Internal validation of the MiSeq FGx Forensic Genomics System: Practical considerations and lessons learned

Bill Hudlow
California Department of Justice

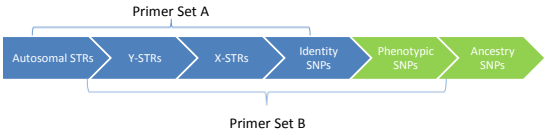
Disclaimer

- The following is a summary of the preliminary results from our internal validation, which is work in progress at the California Department of Justice DNA laboratory. While all validation runs have been completed the data review process is ongoing.
- All studies were conducted as indicated in the ForenSeq Signature Prep Reference Guide with the exception of diluting samples in TE^{-4} buffer instead of water.

Why do we need next generation sequencing?

- Despite the vast array of CE kits available, we sometimes need more…
  - More markers including SNPS!!!
  - More loci (e.g. >24 autosomal STRs)!
  - More reduced size amplicons!
  - More detailed locus information!
  - More nucleic acids, including RNA!

ForenSeq Signature Prep Kit



The ForenSeq kit allows for the simultaneous typing of more than 200 loci that generally have reduced amplicon sizes as compared to their CE counterparts!

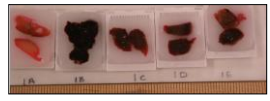
ForenSeq DNA Signature Prep Kit

- Practical Consideration #1, is it cost efficient?
  - Primer Mix A with a 96 sample run without consumables ~$54/sample
    - ~36¢/locus
  - Primer Mix B with a 32 sample run without consumables ~$85/sample
    - ~36¢/locus
  - GlobalFiler list price of $20,000/1,000 reaction kit without consumables ~$20/sample
    - ~83¢/locus
  - YFiler Plus list price of $3,320/100 reaction kit without consumables ~$33/sample
    - $1.22/locus

Missing Persons DNA Program Samples

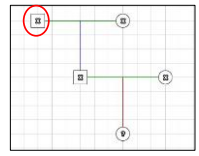
Human Remains

- Generally subjected to one or more types of environmental abuse



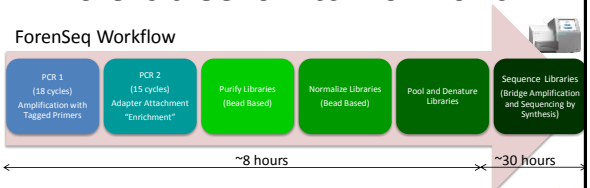
Reference Samples

- Primary/secondary references and reference samples from immediate relatives are not available

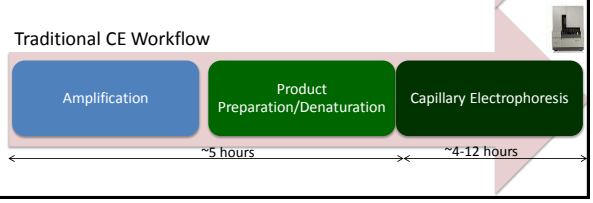


Forensic Genomics Workflows

ForenSeq Workflow

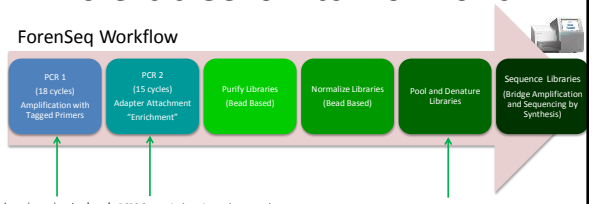


Traditional CE Workflow



Forensic Genomics Workflows

ForenSeq Workflow

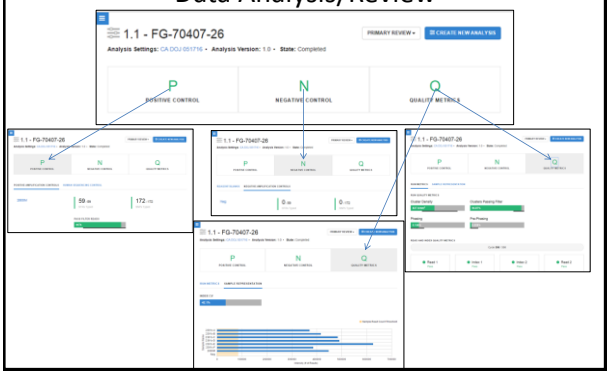


5 uL maximum input volume!
Practical Consideration #2
Is this appropriate for my extraction process and how will I concentrate my samples, if necessary?

PCR2 Set-up is done in product room!
Practical Consideration #3
Do I have dedicated equipment and/or space for this?

8-96 libraries can be pooled and decreasing the number of libraries in the pool will increase the depth of coverage
Practical Consideration #4
Do I need to have different work flows for high quality reference samples and low quality evidence samples?

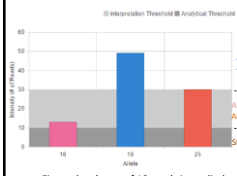
ForenSeq Universal Analysis Software (UAS) Data Analysis/Review



Analysis Parameters AKA Locus Threshold Template

Analytical and Interpretational Thresholds

(2372A Example)



Alleles with reads >IT are shaded in blue and are automatically called
Any allele with reads >AT can be manually edited so they are called and will then be shaded in blue

IT= 4.5% of 650 = 30

Alleles with reads >AT and <IT are shaded in pink and flagged "It" or "Ic", but are not automatically called
Alleles with reads = IT are shaded in orange and flagged "It" or "Ic", but are not automatically called

AT= 1.5% of 650 = 10

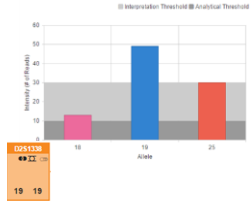
Stutter peaks (not shown) are shaded in brown and are not called

- First, a hard stop of 10 reads is applied to the data
- Second, a minimum of 650 reads is required for the AT and IT calculation and a default value of 650 reads is used when the sum of the reads for the locus is less than 650
- Allele designations will be given to alleles with intensities as low as 10 reads, but are not called when the AT value is greater than 10 (e.g. loci where total read count is >650)

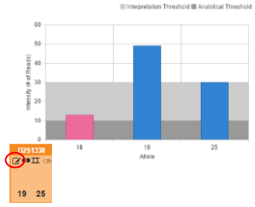
Analytical and Interpretational Thresholds

(2372A Example)

Before manual edit

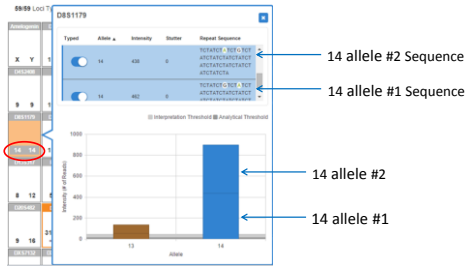


After manual edit



Sequence Variants (AKA Isometric Heterozygotes)

(2372A Example)



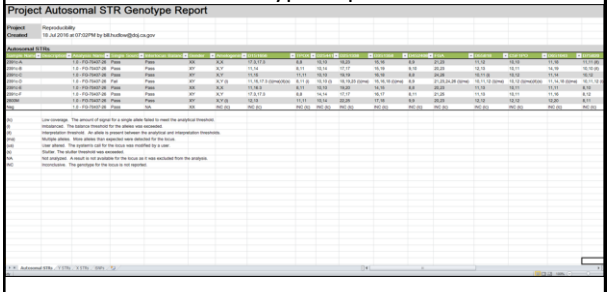
Data Output- ForenSeq UAS GUI

(2800M Positive Control)

Data Output

(NIST 2391c and 2800M Positive Control Data)

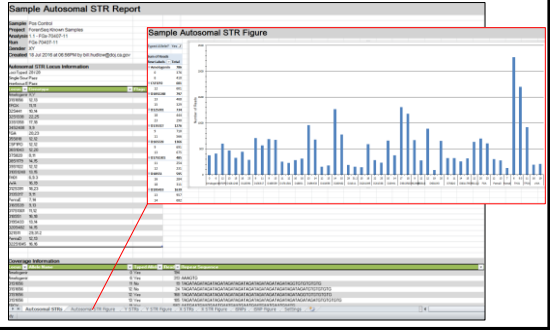
Genotype Reports



Data Output

(2800M Positive Control Data)

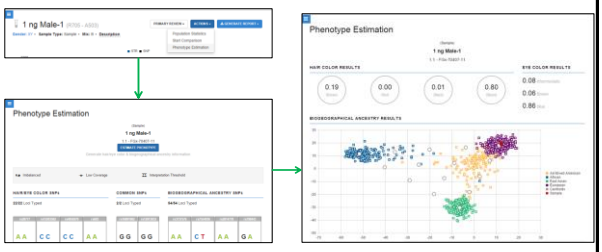
Sample Detail/Summary Reports



Phenotype and Biogeographical Estimates

(NIST 2372A Data)

Phenotype and Biogeographical Ancestry estimates are not automatically generated!



Population Statistics

(NIST 2372A Data)

Population Statistics are not automatically generated!

Pop Stats options include RMP or LR and 2p or 2p-p^2 with a default theta setting of 0.01 (NRC II.4.4a)

Internal Validation Studies

- **QAS 8.3.1 Internal Validation Studies**
  - Known and non probative evidence samples
  - Reproducibility and precision
  - Sensitivity and stochastic studies
  - Mixture Studies
  - Contamination assessment
- **SWGDAM Validation Guidelines for Forensic DNA Analysis Methods**

4. Internal Validation

The internal validation process shall include the studies detailed below. If conducted within the same laboratory, developmental validation studies may satisfy some elements of the internal validation guidelines. The laboratory should evaluate the appropriate sample number and type, based on the methodology and/or application necessary to demonstrate the potential limitations and reliability. The laboratory should determine the suitability of each study based on the methodology and may determine that a study is not necessary. The recommended internal validation studies are summarized in Table 1.

CA DOJ Internal Validation Studies

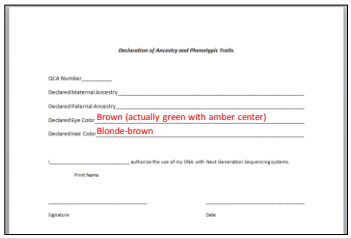
- Known and non probative evidence samples
  - 30 known male samples
  - 7 samples from adjudicated missing persons cases with 7 associated reagent blanks
- Reproducibility and precision
  - Reproducibility study#1-NIST 2391c samples run by analyst #1
  - Reproducibility study #2-NIST2391c samples run by analyst #2
  - Repeatability study-NIST 2391c samples re-run by analyst #1
  - ~~Precision study~~
- Sensitivity and stochastic studies
  - 32 sample sensitivity study run with template quantities ranging from 4 ng to 0.05 ng
  - 8 sample sensitivity study run with the <0.25 ng sequencing libraries from the first sensitivity study
  - Stochastic study with thirty 0.05 ng male samples
- Mixture Studies
  - Two person male/female mixtures with a total of 1 ng template DNA
- Contamination assessment
  - Traditional checkerboard contamination assessment study
  - Carryover study check for run-to-run carryover
- Material Modification Studies
  - Direct amplification of FTA samples
  - Upgrade to ForenSeq Universal Analysis Software version 1.1 (1.1.15190) with re-run of NIST 2391c samples and reanalysis of previously run 2391c samples

Known Samples

- Thirty male buccal samples were contributed by male staff and staff family members along with a completed declaration of Ancestry and Phenotypic Traits form. The sample extracts were typed with the ForenSeq, GlobalFiler and YFiler Plus kits for comparison.

Known Samples

- Thirty male buccal samples were contributed by male staff and staff family members along with a completed declaration of Ancestry and Phenotypic Traits form



Declaration of Ancestry and Phenotypic Traits

QCA Number_____

Declared Maternal Ancestry_____

Declared Paternal Ancestry_____

Declared Eye Color: Brown (actually green with amber center)

Declared Hair Color: Blonde-brown

_____ authorize the use of my DNA with Next Generation Sequencing systems.

Print Name

_____ _____

Signature Date

Known Samples

- Lesson #1, eye and hair color is subjective and we (guys?) either don't know or can't decide on one answer. So, it would probably be best to make this a multiple choice test!

Declaration of Ancestry and Phenotypic Traits

QCA Number_____

Please circle the appropriate answer or give a brief description, if necessary.

Declared Maternal Ancestry: European, East Asian, African, Ad Mixed American, other (_____)

Declared Paternal Ancestry: European, East Asian, African, Ad Mixed American, other (_____)

Declared Eye Color: Brown, Blue, Green, Hazel, other (_____)

Declared Hair Color: Brown, Red, Black, blond, other (_____)

_____ authorize the use of my DNA with Next Generation Sequencing systems.

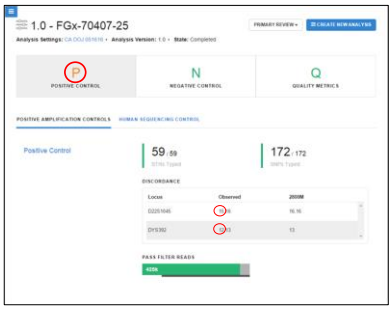
Print Name

_____ _____

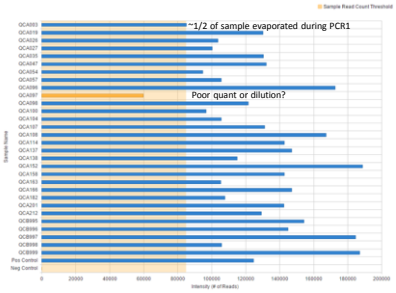
Signature Date

Known Samples-Why Start Here?

ForenSeq UAS-PNQ Page

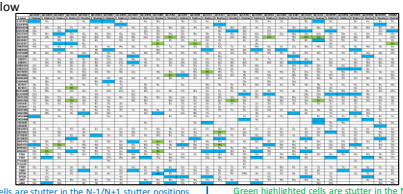


ForenSeq UAS
Known Sample Presentation



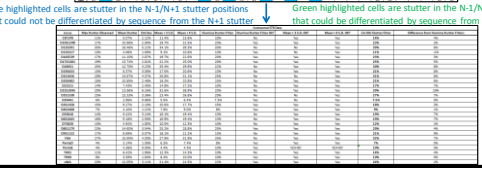
Known Samples Stutter

N-1 Stutter thresholds were derived from the mean stutter and standard deviation values, after removing stutter in the N-1/N+1 positions, for each locus as indicated for the autosomal STR results below



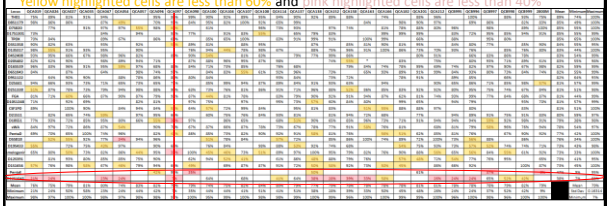
Blue highlighted cells are stutter in the N-1/N+1 stutter positions that could not be differentiated by sequence from the N+1 stutter

Green highlighted cells are stutter in the N-1/N+1 stutter positions that could be differentiated by sequence from the N+1 stutter



Autosomal STR Intralocus Balance

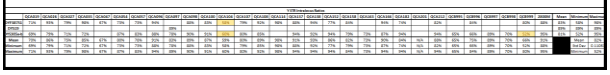
Yellow highlighted cells are less than 60% and pink highlighted cells are less than 40%



Excerpt from ForenSeq DNA Signature Prep Reference Guide

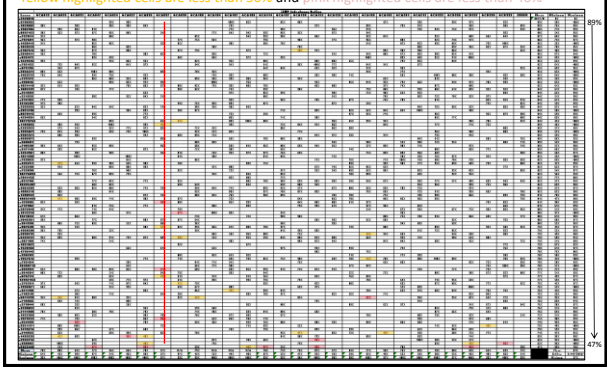
* Interpret the locus D22S1045 with caution. More imbalance in read counts may be seen between alleles of a heterozygote than observed at other loci. Consider multilocus genotype when determining the presence of a DNA mixture.

Y-STR Intralocus Balance



iSNP Intralocus Balance

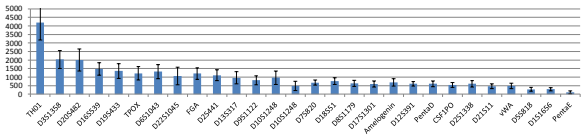
Yellow highlighted cells are less than 50% and pink highlighted cells are less than 40%



Known Samples-Depth of Coverage

Autosomal STR Example

Average Autosomal STRs Depth of Coverage (Mean = 1,004 Reads)

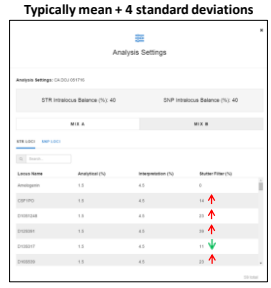


- Similar trends were observed for the other markers in the ForenSeq kit as indicated below
  - Y-STRs with DYS389 on the high end (mean of 1,748 reads) and DYS389II on the low end (mean of 219 reads) and an average of 678 reads
  - X-STRs with DXS7423 on the high end (mean of 1,105 reads) and DXS10103 on the low end (mean of 44 reads) and an average of 749 reads
  - iSNPs with rs338882 on the high end (mean of 2,251 reads) and rs1031825 on the low end (mean of 50 reads) and an average of 450 reads

Updated Stutter Thresholds

Illumina Stutter Thresholds
Mean + 3 standard deviations?

CA DOJ Stutter Thresholds
Typically mean + 4 standard deviations



Known Samples

- Autosomal STR loci- All major allele calls were concordant with the known types
  - However, we also detected minor artifacts (N+1 Stutter, etc.) and observed dropout of one allele at the D22S1045 locus
- Y-STR loci- All major allele calls were concordant with the known types with the possible exception of what appears to be called stutter at the DYS392 locus
  - However, we also detected minor artifacts (N+1 Stutter, etc.), observed dropout of the DYS392 locus in one sample
- All X-STR and iSNP calls were concordant with the known types listed in the ForenSeq DNA Signature Prep Reference Guide

Eye Color Predictions

All Results

	Eye Color Estimation			
Sample	Intermediate	Brown	Blue	Self Declaration
QCA019	4%	1%	95%	Blue
QCA026	12%	83%	5%	Brown
QCA027	5%	3%	92%	Hazel
QCA035	2%	98%	0%	Brown
QCA047	0%	100%	0%	Brown
QCA054	24%	59%	17%	Hazel
QCA057	1%	99%	0%	Brown
QCA096	3%	2%	95%	Blue
QCA097*	1%	99%	0%	Brown
QCA098*	3%	0%	97%	Blue
QCA100*	3%	97%	0%	Hazel
QCA104	4%	2%	94%	Blue
QCA107	22%	66%	12%	Brown (Actually green with amber center)
QCA108	2%	98%	0%	Brown
QCA114	16%	70%	14%	Brown
QCA137	21%	65%	18%	Brown
QCA138	5%	2%	93%	Blue
QCA152	7%	4%	89%	Blue
QCA158	22%	37%	41%	Green
QCA163	0%	100%	0%	Brown
QCA166	9%	88%	2%	Brown
QCA182	22%	66%	12%	Brown
QCA201	5%	3%	92%	Blue
QCA212	8%	88%	4%	Brown
QCB995	4%	2%	94%	Blue
QCB996	0%	100%	0%	Black*
QCB997	6%	3%	92%	Blue
QCB998	0%	100%	0%	Dark Brown or Black*
QCB999	0%	100%	0%	Brown
Percent Concordance				86%

Results by declared eye color

	Eye Color Estimation			
Sample	Intermediate	Brown	Blue	Self Declaration
QCA019	4%	1%	95%	Blue
QCA096	3%	2%	95%	Blue
QCA098*	3%	0%	97%	Blue
QCA104	4%	2%	94%	Blue
QCA138	5%	2%	93%	Blue
QCA152	7%	4%	89%	Blue
QCA201	5%	3%	92%	Blue
QCB995	4%	2%	94%	Blue
QCB997	6%	3%	92%	Blue
Blue Eyes Percent Concordance				100%
Sample	Intermediate	Brown	Blue	Self Declaration
QCA026	12%	83%	5%	Brown
QCA035	2%	98%	0%	Brown
QCA047	0%	100%	0%	Brown
QCA057	1%	99%	0%	Brown
QCA097*	1%	99%	0%	Brown
QCA108	2%	98%	0%	Brown
QCA114	16%	70%	14%	Brown
QCA137	21%	65%	16%	Brown
QCA163	0%	100%	0%	Brown
QCA166	9%	88%	2%	Brown
QCA182	22%	66%	12%	Brown
QCA212	8%	88%	4%	Brown
QCB999	0%	100%	0%	Brown
QCA107	22%	66%	12%	Brown (Actually green with amber center)
QCB996	0%	100%	0%	Black*
QCB998	0%	100%	0%	Dark Brown or Black*
Brown Eyes Percent Concordance				100%
Sample	Intermediate	Brown	Blue	Self Declaration
QCA027	5%	3%	92%	Hazel
QCA054	24%	59%	17%	Hazel
QCA100*	3%	97%	0%	Hazel
QCA158	22%	37%	41%	Green
Indeterminate Eyes Percent Concordance				0%

Hair Color Predictions

All results

	Hair Color Estimation				
Sample	Brown	Red	Black	Blond	Self Declaration
QCA019	14%	10%	1%	75%	Blond
QCA026	47%	0%	11%	42%	Brown
QCA027*	54%	26%	0%	20%	Brown
QCA035	55%	0%	31%	15%	Brown
QCA047	17%	0%	83%	1%	Black
QCA054	59%	0%	27%	14%	Black?**
QCA057	12%	0%	88%	1%	Brown?***
QCA096	14%	16%	1%	68%	Blond
QCA097*	21%	0%	76%	0%	Brown
QCA098*	79%	0%	9%	12%	Blond-Brown
QCA100*	46%	0%	52%	1%	Brown
QCA104	22%	0%	2%	75%	Brown
QCA107	70%	0%	20%	10%	Brown
QCA108	62%	0%	28%	10%	Brown
QCA114	46%	8%	3%	43%	Brown
QCA117	57%	12%	1%	30%	Brown
QCA138	43%	2%	16%	39%	Brown
QCA152	21%	25%	1%	54%	Brown (blond as child)
QCA158	42%	10%	2%	46%	Brown
QCA163	10%	0%	90%	0%	Black
QCA166	21%	0%	78%	1%	Black
QCA182	62%	17%	1%	20%	Brown
QCA201	28%	1%	2%	68%	Blond (Dirty)
QCA212	45%	0%	48%	7%	Brown
QCB995	22%	0%	1%	76%	Blond
QCB996	5%	0%	95%	0%	Black
QCB997	17%	0%	1%	82%	Blond
QCB998	16%	0%	83%	0%	Dark Brown or Black
QCB999	5%	0%	94%	0%	Black
Percent Concordance					76%

Results by declared hair color

	Hair Color Estimation				
Sample	Brown	Red	Black	Blond	Self Declaration
QCA047	17%	0%	83%	1%	Black
QCA054	59%	0%	27%	14%	Black?**
QCA163	10%	0%	90%	0%	Black
QCA166	21%	0%	78%	1%	Black
QCB996	5%	0%	95%	0%	Black
QCB998	16%	0%	83%	0%	Dark Brown or Black
QCB999	5%	0%	94%	0%	Black
Black Hair Percent Concordance					**86%**
Sample	Brown	Red	Black	Blond	Self Declaration
QCA019	14%	10%	1%	75%	Blond
QCA096	14%	16%	1%	68%	Blond
QCB995	22%	0%	1%	76%	Blond
QCB997	17%	0%	1%	82%	Blond
QCA201	28%	1%	2%	68%	Blond (Dirty)
Blond Hair Percent Concordance					100%
Sample	Brown	Red	Black	Blond	Self Declaration
QCA026	47%	0%	11%	42%	Brown
QCA027**	54%	26%	0%	20%	Brown
QCA035	55%	0%	31%	15%	Brown
QCA097**	21%	0%	76%	0%	Brown
QCA098*	79%	0%	9%	12%	Blond-Brown
QCA100**	46%	0%	52%	1%	Brown
QCA104	22%	0%	2%	75%	Brown
QCA107	70%	0%	20%	10%	Brown
QCA108	62%	0%	28%	10%	Brown
QCA114	46%	8%	3%	43%	Brown
QCA117	57%	12%	1%	30%	Brown
QCA138	43%	2%	16%	39%	Brown
QCA158	42%	10%	2%	46%	Brown
QCA182	62%	17%	1%	20%	Brown
QCA212	45%	0%	48%	7%	Brown
QCA152	21%	25%	1%	54%	Brown (blond as child)
QCA057	12%	0%	88%	1%	Brown?***
Brown Hair Percent Concordance					59%

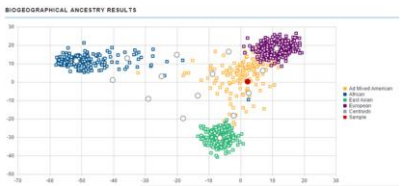
Biogeographical Ancestry Predictions

Results by declared ancestry

	Forensiq	Declared Ancestry	
Sample	BioGeographical Prediction	Maternal	Paternal
QCA019	European	English, Irish, German..	English, Scottish
QCA026	European	Northern European	European
QCA027	European	Puerto Rico Caucasian	Irish/German-Caucasian
QCA035	European	Italian, Irish, Scottish	German, English
QCA054	European	German, English, Dutch...	Irish
QCA096	European	Caucasian-Mixed European	Caucasian-Germanic/English...
QCA098	European	Caucasian (Irish, English...)	Caucasian (French, Irish...)
QCA100	European	German/Irish	Norwegian
QCA107	European	German/English	Italian
QCA108	European	Norwegian, German	German, Swiss
QCA114	European	Caucasian	Caucasian
QCA117	European	Caucasian (Swedish/Irish)	Caucasian (Italian/Irish)
QCA138	European	European (Italian)	European (Irish)
QCA152	European	Polish, Slovak	German, English
QCA158	European	Italian and Irish	Hungarian and British
QCA182	European	Irish and German	Dutch
QCA201	European	German/Danish	Austrian/Danish
QCB995	European	English	German, Dutch, Irish..
QCB997	European	Welsh and German	English
European Percent Concordance			**100%**
QCA047	African	Black	Black
African Percent Concordance			**100%**
QCA057	Ad Mixed American	Mexican	Mexican
Ad Mixed American Percent Concordance			**100%**
QCA097	European	German	Iranian
QCA104	European	Italian/German	Supposedly North African***
QCA166	Ad Mixed American*	Chinese	English
QCA212	Ad Mixed American*	English/German	Japanese
Mixed Ancestry Percent Concordance			**50% or 100%?**
QCA163	East Asian	Asian	Asian
QCB996	East Asian	Chinese	Chinese
QCB998	Ad Mixed American	Asian**	Asian**
QCB999	East Asian	Chinese	Chinese
Asian Percent Concordance			**75%**

So, the phenotype and biogeographical estimates seem to work well for individuals with European ancestry, blond or black hair and blue or brown eyes

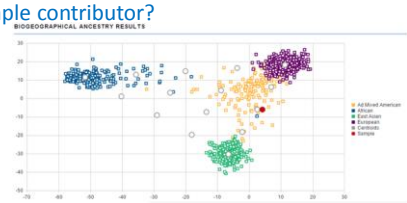
Biogeographical Ancestry Predictions Exception #1



Note: The sample contributor is of mixed ancestry (European/East Asian) and the sample falls roughly in the middle of the European and East Asian centroids

Biogeographical Ancestry Predictions Exception #2

Lesson #2, if a sample falls between two centroids we probably should consider mixed ancestry as a possibility for the sample contributor?



Note: The sample contributor is of mixed ancestry (European/East Asian) and the sample falls roughly in the middle of the European and East Asian centroids

Biogeographical Ancestry Predictions Exception #3

Lesson #3, all population groups should be considered as a possibility when the sample does not fall in one of the three major centroids?

Note: The sample contributor is of Southern Asian ancestry (India) and it is not surprising that they don't fall within the East Asian, African or European centroids

Reproducibility and Precision Studies

- Review of the reproducibility and repeatability results is ongoing and the run-to-run and analyst-to-analyst reproducibility appears to be acceptable

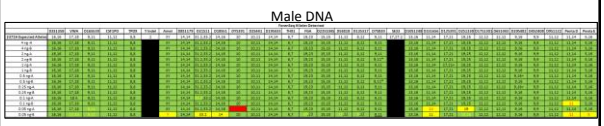
Sensitivity Study #1

- Duplicates of male DNA (NIST 2372A) and female DNA (QCA127) ranging from 4 ng to 0.05 ng were analyzed with the ForenSeq and GlobalFiler kits to determine the relative sensitivity of the assays and to determine if the ForenSeq kit might be more sensitive with female samples where the amplification of the 24 Y-STRs does not occur

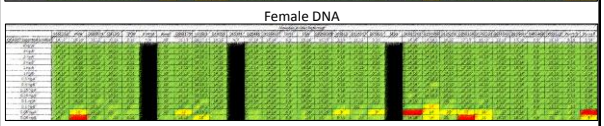
Sensitivity Study #1

NIST 2372A and QCA127 Data

Male DNA



Female DNA



Yellow font indicates the allele was below the AT (1.5%), red font indicates a single allele was detected above IT and red cells indicate complete dropout of the locus

As more locus and allelic dropout was observed in the female DNA sample, the sensitivity of the ForenSeq system does not seem to be increased for female samples where the amplification of the 24 Y-STRs does not occur

Sensitivity Study #1

NIST 2372A Data-32 sample run

Yellow font indicates the allele was below the AT (1.5% for ForenSeq/150 RFU for GlobalFiler), red font indicates a single allele was detected above IT (4.5% for ForenSeq/855 RFU for GlobalFiler) and red cells indicates complete dropout of the locus

Sensitivity Study #1

- Y-STR Results- Complete profiles detected down to 0.1 ng with near complete profiles at 0.05 ng
- X-STR Results- Complete profiles detected down to 0.5 ng with near complete profiles at 0.05 ng as only DXS10103 dropped out
- iSNP Results- Complete profiles detected down to 0.5 ng with near complete profiles down to 0.1 ng and approximately two-thirds of the iSNPs detected in the 0.05 ng samples
- Ancestry and phenotypic SNP results
  - Consistent phenotype estimates down to 0.25 ng
  - Consistent ancestry results down to ~0.1 ng

Sensitivity Study #2

As the amount of each sequencing library loaded onto the MiSeq is inversely proportional to the number of sequencing libraries pooled, a second sensitivity study was conducted to determine the effect of pooling size on the sensitivity of the ForenSeq system. Specifically, we always load a fixed volume (7 uL) of the pooled ForenSeq libraries onto the MiSeq after pooling of 5 uL of each ForenSeq library, which can result in running varied quantities of each library as indicated below

- 32 libraries = 32 x 5 uL = 160 uL with a 7 uL load results in loading 0.0438 uL of each library on the MiSeq
- 8 libraries = 8 x 5 uL = 40 uL with a 7 uL load results in loading 0.175 uL of each library on the MiSeq

Similar increases in sensitivity were observed for the other markers in the ForenSeq kit

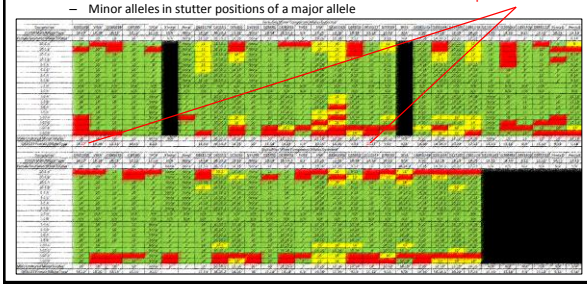
Stochastic Studies

- Review of the stochastic study results is ongoing and the results are similar to those yielded from the previous 0.05 ng samples.

Mixture Studies

- Male/Female DNA mixtures (1 ng total) ranging from 20:1 to 1:20 ratios amplified in duplicate with the ForenSeq and GlobalFiler kits
- Generally, the ability to detect the unshared, minor component alleles was similar between the ForenSeq and GlobalFiler kits with two exceptions
  - The D22S1045 locus
  - Minor alleles in stutter positions of a major allele

Sequence Variants!



Contamination Assessment #1

A partial checkerboard plate consisting of DNA extracts/controls and negative controls was processed with the ForenSeq system to check for contamination as shown below

	1	2	3
A	Negative Control	Negative Control	QC Extract
B	QC Extract	Negative Control	Negative Control
C	Negative Control	Negative Control*	QC Extract
D	QC Extract	Negative Control	Negative Control
E	Negative Control*	Negative Control	QC Extract
F	QC Extract	Negative Control	Negative Control
G	Negative Control	Negative Control	2800M Positive Control
H	QC Extract	Negative Control	Negative Control

*A single allele was detected in the negative controls in wells E1 (rs430046-68 bp-11 reads) and C2 (rs576261-29 bp-14 reads)

Contamination Assessment #2

A second contamination assessment study was completed to check for run-to-run contamination as indicated below

Run #25

Sample	Index 1 (i7)	Index 2 (i5)
2391c-A	R708	A501
2391c-B	R708	A502
2391c-C	R708	A503
2391c-D	R708	A504
2391c-E	R708	A505
2391c-F	R708	A506
2800M	R708	A507
Negative Control	R708	A508

Post Run Wash →

Run #26

Sample	Index 1 (i7)	Index 2 (i5)
2391c-A	R709	A501
2391c-B	R709	A502
2391c-C	R709	A503
2391c-D	R709	A504
2391c-E	R709	A505
2391c-F	R709	A506
2800M	R709	A507
Negative Control	R709	A508

→

Reanalysis of Run #26 "Create new run"

Sample	Index 1 (i7)	Index 2 (i5)
2391c-A	R708	A501
2391c-B	R708	A502
2391c-C	R708	A503
2391c-D	R708	A504
2391c-E	R708	A505
2391c-F	R708	A506
2800M	R708	A507
Negative Control	R708	A508

No alleles were detected for any combination of indices from the previous run!!!

Non-probative Evidence Samples

Seven challenging, non-probative evidence extracts and associated reagent blanks were obtained from the Missing Persons DNA Program and 5 uL was typed with the ForenSeq and GlobalFiler systems (29 cycles with standard 3500 injection parameters).

Sample Information		qPCR Data			ForenSeq Results (5 uL)					GlobalFiler Results (5 uL)		
Sample	Sample Type	ng Amp/ml per ng/ml(E ^180 bp)	ng Amp/ml per ng/ml (67 bp)	Degradation Ratio	Autosomal STRs	Amelogenin	Y STRs	X STRs	SNPs	Autosomal STRs	Amelogenin	Y Indel
1	Oral Swab	0.027	4.475	164	0	XY	18	5	94	0	XY	Yes
2B-1	Reagent Blank	0.000	0.000	N/A-no THO1 ND	0	ND	0	0	0	0	ND	ND
2	Deep Muscle Tissue	0.043	0.105	2	0	XY	14	4	88	0	XY	Yes
3B-2	Reagent Blank	0.000	0.000	N/A-no THO1 ND	0	ND	0	0	0	0	ND	ND
3	Brain	0.000	0.004	N/A-no THO1 ND	0	ND	N/A-Female	3	35	0	X	ND
4B-1	Reagent Blank	0.000	0.000	N/A-no THO1 ND	0	ND	0	0	0	0	ND	ND
4	Tooth	0.002	0.014	8	0	ND	N/A-Female	1	0	0	ND	ND
5B-4	Reagent Blank	0.000	0.000	N/A-no THO1 ND	0	ND	0	0	0	0	ND	ND
5	Skull	0.000	0.005	N/A-no THO1 ND	0	ND	N/A-Female	0	0	0	ND	ND
6B-5	Reagent Blank	0.000	0.000	N/A-no THO1 ND	0	ND	0	0	0	0	ND	ND
6	Tibia	0.003	0.111	32	0	XY	12	2	83	0	XY	Yes
7B-6	Reagent Blank	0.000	0.000	N/A-no THO1 ND	0	ND	0	0	0	0	ND	ND
7	Tooth	0.008	0.020	N/A-no THO1 ND	0	ND	8	1	59	0	ND	Yes
8B-7	Reagent Blank	0.000	0.000	N/A-no THO1 ND	0	ND	0	0	0	0	ND	ND

A single allele was detected in the seventh reagent blank at the D20S482 locus (14 allele with 42 reads)

The ForenSeq Kit yielded more autosomal STR data than the GlobalFiler kit for all samples, in addition to yielding SNP data!

Material Modification Studies

- Direct amplification of FTA punches
  - Add 100 uL TBE buffer to a 1.2 mm FTA punch, shake at 1800 RPM for 2 minutes, centrifuge, remove supernatant and add PCR1 master mix to punch
  - This works great!!!
- ForenSeq UAS 1.1 upgrade
  - As the ForenSeq UAS 1.1 software became available after we started our internal validation studies we needed to perform a material modification study, which was done in two parts
    - The pooled sequencing libraries from our last study (repeatability) was re-run with the 1.1 software to check for concordance
      - All results appear to be concordant
    - The repeatability study was reanalyzed with the 1.1 software
      - All results appear to be concordant with no differences in reads, etc.

Acknowledgements

CA DOJ DNA Laboratory

- Carolyn Weigand
- Daniela Cuenca
- Gunther Scharnhorst
- John Tonkyn
- Karen Tsai
- Melody Duke

Illumina

- Joe Varlaro
- Laura Wachtmann
- Melissa Kotkin