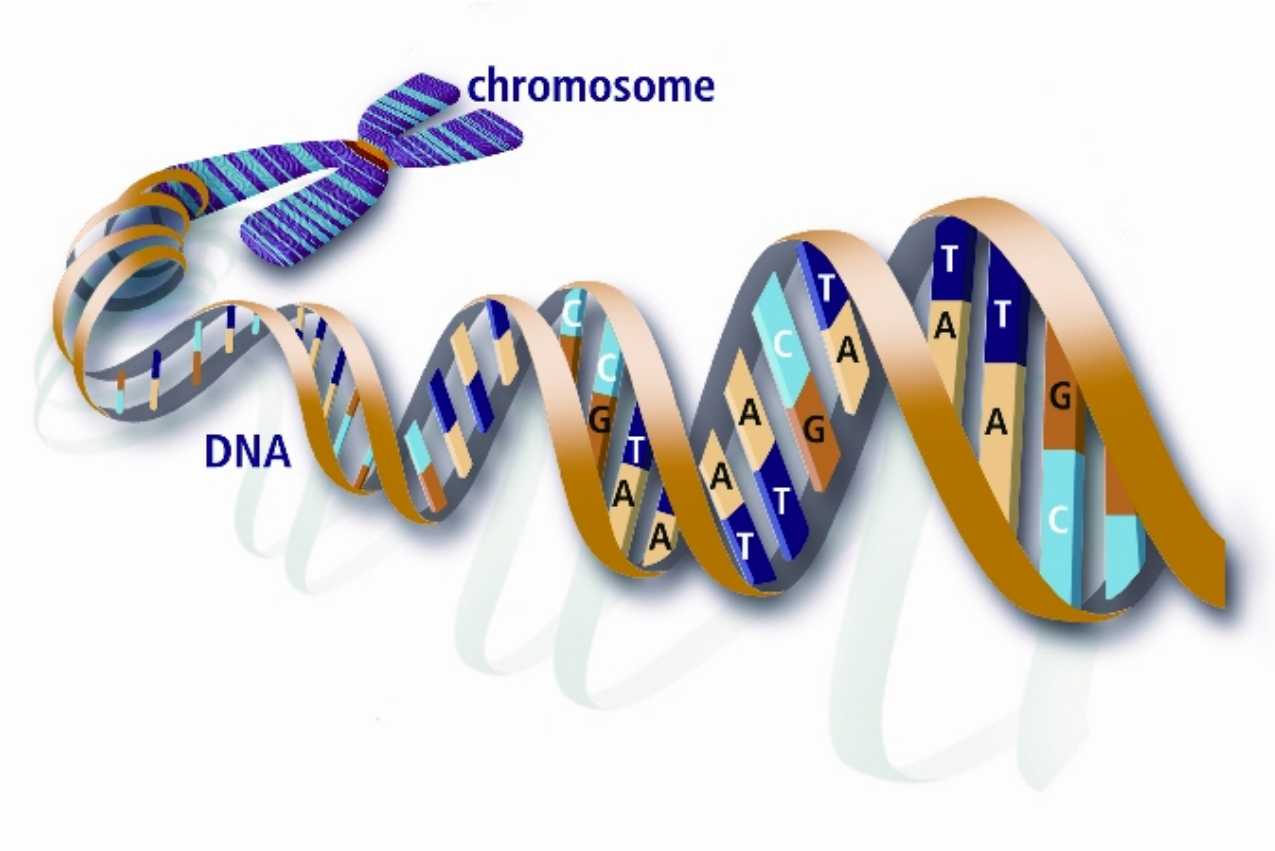
DNA 101:
Basics, Case Files, and Mixture Interpretation

Presented by
Stephanie Berger

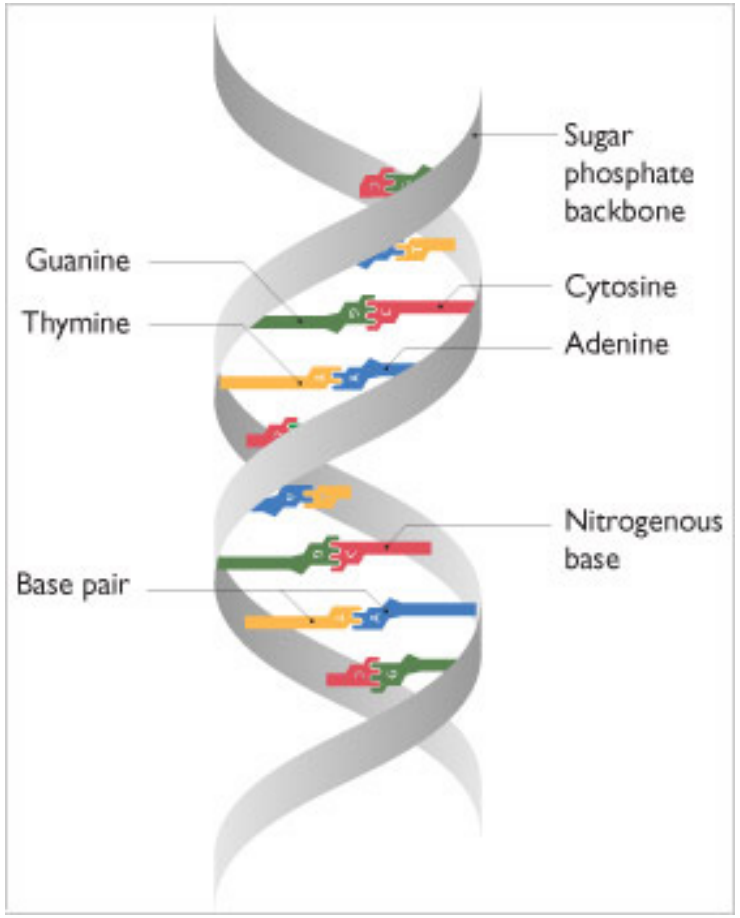
Outline

- Part 1: Basics
  - DNA Structure and Function
  - Testing Procedure
  - Sources of Error
- Part 2: Case Files
  - Raw Data
  - Profiling Results
  - Probability of Inclusion
- Part 3: Mixture Interpretation

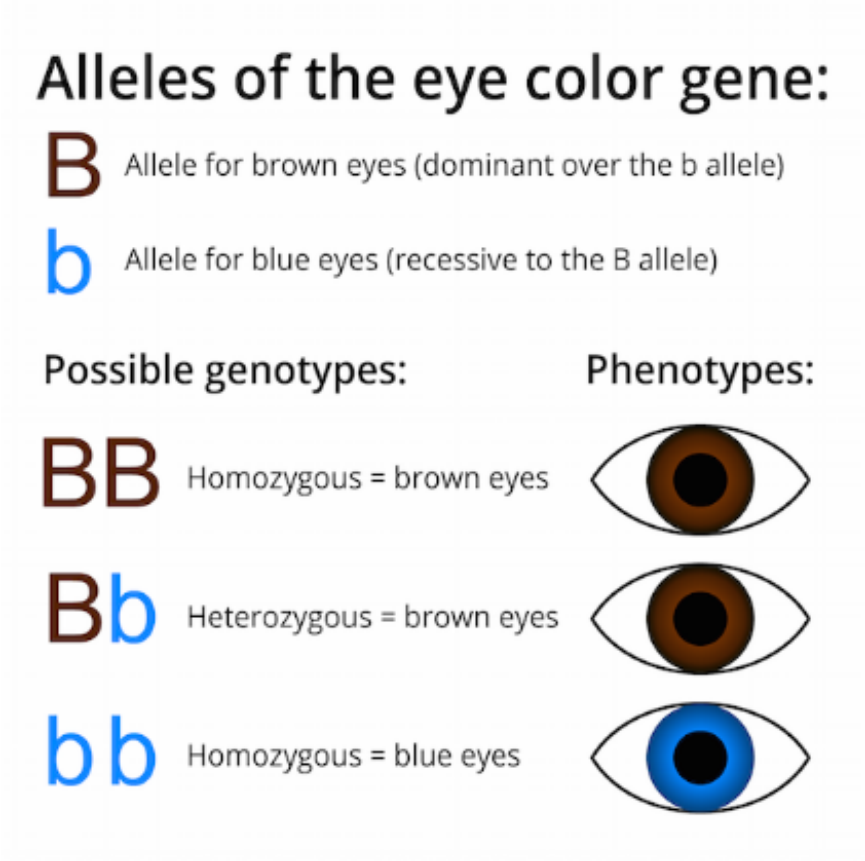


Basics: DNA Structure and Function

- Code
- Sequence of 4 Letters
  - (A)denine
  - (T)hymine
  - (C)ytosine
  - (G)uanine
- A pairs with T
- C pairs with G

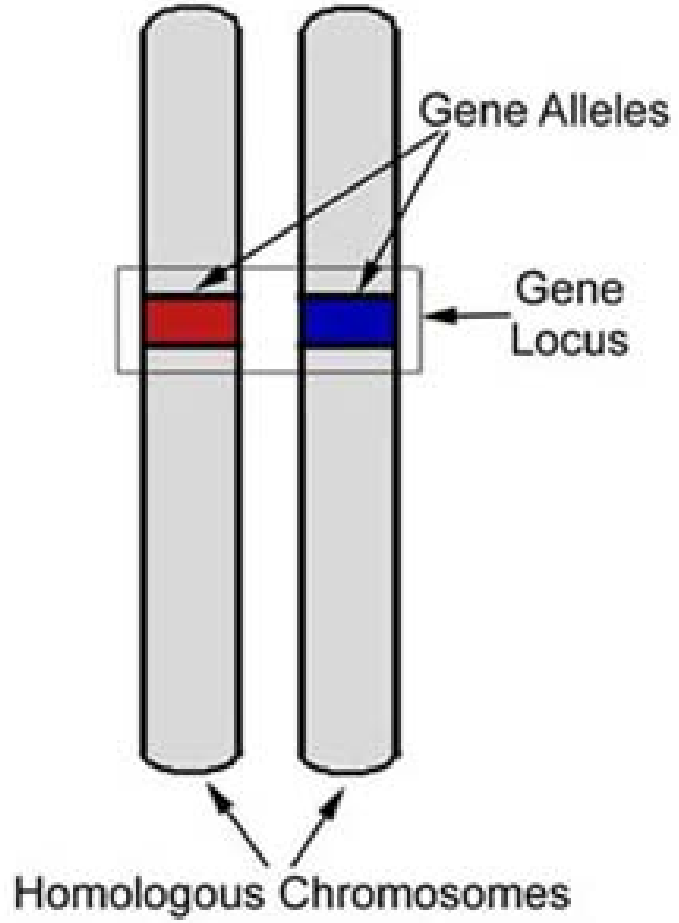


DNA Structure and Function: Alleles

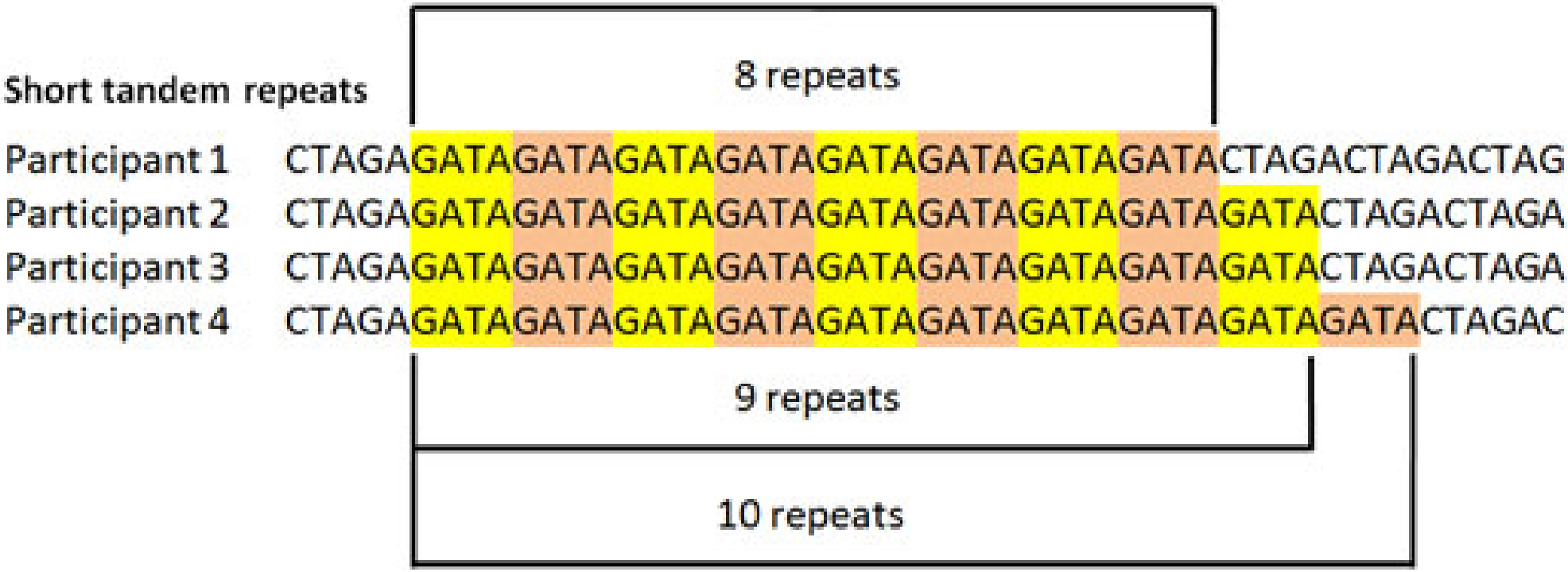


DNA Structure and Function: Loci

- Locus
  - Plural = loci
  - Section of the code with known alleles

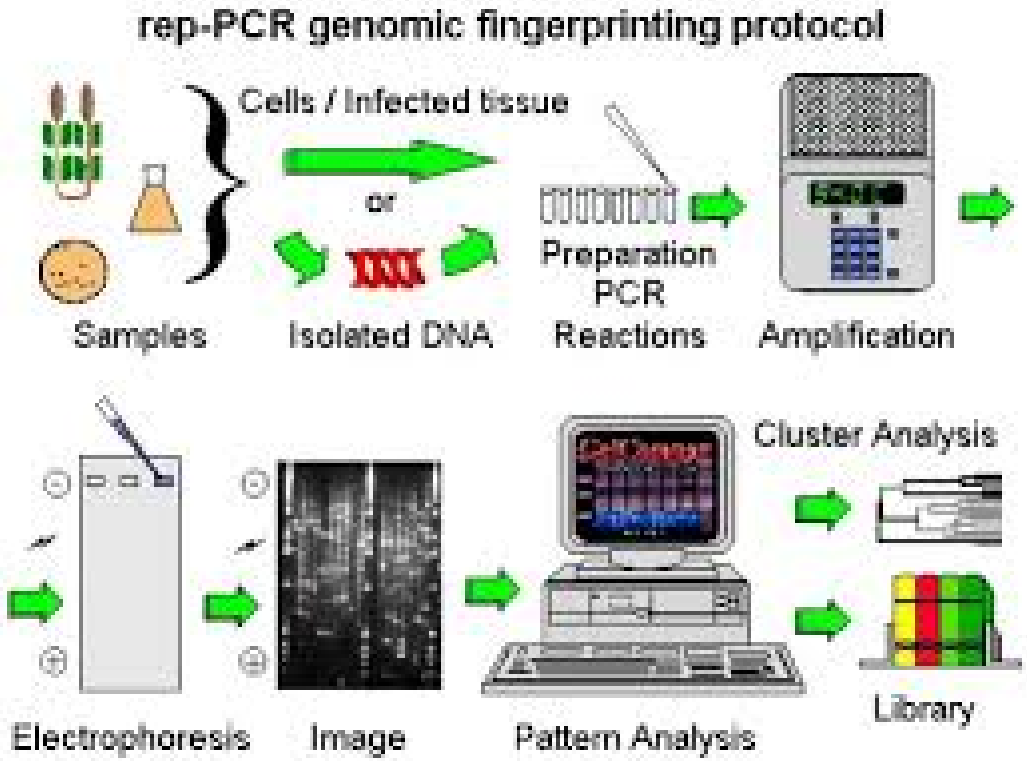


DNA Structure and Function: Short Tandem Repeats



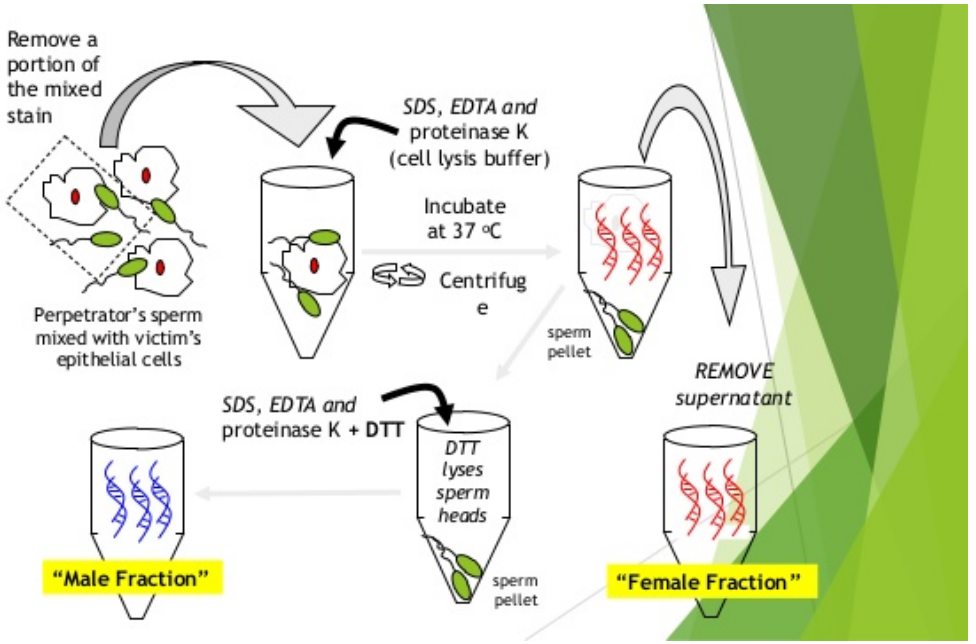
Basics: Testing Procedure

- Steps
  - Step 1: Extraction
  - Step 2: Amplification
  - Step 3: Capillary Electrophoresis
  - Step 4: Comparison
  - Step 5: Combined Probability of Inclusion

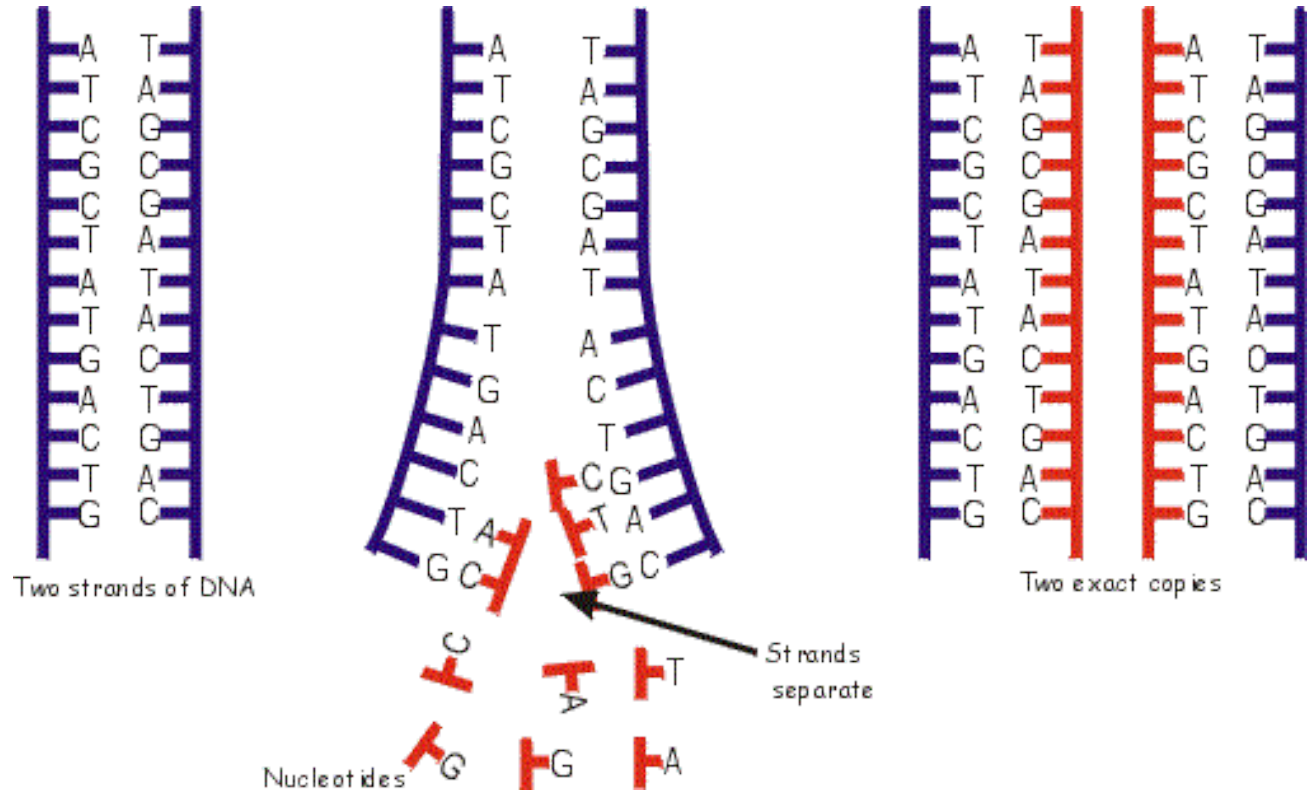


Testing Procedure: Extraction

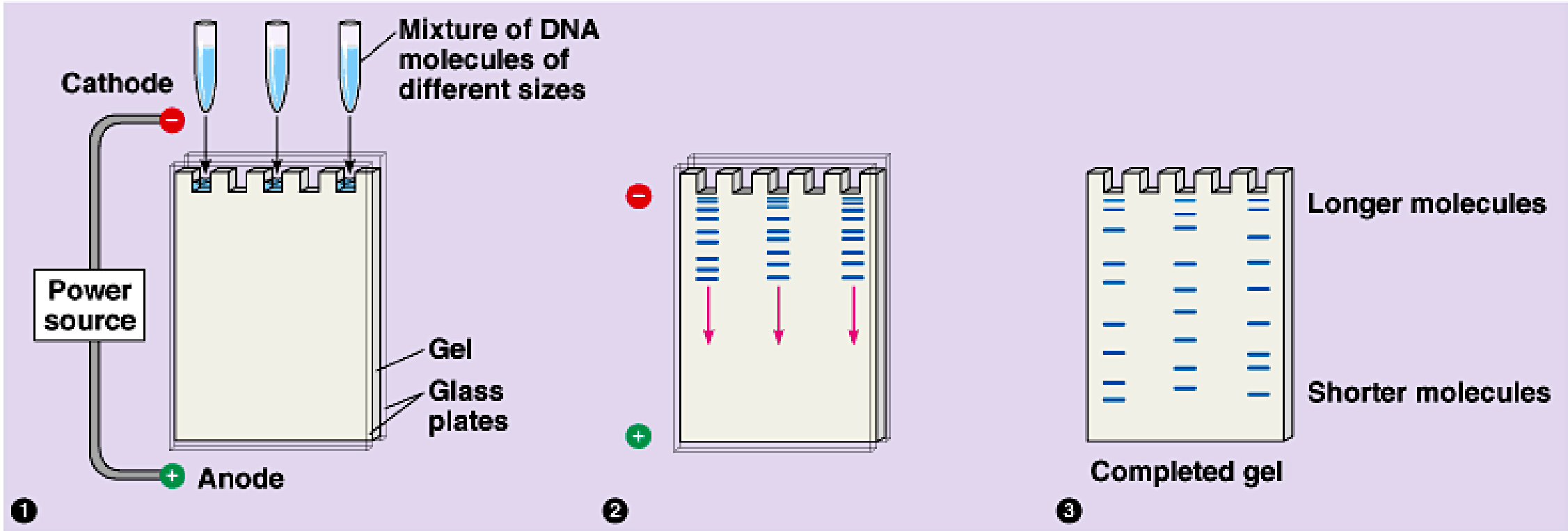
- Sex Cases
  - Separate Sperm from other cells
- Isolate DNA
- Cut DNA into fragments



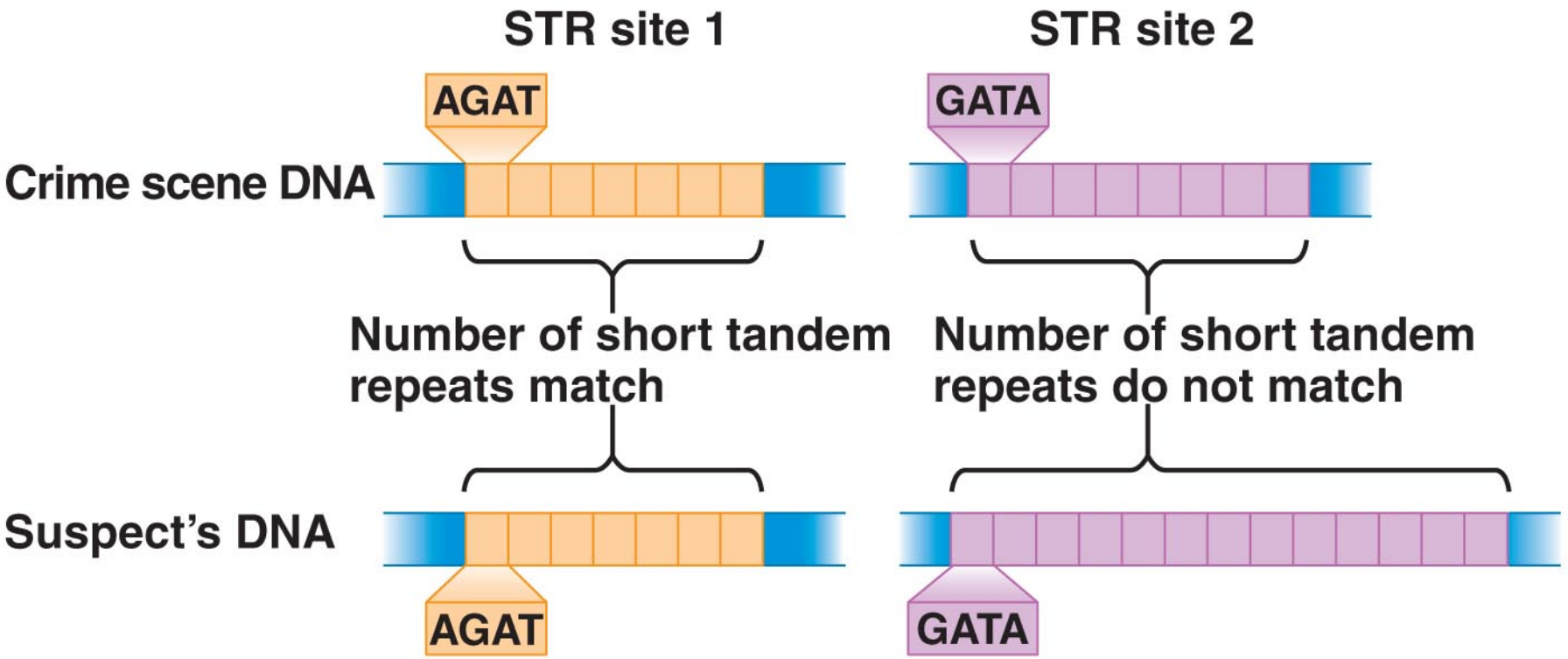
Testing Procedure: Amplification



Testing Procedure: Capillary Electrophoresis



Testing Procedure: Comparison



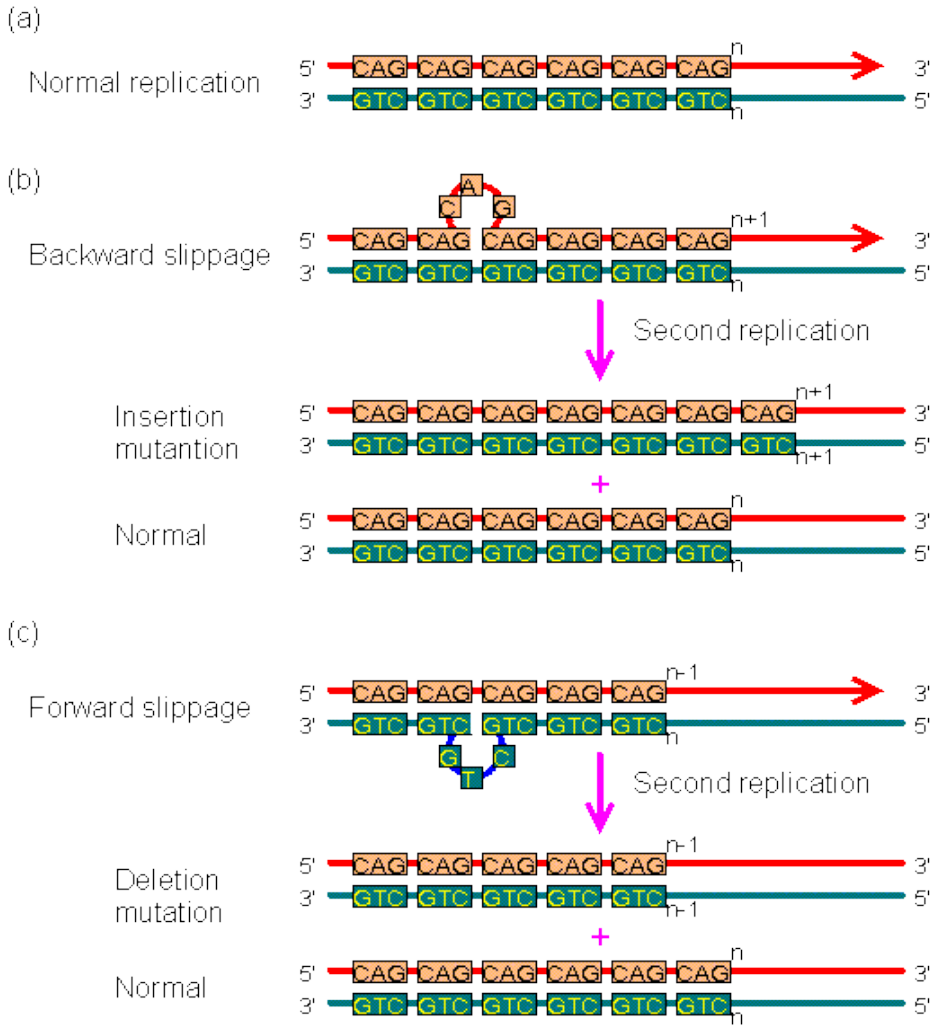
Testing Procedure: Probability of Inclusion

- Frequency of particular allele (at a particular locus) in the population
  - Based on FBI database
- Multiply frequencies of each allele tested
  - Assumes Independence

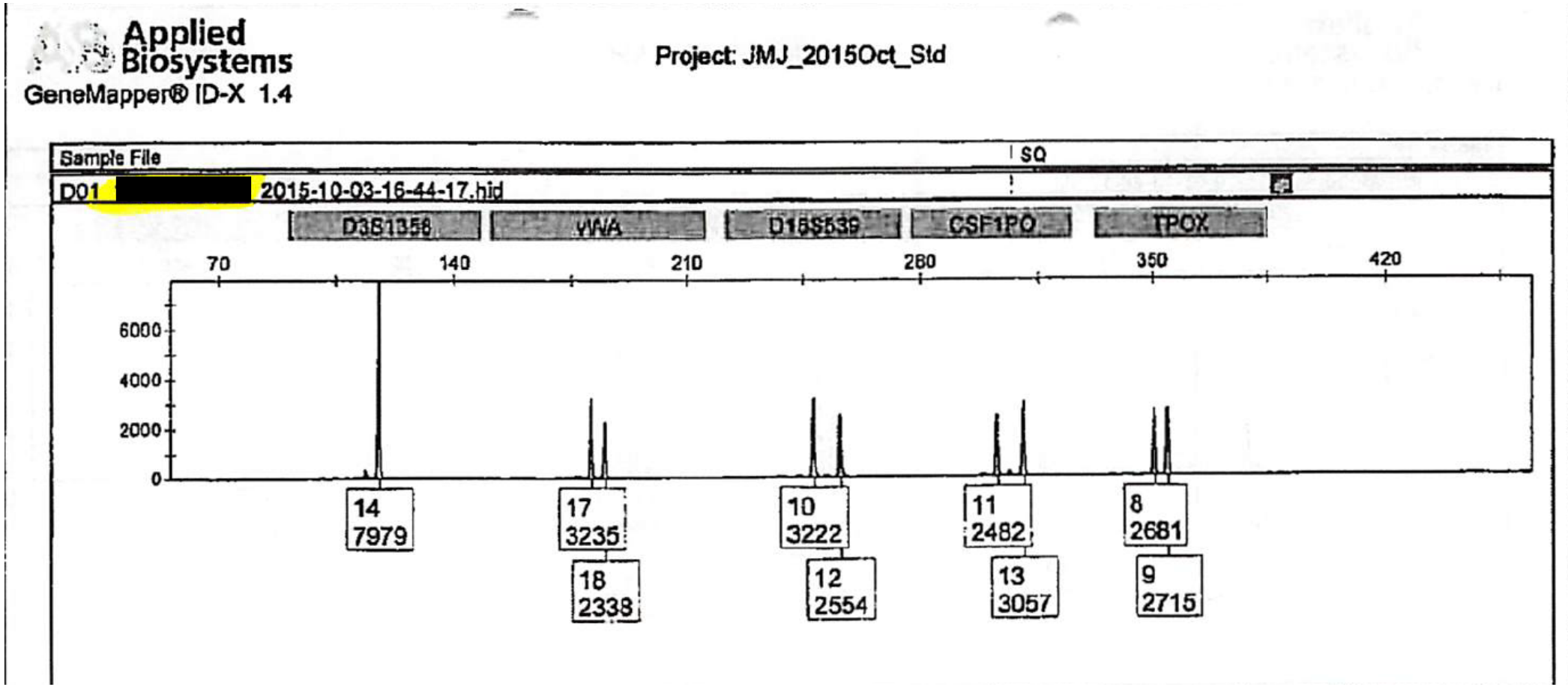
Basics: Sources of Error

- Allelic Dropout
  - An allele is present in the sample, but the test does not detect it
- Stutter
  - Test detects an allele that is not actually present in the sample
  - Software settings filter these out
- Bigger Problem with small or degraded sample

Errors Occur During Amplification



DFS Case File: Raw Data



Case File: Profiling Results

Alabama Department of Forensic Sciences

DNA Profiling Results

Case #: [redacted]

Analyst: EA GA

Date: [redacted]

Sample	Item 3B NS - sex toy	Item 1A - [redacted]	Item 4 - [redacted]	Item 1C1 NS - rectal
D3S1358	16 (14,15)	15,16	14	15,16
vWA	15,16 (17) (18)	15,17	17,18	15,17
D16S539	8,10 (12)	8,12	10,12	8,12
CSF1PO	12 (11) (13)	12,13	11,13	12,13 (11)
TPOX	8,11	11	8,9	11

Case File: Probability of Inclusion

Probability of Inclusion (Short)

Database: \\192.168.2.11\codis\Popstats\POPDATA\FBI\NIST
Theta1: 0.01

	Mixture	*H1*	*H2*
Lab ID:	AL001025Y	AL001025Y	AL001025Y
Specimen ID:	[redacted] (Keyboard)	Keyboard	Keyboard
Comment:			

Allele Frequency

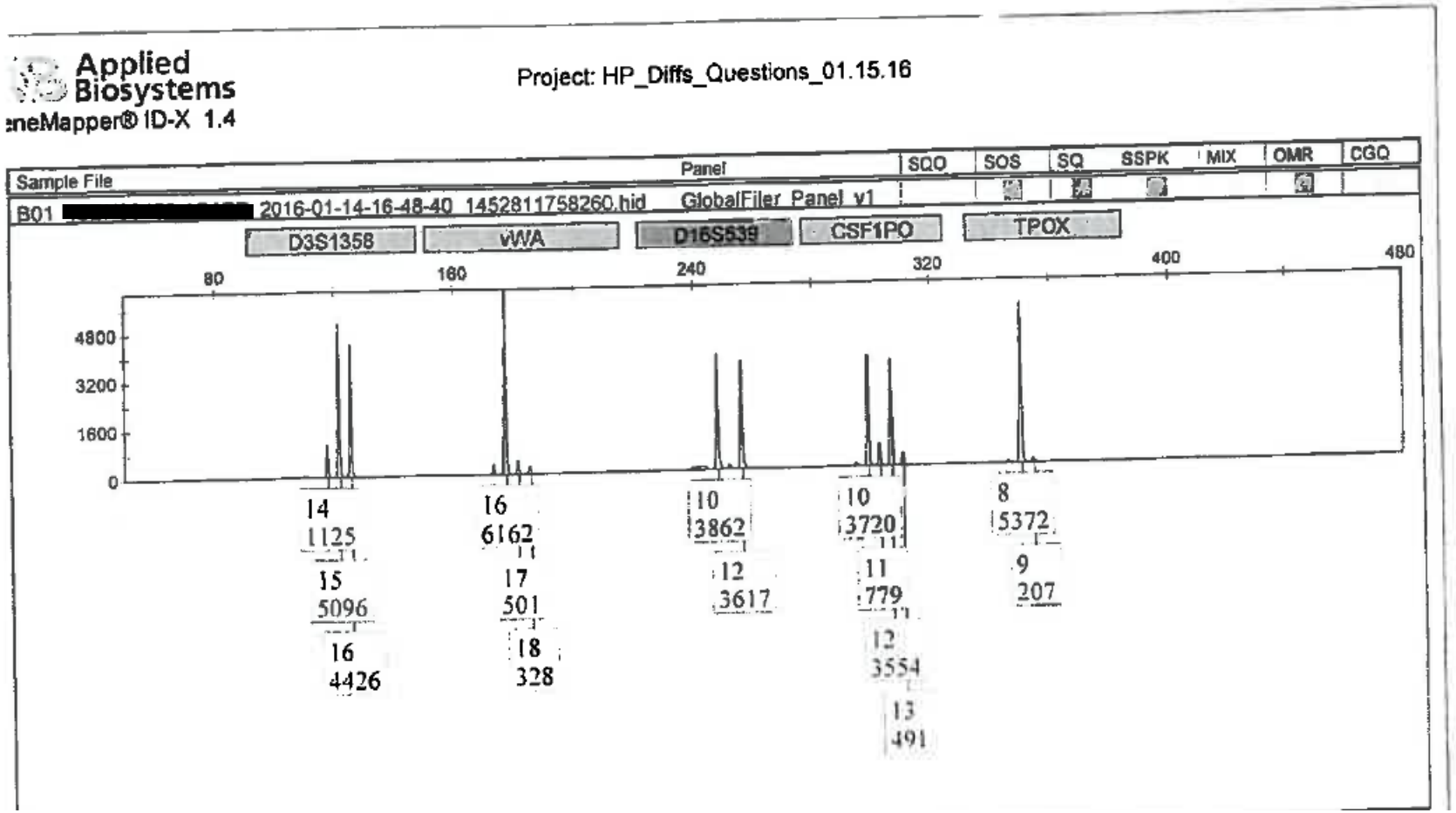
Locus	Allele	COMB	CAU	BLK	HSP	ASN
D3S1358	14	8.7400E-02	1.0660E-01	9.0600E-02	7.8400E-02	2.5800E-02
D3S1358	15	3.0450E-01	2.7290E-01	3.0850E-01	3.2200E-01	3.6600E-01
D3S1358	16	2.8280E-01	2.3820E-01	3.1870E-01	2.7970E-01	3.2990E-01
VWA	15	1.3470E-01	1.0530E-01	1.9150E-01	1.4410E-01	2.5800E-02(M)
VWA	16	2.3020E-01	2.0080E-01	2.5000E-01	2.8390E-01	1.3920E-01
VWA	17	2.6210E-01	2.8390E-01	2.3540E-01	2.4580E-01	3.1440E-01
VWA	18	1.8000E-01	2.0220E-01	1.4910E-01	1.8010E-01	2.0620E-01
D16S539	10	1.0810E-01	5.6800E-02	1.1700E-01	1.5040E-01	1.6490E-01
D16S539	12	2.5680E-01	3.1440E-01	2.0470E-01	2.7750E-01	1.7530E-01
CSF1PO	10	2.3210E-01	2.2020E-01	2.5000E-01	2.3730E-01	2.0100E-01
CSF1PO	11	2.7360E-01	3.0890E-01	2.4850E-01	2.7970E-01	2.1650E-01
CSF1PO	12	3.4460E-01	3.6010E-01	2.9530E-01	3.7500E-01	3.8660E-01
CSF1PO	13	6.5600E-02	8.1700E-02	4.6800E-02	5.9300E-02	8.7600E-02
TPOX	8	4.6620E-01	5.2490E-01	3.6700E-01	4.8520E-01	5.5150E-01
TPOX	9	1.3750E-01	1.2740E-01	1.9590E-01	9.3200E-02	7.7300E-02

Mixture Interpretation

- 5 steps
- Each step involves SUBJECTIVE DETERMINATIONS
- Should be completed BLIND
  - Prevent bias
- Should rely on standard operating procedures
- Comparison AFTER completing all 5 steps

Mixture Interpretation: Step 1

- Identify a mixture
  - 3 or more alleles at a given locus
  - Peak height imbalance between alleles
  - Ratio of male DNA to Human DNA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SSPK
MIX
OMR
CGQ
B01 [redacted] 2016-01-14-16-48-40 1452811758260.hid GlobalFiler Panel v1
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D16S539
CSF1PO
TPOX
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480
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3200
1600
0
14 1125
15 5096
16 4426
16 6162
17 501
18 328
10 3862
12 3617
10 3720
11 779
12 3554
13 491
8 5372
9 207

Mixture Interpretation: Step 2

- Designate Allele Peaks
  - Is a particular peak a true allele or stutter?
  - Software Filters
  - Thresholds
    - Analytic
    - Stochastic
  - Lab should have set standards

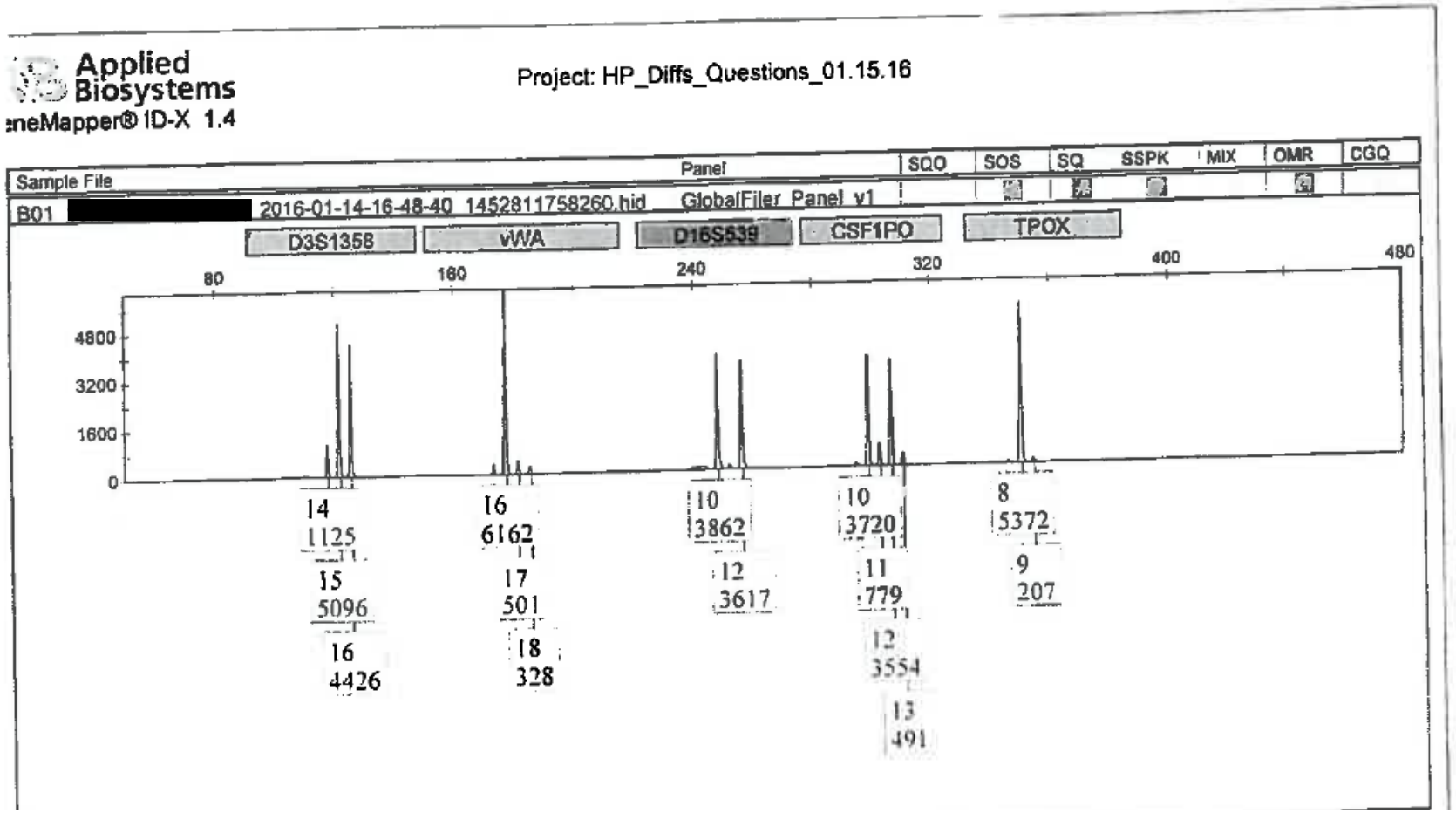
Designating Allelic Peaks (2 Thresholds)

Analytic Threshold (AT)

- Peaks below this threshold should NOT be used
- If an analyst thinks that a peak below this threshold is a true allele, the entire locus should be excluded

Stochastic Threshold (ST)

- Higher than AT
- Peaks below this threshold should only be used to identify mixtures or to exclude an individual
- Any locus with a peak below this threshold should be excluded
- Exclusion of multiple loci should result in an inconclusive report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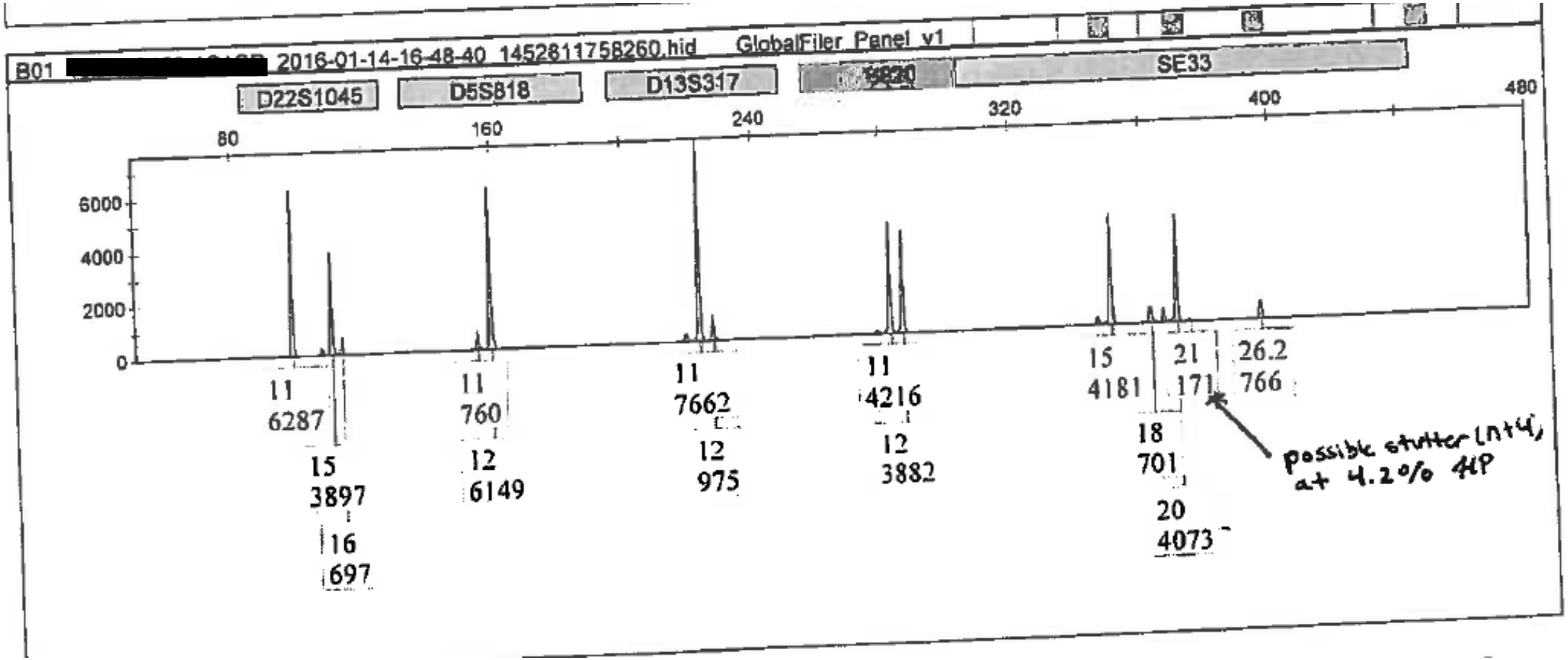
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D5S818
D13S317
SE33
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320
400
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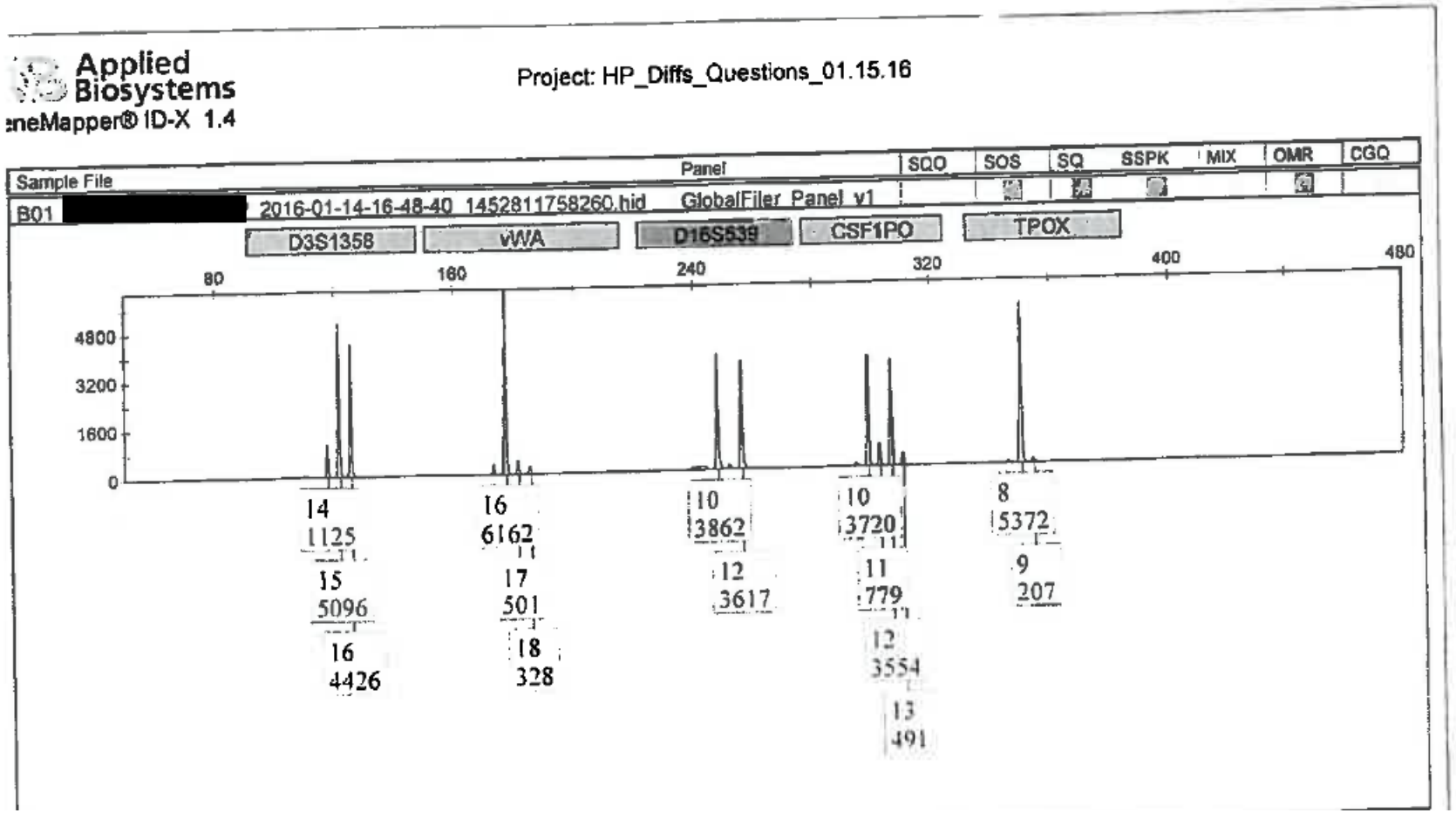
4000
2000
0
11
6287
15
3897
16
697
11
760
12
6149
11
7662
12
975
11
4216
12
3882
15
4181
18
701
20
4073
21
171
26.2
766
possible stutter (n+4) at 4.2% HP

Mixture Interpretation: Step 3

- Identify the Number of Potential Contributors
  - Relies on previous steps
  - Number of alleles at each locus
    - 5 alleles = at least 3 people
    - 7 alleles = at least 4 people, etc.
  - Peak height ratios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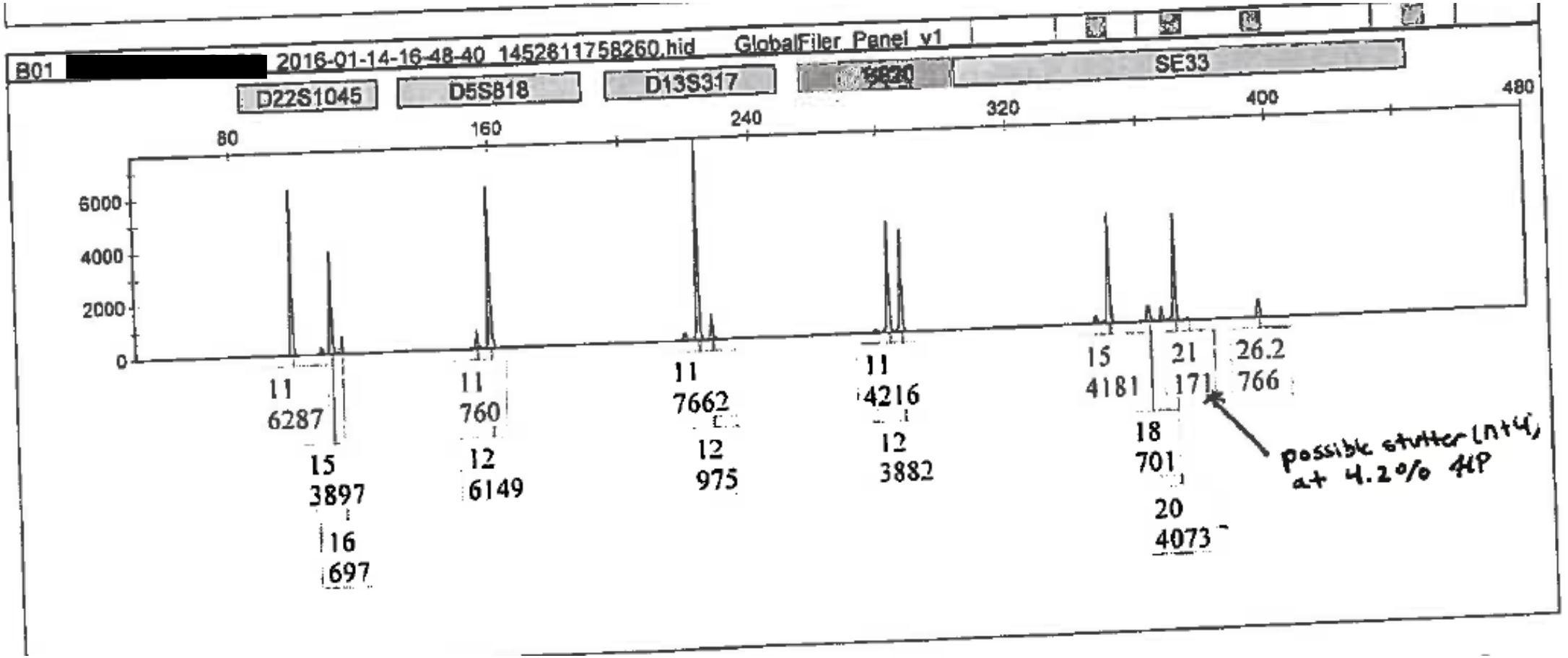
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D5S818
D13S317
SE33
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6287
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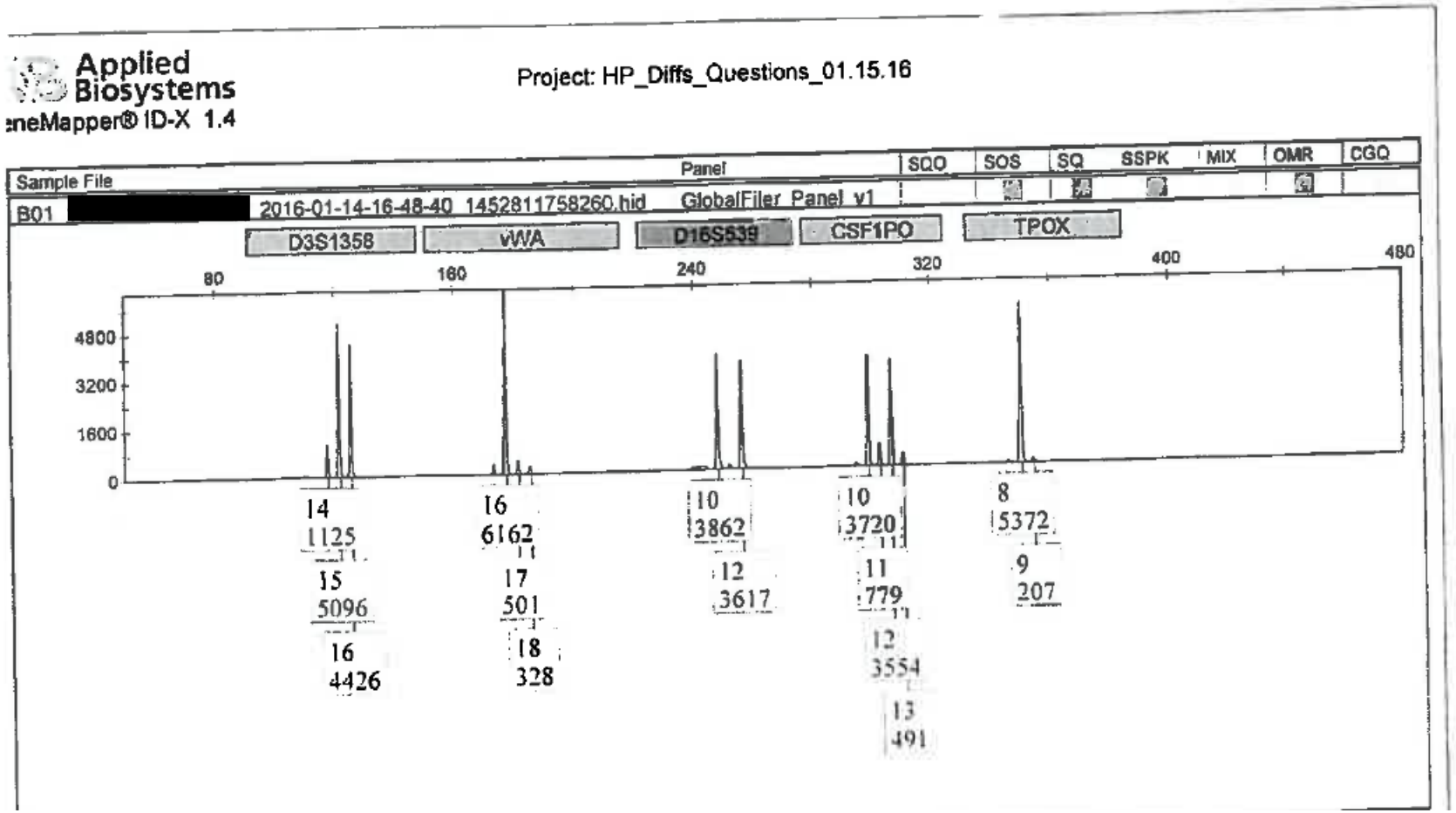
16
697
11
760
12
6149
11
7662
12
975
11
4216
12
3882
15
4181
18
701
20
4073
21
171
26.2
766
possible stutter (n+4) at 4.2% HP

Mixture Interpretation: Step 4

- Estimate the Relative Ratio of the Individuals Contributing to a Mixture
  - Relies on previous steps
  - Start at locus with no allelic overlap
  - Lab should establish set standards
  - Harder to calculate as the number of contributors increases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207

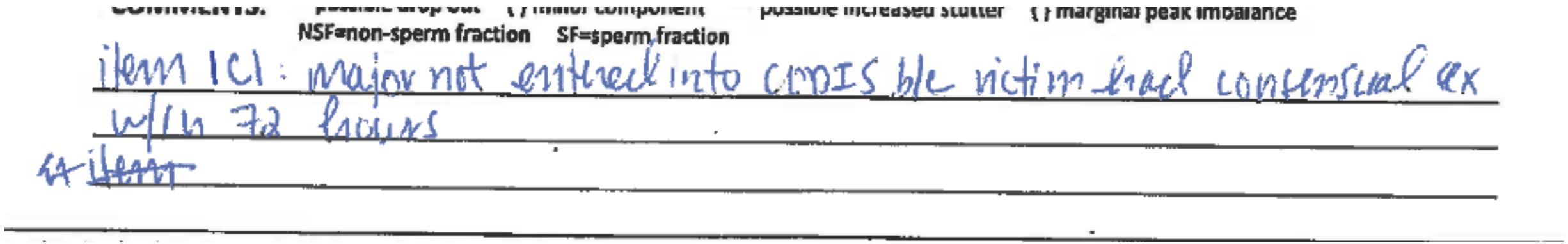
Mixture Interpretation: Step 5

- Compare mixture profile to reference profile
- ALL alleles in the reference profile should be present in the mixture profile, if that individual contributed to the mixture
- Calculate the CPI

Case Study: QW

D2S441	12,14 (11.3)	11.3,14
D19S433	11,12 (13,14)	12,14
TH01	7,9	7,9
FGA	22,24 (21)	21,22
D22S1045	11,15 (16)	11,16
D5S818	12 (11)	11,12
D13S317	11 (12)	11,12
D7S820	11,12	11,12
SE33	15,20 (18,26.2)	18,26.2
D10S1248	14 (15,16)	15,16
D1S1656	14,16.3 (13,15)	13,15
D12S391	17,18 (19)	18,19
D2S1338	20	19,20

Case Study: QW



Case Study: QW

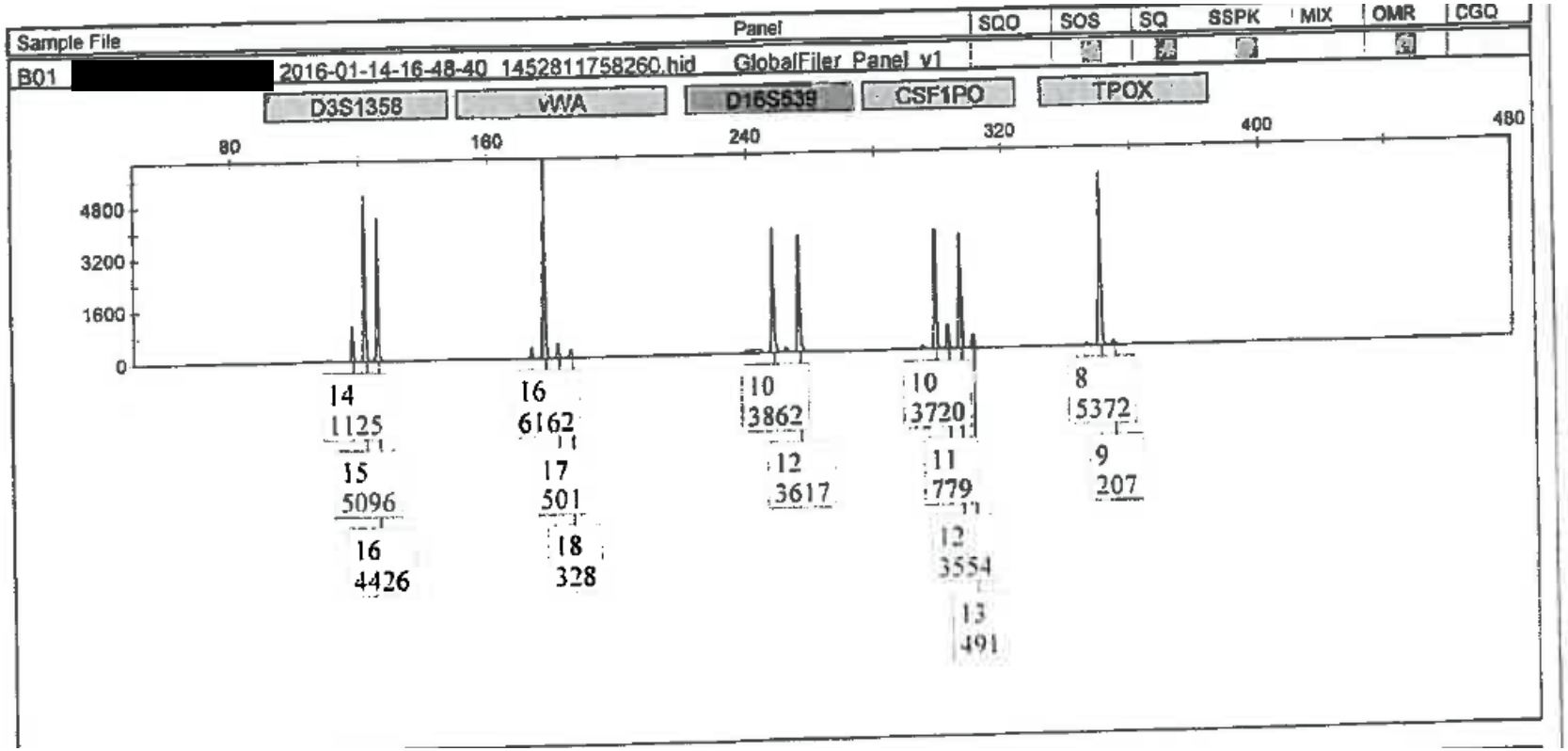
TPOX	9	1.3750E-01	1.2740E-01	1.9590E-01	9.3200E-02	7.7300E-02
AMELOGENIN	X	N/A	N/A	N/A	N/A	N/A
AMELOGENIN	Y	N/A	N/A	N/A	N/A	N/A
D8S1179	13	2.6830E-01	3.2960E-01	2.1930E-01	2.7330E-01	2.0100E-01
D8S1179	14	2.3360E-01	1.6620E-01	2.9390E-01	2.6270E-01	2.0100E-01
D8S1179	15	1.4040E-01	1.0390E-01	1.9010E-01	1.2920E-01	1.2890E-01
D21S11	28	1.6460E-01	1.5930E-01	2.4560E-01	9.9600E-02	5.6700E-02
D21S11	30	2.4760E-01	2.8250E-01	1.6950E-01	2.7330E-01	3.2990E-01
D21S11	31.2	7.7200E-02	9.8300E-02	5.1200E-02	9.9600E-02	3.6100E-02
D18S51	12	9.4100E-02	1.1360E-01	7.6000E-02	1.1440E-01	3.6100E-02
D18S51	17	1.3320E-01	1.3850E-01	1.5200E-01	1.2500E-01	6.7000E-02
D18S51	19	6.1300E-02	4.0200E-02	9.9400E-02	4.6600E-02	4.1200E-02
D2S441	11.3	4.8700E-02	6.0900E-02	4.3900E-02	4.4500E-02	3.0900E-02
D2S441	12	9.9400E-02	4.7100E-02	1.6520E-01	3.6000E-02	2.1650E-01
D2S441	14	2.2680E-01	2.4100E-01	2.6750E-01	2.0550E-01	8.2500E-02
D19S433	11	2.6100E-02	6.9000E-03(M)	6.2900E-02	1.4800E-02	2.5800E-02(M)
D19S433	12	8.3500E-02	7.0600E-02	1.2280E-01	6.5700E-02	3.6100E-02
D19S433	13	2.4710E-01	2.5480E-01	2.4560E-01	2.2250E-01	2.8350E-01
D19S433	14	3.0410E-01	3.6150E-01	2.1050E-01	3.5380E-01	2.9900E-01
TH01	7	2.9490E-01	1.9390E-01	4.0790E-01	2.9660E-01	2.6800E-01
TH01	9	1.6890E-01	1.1910E-01	1.5940E-01	1.4620E-01	4.4330E-01
FGA	21	1.4720E-01	1.7870E-01	1.2280E-01	1.5250E-01	1.0310E-01
FGA	22	1.9740E-01	2.0500E-01	1.9880E-01	1.6530E-01	2.4230E-01
FGA	24	1.3710E-01	1.3430E-01	1.3300E-01	1.4190E-01	1.4950E-01
D22S1045	11	1.2980E-01	1.3990E-01	1.4470E-01	6.3600E-02	2.0100E-01
D22S1045	15	3.2090E-01	3.2130E-01	2.5150E-01	4.2580E-01	3.0930E-01
D22S1045	16	2.9730E-01	3.8230E-01	1.9150E-01	3.4960E-01	2.2680E-01
D5S818	11	3.1520E-01	3.5600E-01	2.3390E-01	3.8980E-01	2.6800E-01
D5S818	12	3.5380E-01	3.8780E-01	3.6990E-01	3.3900E-01	2.0620E-01
D13S317	11	2.9050E-01	3.2550E-01	3.0990E-01	2.1820E-01	2.6800E-01
D13S317	12	3.0450E-01	2.6870E-01	4.1810E-01	2.3520E-01	2.0620E-01
D7S820	11	2.3550E-01	2.0500E-01	2.0320E-01	2.7750E-01	3.6080E-01
D7S820	12	1.3610E-01	1.5930E-01	8.7700E-02	1.5470E-01	1.7530E-01
SE33	15	3.7600E-02	4.0200E-02	4.3900E-02	3.6000E-02	2.5800E-02(M)

Omitted from stat:
- DaS1338

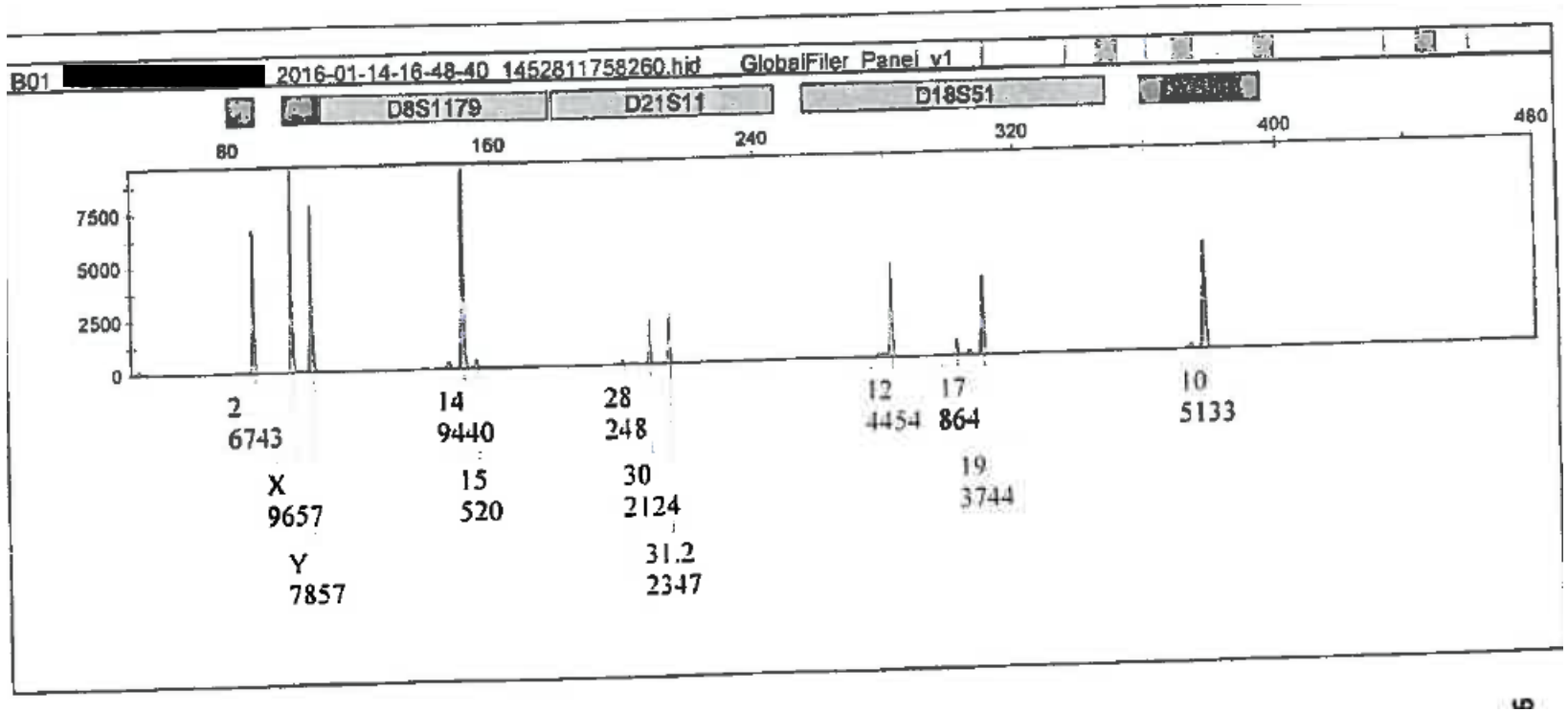
filtered stutter peaks added to stat:
- 15 @ vWA
- 13 @ D8
- 21 @ SE33
- 13 @ D10

Stutter peaks approximately same peak height as minor component ~~4 peaks~~ alleles

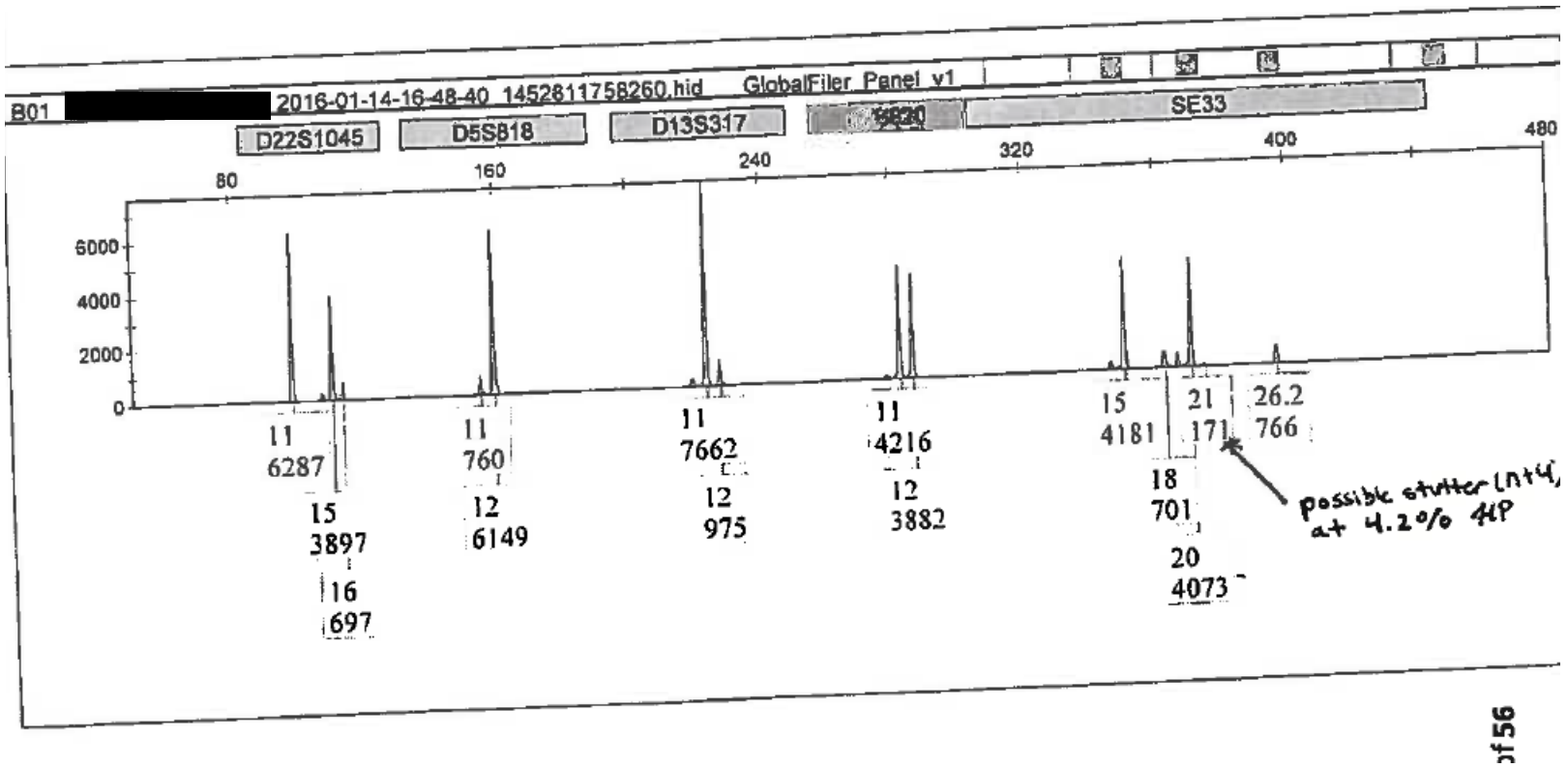
Case Study: QW



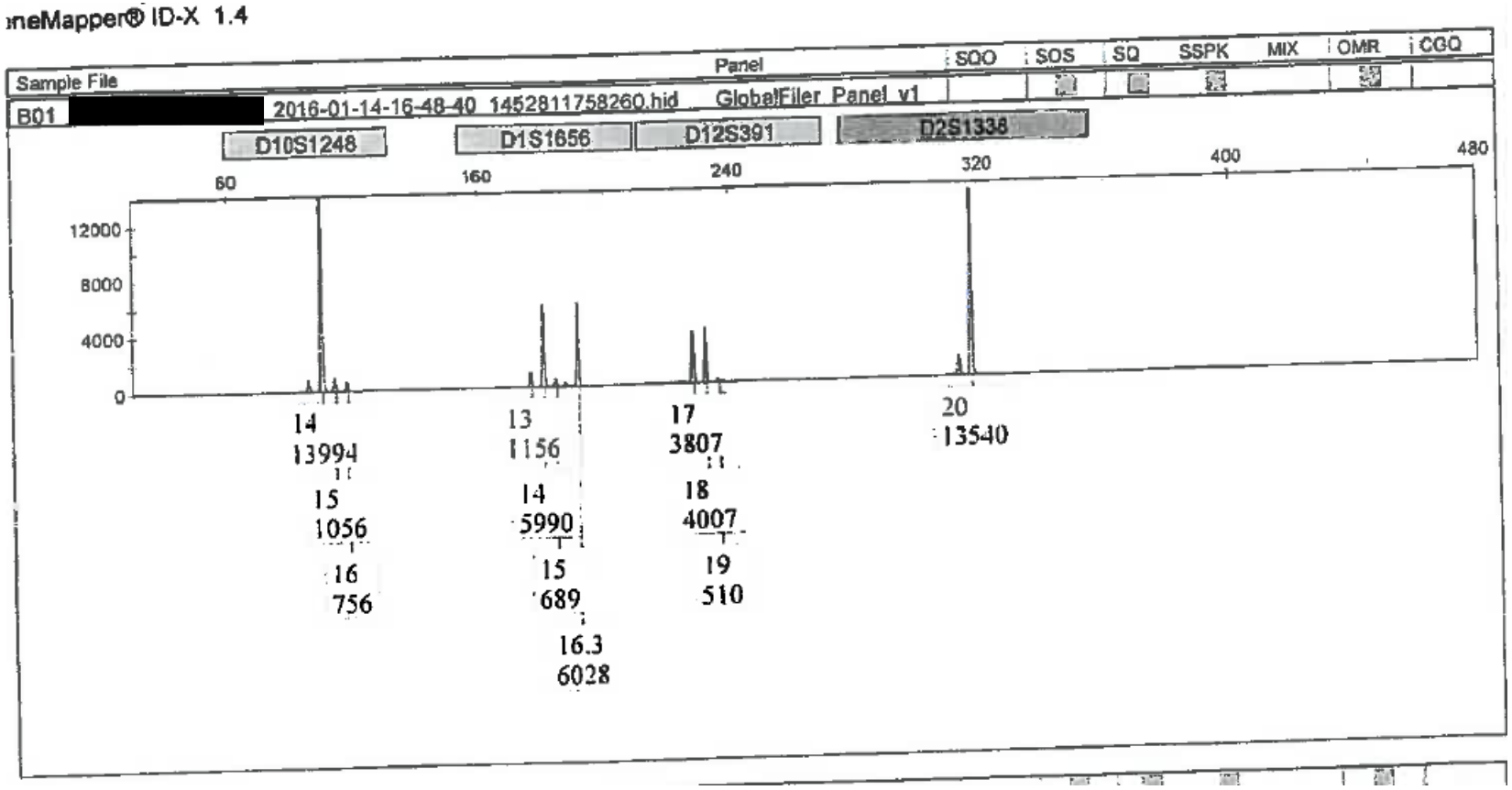
Case Study: QW



Case Study: QW



Case Study: QW



Resources

- National Institute of Standards and Technology (NIST)
  - https://www.nist.gov/topics/dna-biological-evidence
- Scientific Working Group on DNA Analysis Methods (SWGDAM)
  - https://www.swgdam.org/