NIJ National Institute of Justice *Technology Transition Workshop*

Enhanced Resolution and Statistical Power Through SNP Distributions Within the Short Tandem Repeats

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The STR in Forensic Analysis

- **STRs are the mainstay in Human Identity Testing**
- **Allele is described as a nominal repeat number of a sequence motif**
- **Allele distribution typically provides some fairly common alleles and several rarer alleles**
- **Assumptions concerning STR alleles vary among applications**

STR Markers

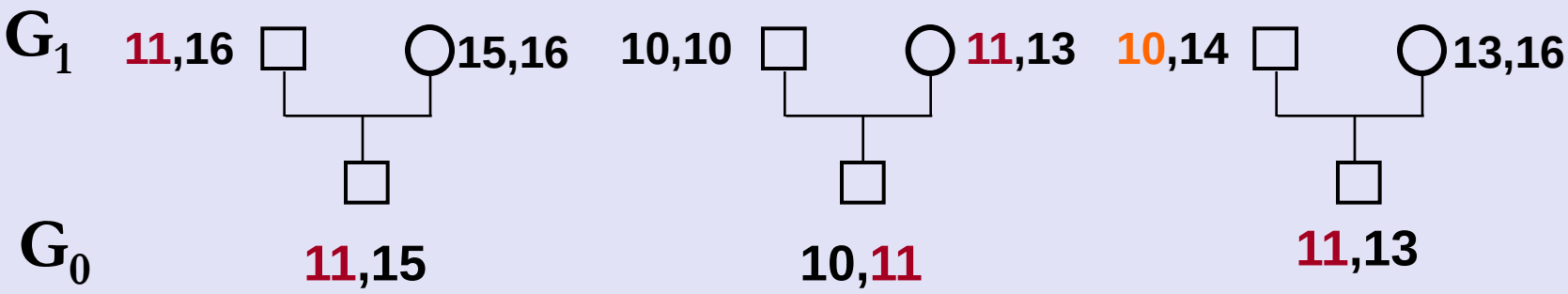
The STR markers we use in routine forensic comparisons are what is considered:

Identical by State

There is no presupposition of ancestral descent attached to them…there doesn't need to be!

STR Markers

History of an 11 Allele



~~Siblings~~ ? Parent-~~Offspring~~ ? Unrelated ?

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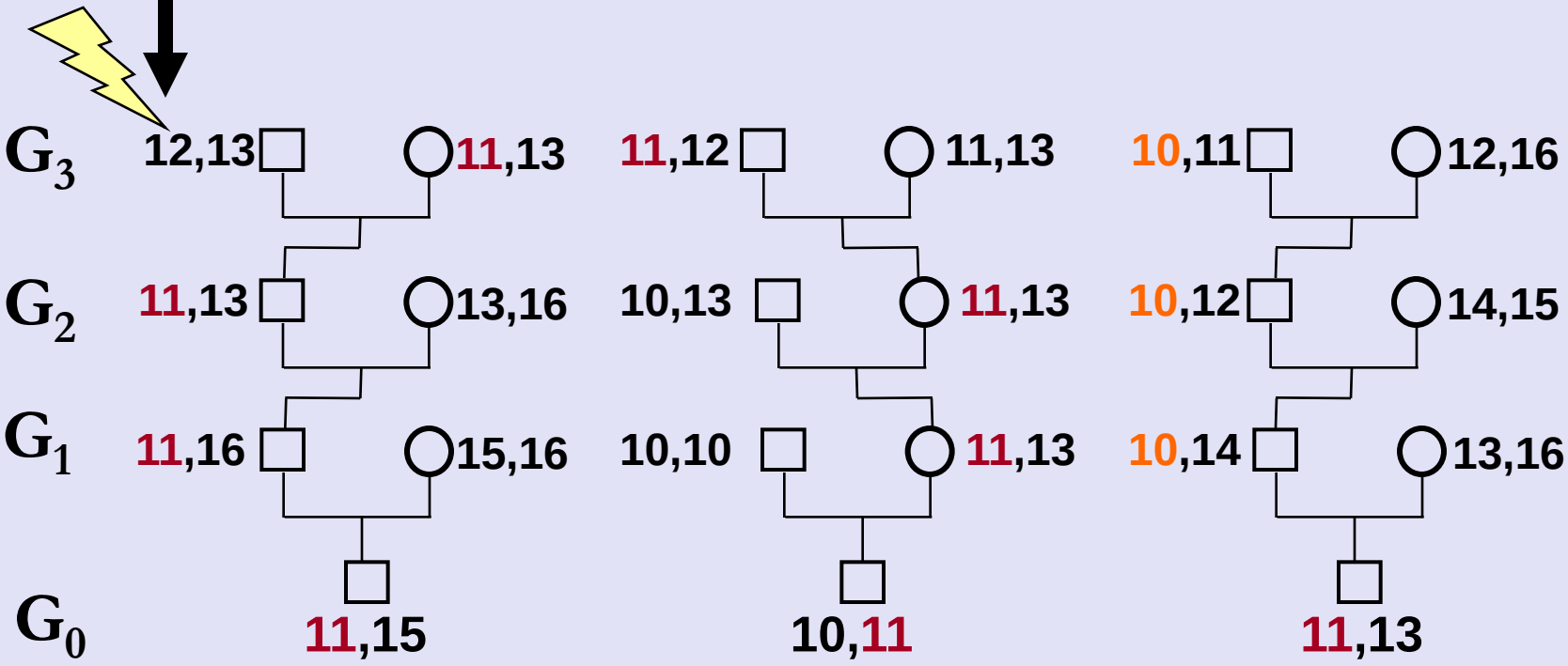
Mutation Process for STR Loci

- **STR loci undergo mutational changes at a frequency of ~ 10^{-3}**
- **The allele changes observed between generations is typically one repeat larger or smaller than the allele observed in the parental generation**
- **This process is analogous to the formation of stutter alleles observed when amplifying STR loci**

STR Markers

History of an 11 Allele



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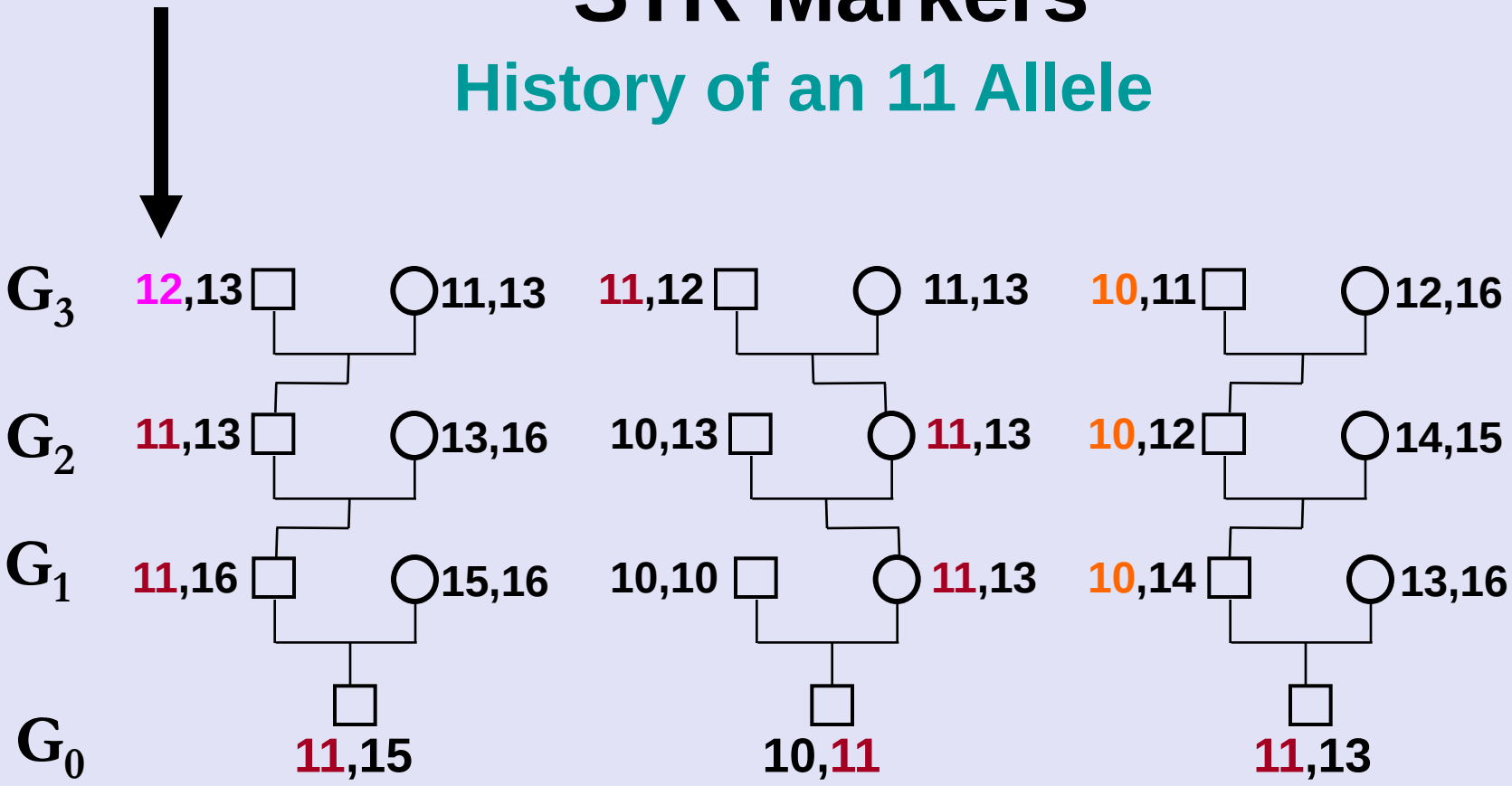
STR Markers

History of an 11 Allele



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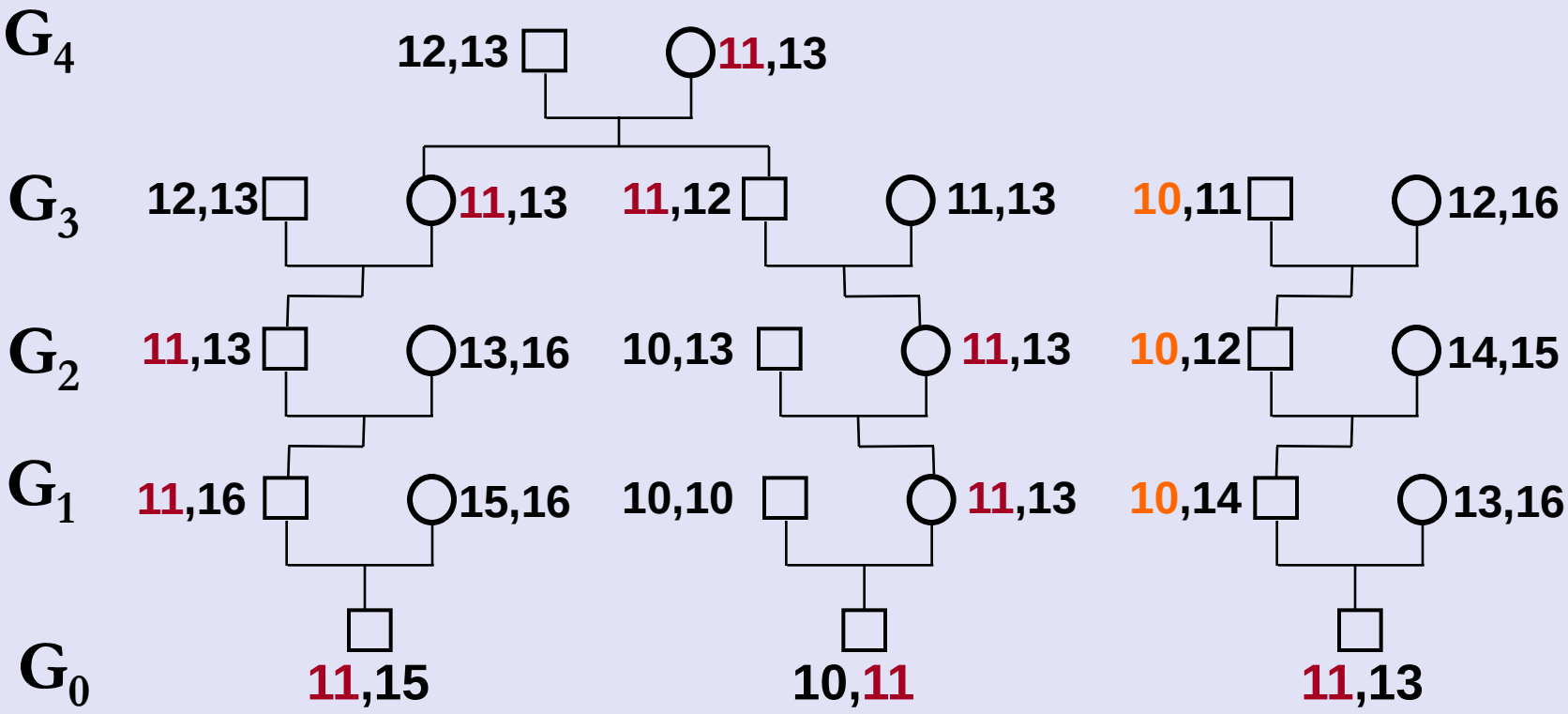
STR Markers

History of an 11 Allele



Third Cousins!

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STR Markers

When comparing alleles between individuals as a result of a database search under the premise of a familial association, we are banking on the shared alleles being

Identical by Descent

This is clearly a fallacy with regard to the way we use autosomal STR markers when conducting an open database search.

Assumptions/Applications

- **Single nucleotide polymorphisms in and around the STR loci are known**
- **Current analytical methods observe some of these polymorphisms as primer binding mutations or microvariants**
- **Categorizing SNP variants would require sequence analysis**
- **Initial thoughts were that these polymorphisms would be relatively rare**

Assumptions/Applications

- **Single nucleotide polymorphisms in and around the STR loci are known**
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- **Categorizing SNP variants wou… sequence analysis**
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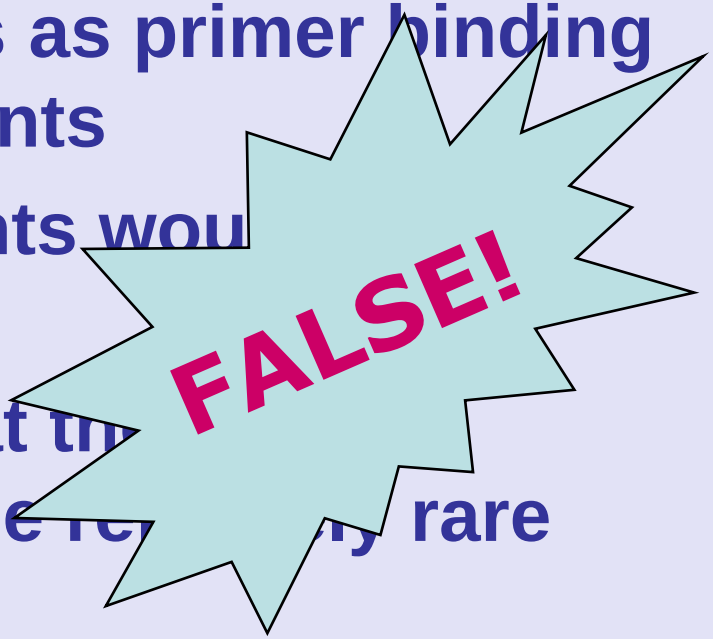

Evaluation of Typing System

- **587 individuals from three US population groups were typed for the 13 CODIS core STR loci**
- **Samples had previously been typed using PowerPlex 16®**
- **Data evaluated for allele distribution as well as Hardy Weinberg Equilibrium and Linkage Disequilibrium**
- **An additional 50 individuals belonging to 2 extensive pedigrees were examined**

SNP Rich STRs

- **Seven of the CODIS core loci contained SNPs within the repeat motifs, which increased the number of commonly observed alleles**

  D3S1358, D5S818, D7S820, D8S1179, D13S317, D21S11, vWA

- **Allele count increased between 4 and 15 alleles dependent on locus and population group**
- **Typing of nominal alleles was concordant with prior PP16® results**

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Locus	Population	STR-SNP Analysis 0n IBIS T5000				STR Only Analysis on IBIS T5000			
		n	Alleles Detected	H_e	H_o	n	Alleles Detected	H_e	H_o
D13S317	Caucasian	181	12	0.8735	0.9061	182	7	0.7820	0.8297
	African Am.	213	12	0.8345	0.7981	214	7	0.7026	0.7056
	Hispanic	193	13	0.8847	0.9016	193	7	0.8241	0.8290
D21S11	Caucasian	181	23	0.8925	0.9006	182	14	0.8390	0.8681
	African Am.	213	33	0.8688	0.8357	214	20	0.8459	0.8178
	Hispanic	193	25	0.8845	0.8446	193	14	0.8335	0.8083
D3S1358	Caucasian	181	18	0.8641	0.8895	182	8	0.7900	0.8077
	African Am.	213	18	0.8907	0.9061	214	8	0.7485	0.7850
	Hispanic	193	18	0.8101	0.8187	193	8	0.7391	0.7409
D5S818	Caucasian	181	15	0.7870	0.8177	182	9	0.6858	0.6703
	African Am.	213	17	0.8396	0.8310	214	9	0.7449	0.7009
	Hispanic	193	13	0.7471	0.7617	193	9	0.6998	0.6891
D7S820	Caucasian	181	15	0.8525	0.8066	182	8	0.8089	0.7857
	African Am.	213	12	0.8117	0.7887	214	8	0.7957	0.7710
	Hispanic	193	14	0.8271	0.7876	193	9	0.7895	0.7358
D8S1179	Caucasian	181	14	0.8554	0.8785	182	10	0.7970	0.8187
	African Am.	213	19	0.8215	0.8122	214	10	0.7860	0.7523
	Hispanic	193	16	0.8581	0.8860	193	9	0.7983	0.8394
vWA	Caucasian	181	22	0.8471	0.8674	182	10	0.8152	0.8022
	African Am.	213	26	0.8882	0.8920	214	11	0.8166	0.8224
	Hispanic	193	16	0.7955	0.7979	193	7	0.7692	0.7876

TABLE COURTESY OF JOHN V. PLANZ, PH.D.

Locus	Population	STR-SNP Analysis 0n IBIS T5000				STR Only Analysis on IBIS T5000			
		n	Alleles Detected	H_e	H_o	n	Alleles Detected	H_e	H_o
D21S11	Caucasian	181	23	0.8925	0.9006	182	14	0.8390	0.8681
	African Am.	213	33	0.8688	0.8357	214	20	0.8459	0.8178
	Hispanic	193	25	0.8845	0.8446	193	14	0.8335	0.8083
D3S1358	Caucasian	181	18	0.8641	0.8895	182	8	0.7900	0.8077
	African Am.	213	18	0.8907	0.9061	214	8	0.7485	0.7850
	Hispanic	193	18	0.8101	0.8187	193	8	0.7391	0.7409
D5S818	Caucasian	181	15	0.7870	0.8177	182	9	0.6858	0.6703
	African Am.	213	17	0.8396	0.8310	214	9	0.7449	0.7009
	Hispanic	193	13	0.7471	0.7617	193	9	0.6998	0.6891

TABLE COURTESY OF JOHN V. PLANZ, PH.D.

- **Additional alleles resulting from SNP variations were not observed in D16S539, TH01, and TPOX**
- **CSF1P0, FGA and D18S51 yielded only 3-5 SNPs among the STR repeats, however these were too low in frequency to substantially affect overall allele distributions**

Locus	Population	STR-SNP Analysis 0n IBIS T5000				STR Only Analysis on IBIS T5000			
		n	Alleles Detected	H_e	H_o	n	Alleles Detected	H_e	H_o
	Caucasian	181	7	0.7334	0.7348	182	7	0.7346	0.7363
CSF1PO	African Am.	213	11	0.7826	0.8028	214	9	0.7807	0.8037
	Hispanic	193	9	0.7242	0.7824	193	9	0.7242	0.7824
	Caucasian	181	7	0.7756	0.7845	182	7	0.7766	0.7802
D16S539	African Am.	213	9	0.8037	0.7653	214	9	0.8025	0.7617
	Hispanic	193	8	0.7769	0.7617	193	7	0.7768	0.7617
	Caucasian	181	7	0.7737	0.7845	182	7	0.7732	0.7857
TH01	African Am.	213	6	0.7363	0.7324	214	6	0.7348	0.7290
	Hispanic	193	6	0.7414	0.7306	193	6	0.7401	0.7306
	Caucasian	181	6	0.6509	0.6575	182	6	0.6524	0.6538
TPOX	African Am.	213	7	0.7692	0.7700	214	7	0.7679	0.7757
	Hispanic	193	7	0.6139	0.6114	193	7	0.6118	0.6062
	Caucasian	181	15	0.8632	0.8729	182	13	0.8625	0.8736
FGA	African Am.	213	27	0.8747	0.8920	214	22	0.8729	0.8879
	Hispanic	193	18	0.8806	0.9016	193	15	0.8784	0.9016
	Caucasian	181	14	0.8788	0.8729	182	13	0.8772	0.8736
D18S51	African Am.	213	23	0.8847	0.9249	214	18	0.8818	0.9206
	Hispanic	193	19	0.8755	0.8860	193	15	0.8710	0.8756

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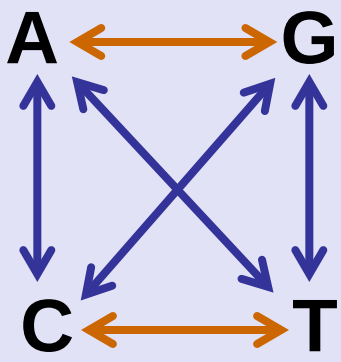
Allele Nomenclature

The inclusion of various SNP possibilities within the nominal repeat structure of the STR alleles required specific nomenclature to allow for unambiguous naming of the alleles for analysis and databasing.

In addition to the nominal allele (based on repeats), a suffix code was added that describes the specific type of polymorphism (Transition or Transversion) and number of SNPs observed in the fragment.



Transitions

Transversions

1 G→A	2 A→G	3 C→T	4 T→C
5 C→G	6 G→C	7 T→G	8 G→T
9 A→T	10 T→A	11 A→C	12 C→A

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12 Allele containing a A→G SNP = 12S2

12 Allele containing a A→G & T→C SNP = 12S2S4

12 Allele containing 2 A→G SNPs = 12S2.2

S1 G→A	S2 A→G	S3 C→T	S4 T→C
S5 C→G	S6 G→C	S7 T→G	S8 G→T
S9 A→T	S10 T→A	S11 A→C	S12 C→A

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Using the mass analysis methodology, allele designations are based on sequence base composition.

Unlike fragment analysis with electrophoresis and allelic ladders, allele designations are made against a reference DNA sequence specified for the locus.

D8S1179 Simple TCTA repeat

Reference Sequence – G08710 (12 Repeats)

```
LOCUS       G08710                  340 bp    DNA     linear   STS 05-FEB-1997
DEFINITION  human STS CHLC.GATA7G07.P6384 clone GATA7G07, sequence tagged site.
ACCESSION   G08710
VERSION     G08710.1  GI:939260
KEYWORDS    STS; STS sequence; primer; sequence tagged site.
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 340)
  AUTHORS   Murray,J., Sheffield,V, Weber,J.L., Duyk,G. and Buetow,K.H.
  TITLE     Cooperative Human Linkage Center
  JOURNAL   Unpublished (1995)
COMMENT     Synonyms: GATA7G07, CHLC.GATA7G07.#T6383
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                     /organism="Homo sapiens"
                     /mol_type="genomic DNA"
                     /db_xref="taxon:9606"
     STS             17..193
     primer_bind     17..41
     primer_bind     complement(169..193)
ORIGIN
        1 tggcaactta tatgtatttt tgtatttcat gtgtacattc gtatctatct atctatctat
       61 ctatctatct atctatctat ctatctatct attccccaca gtgaaaataa tctacaggat
      121 aggtaaataa attaaggcat attcacgcaa tgggatacgn tacagtgatg aaaatgaact
      181 aattatagct acgtgaaact atactcatgn acacaatttg gtaaaagaaa ctgggaacaa
      241 gaatacatac ggtttttgnc agctgtgcta ttttacattc ccaacaacaa tgcacagggt
      301 ttcagnttct ccacatnctt gtcaacattn tgttatttttg
//
```

G08710 REFERENCE SEQUENCE COURTESY OF THE NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION AND THE NATIONAL LIBRARY OF MEDICINE http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?list_uids=939260&db=nuccore&dopt=gb

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ACCESSION   G08710
VERSION     G08710.1  GI:939260
KEYWORDS    STS; STS sequence; primer; sequence tagged site.
SOURCE      Homo sapiens (human)
```

```
   1 tggcaactta tatgtatttt tgtatttcat gtgtacattc gtatctatct atctatctat
  61 ctatctatct atctatctat ctatctatct attccccaca gtgaaaataa tctacaggat
 121 aggtaaataa attaaggcat attcacgcaa tgggatacgn tacagtgatg aaaatgaact
 181 aattatagct acgtgaaact atactcatgn acacaatttg gtaaaagaaa ctgggaacaa
 241 gaatacatac ggtttttgnc agctgtgcta ttttacattc ccaacaacaa tgcacagggt
 301 ttcagnttct ccacatnctt gtcaacattn tgttattttg
```

```
     STS             17..193
     primer_bind     17..41
     primer_bind     complement(169..193)
ORIGIN
        1 tggcaactta tatgtatttt tgtatttcat gtgtacattc gtatctatct atctatctat
       61 ctatctatct atctatctat ctatctatct attccccaca gtgaaaataa tctacaggat
      121 aggtaaataa attaaggcat attcacgcaa tgggatacgn tacagtgatg aaaatgaact
      181 aattatagct acgtgaaact atactcatgn acacaatttg gtaaaagaaa ctgggaacaa
      241 gaatacatac ggtttttgnc agctgtgcta ttttacattc ccaacaacaa tgcacagggt
      301 ttcagnttct ccacatnctt gtcaacattn tgttattttg
//
```

G08710 REFERENCE SEQUENCE COURTESY OF THE NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION AND THE NATIONAL LIBRARY OF MEDICINE http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?list_uids=939260&db=nuccore&dopt=gb

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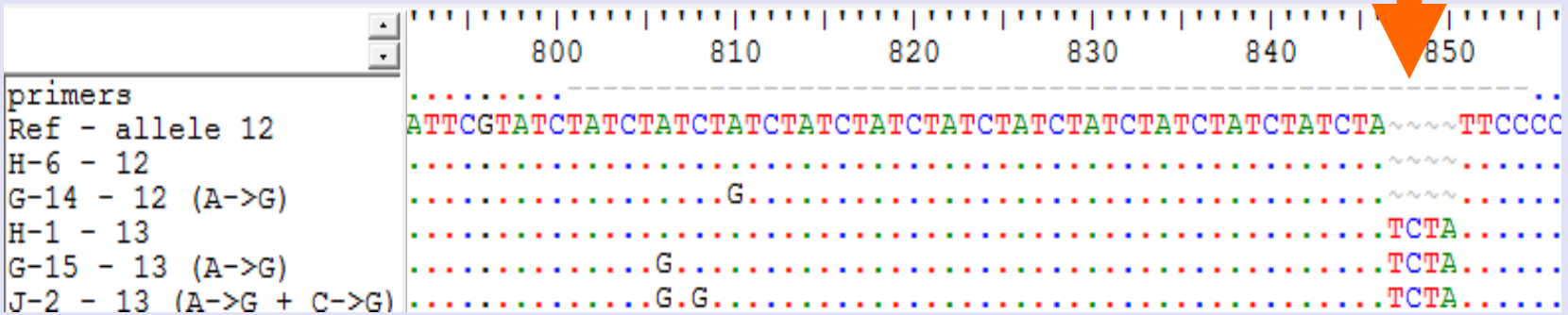
D8S1179

12 Allele represented by reference

12 & 13 Alleles containing various SNPs



Length variations consist of repeat motifs added to the end of reference sequence.

IMAGE COURTESY OF JOHN V. PLANZ, PH.D.

D8S1179

12 Allele containing a A→G SNP = **12S2**

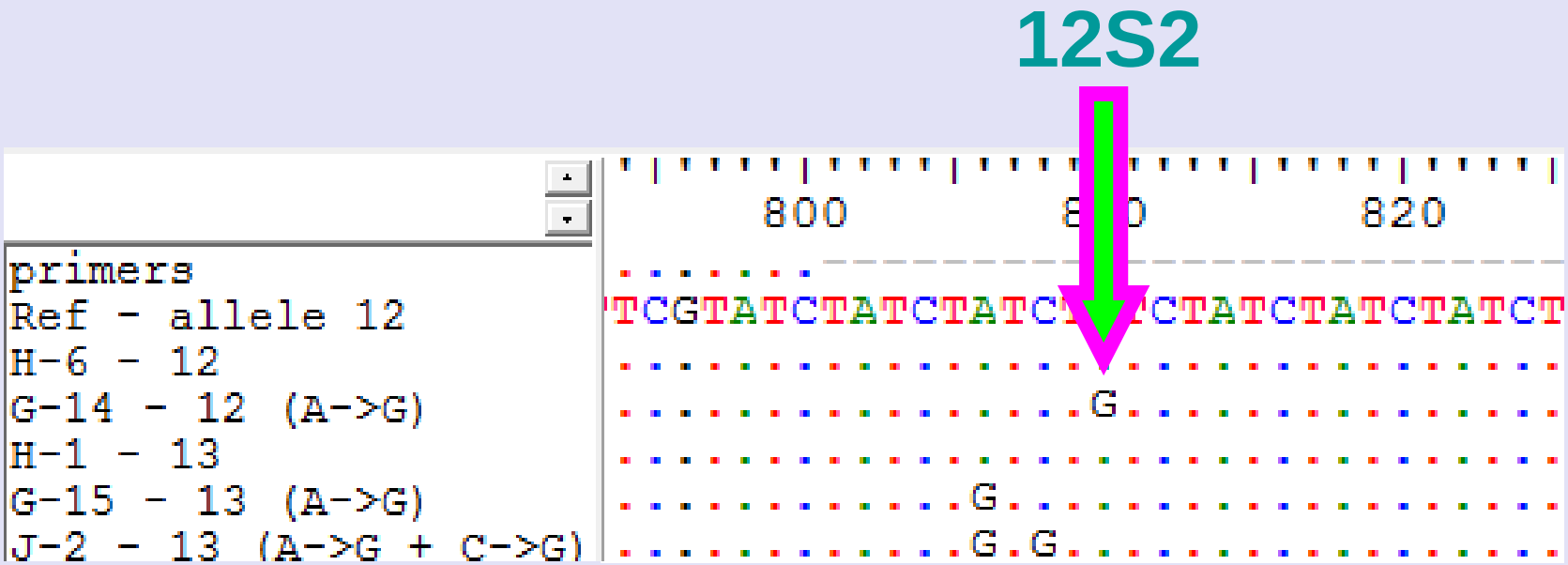


IMAGE COURTESY OF JOHN V. PLANZ, PH.D.

D8S1179

12 Allele containing a A→G SNP = **12S2**

13 Allele containing a A→G SNP = **13S2**

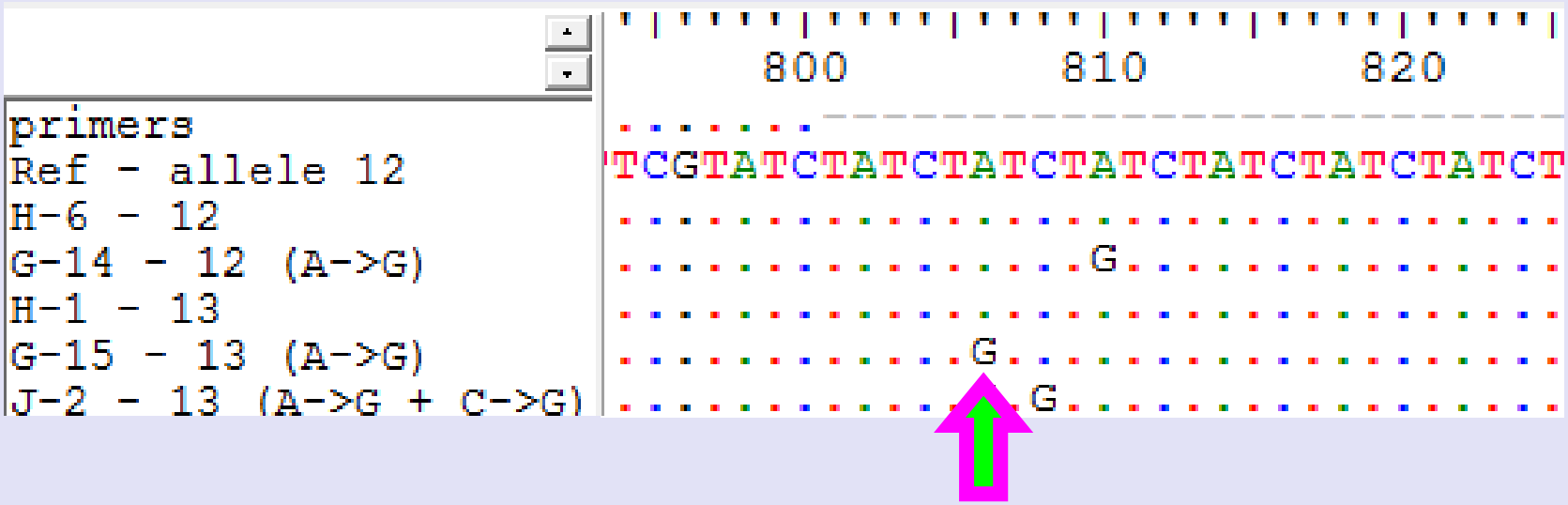


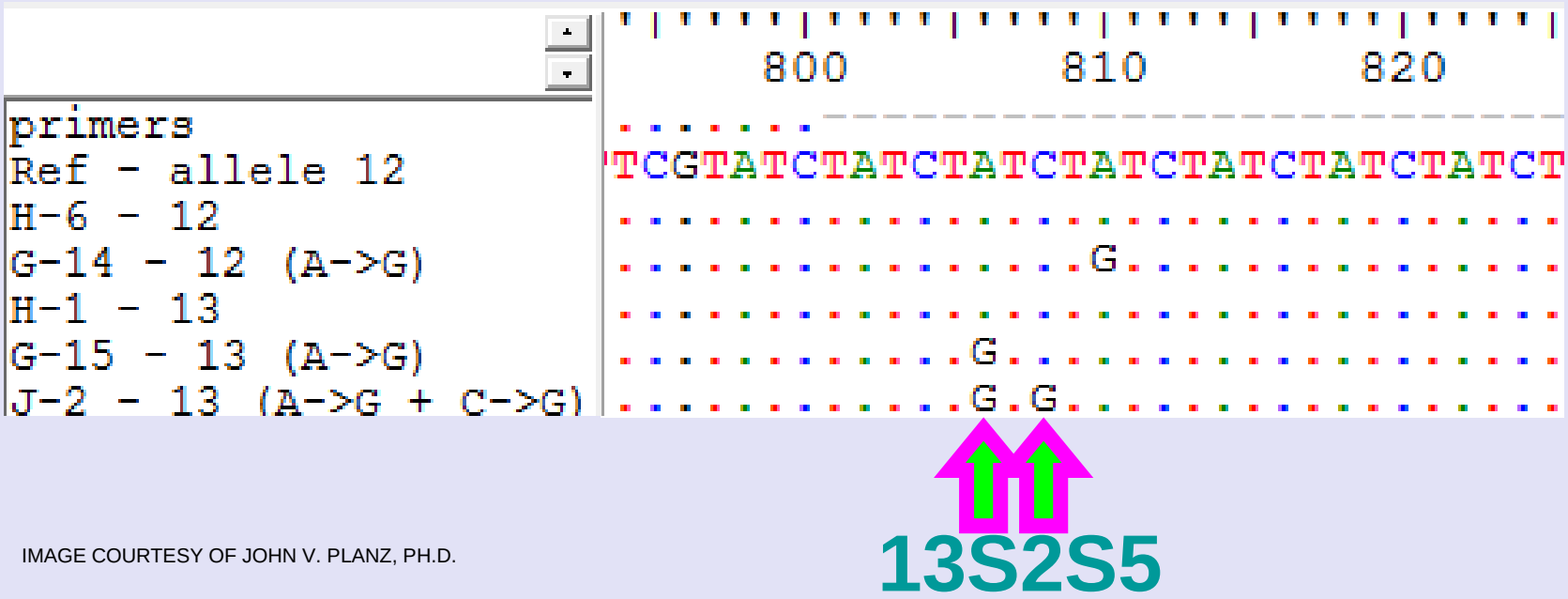
IMAGE COURTESY OF JOHN V. PLANZ, PH.D.

D8S1179

12 Allele containing a A→G SNP = **12S2**

13 Allele containing a A→G SNP = **13S2**

13 Allele containing a A→G & C→G SNP = **13S2S5**



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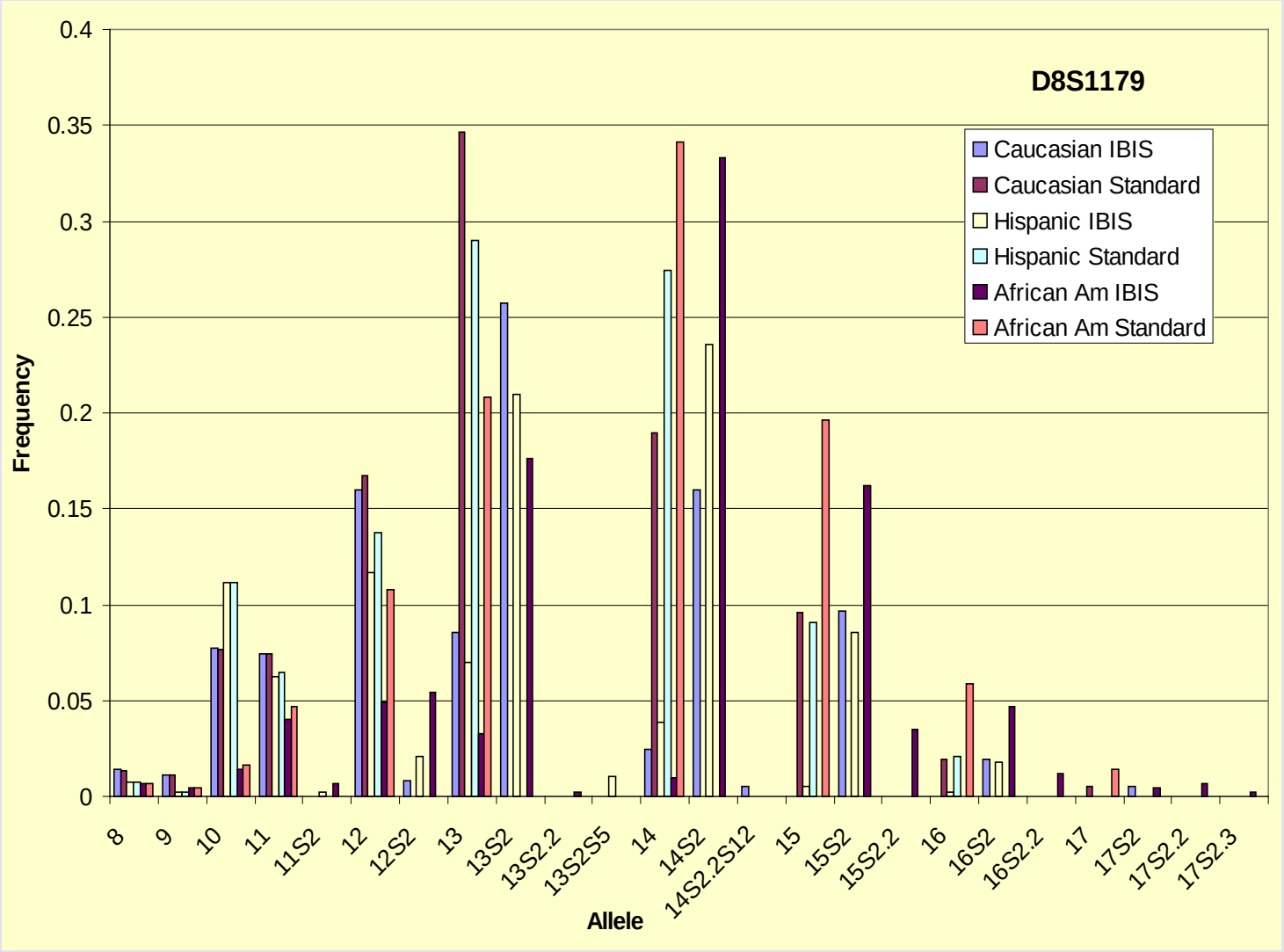
D8S1179



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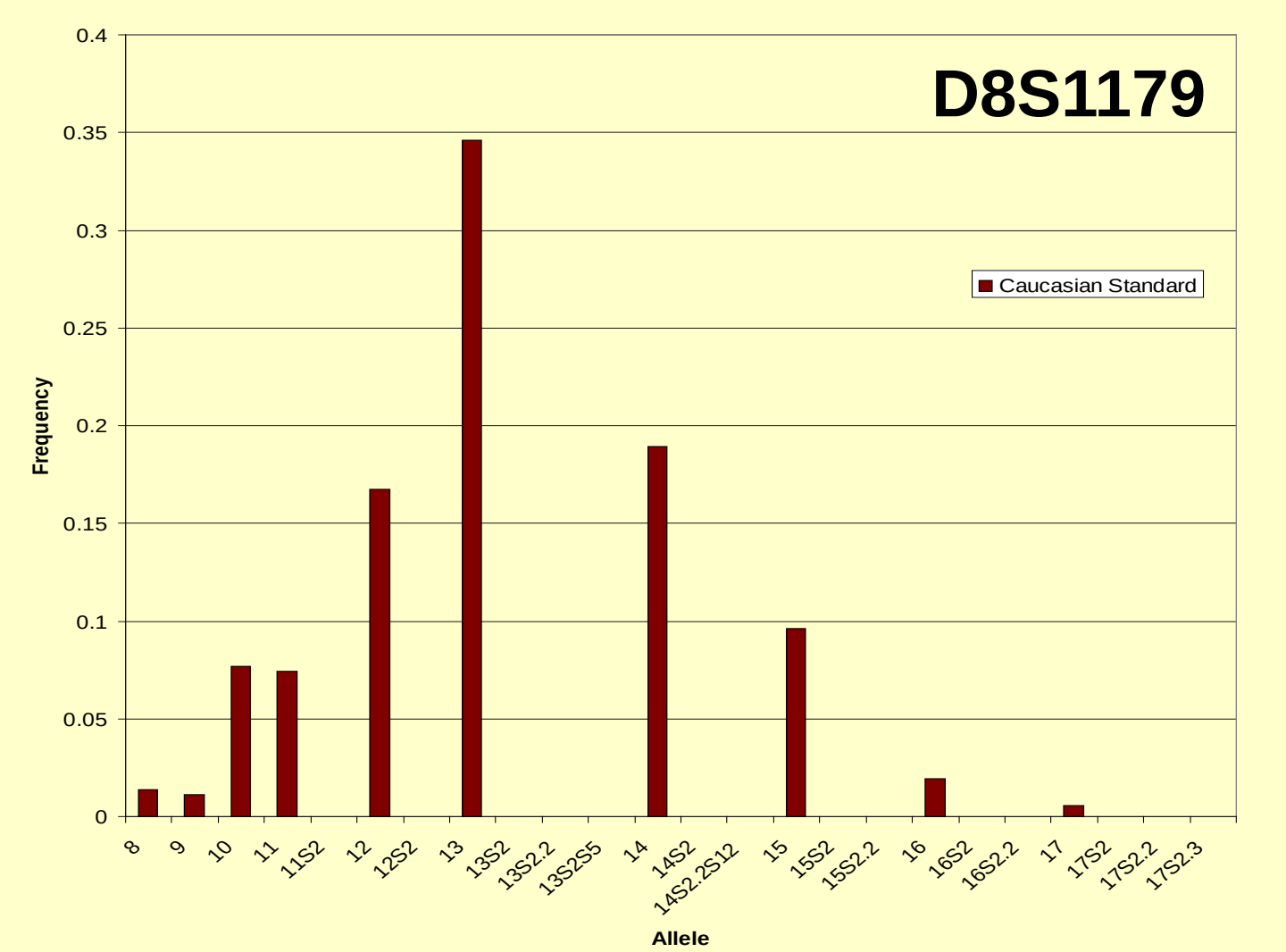

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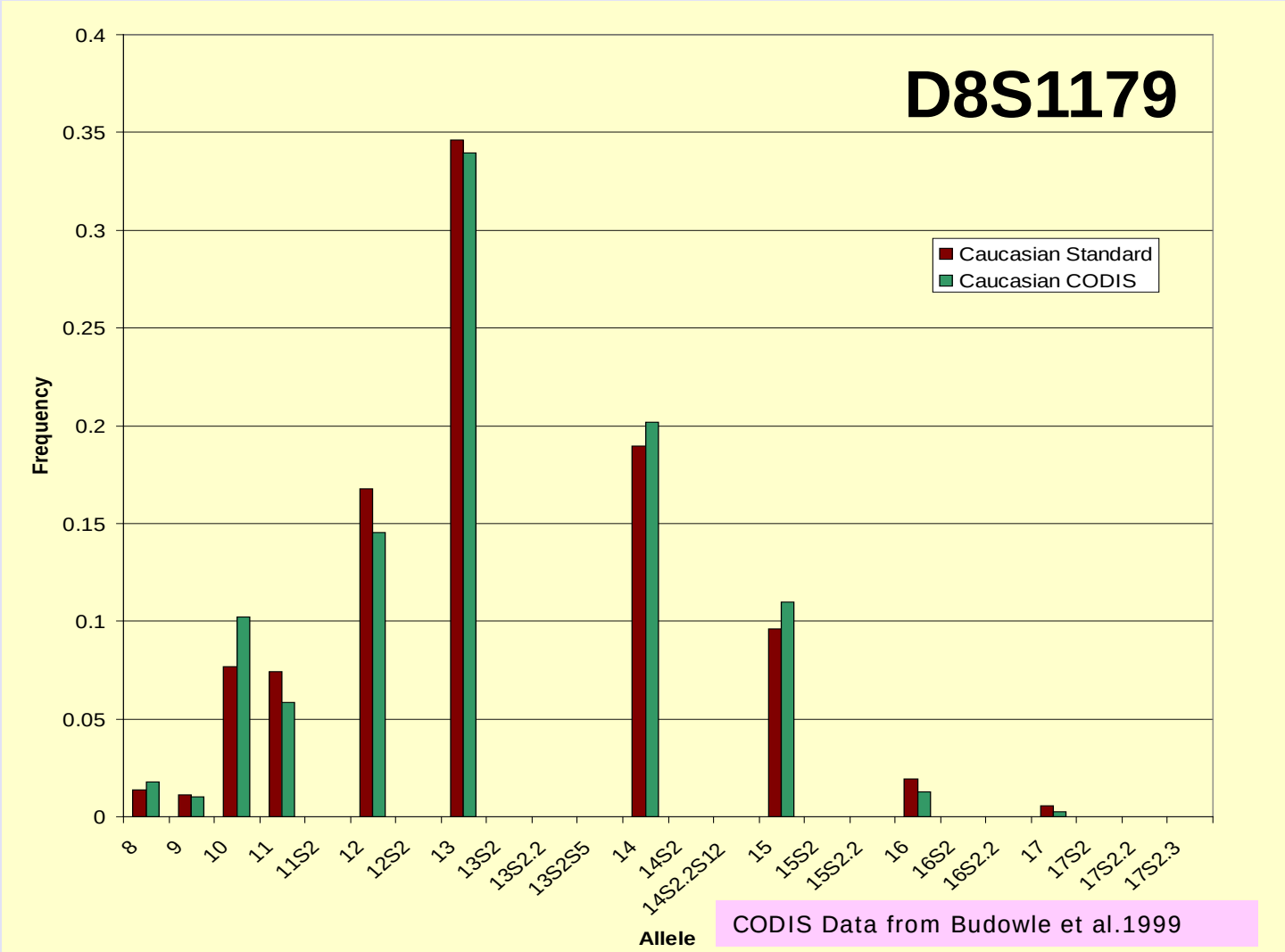

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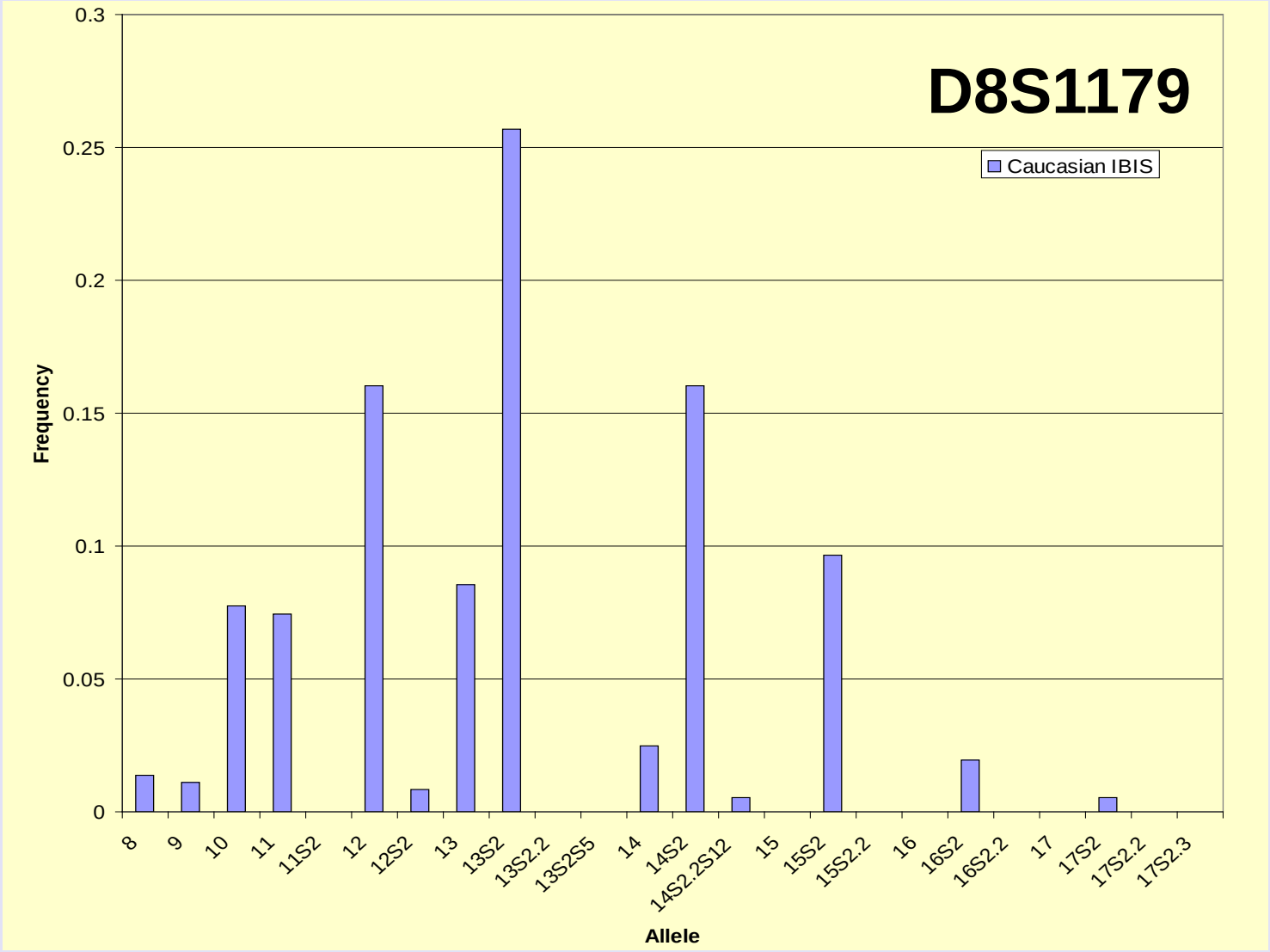

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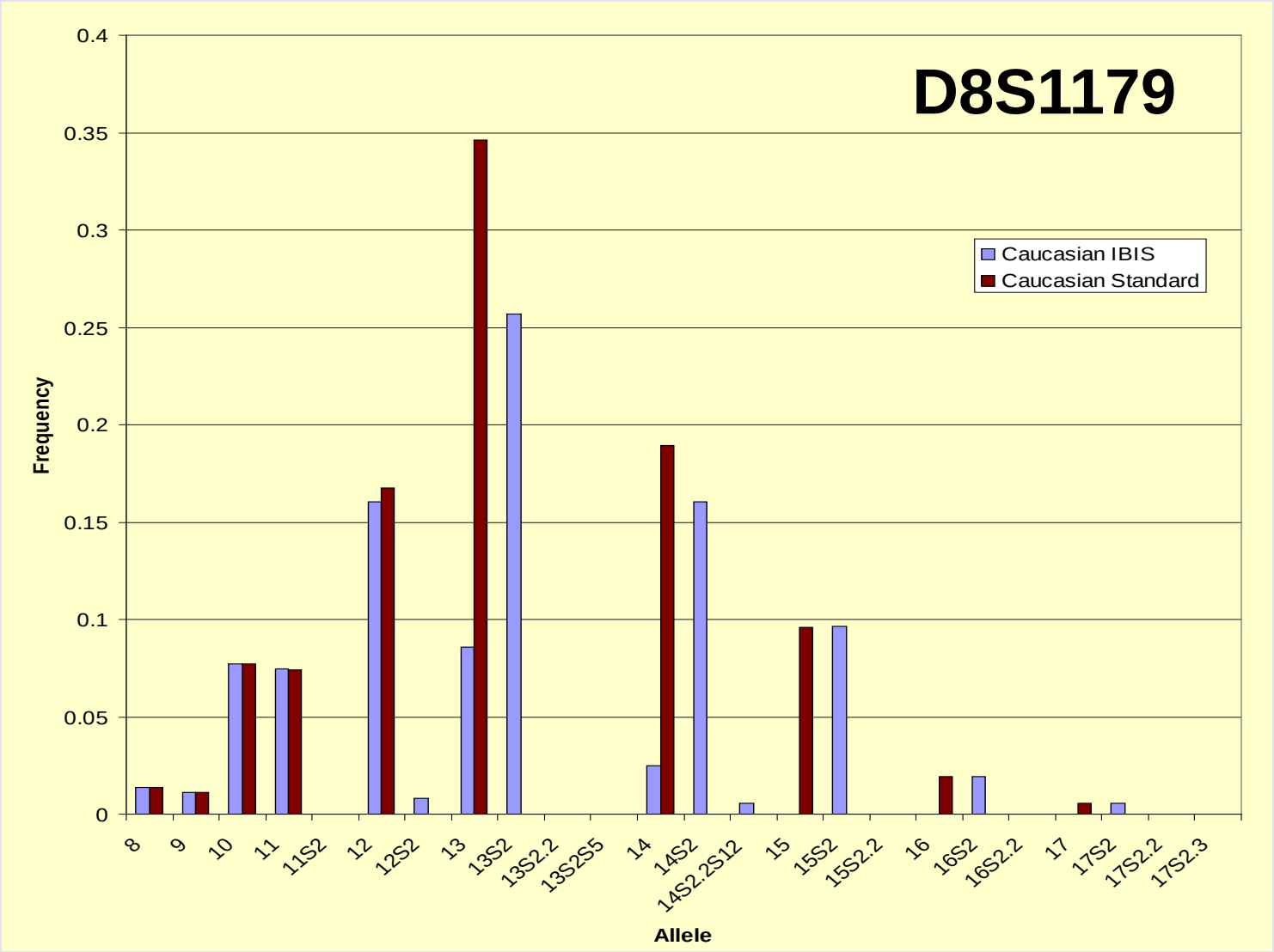

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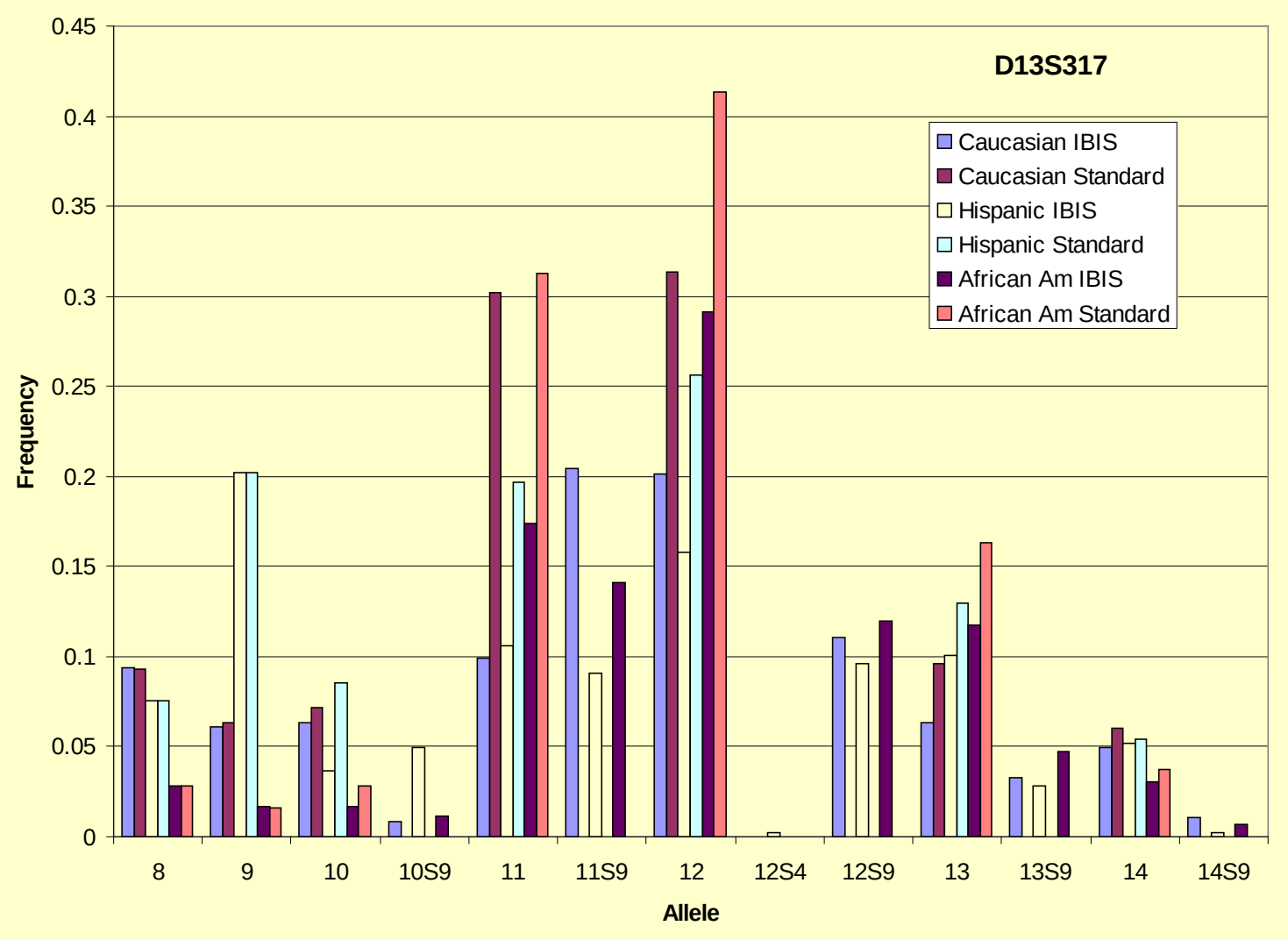
D13S317



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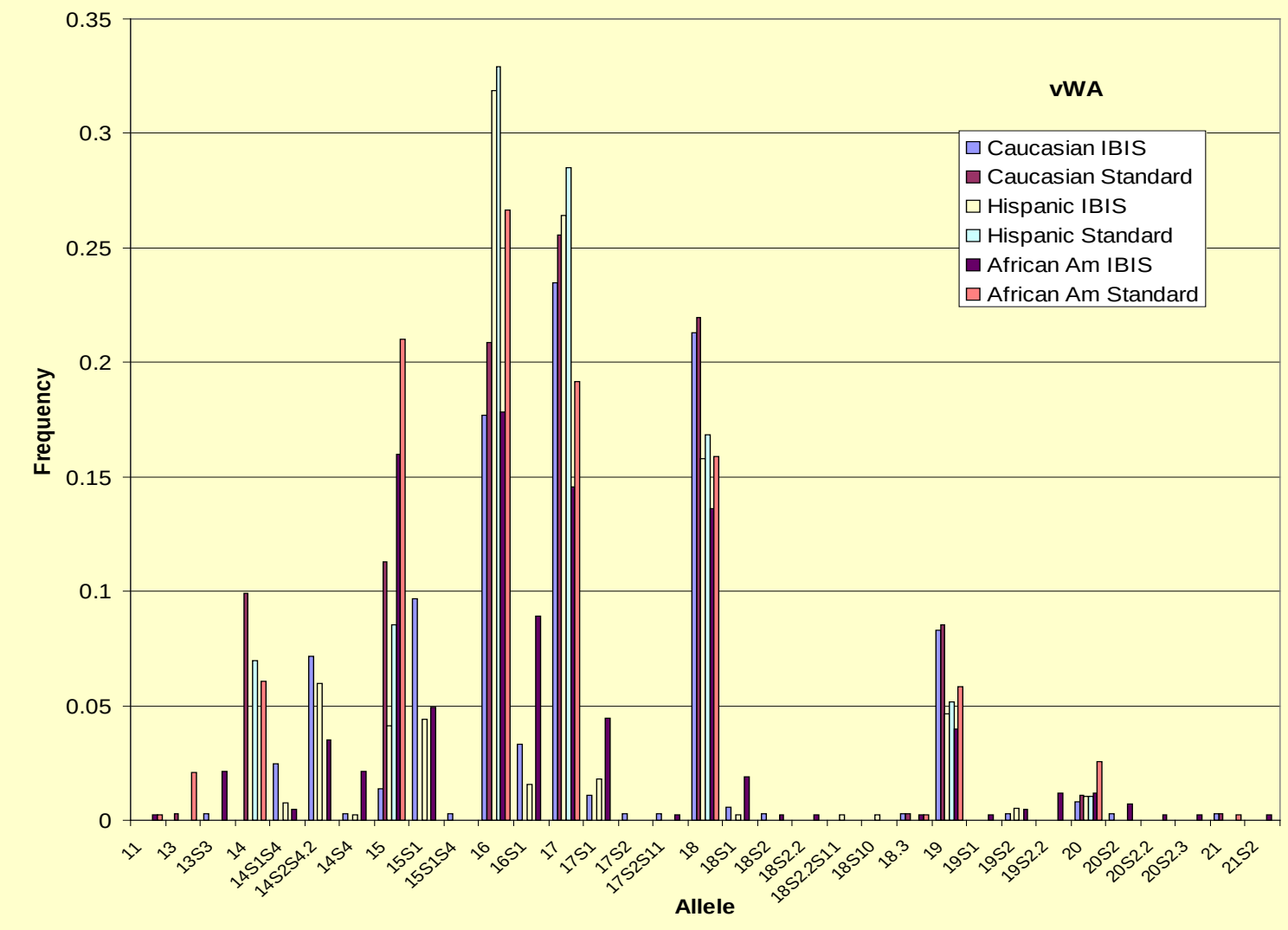
vWA



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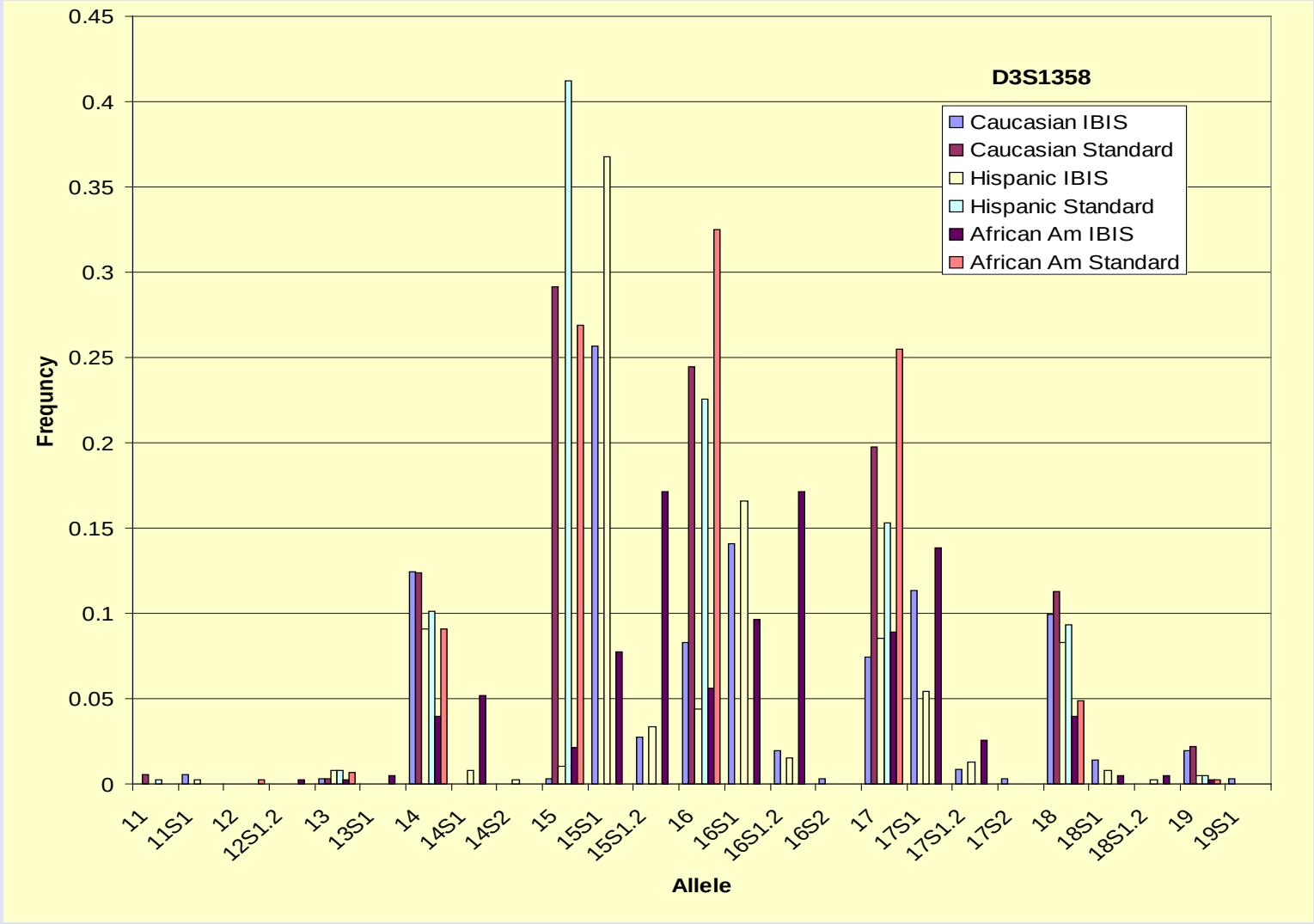
D3S1358



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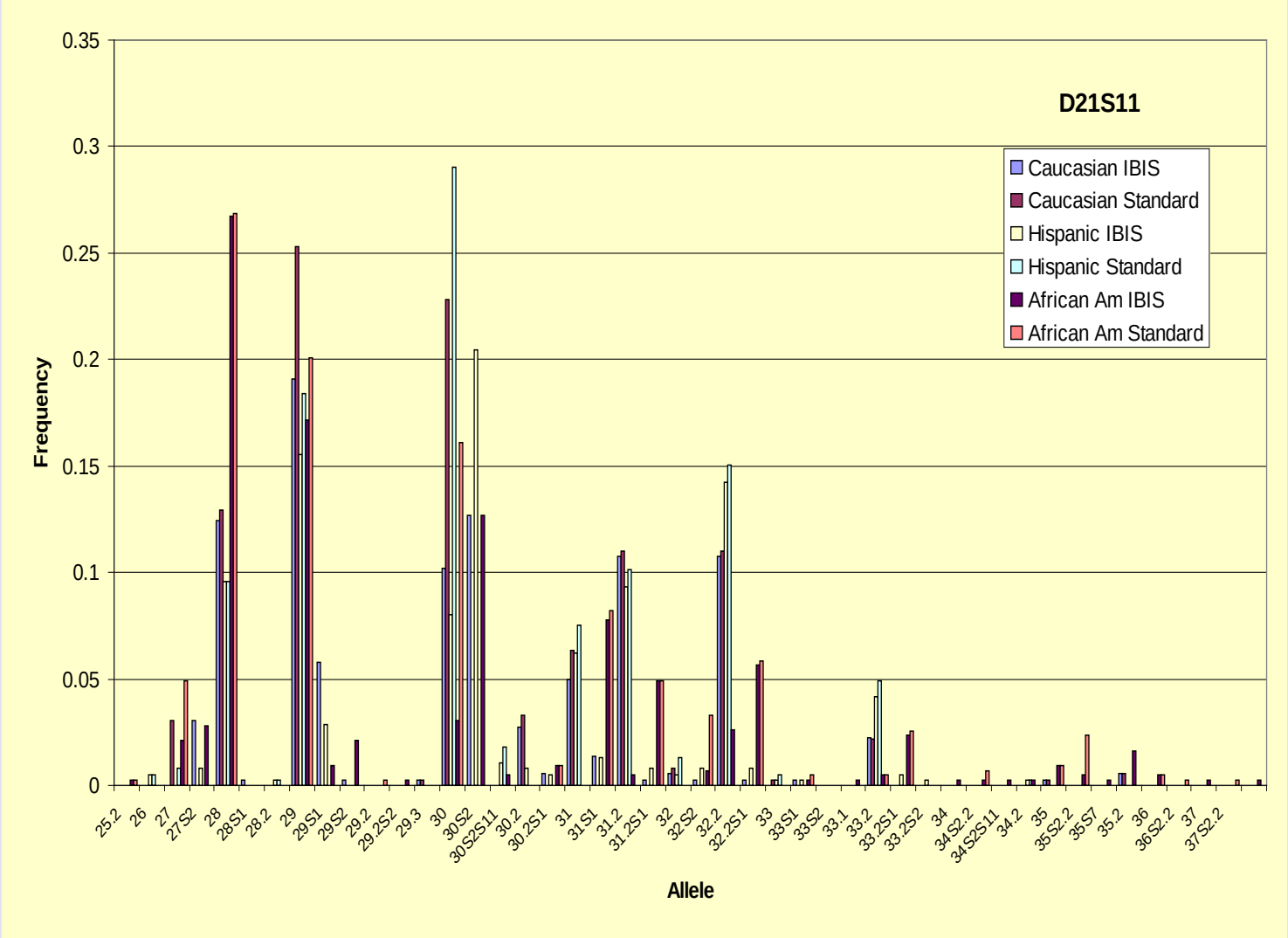
D21S11



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SNPs within STRs open an expanded realm of applications and interpretational power

Identity Testing

- Increased PE & PD
- Overall reduction of homozygotes
- More information without changing the locus panel
- Complete reverse compatibility with existing databases

Relationship Testing

- SNPs can be tracked within a lineage
- STR/SNP combo becomes a haplotype
- Allele transfer in mutation cases can be verified

Discriminatory Power

Capture of the SNPs within the STRs increases the Combined Power of Discrimination of the 7 loci to approximate that of 10 regularly typed STR loci.

Random match probability for 50 individuals selected from each population group decreased on average 10^{-2} to 10^{-4} for the panel of 7 loci as compared to standard STR profile statistics.

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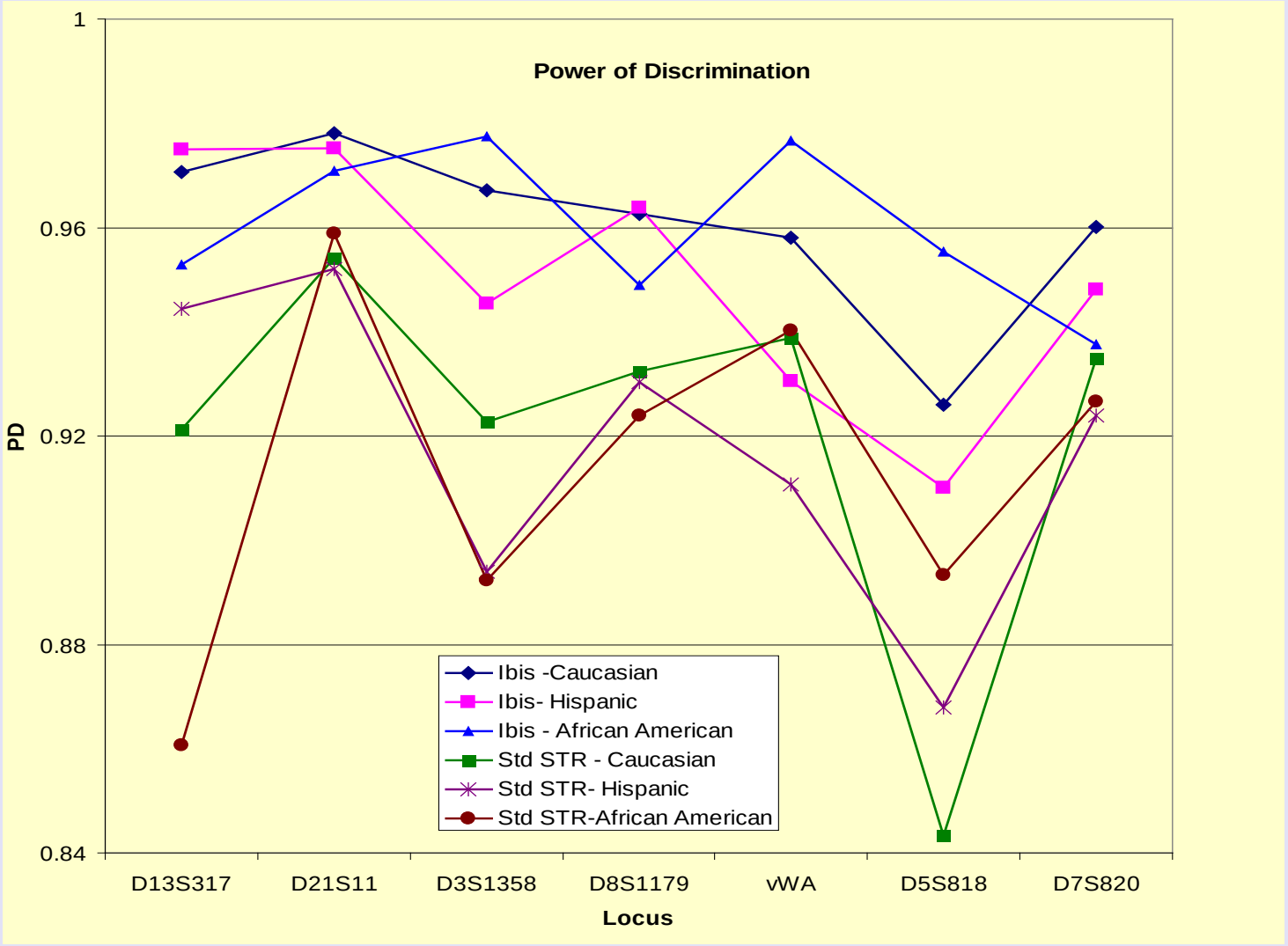

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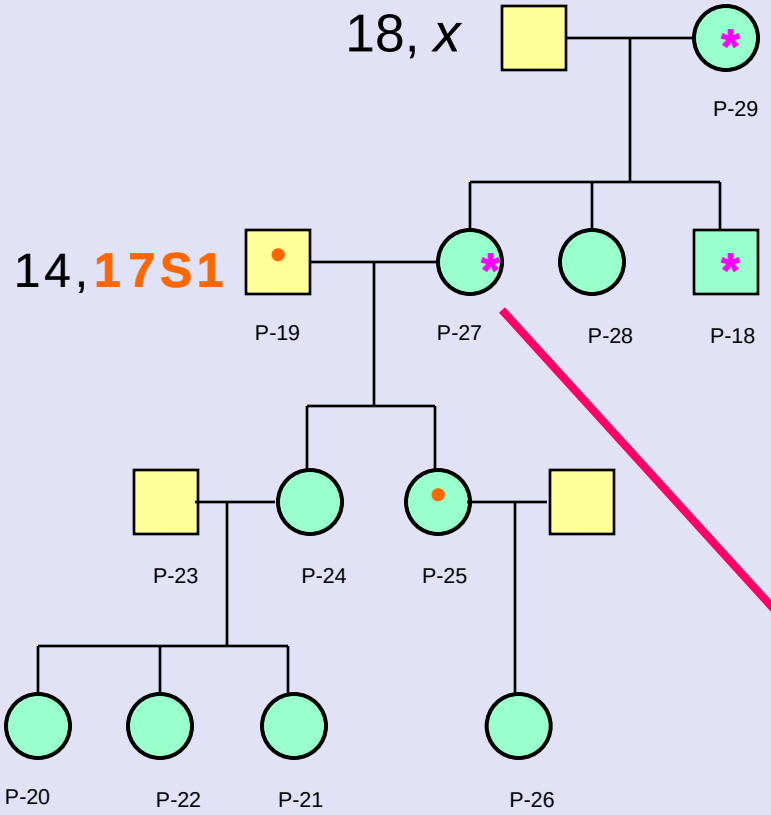
Uses in Relationship Testing

- **SNPs within STR repeats allow individual alleles to be tracked through a pedigree**
- **This enhances the ability to successfully associate relatives in mass disaster and missing persons scenarios**
- **As the loci have increased discriminatory capacity, alleles that match in low stringency "familial" searches have a greater probability of hitting a relative, reducing fortuitous associations obtained in pairwise database searches**

D3S1358



Sample	Genotype
P-18	18, 18S1
P-19	14, 17S1
P-20	14, 18
P-21	14, 14
P-22	14, 14
P-23	14, 15S1
P-24	14, 18
P-25	17S1, 18
P-26	18, 18
P-27	18, 18S1
P-28	16, 18
P-29	16, 18S1

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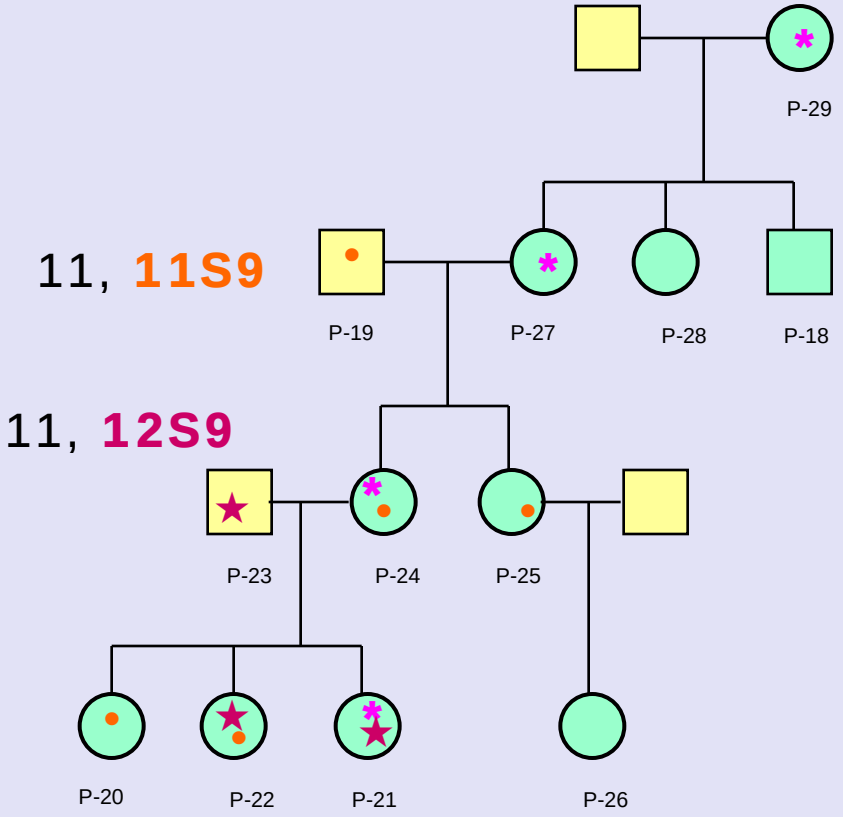
D13S317



Sample	Genotype
P-18	12, 12
P-19	11, 11S9
P-20	11, 11S9
P-21	10S9, 12S9
P-22	11S9, 12S9
P-23	11, 12S9
P-24	10S9, 11S9
P-25	11S9, 12
P-26	9, 12
P-27	10S9, 12
P-28	12, 12
P-29	10S9, 12

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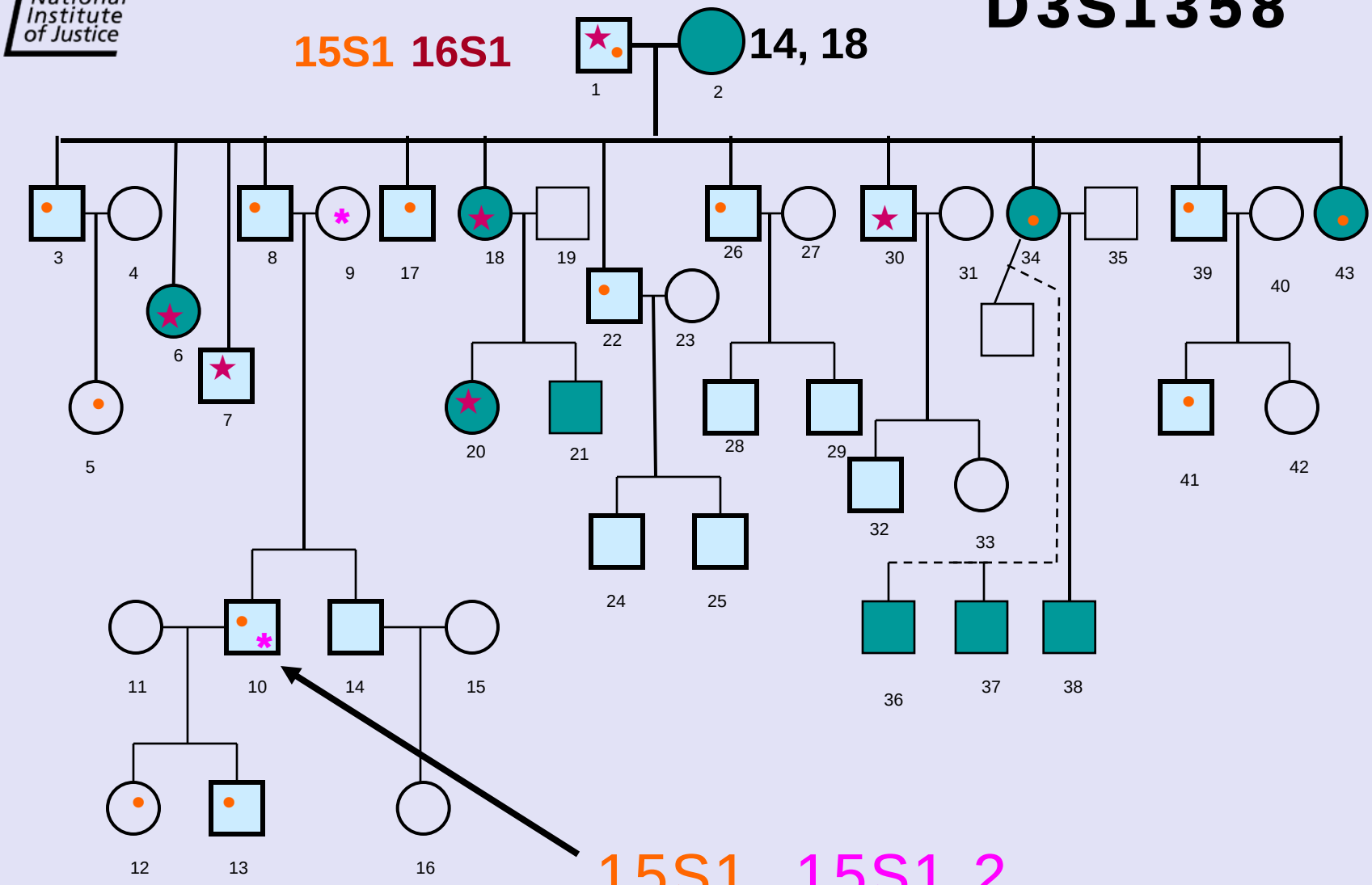
D3S1358



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Much to do!

- **Expand database size to 400-500 individuals per population group**
- **Sequence a number of individuals sharing a common allele to evaluate effect on population structure**
- **Type and sequence a number of parentage trios exhibiting single non-matching systems in SNP containing loci**
- **Evaluate additional STRs (i.e. NCO's, Y STRs, X STRs) for discriminatory value**

Special Thanks to:

Tom Hall
Kristin Lowery
Suzanne Gonzalez
Pamela Marshall
Elizabeth Feller
Shubra Nandi
Jennifer Thomas

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