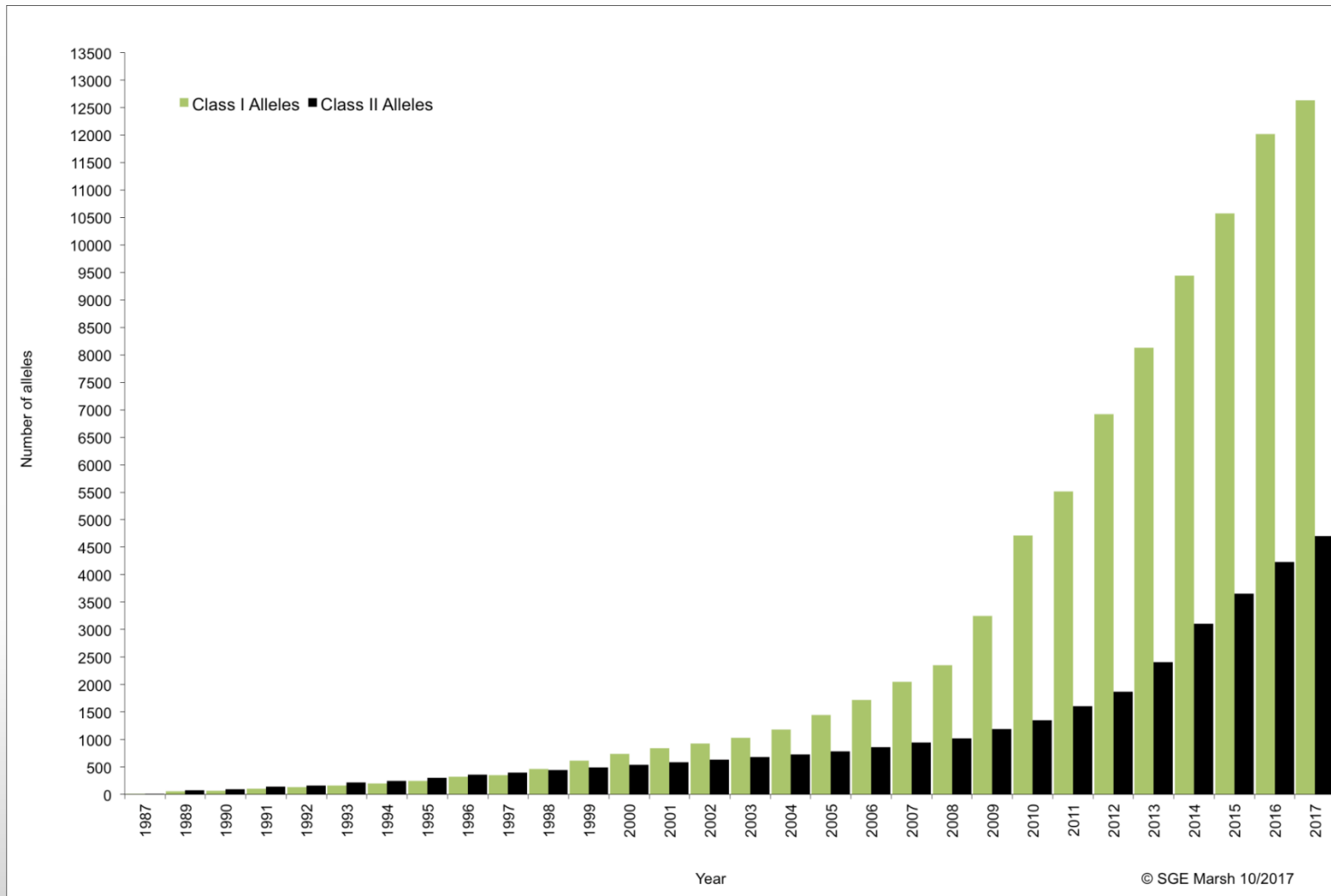
HLA Class I	Alleles	7,353
HLA Class II	Alleles	2,202
Other non-HLA	Alleles	155



Polymorphism is expressed in the antigen-binding groove  
It represents the consequence of selective pressure, related to the role of HLA molecules in the presentation of infectious agents in the different areas of the world

POLYMORPHISM HELP TO ENSURE THE SURVIVAL OF THE SPECIES AGAINST MAJOR PATHOGEN EPIDEMICS

# Graph showing numbers of alleles named by year from 1987 to end of March 2017



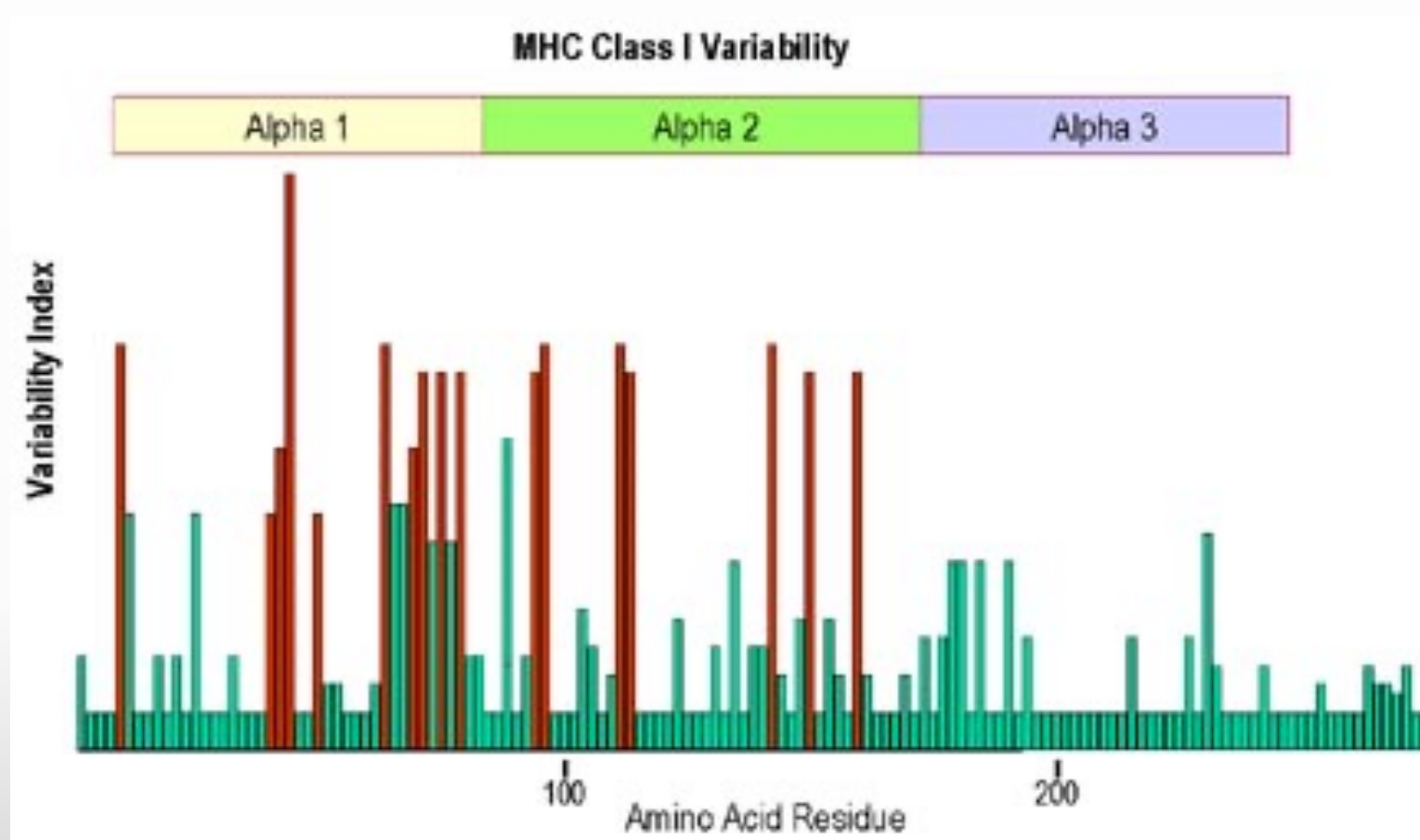
# And the numbers are growing...

*As of March 2022*

There are currently 34145 HLA and related alleles described by the HLA nomenclature and included in the IPD-IMGT/HLA Database

# MHC Class I polymorphism

The high polymorphism characterizes especially the I class of HLA molecules







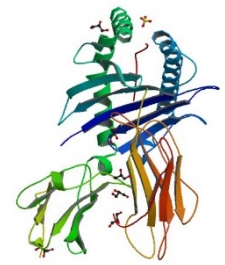
**The distribution and frequency of HLA antigens vary greatly among different ethnic groups.**

**It has been postulated that this diversity of HLA polymorphism has evolved under unique selective pressure in different geographic areas.**

**This could be related to the role of the HLA molecules in the presentation of prevalent infectious agents in the different areas of the world.**

*The HLA System: Genetics, Immunology, Clinical Testing, and Clinical Implications*  
*Sung Yoon Choo, 2007*

# HLA Keypoints!



Some key point concepts!

There are two groups of MHC genes structurally and functionally distinct:

**Class I** → recognition by CD8+ T cells  
of endogenous antigens synthesized within the target cell  
(cellular, transformed or virus-induced proteins)

**Class II** → recognition by CD4+ T cells  
extracellular exogenous proteins are endocytosed  
and undergo degradation in the acid endosomal compartment

**Both HLA classes, I and II, are responsible for the compatibility of the tissues of genetically different individuals and for the rejection of the transplant**

**MHC genes are codominantly expressed in each individual**

**Monozygotic twins have the same histocompatibility molecules on their cells**

# HLA expression

Tissue	MHC class I	MHC class II
T cells	+++	+/-
B cells	+++	+++
Macrophages	+++	++
Other APC	+++	+++
Thymus epithelium	+	+++
Neutrophils	+++	-
Hepatocytes	+	-
Kidney	+	-
Brain	+	-
Erythrocytes	-	-

The pattern of expression reflects the function of MHC molecules:

- **Class I** is involved in the regulation of anti-viral immune responses
- **Class II** is involved in regulation of the cells of the immune system

**Erythrocytes can not support pathogens replication - hence no MHC class I !!!**

**But, some pathogens exploit this - e.g. *Plasmodium* species.**

Since the class I HLA function is related to the answer to viral infection, their presence can be revealed only in case of nuclear activity: hence their absence on red cells!! But, some pathogens exploit this - e.g. *Plasmodium* species.

# Structure of HLA molecules

HLA molecules are glycoproteins, heterodimers (two chains)

Structure of HLA molecules of both classes enables antigen binding and contact with T cell receptors

Two functional parts:

- ✓ Polymorphic (predominantly in the cleft)
- ✓ Non polymorphic part of the molecule contain binding sites for the T cell molecules CD4 and CD8

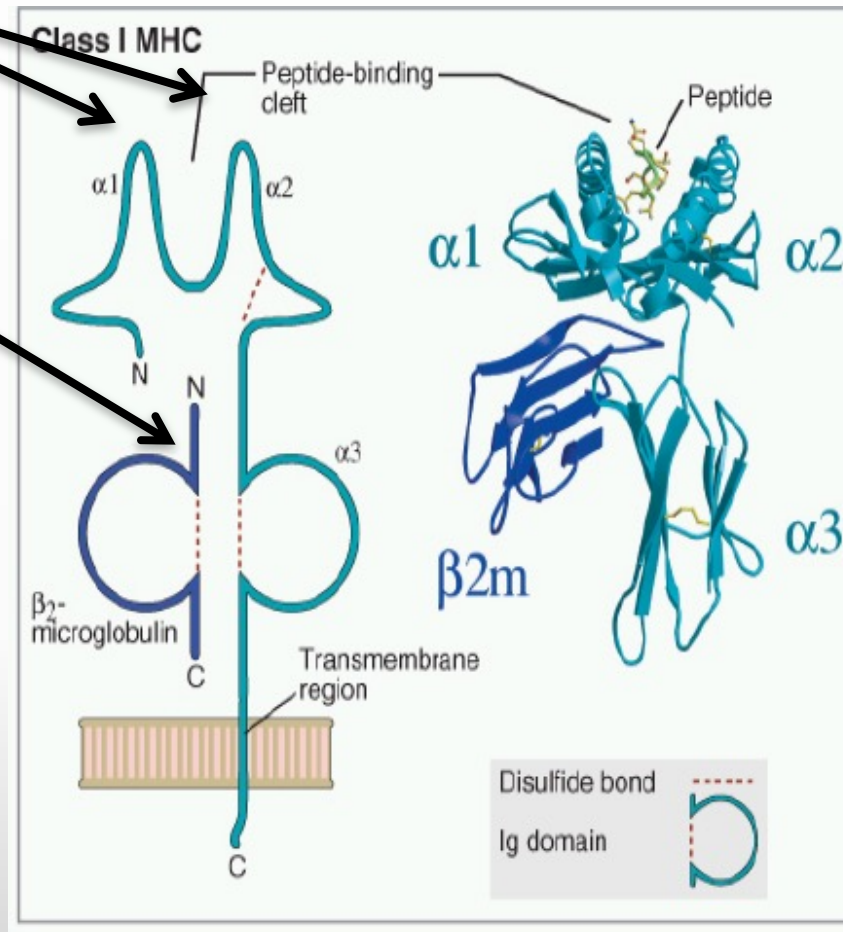
# HLA class I molecules

Glycoproteins, heterodimers (two chains)

Structure of HLA molecules of both classes enables antigen binding and contact with T cell receptors

Polymorphic sites

Binding site of CD8



Class I molecules consist of glycosylated heavy chains non-covalently bound to β<sub>2</sub> microglobulin (extracellular).

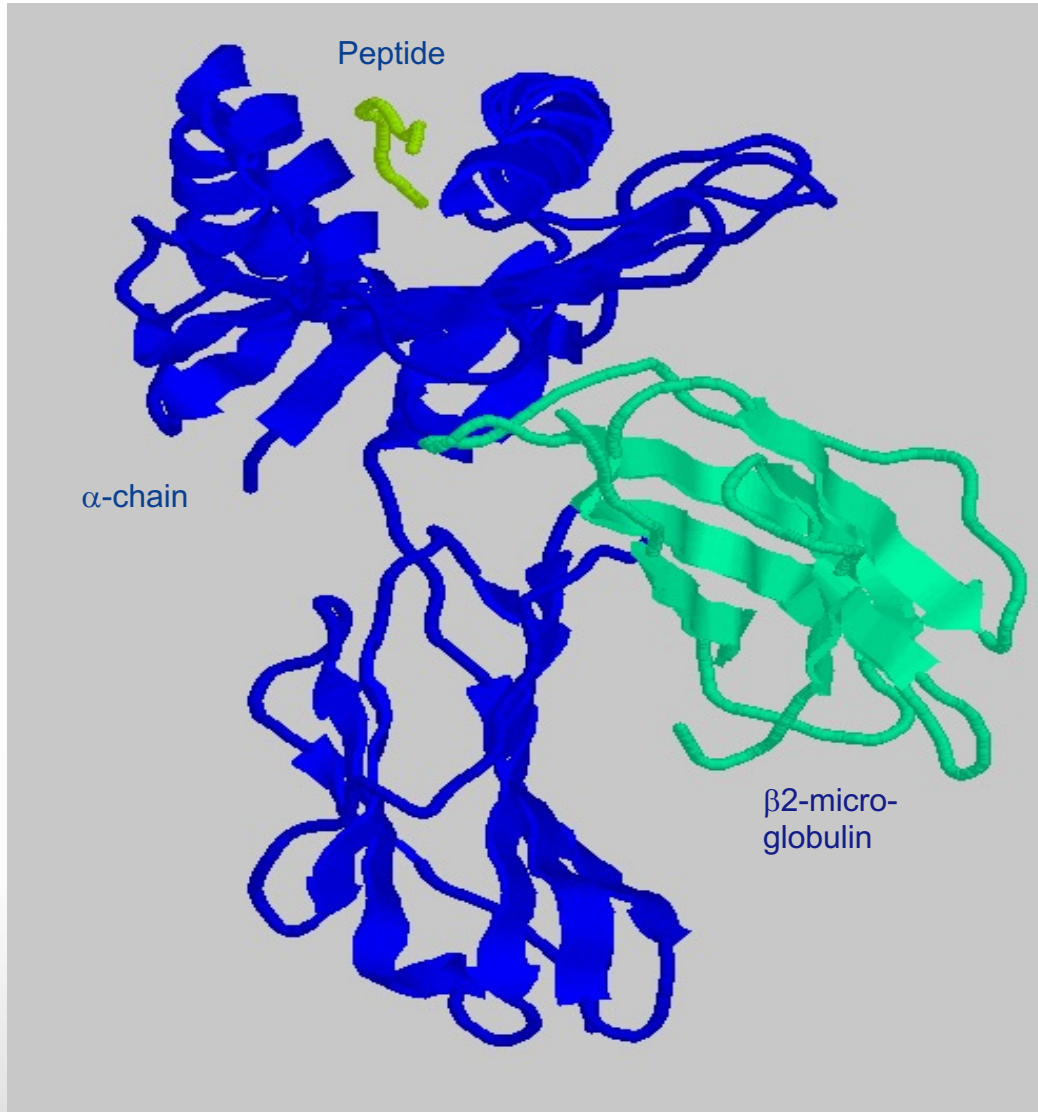
Human β<sub>2</sub> m is invariant and its gene was mapped to chromosome 15.

The class I heavy chain has three extracellular domains (α<sub>1</sub>, α<sub>2</sub>, α<sub>3</sub>), a transmembrane region and an intracytoplasmic domain.

The α<sub>1</sub> and α<sub>2</sub> domains contain variable amino acid sequences and these domains determine the antigenic specificities of the HLA class I molecules.

α<sub>3</sub> domain & β<sub>2</sub>m have structural & amino acid sequence homology with Ig C domains Ig GENE SUPERFAMILY

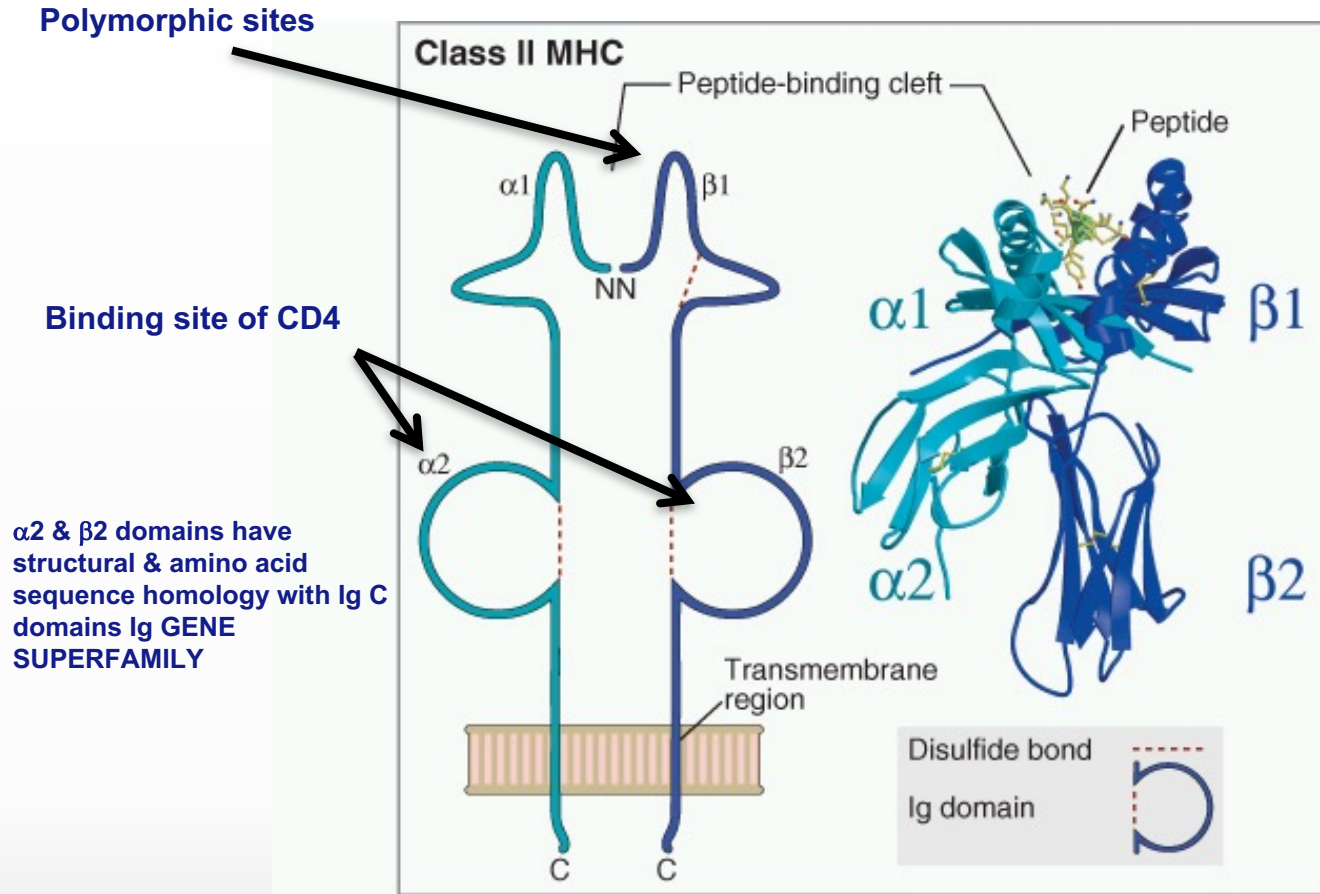
# Structure of MHC class I molecules



$\alpha 1$  and  $\alpha 2$  domains form two segmented  $\alpha$ -helices on eight anti-parallel  $\beta$ -strands to form an antigen-binding cleft.



# HLA class II molecules



Class II molecules are heterodimers of two non covalently associated glycosylated polypeptide chains;  $\alpha$  and  $\beta$ .

The  $\alpha$  and  $\beta$  chains are transmembrane, and they have the same overall structure:

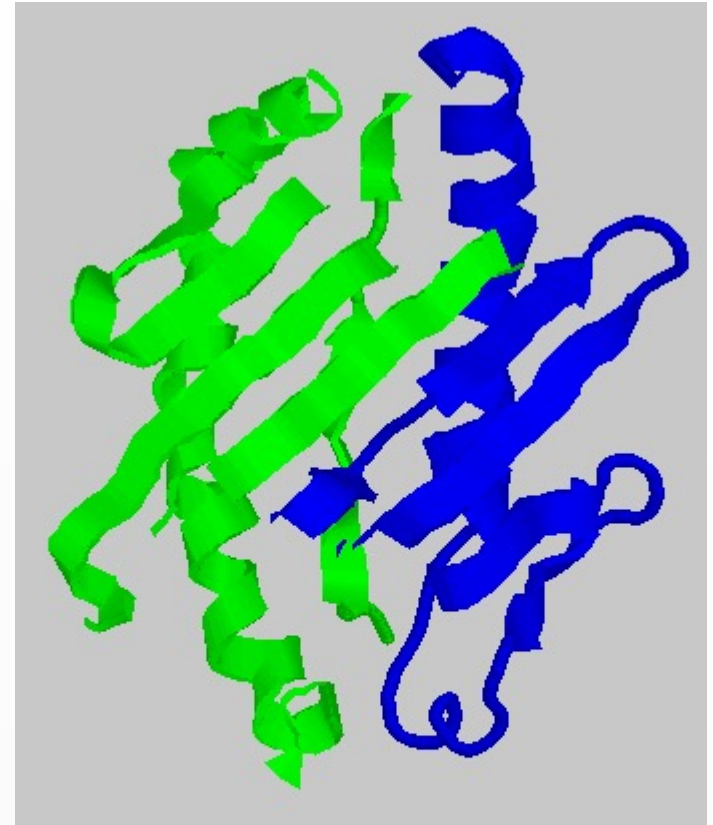
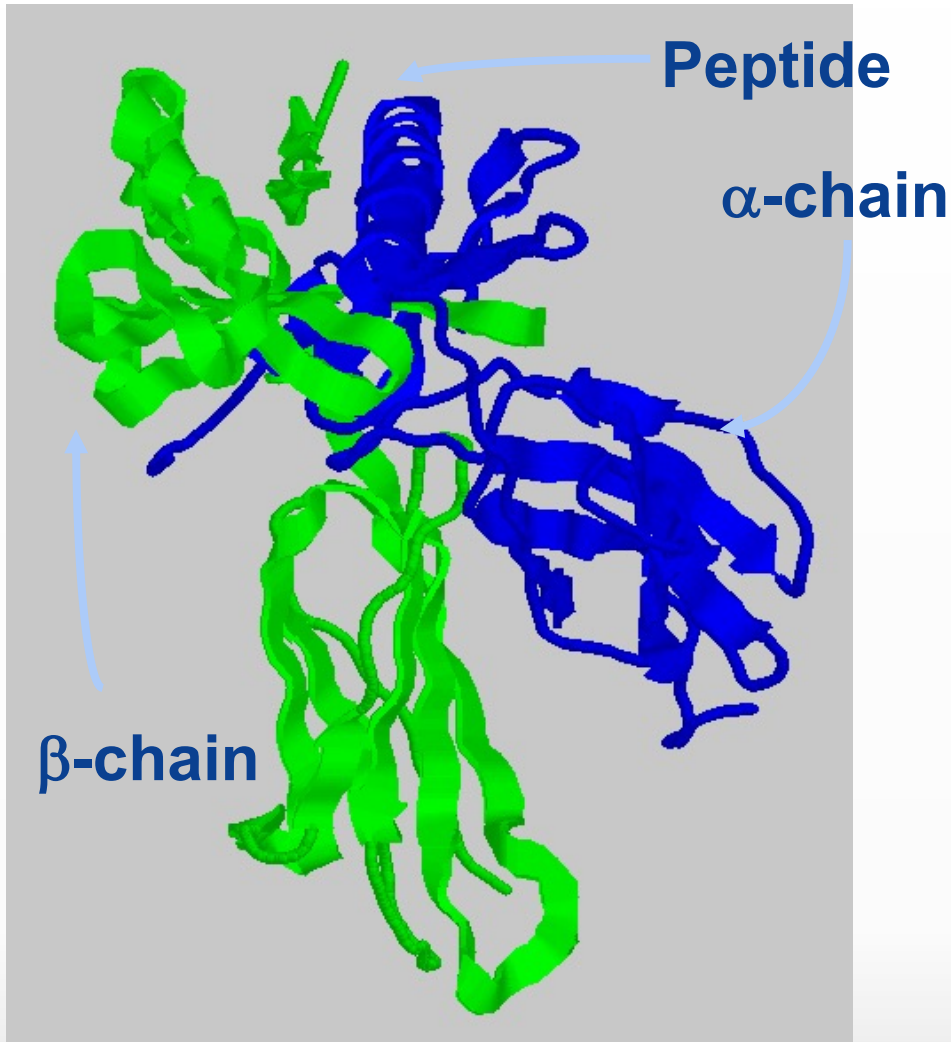
an extracellular portion composed of two domains is anchored on the membrane by a short transmembrane region and a cytoplasmic domain.

$\alpha 1$  and  $\beta 1$  domains form an antigen-binding groove.

Both ends of the class II groove are more open and longer peptides can be accommodated

Polymorphism of class II molecules occur in the first aminoterminal  $\beta 1$  domain.

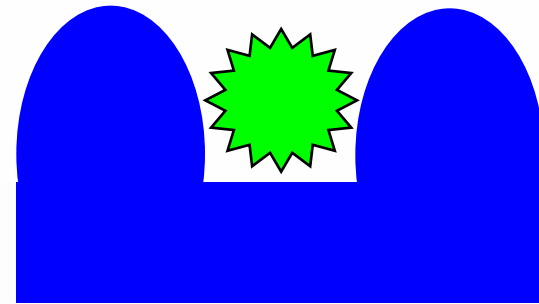
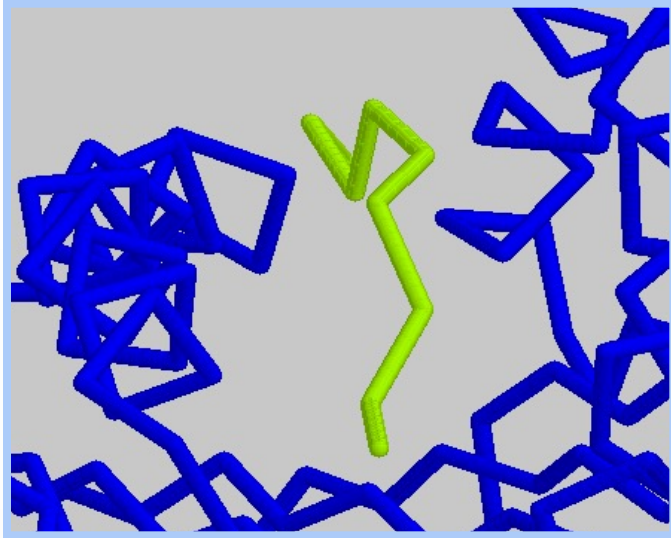
# MHC class II molecule structure



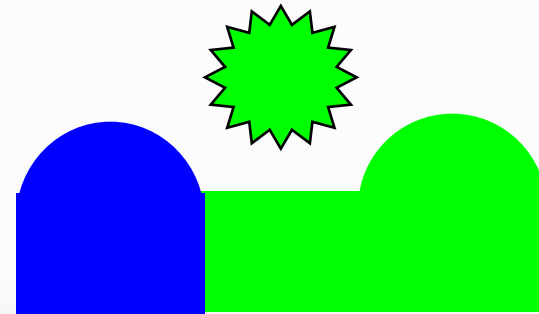
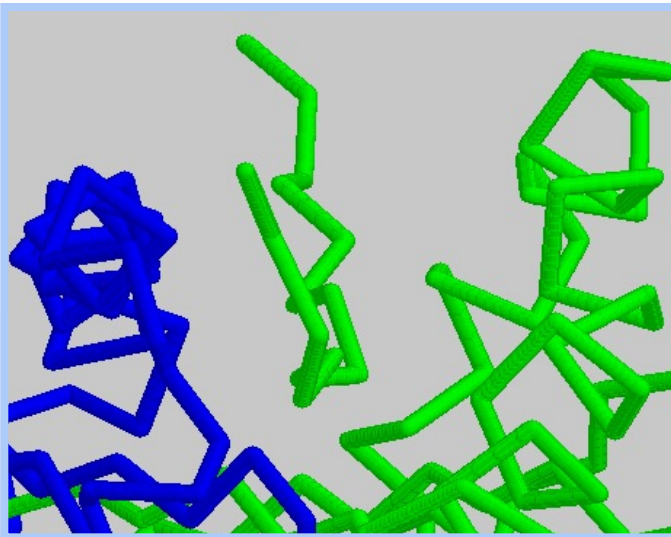
Cleft is made of both  
 $\alpha$  and  $\beta$  chains

Class II groove is more open and can accommodate longer peptides

# Cleft geometry



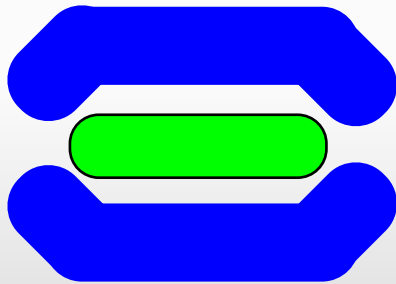
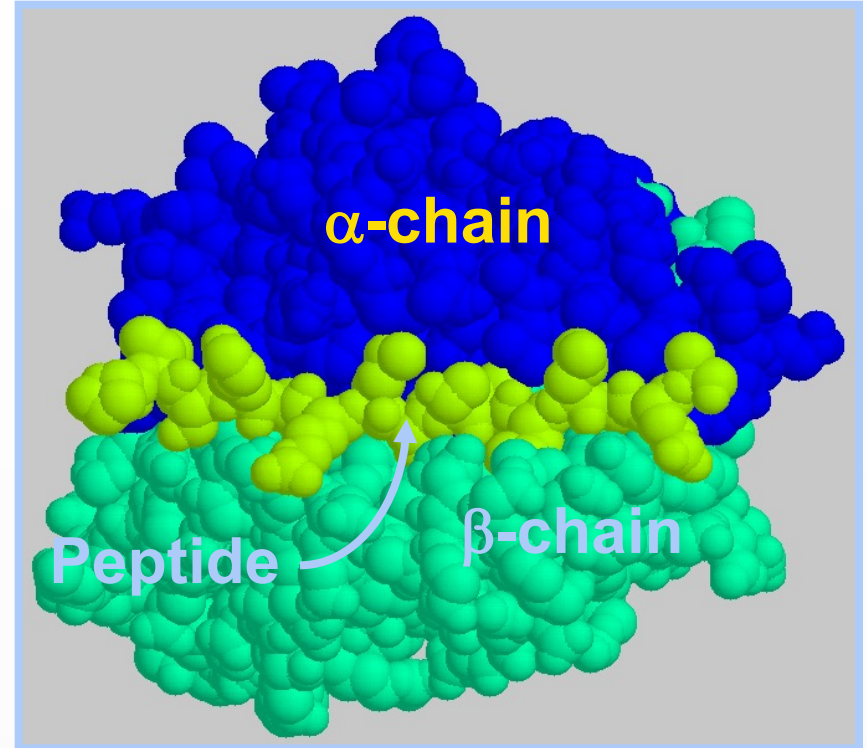
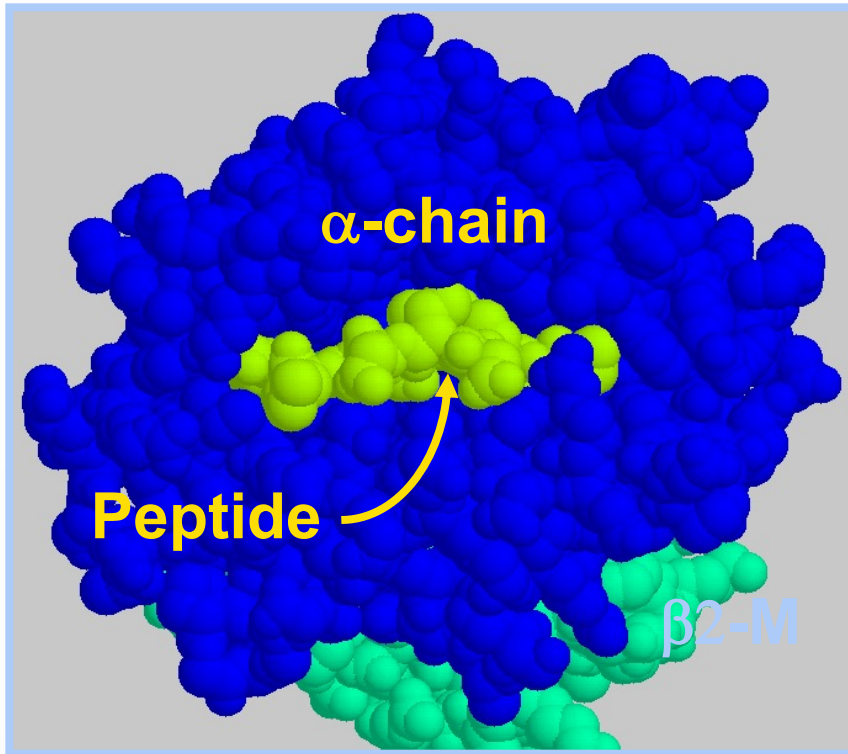
MHC class I



MHC class II

Peptide is held in the cleft by non-covalent forces

# Cleft geometry



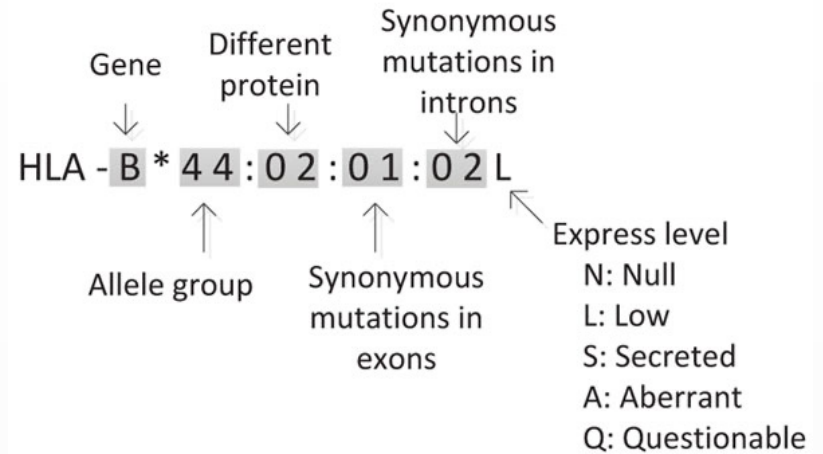
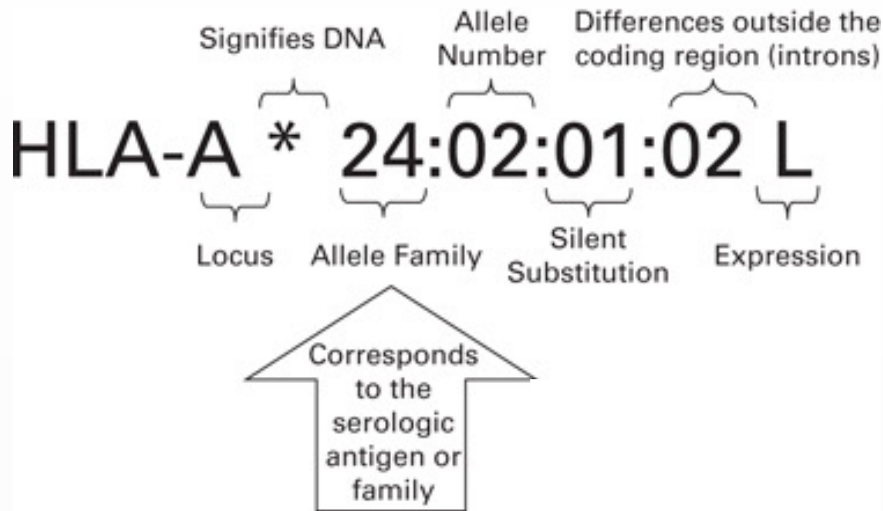
MHC class I accommodate peptides of 8-10 amino acids



MHC class II accommodate peptides of >13-30 amino acids



# HLA nomenclature



Low Resolution

HLA-A\*68:02:01:02

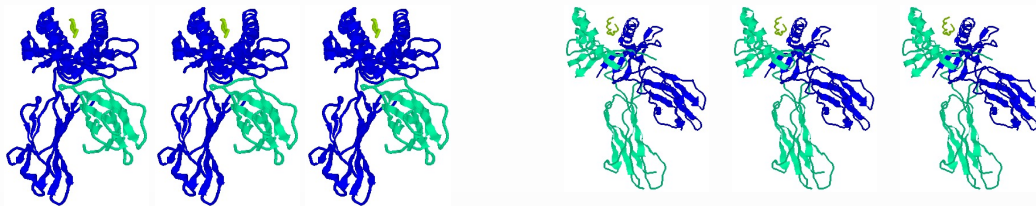
High Resolution



# MHC-binding peptides

Each human usually expresses:

- ✓ 3 types of MHC class I (A, B, C)
- ✓ 3 types of MHC class II (DR, DP, DQ)



The number of different T cell antigen receptors is estimated to be **1,000,000,000,000,000**

Each of which may potentially recognise a different peptide antigen

**How can 6 molecules have the capacity to bind to 1,000,000,000,000,000 different peptides?**

# Eluted peptides from MHC class I molecules have different sequences but contain motifs

The answer is the polymorphism!

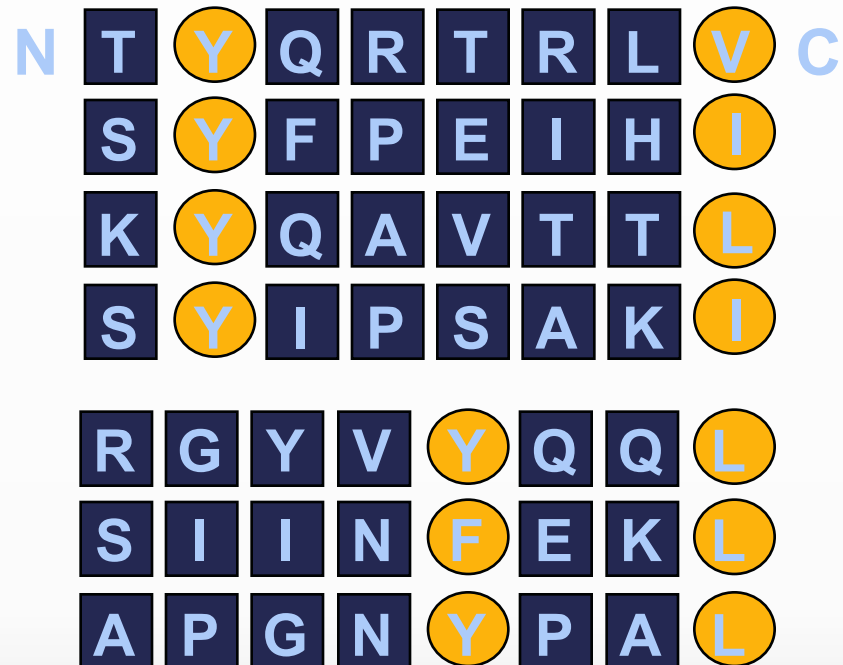
Different types of MHC molecule bind peptides with different patterns of conserved amino acids

Peptides bound to a particular type of MHC class I molecule have conserved patterns of amino acids

A common sequence in a peptide antigen that binds to an MHC molecule is called a MOTIF

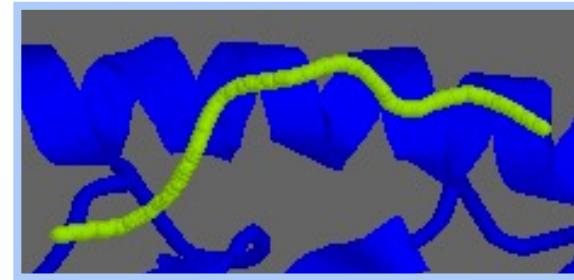
Amino acids common to many peptides tether the peptide to structural features of the MHC molecule

ANCHOR RESIDUES

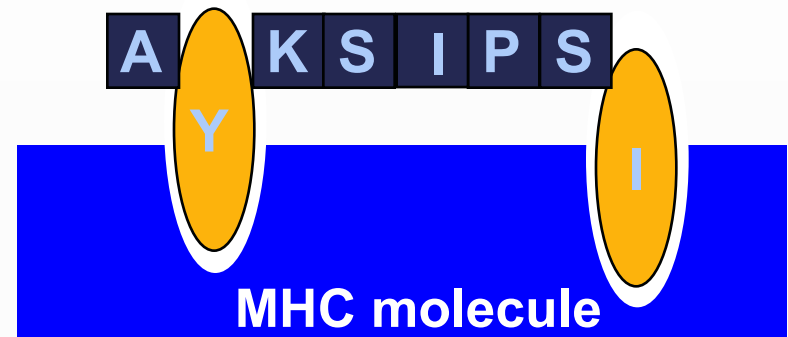
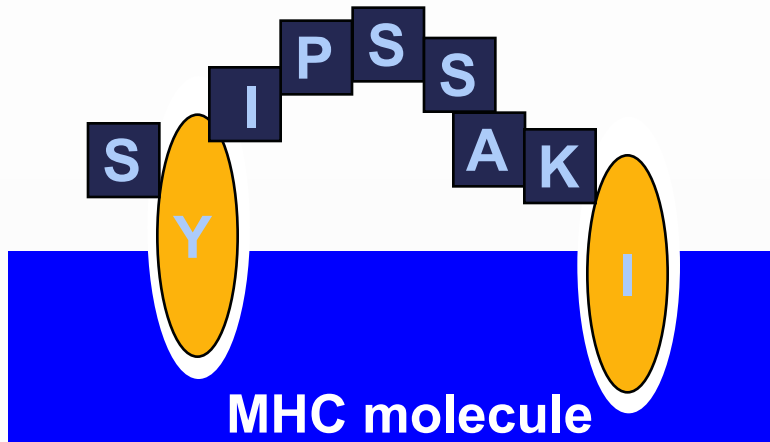


# MHC molecules can bind peptides of different length

And here, the mechanism of MOTIF and ANCHOR is shown!!



Arched peptide



Complementary anchor residues & pockets provide **the broad specificity** of a particular type of MHC molecule for peptides

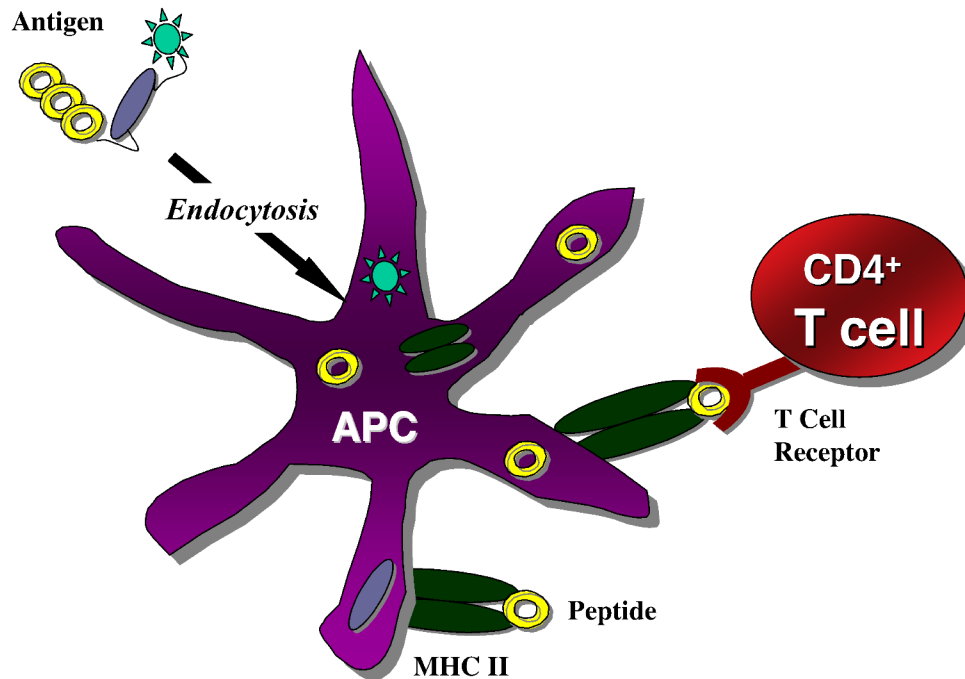
**Peptide sequences and numbers of amino acids between anchors can vary**



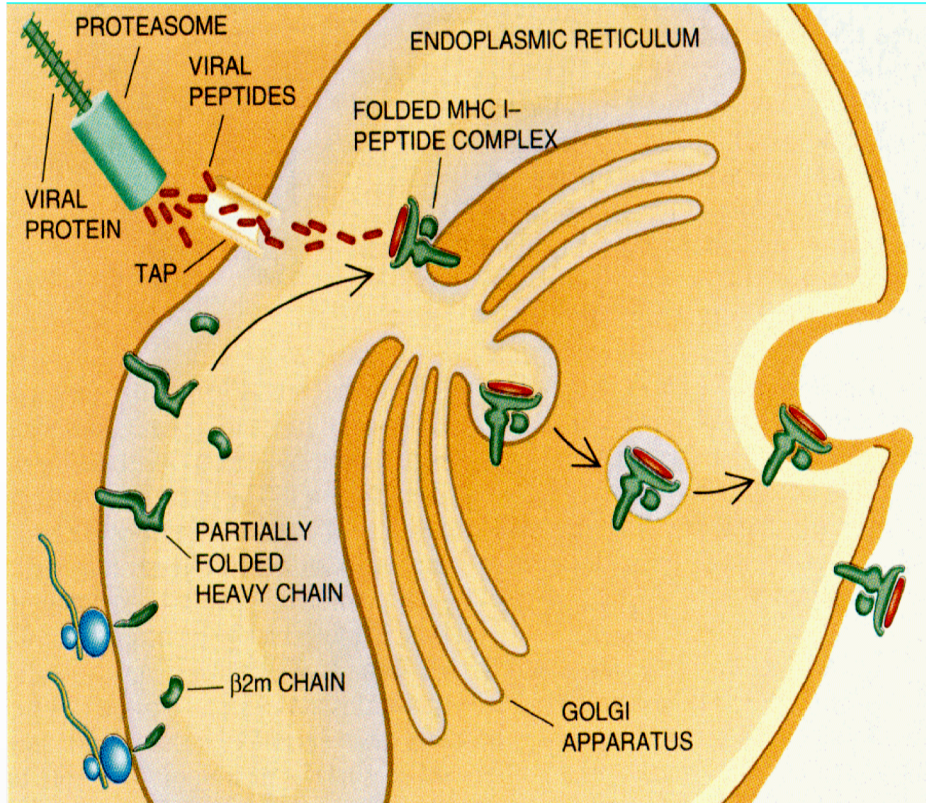


# Role of HLA in the immune response

## T cell Recognition of Antigen on an APC



# INTRACELLULAR ANTIGENS ARE PRESENTED BY MHC CLASS I MOLECULES



Class I molecules present intracellular antigens that are processed in the cytoplasm and pumped into the endoplasmic reticulum, where new HLA molecules are being assembled. Processing of the antigens is performed by the proteasome

Peptides that are processed in this manner are transported into the endoplasmic reticulum by TAP (transporter associated with antigen).

The newly synthesized HLA molecule is maintained in a partially folded conformation by calnexin (not shown). When  $\beta_2$ -microglobulin binds to the HLA

Peptides are processed by the proteasome and transported into the endoplasmic reticulum where new HLA molecules are being assembled by TAP. The newly synthesized HLA molecule is maintained in a partially folded conformation by *calnexin*; When  $\beta_2$ -microglobulin binds to the HLA molecule, the complex dissociates from calnexin. Finally, the HLA molecule is then transported through the Golgi complex to the cell surface.

# MHC Class I pathway

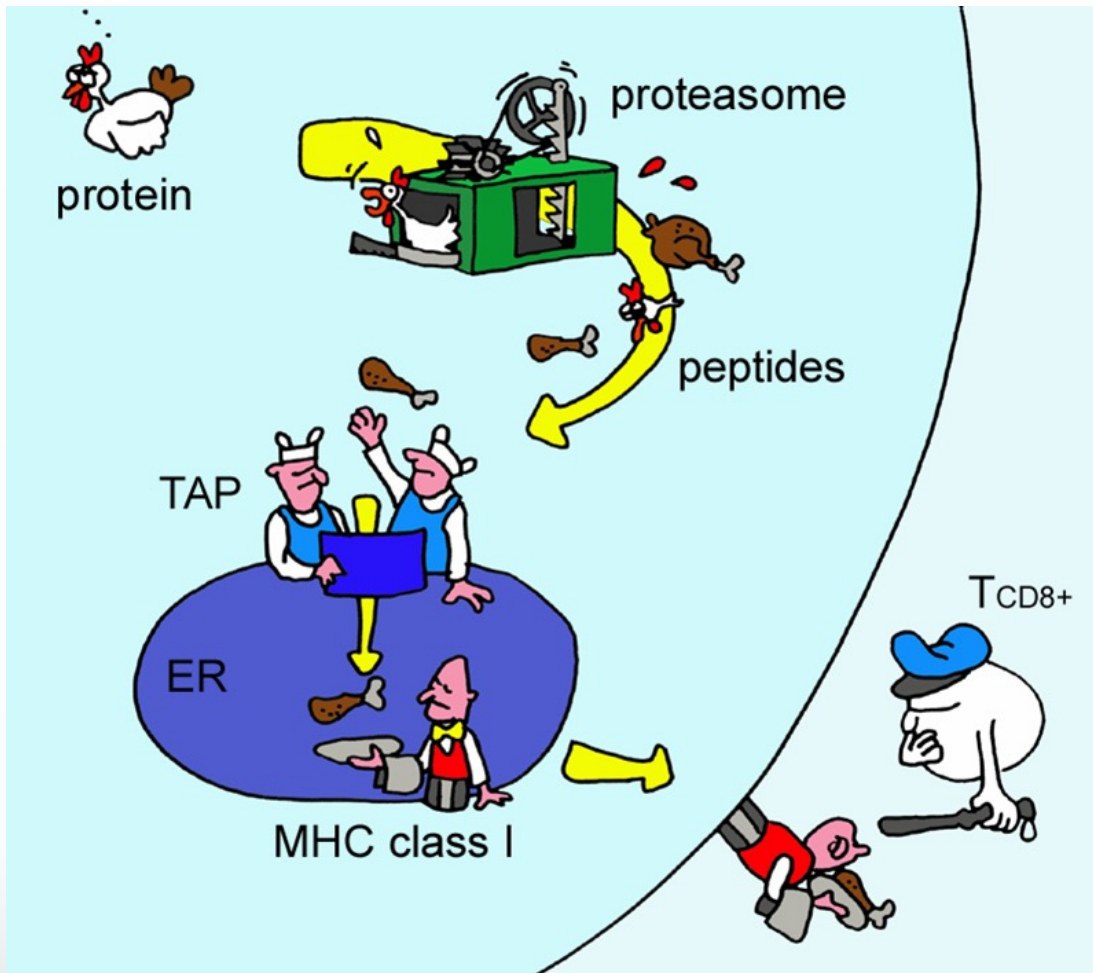
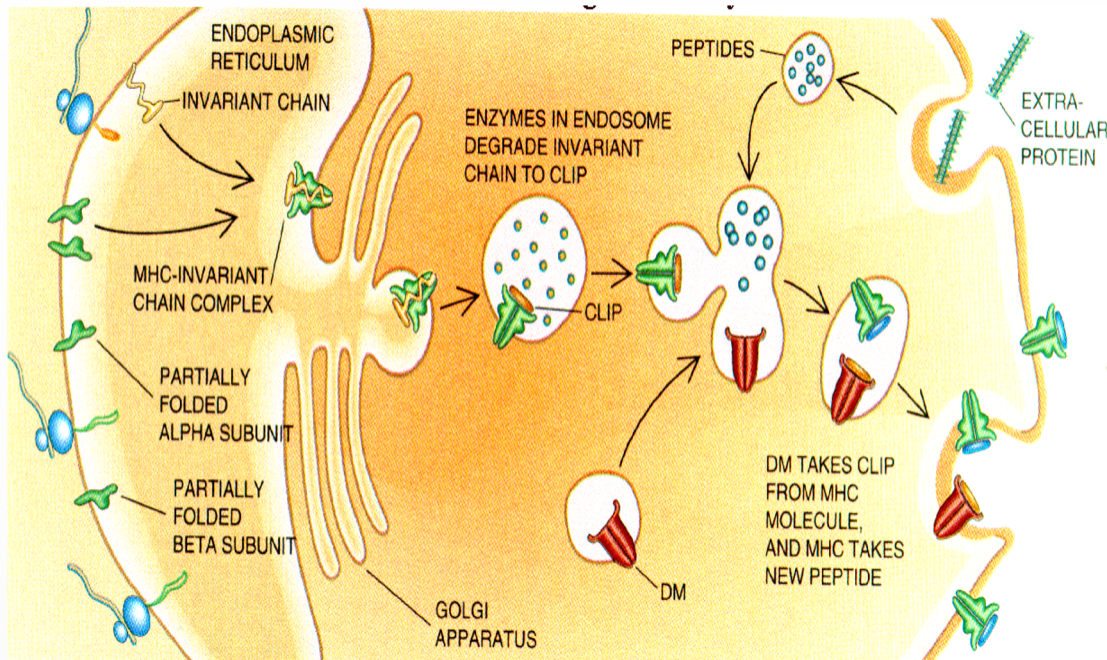


Figure by Eric A.J. Reits

*like a waiter presenting a dinner to the costumers*



# Extracellular antigens are presented by MHC class II molecules



Class II molecules are also assembled in the endoplasmic reticulum but associate with a third protein known as the invariant chain, which prevents peptide binding.

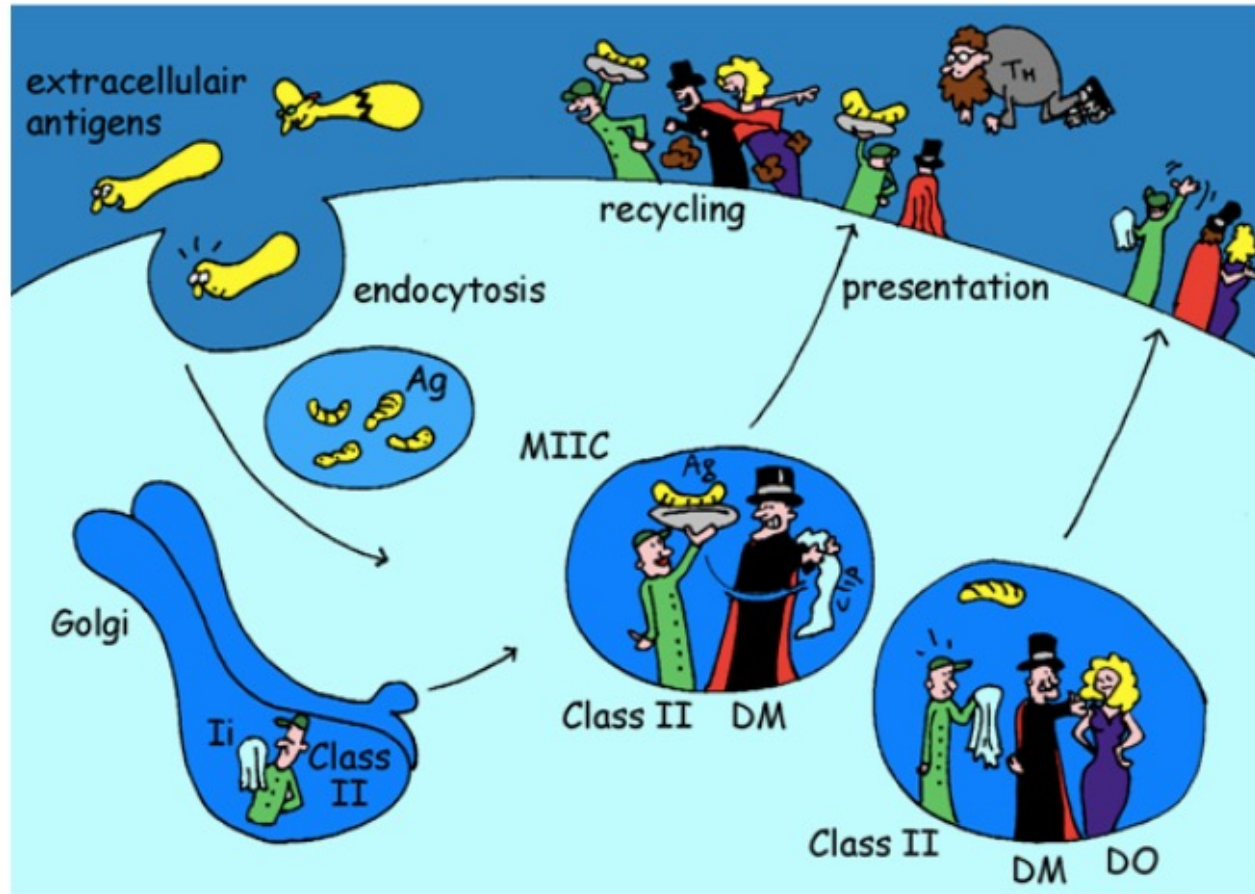
The invariant chain is processed within endosomes to CLIP (class II-associated invariant chain peptide).

In the presence of HLA-DM molecules, CLIP dissociates from HLA-DR or DQ molecules and allows binding of new peptides that have been endocytosed from the extracellular environment.

Thus, MHC class II molecules differ from MHC class I molecules because they preferentially present extracellular antigens rather intracellular ones.

HLA-DM serves to 'edit' peptide binding, promoting association with high affinity peptides over lower affinity peptides.

# MHC Class II pathway

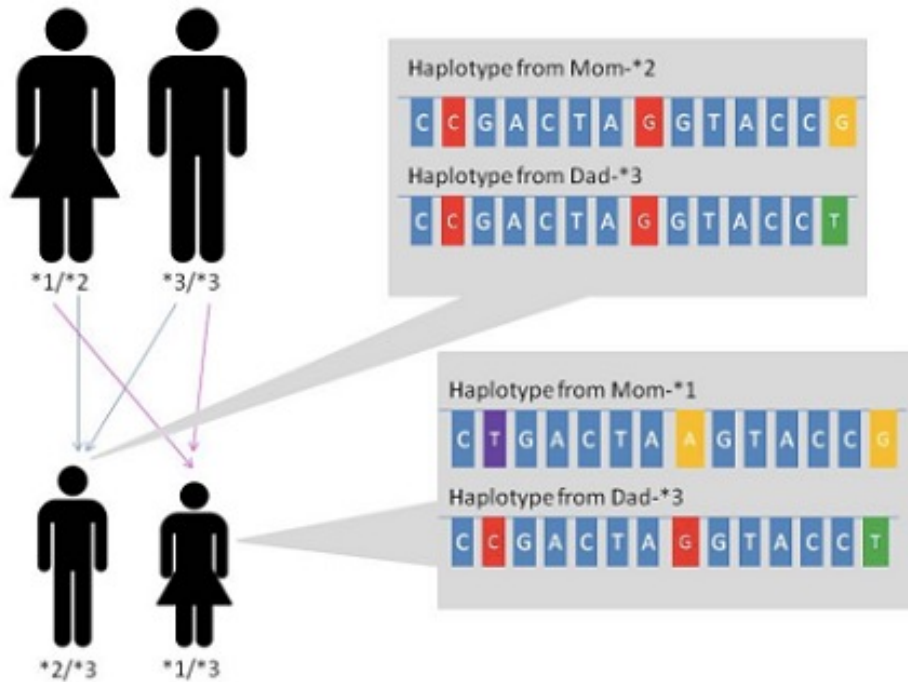


# **What's a *haplotype*?**

**Genes in the MHC are tightly linked and usually inherited in a unit called an MHC haplotype.**

**Each individual inherits in a mendelian fashion one haplotype from their father and one from the mother**

# What's a *haplotype*?

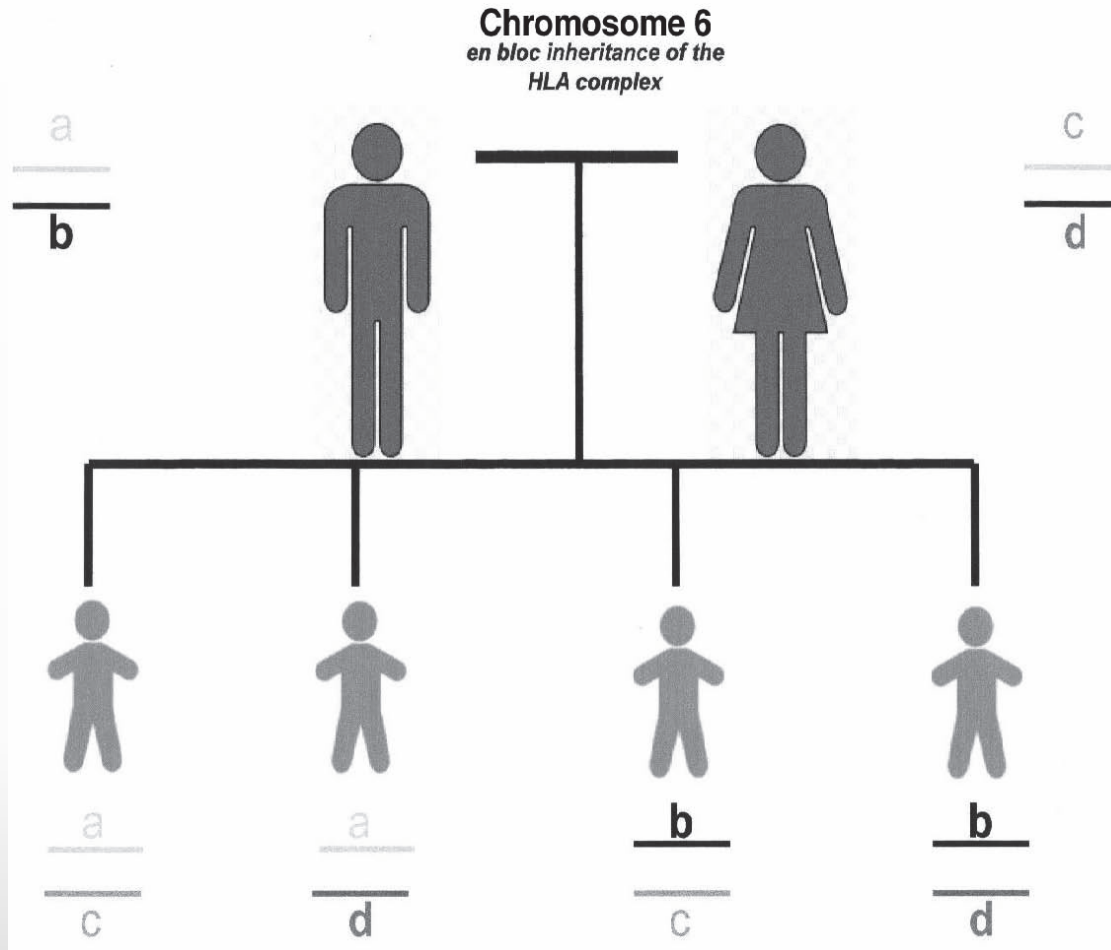


Genes in the MHC are closely linked and the HLA haplotype is inherited in a mendelian fashion from each parent

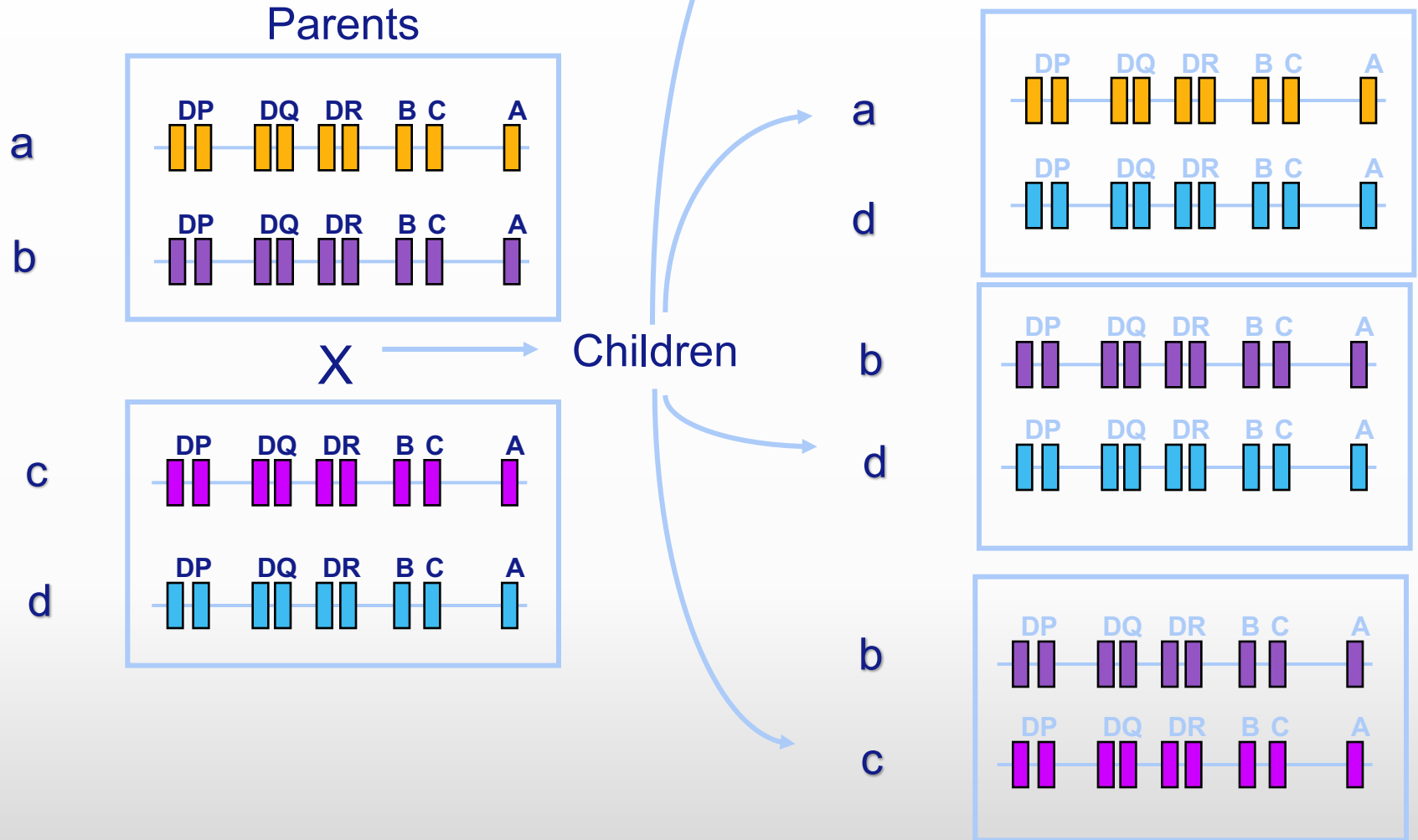
Since a person has two copies of each gene – one from their mother and one from their father – they will inherit a haplotype from each parent. **Each person's haplotype is written as two haplotype numbers separated by a slash.**



Here all the possible combinations



# Inheritance of MHC haplotypes



# Linkage Disequilibrium

But sometimes something can occur...

***Linkage disequilibrium*** is the occurrence of some combination of alleles in a population more often or less often than would be expected from a random formation of haplotypes from alleles based on their frequencies

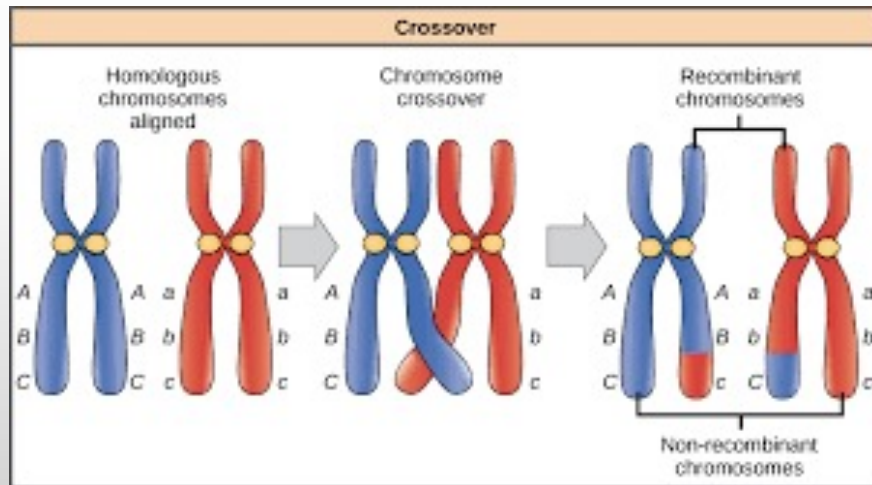
Linkage disequilibrium can be caused by evolutionary factors such as natural selection and genetic drift.

In Caucasian HLA-A1, B8, DR17 is the most common HLA haplotype (frequency 5%)

# Errors in the inheritance of haplotypes generate polymorphism in the MHC by gene conversion and recombination

**CROSS OVER:** The genes of the HLA region occasionally demonstrate chromosome crossover, in which segments containing linked genetic material are exchanged between the two chromosomes during meiosis or gametogenesis.

The recombinant chromosomes are then transmitted as **NEW HAPLOTYPES** to the offspring.



Crossover frequency is related partly to the **PHYSICAL DISTANCE** between the genes and partly to the **RESISTANCE OR SUSCEPTIBILITY** of specific A, B, and DR antigens to **RECOMBINATION**.

# Example

## Haplotype Inheritance

Father	
<u>a</u>	<u>b</u>
A*0101	A*2902
Cw*0701	Cw*1601
B*0801	B*4403
DRB1*0301	DRB1*0701
DQB1*0201	DQB1*0202

Mother	
<u>c</u>	<u>d</u>
A*0301	A*0201
Cw*0702	Cw*0501
B*0702	B*4402
DRB1*1501	DRB1*0401
DQB1*0602	DQB1*0301

Patient	
<u>a</u>	<u>c</u>
A*0101	A*0301
Cw*0701	Cw*0702
B*0801	B*0702
DRB1*0301	DRB1*1501
DQB1*0201	DQB1*0602

Sibling 1	
<u>a</u>	<u>d</u>
A*0101	A*0201
Cw*0701	Cw*0501
B*0801	B*4402
DRB1*0301	DRB1*0401
DQB1*0201	DQB1*0301

Sibling 2	
<u>b</u>	<u>d</u>
A*2902	A*0201
Cw*1601	Cw*0501
B*4403	B*4402
DRB1*0701	DRB1*0401
DQB1*0202	DQB1*0301

Sibling 3	
<u>a</u>	<u>c</u>
A*0101	A*0301
Cw*0701	Cw*0702
B*0801	B*0702
DRB1*0301	DRB1*1501
DQB1*0201	DQB1*0602

Sibling 4	
<u>a</u>	<u>b</u>
A*0101	A*2902
Cw*0701	Cw*1601
B*0801	B*4403
DRB1*0301	DRB1*0701
DQB1*0201	DQB1*0202

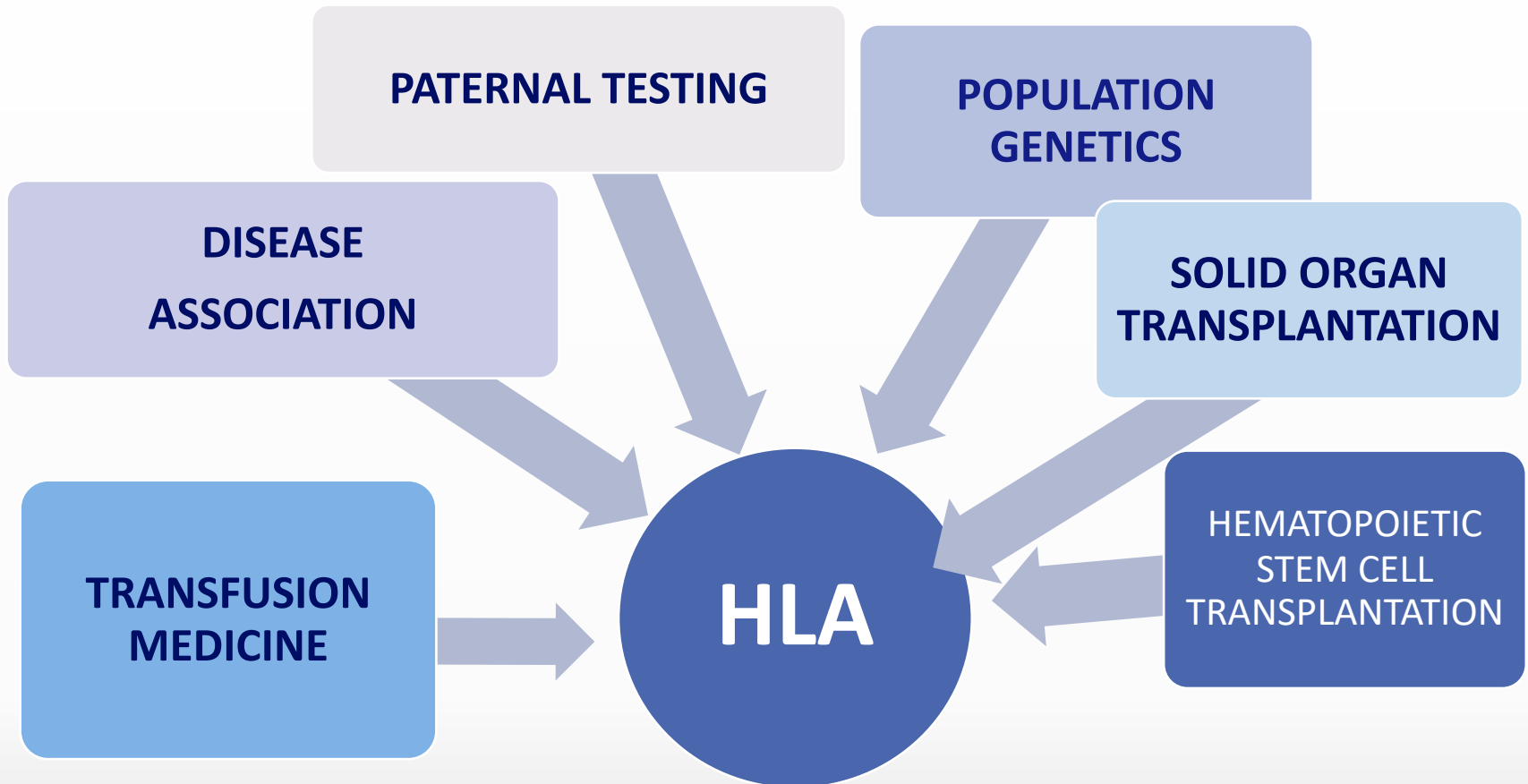
Sibling 5 (paternal recombination)	
<u>a/b</u>	<u>c</u>
A*2902	A*0301
Cw*0701	Cw*0702
B*0801	B*0702
DRB1*0301	DRB1*1501
DQB1*0201	DQB1*0602

Sibling 5 (paternal recombination)	
<u>c</u>	
A*0301	
Cw*0702	
B*0702	
DRB1*1501	
DQB1*0602	

Sibling 5 (paternal recombination)	
<u>a/b</u>	<u>c</u>
A*2902	A*0301
Cw*0701	Cw*0702
B*0801	B*0702
DRB1*0301	DRB1*1501
DQB1*0201	DQB1*0602

# The HLA system in clinical practice

The HLA system takes a role in the various medical fields



# The HLA system

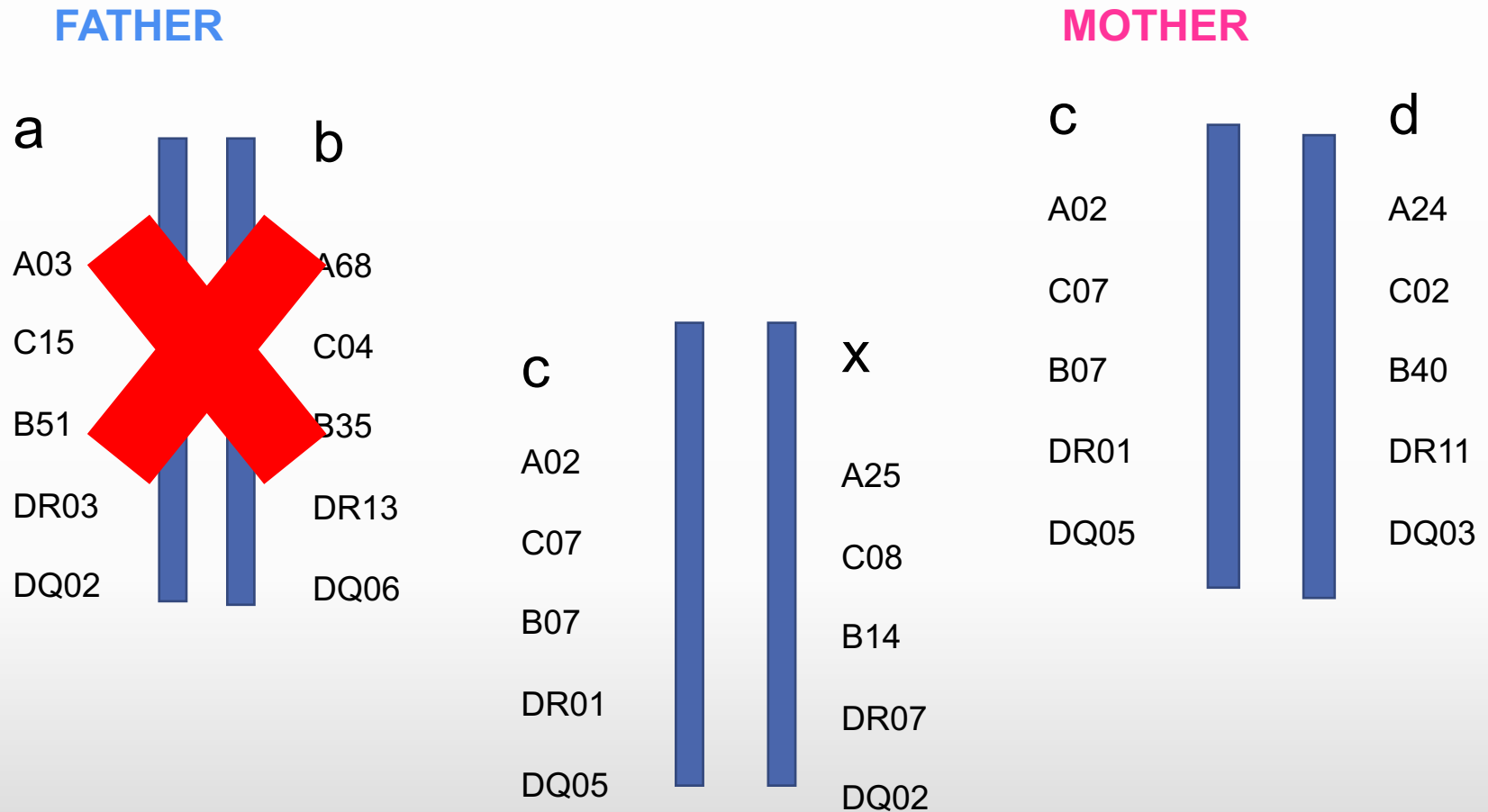
## PATERNAL TESTING

- Regarding paternity testing, it doesn't provide certainties because, in many cases, it involves paternal HLA haplotypes that are common in some ethnic groups.
- The high diffusion of HLA haplotypes in a given population limits its use ...it could only be used for paternity exclusion

During the past decade, STR loci have become a valuable tool in paternity testing because of their high polymorphism and heterozygosity.

# The HLA system

here is an example!!! the HLA test allowed us to exclude the paternity





# **The HLA system in clinical practice**

## **DISEASE ASSOCIATION**

# Genetic tests in genetic diseases

Certain diseases are associated with the HLA system, in particular in case of autoimmune diseases

Single gene



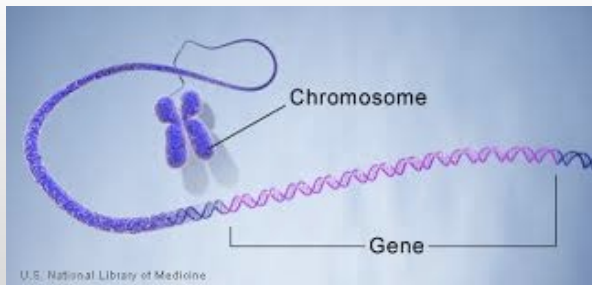
Diagnostic

Major gene + Additive



Genetic risk Complex trait

(none of the genes is necessary or sufficient for the developing of the disease)



In case of single gene association the gene identification is diagnostic

In the case of a complex trait, many genes are associated, none with a necessary association: in this case, the genetic defines a risk and not a diagnosis!

# HLA and disease association

Over the last several decades, population studies have identified a long list of human diseases that are significantly most common among individuals carrying particular HLA alleles.

## Some examples:

- ✓ Ankylosing spondylitis, 90% patients, B\*27:02, B\*27:05
- ✓ Narcolepsy, 90% patients, DQB1\*06:02
- ✓ Rheumatoid Arthritis, 90% patients, DRB1\*01:01, \*04:01/04/05
- ✓ Coeliac Disease 80% patients DQA1\*05,DQB1\*02

# Is HLA typing diagnostic?

- **No diseases** are associated 100% with HLA antigens
- HLA typing has a **limited diagnostic value** and only assesses a person's risk of developing the disease.
- Analysis of HLA susceptibility genes has mostly **negative predictive value**, since **the absence of risk alleles** makes highly unlikely the development of the disease (but does not exclude it!)



# What about the mechanism?



The exact mechanisms underlying the most HLA-disease association are not well understood and other genetic and environmental factors may play roles as well

# The HLA system in clinical practice

Significance of HLA  
in Blood Transfusion and Transplantation



# Significance of HLA in Blood Transfusion and Transplantation

## HLA and Transfusion, Alloimmunization

- Refractoriness to platelets
- Transfusion-Associated Graft-Versus-Host Disease (TA-GVHD)
- Transfusion-Related Acute Lung Injury (TRALI)

In the case of TA GVHD, T allogeneic lymphocytes of not leuco-depleted/irradiated blood components transfused to immunosuppressed individuals leading to an immune attack against the recipient cell, with a fatal outcome

It is important to irradiate blood products to be given to immunodeficient individuals, such as hematologic patients who received chemotherapy or HSCT, congenital immunodeficiencies

# Significance of HLA in Blood Transfusion and Transplantation

## **HLA and Transfusion, Alloimmunization**

- Refractoriness to platelets
- Transfusion-Associated Graft-Versus-Host Disease (TA-GVHD)
- Transfusion-Related Acute Lung Injury (TRALI)

## **Transplantation**

- HSCT (bone marrow and PBSC)
- Cord blood
- Solid organ



# THE HLA SYSTEM IN TRANSFUSION THERAPY

Antibodies directed against



HLA Antigens

## CAUSES OF IMMUNIZATION:

- PREGNANCIES
- BLOOD TRANSFUSIONS
- PREVIOUS TRANSPLANTATION

## CONSEQUENCES OF IMMUNIZATION:

- SOLID ORGAN TRANSPLANTATION (hyperacute, acute and chronic graft rejection)
- HSCT (primary graft failure and delayed engraftment)
- TRANSFUSION MEDICINE (transfusion refractoriness; TRALI)

# THE HLA SYSTEM IN TRANSFUSION THERAPY

HLA antibodies → Platelets Transfusion Refractoriness

IMMUNOLOGICAL DESTRUCTION CAUSED BY ANTIBODIES DIRECTED AGAINST HLA CLASS I ANTIGENS



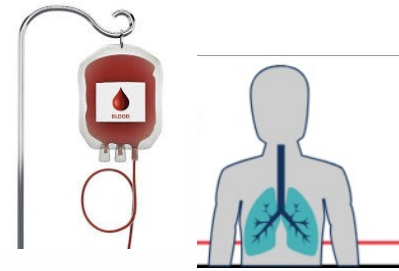
**Platelet transfusion therapy plays a significant role in the management of patients with haematological and oncological disorders.**

Approximately 30–50% of transfusion-dependent patients become refractory to platelet transfusion.

IF THE SPECIFICITY OF THE PATIENT'S ANTIBODIES CAN BE DETERMINED, PLATELETS DONORS WHO ARE NEGATIVE FOR CORRESPONDING HLA ANTIGENS CAN BE SELECTED



# Transfusion related acute lung injury (TRALI)

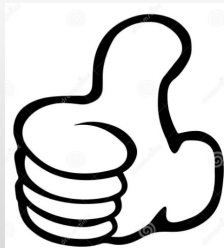


**THE PRESENCE (IN PLASMA PRODUCTS) OF DONOR ANTIBODIES DIRECTED AGAINST RECIPIENT HLA ANTIGENS COULD CAUSE THE TRALI.**

**TRALI is a rare but life-threatening complication of blood transfusion and can be clinically indistinguishable from adult respiratory distress syndrome.**

**This reaction normally develops within 2h following the administration of plasma-containing blood components.**

**ANTIBODIES ARE MOST COMMONLY FOUND IN THE DONATIONS OF MULTIPAROUS WOMEN**



**USE OF MALE DONORS FOR PLASMA CONTAINING PRODUCTS**

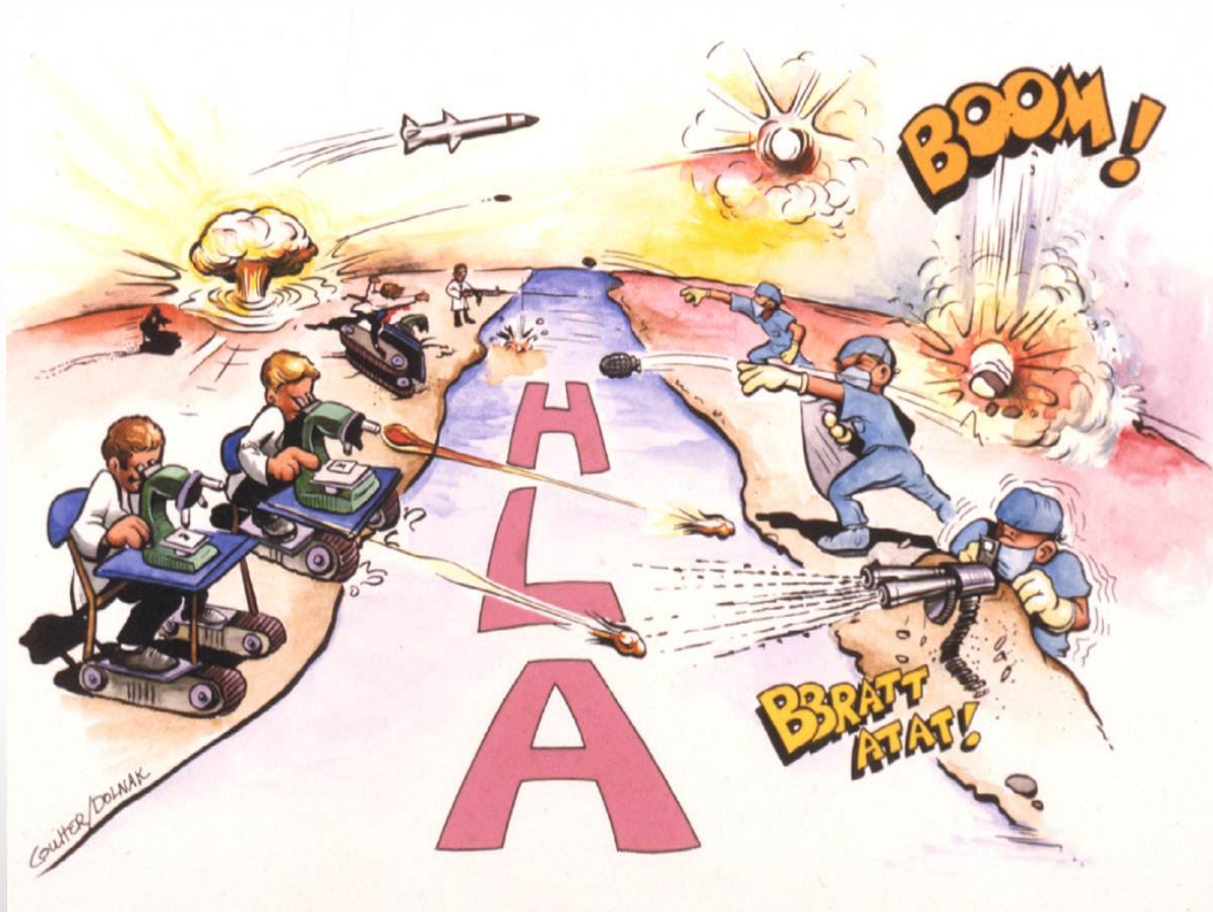
# How to reduce HLA immunization?



**PREFERENTIAL USE OF MALE DONORS FOR PLASMA CONTAINING PRODUCTS**

**DEVELOPMENT OF CELLULAR COMPONENTS LACKING EXPRESSION OF THESE ALLOANTIGENS (LEUCOCYTE DEPLETION, UV IRRADIATION)**

# HLA and transplantation

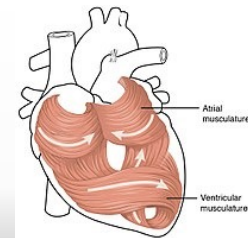


# HLA and Transplantation Solid organ

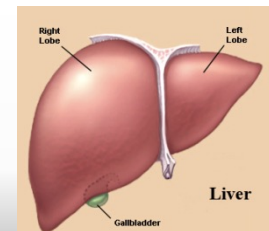
**Lung: Class II HLA match**



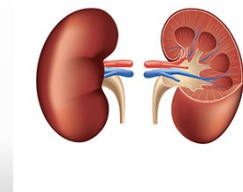
**Heart: Class I HLA match on case-by-case basis, no class II**



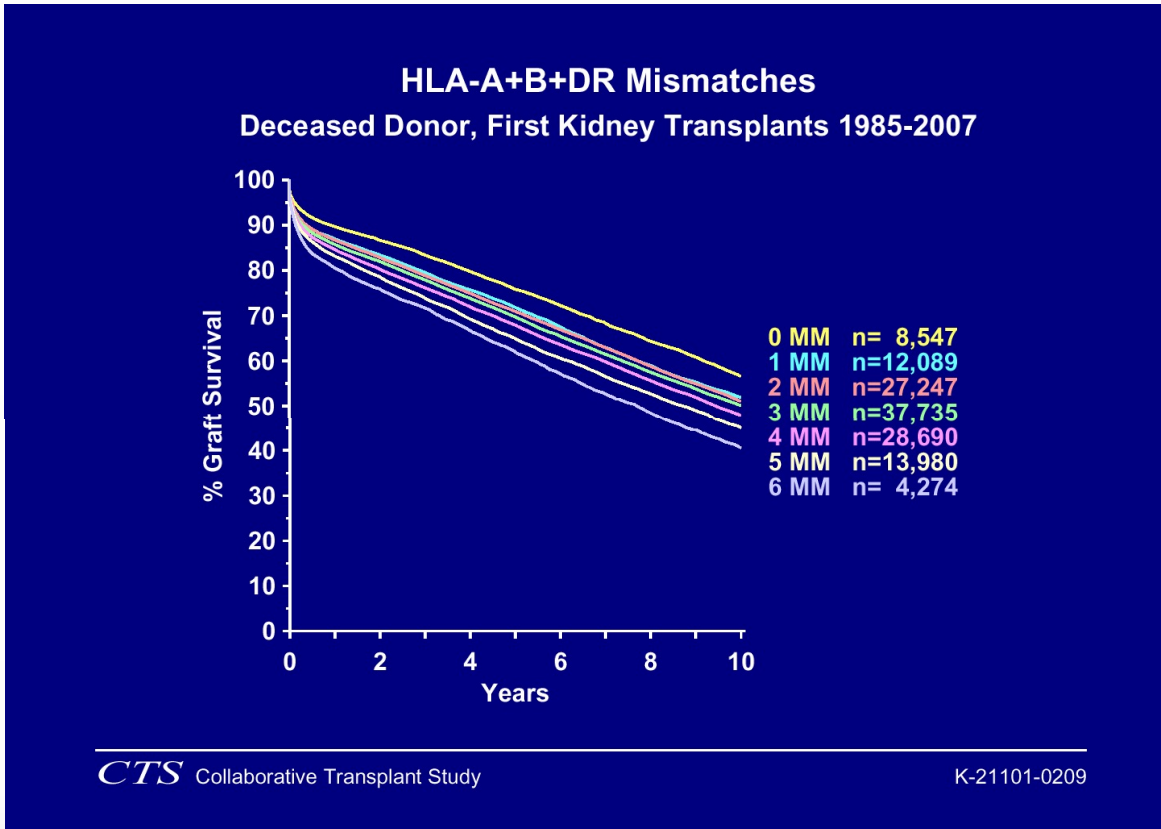
**Liver: No HLA typing required**



**Kidney: Class I and II HLA matching but OK if only 1 or 2 out of 6 matching HLA alleles because prognosis depends more on the timing of transplant**



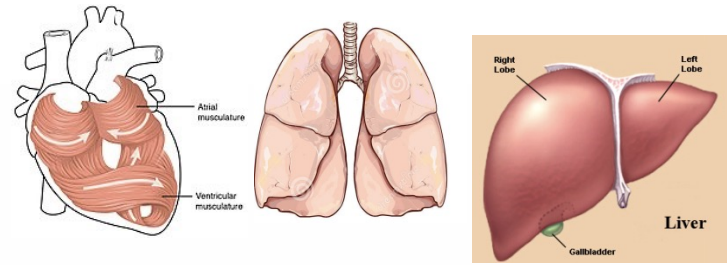
# What is the magnitude of the HLA matching effect in kidney transplantation?



Even when a full HLA match is not required, the incompatibility has a big consequence in terms of graft survival, as shown in this picture for kidney transplantation!!!

In the case of no MM, the probability to observe a good graft outcome is higher!!!

# Heart, Lung and Liver Transplantation



## So why don't we do it routinely?

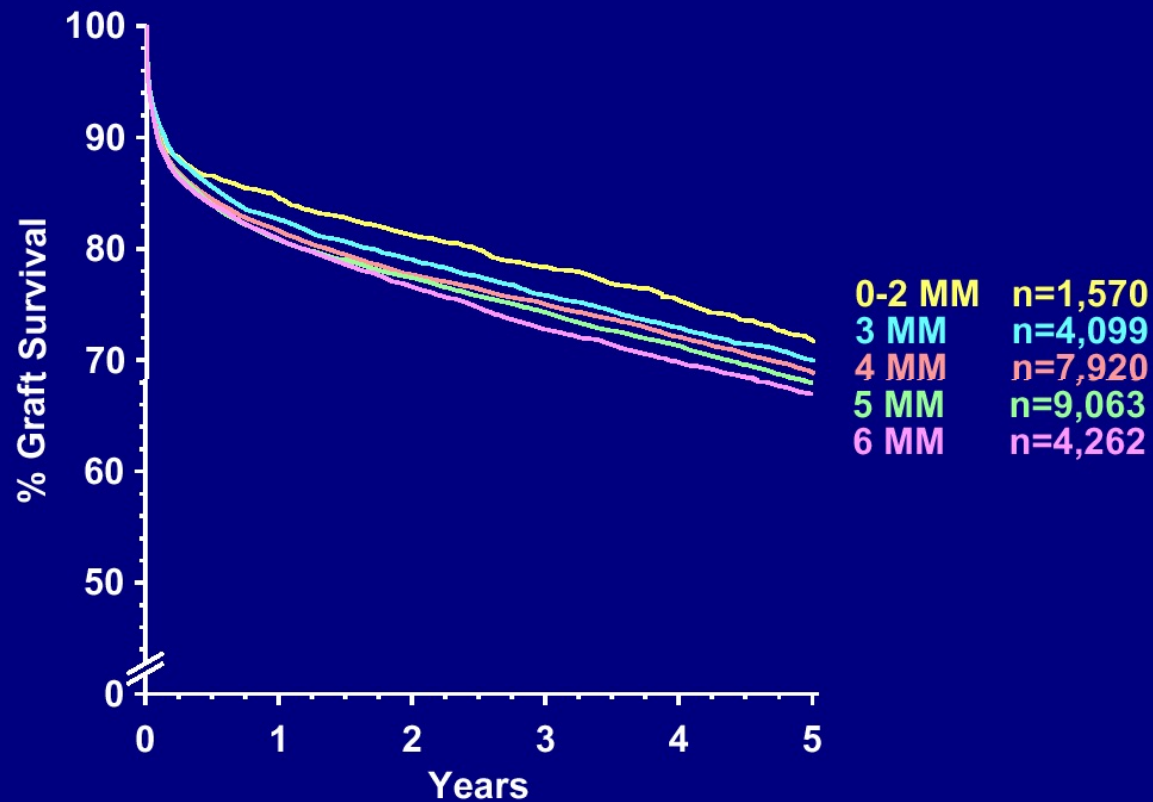
HLA matching is not applied to heart, lung and liver transplants for the following reasons:

- ✓ The patient pool is too small for matching.  
Cold ischaemia time is too short. Hearts must be transplanted within 6-7 hours compared with 24 hours for kidneys. Long-distance organ sharing, therefore, is not feasible.
  - ✓ Most heart, lung and liver transplants are in clinical urgency.  
This overrides any matching considerations.
- ....But this does not mean HLA matching does not have an effect.



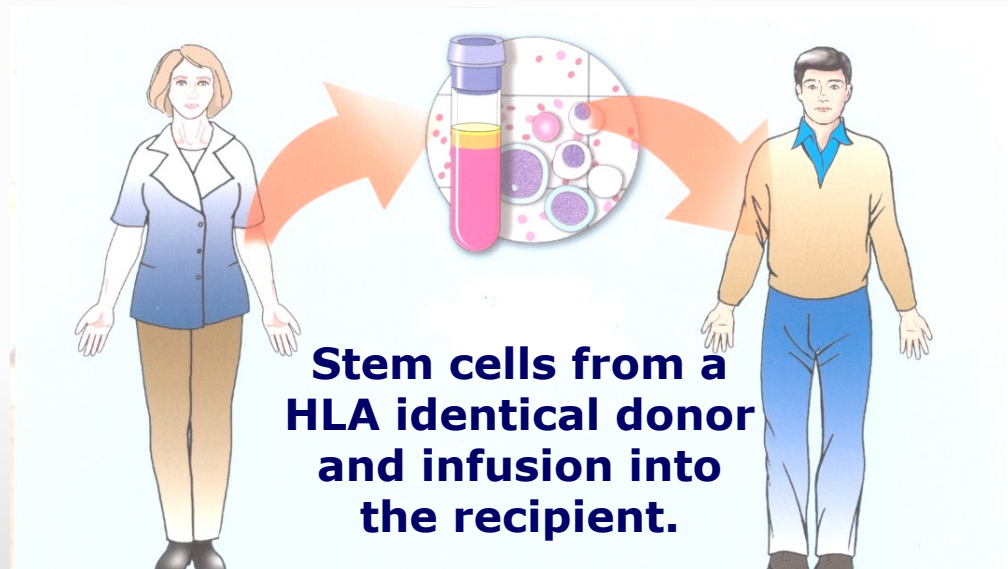
# HLA-A+B+DR Mismatches

## First Orthotopic Heart Transplants 1985-2007



# Hematopoietic Stem cell Transplantation (HSCT)

- Replacement of patient stem cell compartment with one obtained from a healthy donor
- Reconstitution of a new immune system able to recognise recipient tissues as non-self.



# Hematopoietic Stem cell Transplantation (HSCT)

*HLA genotypically identical sibling donors are the gold standard for transplantation purposes, but only 30% patients have such a donor.*

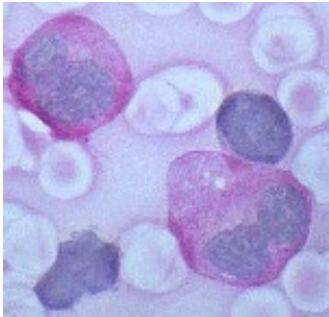
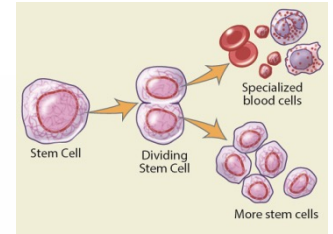
*For the remaining 70% patients alternative sources of stem cells are a **matched unrelated adult volunteer donor**, a **haploidentical donor** or a **cord blood unit**.*

The probability of identifying a matched donor depends on the ethnic group



*In most European populations a 10/10 matched donor can be found for at least 50% of patients and an additional 20-30% patients may have a 9/10 matched donor.*

# HSCT: Types of transplan



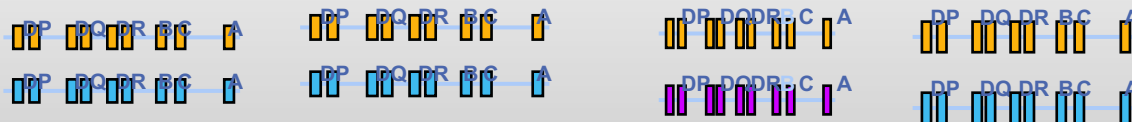
**ALLOGENEIC**

**Related**

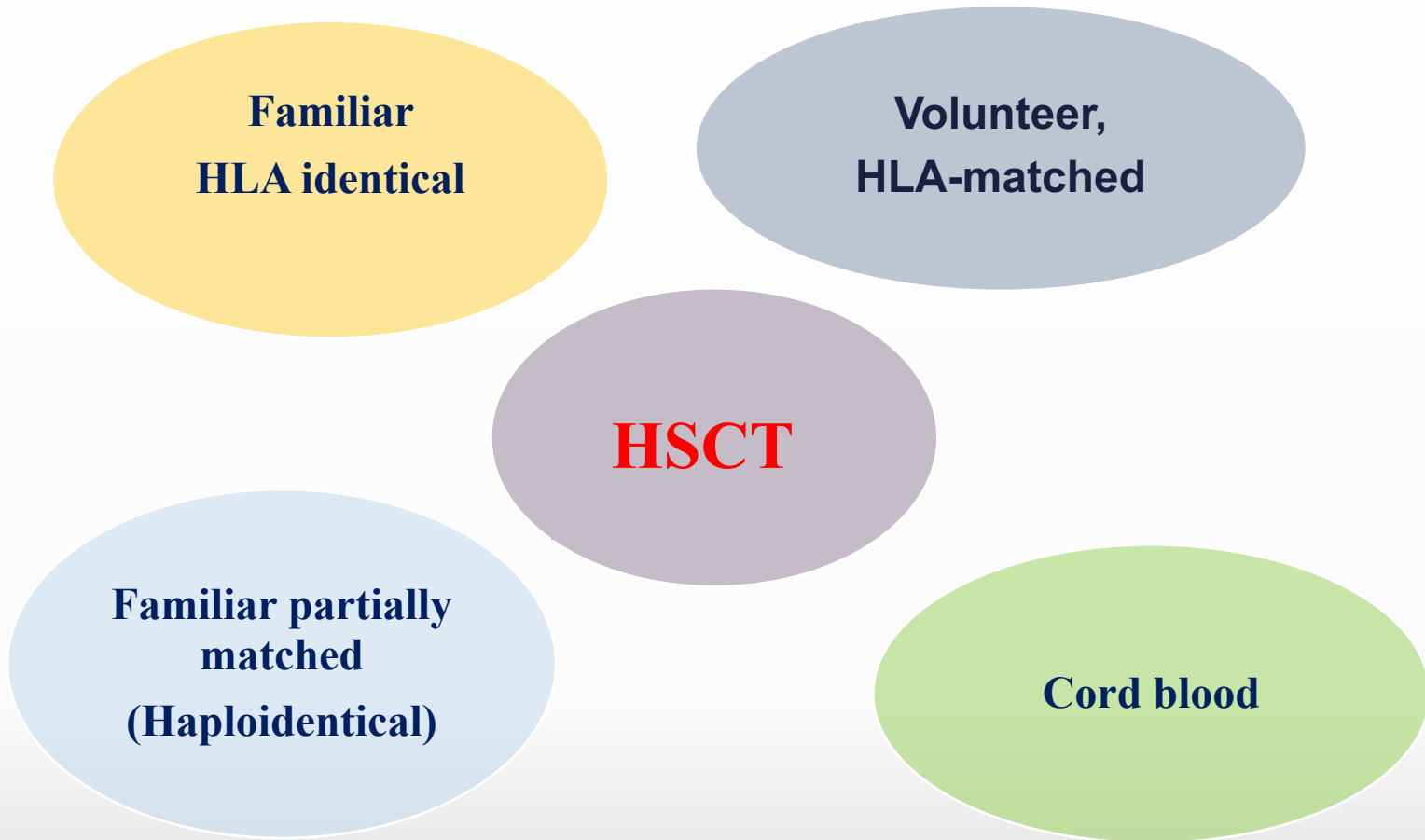
**Unrelated**

**Sibling**

**Haploidentical**



## HSCT: Donor Sources



# Haematopoietic Stem Cell Transplantation (HSCT) : STEM CELL SOURCES

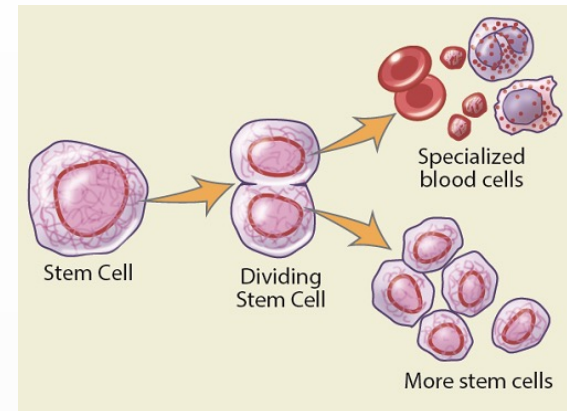


- Here different sources are shown:
- ✓ apheretic collection requiring G-CSF administration to mobilise HSC
  - ✓ cord blood donation;
  - ✓ bone marrow aspiration by the donor iliac crest

# Haematopoietic Stem Cell Transplantation (HSCT)

## Types of transplants:

- Bone Marrow
- PBSC (GCSF mobilized stem cells )
- cord blood



**From an immunological perspective what is the important difference between solid organ and HSCT?**



# Haematopoietic Stem Cell Transplantation (HSCT)

## DOUBLE BARRIER :

Unlike solid organ transplantation, HSCT involves **the transfer of donor immunocompetent cells** which can **recognize HLA differences in the recipient.**

This leads to a **rejection reaction** directed against the recipient.

This is called **graft versus host disease (GVHD).**

GVHD targets the skin, gut and liver and can be life-threatening.





# HLA disparities may cause ...



## Host versus graft (HVG) reaction

*Host T cells attack transplanted donor stem cells (leads to graft failure)*



## Graft versus host (GVH) reaction (30-60%)

*Donor T cells attack cells of the host (leads to host tissue damage, GvHD)*



## Graft versus leukaemia (GVL) effect

Donor T cells attack host leukemia cells (may lead to cure of the malignancy)



# Haematopoietic Stem Cell Transplantation (HSCT)

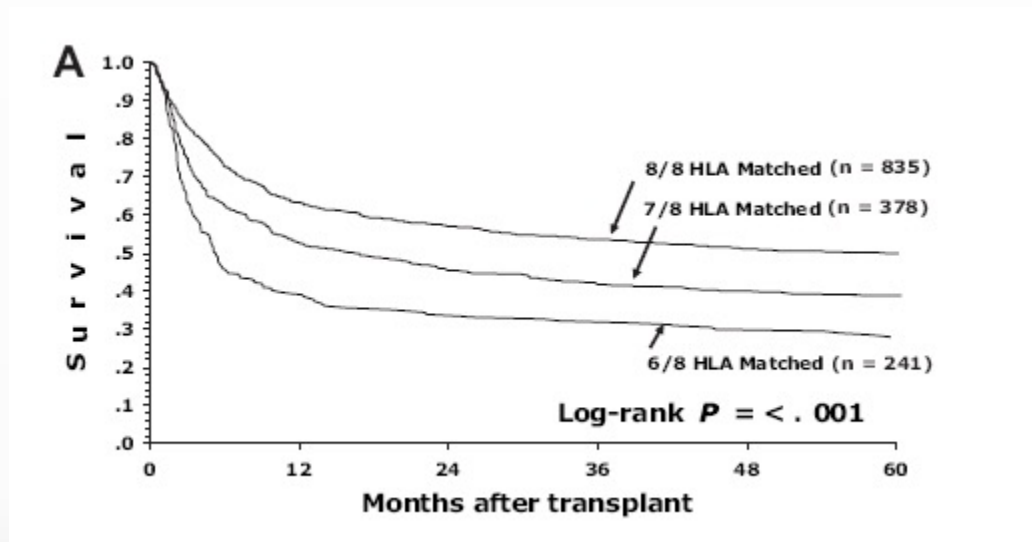
To minimize GVHD risk, donors and recipients should be matched at the allele level at all HLA loci A,B,C,DR,DQ,DP.

If a compatible donor is not found within the family, bone marrow registries are searched.



# High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation

Stephanie J. Lee,<sup>1</sup> John Klein,<sup>2</sup> Michael Haagenson,<sup>3</sup> Lee Ann Baxter-Lowe,<sup>4</sup> Dennis L. Confer,<sup>5</sup> Mary Eapen,<sup>2</sup> Marcelo Fernandez-Vina,<sup>6</sup> Neal Flomenberg,<sup>7</sup> Mary Horowitz,<sup>2</sup> Carolyn K. Hurley,<sup>8</sup> Harriet Noreen,<sup>9</sup> Machteld Oudshoorn,<sup>10</sup> Effie Petersdorf,<sup>1</sup> Michelle Setterholm,<sup>5</sup> Stephen Spellman,<sup>5</sup> Daniel Weisdorf,<sup>11</sup> Thomas M. Williams,<sup>12</sup> and Claudio Anasetti<sup>13</sup>

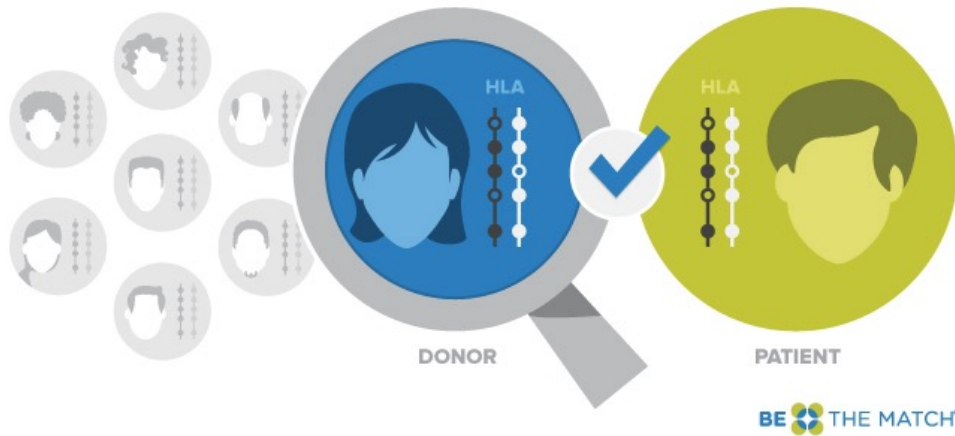


Blood 2007

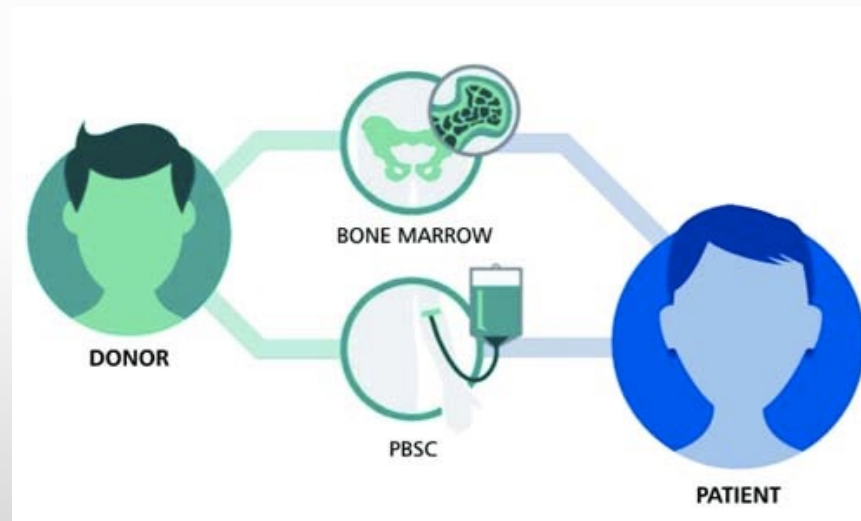
# BONE MARROW REGISTRIES



Matching **donors** with **patients**.



The best available unrelated donor is the donor with the lower number differences in the HLA system!!!!



# What is the probability of finding a MUD?

- ✓ Sibling 1:4
- ✓ Extended family: cousin, uncle etc 1:10,000
- ✓ Unrelated 1:1,000,000 Worldwide Registry of Bone Marrow Donors

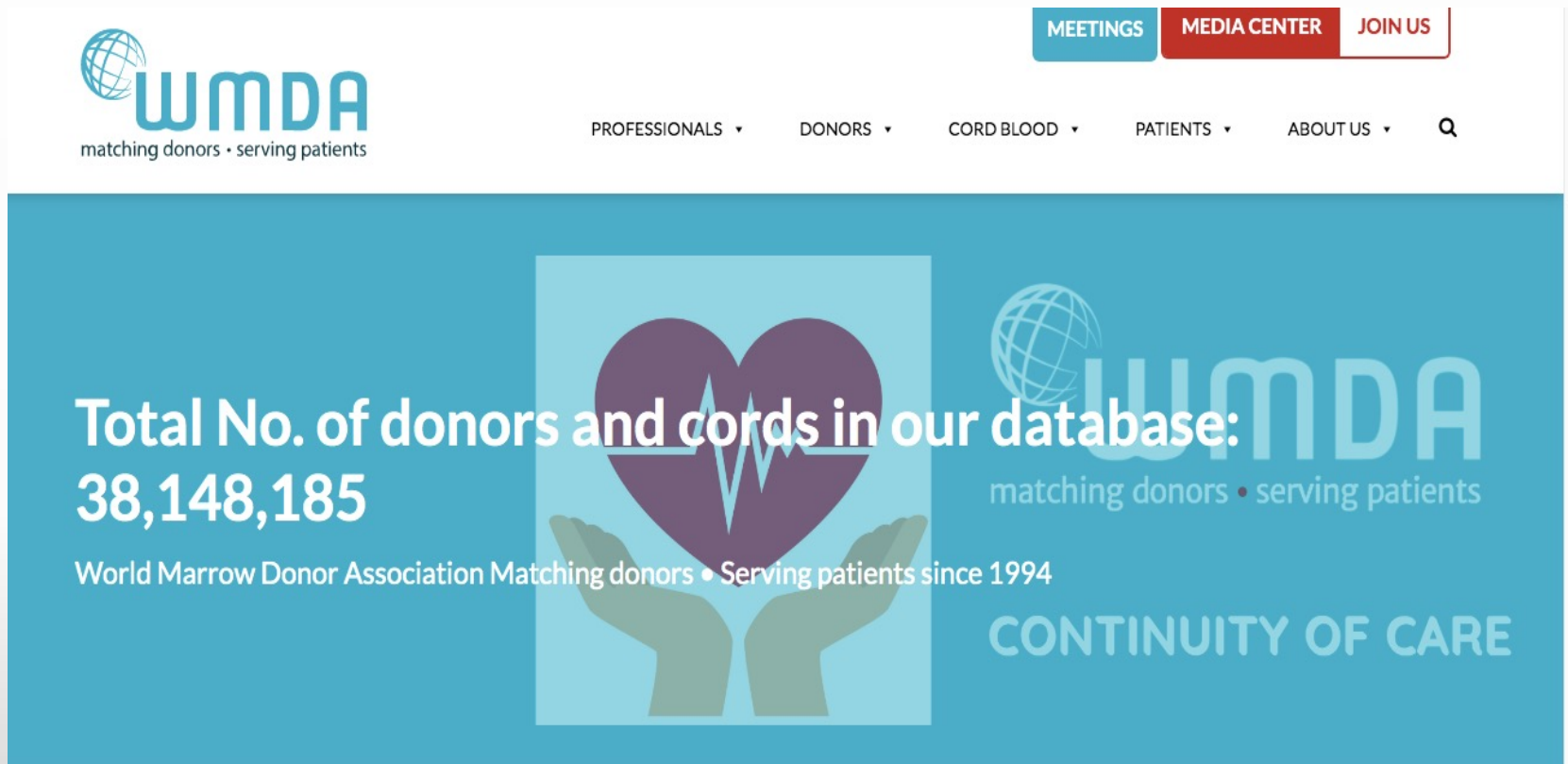
**2018: OVER 27 MILLION DONORS  
ARE NOW REGISTERED IN THE  
INTERNATIONAL DATABASE**



# Today

The global database of volunteer donors was founded in the Netherlands in 1988.

Today, WMDA is the world's largest hematopoietic cell database, listing more than 30 million stem cell donors and over 750,000 cord blood units



The image shows a screenshot of the WMDA website homepage. At the top left is the WMDA logo, which consists of a globe icon and the text "WMDA" in a bold, sans-serif font, with the tagline "matching donors • serving patients" underneath. To the right of the logo is a navigation menu with three buttons: "MEETINGS" (teal), "MEDIA CENTER" (red), and "JOIN US" (white with a red border). Below these buttons is a secondary navigation menu with links for "PROFESSIONALS", "DONORS", "CORD BLOOD", "PATIENTS", and "ABOUT US", each followed by a small downward arrow, and a search icon on the far right. The main content area has a teal background. On the left, it features the text "Total No. of donors and cords in our database:" followed by the large number "38,148,185". Below this is the text "World Marrow Donor Association Matching donors • Serving patients since 1994". In the center is a graphic of two hands holding a heart with a white ECG line. On the right, there is a large, semi-transparent WMDA logo with the tagline "matching donors • serving patients" and the text "CONTINUITY OF CARE" in a bold, white, sans-serif font.

**WMDA**  
matching donors • serving patients

MEETINGS MEDIA CENTER JOIN US

PROFESSIONALS ▾ DONORS ▾ CORD BLOOD ▾ PATIENTS ▾ ABOUT US ▾ 🔍

**Total No. of donors and cords in our database:**  
**38,148,185**

World Marrow Donor Association Matching donors • Serving patients since 1994

**CONTINUITY OF CARE**

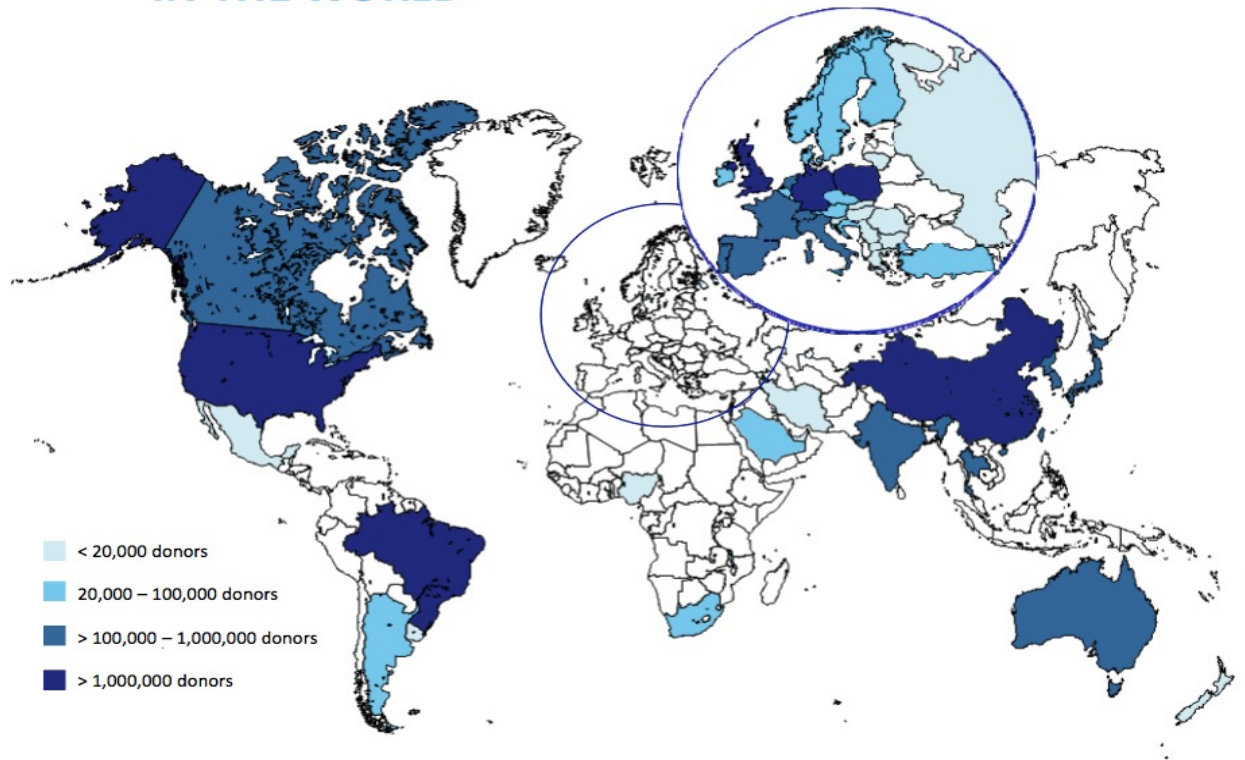


# Probability of finding HLA-identical Donor

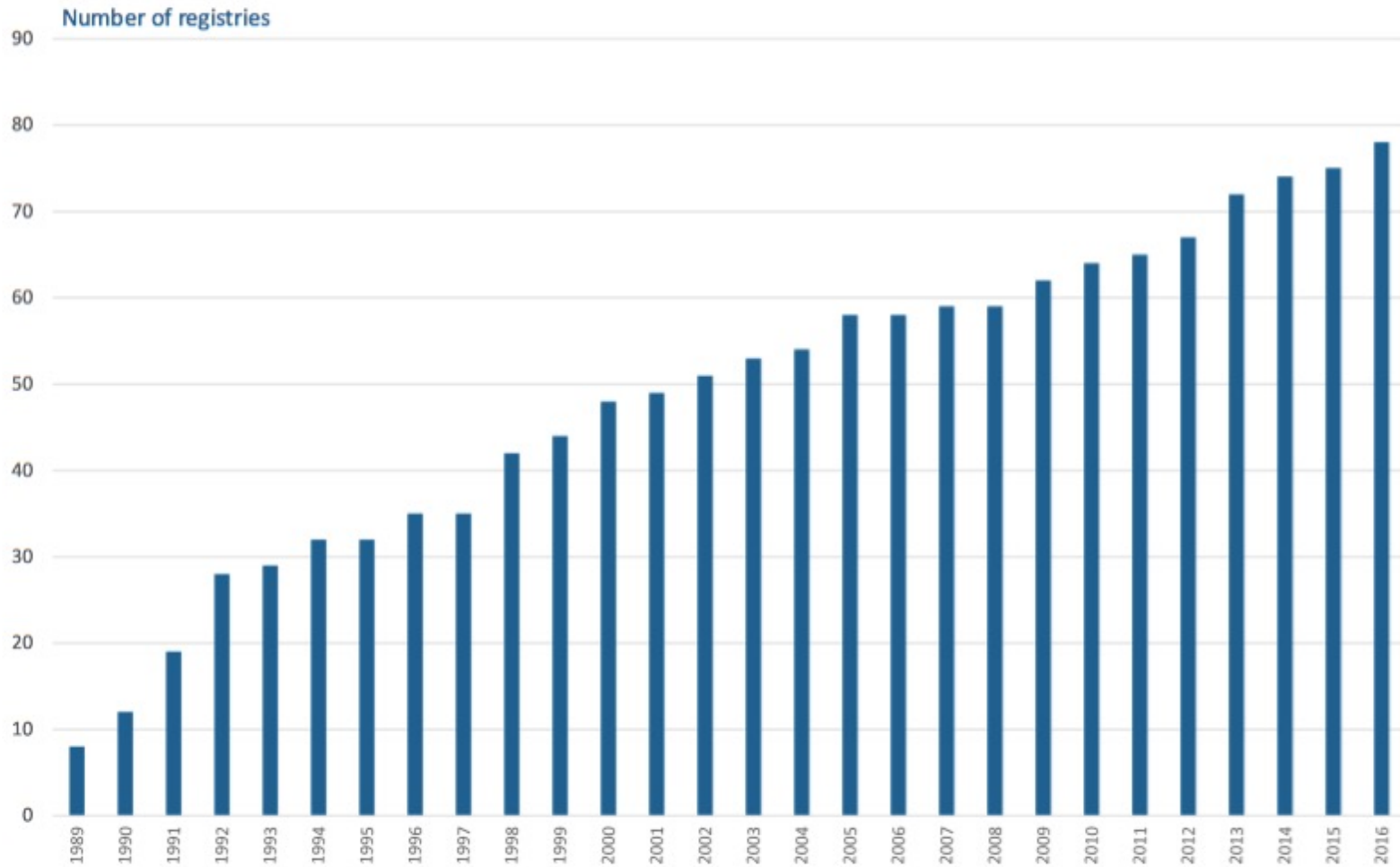
WMDA participants consist of 75 hematopoietic cell donor registries from 53 countries, and 53 cord blood banks from 36 countries.



## DISTRIBUTION OF STEM CELL DONORS IN THE WORLD



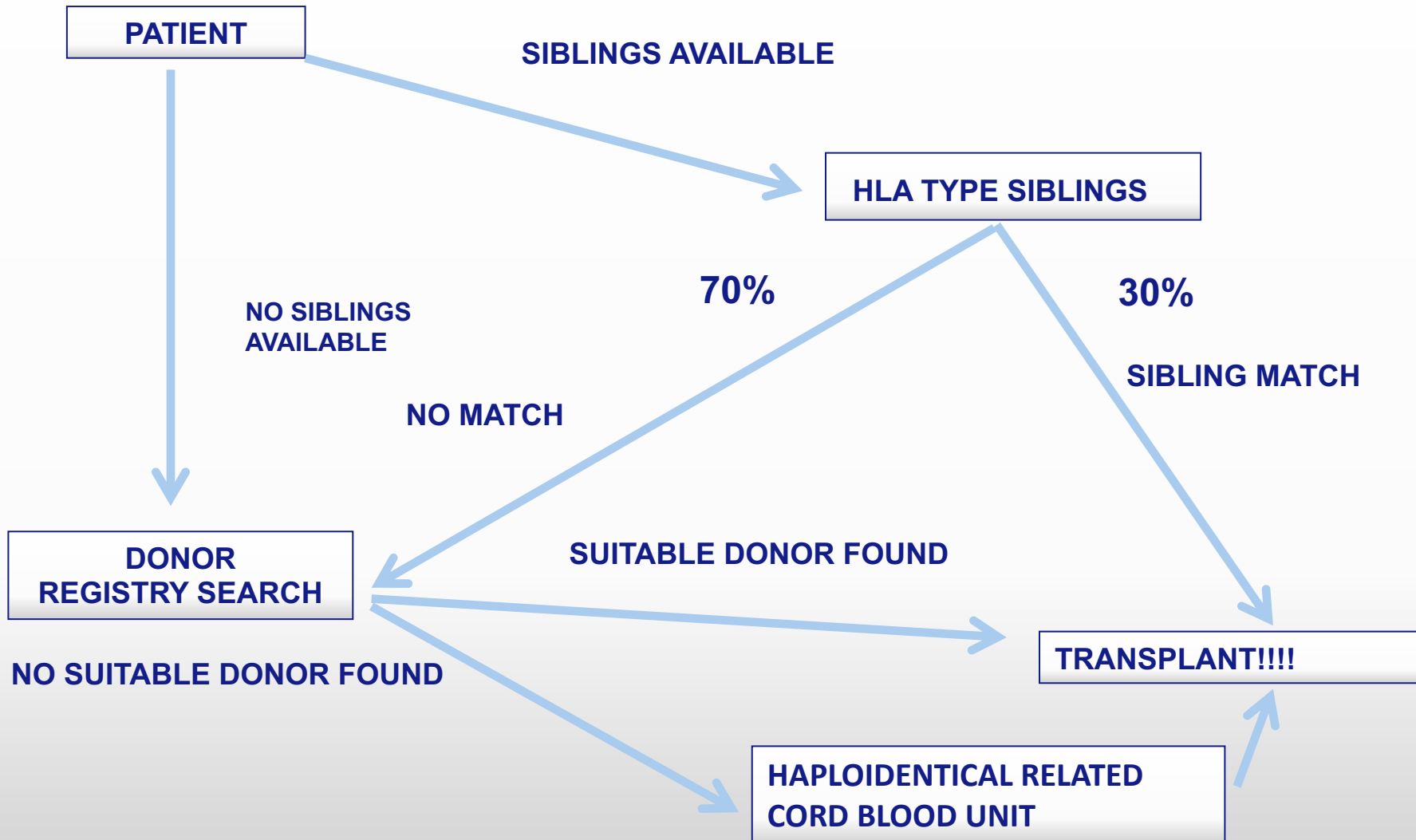
# PARTICIPATING STEM CELL DONOR REGISTRIES





# Donor search approach

Here we can see the HLA based approach in the stem cell donor selection!  
The HLA is the basis!



# The path towards transplant

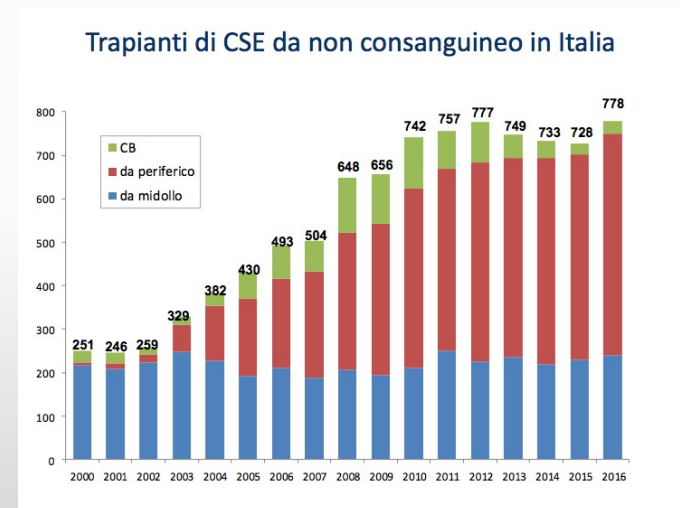
- All tests must be performed according to the **EFI/ASHI/ASEATTA** rules
- Only laboratories with EFI/ASHI/ASEATTA accreditation can perform immunogenetic tests for transplantation



# The numbers

- Donors in Italy (IBMDR): 498.557
- Cord blood units in Italy: 34.453
- Donors in the world: 30.843.592
- Cord Blood Units in the world: 736.332

<https://www.bmdw.org/26-bmdw/news-and-publications/statistics/>



# HLA typing techniques

# HLA typing techniques



## Serology

Microlymphocytotoxicity  
Test

## Molecular Biology

PCR-SSO

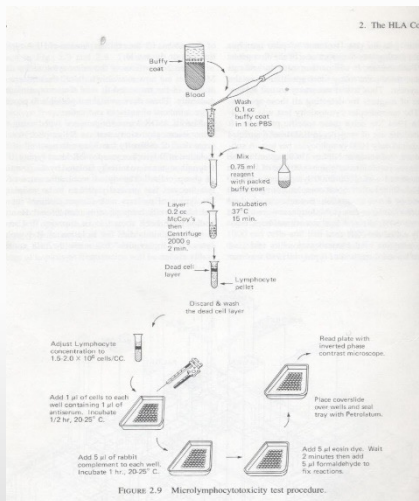
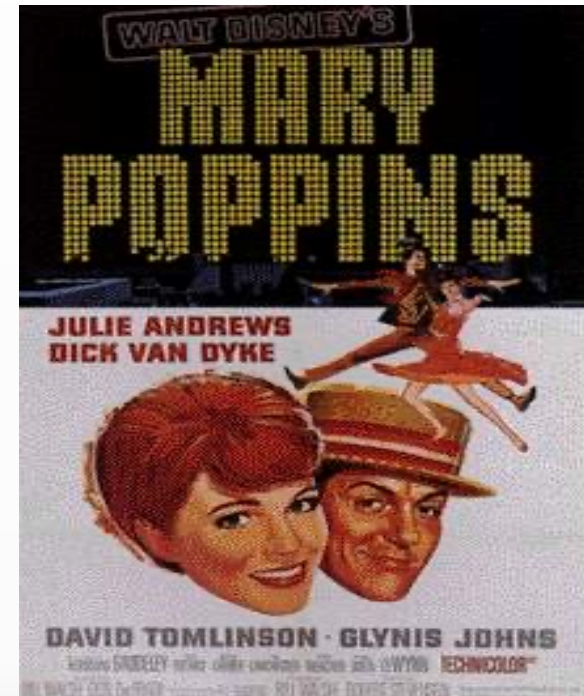
PCR-SSP

PCR-SBT

# What happened in 1964 ?

- Vietnam war
- Nelson Mandela sentenced to life imprisonment
- Martin Luther King Nobel Prize for peace
- Jean Paul Sartre Nobel Prize for literature
- Tokyo Olympic Games
- My fair lady best movie of the year
- And....

*Mary Poppins*  
W. Disney



*The microlymphocytotoxicity test*  
*P.I. Terasaki*

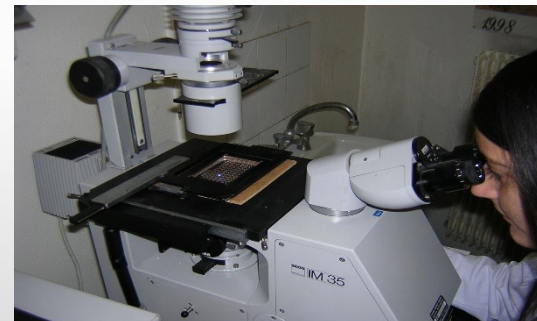
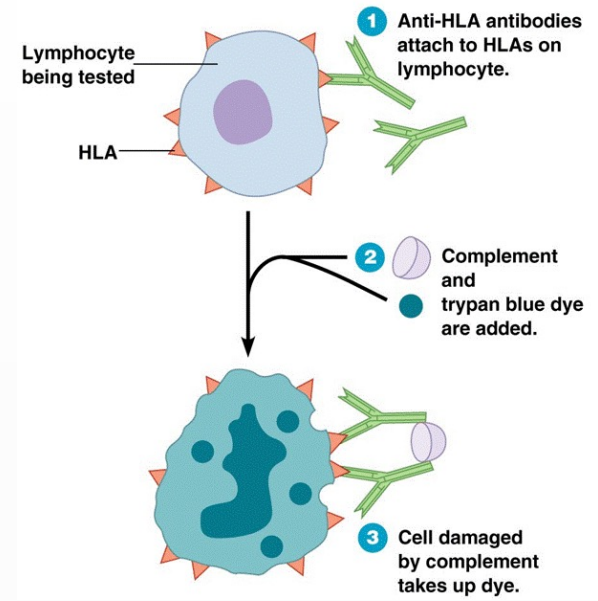
# Principle of microlymphocytotoxicity test

Lymphocytes are tested with a panel of sera containing well characterized **HLA-specific alloantibodies**.

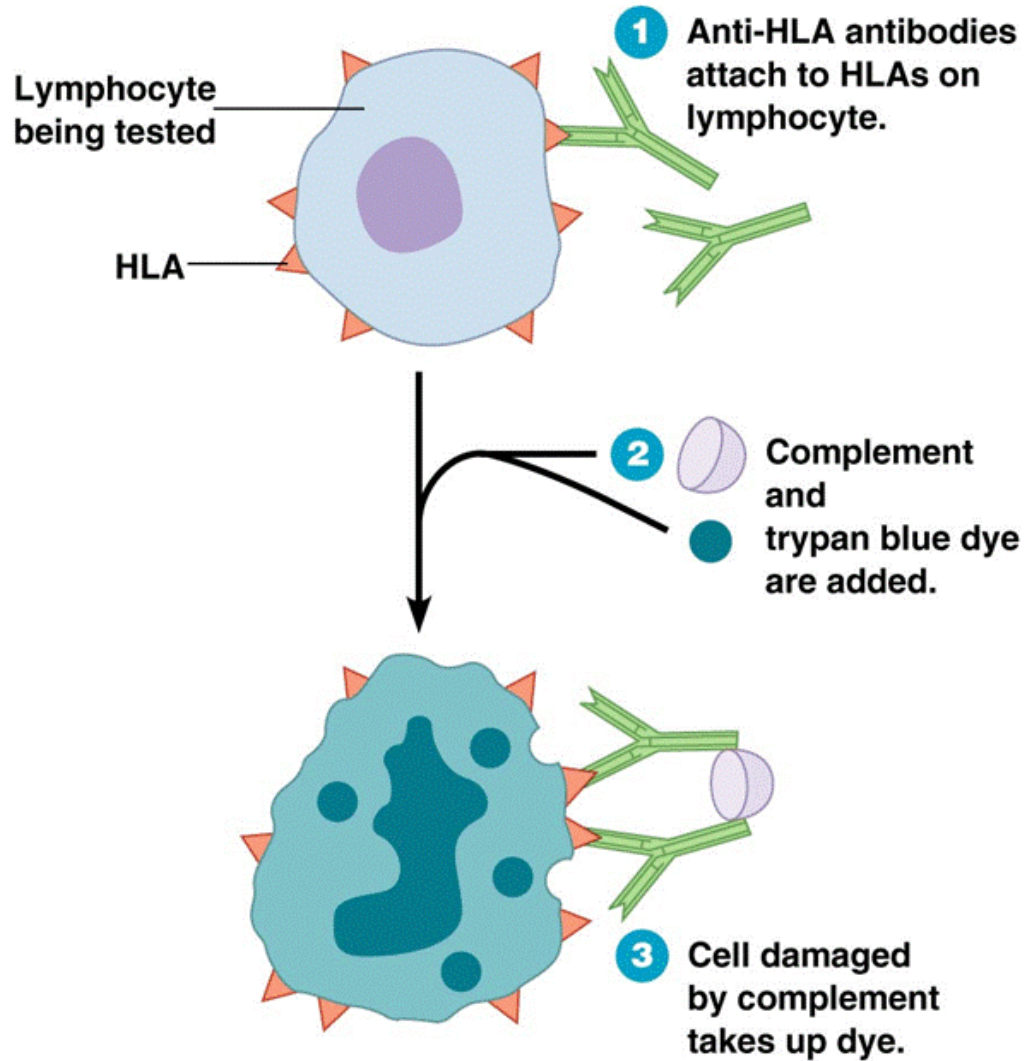
After a short incubation, rabbit serum is added as a source of complement; **the complement cascade is activated through the membrane attack complex**, leading to lymphocytotoxicity.

Cells that have no attached antibody, activated complement, or damage to the membrane keep the vital dyes from penetrating; **however, cells with damaged membranes allow the dye (fluorochrome ethidium bromide) to enter**.

**The cells are examined for dye exclusion or uptake under phase contrast microscopy.** If a fluorescent microscope is available, fluorescent vital dyes can also be used.

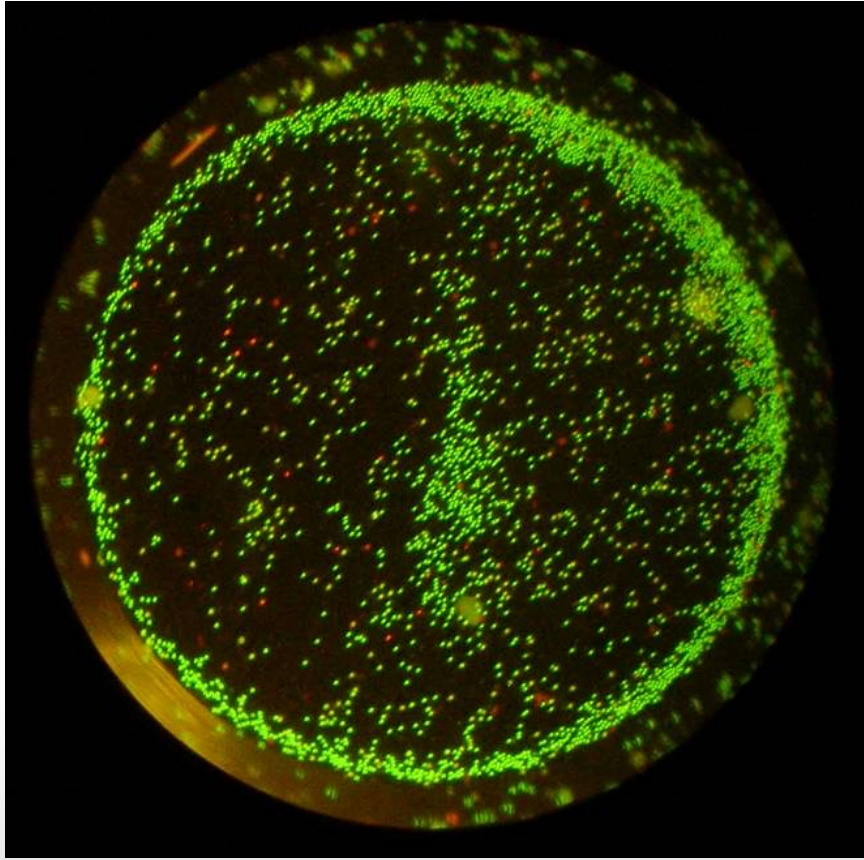


# *Principle of microlymphocytotoxicity test*

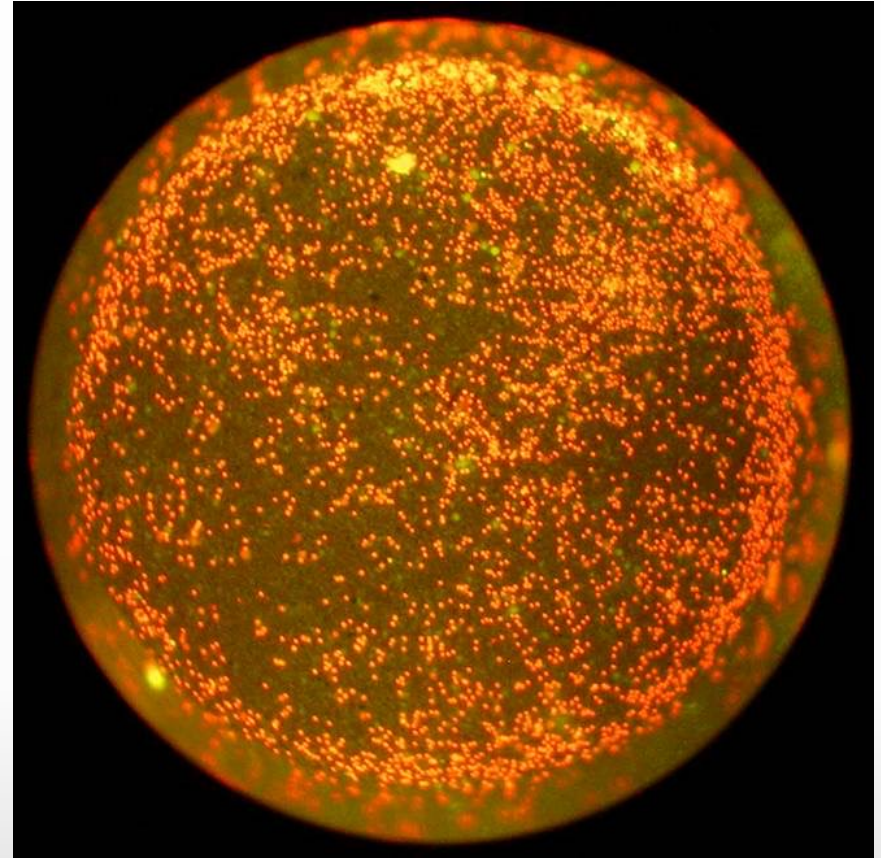




# *The microlymphocytotoxicity test*



**Negative**

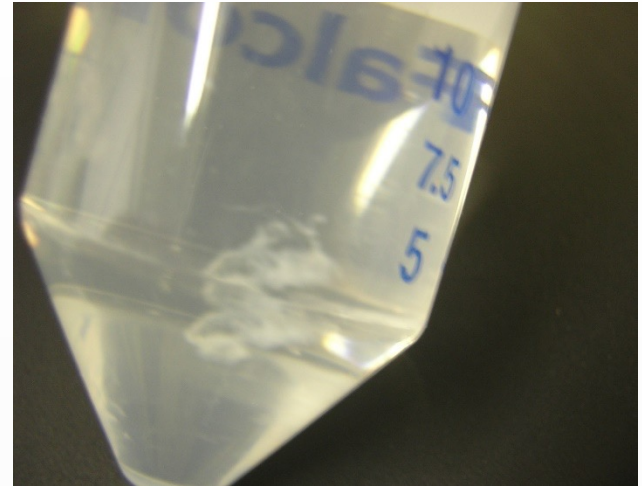


**Positive**

# Molecular Biology

PCR (polymerase chain reaction): is a technique in molecular genetics that permits the analysis of any short sequence of DNA (or RNA) even in samples containing only minute quantities of DNA or RNA.

PCR is used to reproduce (amplify) selected sections of DNA or RNA for analysis



# PCR



## PCR-SSP

*Sequence-Specific-Primers*

*Oligonucleotides are complementary to specific sequences of a certain allele or groups of alleles*



## PCR-SSO

*Sequence-Specific-Oligotyping*

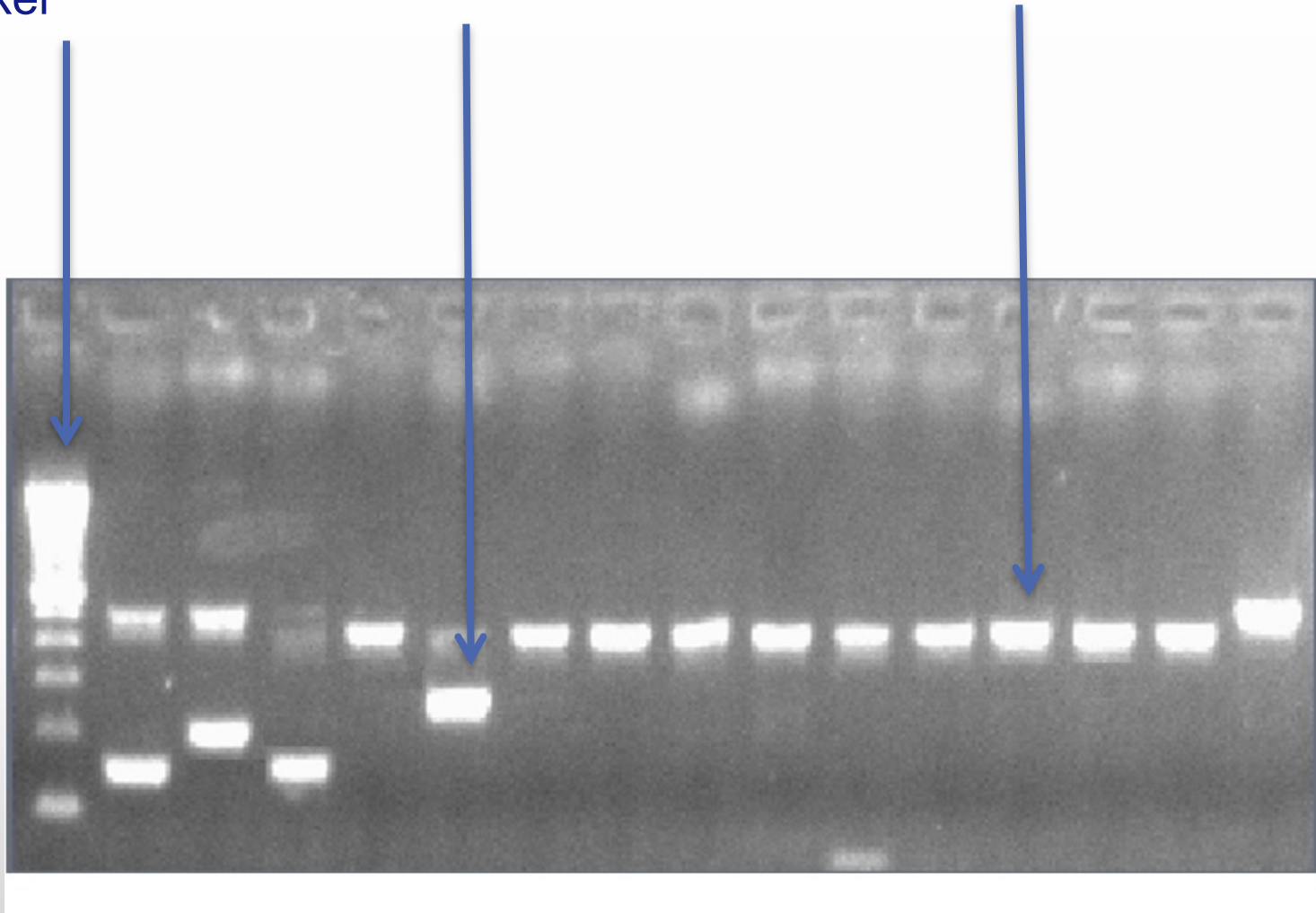
*Oligonucleotides recognize common sequences to different alleles of a certain locus*

# ***DNA picture***

DNA weight  
marker

Amplified DNA

Control DNA

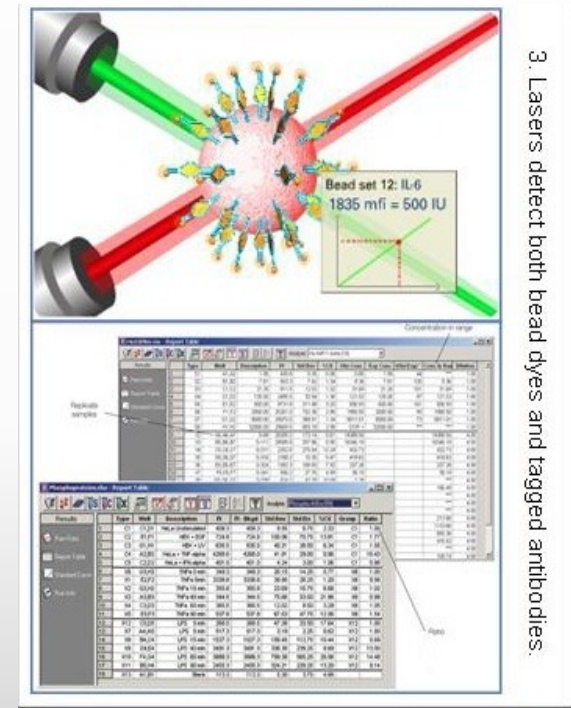
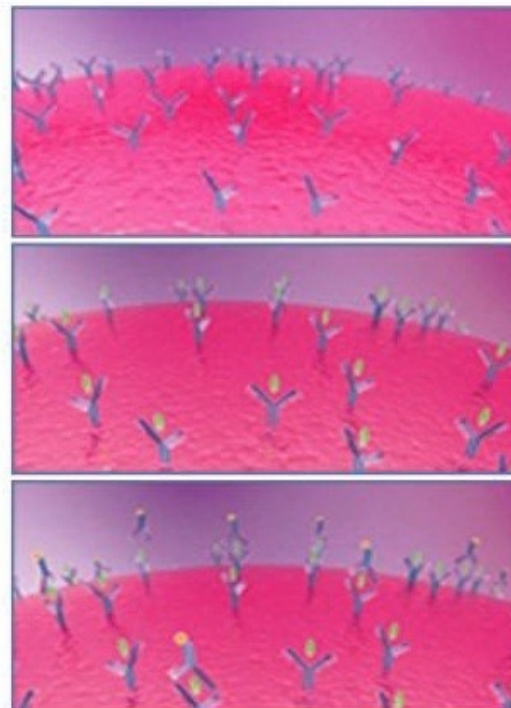
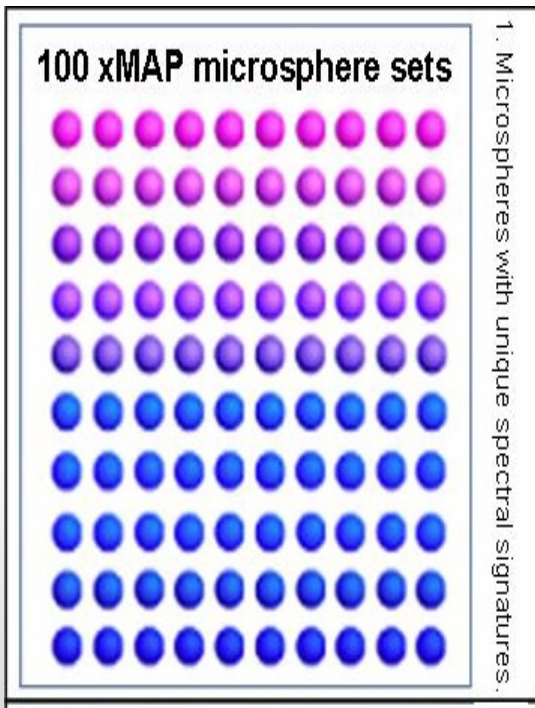




# THE Luminex Technology

The luminex technology use HLA coated beads; is a cytofluorimetric method!

Assays based on the xMAP® technology use a liquid suspension array with up to 100 uniquely colour-coded bead sets. Each of the 100 beads are internally labelled with a specific ratio of two fluorophores to assign it a unique spectral address. The beads are then conjugated with different biomolecules (including RNA, DNA, enzyme substrates, receptors, antigens, and antibodies), allowing the capture of specific analytes from the sample. A fluorescently-labelled reporter molecule is then added to the sample in order to detect and quantitate each captured analyte. The beads are drawn through a flow cell where two lasers excite each bead. Fluorescent signals are recorded, translating the signals into data for each bead-based assay.

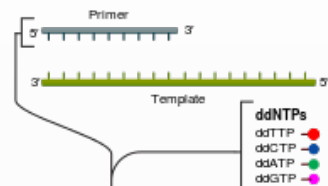


# HLA Sequencing

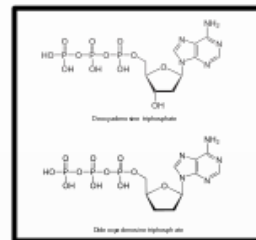
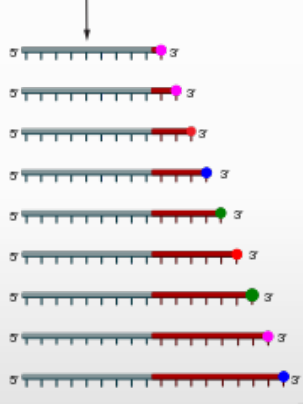
**Sanger sequencing** is a method of DNA sequencing based on the selective incorporation of chain-terminating dideoxynucleotides (instead of deoxynucleotides) by DNA polymerase during in vitro DNA replication.

## ① Reaction mixture

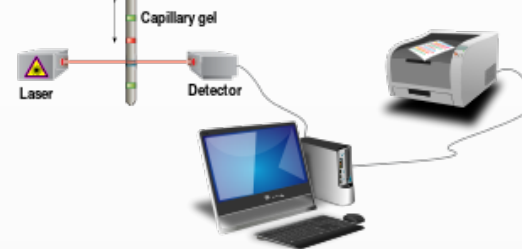
- ▶ Primer and DNA template
- ▶ DNA polymerase
- ▶ ddNTPs with fluorochromes
- ▶ dNTPs (dATP, dCTP, dGTP, and dTTP)



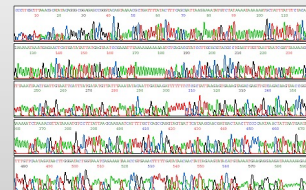
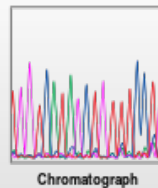
## ② Primer elongation and chain termination



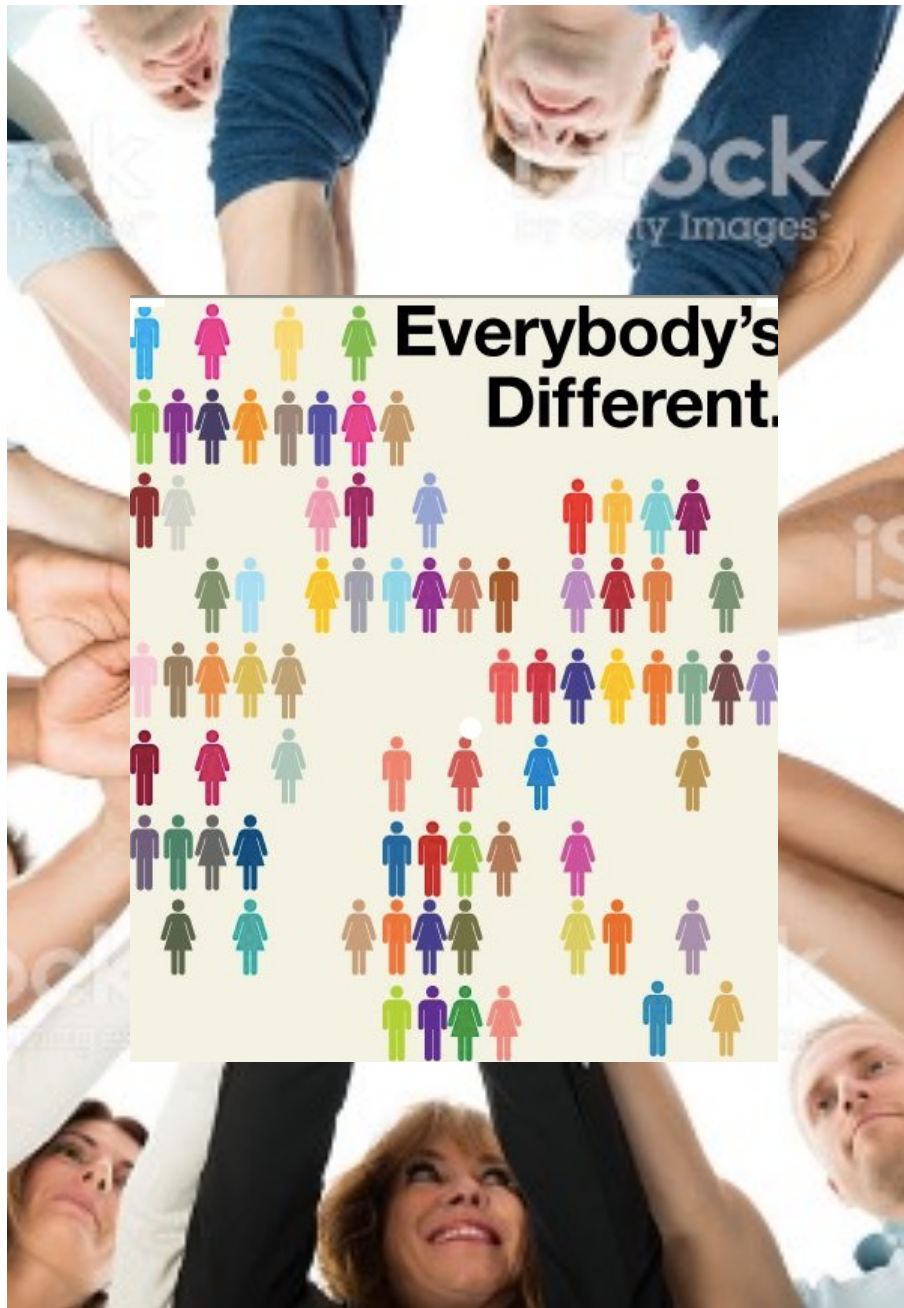
## ③ Capillary gel electrophoresis separation of DNA fragments



## ④ Laser detection of fluorochromes and computational sequence analysis



The Sanger (chain-termination) method for DNA sequencing. (1) A primer is annealed to a sequence, (2) Reagents are added to the primer and template, including: DNA polymerase, dNTPs, and a small amount of all four dideoxynucleotides (ddNTPs) labeled with fluorophores. During primer elongation, the random insertion of a ddNTP instead of a dNTP terminates synthesis of the chain because DNA polymerase cannot react with the missing hydroxyl. This produces all possible lengths of chains. (3) The products are separated on a single lane capillary gel, where the resulting bands are read by an imaging system. (4) This produces several hundred thousand nucleotides a day, data which require storage and subsequent computational analysis.







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[Sung Yoon Choo](#)

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### HLA TESTING IN THE MOLECULAR DIAGNOSTIC LABORATORY

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Virchows Arch 474, 139–147 (2019)

<https://doi.org/10.1007/s00428-018-2501-3>

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