Human Leukocyte Antigens - HLA

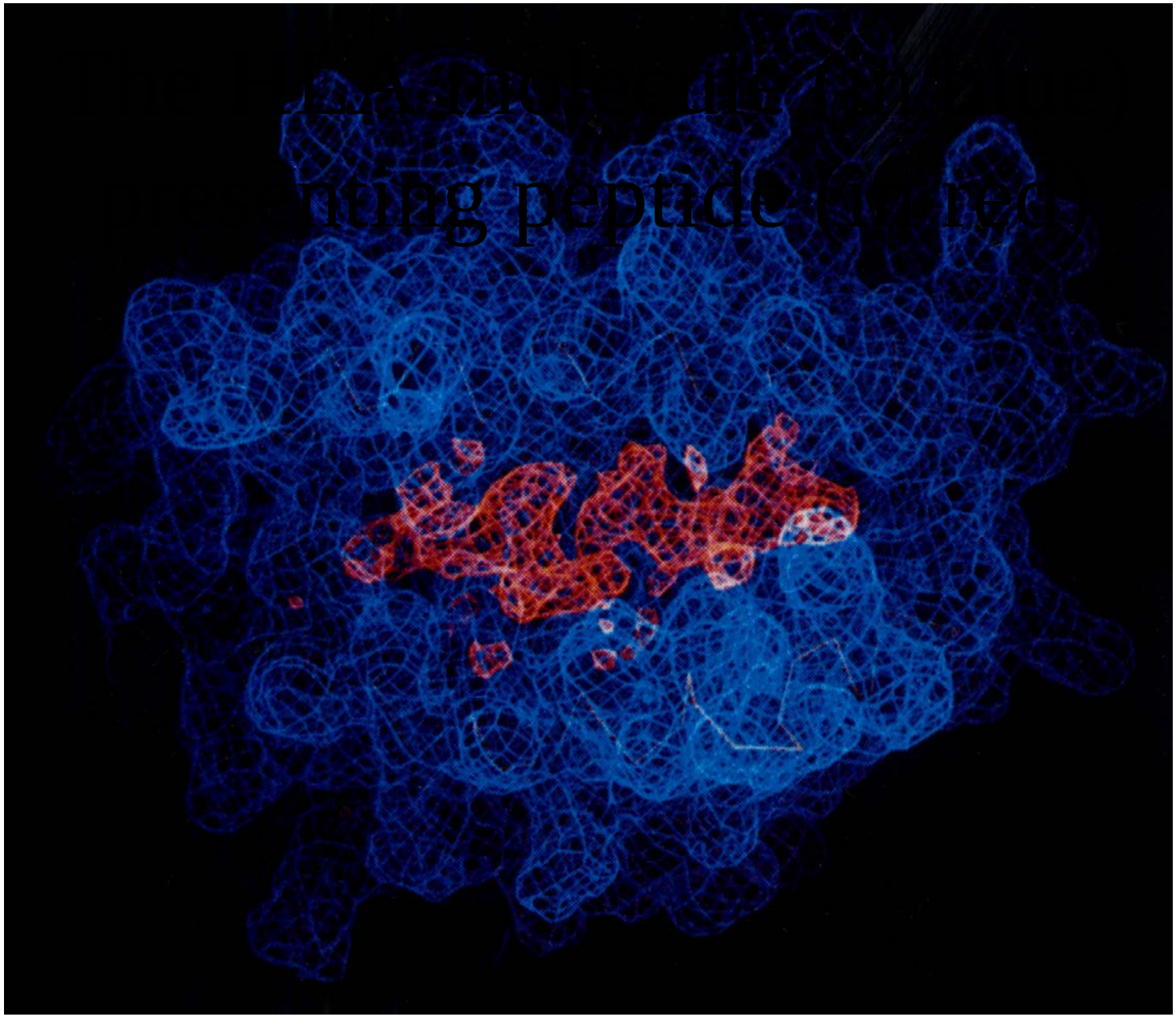
Human **L**eukocyte **A**ntigens (HLA) are cell surface proteins involved in immune function.

- HLA molecules present antigenic peptides to generate immune defense reactions.
  - HLA-class I antigens
    - » Present intra-cellular derived peptides (viruses).
    - » Expressed on most cells in the body, including B-cells, T-cells, fibroblasts, etc.
  - HLA-class II antigens
    - » Present extra-cellular derived peptides (bacteria).
    - » Restricted cellular expression: B-cells and activated T-cells.

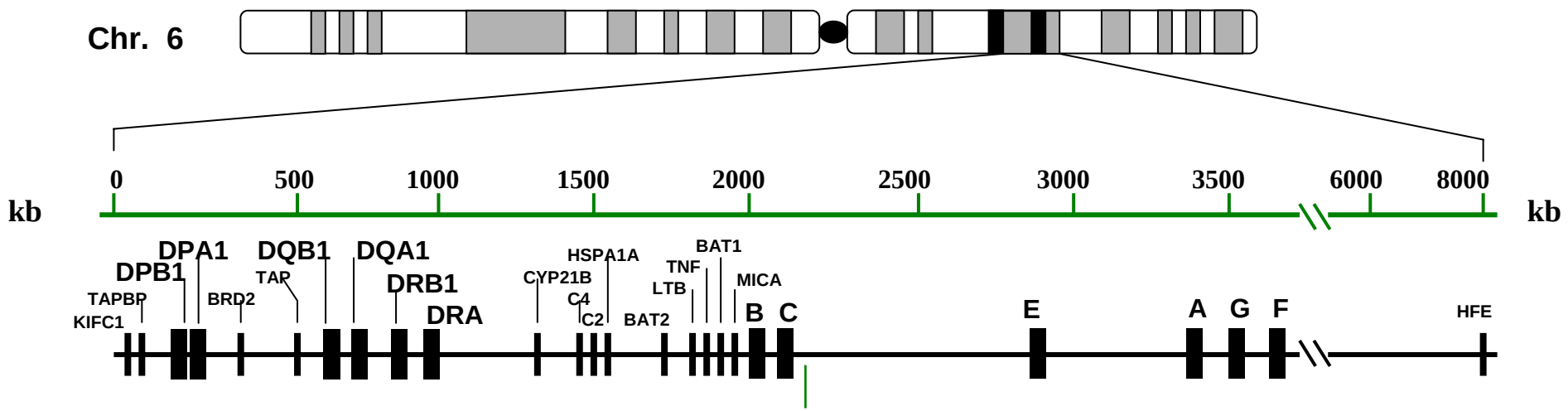
The HLA Complex or the MHC

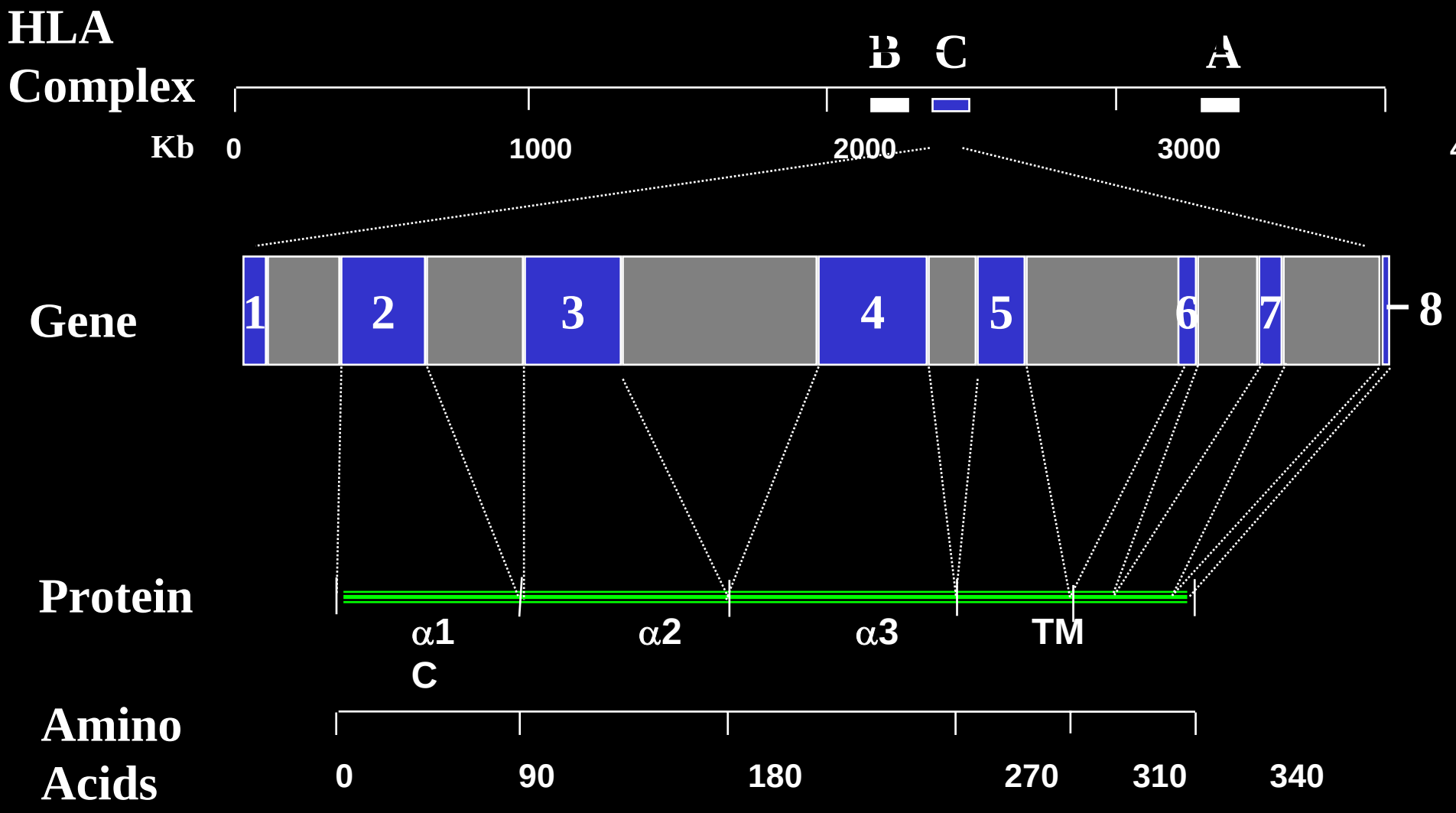
The HLA complex is defined by the genes of the **M**ajor **H**istocompatibility **C**omplex (MHC)

- Chromosome 6: 6p21 (3000 kb)
- HLA genes are characterized by extraordinary polymorphism with >1980 unique known alleles.
  - HLA alleles are not randomly distributed, certain alleles are frequent while others are infrequent or rare.
  - HLA allele frequencies differ among different human populations.
- Classical HLA genes:
  - Class I: HLA-A, B, C
  - Class II: HLA-DRB, DQB1
- Most of the nucleotide substitutions by which HLA alleles differ result in changes in the HLA cell surface proteins at positions that impact either peptide presentation or interactions with the T-cell receptor.



Human Major Histocompatibility Complex




HLA
Complex
B
C
A
Kb
0
1000
2000
3000
Gene
1
2
3
4
5
6
7
8
Protein
α1
α2
α3
TM
C
Amino
Acids
0
90
180
270
310
340

HLA Inheritence

The HLA genes that are inherited by an individual on a single chromosome constitute a “haplotype”.

- Each individual has a 1 in 4 chance of inheriting the same HLA chromosomes/haplotypes from their parents.
- HLA haplotype frequencies differ among different human populations.
  - Frequent Caucasian haplotypes are:
    - A*0101-Cw*0701-B*0801-DRB1*0301-DRB3*0101-DQB1*0201.
    - A*0301-Cw*0702-B*0702-DRB1*1501-DRB5*0101-DQB1*0602.
  - Frequent haplotypes in African Americans:
    - A*3001-Cw*1701-B*4201-DRB1*0302-DRB3*0101-DQB1*0402.
    - A*0301-Cw*0401-B*5301-DRB1*0804-DQB1*0301.
  - New haplotypes are developed by recombination events.

Example of HLA Family Study

Haplotype Inheritance

Father

a	b
A*0101	A*2902
Cw*0701	Cw*1601
B*0801	B*4403
DRB1*0301	DRB1*0701
DQB1*0201	DQB1*0202

Mother

c	d
A*0301	A*0201
Cw*0702	Cw*0501
B*0702	B*4402
DRB1*1501	DRB1*0401
DQB1*0602	DQB1*0301

Patient

a	c
A*0101	A*0301
Cw*0701	Cw*0702
B*0801	B*0702
DRB1*0301	DRB1*1501
DQB1*0201	DQB1*0602

Sibling 1

a	d
A*0101	A*0201
Cw*0701	Cw*0501
B*0801	B*4402
DRB1*0301	DRB1*0401
DQB1*0201	DQB1*0301

Sibling 2

b	d
A*2902	A*0201
Cw*1601	Cw*0501
B*4403	B*4402
DRB1*0701	DRB1*0401
DQB1*0202	DQB1*0301

Sibling 3

a	c
A*0101	A*0301
Cw*0701	Cw*0702
B*0801	B*0702
DRB1*0301	DRB1*1501
DQB1*0201	DQB1*0602

Sibling 4

b	c
A*2902	A*0301
Cw*1601	Cw*0702
B*4403	B*0702
DRB1*0701	DRB1*1501
DQB1*0202	DQB1*0602

Sibling 5 (paternal recombination)

a/b	c
A*2902	A*0301
Cw*0701	Cw*0702
B*0801	B*0702
DRB1*0301	DRB1*1501
DQB1*0201	DQB1*0602

HLA and Hematopoietic Cell Transplantation

- Donor (Graft) recognizes recipient HLA disparity.
  - Patient has HLA antigens not present in the donor.
    - Patient: A*0101, **0201**
    - Donor: A*0101, **0301**
  - Disparity can result in Graft vs Host (GVH) disease.
  - Disparity can result in Graft vs Leukemia (GVL) effects.
- Recipient recognizes HLA disparity in donor graft.
  - Donor has HLA antigens not present in the patient.
    - Patient: A*0101, 0101
    - Donor: A*0101, **0301**
  - Disparity can result in Graft failure or rejection .

HLA and KIR

Certain of the HLA-B and HLA-C proteins function as ligands for natural killer cell receptors (KIR).

- KIR: Killer Immunoglobulin-like Receptors.
  - Encoded on chromosome 19.
  - Multi-gene family.
  - Highly polymorphic.
  - Inherited independently of HLA genes.
- There is a growing body of evidence that interactions between HLA and KIR molecules play a role in hematopoietic cell transplantation.

HLA Typing Methods

- Serology based typing
- Molecular based typing
  - Medium resolution (SSOP)
  - High resolution (SBT)

Serology Based Typing

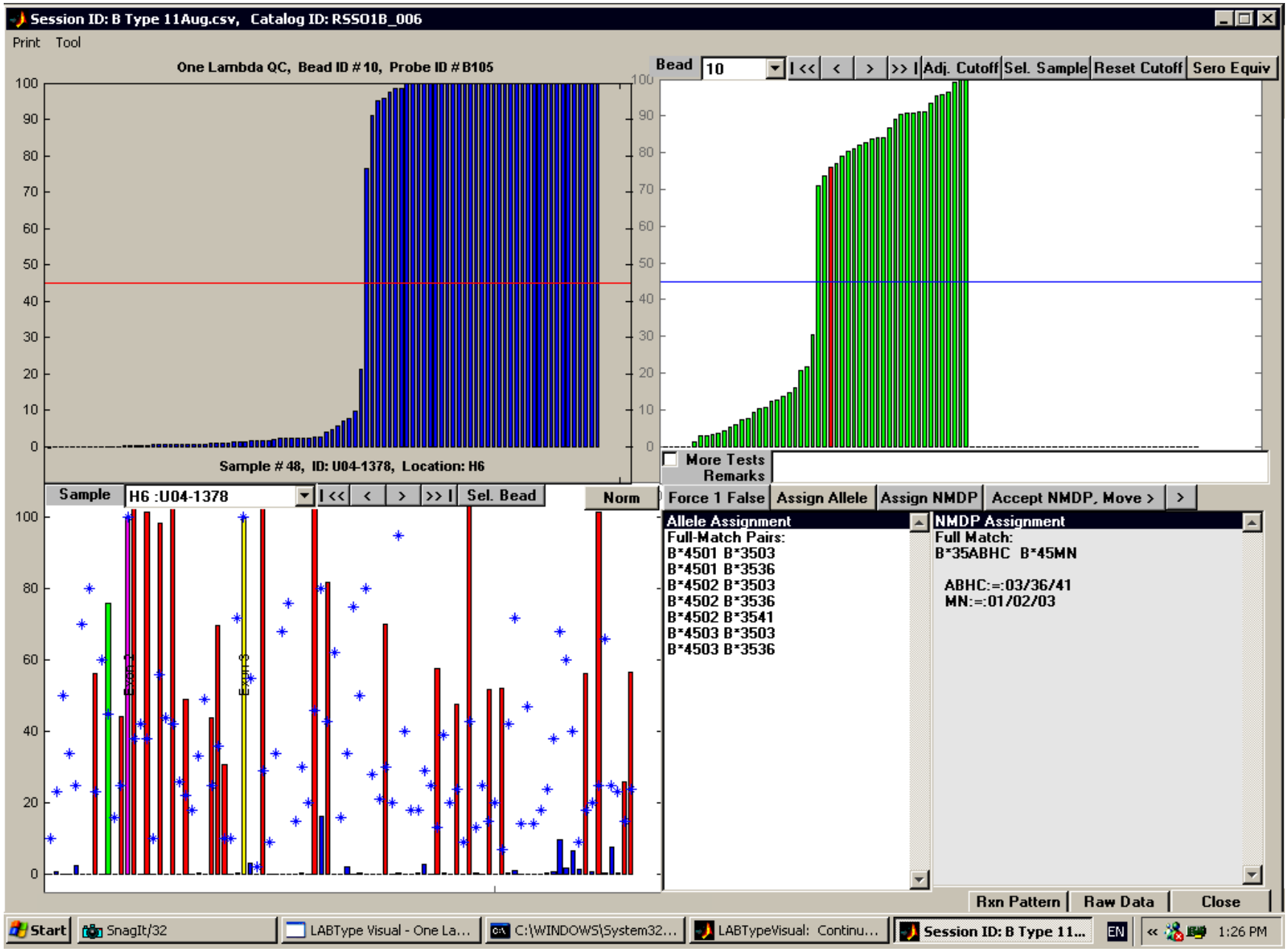
- A panel of known anti-HLA antibodies are incubated with viable lymphocytes of unknown HLA type.
- The HLA type of the sample is determined from the pattern of cell killing (cytotoxicity) that results from the antigen-antibody reactions.
- Advantages
  - Rapid
  - Assesses HLA cell surface expression
- Disadvantages
  - Limited detection of HLA polymorphism – low resolution
  - Requires viable cells
  - Requires HLA cell surface expression

Medium resolution HLA Typing (SSOP)

SSOP: **S**equence **S**pecific **O**ligonucleotide **P**robe.

- Genomic DNA is PCR amplified with primers that amplify all of the known alleles at each HLA locus (-A, -B, -C, DRB, -DQB).
- PCR amplified sample DNA is hybridized with panels of probes directed against known polymorphisms.
- HLA types are determined from the patterns of probe reactions.
- SSOP provides partial sequence information.
  - Intermediate resolution.
  - Useful as a screening test.
    - Identifies family members that have inherited the same HLA chromosomes.
    - Identifies unrelated donors likely to be matched with the patient.
- Also referred to as microarray typing

Software – LABType Visual



High resolution HLA typing
Sequence **B**ased **T**yping (SBT)

Sample DNA is amplified by Polymerase Chain Reaction (PCR)

- Each HLA locus is amplified with either generic primers or allele specific primers.

Amplified DNA is used as a template for sequencing reactions using fluorescent dye-labeled dideoxynucleotides (ddATP, ddTTP, ddCTP, ddGTP).

Fluorescence labeled sequence fragments are analyzed by capillary gel electrophoresis, based on fragment size separation.

Sample sequence is compared with known HLA allele sequences to assign the HLA type.

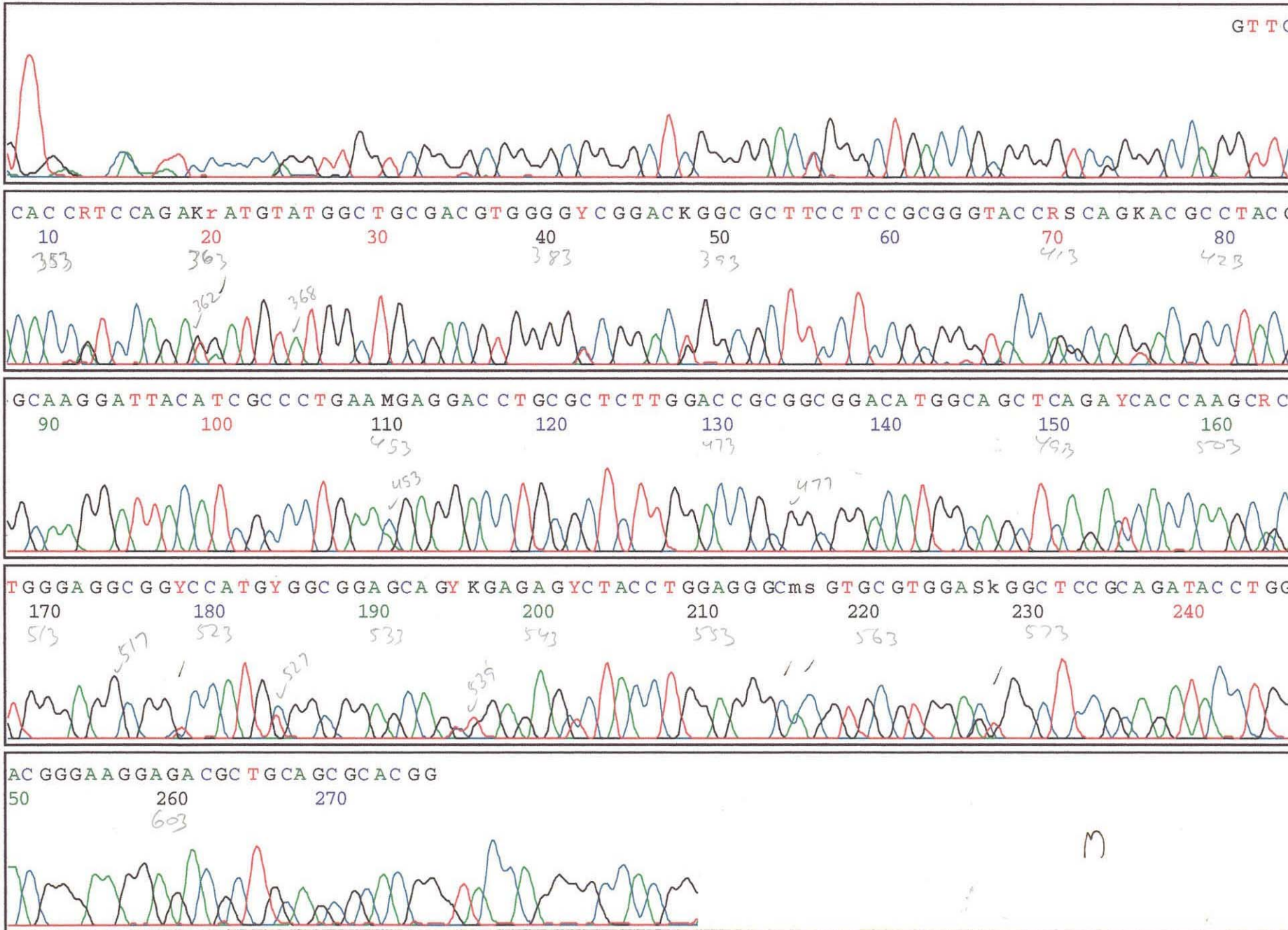
- Method uses HLA sequencing analysis software.
- Method provides HLA allele level typing to determine allele matching and to detect rare or novel alleles.

ABI PRISM™

HLA-A*0101/04N, 0201/09/43N, Exon 3 F

Signal G:437 A:247 T:110 C:309
DT {BD Set Any-Primer}
BDT 377 #2 (11/17/00)
Points 1010 to 4750 Pk 1 Loc: 1010

Wed, Aug 28, 2002
Tue, Aug 27, 2002
Spacing: 10.4



HLA Typing Ambiguities

Alleles may not be resolved because the typing method used does not test for every known polymorphism.

- For example, Class I sequencing of exons 2 and 3 may give these ambiguous results:
  - B*4402/*4419N/*4427 (coded as B*44PYV)
    - B*4402 is the most common allele.
    - B*4419N has a single base substitution in exon 1 – not analyzed
    - B*4427 has a single base substitution in exon 4 – not analyzed.
  - Cw*0401/*0409N (coded as Cw*04KBG)
    - Cw*0401 is the most common allele.
    - Cw*0409N has single base deletion in exon 7 – not analyzed.
- For example, Class II sequencing of exon 2 may give
  - DRB1*1201/*1206 (coded as DRB1*12AG)
    - DRB1*1201 is the common allele.
    - DRB1*1206 has a single base substitution exon 3 – not analyzed

Other HLA Typing Ambiguities

Typing may not be resolvable because certain allele combinations produce identical sequences, known as Cis/Trans ambiguities

- For example, these DRB1 allele combinations have identical sequences:

  DRB1*1301/*1101 = DRB1*1104/*1302 = DRB1*1102/*1305

- These Class I allele combinations have identical sequences:

  A*0201/* 0205 = A*0202/*0206

  B*4402/*5601 = B*4403/*5613 = B*4409/*5607

Allo-Immune Testing

Detection and characterization of recipient sensitization to HLA antigens.

- Patients with antibodies against a donor's HLA antigens have an increased risk of rejection.
- **PRA** (**P**anel **R**eactive **A**ntibody) testing determines if the patient has antibodies against HLA antigens
  - Step 1: Screening test.
    - » Patient serum is incubated with a panel of known HLA antigens to determine if HLA antibodies are present in patient.
  - Step 2: Antibody Identification
    - » Patient serum is incubated with individual HLA antigens to define the specificities of the patient's HLA antibodies.
- Crossmatch testing: Patient serum is incubated with donor cells.
  - Serology cross match.
    - » Presence of donor specific antibodies in patient serum is determined from the pattern of cell killing (cytotoxicity) that results from the antigen-antibody reactions.
  - Flow Cytometry (FACS) crossmatch
    - » This method further characterizes antibody type - IgG or IgM – against the donor

References

- HLA information websites
  - http://www.ebi.ac.uk/imgt/hla/
  - http://anthonynolan.com/HIB/data.html
  - http://bioinformatics.nmdp.org
- Publications

1. Impact of HLA class I and class II high-resolution matching on outcomes of unrelated donor bone marrow transplantation: HLA-C mismatching is associated with a strong adverse effect on transplantation outcome. Flomenberg N, Baxter-Lowe LA, Confer D, Fernandez-Vina M, Filipovich A, Horowitz M, et al Blood 104:1923-1930, 2004.
2. Limits of HLA mismatching in unrelated hematopoietic cell transplantation. Petersdorf EW, Anasetti C, Martin PJ, Gooley T, Radich J, Malkki M, et al. Blood 104:2976-2980, 2004.
3. Inhibitory killer Ig-like receptor genes and human leukocyte antigen class I ligands in haematopoietic stem cell transplantation. Dupont B, Hsu KC. Current Opinion in Immunology 16:634-643, 2004.
4. National Marrow Donor Program HLA-Matching Guidelines for Unrelated Marrow Transplants. Hurley CK, Baxter-Lowe LA, Logan B, Karanes C, Anasetti C, Weisdorf K, Confer DL. Biology of Blood and Marrow Transplantation 9:610-615, 2003.
5. History of DNA typing for the human MHC. Middleton D. Reviews in Immunogenetics 1:135-156, 1999.