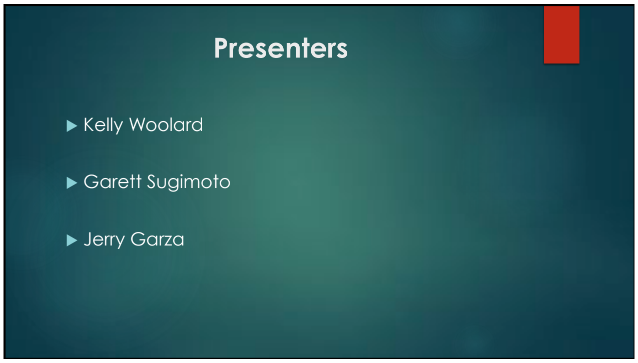
Kern Regional Crime Laboratory

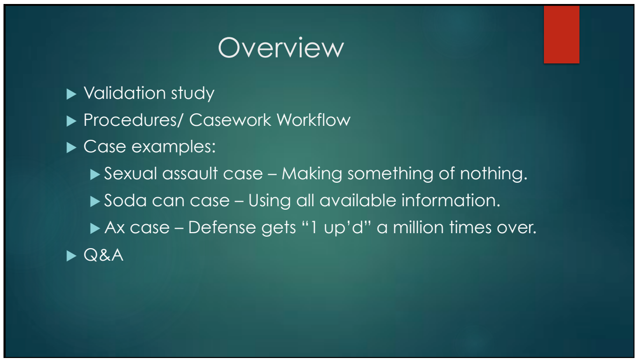
Laboratory Director: Dr. Kevin W. P. Miller

TRUEALLELE® WORK AND WORKFLOW:
KERN COUNTY'S FIRST CASES
APRIL 23, 2014



Presenters

- Kelly Woolard
- Garett Sugimoto
- Jerry Garza



Overview

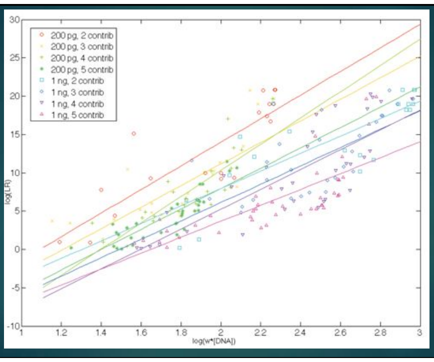
- Validation study
- Procedures/ Casework Workflow
- Case examples:
  - Sexual assault case – Making something of nothing.
  - Soda can case – Using all available information.
  - Ax case – Defense gets "1 up'd" a million times over.
- Q&A

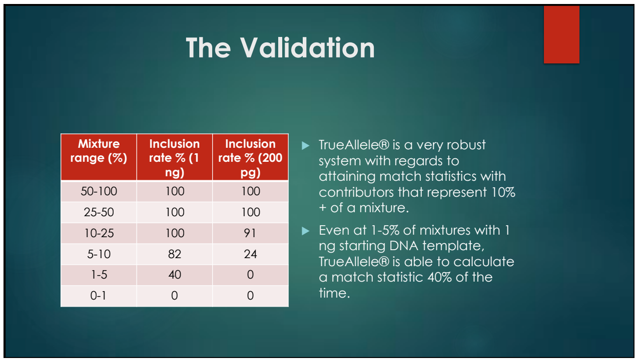
The Validation

- Reasons for validating the TrueAllele® system:
  - Is it more informative than manual interpretation?
  - Is it a more consistent method of mixture interpretation?
- Validation set up:
  - Mixtures were set up consisting of 2, 3, 4 and 5 contributors.
  - 10 mixtures were prepared per mixture group using 5 known references. Each of the mixtures within each mixture group were amplified with 1ng of DNA template and with 200 pg of DNA template.
  - The known references were chosen randomly as were the mixture ratios to better approximate casework samples.
  - 40 total mixtures.

The Validation

- Results:
  - TrueAllele® was a more informative, reproducible and consistent method of mixture interpretation.
  - TrueAllele® had little interpretation variance across the mixture samples containing 2-5 contributors and 200pg-1.0ng of DNA template. There were no significant differences in the regression line slopes between all the samples regardless of the number of contributors and target amount of DNA for amplification.

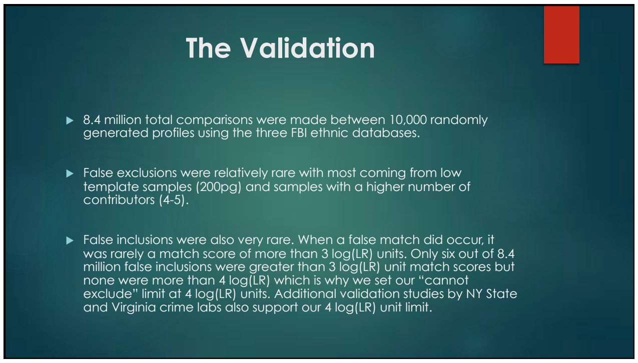




The Validation

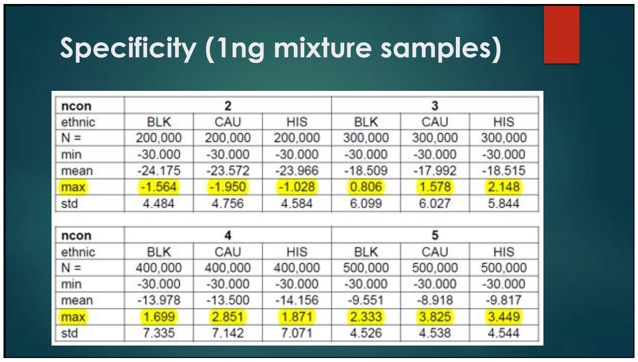
Mixture range (%)	Inclusion rate % (1 ng)	Inclusion rate % (200 pg)
50-100	100	100
25-50	100	100
10-25	100	91
5-10	82	24
1-5	40	0
0-1	0	0

- TrueAllele® is a very robust system with regards to attaining match statistics with contributors that represent 10% + of a mixture.
- Even at 1-5% of mixtures with 1 ng starting DNA template, TrueAllele® is able to calculate a match statistic 40% of the time.



The Validation

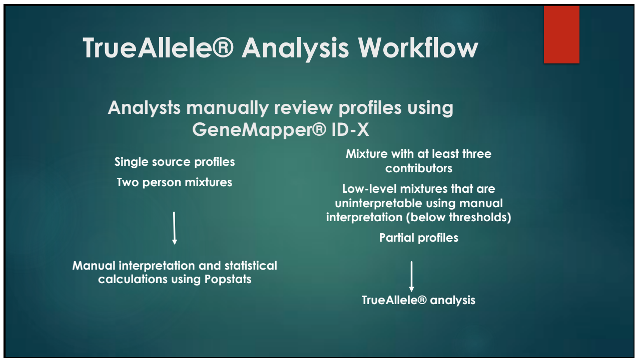
- 8.4 million total comparisons were made between 10,000 randomly generated profiles using the three FBI ethnic databases.
- False exclusions were relatively rare with most coming from low template samples (200pg) and samples with a higher number of contributors (4-5).
- False inclusions were also very rare. When a false match did occur, it was rarely a match score of more than 3 log(LR) units. Only six out of 8.4 million false inclusions were greater than 3 log(LR) unit match scores but none were more than 4 log(LR) which is why we set our "cannot exclude" limit at 4 log(LR) units. Additional validation studies by NY State and Virginia crime labs also support our 4 log(LR) unit limit.



Specificity (1ng mixture samples)

ncon	**2**			**3**		
ethnic	BLK	CAU	HIS	BLK	CAU	HIS
N =	200,000	200,000	200,000	300,000	300,000	300,000
min	-30.000	-30.000	-30.000	-30.000	-30.000	-30.000
mean	-24.175	-23.572	-23.966	-18.509	-17.992	-18.515
max	-1.564	-1.950	-1.028	0.806	1.578	2.148
std	4.484	4.756	4.584	6.099	6.027	5.844

ncon	**4**			**5**		
ethnic	BLK	CAU	HIS	BLK	CAU	HIS
N =	400,000	400,000	400,000	500,000	500,000	500,000
min	-30.000	-30.000	-30.000	-30.000	-30.000	-30.000
mean	-13.978	-13.500	-14.156	-9.551	-8.918	-9.817
max	1.699	2.851	1.871	2.333	3.825	3.449
std	7.335	7.142	7.071	4.526	4.538	4.544



TrueAllele® Analysis Workflow

Analysts manually review profiles using GeneMapper® ID-X

Single source profiles

Two person mixtures

↓

Manual interpretation and statistical calculations using Popstats

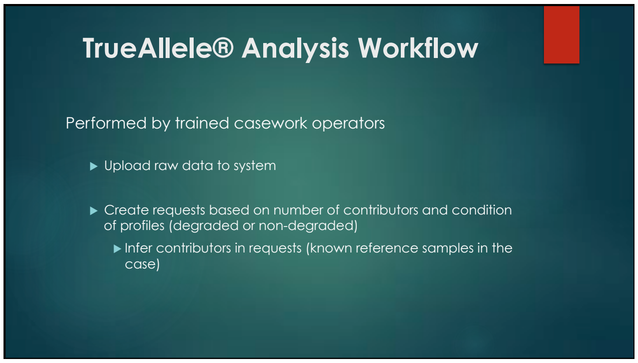
Mixture with at least three contributors

Low-level mixtures that are uninterpretable using manual interpretation (below thresholds)

Partial profiles

↓

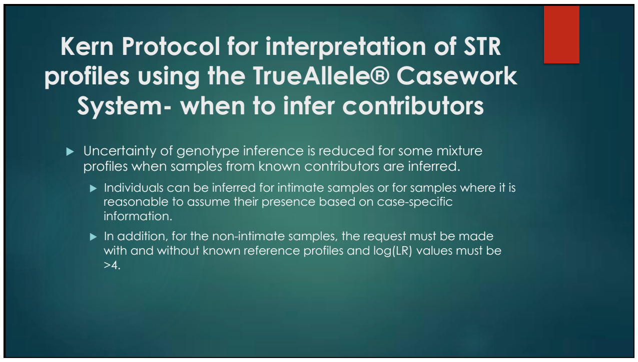
TrueAllele® analysis



TrueAllele® Analysis Workflow

Performed by trained casework operators

- Upload raw data to system
- Create requests based on number of contributors and condition of profiles (degraded or non-degraded)
    - Infer contributors in requests (known reference samples in the case)



Kern Protocol for interpretation of STR profiles using the TrueAllele® Casework System- when to infer contributors

- Uncertainty of genotype inference is reduced for some mixture profiles when samples from known contributors are inferred.
    - Individuals can be inferred for intimate samples or for samples where it is reasonable to assume their presence based on case-specific information.
    - In addition, for the non-intimate samples, the request must be made with and without known reference profiles and log(LR) values must be >4.

Kern Regional Crime Laboratory Interpretation Protocol (Naming Requests)

- Example: 12CL12345_D9947X_Q_ncon3_D_100K+D9948X_copy
- When copy requests are made, leave the "_copy" at the end of the request name

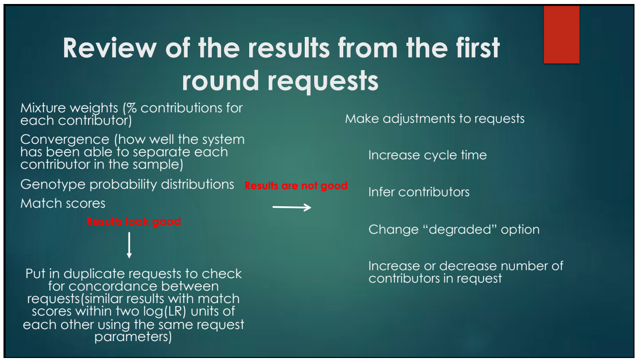
YYCL#####	**Laboratory Number**
X####X	**Unique ID number (DNA number)**
Q/K	**Questioned or known item**
Ncon#	**Number of assumed contributors**
N/D	**X-degraded option off** **D- degraded option on**
###K	**Number of cycles, K= thousand**
+X####X	**DNA number(s) of known reference profiles inferred to the mixture**

Processing Time

- Depends on several factors
  - Number of processors
  - Type of request (degraded vs. non-degraded)
  - Number of cycles
  - Number of contributors in the mixture
  - Nature of the evidence sample
  - Total number of samples and requests made in the case

Approximate Request Time

- 500 cycles **~15** minutes (known reference samples)
- 50,000 cycles **~6 to 12** hours (two person mixtures)
- 100,000 cycles **~1** day (three person mixtures and above)
- 200,000 cycles **~2+** days (nasty three person mixtures and above or difficult samples)



Review of the results from the first round requests

Mixture weights (% contributions for each contributor)

Convergence (how well the system has been able to separate each contributor in the sample)

Genotype probability distributions

Match scores

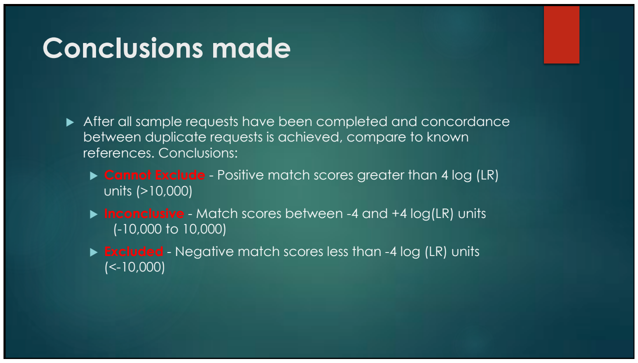
Results look good

Put in duplicate requests to check for concordance between requests(similar results with match scores within two log(LR) units of each other using the same request parameters)

Results are not good →

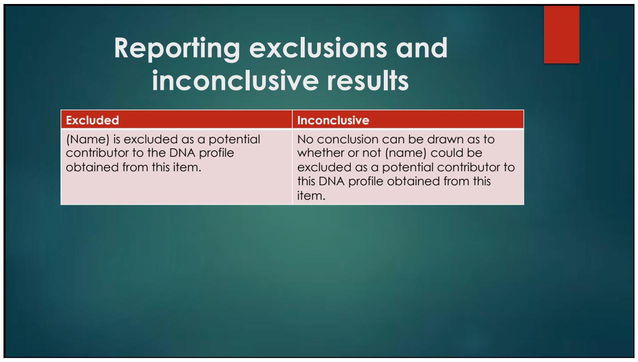
Make adjustments to requests

- Increase cycle time
- Infer contributors
- Change "degraded" option
- Increase or decrease number of contributors in request



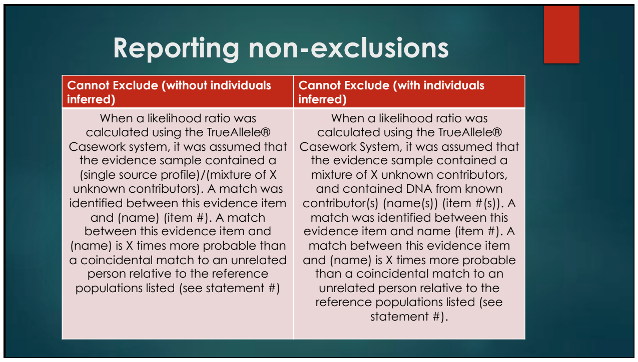
Conclusions made

- After all sample requests have been completed and concordance between duplicate requests is achieved, compare to known references. Conclusions:
  - **Cannot Exclude** - Positive match scores greater than 4 log (LR) units (>10,000)
  - **Inconclusive** - Match scores between -4 and +4 log(LR) units (-10,000 to 10,000)
  - **Excluded** - Negative match scores less than -4 log (LR) units (<-10,000)



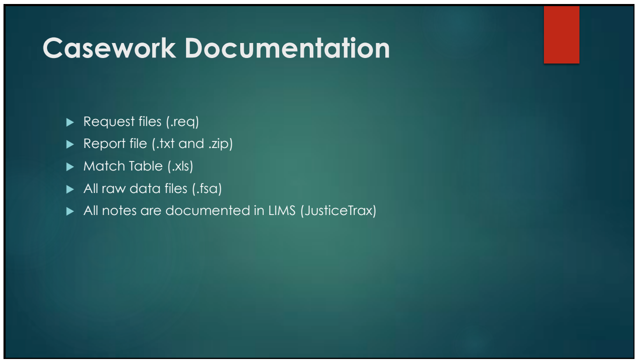
Reporting exclusions and inconclusive results

Excluded	Inconclusive
(Name) is excluded as a potential contributor to the DNA profile obtained from this item.	No conclusion can be drawn as to whether or not (name) could be excluded as a potential contributor to this DNA profile obtained from this item.



Reporting non-exclusions

Cannot Exclude (without individuals inferred)	Cannot Exclude (with individuals inferred)
When a likelihood ratio was calculated using the TrueAllele® Casework system, it was assumed that the evidence sample contained a (single source profile)/(mixture of X unknown contributors). A match was identified between this evidence item and (name) (item #). A match between this evidence item and (name) is X times more probable than a coincidental match to an unrelated person relative to the reference populations listed (see statement #)	When a likelihood ratio was calculated using the TrueAllele® Casework System, it was assumed that the evidence sample contained a mixture of X unknown contributors, and contained DNA from known contributor(s) (name(s)) (item #(s)). A match was identified between this evidence item and name (item #). A match between this evidence item and (name) is X times more probable than a coincidental match to an unrelated person relative to the reference populations listed (see statement #).



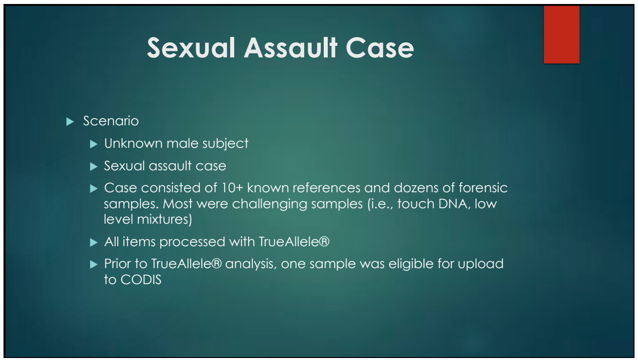
Casework Documentation

- Request files (.req)
- Report file (.txt and .zip)
- Match Table (.xls)
- All raw data files (.fsa)
- All notes are documented in LIMS (JusticeTrax)

JusticeTrax® Meets TrueAllele®

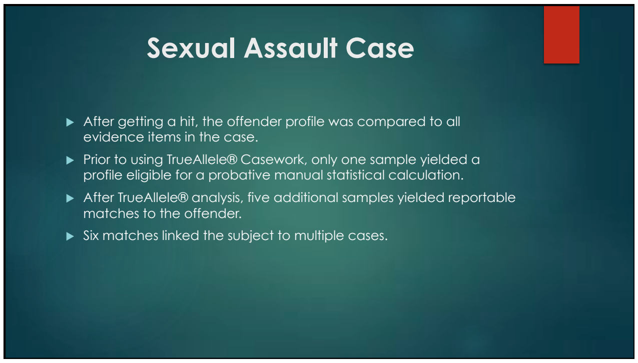
Case Request(0001) - Examination Results
- 001 Sealed bag labeled as - ME1
  - 001.A One (1) aluminum can labeled - LO-CARB MONSTER ENERGY
    - 001.A.01 Cutting from swab of upper portion of aluminum can housed in...

RequestName	Date	MixtureWeightsSimilar	GenotypeConcordant	Convergence	StandardDeviation
		-Select-	-Select-		



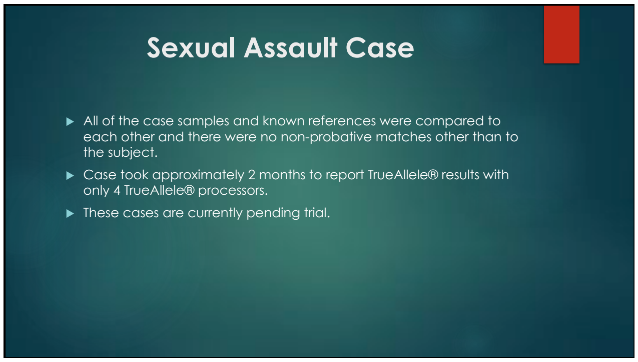
Sexual Assault Case

- Scenario
  - Unknown male subject
  - Sexual assault case
  - Case consisted of 10+ known references and dozens of forensic samples. Most were challenging samples (i.e., touch DNA, low level mixtures)
  - All items processed with TrueAllele®
  - Prior to TrueAllele® analysis, one sample was eligible for upload to CODIS



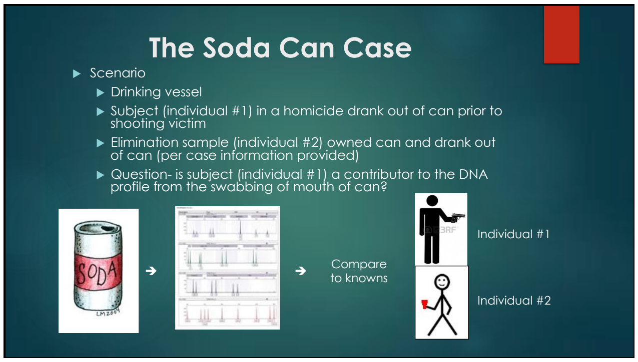
Sexual Assault Case

- After getting a hit, the offender profile was compared to all evidence items in the case.
- Prior to using TrueAllele® Casework, only one sample yielded a profile eligible for a probative manual statistical calculation.
- After TrueAllele® analysis, five additional samples yielded reportable matches to the offender.
- Six matches linked the subject to multiple cases.



Sexual Assault Case

- All of the case samples and known references were compared to each other and there were no non-probative matches other than to the subject.
- Case took approximately 2 months to report TrueAllele® results with only 4 TrueAllele® processors.
- These cases are currently pending trial.



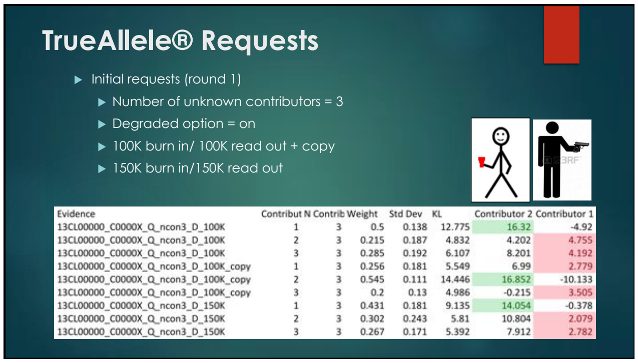
The Soda Can Case

- Scenario
  - Drinking vessel
  - Subject (individual #1) in a homicide drank out of can prior to shooting victim
  - Elimination sample (individual #2) owned can and drank out of can (per case information provided)
  - Question- is subject (individual #1) a contributor to the DNA profile from the swabbing of mouth of can?

➔ ➔ Compare to knowns

Individual #1

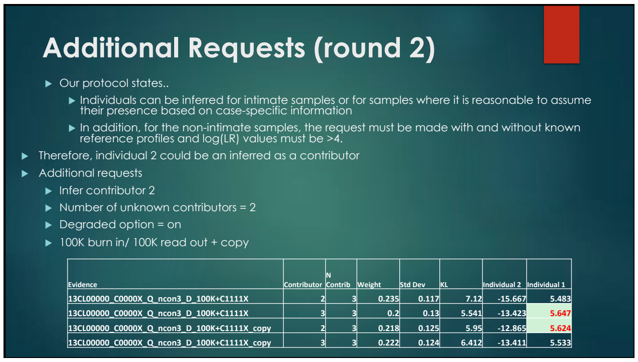
Individual #2



TrueAllele® Requests

- Initial requests (round 1)
  - Number of unknown contributors = 3
  - Degraded option = on
  - 100K burn in/ 100K read out + copy
  - 150K burn in/150K read out

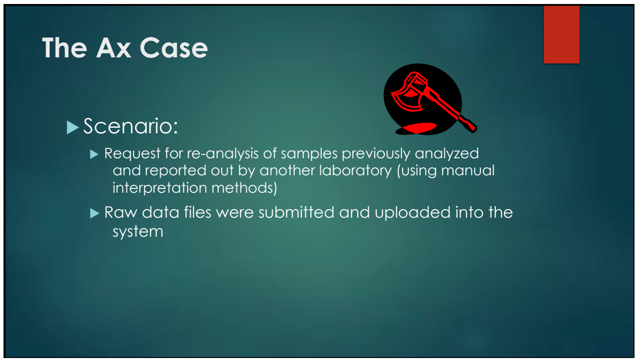
Evidence	Contribut	N Contrib	Weight	Std Dev	KL	Contributor 2	Contributor 1
13CL00000_C0000X_Q_ncon3_D_100K	1	3	0.5	0.138	12.775	16.32	-4.92
13CL00000_C0000X_Q_ncon3_D_100K	2	3	0.215	0.187	4.832	4.202	4.755
13CL00000_C0000X_Q_ncon3_D_100K	3	3	0.285	0.192	6.107	8.201	4.192
13CL00000_C0000X_Q_ncon3_D_100K_copy	1	3	0.256	0.181	5.549	6.99	2.779
13CL00000_C0000X_Q_ncon3_D_100K_copy	2	3	0.545	0.111	14.446	16.852	-10.133
13CL00000_C0000X_Q_ncon3_D_100K_copy	3	3	0.2	0.13	4.986	-0.215	3.505
13CL00000_C0000X_Q_ncon3_D_150K	1	3	0.431	0.181	9.135	14.054	-0.378
13CL00000_C0000X_Q_ncon3_D_150K	2	3	0.302	0.243	5.81	10.804	2.079
13CL00000_C0000X_Q_ncon3_D_150K	3	3	0.267	0.171	5.392	7.912	2.782



Additional Requests (round 2)

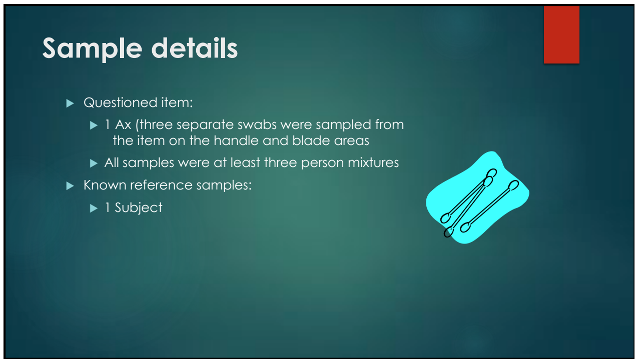
  - Our protocol states..
    - Individuals can be inferred for intimate samples or for samples where it is reasonable to assume their presence based on case-specific information
    - In addition, for the non-intimate samples, the request must be made with and without known reference profiles and log(LR) values must be >4.
- Therefore, individual 2 could be an inferred as a contributor
- Additional requests
  - Infer contributor 2
  - Number of unknown contributors = 2
  - Degraded option = on
  - 100K burn in/ 100K read out + copy

Evidence	Contributor	N Contrib	Weight	Std Dev	KL	Individual 2	Individual 1
13CL00000_C0000X_Q_ncon3_D_100K+C1111X	2	3	0.235	0.117	7.12	-15.667	5.483
13CL00000_C0000X_Q_ncon3_D_100K+C1111X	3	3	0.2	0.13	5.541	-13.423	5.647
13CL00000_C0000X_Q_ncon3_D_100K+C1111X_copy	2	3	0.218	0.125	5.95	-12.865	5.624
13CL00000_C0000X_Q_ncon3_D_100K+C1111X_copy	3	3	0.222	0.124	6.412	-13.411	5.533



The Ax Case

- Scenario:
  - Request for re-analysis of samples previously analyzed and reported out by another laboratory (using manual interpretation methods)
  - Raw data files were submitted and uploaded into the system

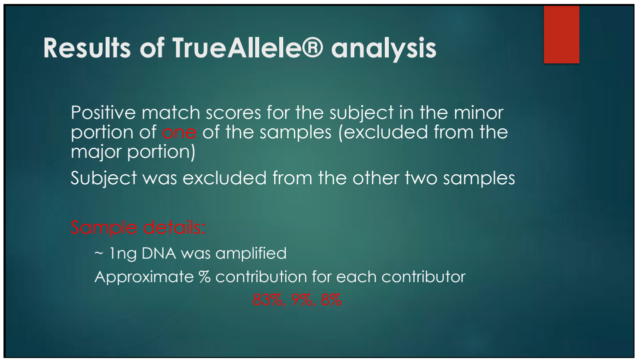


Sample details

- Questioned item:
  - 1 Ax (three separate swabs were sampled from the item on the handle and blade areas
  - All samples were at least three person mixtures
- Known reference samples:
  - 1 Subject

Requests

- Three person requests at 100,000 cycles non-degraded
- Three person requests at 100,000 cycles degraded*
- Four person requests at 100,000 cycles non-degraded
- Four person requests at 100,000 cycles degraded



Results of TrueAllele® analysis

Positive match scores for the subject in the minor portion of one of the samples (excluded from the major portion)

Subject was excluded from the other two samples

Sample details:

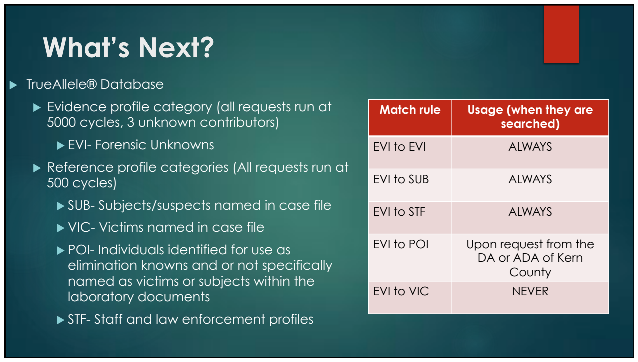
~ 1ng DNA was amplified

Approximate % contribution for each contributor

83%, 9%, 8%

Comparison of results

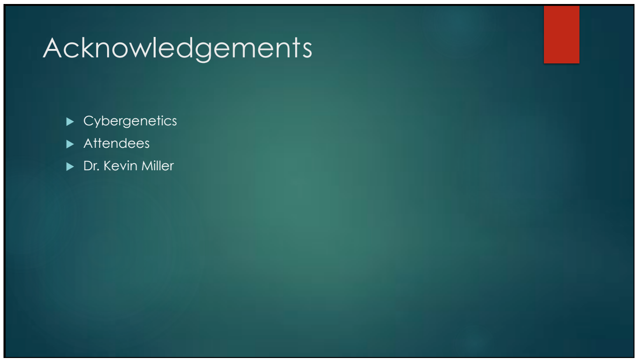
Manual Interpretation	TrueAllele® Analysis
Best statistic in a population group:	Best statistic in a population group:
1 in 8	2.4 Million



What's Next?

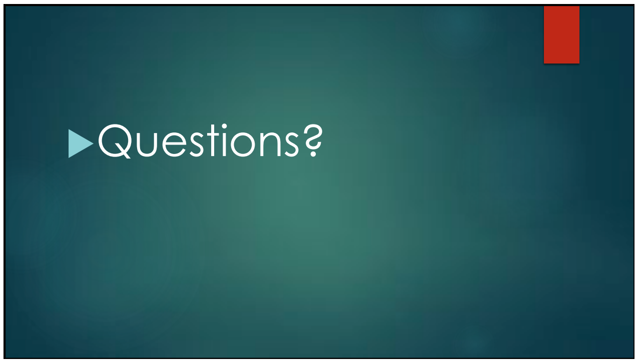
- TrueAllele® Database
  - Evidence profile category (all requests run at 5000 cycles, 3 unknown contributors)
    - EVI- Forensic Unknowns
  - Reference profile categories (All requests run at 500 cycles)
    - SUB- Subjects/suspects named in case file
    - VIC- Victims named in case file
    - POI- Individuals identified for use as elimination knowns and or not specifically named as victims or subjects within the laboratory documents
    - STF- Staff and law enforcement profiles

Match rule	Usage (when they are searched)
EVI to EVI	ALWAYS
EVI to SUB	ALWAYS
EVI to STF	ALWAYS
EVI to POI	Upon request from the DA or ADA of Kern County
EVI to VIC	NEVER



Acknowledgements

- Cybergenetics
- Attendees
- Dr. Kevin Miller



- Questions?