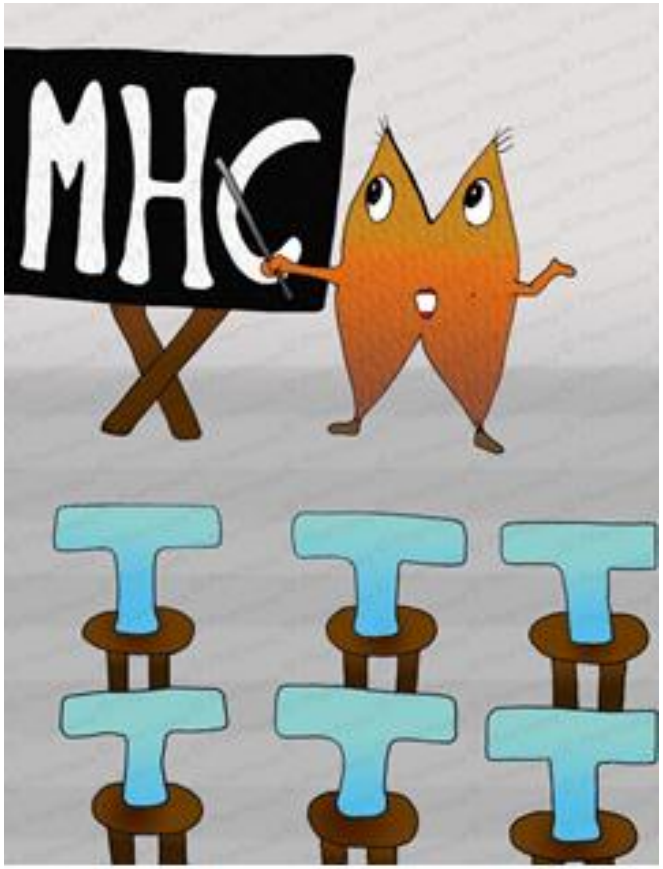
HLA and Typing

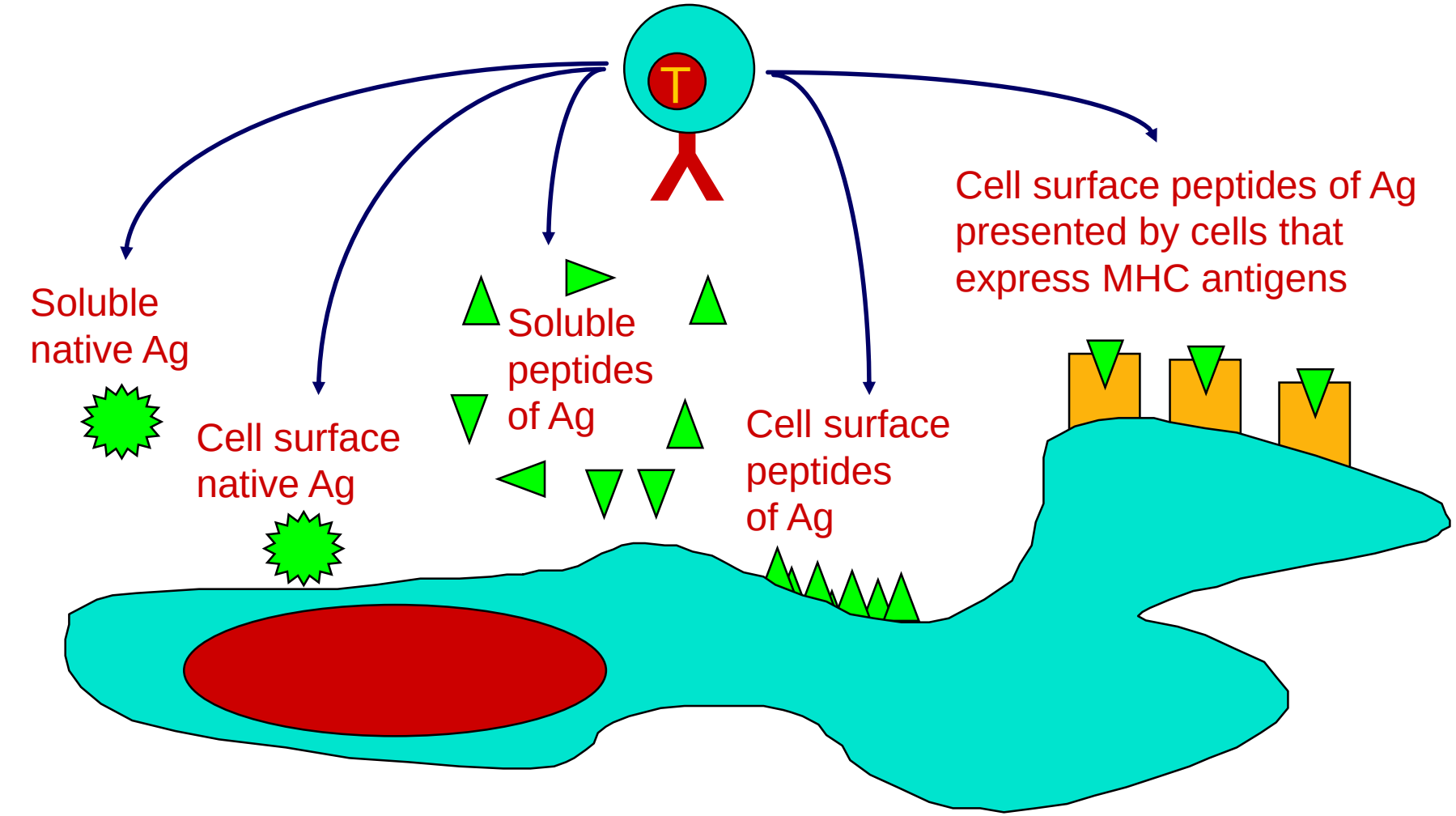


Reza Jafari-Shakib

MD, Ph.D in immunology

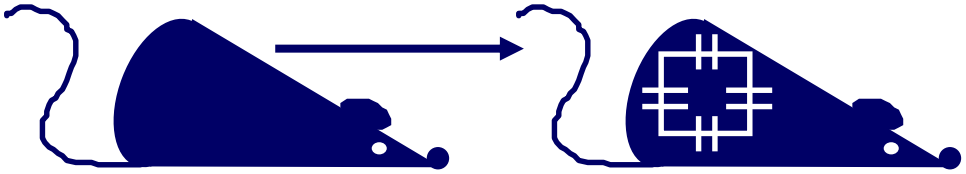
Associate Professor in Guilan University of Medical Sciences

Antigen recognition by T cells requires peptide antigens and presenting cells that express MHC molecules



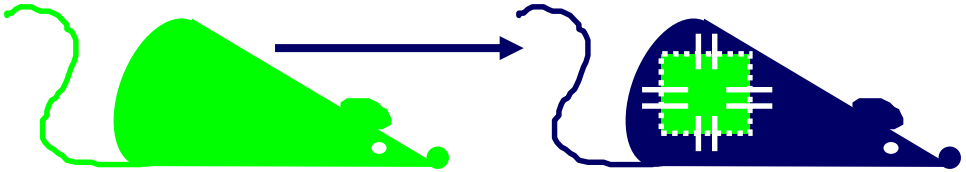
Genetic basis of transplant rejection

Inbred mouse strains - all genes are identical



ACCEPTED

Skin from an inbred mouse grafted onto the same strain of mouse



REJECTED

Skin from an inbred mouse grafted onto a different strain of mouse

Transplantation of skin between strains showed that rejection or acceptance was dependent upon the genetics of each strain

Discovery of MHC

• The recognition of a graft as self or foreign is an inherited trait. The genes responsible for causing a grafted tissue to be perceived as similar to or different from one's own tissues were called **histocompatibility genes** (genes that determine tissue compatibility between individuals), and the differences between self and foreign were attributed to polymorphisms among different histocompatibility gene alleles.

• The genetic region that is primarily responsible for rapid graft rejection and contained several linked genes was named the **major histocompatibility complex (MHC)**.

• Other genes that contribute to graft rejection to a lesser degree are called **minor histocompatibility genes.**

• MHC is called HLA (Human Leukocyte Antigens) in human.

• For almost 20 years after the MHC was discovered, its only documented role was in graft rejection. In the 1960s and 1970s, it was discovered that MHC genes are of *fundamental importance for all immune responses to protein antigens*.

Genetic definition

• Some genes are represented by only one normal nucleic acid sequence in all the members of a species (except for relatively rare mutations); such genes are said to be **nonpolymorphic**, and the gene sequence is usually present on both chromosomes of a pair in every member of the species.

• By contrast, alternate forms, or variants, of other genes are present at stable frequencies in different members of the population. Such genes are said to be **polymorphic**, and each common variant of a polymorphic gene is called an **allele**. For polymorphic genes, an individual can have the same allele at that genetic locus on both chromosomes of the pair and would be said to be **homozygous**, or an individual can have two different alleles, one on each chromosome, and would be termed **heterozygous**.

TABLE 15.3 Association of Human Leukocyte Antigen Alleles With Autoimmune Disease

Disease	HLA Allele	Odds Ratio[a]
RA (anti-CCP Ab positive)[b]	*DRB1, 1 SE* allele[c]	4
	DRB1, 2 SE alleles	12
T1D	*DRB1*0301-DQA1*0501-DQB1*0201* haplotype	4
	*DRB1*0401-DQA1*0301-DQB1*0302* haplotype	8
	*DRB1*0301/0401* heterozygotes	35
Multiple sclerosis	*DRB1*1501*	3
SLE	*DRB1*0301*	2
	*DRB1*1501*	1.3
AS	*B27* (mainly *B*2705* and *B*2702*)	100
Celiac disease[d]	*DQA1*0501-DQB1*0201* haplotype	7

HLA Disease associations

Olerup SSP kits for the screening of HLA-alleles associated with diseases.

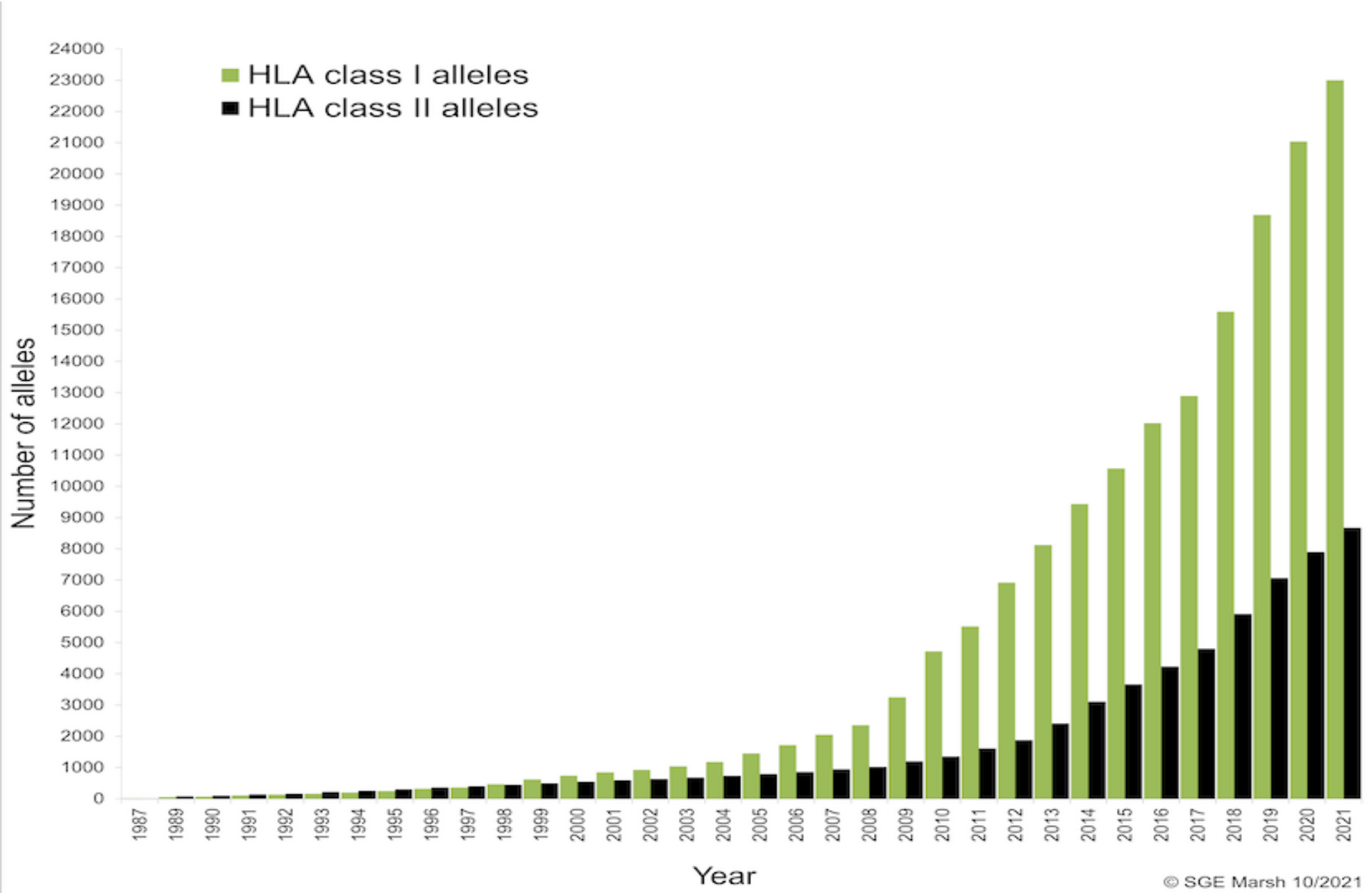
There are autoimmune conditions with a strong association with HLAs, such as: Ankylosing Spondylitis associated to HLA-B*27; Narcolepsy a neurological disease closely associated with the HLA-DQB1*06:02 and over 95% of patients with Coeliac Disease have some isoform of HLA-DQ2 or DQ8.

Drug hypersensitivity: Abacavir is a nucleoside reverse transcriptase inhibitor used in conjunction with other antiretroviral agents in the treatment of HIV infection. Hypersensitivity to abacavir is immunologically mediated, driven by conventional MHC-I antigen presentation and activation of HLA-B*57:01.

All kits are available in 2 formats: with and without Taq Polymerase. All master mixes contain cresol red, glycerin , PCR buffer and dNTPs.

Ankylosing Spondylitis →
Coeliac Disease →
Narcolepsy →
Abacavir →

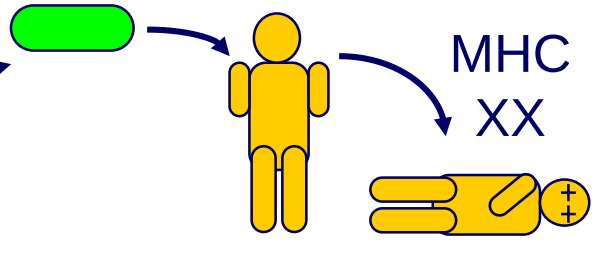
Graph showing numbers of alleles from 1987 to 2021



Numbers of HLA Alleles	Apr 2012	Oct 2015	Dec 2017	Sep 2018	Mar 2020	Sep 2020	Sep 2021
HLA Class I Alleles	5,880	10,297	12,893	14,800	19,587	20,597	23,002
HLA Class II Alleles	1,647	3,543	4,802	5,288	7,302	7,723	8,673
HLA Alleles	7,527	13,840	17,695	20,088	26,889	28,320	31,675
Other non-HLA Alleles	143	175	179	184	384	466	655

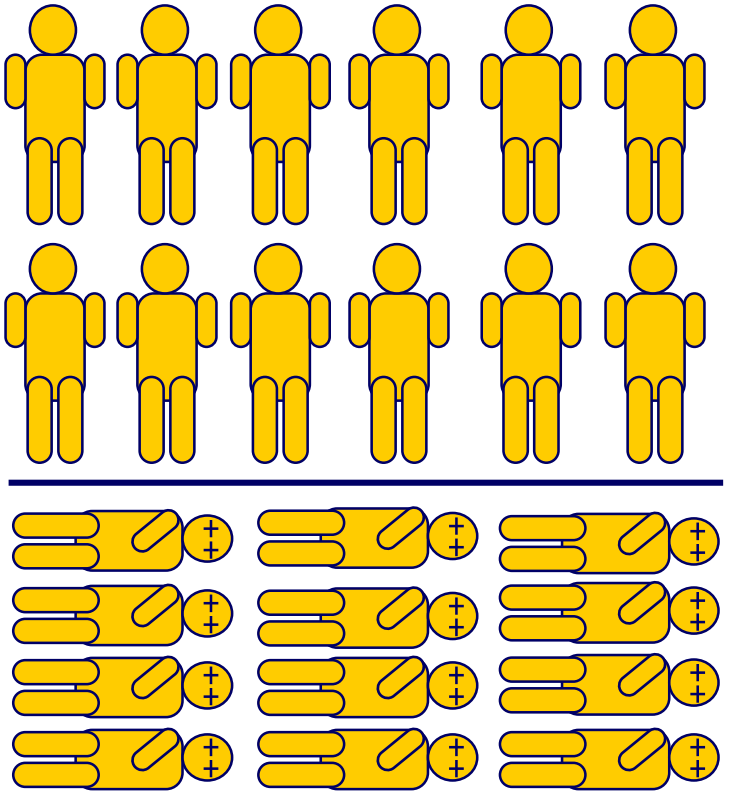
Data from http://hla.alleles.org/nomenclature/stats.html

Problem……if MHC molecules aren't polymorph



Pathogen that mutates MHC X - binding antigens in order to survive

Survival of individual threatened



Population threatened with extinction

Why so many different HLAs in human population?

- HLA heterozygosity may influence survival from HIV infection
  - **Red line – completely heterozygous for all HLA Class I loci**
  - **Yellow line – homozygous for one of the HLA Class I loci**
  - **Blue line – homozygous for two or three HLA Class I loci**

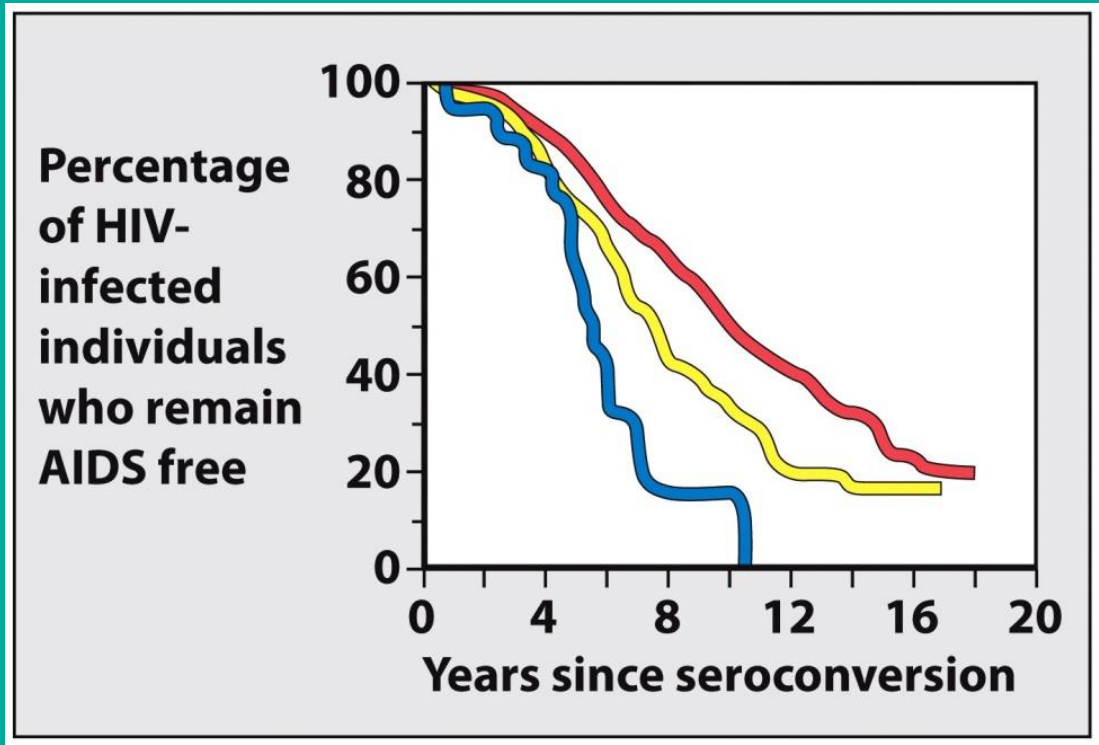


Figure 5.35 The Immune System, 3ed. (© Garland Science 2009)

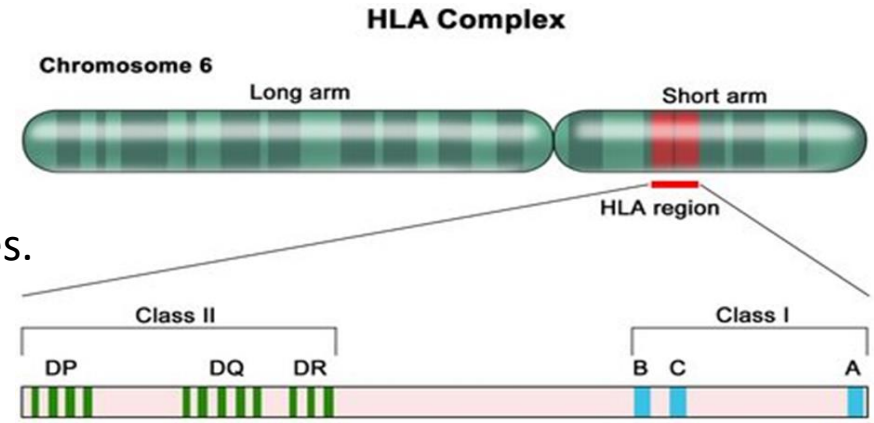
Peptide-Binding Motifs Encoded by Different HLA Alleles Influence the Number of Peptides in a Protein That can be Recognized by a HLA Molecule (*e.g.*, HIV Envelope Protein)

Allele designation HLA-B*27:05 HLA-B*35:01 HLA-B*07:02 Peptide-binding motif	HLA-B*27:05 XRXXXXXX[KRYL]	HLA-B*35:01 XPXXXXXXY	HLA-B*07:02 XPXXXXXXL
Peptides from the HIV envelope protein able to bind to each allotype	IRGKVQKEY IRPVVSTQL TRPNNNTRK IRIQRGPGR SRAKWNNTL LREQFGNNK FRPGGGDMR WRSELYKYK KRRVVQREK ARILAVERY ERDRDRSIR LRSLCLFSY TRIVELLGR CRAIRHIPR IRQGLERIL	None	DPNPQEVVL KPCVKLTPL RPVVSTQLL SPLSFQTHL IPRRIRQGL
Number of peptides bound	15	0	5

HLA-B*27:05 is one of the widely reported alleles associated with resistance to HIV
Previous longitudinal studies demonstrated that HLA-B*35 was significantly associated with rapid progression to AIDS.

Human MHC

- In humans, the MHC is located on the short arm of chromosome 6, and β2-microglobulin is encoded by a gene on chromosome 15.
- HLA extends about 3500 kilobases (kb). (For comparison the size of the entire genome of the bacterium *Escherichia coli is approximately 4500kb)*
- Many of the proteins involved in the processing of protein antigens and the presentation of peptides to T cells are encoded by genes located within the MHC.
- There are two main types of MHC gene products, called class I MHC molecules and class II MHC molecules
- **Major** HLA molecules
  - Class I – HLA-A, HLA-B, HLA-C
  - Class II – HLA-DP, HLA-DQ, HLA-DR
- Most individuals are heterozygous for MHC genes.



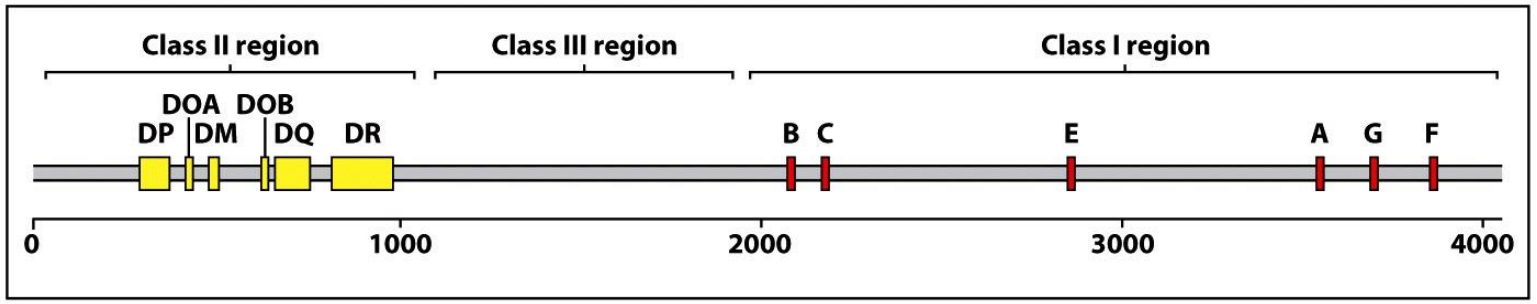
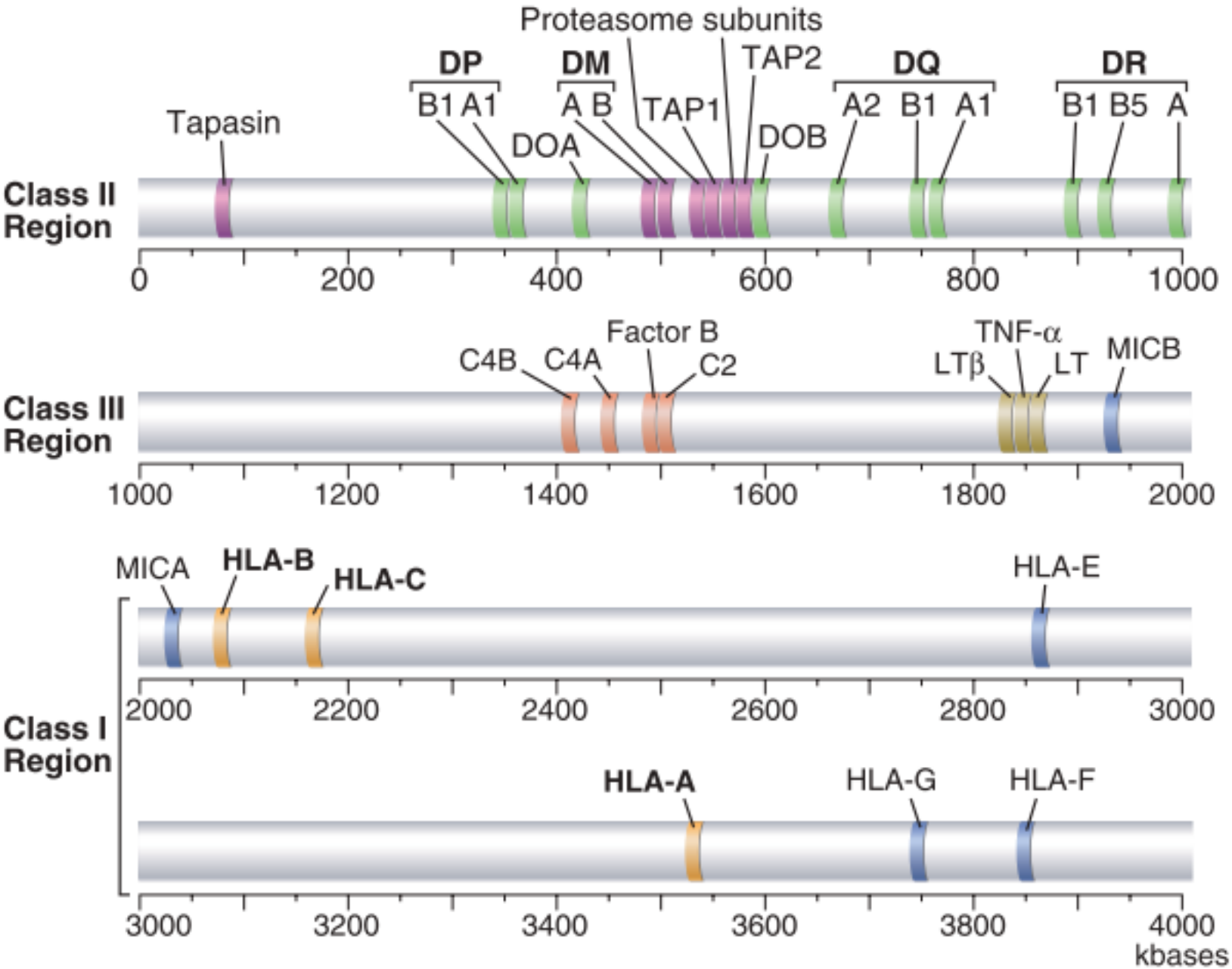
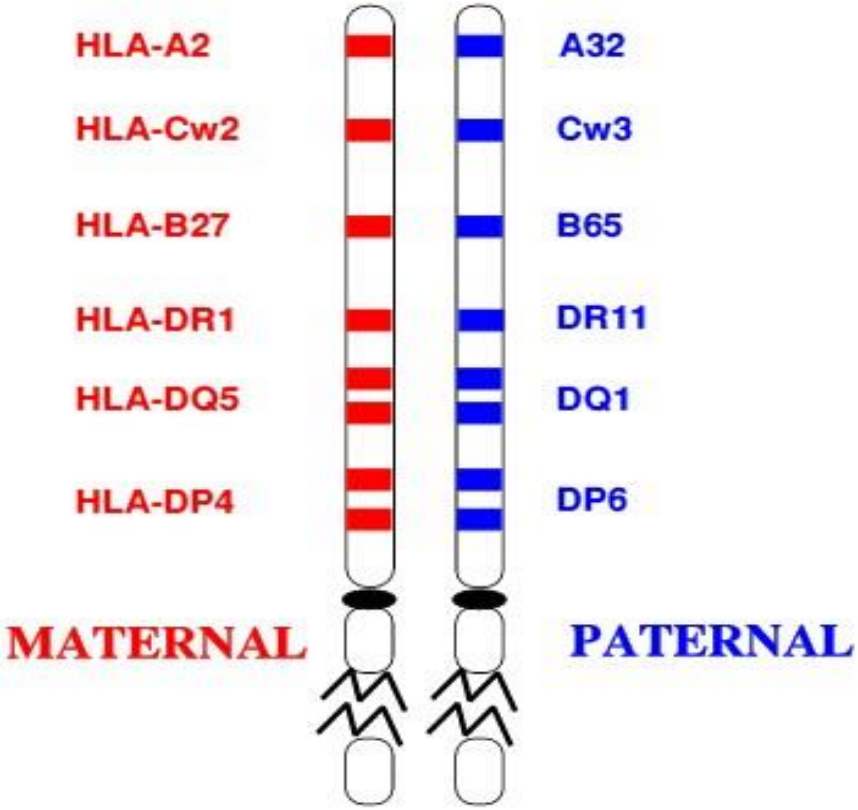


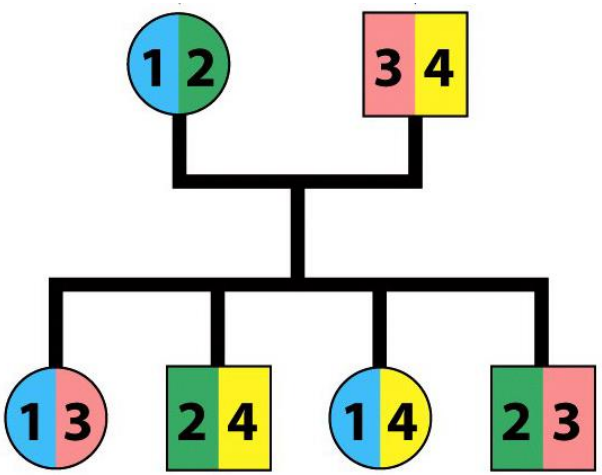
Figure 5.26 The Immune System, 3ed. (© Garland Science 2009)

Map of the Human MHC



Most Humans are heterozygous at the MHC

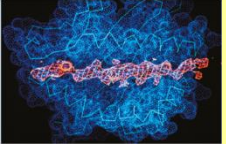


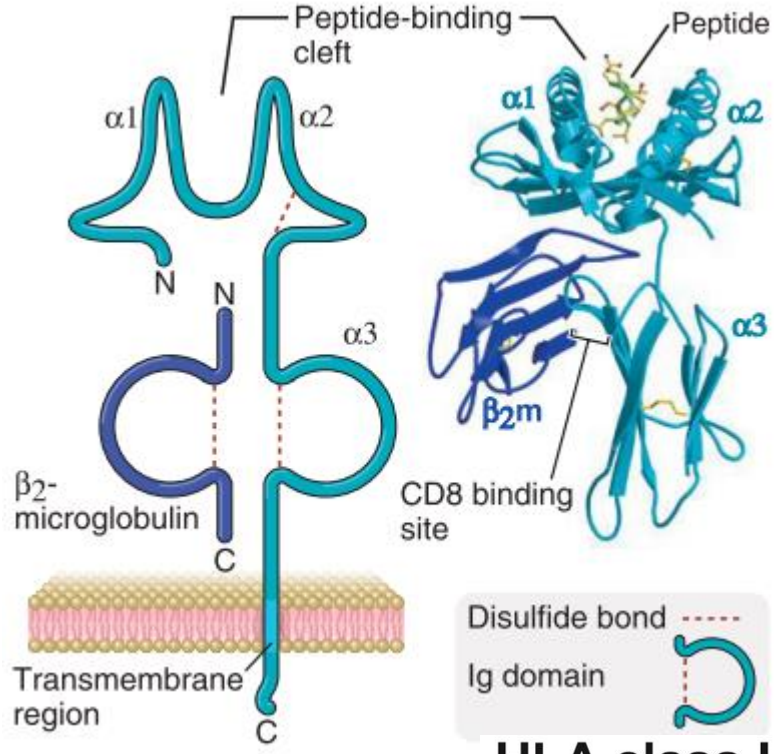


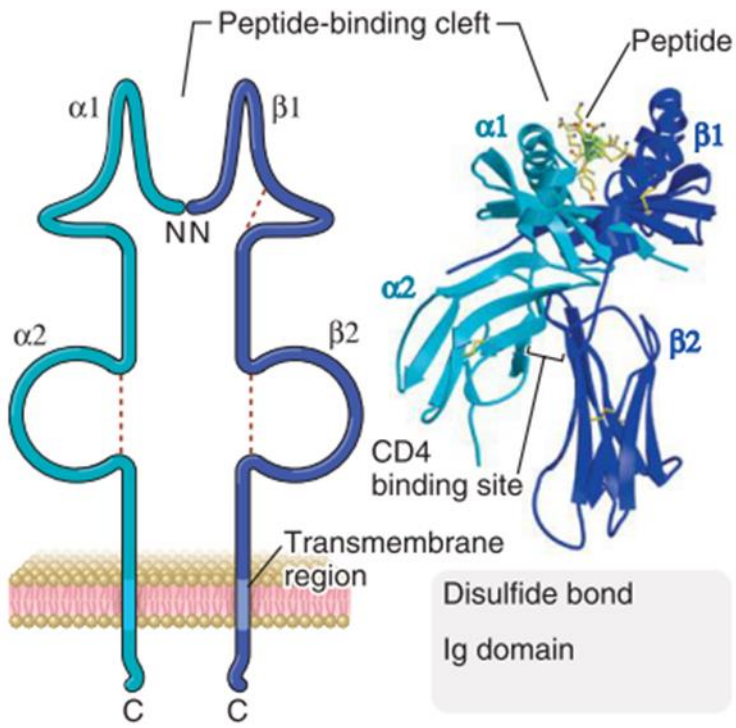
• MHC genes are codominantly expressed in each individual. In other words, for a given MHC gene, each individual expresses the alleles that are inherited from each of the two parents.

•The set of MHC alleles present on each chromosome is called an MHC haplotype.

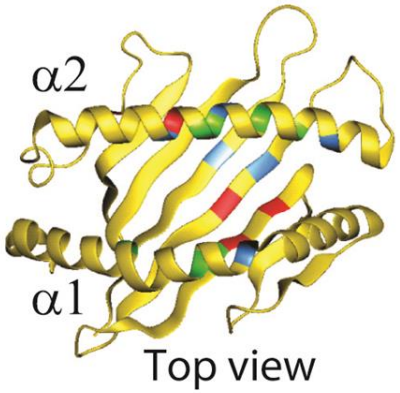
Structure of a Class I, II MHC Molecule



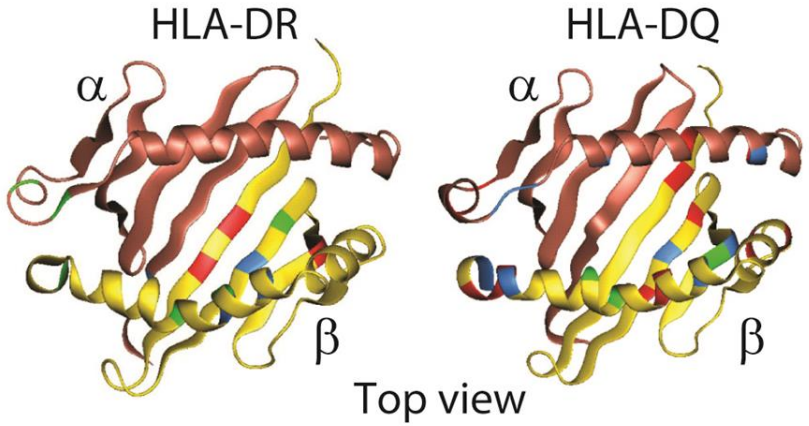




HLA class I



HLA class II



MHC variability

•The polymorphic amino acid residues of MHC molecules are located in and adjacent to the peptide binding cleft.

• This portion of the MHC molecule binds peptides for display to T cells, and the antigen receptors of T cells interact with the displayed peptide and with the α-helices of the MHC molecules.

•The nonpolymorphic Ig-like domains of MHC molecules contain binding sites for the T cell molecules CD4 and CD8.

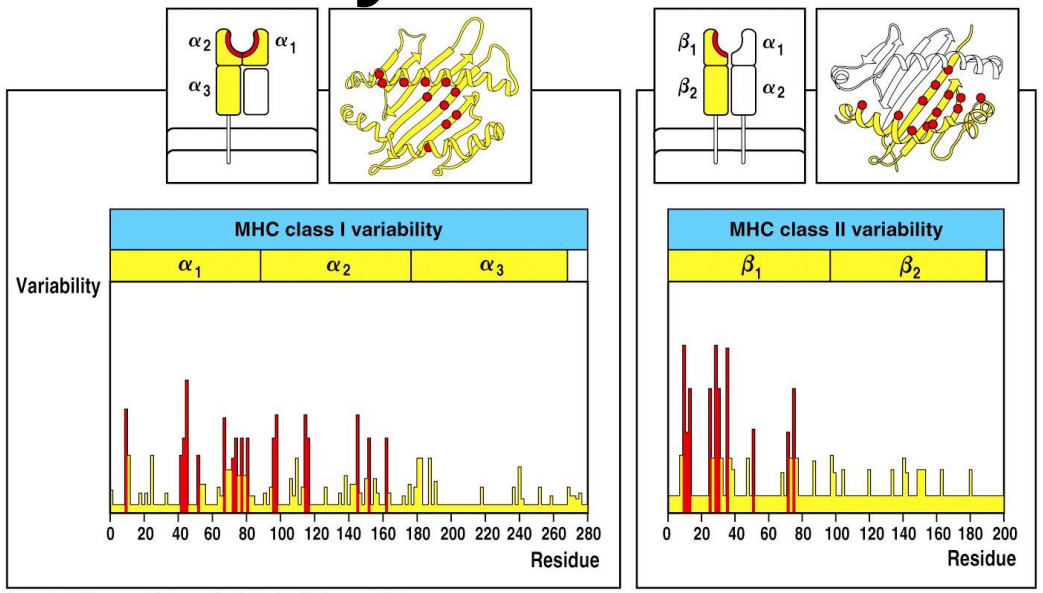
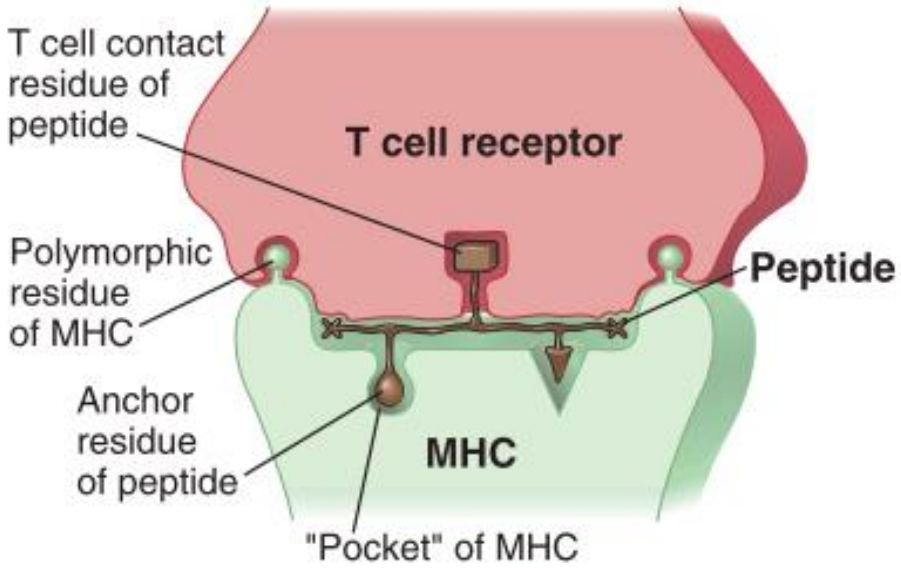


Figure 5-16 Immunobiology, 6/e. (© Garland Science 2005)



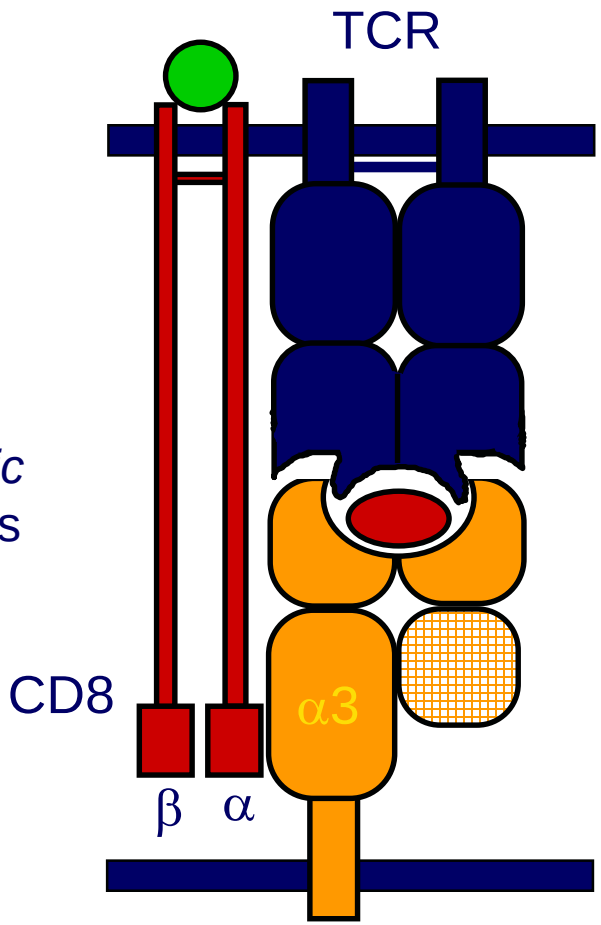
Peptide characteristics

- All proteins that are immunogenic in an individual must generate peptides that can bind to the MHC molecules of that individual.
- Each class I or class II MHC molecule has a single peptide-binding cleft that binds one peptide at a time, but each MHC molecule can bind many different peptides.
- It is not surprising that a single MHC molecule can bind multiple peptides because each individual contains only a few different MHC molecules and these must be able to present peptides from the enormous number of protein antigens that one is likely to encounter.
- The residues of a peptide that bind to MHC molecules are distinct from those that are recognized by T cells.

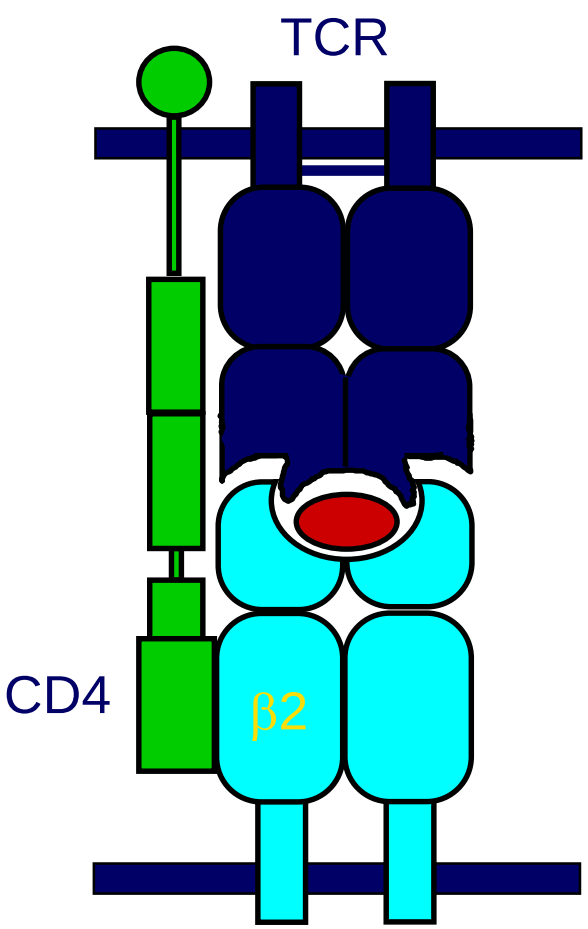
	MHC molecule	Amino acid sequence of peptide-binding motifs and bound peptides	Source of bound peptide
		Position in peptide sequence: N — 1 2 3 4 5 6 7 8 9 — C	
Class I	HLA-A*0201	Peptide-binding motif: [ ] (L/M) [ ] [ ] [ ] (V) [ ] [ ] (V/L)	
		Bound peptide: I L K E P V H G V	HIV reverse transcriptase
	HLA-B*2705	Peptide-binding motif: [ ] (R) [ ] [ ] [ ] [ ] [ ] [ ] (R/K)	
		Bound peptide: S R Y W A I R T R	Influenza A nucleoprotein
Class II	HLA-DRB1*0401	Self peptide: G V Y F Y L Q W G R S T L V S V S	Igκ light chain
	HLA-DQA1*0501 HLA-DQB1*0301	Self peptide: I P E L N K V A R A A A	Transferrin receptor

T cell co-receptor molecules

Class I MHC molecules present peptides to *CD8+cytotoxic* T lymphocytes (CTLs)



MHC Class I

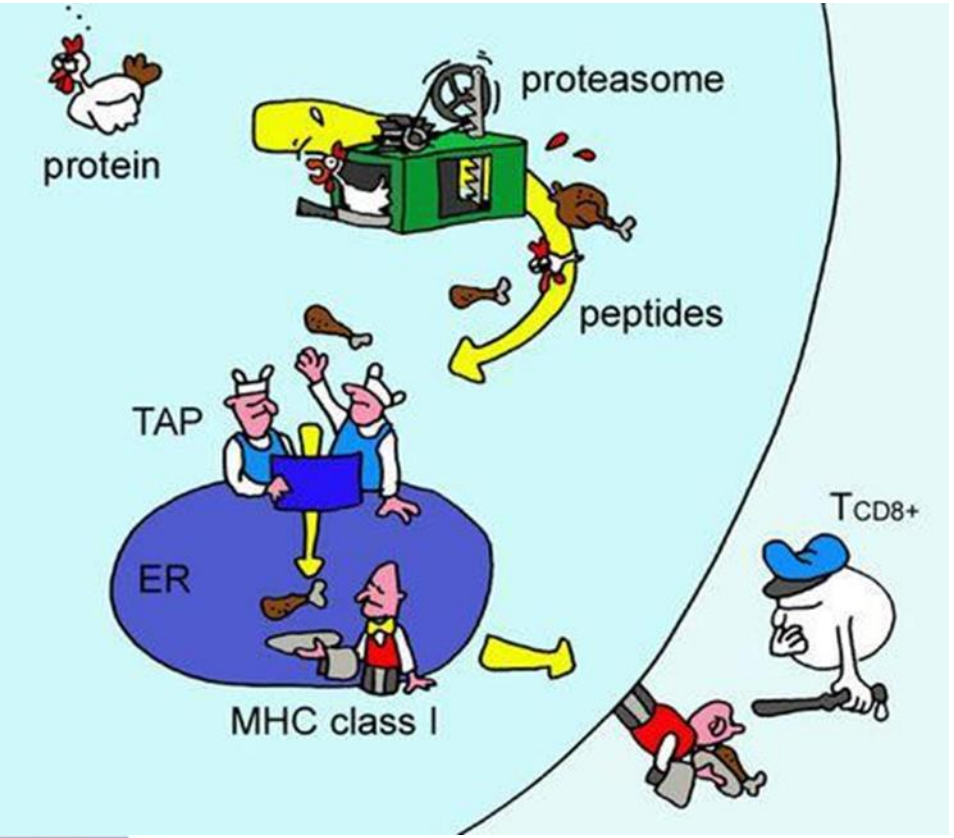


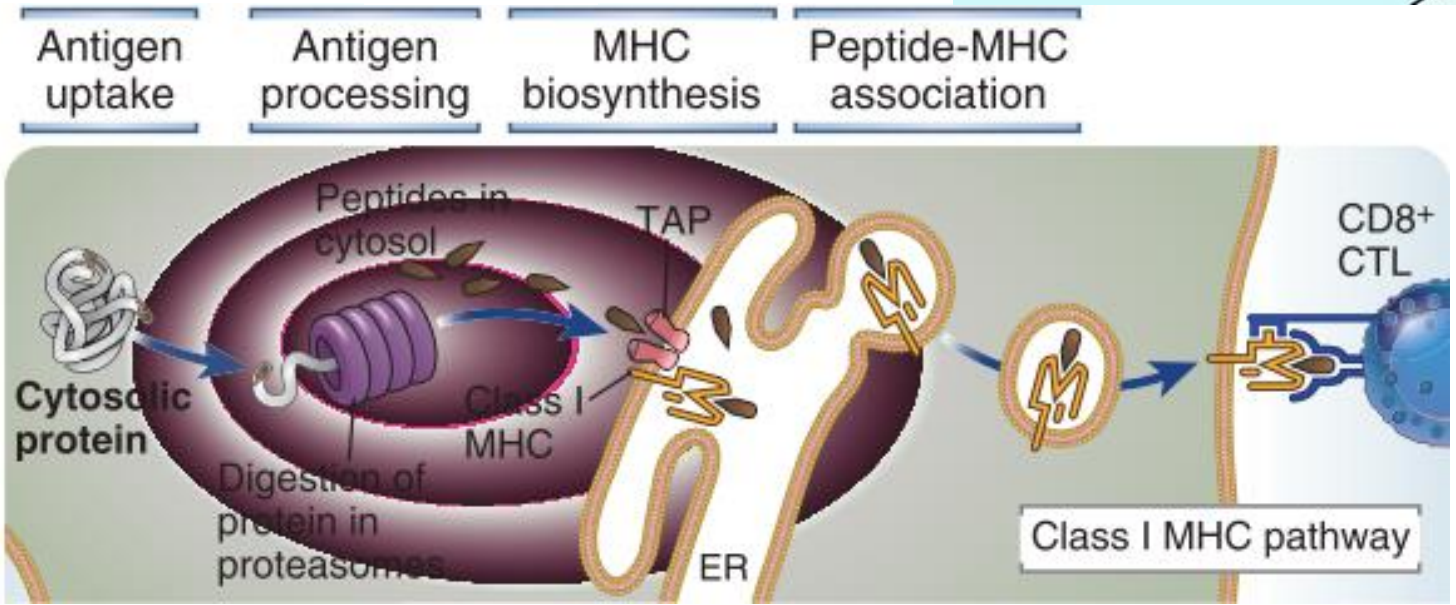
MHC Class II

class II MHC molecules present peptides to CD4+helper T cells

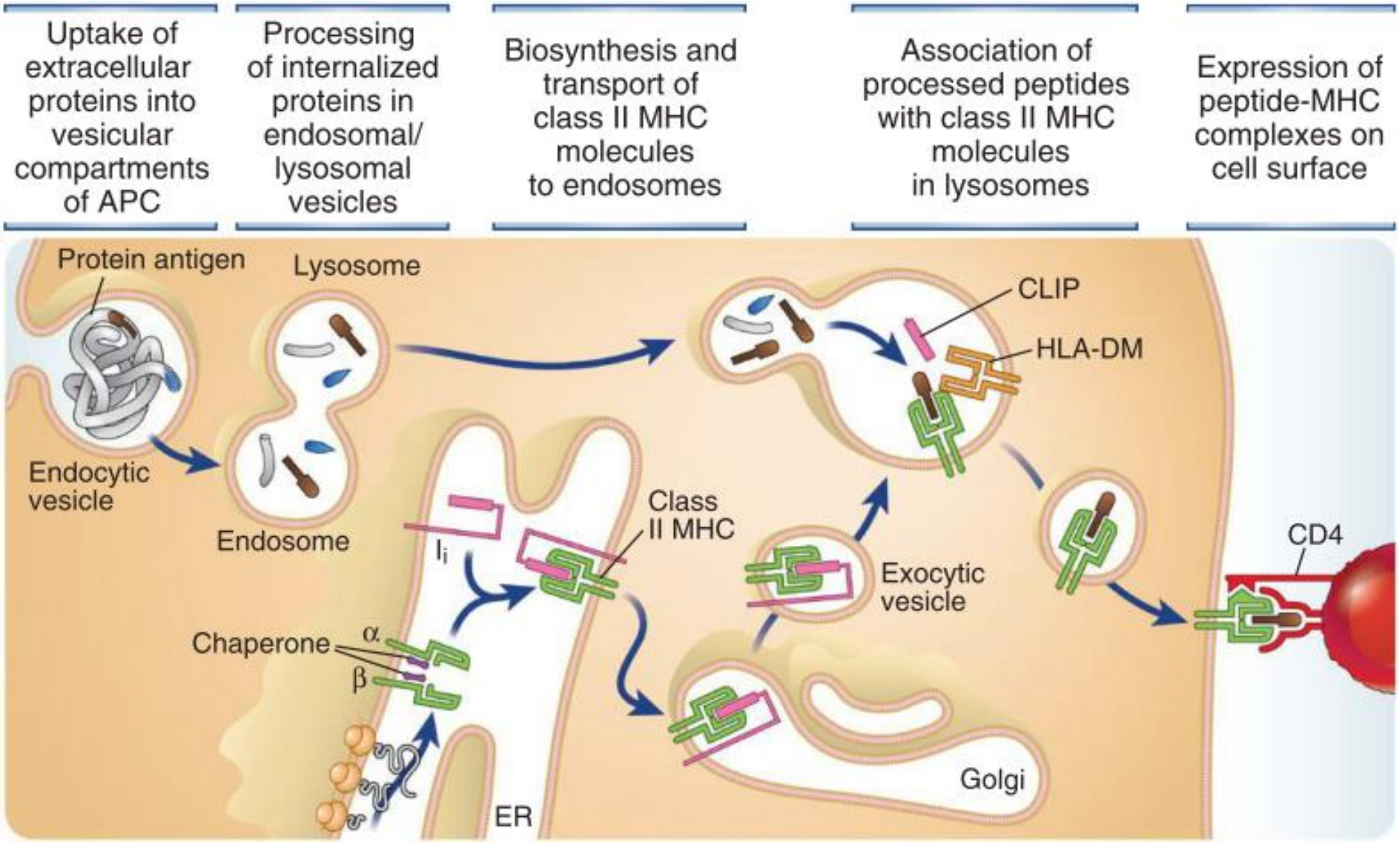
CD4 and CD8 are expressed on distinct subpopulations of mature T lymphocytes and participate, together with antigen receptors, in the recognition of antigen; that is, CD4 and CD8 are T cell "**coreceptors**". CD4 binds selectively to class II MHC molecules and CD8 binds to class I molecules

Protein antigen in cytosol --> class I MHC – CTLs





Pathways of antigen processing



Distribution of MHC Class I and Class II

- Related to cell “needs”
- **Class I** - all nucleated cells (excludes red blood cells) - can be infected with viruses and become tumor cells
- **Class II-** few cells are specialised to take up extracellular antigens, and so the distribution of MHC class II expression is restricted
  - B-lymphocytes
  - Macrophages
  - Dendritic cell (DC)
  - Cells in thymus involved in T-cell development
- *The expression of MHC molecules is increased by Cytokines produced during both innate and adaptive immune responses.*

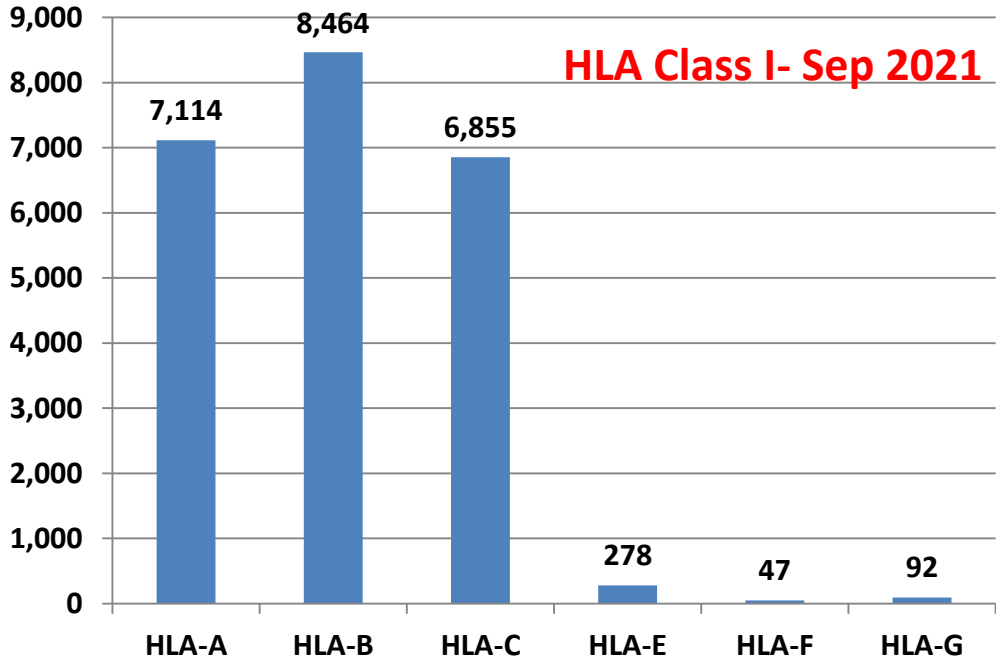
Tissue	MHC class I	MHC class II
Lymphoid tissues		
T cells	+ + +	+*
B cells	+ + +	+ + +
Macrophages	+ + +	+ +
Other antigen-presenting cells (eg Langerhans' cells)	+ + +	+ + +
Epithelial cells of the thymus	+	+ + +
Other nucleated cells		
Neutrophils	+ + +	–
Hepatocytes	+	–
Kidney	+	–
Brain	+	– †
Non-nucleated cells		
Red blood cells	–	–

Figure 3-19 Immunobiology, 6/e. (© Garland Science 2005)

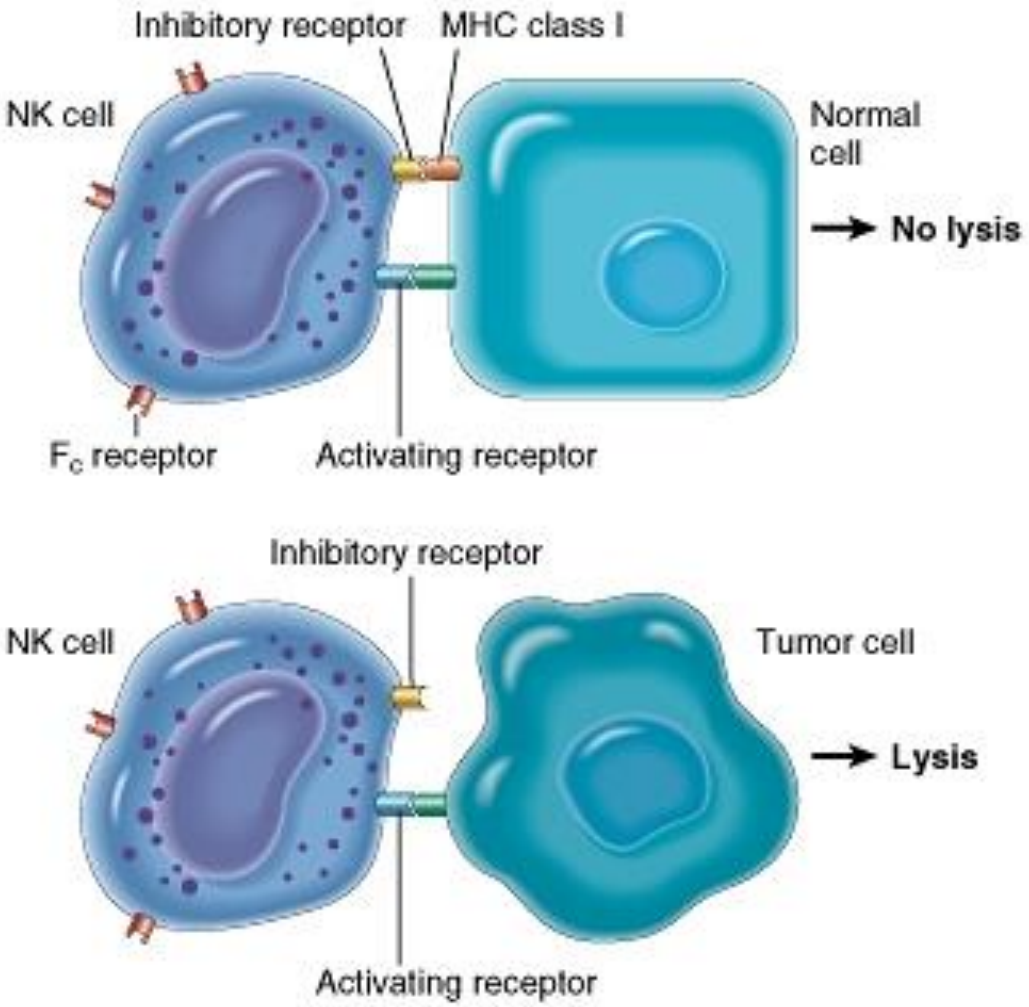
Nonclassic HLA-E, HLA-F, and HLA-G

Within MHC class I region, there are many genes that are called class I-like because they resemble class I genes but exhibit little or no polymorphism. They are called class Ib molecules, to distinguish them from the classical polymorphic class I molecules (i.e. HLA-A, HLA-B, HLA-C).

The HLA nonclassic molecules E, F, and G are less polymorphic and have different functions and more limited tissue distribution compared with their classic HLA class I counterparts.

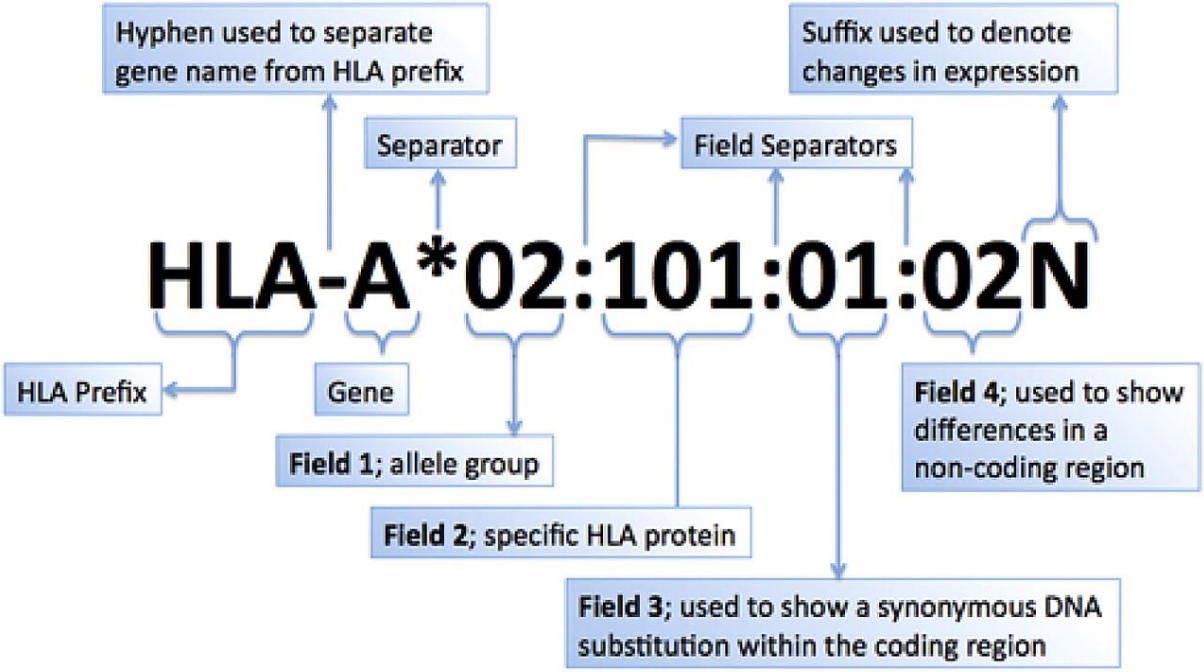


Role of MHC class I in NK inactivation



HLA Typing Methods

- HLA may be serologically typed (to determine the phenotype) or typed by DNA molecular analysis.
- Each serologically defined HLA antigenic specificity may be encoded by a number of different HLA alleles. For some HLA loci (HLA-B), as many as 250 alleles have been identified by serologic assays.
- Conversely many HLA alleles have no determined serologically defined antigen.
- Direct DNA-based typing techniques have all but replaced serological methods in routine HLA typing.



*A*01:17* has not been found to have synonymous DNA substitutions or differences in the noncoding region; thus, fields 3 and 4 have not been assigned for this allele.

Low resolution

A DNA-based typing result at the level of the digits composing the first field in the DNA-based nomenclature. Examples include: A*01; A*02. If the resolution corresponds to a serologic equivalent, this typing result should also be called low resolution.

High resolution

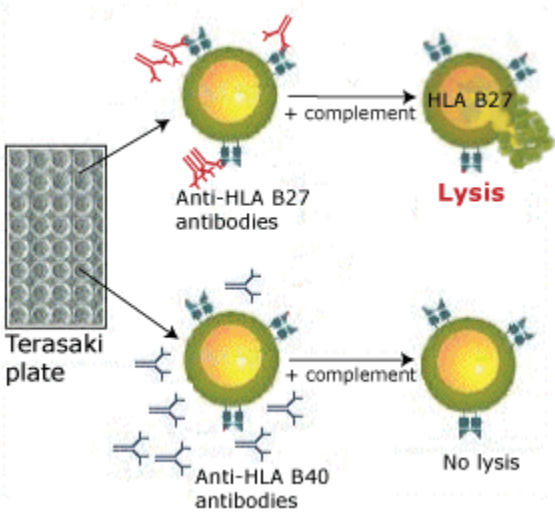
The two fields (XX:XX) indicate one or more nucleotide substitutions that change the HLA protein coding sequence and are often referred to as “high-resolution typing.”

DNA typing level

Low	*Intermediate*	*High*
A*02 B*44 DRB1*04	A*0201/0209 B*4402/4405 DRB1*0403/0407	A*0201 B*4402 DRB1*0403
Kidney Tx		***Stem cell Tx***

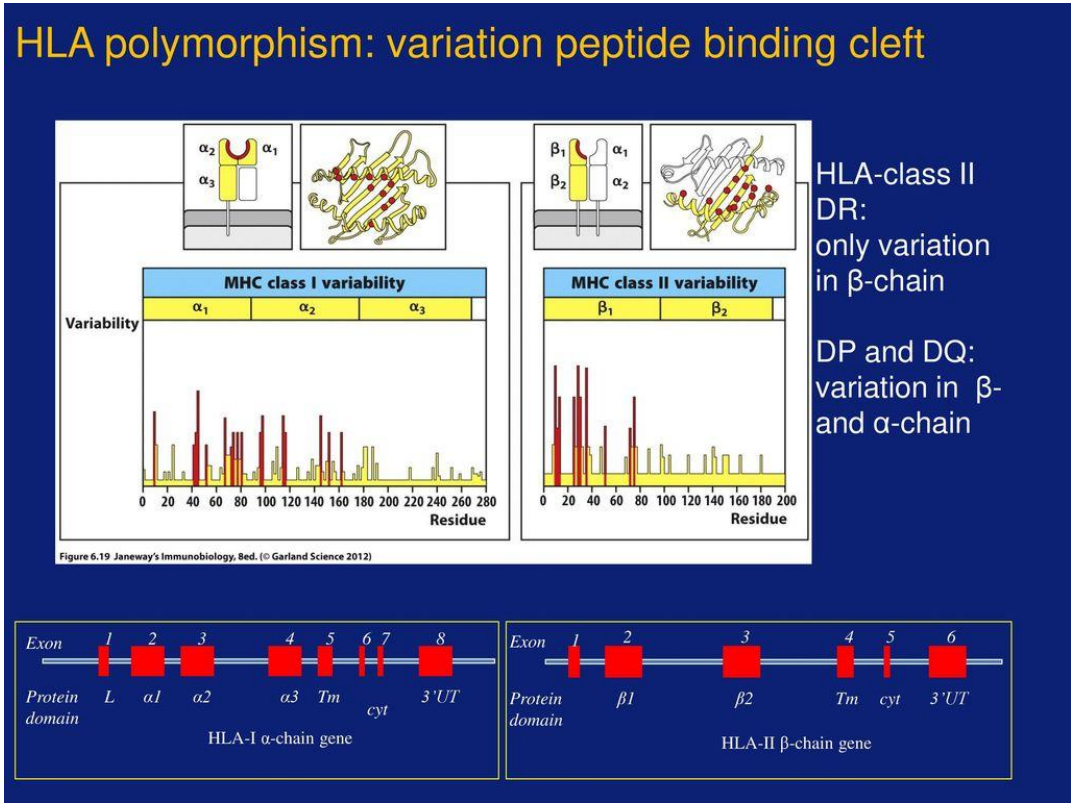
Microlymphocytotoxicity test

- Lymphocytes are tested with a panel of sera containing well characterized **HLA-specific alloantibodies**.
- Each serum is placed in a microtiter well of a **Terasaki plate**.
- A complement-dependent cytotoxicity (CDC) test or microlymphocytotoxicity assay for HLA typing has been developed in the 60's.



DNA-Based Typing Techniques: SSO, SSP, and SBT

- The techniques primarily in use today in clinical immunogenetics laboratories are **SSP**, **SSO**, and **SBT**. The genomic regions analyzed are usually exon 2 and 3 of class I and exon 2 of class II genes.
- However, this rather limited genomic characterization generates many typing ambiguities.



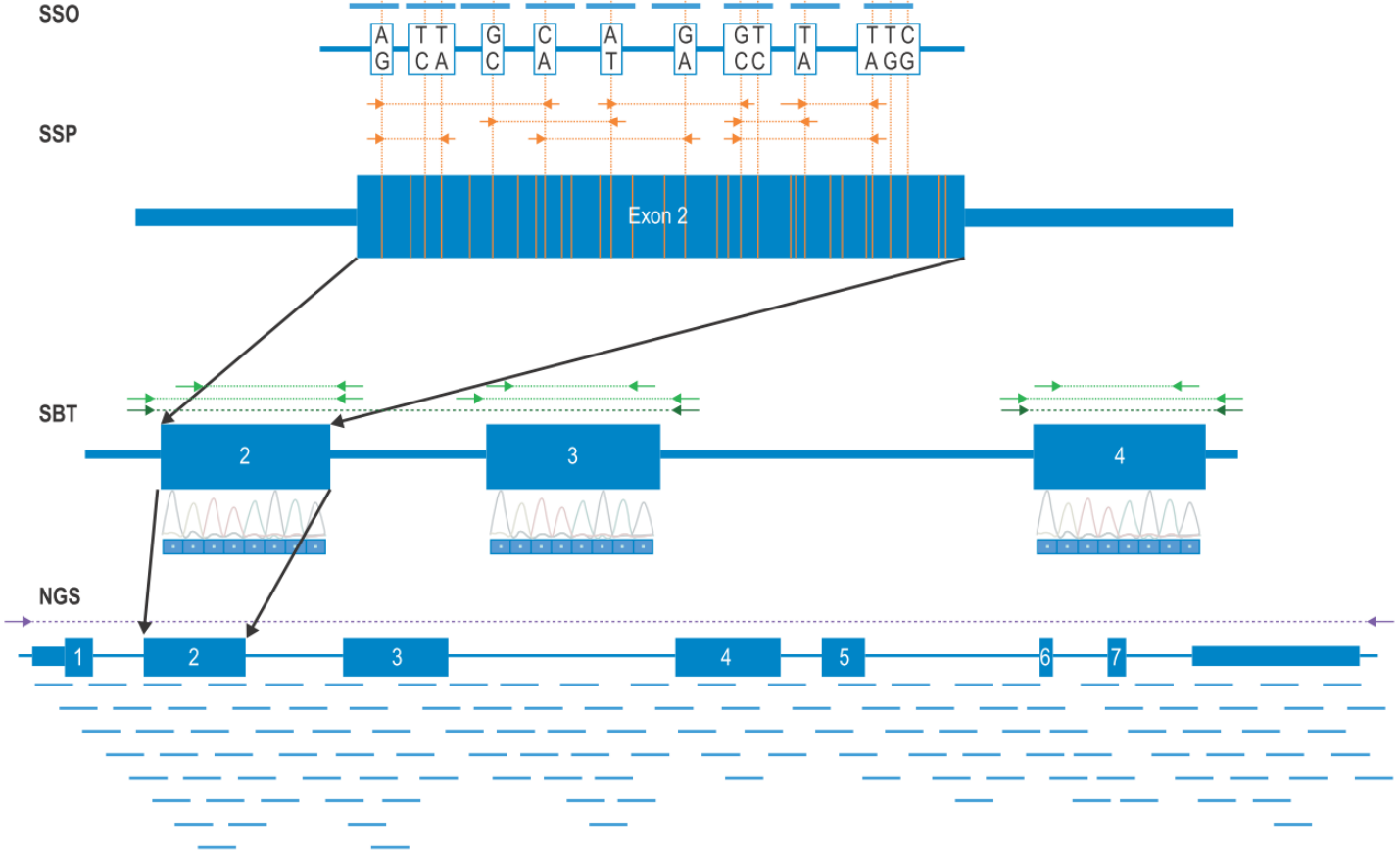
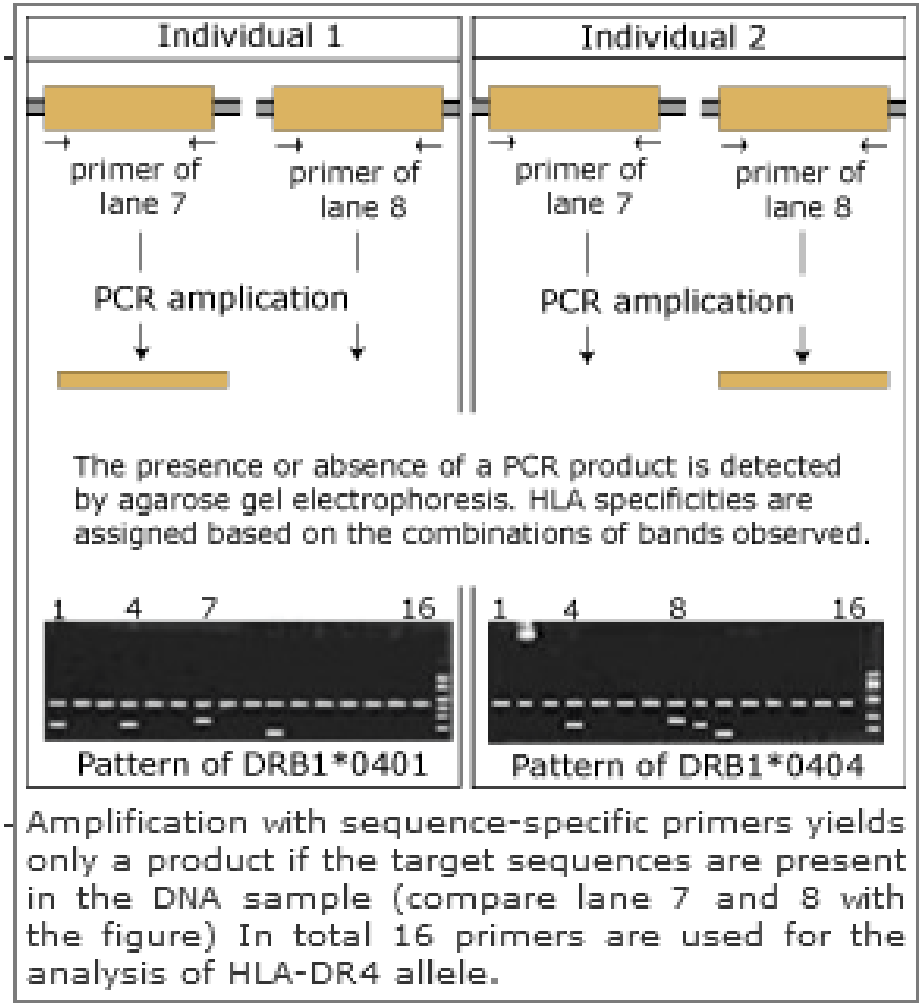


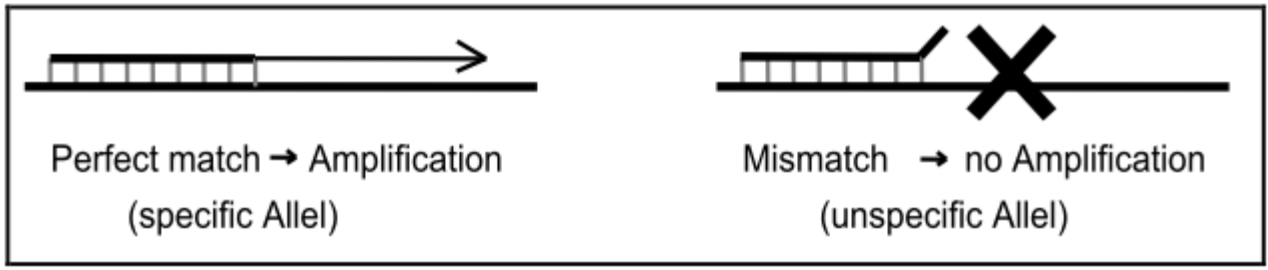
FIG 5.5 Examples of Molecular Human Leukocyte Antigen (HLA) Typing Techniques and Their Methods of Interrogating the HLA Genes. For any given HLA gene (dark blue rectangles), SSOs of ≈20 bp (light blue lines) can provide single-nucleotide resolution of haplotype differences (polymorphic differences, red lines in exon 2). This requires a complex panel of oligonucleotide probes to discern differences between HLA alleles. This probe set is static and therefore cannot adjust to novel alleles. SSPs (orange arrows) can provide haplotype-specific or allele-specific resolution of nucleotide differences and additionally provide some level of phasing between polymorphic sites. As with SSOs, these oligonucleotide sets are complex and static, limiting their flexibility. SBT provides whole-exon information on the polymorphic content of the HLA allele (amplification primers [dark green] and sequencing primers [light green arrows]) but cannot discern phasing, as this method generally does not rely on allele-specific primers for amplification as a first step. Next-generation sequencing (NGS) provides whole-gene amplification (amplification primers, purple arrows) and detection of polymorphic content for any HLA allele (known or unknown) and provides significant phasing between polymorphic sites that are within the read lengths of the system being used (usually between 200 and 1000 bp). This is accomplished through the alignment of thousands of short overlapping reads that are combined to form a single consensus sequence (blue lines).

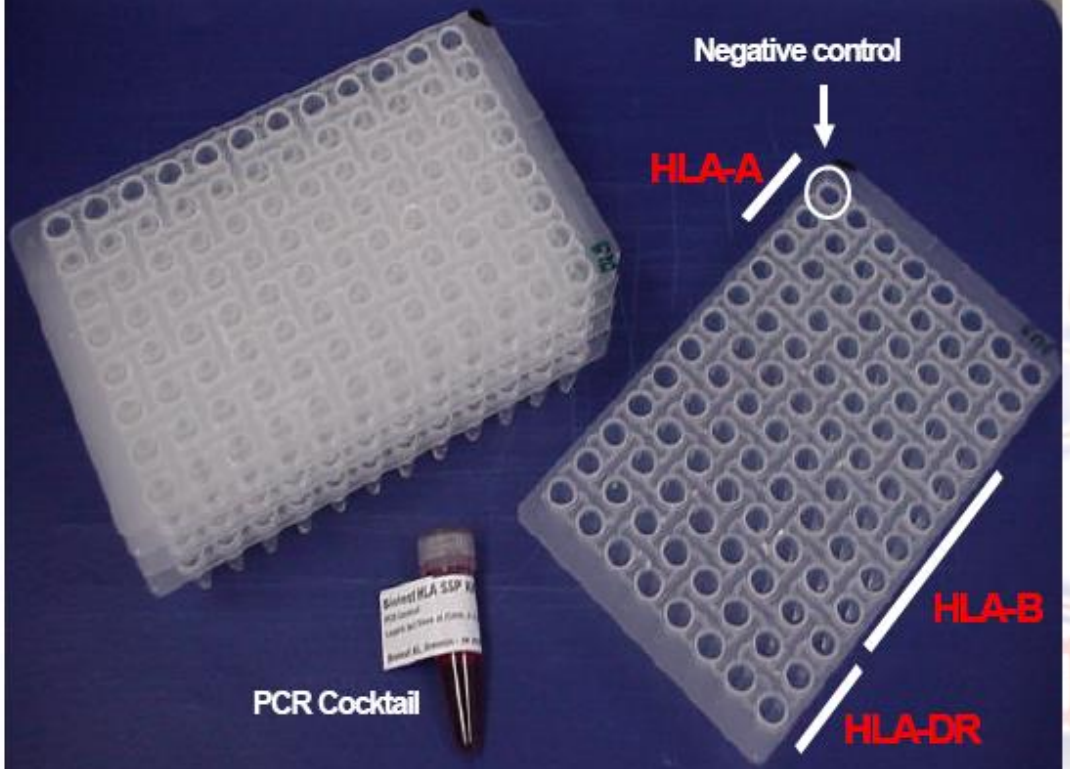
SSP (sequence-specific primers)

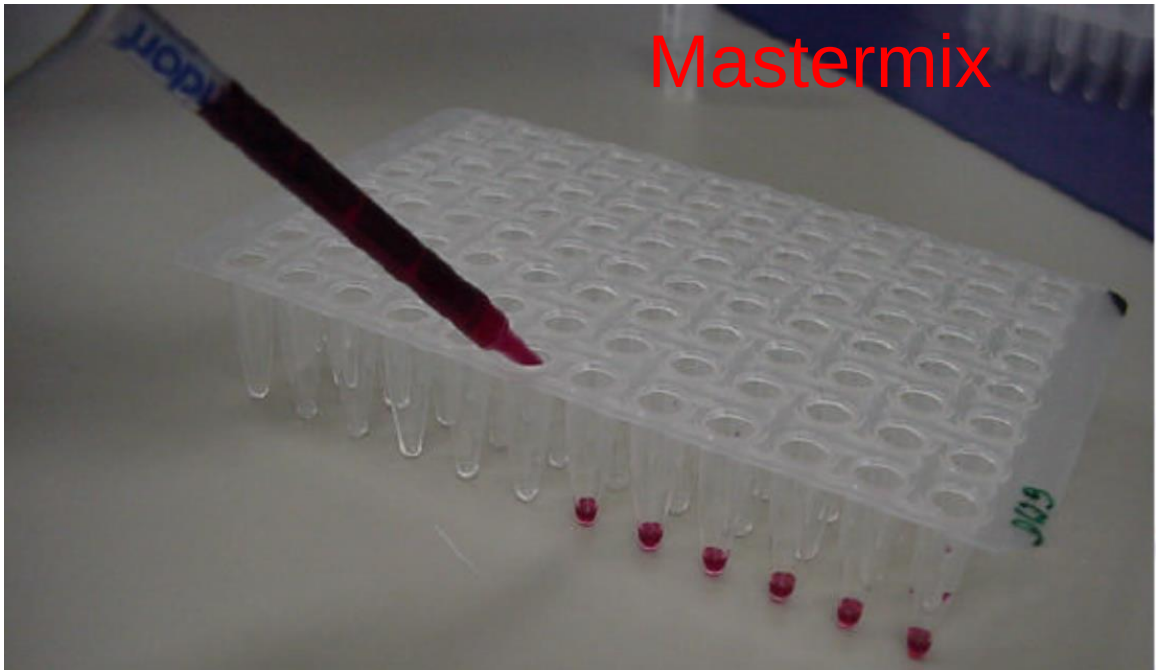
SSP uses panels of specific primer sets that overlap polymorphic sites. Perfectly matched primers produce an amplification product, whereas mismatched primers do not. The pattern of amplification from multiple primer sets determines the HLA allele.



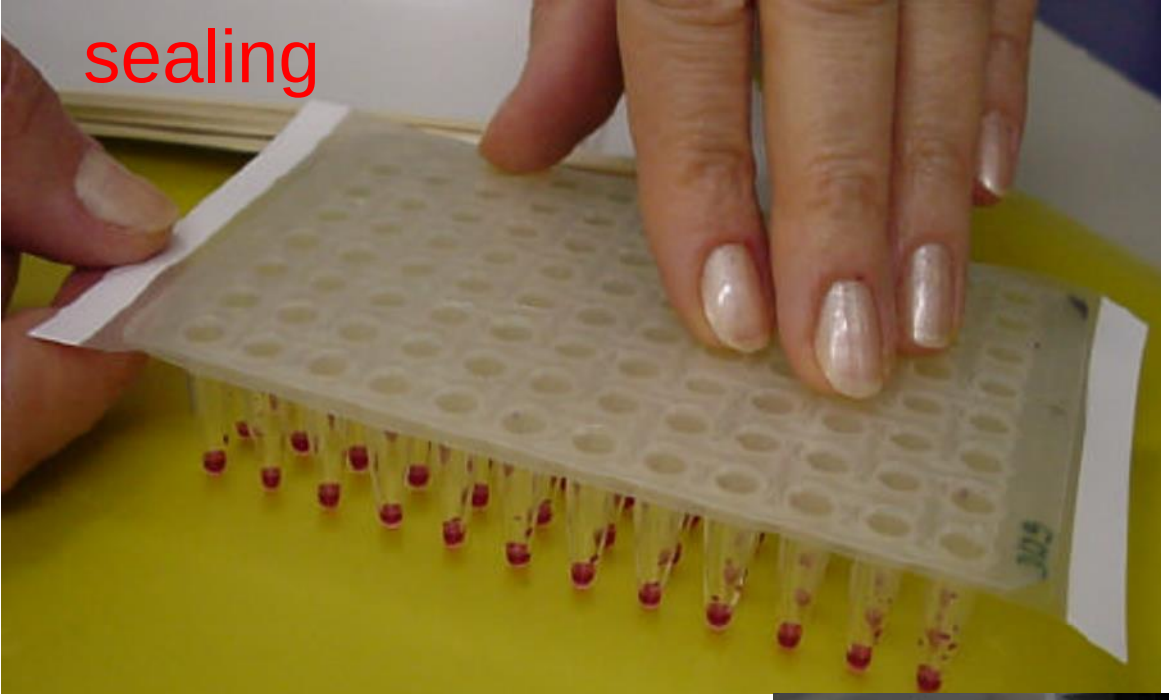
Amplification with sequence-specific primers yields only a product if the target sequences are present in the DNA sample (compare lane 7 and 8 with the figure) In total 16 primers are used for the analysis of HLA-DR4 allele.



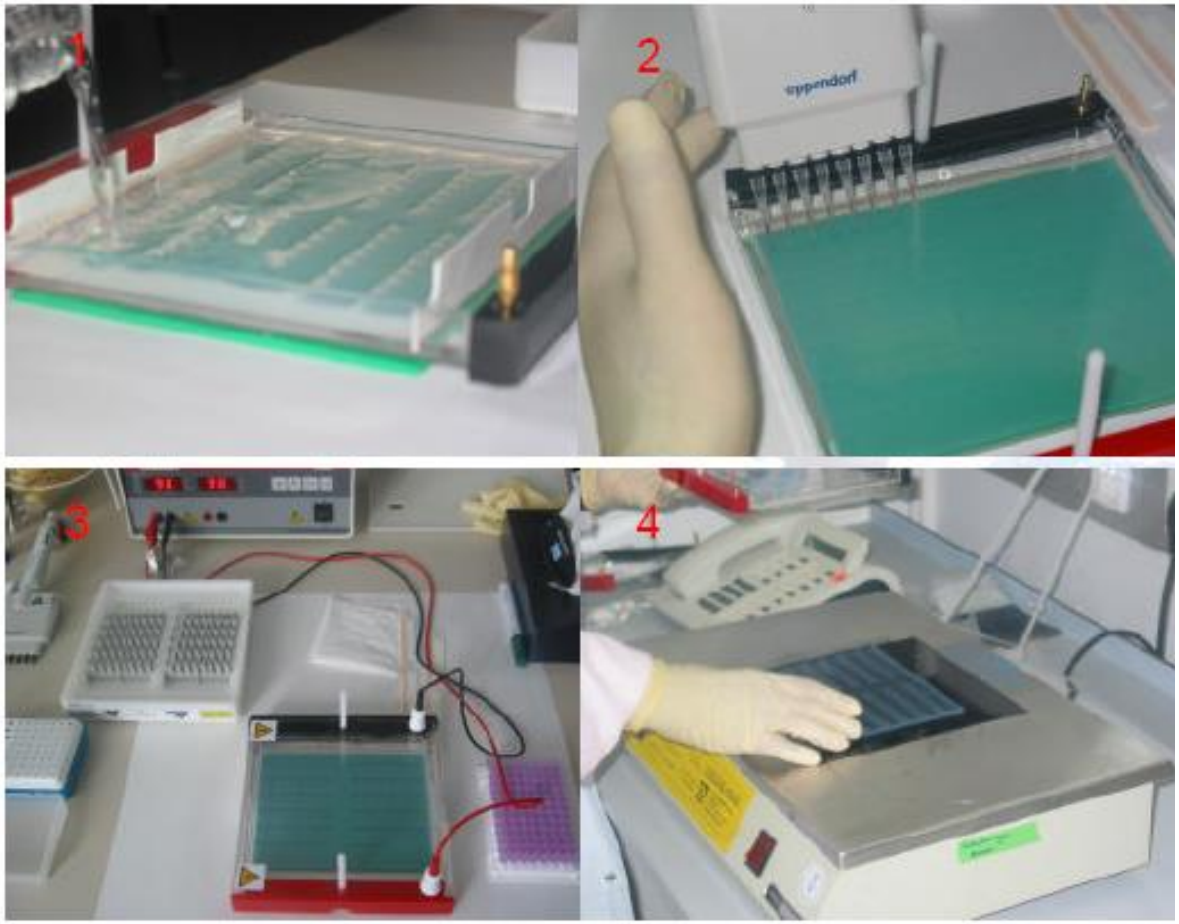

Negative control
HLA-A
HLA-B
HLA-DR
PCR Cocktail


Mastermix

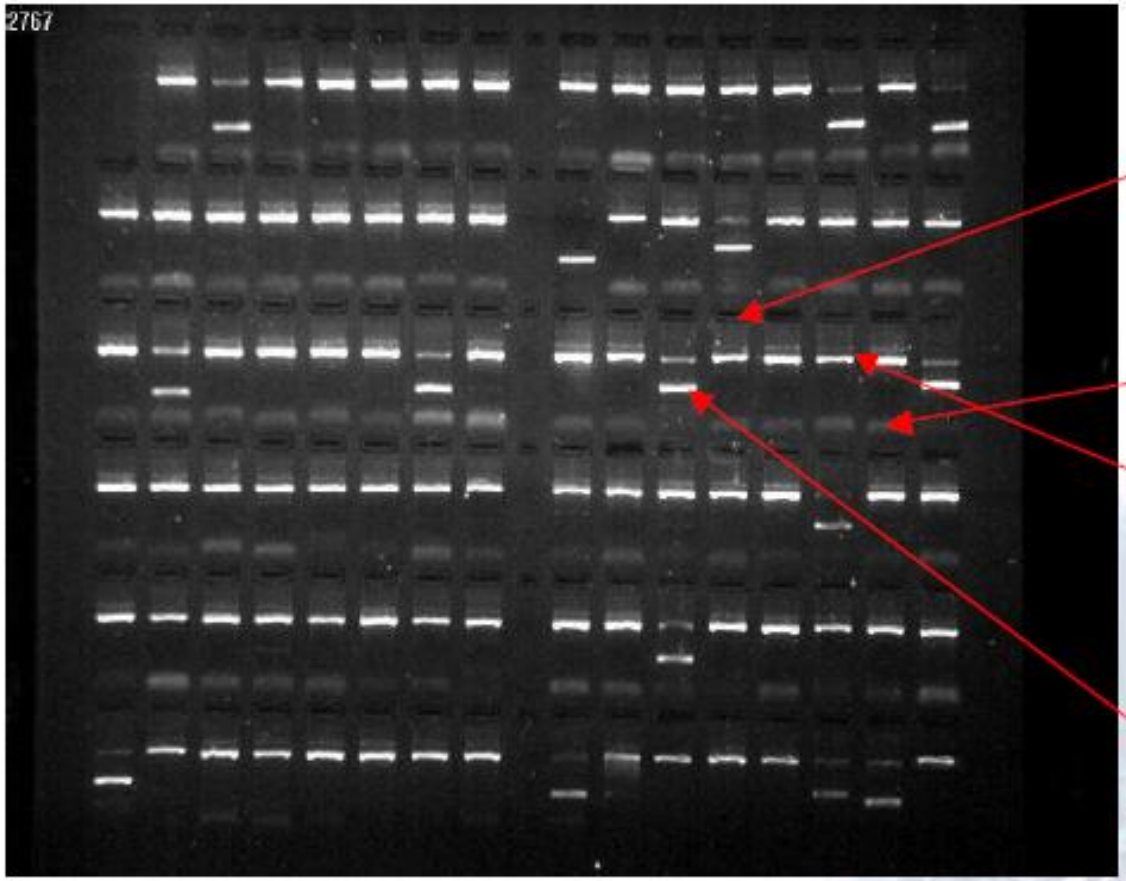
sealing



Gel electrophoresis



Example for a gel picture

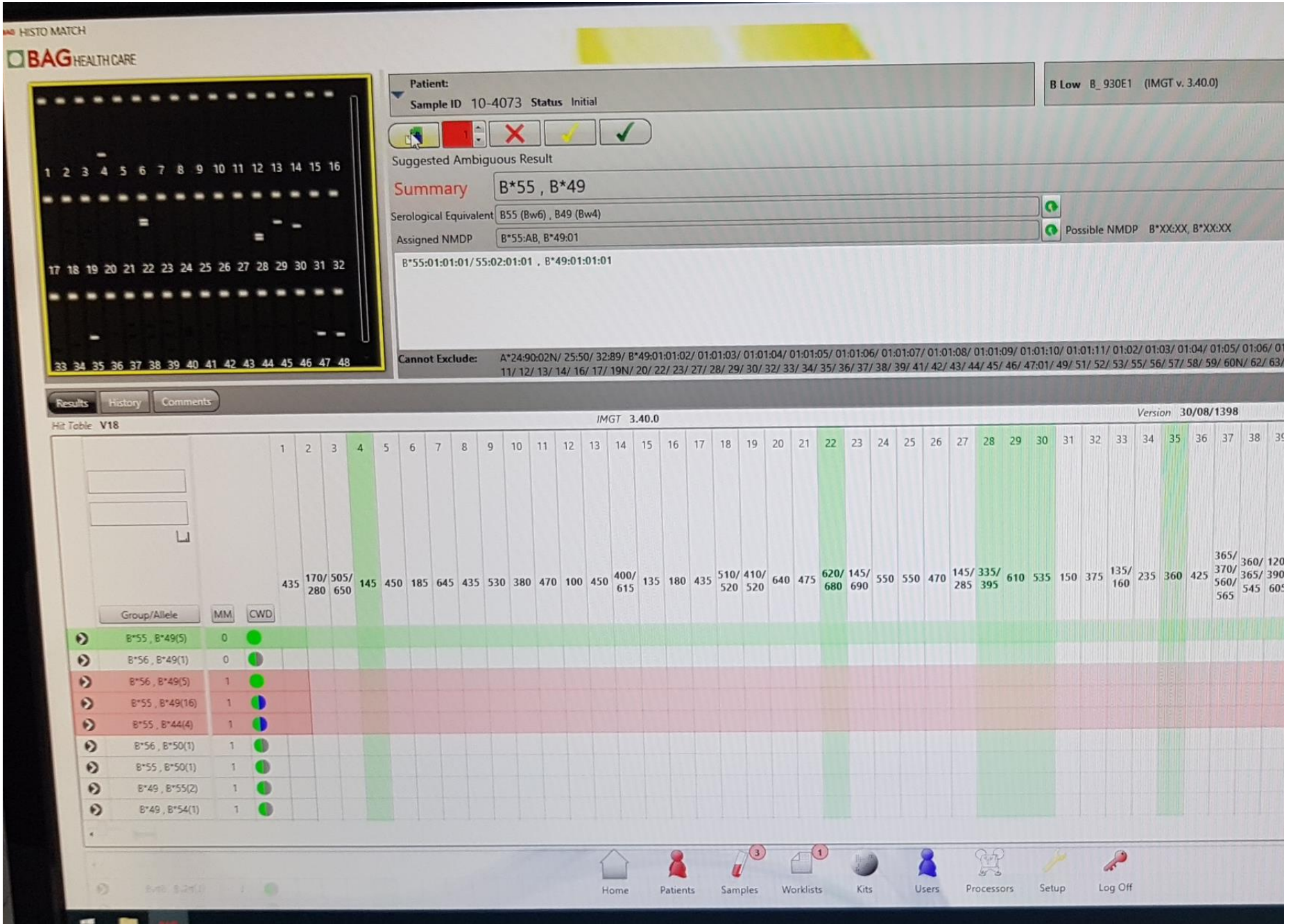


Sample application point

Primer band

Control band

Primermix-specific band


HISTO MATCH
BAG HEALTH CARE
Patient:
Sample ID 10-4073 Status Initial
B Low B_930E1 (IMGT v. 3.40.0)
Suggested Ambiguous Result
Summary B*55 , B*49
Serological Equivalent B55 (Bw6) , B49 (Bw4)
Assigned NMDP B*55:AB, B*49:01
Possible NMDP B*XX:XX, B*XX:XX
B*55:01:01:01/ 55:02:01:01 , B*49:01:01:01
Cannot Exclude: A*24:90:02N/ 25:50/ 32:89/ B*49:01:01:02/ 01:01:03/ 01:01:04/ 01:01:05/ 01:01:06/ 01:01:07/ 01:01:08/ 01:01:09/ 01:01:10/ 01:01:11/ 01:02/ 01:03/ 01:04/ 01:05/ 01:06/ 01:11/ 12/ 13/ 14/ 16/ 17/ 19N/ 20/ 22/ 23/ 27/ 28/ 29/ 30/ 32/ 33/ 34/ 35/ 36/ 37/ 38/ 39/ 41/ 42/ 43/ 44/ 45/ 46/ 47:01/ 49/ 51/ 52/ 53/ 55/ 56/ 57/ 58/ 59/ 60N/ 62/ 63/
Results
History
Comments
Hit Table V18
IMGT 3.40.0
Version 30/08/1398
Group/Allele
MM
CWD
B*55 , B*49(5)
B*56 , B*49(1)
B*56 , B*49(5)
B*55 , B*49(16)
B*55 , B*44(4)
B*56 , B*50(1)
B*55 , B*50(1)
B*49 , B*55(2)
B*49 , B*54(1)
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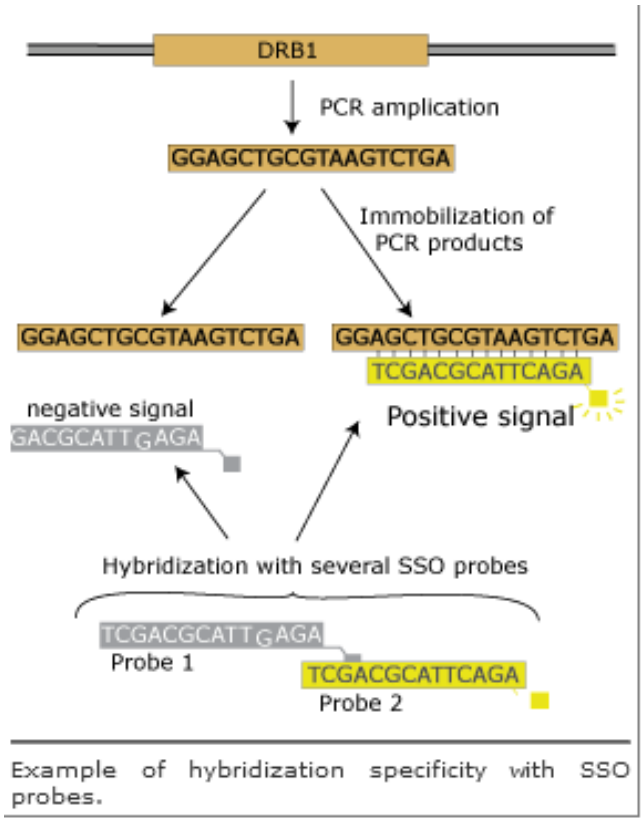
sequence-specific oligonucleotides probes (SSOP or SSO)

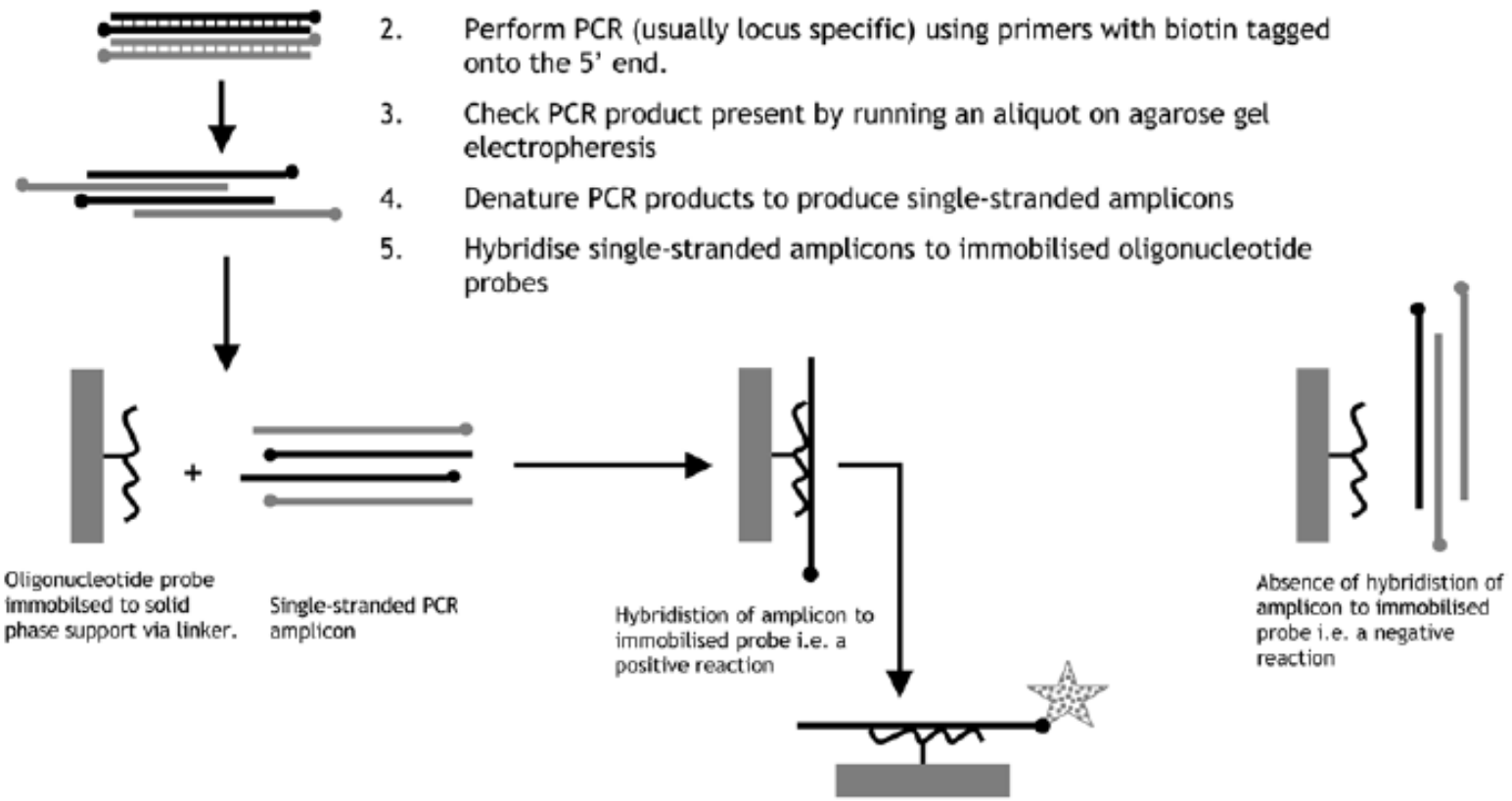
SSO interrogates polymorphic differences using panels of individual DNA oligo probes that differentially hybridize to the target of interest. The probe either perfectly matches or mismatches the target's polymorphic sites. The hybridization pattern of the oligos is compared with an expected pattern, based on the sequence database of HLA alleles, and is interpreted as an HLA type.



Example of hybridization specificity with SSO probes.

PCR using sequence-specific oligonucleotides (PCR-SSO) simplified.

1. Extract DNA
2. Perform PCR (usually locus specific) using primers with biotin tagged onto the 5' end.
3. Check PCR product present by running an aliquot on agarose gel electropheresis
4. Denature PCR products to produce single-stranded amplicons
5. Hybridise single-stranded amplicons to immobilised oligonucleotide probes



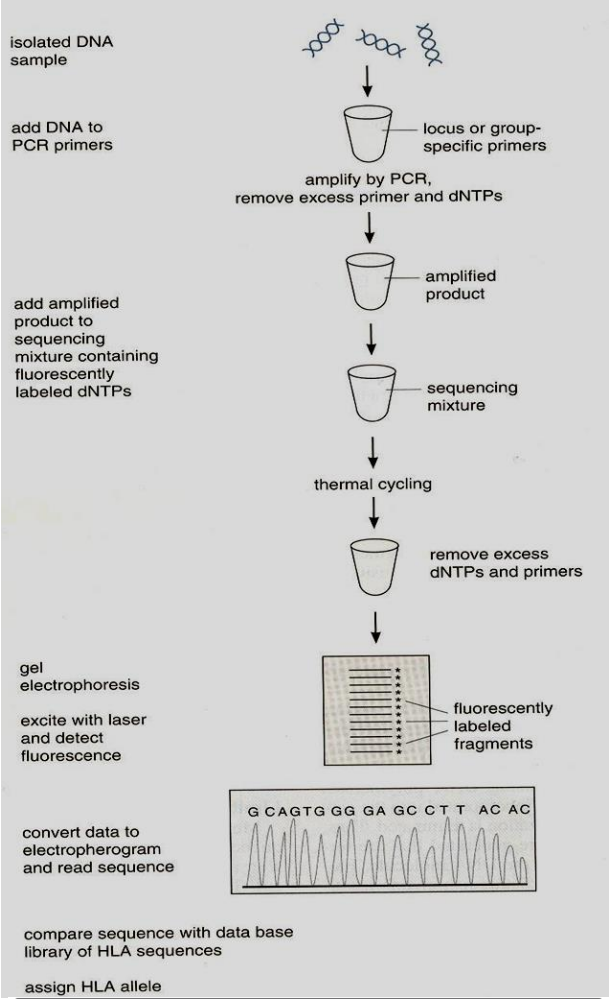
6. Addition of streptavidin conjugated enzyme which emits colour after addition of substrate allows identification of positive reactions
7. Pattern of positive and negative reactions are interpreted to give HLA type

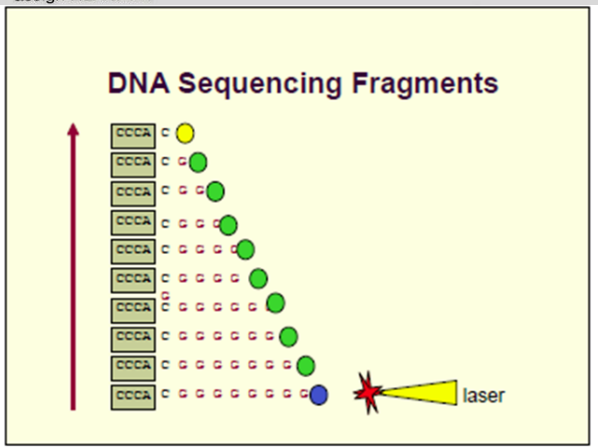
HLA locus	No. probes
HLA-A	61
HLA-B	96
HLA-DRB1	70

Example of number of probes used to obtain medium level resolution in a commercially available kit.

Sequence-based typing (SBT)

In SBT, specific gene regions, usually exons, are amplified and sequenced through a process of polymerase-based extension of specific-sequencing primers using fluorescently labeled nucleotides, indicating allelic differences base by base.





Next-Generation Sequencing (NGS)

- Protocols utilizing NGS technology are on the rise because they provide the means for the complete characterization of these genes and the elimination of ambiguities in a cost-effective manner.
- Although HLA typing by NGS has been introduced only recently, it is likely that this new method will transform the way HLA typing is performed in the coming years.

Thanks for your attention