HLA IN TRANSPLANTATION: PAST, PRESENT AND FUTURE

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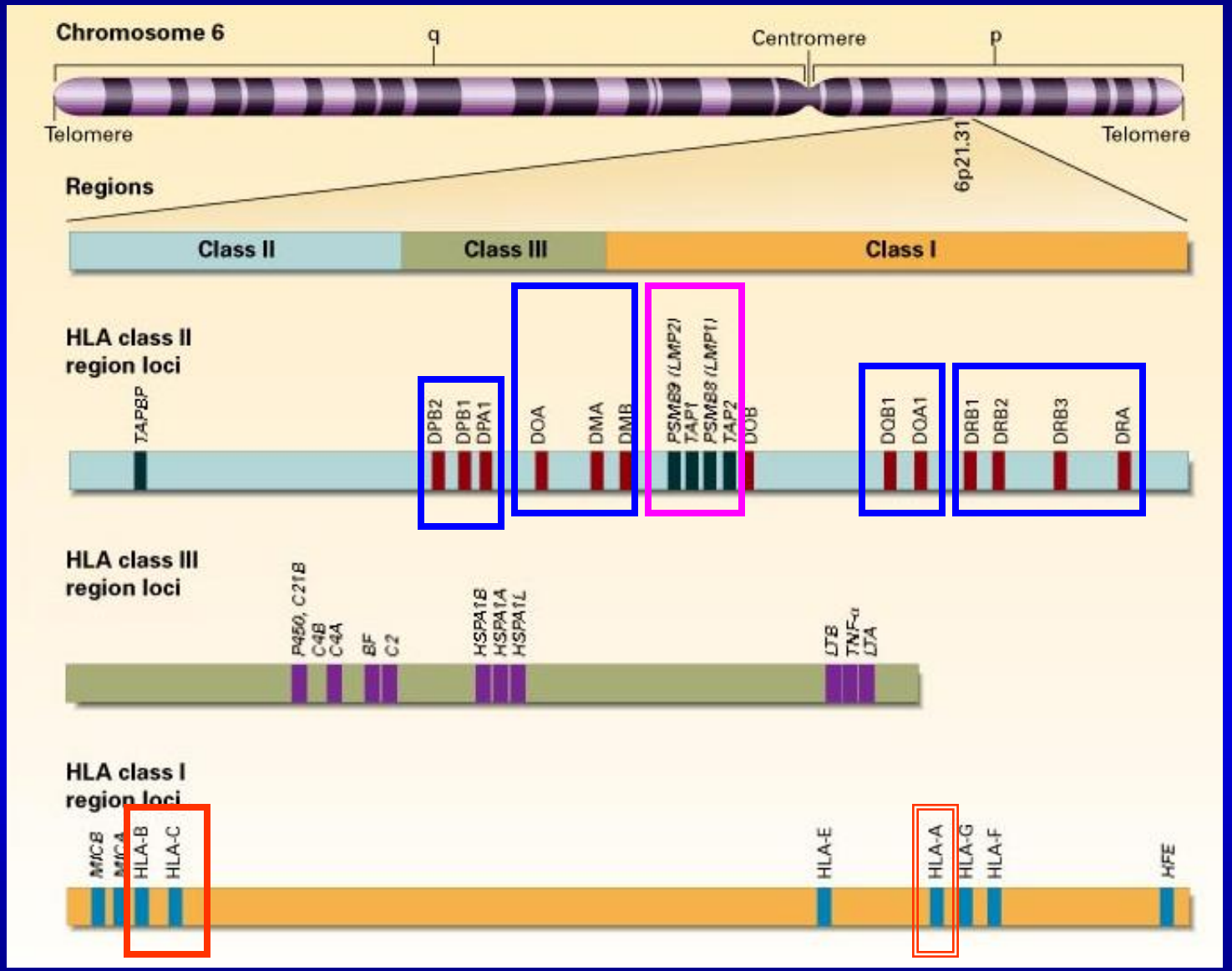
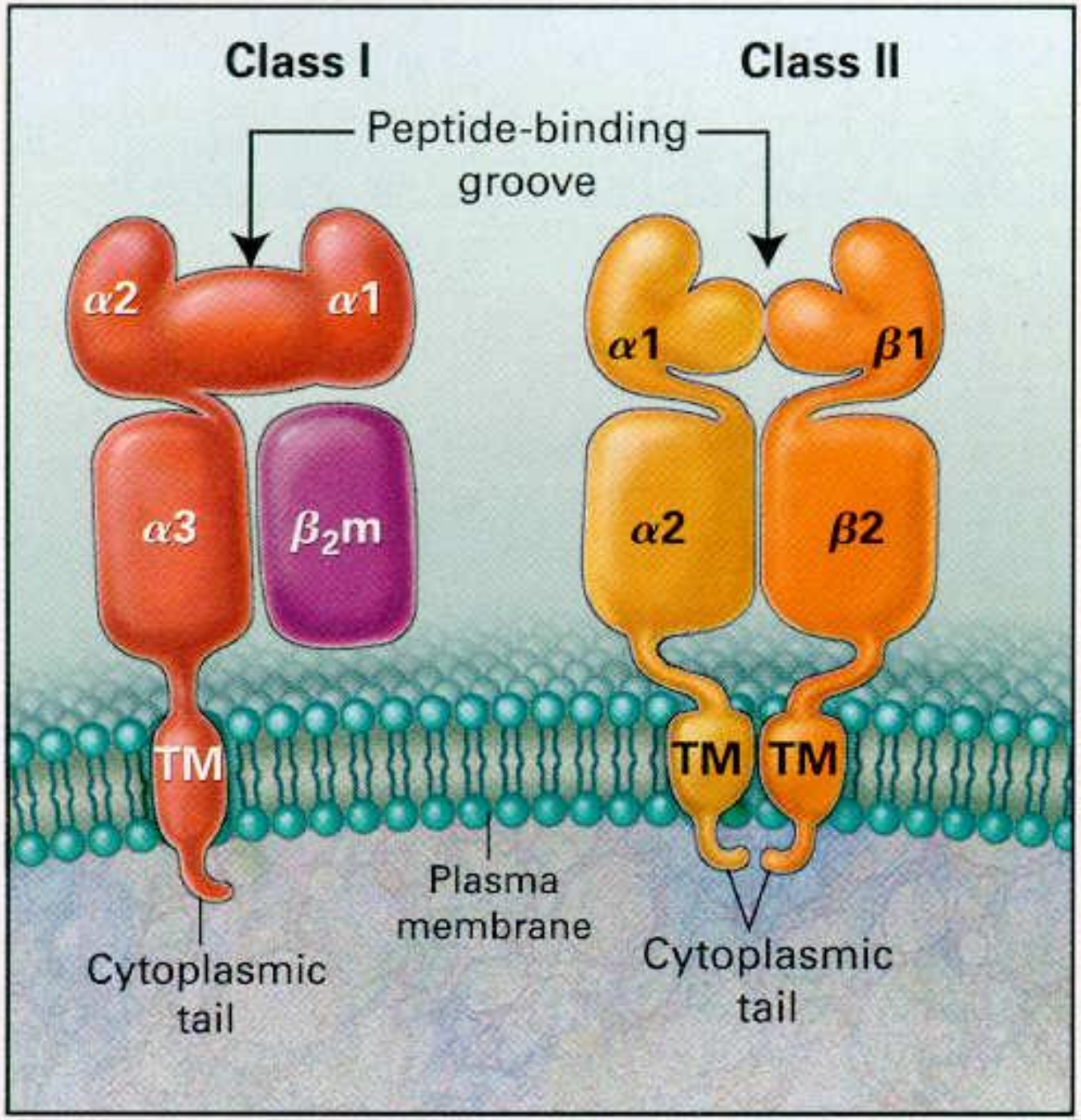
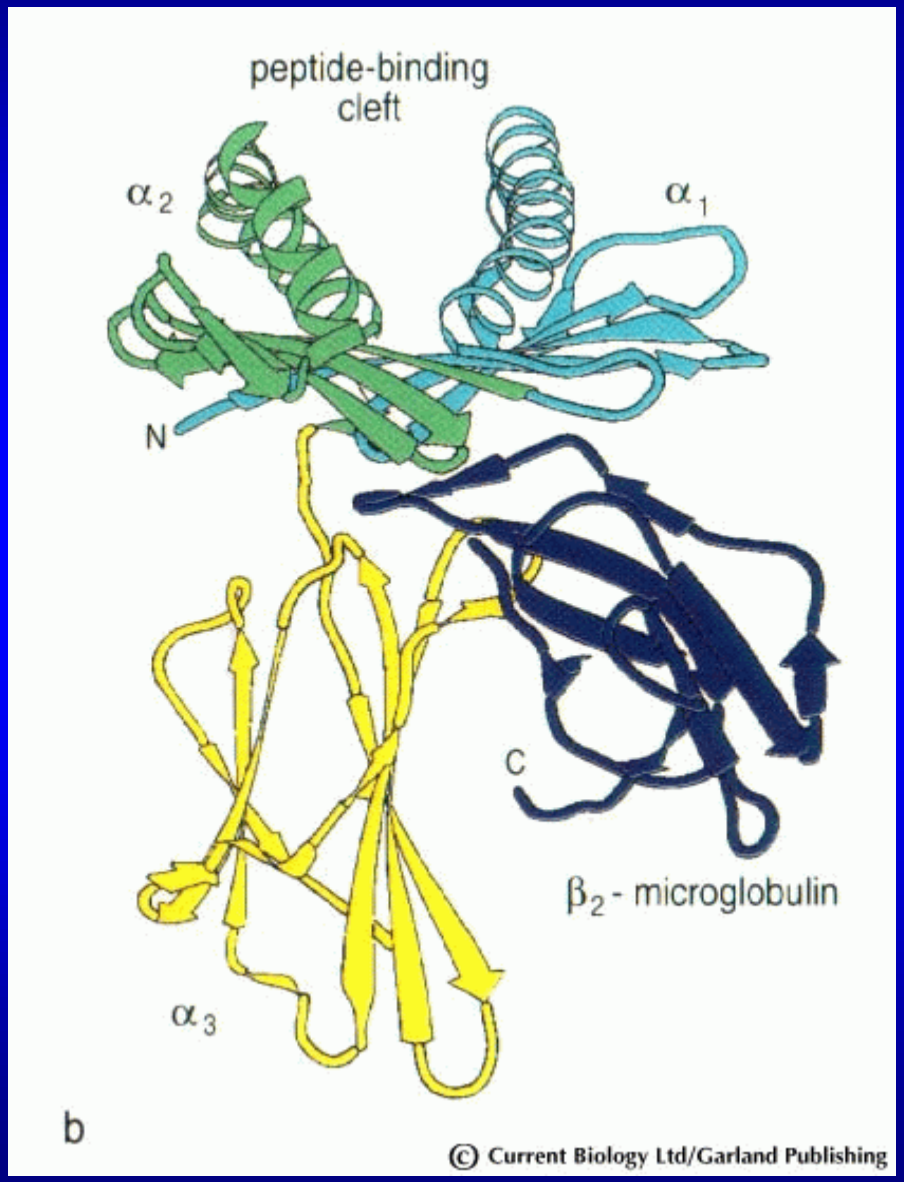
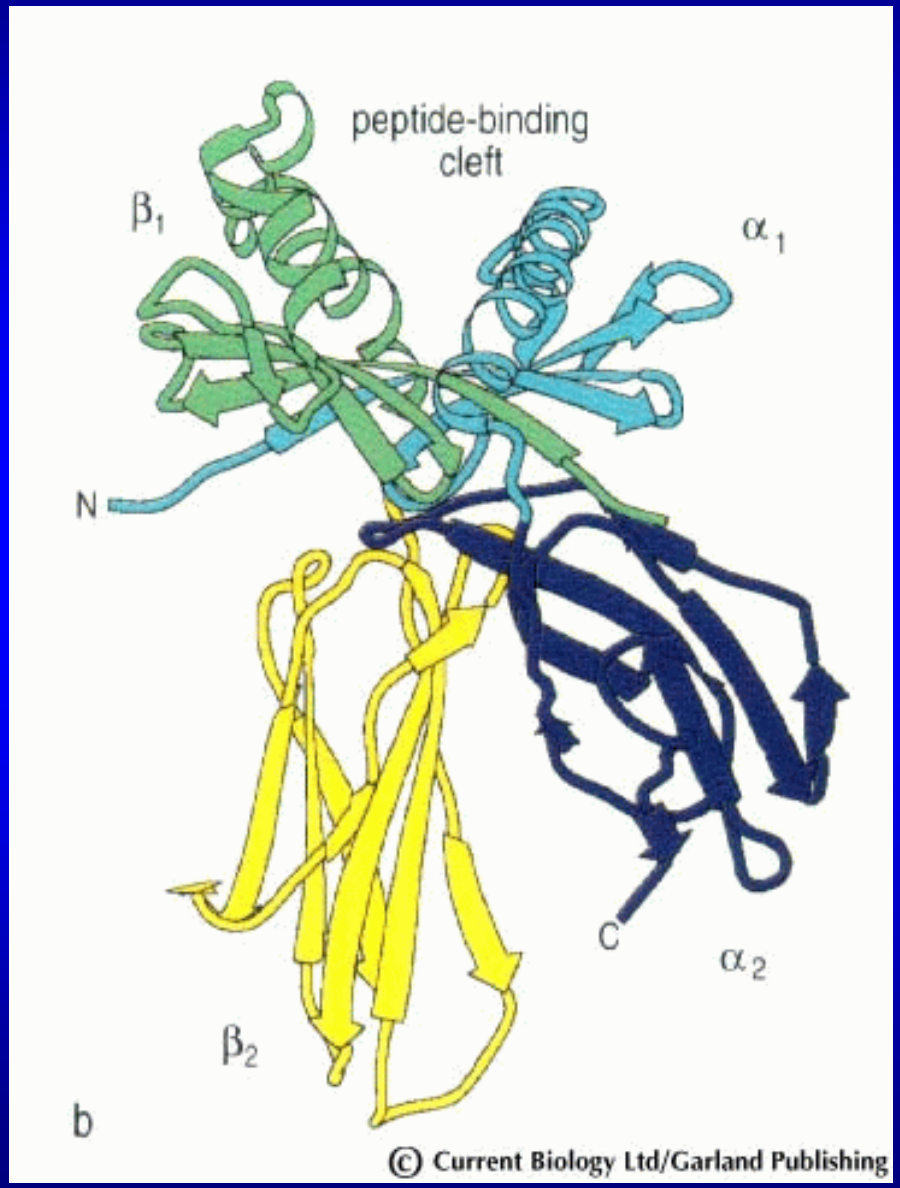

Chromosome 6
q
Centromere
p
Telomere
Telomere
6p21.31
Regions
Class II
Class III
Class I
HLA class II region loci
TAP2P
DPB2
DPB1
DPA1
DOA
DMA
DMB
PSMB9 (LMP2)
TAP1
PSMB8 (LMP7)
TAP2
DOB
DOB1
DOA1
DRB1
DRB2
DRB3
DRA
HLA class III region loci
P450, C21B
C4B
C4A
BF
C2
HSPA1B
HSPA1A
HSPA1L
LTB
TNF-α
LTA
HLA class I region loci
MICB
MICA
HLA-B
HLA-C
HLA-E
HLA-A
HLA-G
HLA-F
HFE


Class I
Class II
Peptide-binding groove
α2
α1
α1
β1
α3
β2m
α2
β2
TM
TM
TM
Plasma membrane
Cytoplasmic tail
Cytoplasmic tail

Class I

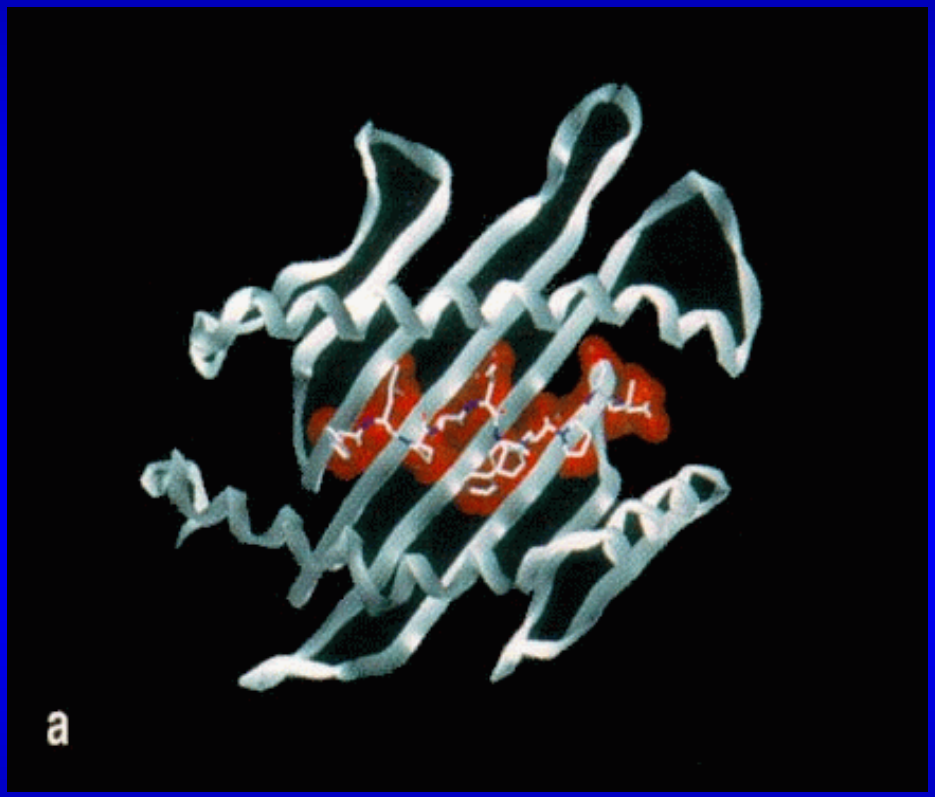


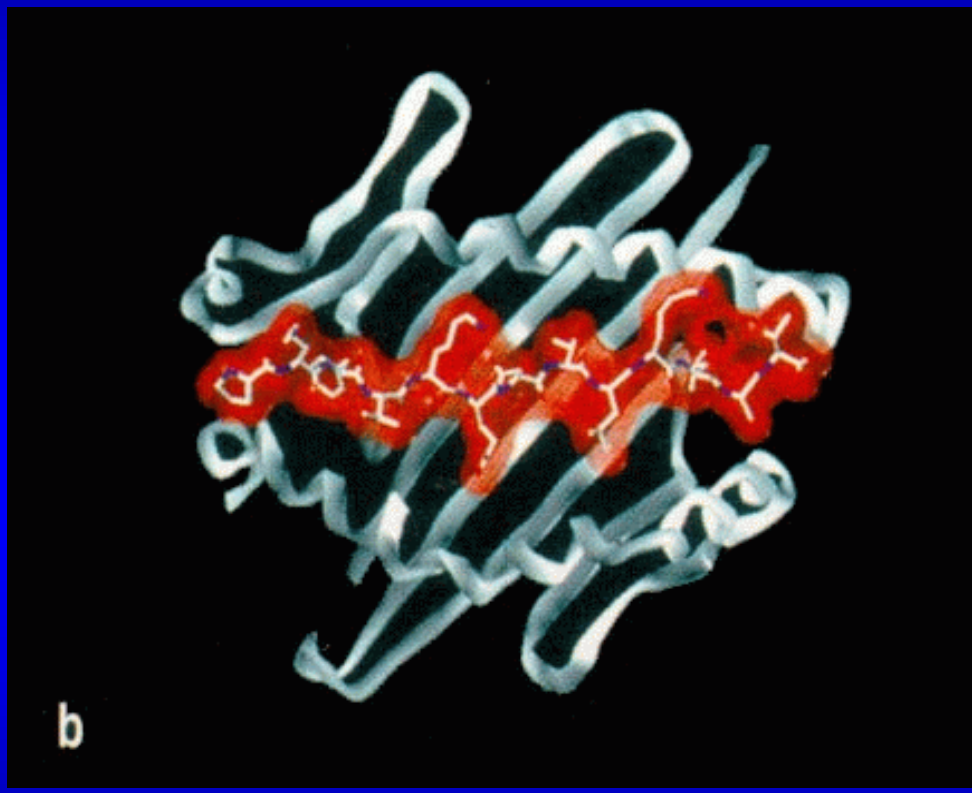
Class II

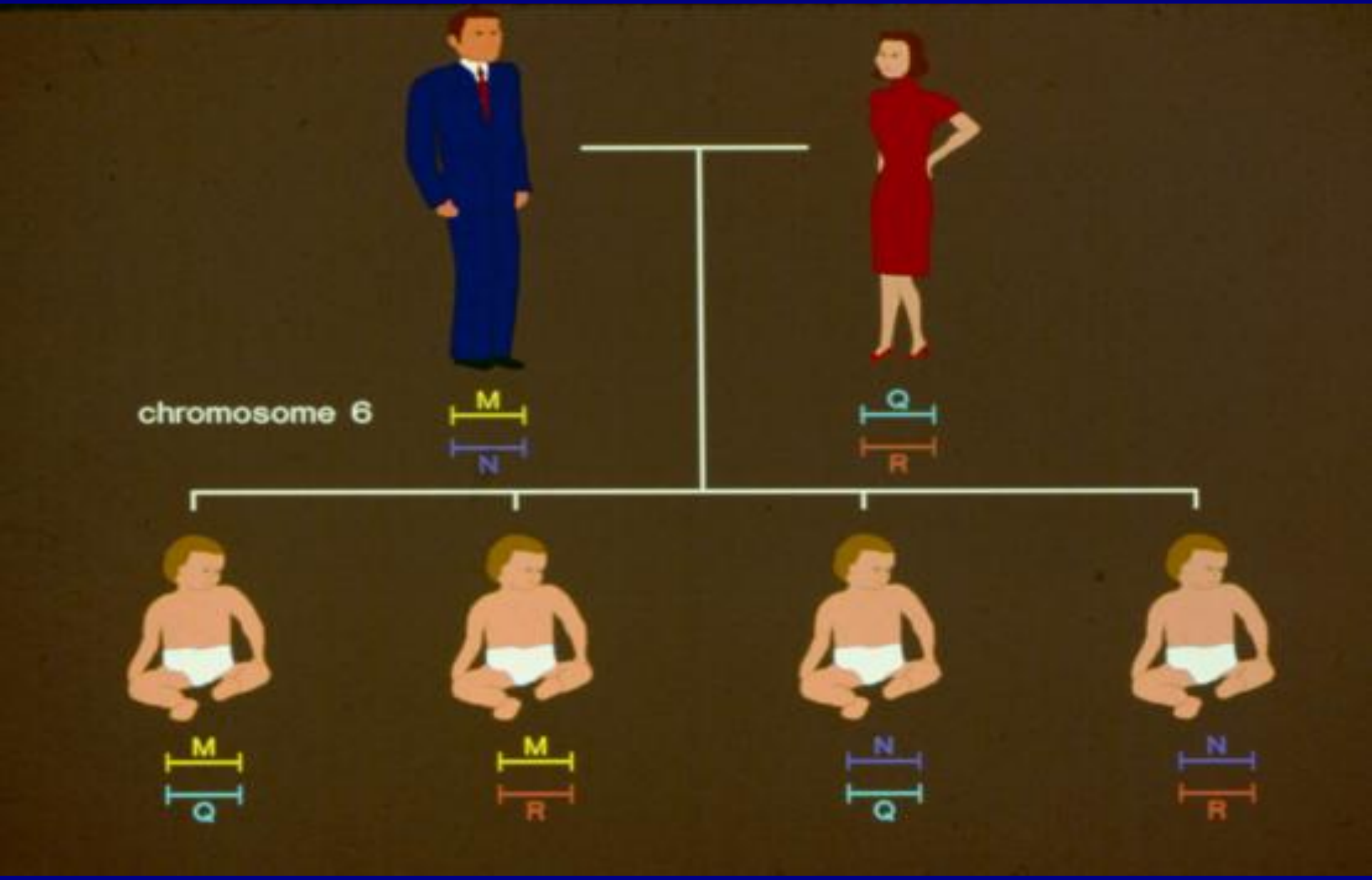


Class I

Class II






chromosome 6
M
N
Q
R
M
Q
M
R
N
Q
N
R

DEFINITIONS

PHENOTYPE: VISABLE TRAITS WHICH CAN BE ASCERTAINED IN A SINGLE INDIVIDUAL

GENOTYPE: GENETIC PROFILE OF INHERITED TRAITS

HAPLOTYPE ASSIGNMENT OF GENES TO MATERNAL OR PATERNAL CHROMOSOME

A1, B7, B8	A1, A1, B7, B8	A1 B7 A1 B8

Identification of HLA Antigens / Alleles

- Serology - Tissue source: lymphocytes

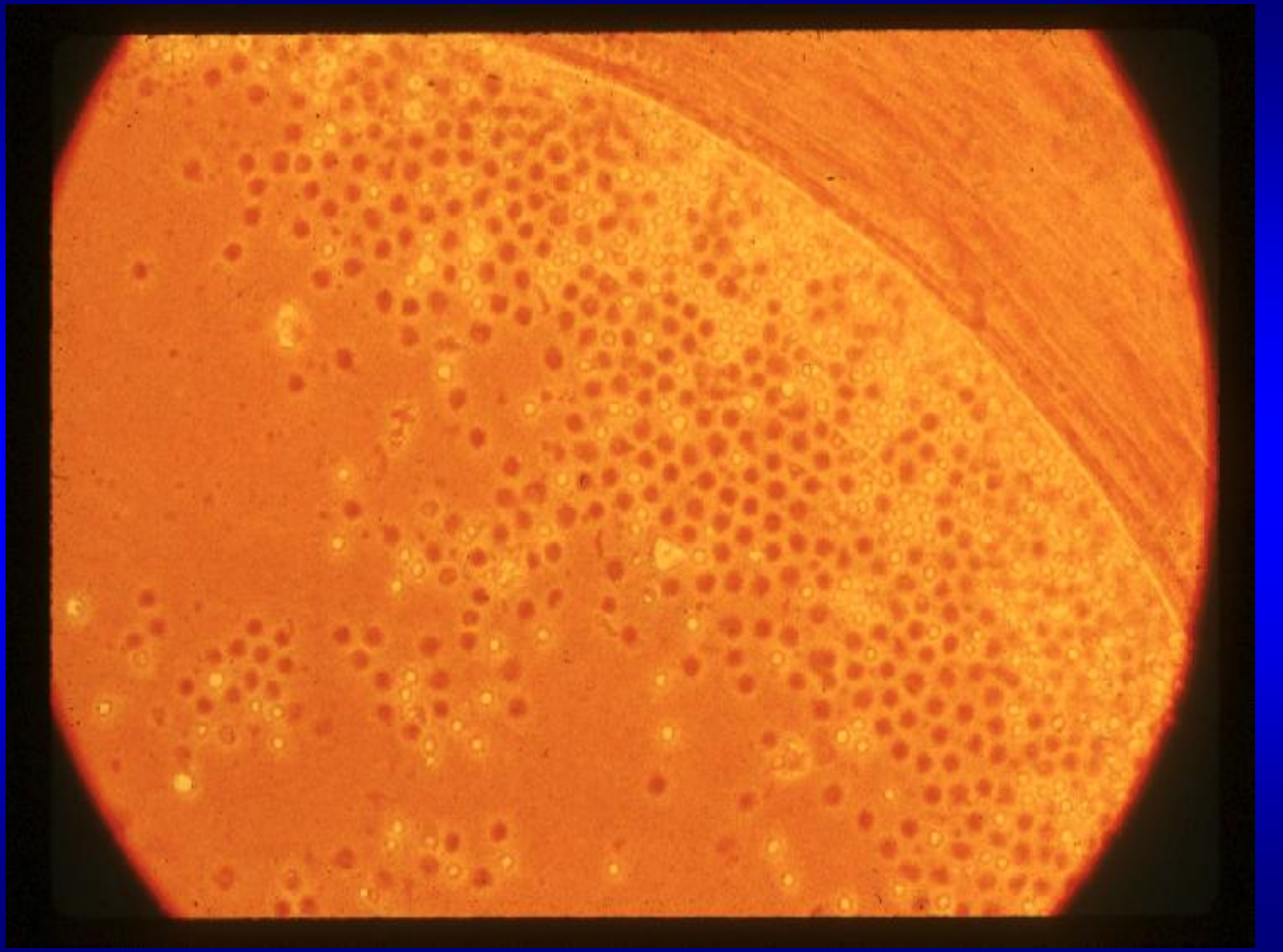
  CDC - Complement-Dependent Cytotoxicity

- Molecular - Tissue source - any nucleated cell

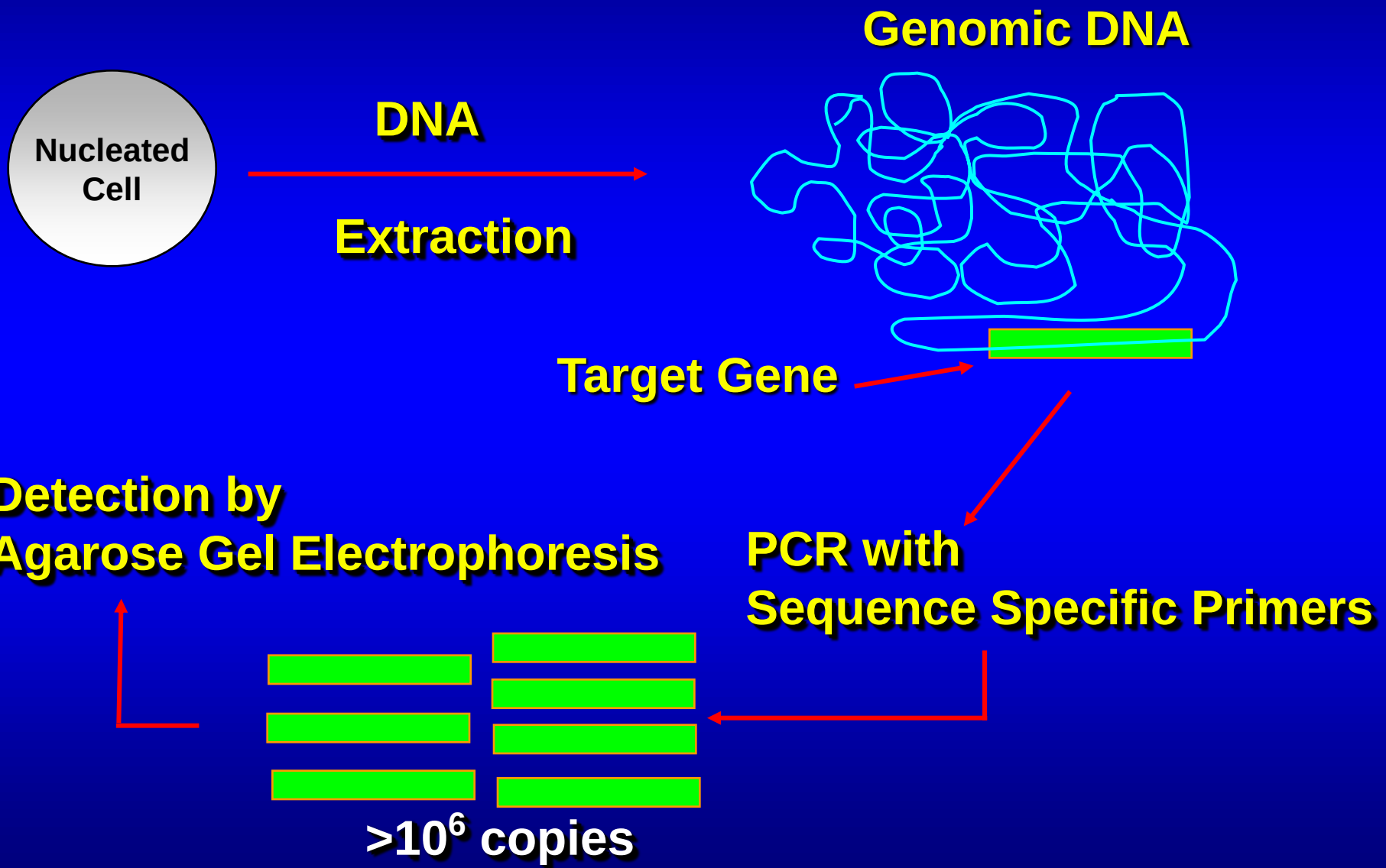
  SSP - Sequence specific primer

  SSOP - Sequence specific probe

  SBT - Sequence based typing

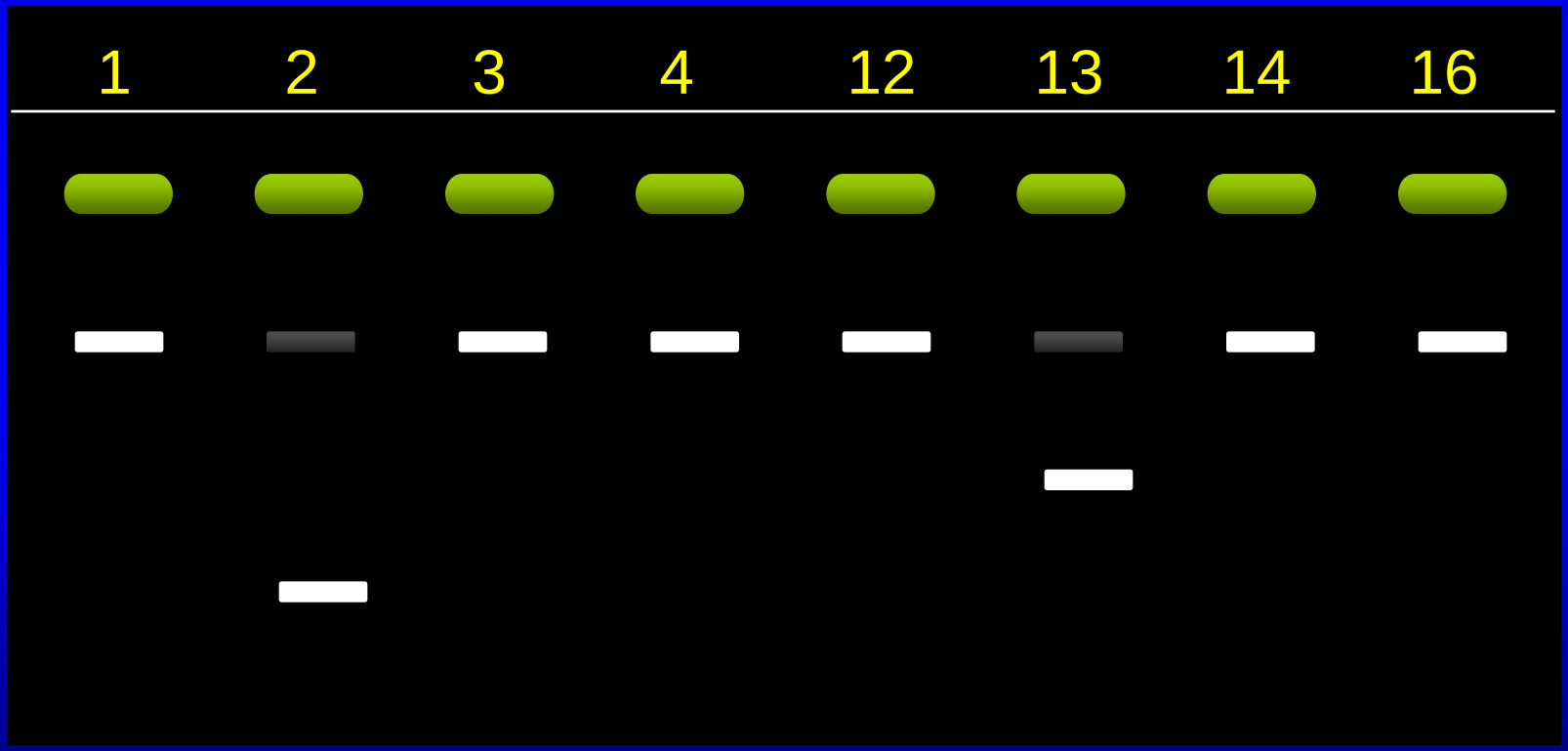


Sequence Specific PCR (SSP)



Sequence Specific PCR (SSP)

DR Specificities



HLA ANTIGENS

	A	B	C	DR	DQ
1991	26	35	14	56	13
1995	59	122	35	152	25
1997	85	188	42	221	32

Bunce, et al. Transplantation 64:1505, 1997

April, 2004

Class I alleles: 1012
Class II alleles: 493

HLA Nomenclature

Serology		Molecular
Parent	Splits	Alleles
A2	--	*0201 - *0254
A9	A23	*2301 - *2306
	A24	*2402 - *2428
B15	B62, B63	*1501 - *1564
B17	B57	*5701 - *5707
	B58	*5801 - *5806
DR7	--	*0701 /03 /04
DR6	DR13	*1301 - *1347
	DR14	*1401 - *1440

HLA Nomenclature

Serology → Molecular

Proteins → Genes

Antigens → Alleles

Few → Many

Moderately Complex →

Clinical Histocompatibility testing

- Class I typing - A, B, C loci
  –DNA
- Class II Typing - DR, DQ
  –DNA, sequence-specific primer amplification by PCR, or Direct Sequencing
- Cross-matching
- Antibody Screening – Pre and Post (DSA)

Antibody Detection Methods

-Cytotoxicity

-Luminex

-Flow Cytometry

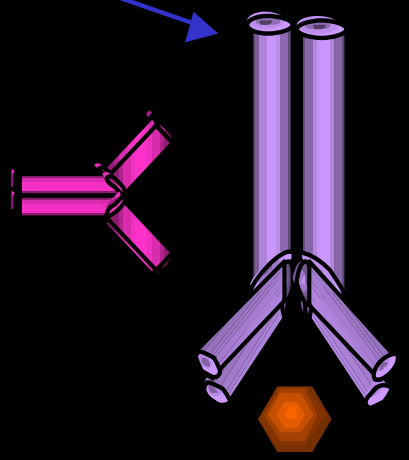
COMPLEMENT-DEPENDENT (CDC) LYMPHOCYTOTOXICITY ASSAYS

	CDC	AMOS Modified	Antiglobulin Augmented (AHG)
Stage 1 Ag-Ab	Cells + Serum		
Interaction	30 min	Wash	Wash
Stage 2 C' Mediated	Complement	X1 X2	X3 Add AHG
Cell Injury	60 min	or	2 min
Stage 3 Visualize Injury	Vital Stain	X3	

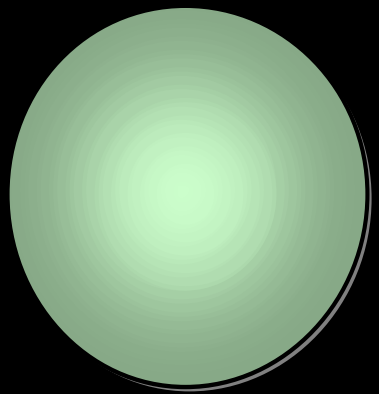
LABScreen™ PRA

Alloantibody

PE anti-IgG

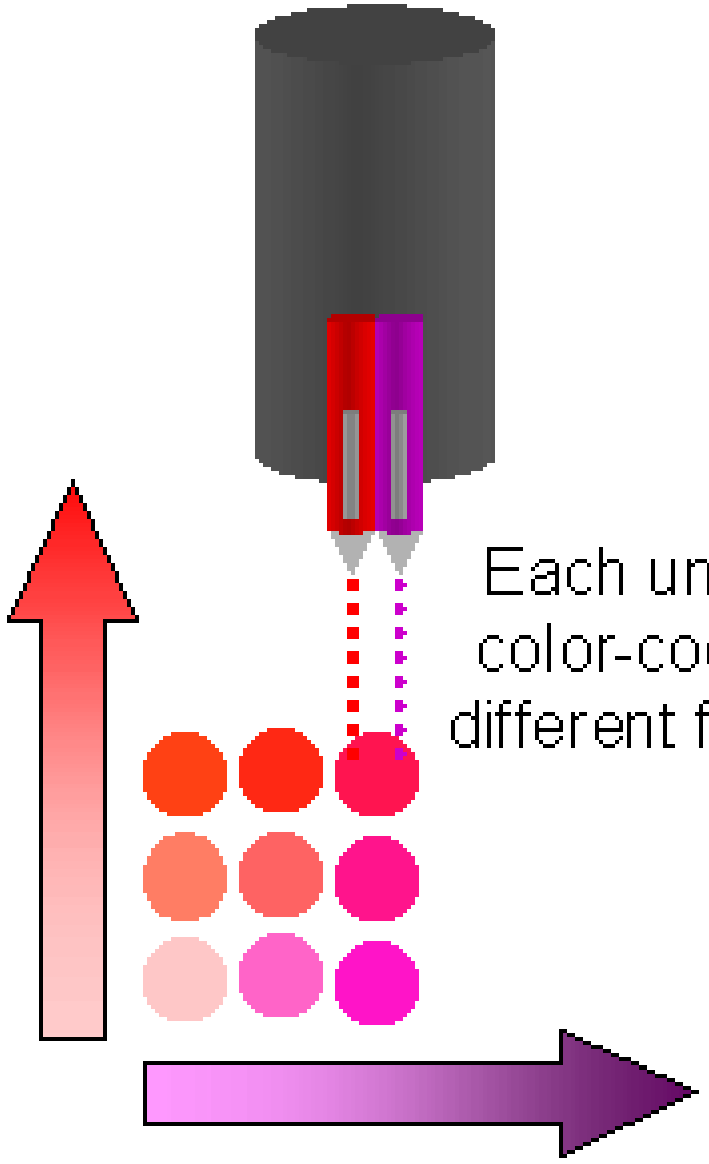


Purified Antigen
Coated Beads




Luminex100
Acquisition
DELL

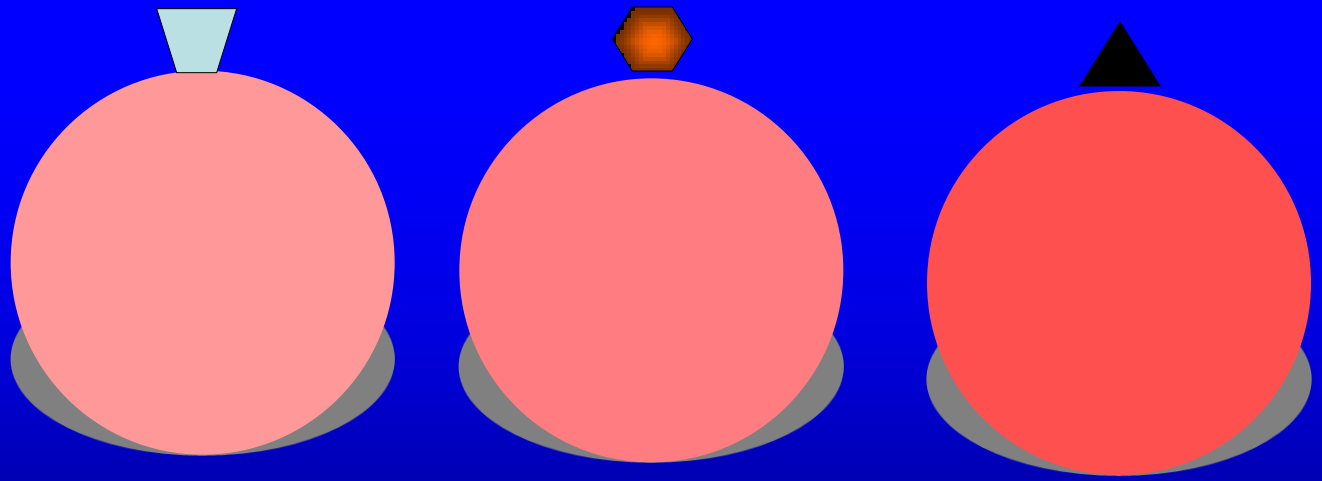
Each Color-code Is A Precise Blend of Two Colors



Each unique microsphere is color-coded using a blend of different fluorescent intensities of two dyes.

Principle of LABScreen Technology

Substrate

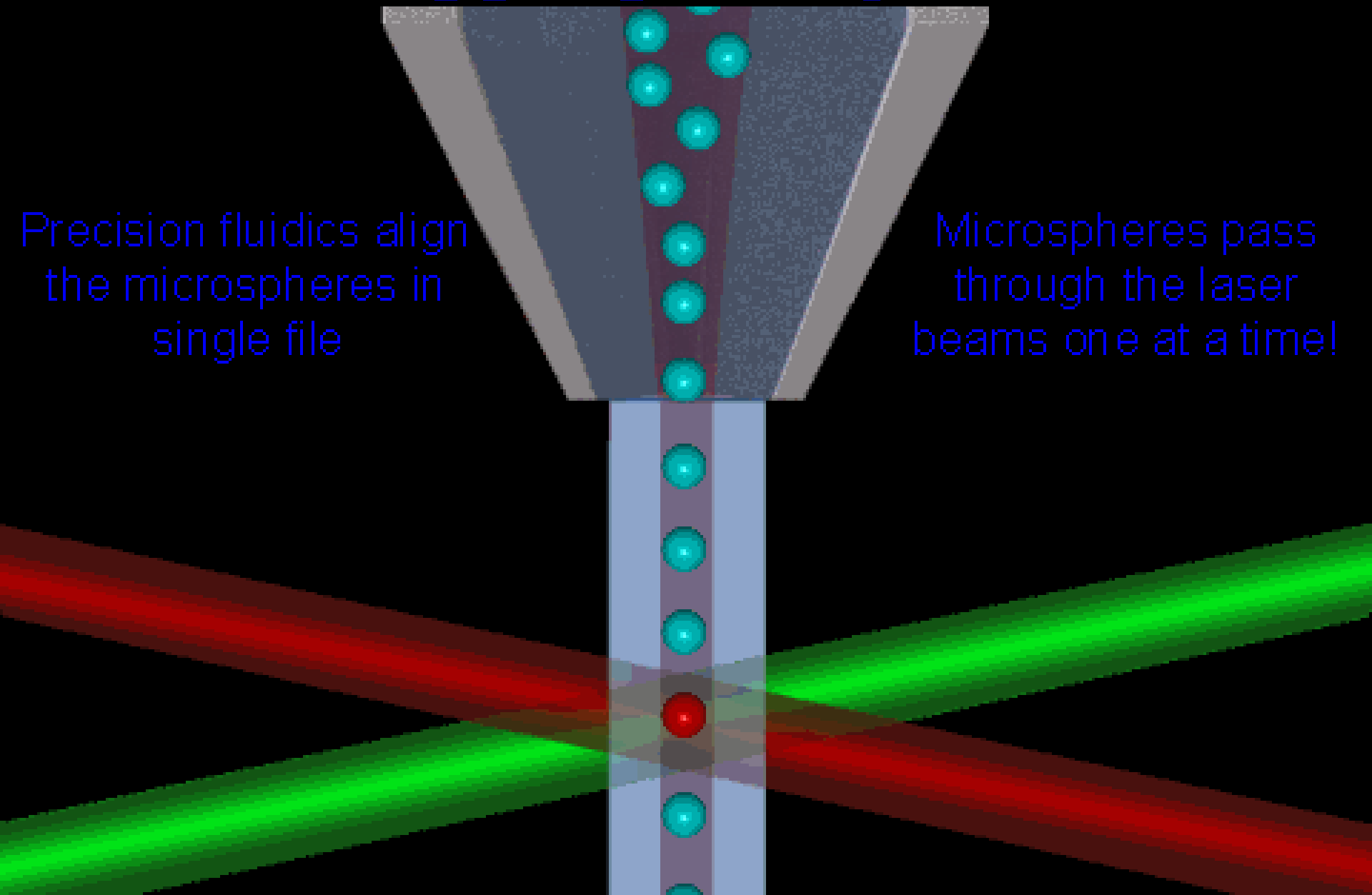


Dual-colored bead

Color-coded Measurements

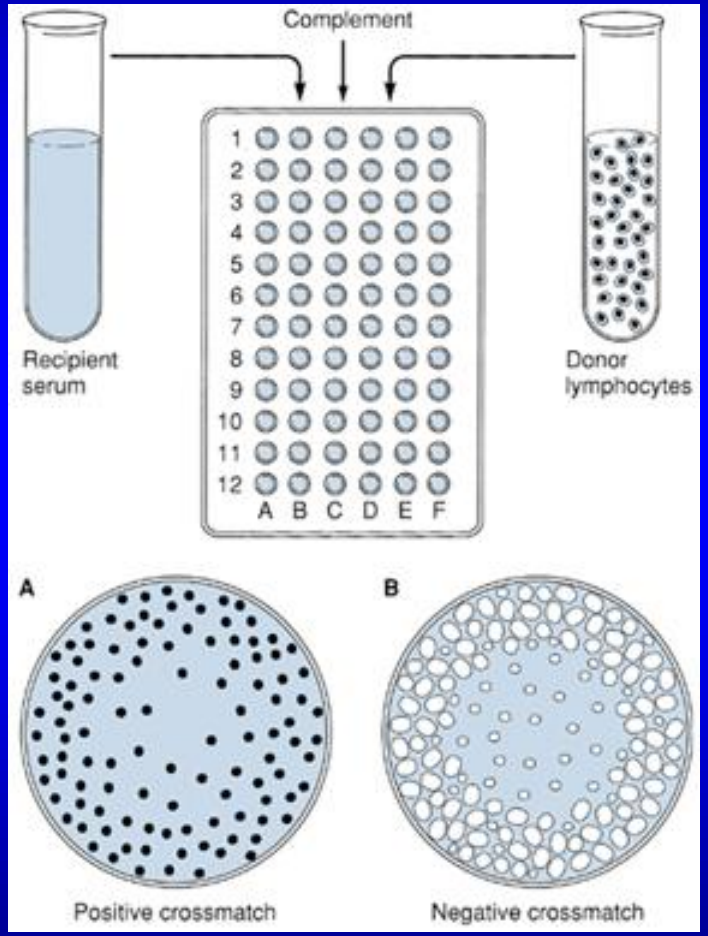
Luminex measures all-in-one!

Digging Deeper:



Cross-Matching

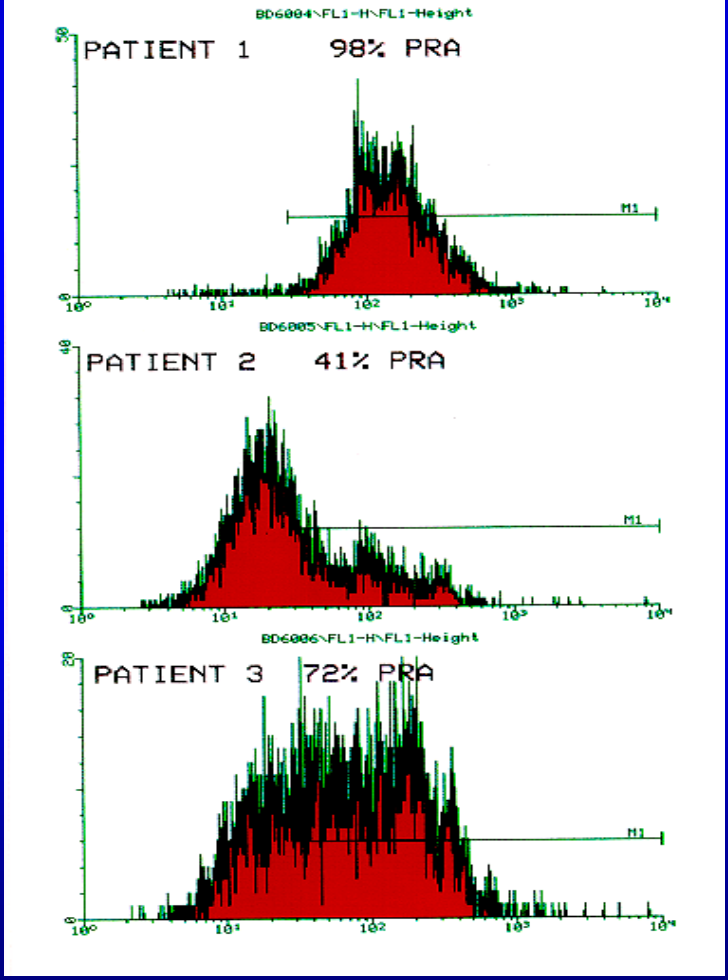
- NIH Microlymphocytotoxicity
- Amos wash
- Extended incubation NIH
- 3-wash
- Antiglobulin
- Flow cytometry

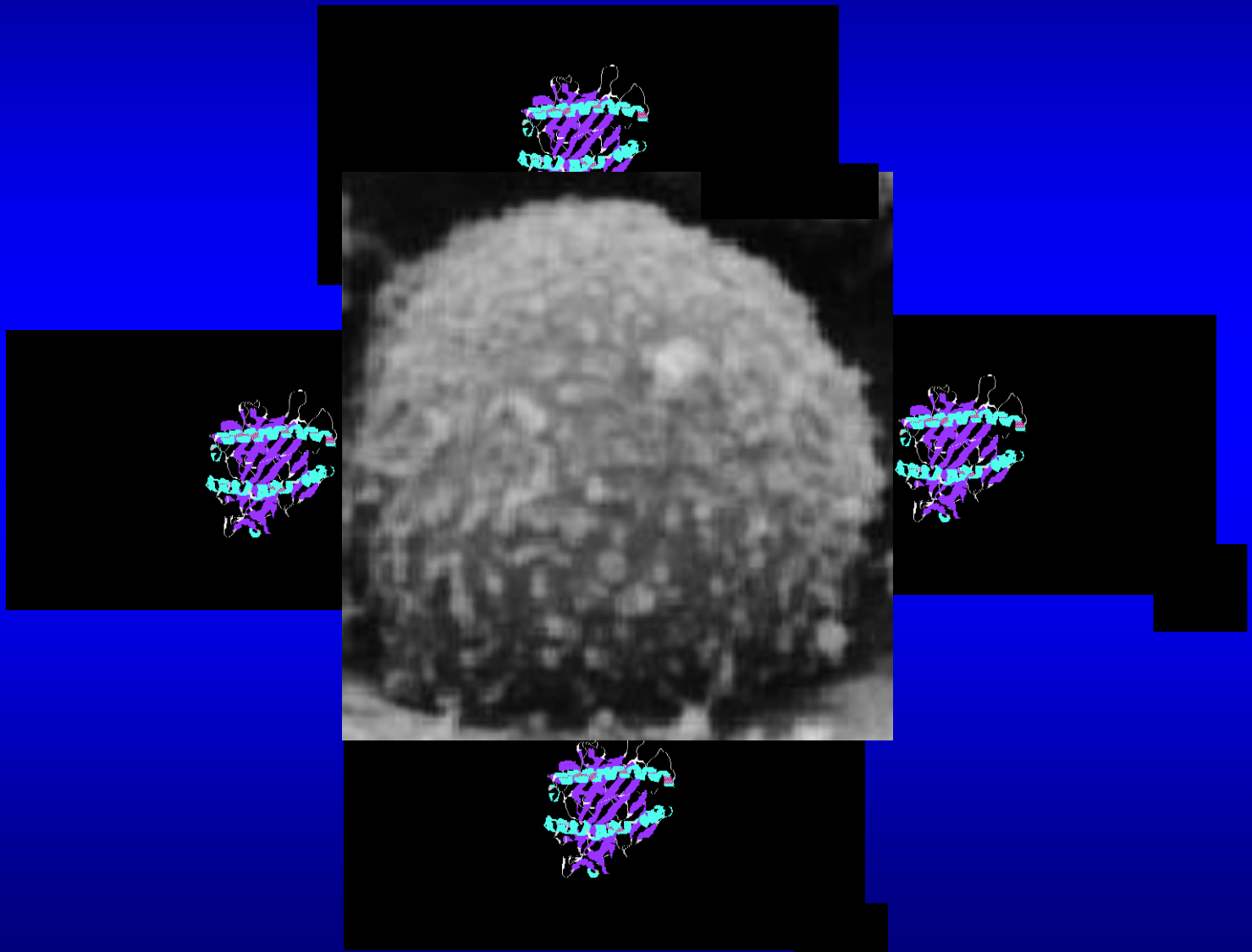


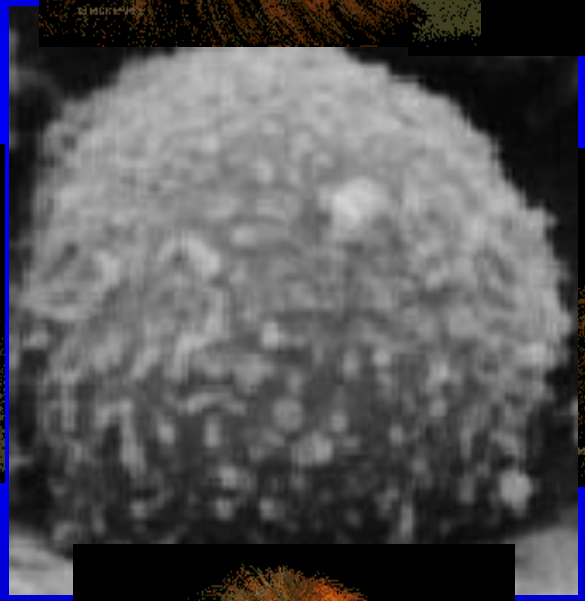
FlowPRA™ Screening Test

Data Analysis

% PRA is represented by the percentage of events shifted to the right of the cut-off point

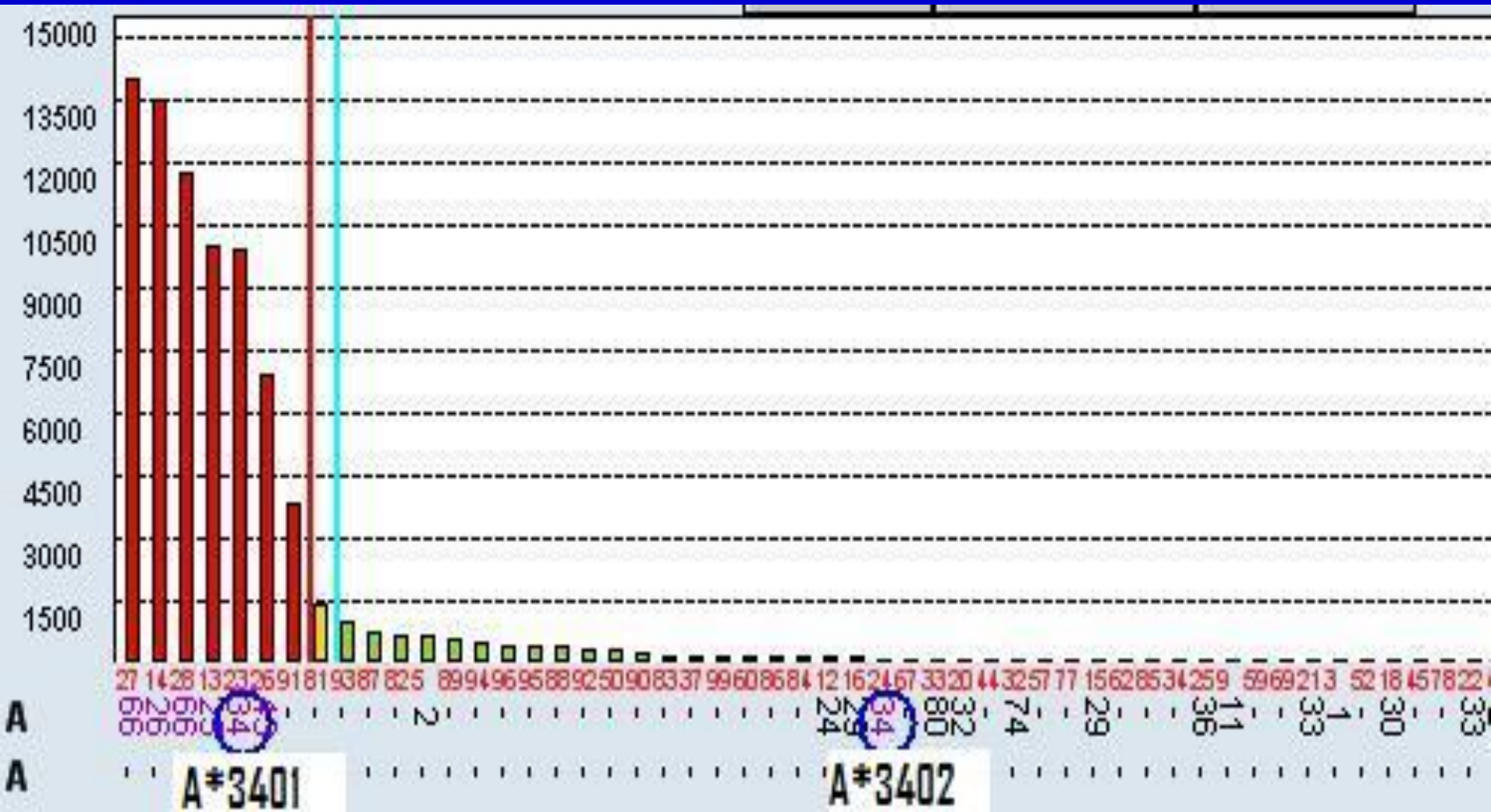






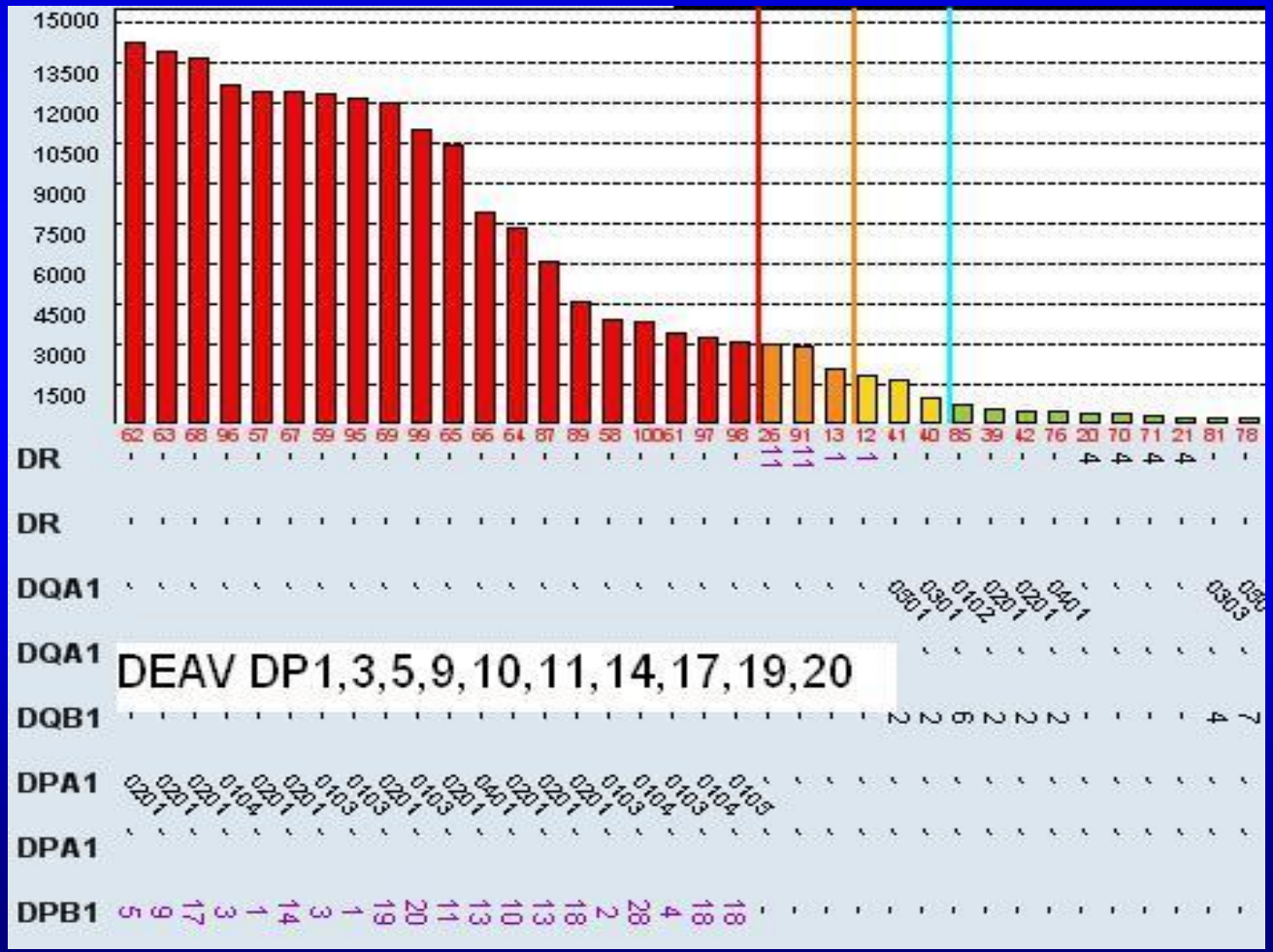
The cell surface is a jungle!

70% of labs called A34

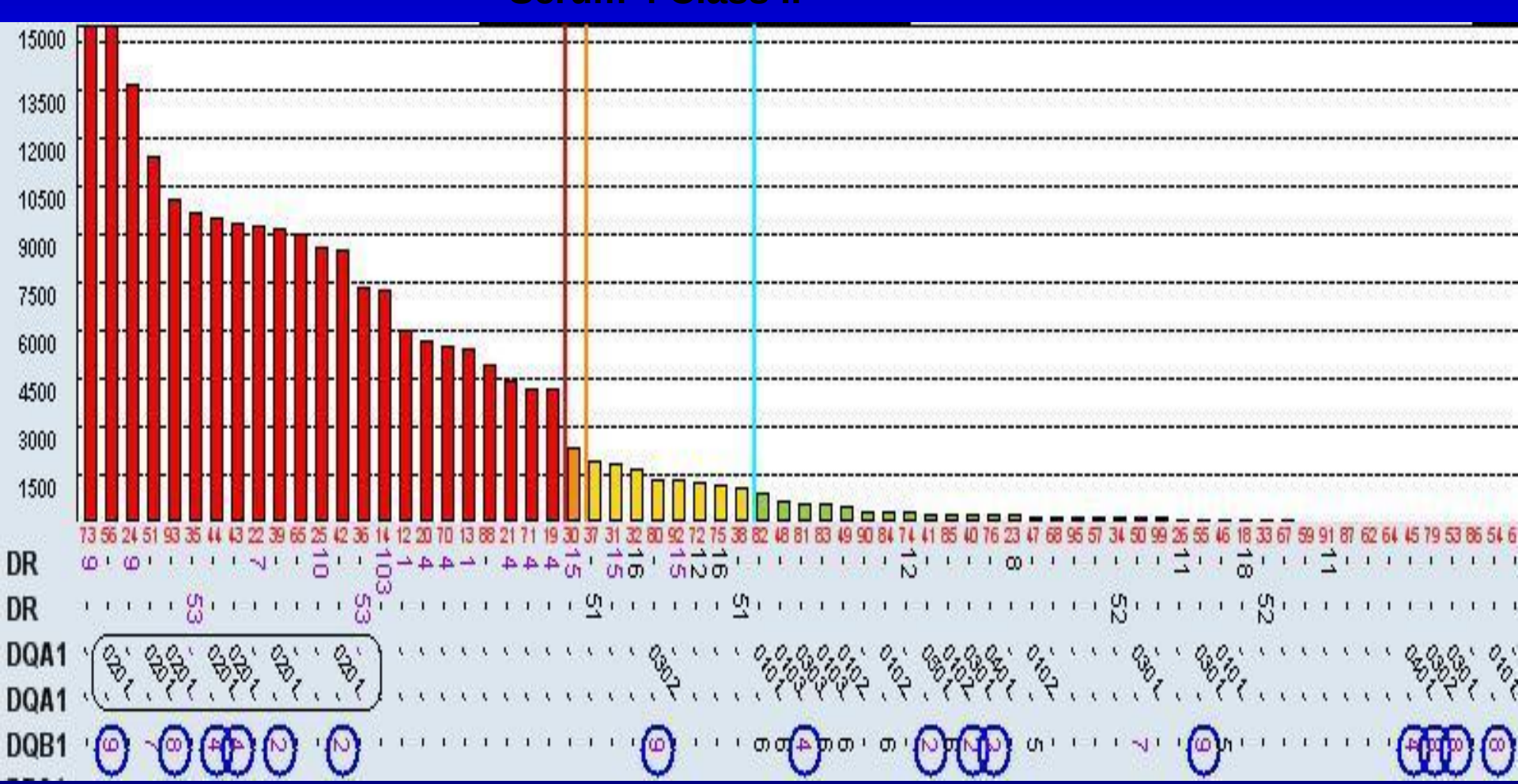


Several labs commented on antibody to epitope DP DEAV 85-87

Serum 5 Class II



More labs reported DQA1*0201 (66%) than the highest DQB1 specificity (DQ9 at 34%)



Cadaver Donor Virtual Crossmatch Worksheet

Cadaver Donor Virtual Crossmatch Worksheet

Donor ID: Donor Phenotype: Tech: Date:

Last Name	First Name	Date of Birth	Sample Date of most recent Luminex	Antibodies as detected by Luminex	Unacceptable Antigens Listed in UNOS	Notes	Mismatched Antigens	Date updated and Tech. Initials	Current Serum Date	Mis Match

Cadaver Donor Virtual Crossmatch Worksheet

Donor ID: YHX261 **Donor Phenotype: A30, 31; B8, 13; Cw6, 7; DR7, 17; DR52, 53; DQ2, -** **Tech: Lauren** **Date: 8-24-11**

Last Name	First Name	Date of Birth	Sample Date of most recent Luminex	Antibodies as detected by Luminex	Unacceptable Antigens Listed in UNOS	Notes	Mismatched Antigens	Date updated and Tech. Initials	Current Serum Date	Mis Match
SLU				44%						
SLU				0%						
SLU				55%						
SLU				0%						
SLU				0%						
Barnes		12/17/1944	1/30/2011	Cw1,12,15	B37; Cw1,12,15		no prev. tx	2/14/11paw		
SLU				77%						
SLU				72%						
Barnes		11/26/1945	6/1/2011	n	DR51	**NOT IN UNOS:** 12/10/09 luminex identified DRB3*0202 (pt is DR52); 11/01/2010 Luminex identified DRB1*1301 (Pt is DR13) and DP11	no prev. tx	7/5/11 jj		
SLU				0%						
SLU				0%						
SLU				0%						
Barnes		1/1/1949	4/4/2011	B45, 76, DP11	B45, 76, DR1, DR51, DQ4	**NOT in UNOS**: DP11; DRB3*0202 - patient is DR52 (identified by Luminex 12-28-09)	no prev. tx	4/20/11 lw		
SLU				0%						
Barnes		1/3/1947	7/22/2011	A2, 28(68, 69); B17(57, 58); DRB1*04:04; DQB1*03:02/DQA1*02:01; DQB1*03.02/DQA1*03:01	A2, A28, A68, A69, B17, B55, B57, B58, DQ8, DR4		no prev. tx	7/27/11 jj		
Barnes		9/4/1937	3/2/2011	A36,DQA1*0503,DQA1*0601(assoc. with DQ7),DQA1*0501,DQA1*0401(assoc. with DQ2): PT is DQ2	A36,DQ7	**DQ2 is not in unos: pt is DQ2**	no prev. tx	03/28/2011hsp		

ALTERNATIVES TO LIVING DONATION

- ABO INCOMPATIBLE TRANSPLANTS
- DESENSITIZATION WITH IVIG/PLASMAPHERESIS FOR THE HIGHLY SENSITIZED PATIENTS
- PAIRED KIDNEY EXCHANGE PROGRAMS

BRAIN TRANSPLANTATION

WHO IS THE RECIPIENT AND WHO IS THE DONOR?