Probabilistic Genotyping:
A Perspective from the Denver Crime Laboratory

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Why are we changing?

- 1/14/2010 SWGDAM Interpretation Guideline 4.6.3
- RMP, CPI, restricted CPI & $2p - p^2$
- Many DNA profiles not suitable for CPI, no conclusions given
- Problem: how to give statistical weight to complex mixtures with dropout and drop-in?
- Other steps of DNA testing optimized for maximum sensitivity
- End product is data that may appear probative, but current tools don’t allow statistical weight to be given

What prompted us to change?

- Communication with the NYC OCME laboratory about FST in late 2012
- April 2013 NIST webinar about DNA mixtures
- Examined the options available:
    - Lab Retriever
    - LRmix
    - TrueAllele®
    - STRMix™ was not available for purchase last year
- Downloaded the first two, which are both free and open source

Why Lab Retriever?

- Scientifically sound
- Produces quality results for single source, 2 & 3 person mixtures
- Availability and cost
- Supported by published scientific literature
- Formulas used are published & open source
- Ease of use
- Speed of analysis
- Training from developers
- Court admissibility support

Training

- DNA analysts (9 of us) read the following:
  - 2012. Lohmueller and Rudin, Calculating the weight of evidence in low-template forensic DNA casework
  - 2009. Balding and Buckleton, Interpreting low template DNA profiles
  - 2009. Tvedebrink et al., Estimating the probability of allelic drop-out of STR alleles in forensic genetics
  - 2012. Mitchell et al., Validation of a DNA mixture statistics tool incorporating allelic drop-out and drop-in

Training

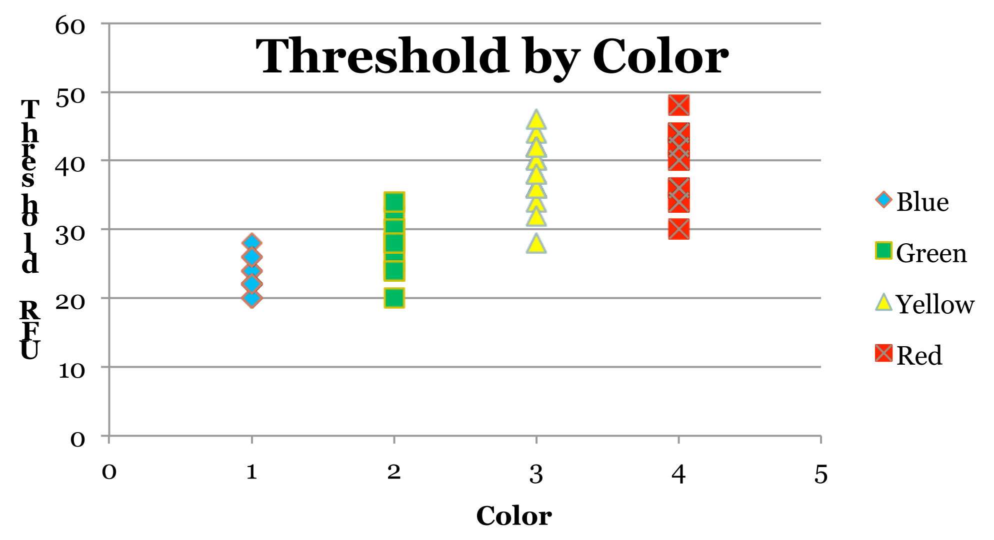
- Hosted 3-day on-site training with Norah Rudin, Keith Inman and by Skype Kirk Lohmueller
- Topics covered:
  - Analytical thresholds
  - Estimating probability of dropout
  - Construction of an LR with dropout
  - Preparing data and running Lab Retriever
  - Performing the calculations by hand
  - Stutter considerations
  - Each of the 9 analysts brought a real case to work
  - Internal validation
  - Reporting, court testimony

Validation

1. New color specific analytical thresholds to maximize sensitivity

   2012. Bregu et al., Analytical Thresholds and Sensitivity: Establishing RFU Thresholds for Forensic DNA Analysis

   2012. Rakay et al., Maximizing allele detection: Effects of analytical threshold and DNA levels on rates of allele and locus drop-out



Validation

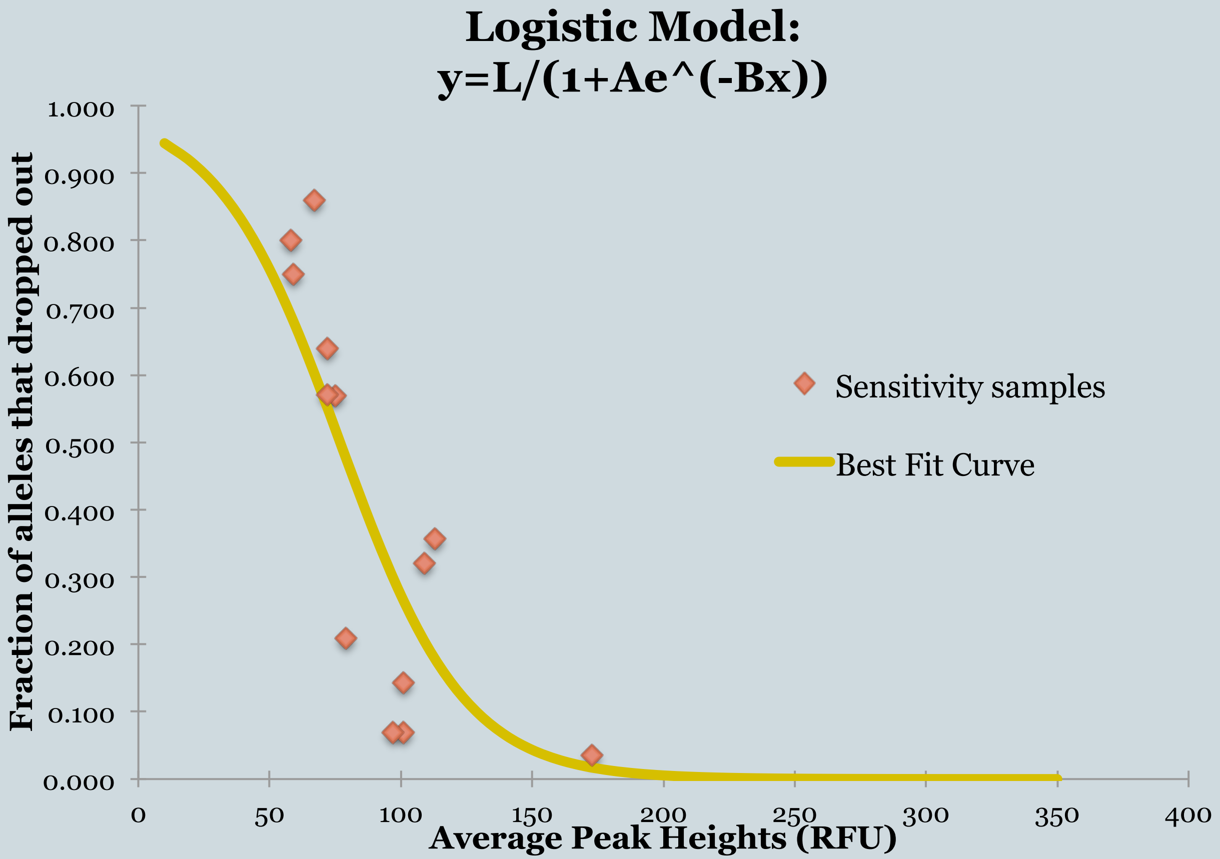
2. Create a probability of dropout curve using previous sensitivity validation data. Plot RFU of detected alleles against % dropout. Logistic regression in MS Excel.

Note 1: Can do all loci together or locus-specific curves

Note 2: Can use quantitation value or input DNA (pg) into the PCR instead of RFU.

Note 3: 2012. Tvedebrink et al., Statistical model for degraded DNA samples and adjusted probabilities for allelic drop-out.

Probability of dropout curve



Validation

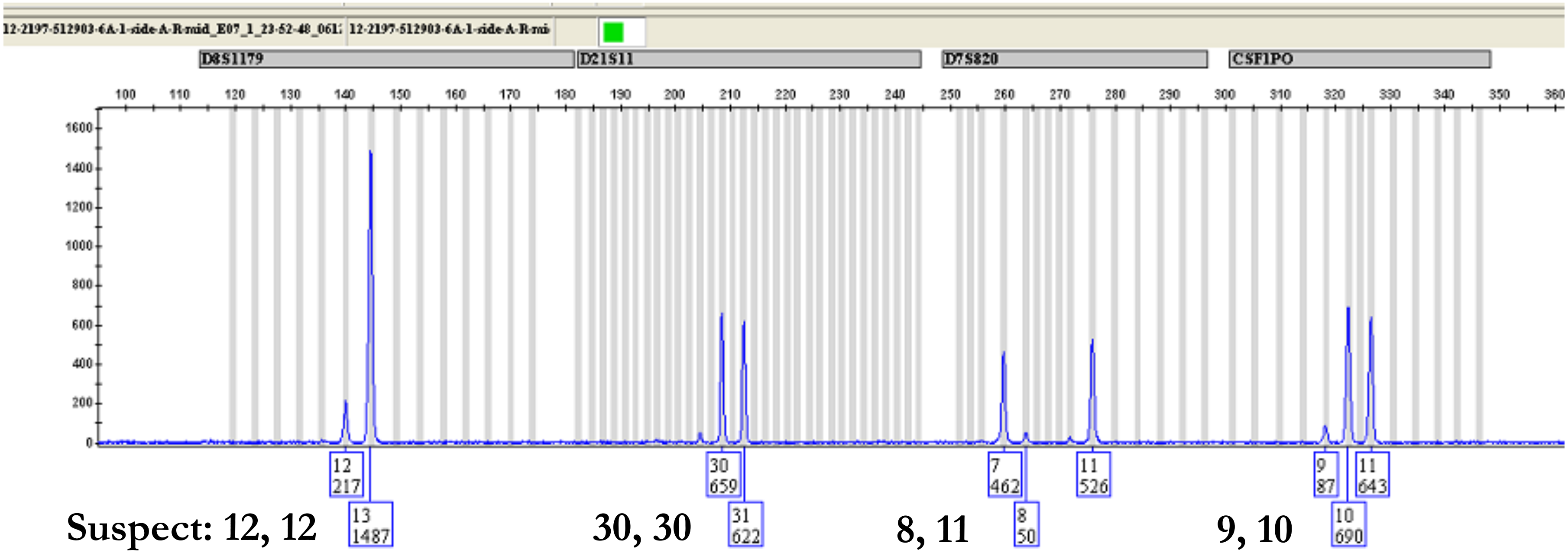
3. Validate drop-in rates (expected ≤1%)
4. Test DNA profiles in Lab Retriever to known contributors and <u>non</u>-contributors
5. Perform likelihood ratio calculations on 3-5 samples to verify calculations
6. Test major/minor mixtures with stutter filters on and off
7. Write SOP, reporting guidelines

Implementation

- Lab Retriever will be used
  - By all DNA analysts
  - For 2 & 3 person mixtures where major/minor cannot be interpreted
  - For future cases
- Selecting hypotheses can be challenging
- Limitations to Lab Retriever
  - Not currently capable of 2 simultaneous suspects, but can do 1 assumed, 1 unknown
  - How to treat possible masked alleles in stutter positions
  - Cannot calculate 4 & 5 person mixtures unless assumed contributor
  - Does not analyze peak heights as fully continuous models do

Case example

- Burglary/sex assault 5/20/2012
- Victim’s head covered with a sheet, bound, held at knifepoint
- DNA evidence – bedding, scarf, stocking cap and nail scrapings

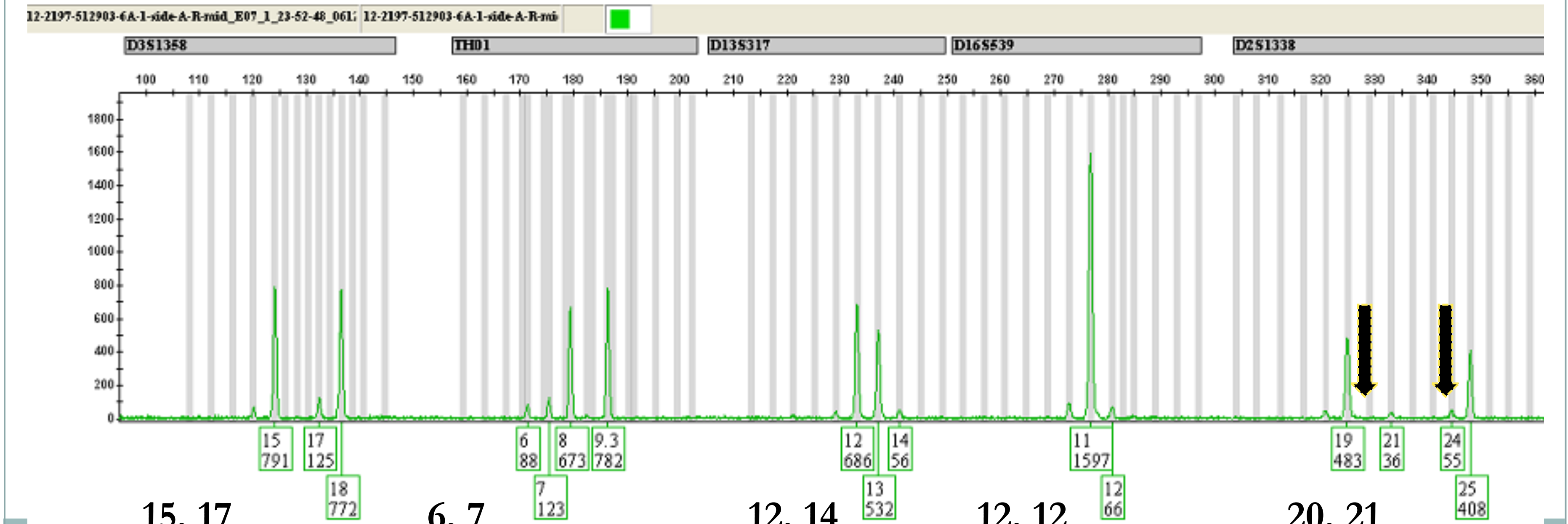


Suspect: 12, 12

30, 30

8, 11

9, 10



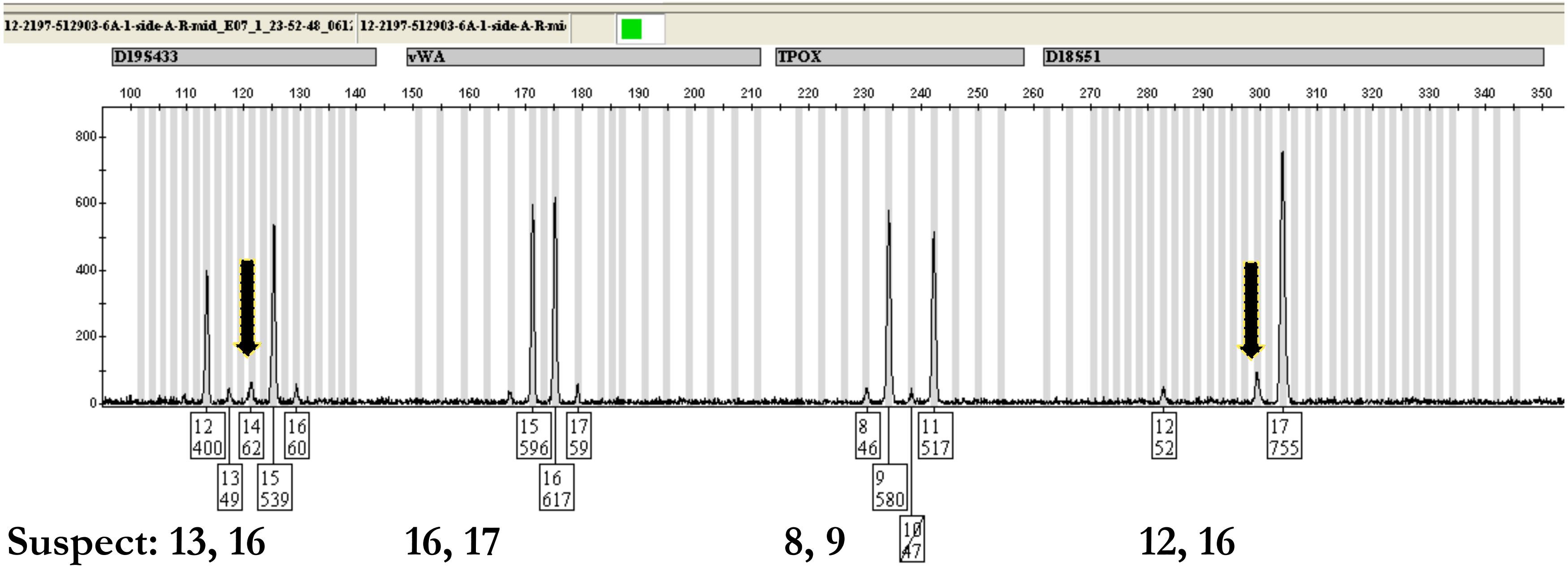
15, 17

6, 7

12, 14

12, 12

20, 21

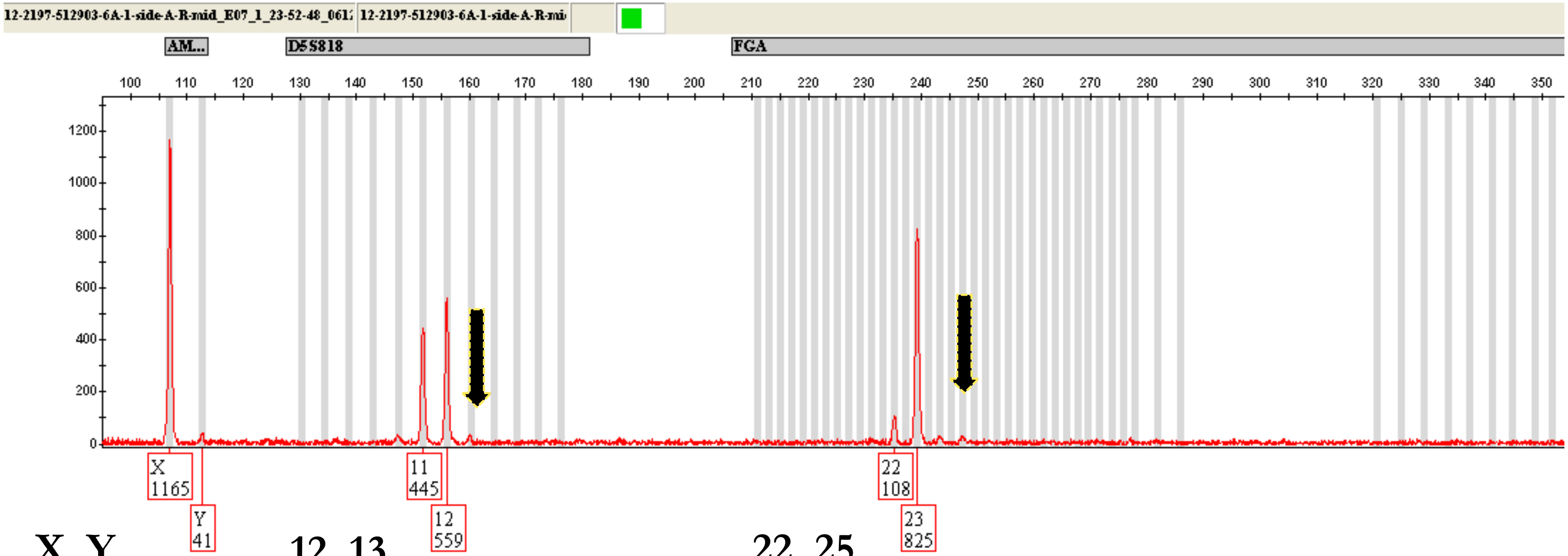


Suspect: 13, 16

16, 17

8, 9

12, 16



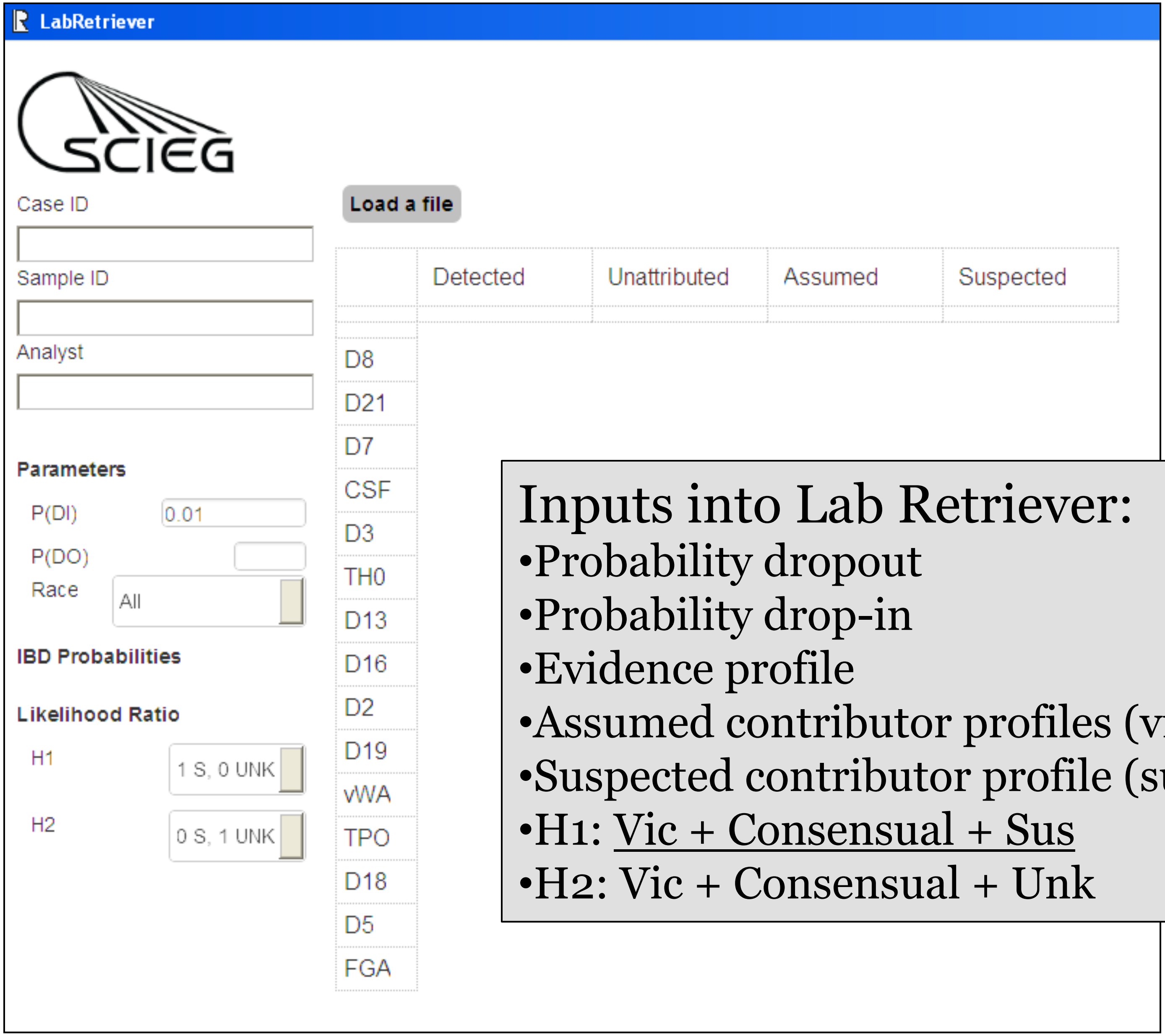
X, Y

12, 13

22, 25

Lab Retriever Analysis

Locus	**6A-1 Comforter (RFU)**
D8S1179	217
D7S820	50
CSF1PO	87
D3S1358	125
TH01	88
TH01	123
D13S317	56
D16S539	66
D2S1338	36
D19S433	49
D19S433	60
vWA	59
TPOX	46
D18S51	52
FGA	108
	81.5 AVE



Case ID

Sample ID

Analyst

Parameters

P(DI) 0.01

P(DO)

Race All

IBD Probabilities

Likelihood Ratio

H1 1 S, 0 UNK

H2 0 S, 1 UNK

	Detected	Unattributed	Assumed	Suspected
D8				
D21				
D7				
CSF				
D3				
TH0				
D13				
D16				
D2				
D19				
vWA				
TPO				
D18				
D5				
FGA				

Inputs into Lab Retriever:

- Probability dropout
- Probability drop-in
- Evidence profile
- Assumed contributor profiles (victim + CP)
- Suspected contributor profile (suspect)
- H1: Vic + Consensual + Sus
- H2: Vic + Consensual + Unk

Locus	Detected – 6A1 Comforter	Assumed - Victim + Consensual	Unattributed	Suspected - Suspect	AA	CAU	HIS
D8	12 13	13	12	12	9.2	5.9	8.2
D21	30 31	30 31		30	3.4	2.5	2.6
D7	7 8 11	11 7	8	8 11	2.9	5.1	5.6
CSF	9 10 11	10 11	9	9 10	18.0	39.0	26.4
D3	15 17 18	15 18	17	15 17	3.8	3.3	3.6
TH0	6 7 8 9.3	9.3 8	6 7	6 7	8.9	10.5	7.9
D13	12 13 14	12 13 14		12 14	1.2	1.6	2.1
D16	11 12	11 12		12	2.1	1.8	2.1
D2	19 21 24 25	19 24 25	21	20 21	1.0	2.9	3.2
D19	12 13 14 15 16	12 14 15	13 16	13 16	101.0	31.8	61.3
vWA	15 16 17	15 16 17		16 17	1.6	1.8	1.7
TPO	8 9 11	8 9 11		8 9	1.5	1.2	1.3
D18	12 17	17	12	12 16	12.8	8.5	9.2
D5	11 12	11 12		12 13	**0.4**	**0.2**	**0.3**
FGA	22 23	23	22	22 25	1.0	0.9	1.4
Final Likelihood Ratios					**1.42E+08**	**1.10E+08**	**6.07E+08**