Cleveland Clinic

The HLA System

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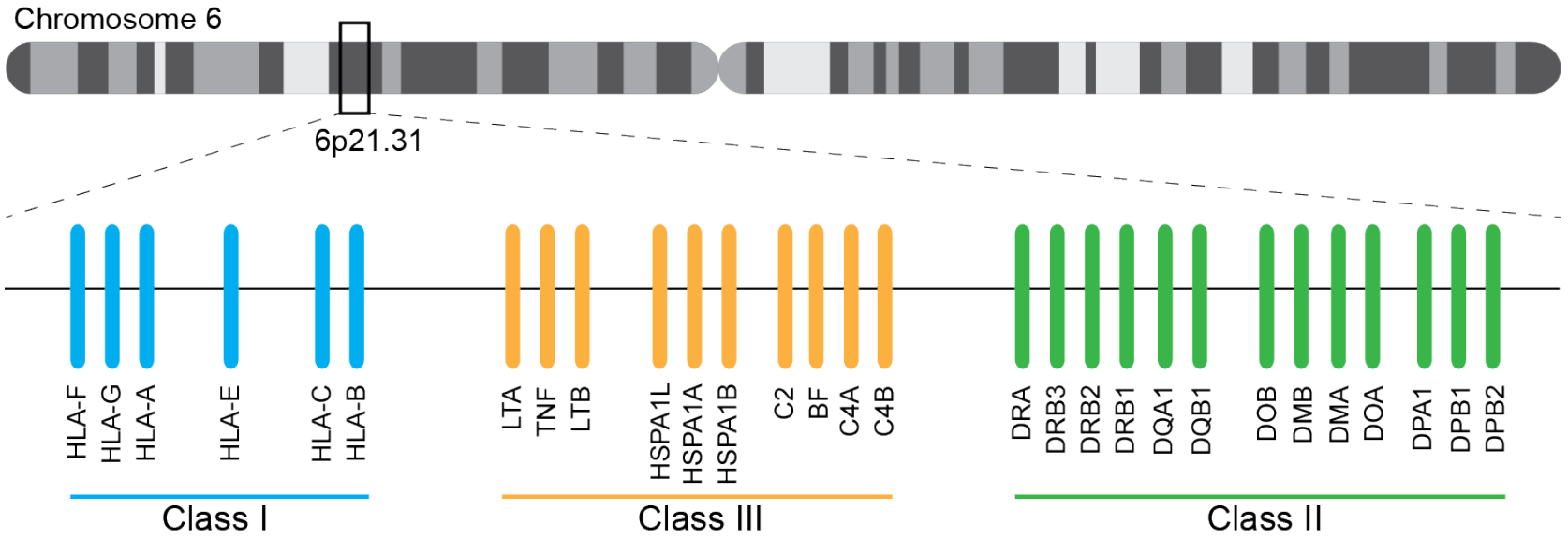
HLA Genetic Region

- Series of closely linked genes
- Determine Major Histocompatibility Factors- surface antigens recognizing foreign tissues
- Also called MHC- Major histocompatibility complex
- 35-40 genes
- On 3 regions of chromosome 6



HLA Classes

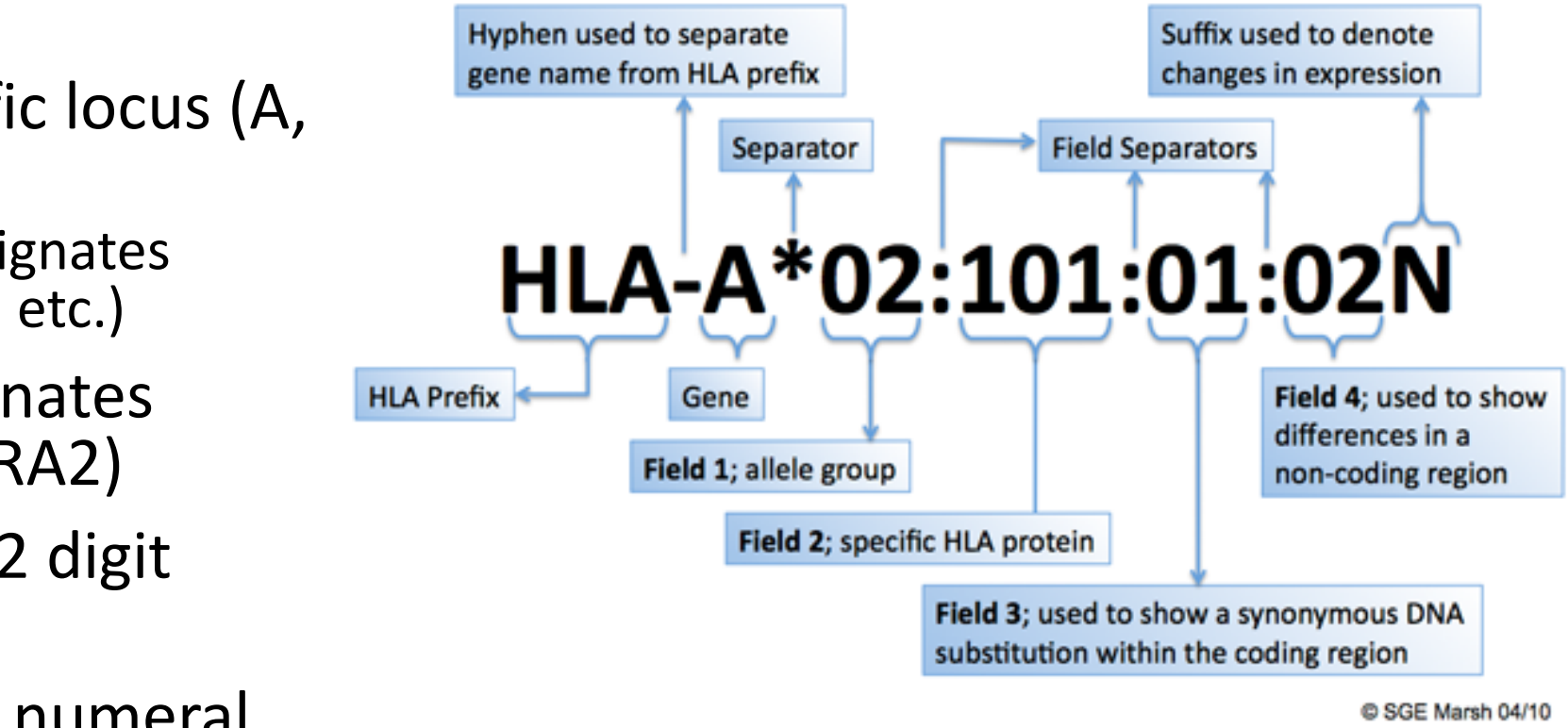
Class I	Class II	Class III
• Classic Transplant molecules • HLA-A, HLA-B, HLA-C	• HLA-DR • HLA-DP • HLA-DQ • All consist of alpha and beta chains	• Encode diverse molecules • C2, C4, Bf, 21-hydroxylase, tumor necrosis factor

HLA Genes

- Highly polymorphic (many different forms)
- Several alleles at each locus
- Antigenic specificity is designated by numbers
- Ex. HLA-A2, HLA-B7
- Alleles display codominantly

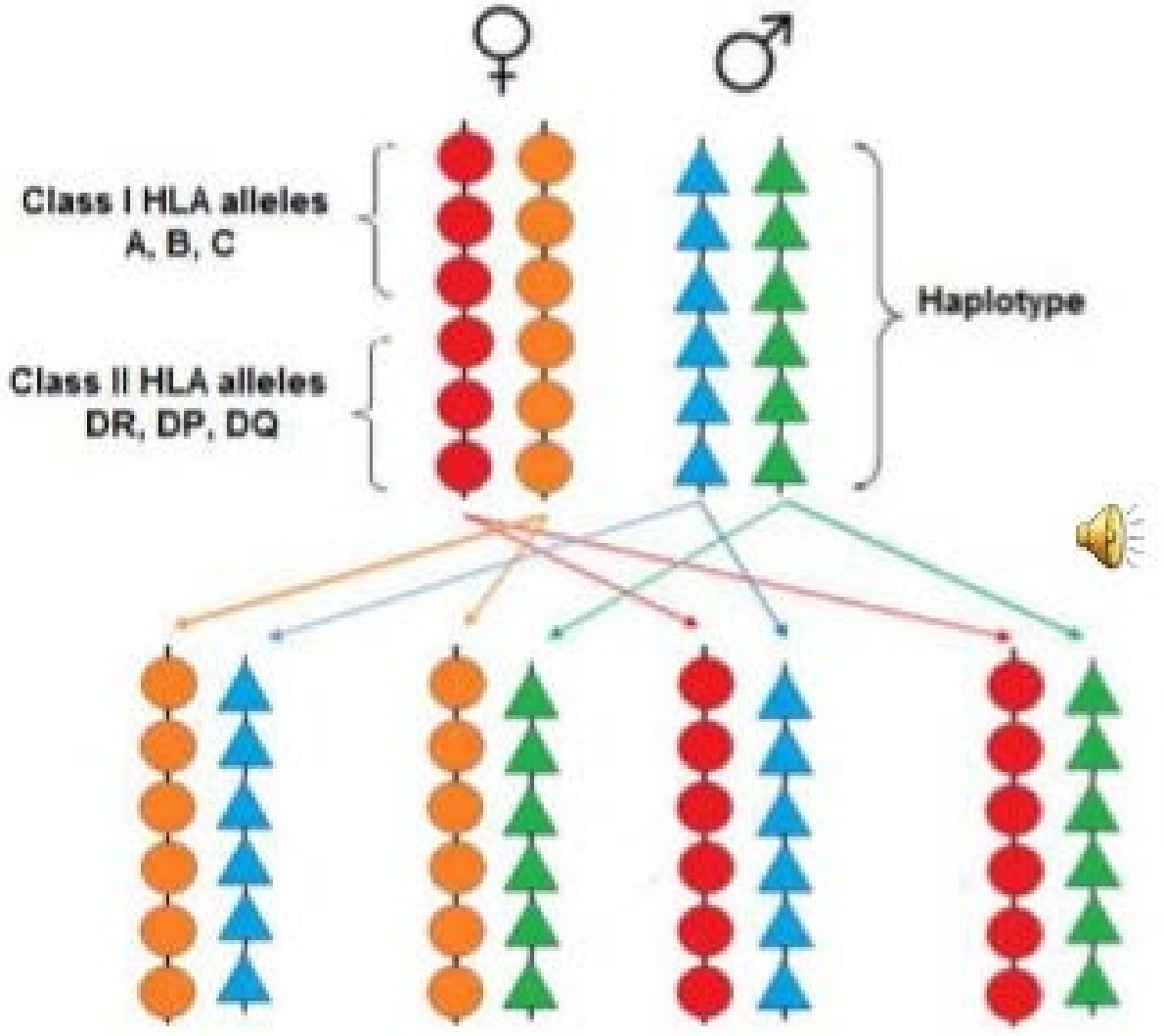
HLA Nomenclature

1. HLA prefix- designates MHC gene complex
2. Capital letter- specific locus (A, B, C, D, etc.)
   - D genes= 2nd letter designates subregions (DR, DQ, DO, etc.)
3. Class II- A or B designates alpha or beta (ex. DRA2)
4. Broad allele family- 2 digit numeral
5. After ':' gives 2 digit numeral for specific allele/protein



Inheritance

- Physical linkage of HLA genes on chromosome
  - All genes on chromosome usually inherited together
- Haplotype = entire set of A, B, C, DR, DQ, DP genes
- Inherit one whole set from mother and one from father
  - Usually 4 combinations possible
- Siblings = highest chance of match



Terms

- Phenotype- antigens on surface
- Genotype- association of alleles on two chromosomes
- Haplotype- allelic makeup of a single chromosome

Phenotype,genotype and haplotype of HLA

Tester	A	B	C
	A1 / B8 | | A2 / B12	A1 / B8 | | A1 / B12	A1 / B8 | | A1 / B8
Phenotype	HLA-A1、2: B8、12	HLA-A1: B8、12	HLA-A1: B8
Genotype	HLA-A1、A2 HLA-B8、B12	HLA-A1、A1 HLA-B8、B12	HLA-A1、A1 HLA-B8、B8
Haplotype	HLA-A1 - B8/A2 - B12	HLA-A1 - B8/A1 - B12	HLA-A1 - B8/A1 - B8

HLA Gene Products

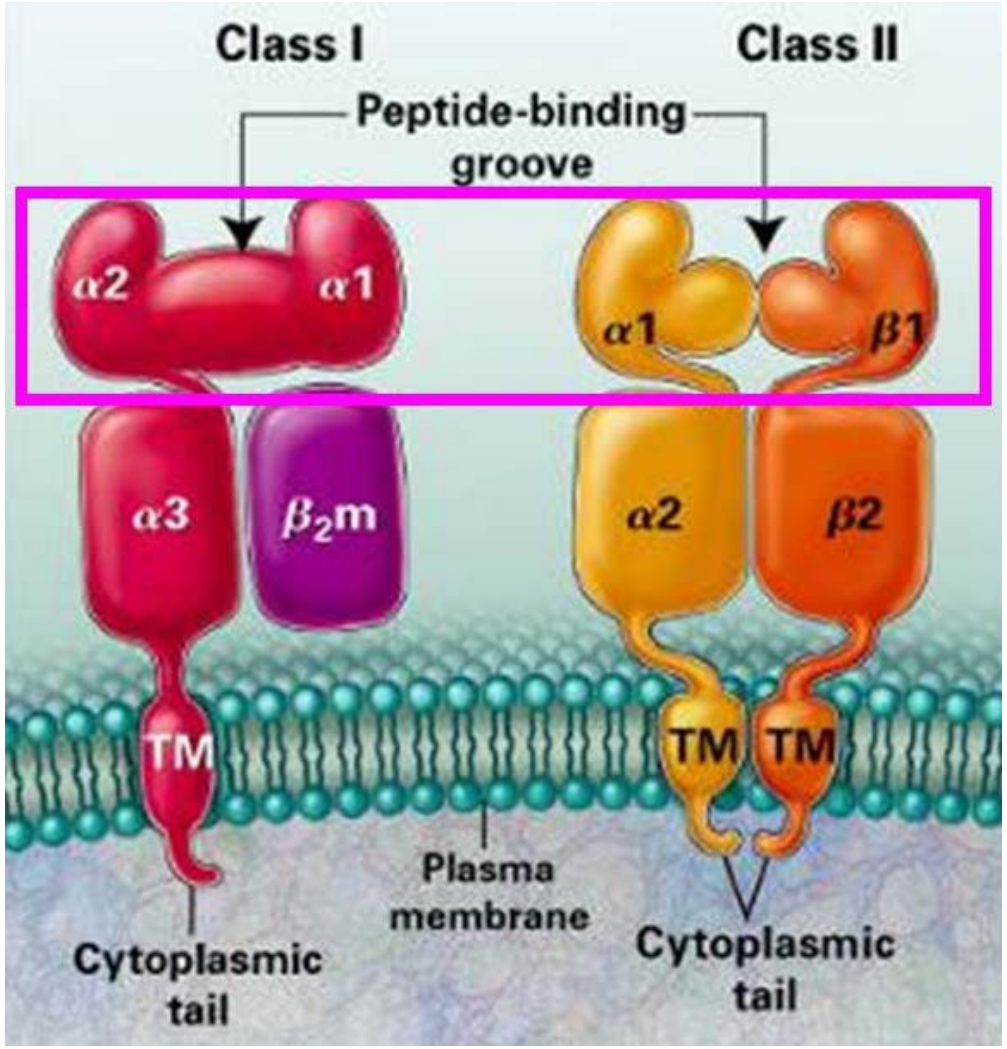
- Globular glycoproteins with 2 non-covalently linked chains

Class I

- One heavy chain + β2-microglobulin
- Heavy chain folds into 3 domains
- Heavy chain inserts into membrane

Class II

- Two similar sized chains
- Both chains insert into membrane
- Fold into 2 domains: α and β



HLA Antigen Locations

Class I

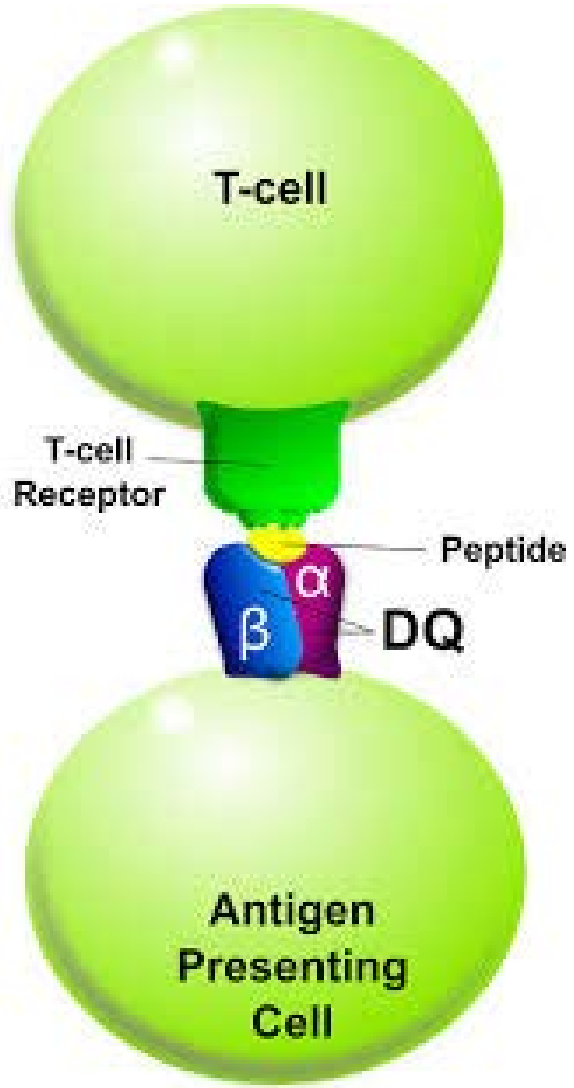
- All nucleated cells
- Dendritic cells
- Platelets

Class II

- B lymphocytes
- Activated T lymphocytes
- Macrophages
- Monocytes
- Endothelial Cells

Function

- HLA I and II- Discrimination at a molecular, cellular, and species level between self vs. non-self
- Present foreign proteins to T cells
- Evolved to present a large range of foreign antigens



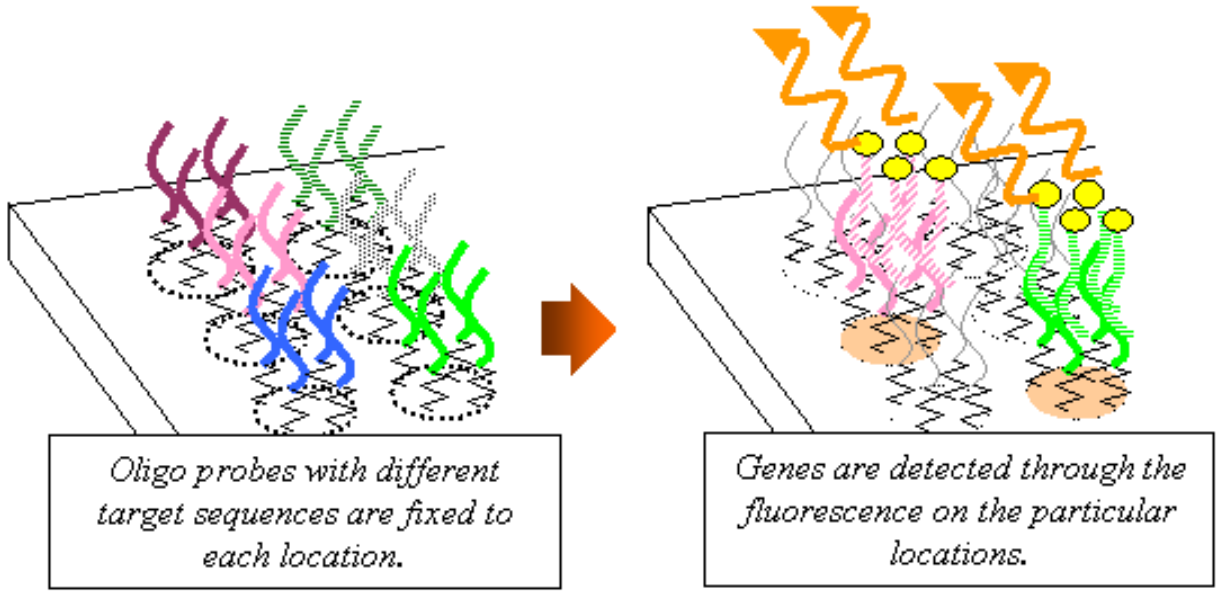
Antibodies to HLA

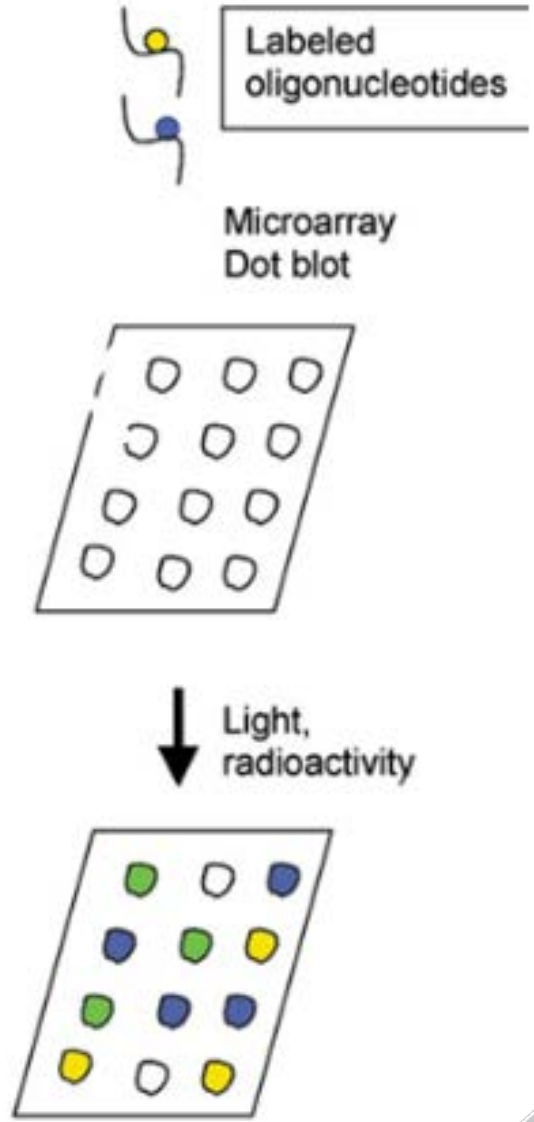
- Majority are IgG
- Two groups:
  - Private antibodies: detect single HLA gene product
  - Public antibodies: detect more than one HLA product
- Can have crossreactivity:
  - Antiserum directed against one HLA antigen reacts with other antigens as well
- Associated with:
  - Accelerated graft rejection
  - Poor response to platelet transfusion
  - TRALI (Pulmonary infiltrates/respiratory distress)

Molecular Genotyping

Sequence-Specific Oligonucleotides (SSO)

- PCR amplification with primers flanking chosen sequence
- Amplified DNA denatured and hybridized with oligonucleotide probe for allele-defining DNA
- Probes are enzymatic or fluorescent

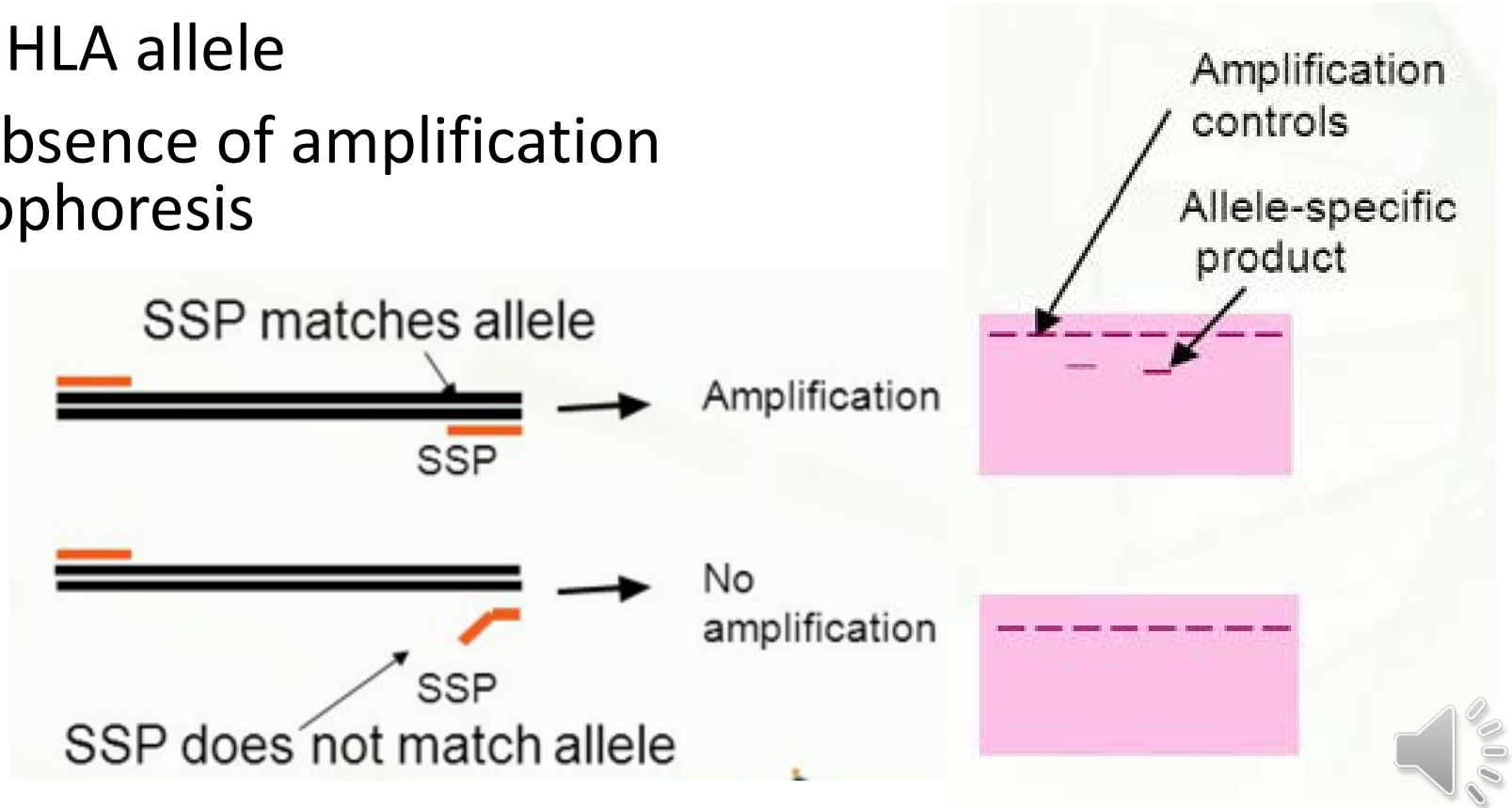




Molecular Genotyping

Sequence-Specific Primers (SSP)

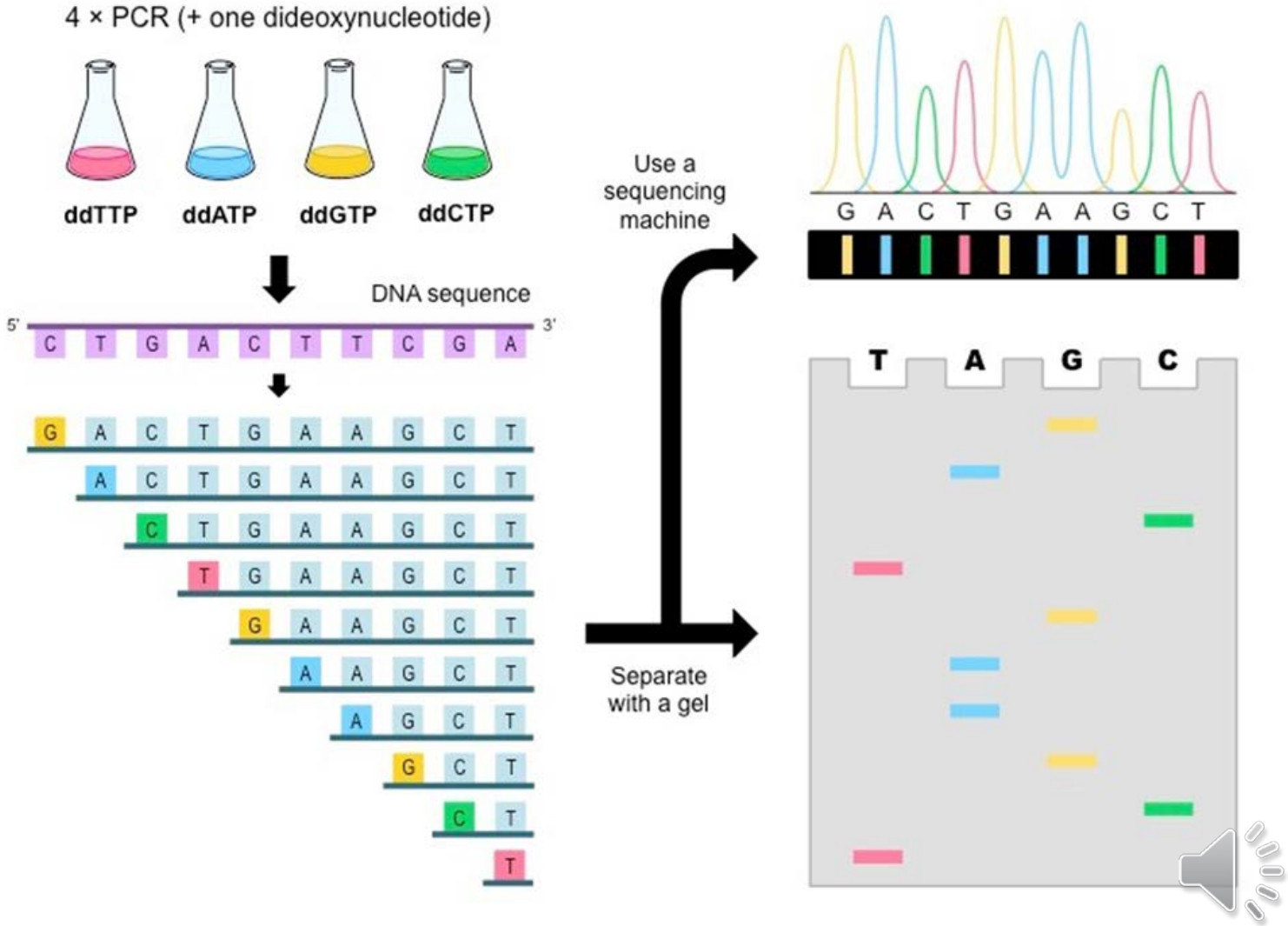
- Primers for PCR amplification target specific DNA sequences
- Sequences identify an HLA allele
- Look for presence or absence of amplification products by gel electrophoresis



Molecular Genotyping

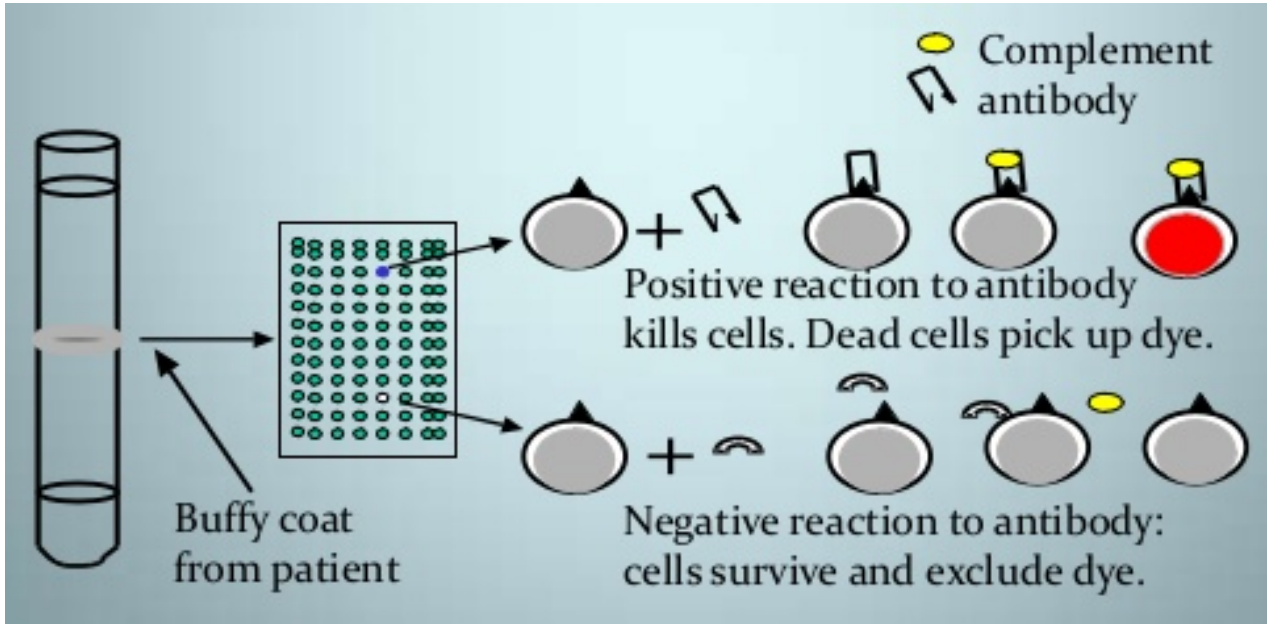
Sequence Based Typing

- Determine nucleotide sequence either by molecular cloning or PCR
- Incorporate ddNTPs labeled with fluorescent dyes
- ddNTPs lack hydroxyl group- terminate chain
- Use the dye and gel to determine the sequence
- Heterozygotes will have mixed fluorescent signals at one position



Microlymphocytotoxicity

- Identify HLA antibodies and Crossmatches
- Incubate serum (antibodies) with leukocytes (donor/reagent), exogenous complement, and fluorescent dye
- Antibody present:
  - Binds leukocytes
  - Activate complement
  - Forms holes
  - Holes allow dye to enter
- Antibody not present – dye can't enter cell
- >40% take up dye = positive reaction

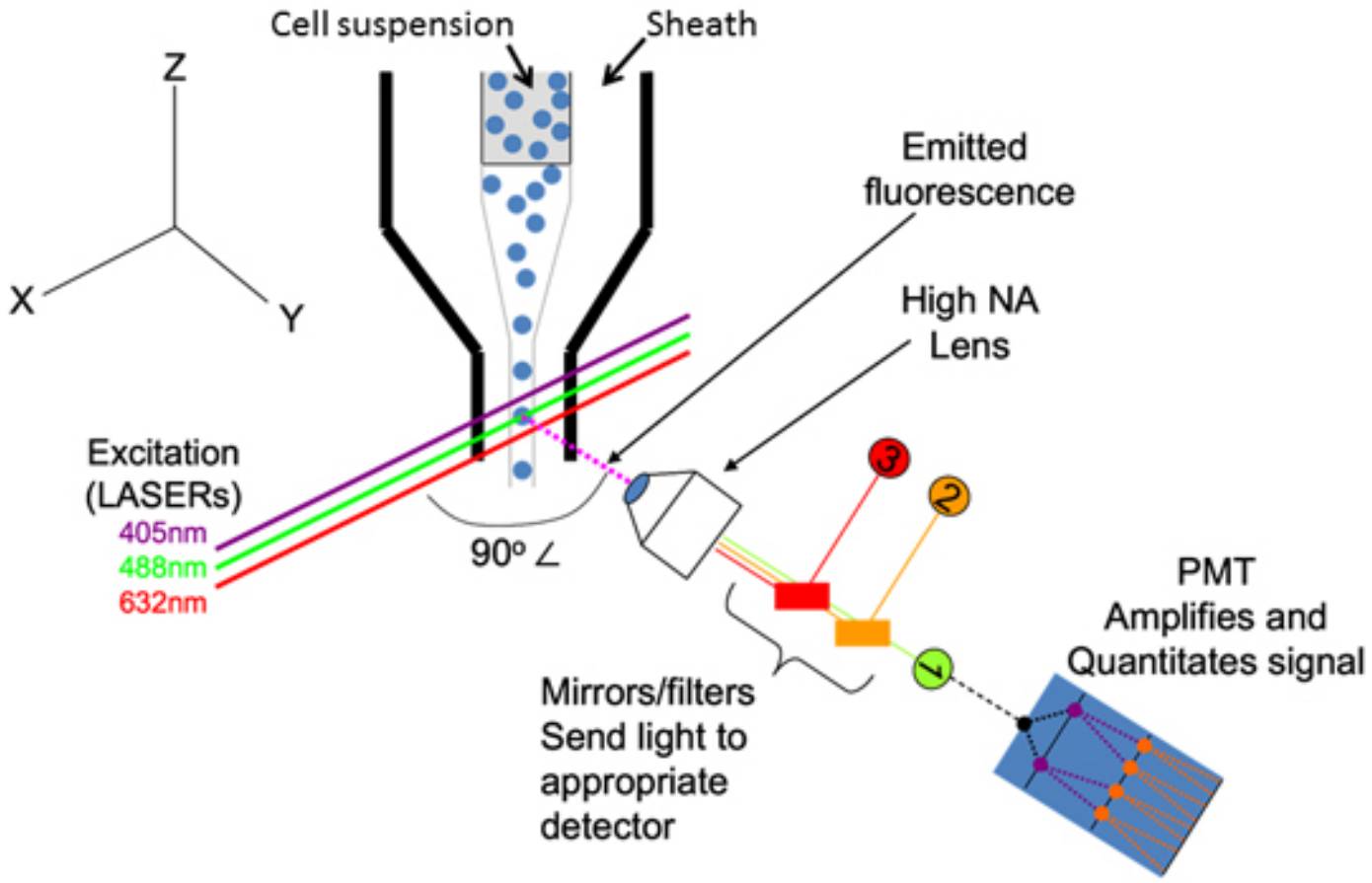


Disadvantages of Cytotoxicity Testing

- Need cells for testing
- Frozen lymphocytes are more fragile
- Low titers may not be identifiable
- To increase sensitivity:
  - Increase incubation time
  - Add wash step
  - Add DTT
  - Add AHG reagent

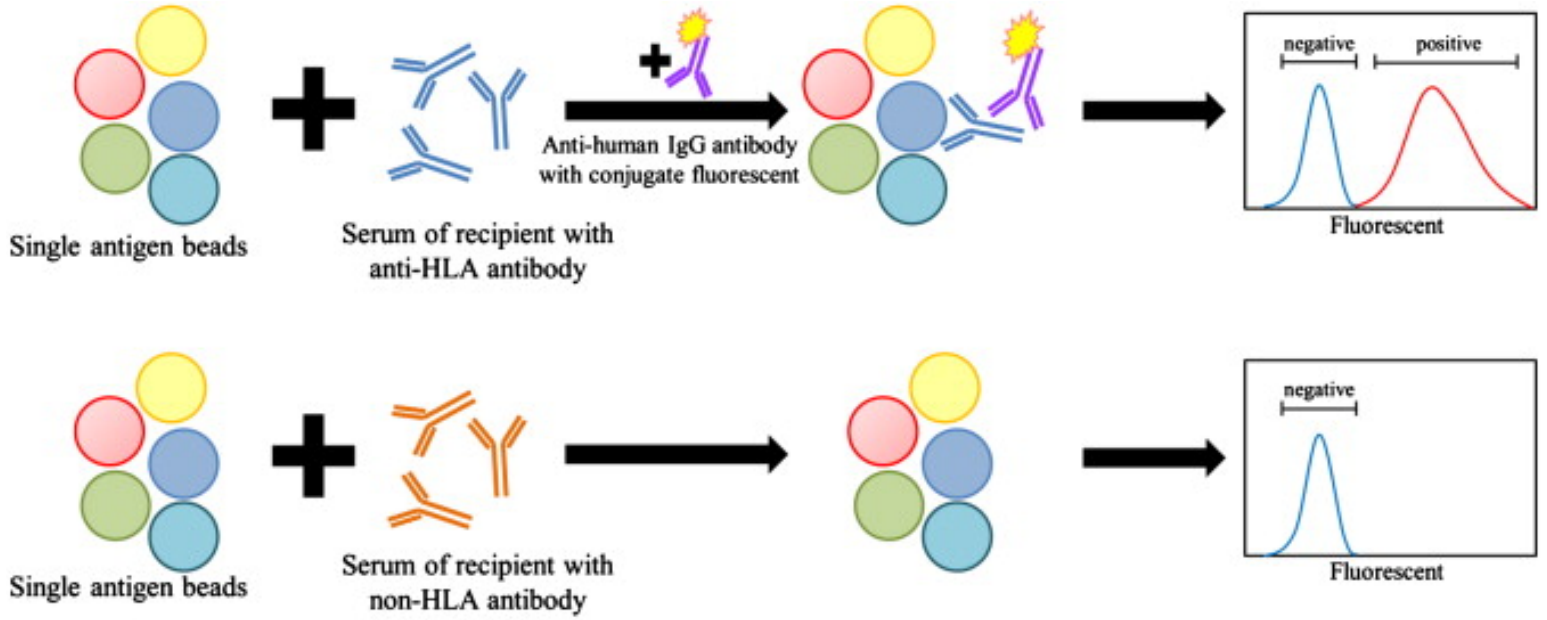
Flow Cytometry

- Antibody detection
- Incubate serum with donor lymphocytes
- Wash lymphocytes and add fluorophore-labeled anti-immunoglobulin
- Flow cytometer uses laser to emit fluorescence and quantitates



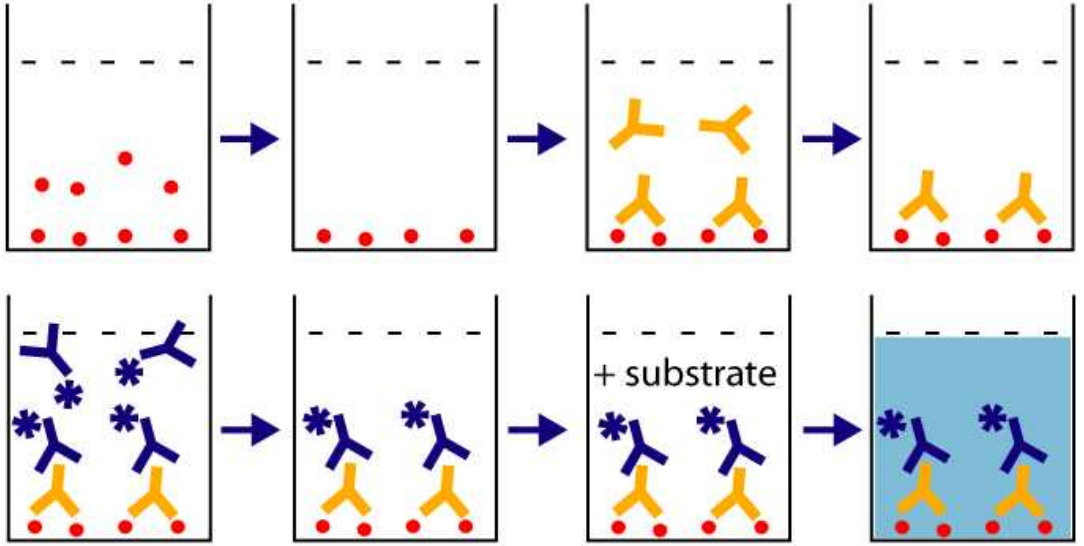
Multiplex Single-Antigen Bead Immunoassay (SAB)

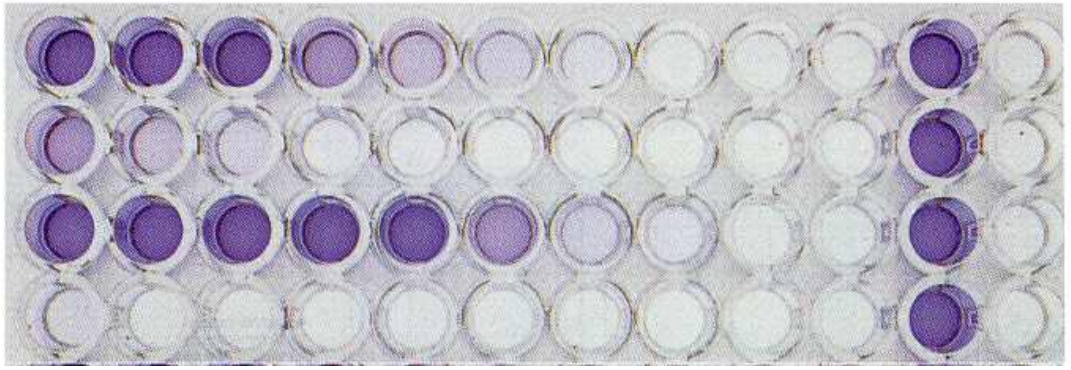
- Use recombinant individual HLA proteins
- Test serum against proteins bound to microbeads
- Anti-Ig binds to antibody and labels microbead with fluorophore – measured by flow cytometer
- Define antigen specificity of anti-HLA antibodies
- More sensitivity than previous ELISA methods



ELISA

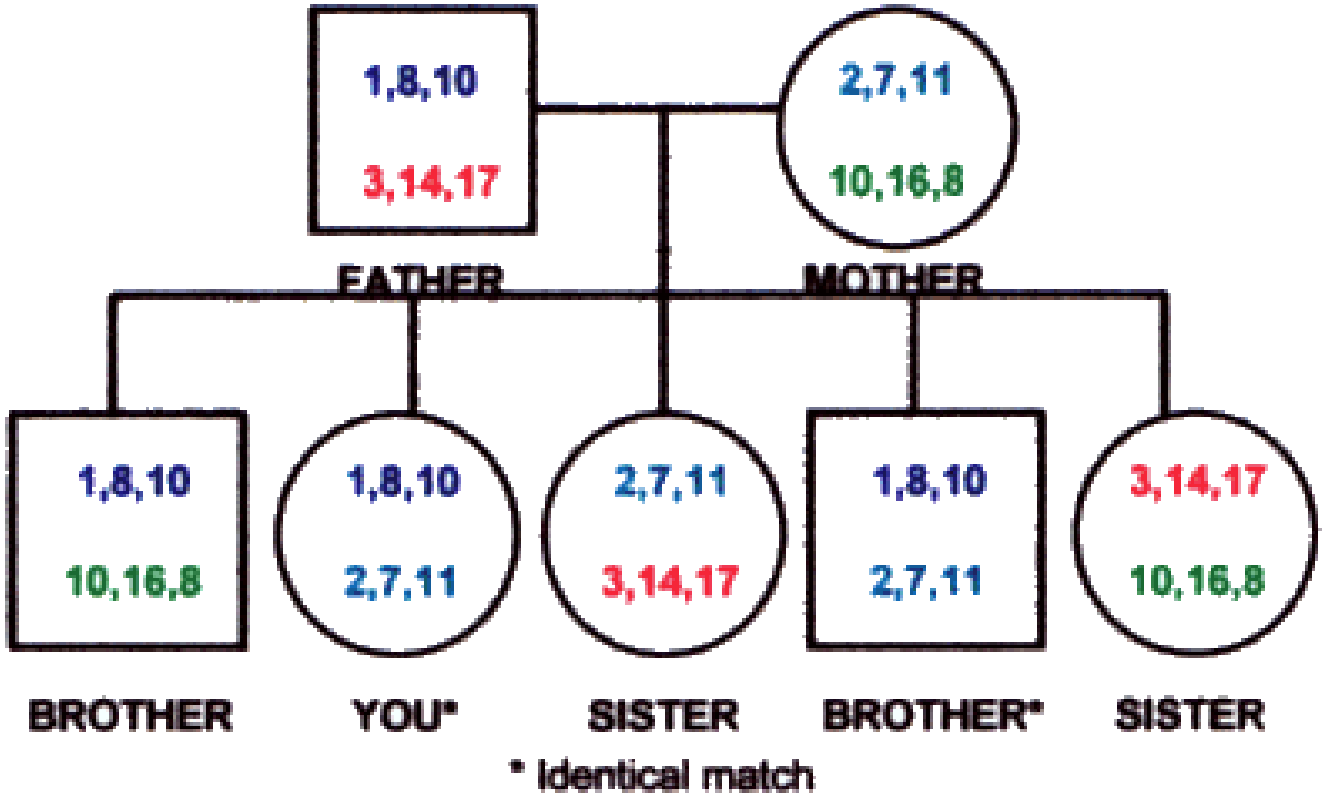
- Test for antibodies to platelet glycoproteins (HLA and HPA antigens)
- Separate microwell coated with different glycoproteins
- Can also have antibodies in microwells to detect antigens





Clinical Significance of HLA System- Paternity

- HLA typing helps determine paternity
- More common to use DNA typing today



Clinical Significance of HLA System- Disease Association

- HLA antigens associated with disease susceptibility more than any other genetic marker

Disease	Associated alleles	Frequency in		Relative risk
		patients	control	
Ankylsoing spondylitis	B27	9	9	87.4
Reiter's disease	B27	79	9	37.0
Acute anterior uveitis	B27	52	9	10.4
Psoriasis vulgaris	CW6	87	33	13.3
Dermatitis herpetiformis	DR3	85	26	15.4

Clinical Significance of HLA System- Platelet Transfusion

- Class I expressed on platelets
- Alloimmunization to HLA= refractoriness to platelets
  - Refractoriness- failure to achieve a rise in circulating platelet count 1 hour after transfusion
- Match HLA antigens in those people who are refractory

Clinical Significance of HLA System- TRALI

- Transfusion-Related Acute Lung Injury
- Strong association with HLA antibodies in blood products
- 50-89% of TRALI cases due to HLA
- Antibodies activate neutrophils in lung, releasing cytokines causing pulmonary edema
- Symptoms: Fever, hypoxemia (low oxygen in blood), pulmonary edema

Clinical Significance of HLA System-Transplantation

- Hematopoietic Stem Cell Transplant (Bone Marrow) and Kidney:
  - HLA matched ahead of time when possible
  - Reduces Graft-Vs-Host Disease (GVHD)
- Liver, Pancreas
  - Better survival when HLA matched
- Heart and Lung
  - No time to HLA type
  - Can only last so long without blood supply
  - Only match if patient has HLA antibodies

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Every life deserves world class care.