BE THE MATCH

CREATING TOMORROW'S SOLUTIONS, TOGETHER
PREPARING THE TRANSPLANT COMMUNITY TO SERVE EVERY PATIENT IN NEED.
2012 COUNCIL MEETING

The ABCs of HLA / Friending HLA

Carolyn Hurley, PhD

Financial Disclosure: None

Learning Objectives

- Describe HLA nomenclature, typing methods and matching
- Utilize HLA as seen in Traxis, on laboratory reports and how it is matched

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Friending HLA

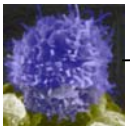
NMDP Council meeting, Fall 2012
Carolyn Hurley

Outline of Topics

- HLA genes, alleles & inheritance
- HLA names
- Resolution of testing
- P and G names for HLA
- How does HLA typing work?
- Understanding search & typing reports

HLA – Genes, Alleles, & Inheritance

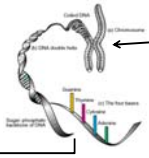
From Cell to DNA Sequence



Human cell

23 pairs chromosome carry genetic information

1 from mom
1 from dad

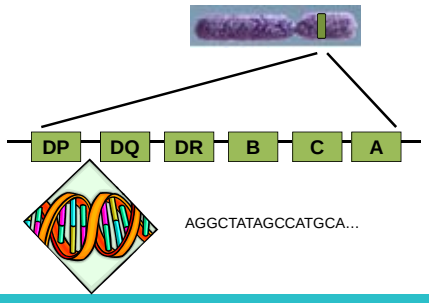


Chromosome--Unit of Inheritance

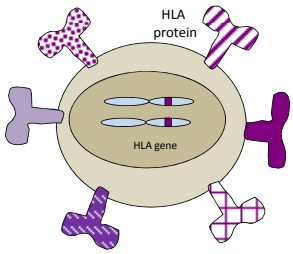
CAGGAGCAGAGGGGTCAGGGCGAAGTCCC
AGGGCCCCAGGCGTGGCTCTCAGGGTCTCA
GAGCCCCGAAGGTCGGTGTATGGATTGGGG
AGTCCCAGCCTTGGGGATTCCCCAACTCCG
CAGCCTCTCCCAACGGTCCTTCATCCTGGAT
ACTCACGACGCGGACCCAGTTCTCACTCCC
ATTGGGTGTCGGGTTTCCAGAGATAAGTGTC
GTCGCGGTCGCTGTTCTAAAGTCCGCACGC
ACCCTAGGGACTCAGGCAAGAGCCACTCTC

"Recipes" for proteins

Chromosome 6 Carries Genes for the Six HLA Proteins



Genes Specify HLA Proteins Found on the Surfaces of Cells in Body



6 HLA proteins

HLA-A HLA-DR
HLA-B HLA-DQ
HLA-C HLA-DP

HLA proteins help immune system recognize pathogens

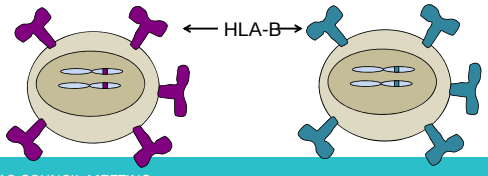
The "Recipes" for an HLA Protein Vary—Alleles Are Variants of a Gene

Allele 2

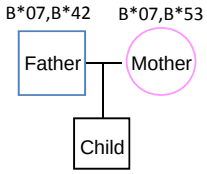
CAGGAGCAGAGGGGTCAGGGCGA
AGTCCCAGGGCCCCAGGCGTGGC
TCTCAGGGTCTCAGAGCCCCGAA
GGTCGGTGTATGGATTGGGGAGTC
CCAGCCTTGGGGATTCCCCAACTC
CGCAGCCTCTCCCAACGGTCCTTC
ATCCTGGATACTCACGACGCGGAC
CCAGTTCTCACTCCCATTGGGTGT

Allele 4

CAGGAGCAGAGGGGTCAGGGCGA
AGTCCCAGGGCCCCACGCGTGGC
TCTCAGGGTCTCAGAGCCCCGAA
GGTCGGTGTATGGATTGGGGAGTC
CCAGCGATGGGGATTCCCCAACTC
CGCAGCCTCTCCCAACGGTCCTTC
ATCCTGGATACTCACGACGCGGAC
CCAGTTCTCACTCCAATTGGGTGT



One Copy of Each HLA Gene (i.e. One Allele) Inherited from Each Parent



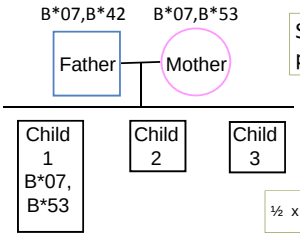
Heterozygous **genotypes** possible

B*07,B*53
B*42,B*07
B*42,B*53

Homozygous **genotype** possible

B*07,B*07

Each Sib Has 25% Chance of Inheriting Same 2 HLA Alleles as Child 1

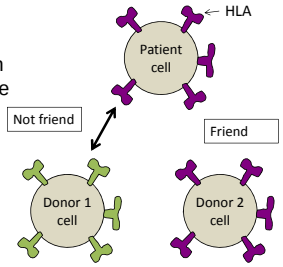


Same alleles = perfect match

½ x ½ = ¼ or 25%

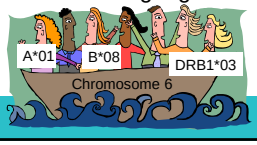
HLA in Transplantation

- HLA = major histocompatibility molecules
- Goal is to select a donor with the same HLA proteins as the patient
- To prevent/reduce immune response to foreign HLA
- To restore immune function



Why Do We Type the Family of a Patient ?

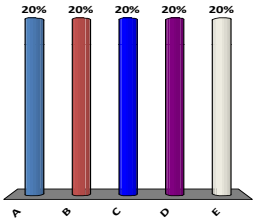
- To find a matched sibling; each sib has 25% chance to match
- Confirm HLA assignments of patient by comparing them with others in family who have inherited the same assignments
- Identify "haplotype" of patient i.e. what alleles of HLA-A/B/C/DR/DQ/DP are traveling together on a chromosome



Test Your Knowledge

Two siblings are HLA-typed as follows:
Sib 1: A*01, A*68 Sib 2: A*02, A*24
Can this happen? If so, what might be the HLA types of their parents?

a) Can't happen, not sibs
b) Parent 1--A*01,*24 and Parent 2--A*02,*68
c) Parent 1--A*01,*02 and Parent 2--A*68,*24
d) B and C can both be right
e) Can't tell, need more info.



Introducing Ourselves – HLA Names

HLA Gene Names

- HLA-A
- HLA-B
- HLA-C
- HLA-DRA, HLA-DRB1
- HLA-DQA1, HLA-DQB1
- HLA-DPA1, HLA-DPB1

Not usually typed

HLA Gene Sequence Defines an Allele

```
                10        20        30        40        50        60        70        80        90       100
|ATGGCCGTCA TGGCGCCCCG AACCCTCCTC CTGCTACTCT CGGGGGCCCT GGCCCTGACC CAGACCTGGG CGG|GTGAGTG CGGGGTCGGG AGGGAAACCG

               110       120       130       140       150       160       170       180       190       200
CCTCTGCGGG GAGAAGCAAG GGGCCCTCCT GGCGGGGGCG CAGGACCGGG GGAGCCGCGC CGGGAGGAGG GTCGGGCAGG TCTCAGCCAC TGCTCGCCCC

               210       220       230       240       250       260       270       280       290       300
CAG|GCTCCCA CTCCATGAGG TATTTCTTCA CATCCGTGTC CCGGCCCGGC CGCGGGGAGC CCCGCTTCAT CGCCGTGGGC TACGTGGACG ACACGCAGTT

               310       320       330       340       350       360       370       380       390       400
CGTGCGGTTC GACAGCGACG CCGCGAGCCA GAAGATGGAG CCGCGGGCGC CGTGGATAGA GCAGGAGGGG CCGGAGTATT GGGACCAGGA GACACGGAAT

               410       420       430       440       450       460       470       480       490       500
ATGAAGGCCC ACTCACAGAC TGACCGAGCG AACCTGGGGA CCCTGCGCGG CTACTACAAC CAGAGCGAGG ACG|GTGAGTG ACCCCGGCCC GGGGCGCAGG

               510       520       530       540       550       560       570       580       590       600
TCACGACCCC TCATCCCCCA CGGACGGGCC AGGTCGCCCA CAGTCTCCGG GTCCGAGATC CACCCCGAAG CCGCGGGACT CCGAGACCCT TGTCCCGGGA

               610       620       630       640       650       660       670       680       690       700
GAGGCCCAGG CGCCTTTACC CGGTTTCATT TTCAGTTTAG GCCAAAAATC CCCCCGGGTT GGTCGGGGCG GGGCGGGGCT CGGGGGACTG GGCTGACCGC

               710       720       730       740       750       760       770       780       790       800
GGGGTCGGGG CCAG|GTTCTC ACACCATCCA GATAATGTAT GGCTGCGACG TGGGGCCGGA CGGGCGCTTC CTCCGCGGGT ACCGGCAGGA CGCCTACGAC

               810       820       830       840       850       860       870       880       890       900
GGCAAGGATT ACATCGCCCT GAACGAGGAC CTGCGCTCTT GGACCGCGGC GGACATGGCA GCTCAGATCA CCAAGCGCAA GTGGGAGGCG GTCCATGCGG

               910       920       930       940       950       960       970       980       990
CGGAGCAGCG GAGAGTCTAC CTGGAGGGCC GGTGCGTGGA CGGGCTCCGC AGATACCTGG AGAACGGGAA GGAGACGCTG CAGCGCACGG |GTACCA[...]
              2910      2920      2930      2940      2950      2960      2970      2980      2990      3000
TGA|GACAGCT GCCTTGTGTG GGACTGAGAG GCAAGAGTTG TTCCTGCCCT TCCCTTTGTG ACTTGAAGAA CCCTGACTTT GTTTCTGCAA AGGCACCTGC
```

= HLA-A*01:01:01:01

Allele Names Have From 3 to 5 Parts
A*01:01:01:01

1	Gene *	HLA-A*
2	Family :	01:
3	Order discovered :	01:
4	Silent variation in exons :	01:
5	Variation outside gene	01

Most important part of name

Different numbers = different protein sequences = non-self

Some Alleles Have a Letter in Their Name
B*15:01:01:02N

1	Gene *	HLA-B*
2	Family :	15:
3	Order discovered :	01:
4	Silent variation in exons :	01:
5	Variation outside gene	02N

Longer names important if they include letter;
N = Not expressed as a protein (null)

Matching at Protein Level Most Important

Not match
- Different DNA & protein
  - HLA-B*08:01:01
  - HLA-B*07:02:01

Match
- Different DNA, same protein
  - HLA-B*08:01:01
  - HLA-B*08:01:02

Not match
- Different DNA, different at protein level, one not expressed
  - HLA-B*15:01:01:01
  - HLA-B*15:01:01:02N

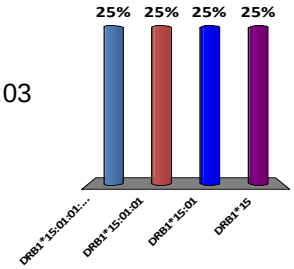
Shorter Allele Names

- B*07 includes over 130 alleles whose names begin with B*07
- B*07:02 includes 20 alleles (e.g. B*07:02:01, B*07:02:02, B*07:02:03, …)
- http://hla.alleles.org is the official website for HLA names

Test Your Knowledge

Which part of the HLA name is the most important for determining a "match" for DRB1*15:01:01:03 ?

a) DRB1*15:01:01:03
b) DRB1*15:01:01
c) DRB1*15:01
d) DRB1*15



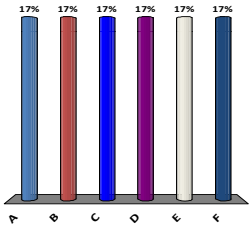
Test Your Knowledge

Which donors are potentially matched to a patient typed as DRB1*15:01:01:03 ?

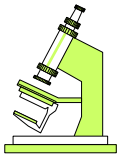
Potentially means possibly expressing the same HLA protein (product of an allele)

Hint: HLA assignments without the * are serologic types

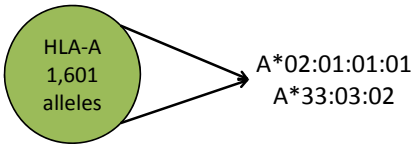
a) DRB1*15:01:01
b) DRB1*15:01
c) DRB1*15:02
d) DR15
e) A, B, & D but not C
f) All of the above



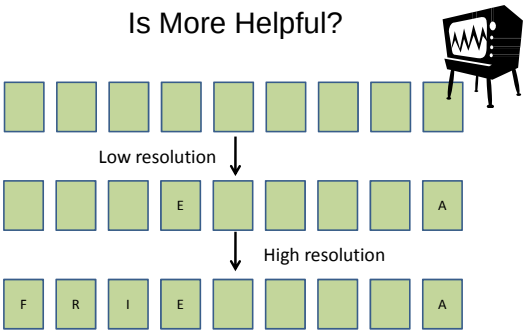
Resolution of Testing



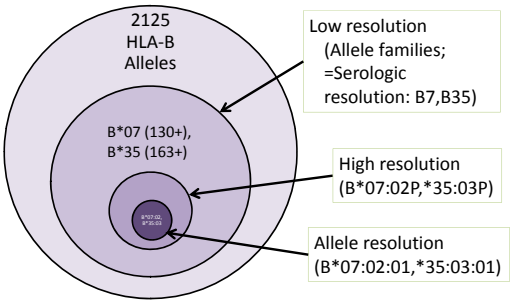
- Definition: ability to identify specific alleles, ability to distinguish alleles from one another by testing



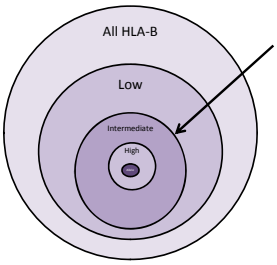
Wheel of Fortune—What Resolution Is More Helpful?



Narrowing Down the HLA Alleles (Typing Resolution)



Narrowing Down the HLA Alleles (Typing Resolution)



<u>Intermediate</u>

- No standard meaning, lab defined
- NMDP allele codes
- B*07:02:01G, *35:03:01G

NMDP Multi-Allele Codes

2 or More Letters Following First Digit Field

Assignment	Alleles included
A*02:BERE	A*02:01, A*02:09, A*02:66, A*02:75
B*13:DS	B*13:01, B*13:02, B*13:03, B*13:04, B*13:08
DRB1*04:AUX	DRB1*04:01, DRB1*04:24

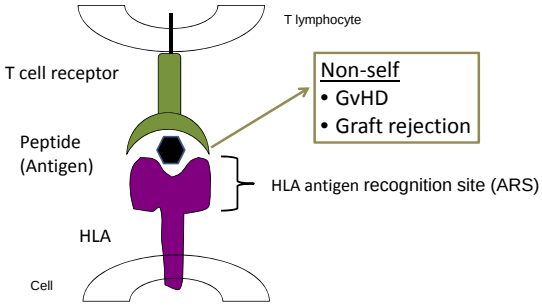
- Random set letters, must look up to identify alleles included
  - http://bioinformatics.nmdp.org/HLA/Allele_Codes/DNA_Type_Lookup
- Assigned by NMDP upon request, used by registries world-wide

http://en.wikipedia.org

Group Names
Herd of cows
Flock of birds
Pride of lions
Colony of ants
School of fish
Pack of dogs
Pod of dolphins
Mob of emus
Gaggle of geese
Bloat of hippos
Smack of jellyfish

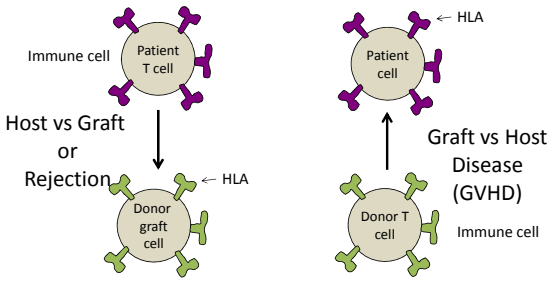
A Plague of Alleles: Ps & Gs

Defining Important Region



Non-self
- GvHD
- Graft rejection

Immune Responses Involving Graft and Recipient (Host)



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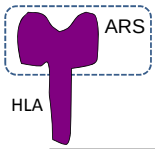
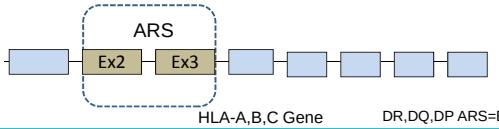
Minding Our Ps & Gs



P group: Same protein sequence in ARS
A*02:07P = A*02:07:01, 02:07:02, 02:07:03, 02:265

G group: Same DNA sequence in ARS exons
A*02:07:01G = A*02:07:01, 02:07:02, 02:15N, 02:265



HLA-A,B,C Gene

DR,DQ,DP ARS=Ex2 only

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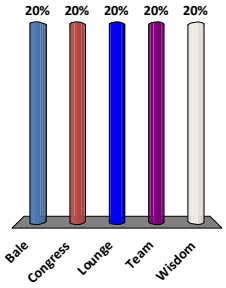
Testing Your Knowledge

What is a collection of wombats called?

a) Bale
b) Congress
c) Lounge
d) Team
e) Wisdom

http://www.wombats.net

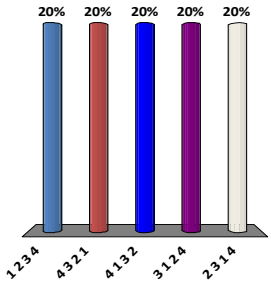


Testing Your Knowledge

Rank these HLA types from low to high resolution ..

1. DRB1*13
2. DRB1*13:01:03
3. DRB1*13: AB
4. DR6

a) 1 2 3 4
b) 4 3 2 1
c) 4 1 3 2
d) 3 1 2 4
e) 2 3 1 4



How Does HLA Typing Work?

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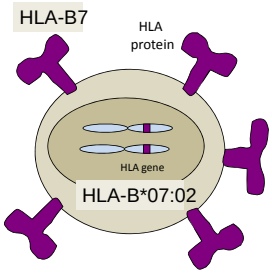
Detection of HLA "Types"



Proteins on cell surface

- Typing method: Serology – antibody assay

Genes/DNA

- Typing method: Molecular biology-DNA assay

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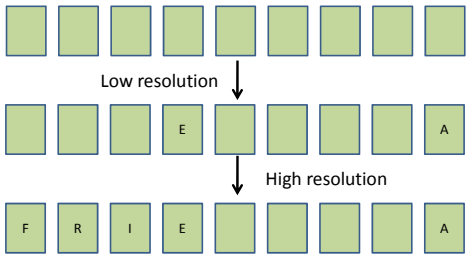
Wheel of Fortune—What HLA Allele(s) Does This Person Have ?



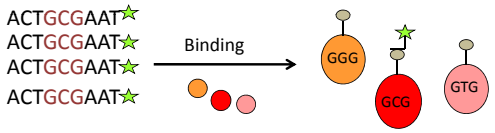
Detecting Presence / Absence of Specific Short DNA Sequences

DNA from blood or buccal swab

ACTGCGAAT

SSOP* assay

Amplify HLA gene (PCR**)



Label

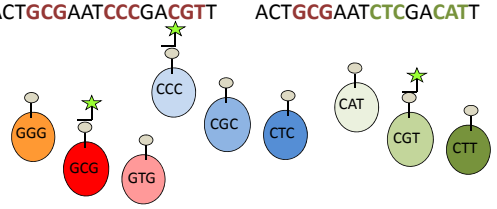
*Sequence specific oligonucleotide probe (synthetic DNA)
**Polymerase chain reaction

Testing Your Knowledge What Allele Is Present?

Answer: a

Allele 1:
ACTGCGAATCCCGACGTT

Allele 2:
ACTGCGAATCTCGACATT



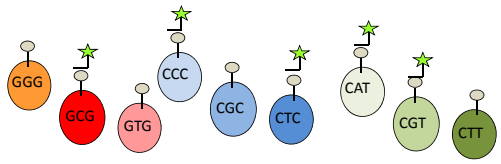
a) Allele 1 only, b) Allele 2 only, c) Alleles 1+2

Usually 2 Out of Many Alleles Are Present

(2,125 alleles for HLA-B)

Allele 1:
ACTGCGAATCCCGACGTT

Allele 2:
ACTGCGAATCTCGACATT



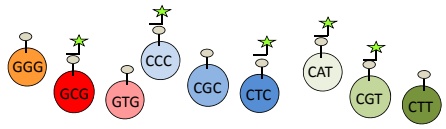
A Typing with More Than One Interpretation

Allele 1:
ACTGCGAATCCCGACGTT

Allele 2:
ACTGCGAATCTCGACATT

Allele 7:
ACTGCGAATCTCGACGTT

Allele 15:
ACTGCGAATCCCGACATT



Alleles 1,2 OR Alleles 7,15 (alternative genotypes)

Allele Codes for Alternative Genotypes

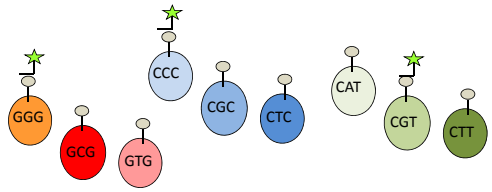
- Expect 2 alleles for each gene
- Depending on typing methods, there may be more than 1 possible genotype
  - A*03:01, A*11:01 Or
  - A*03:02, A*11:04 Or
  - A*03:04, A*11:07
- Assign NMDP allele code to shorten this result for reporting
  - A*03:MS, A*11:MW
- On search report, potential donors usually have many alternative genotypes

Testing Your Knowledge
What Allele Is Present?

Answer: d

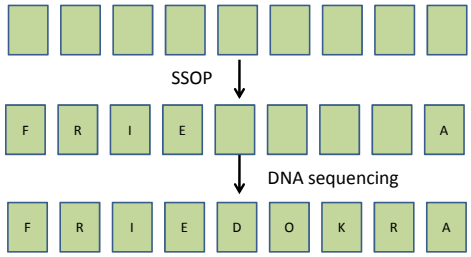
Allele 1:
ACTGCGAATCCCGACGTT

Allele 2:
ACTGCGAATCTCGACATT

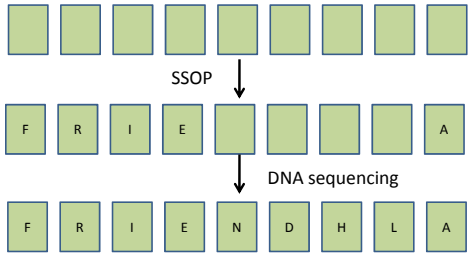


a) Allele 1 only, b) Allele 2 only, c) Alleles 1+2, d) omg omg omg

DNA Sequencing for HLA Typing



DNA Sequencing for HLA Typing



Summary Typing Methods

- HLA typing of DNA identifies DNA sequences present and absent to identify what alleles might be present
- Several different assays can be used
  - SSOP (binding of PCR amplified DNA to probes)
  - SSP (pairs of "probes" in PCR assay)
  - DNA sequencing (more complete information on DNA sequence present)
- Each gene is assayed--expect 2 alleles if heterozygous, 1 is homozygous
- Assay may be unable to discriminate between pairs of alleles (genotypes) so alternative assignments

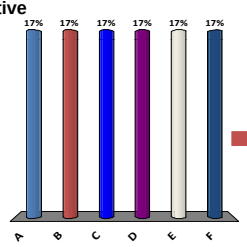
Test Your Knowledge

Alleles

- 1--AGCG**TAA**CGTTA**CAC**AGT
- 2--AGCG**TTT**CGTTA**CGC**AGT
- 3—-AGCG**TTT**CGTTA**CAC**AGT
- 4--AGCG**TAA**CGTTA**CGC**AGT

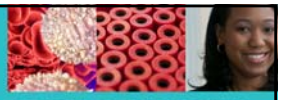
Probes positive

- **TAA +**
- **TTT+**
- **CAC+**
- **CGC+**



What's the HLA genotype?

a) Alleles 1,2
b) Alleles 3,4
c) Alleles 1,3
d) Alleles 2,4
e) Alleles 1,2 or Alleles 3,4
f) Alleles 1,3 or Alleles 2,4



CREATING TOMORROW'S SOLUTIONS, TOGETHER

PREPARING THE TRANSPLANT COMMUNITY TO SERVE EVERY PATIENT IN NEED.

2012 COUNCIL MEETING

Understanding HLA Typing results & Search Reports