NMS LABS

Utilizing Quantifiler® Trio in Conjunction with an External Standard Curve to Screen Sexual Assault Samples from Male DNA

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Forensic Scientist II

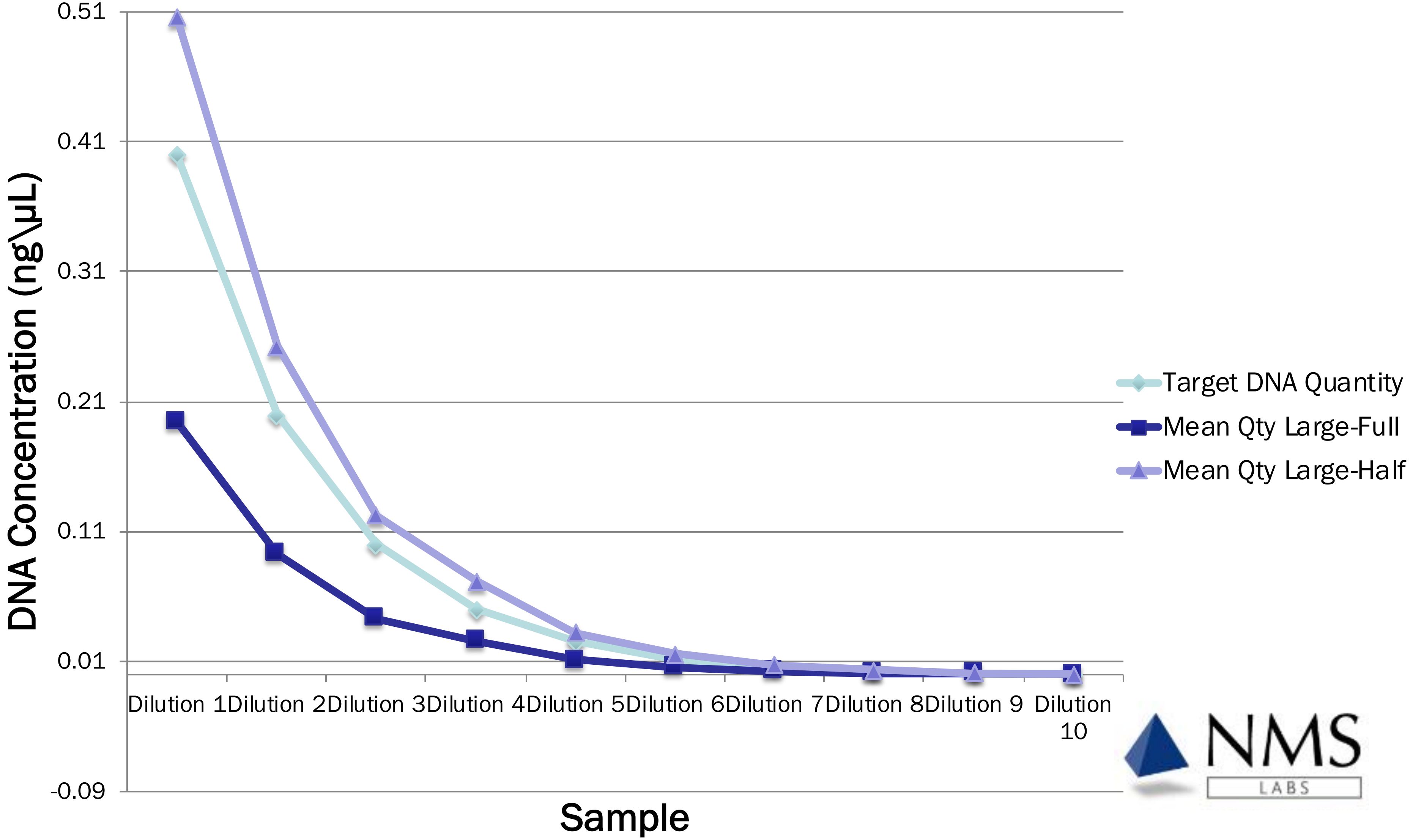
Evaluation of Trio for casework

- Full vs. Half Scale
  - Accuracy
    - Large or Small Autosomal Target, Y-Chromosome Target
  - Sensitivity
- Quality of Sample
  - Degradation Index
  - Inhibition
- Determination of Male:Female ratios in mixed samples

Sensitivity/Accuracy

- Comparison of full vs. half-scale reactions
- Two series of ten 2-fold dilutions
  - Extracted male DNA
  - Range: 0.40 ng/µL to 0.000782 ng/µL
  - Quantified in triplicate
  - Amplified using average quantitation values targeting 0.5 ng
    - AmpFlSTR® Yfiler®
    - PowerPlex® 16HS

Large Autosomal Target



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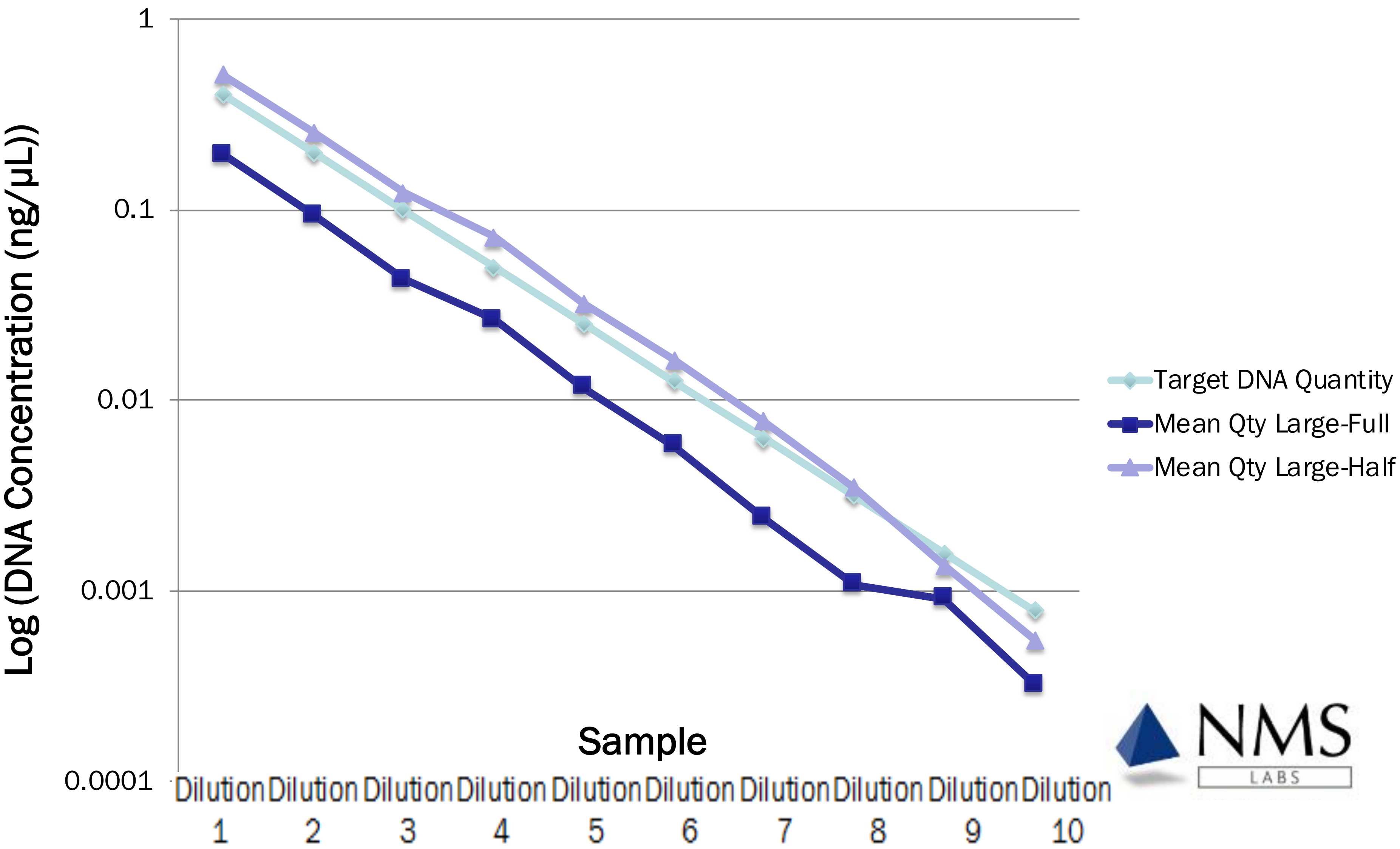

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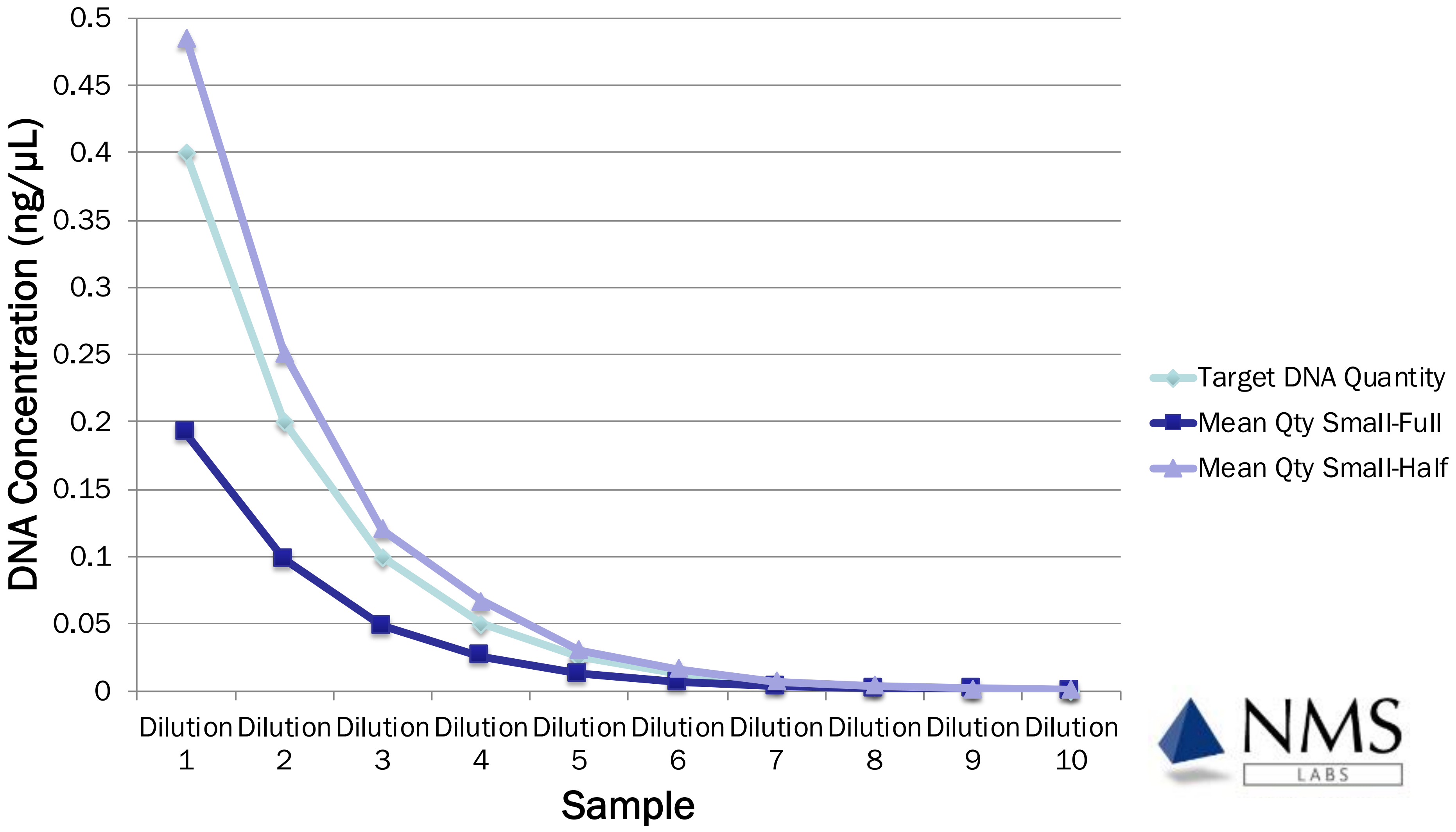
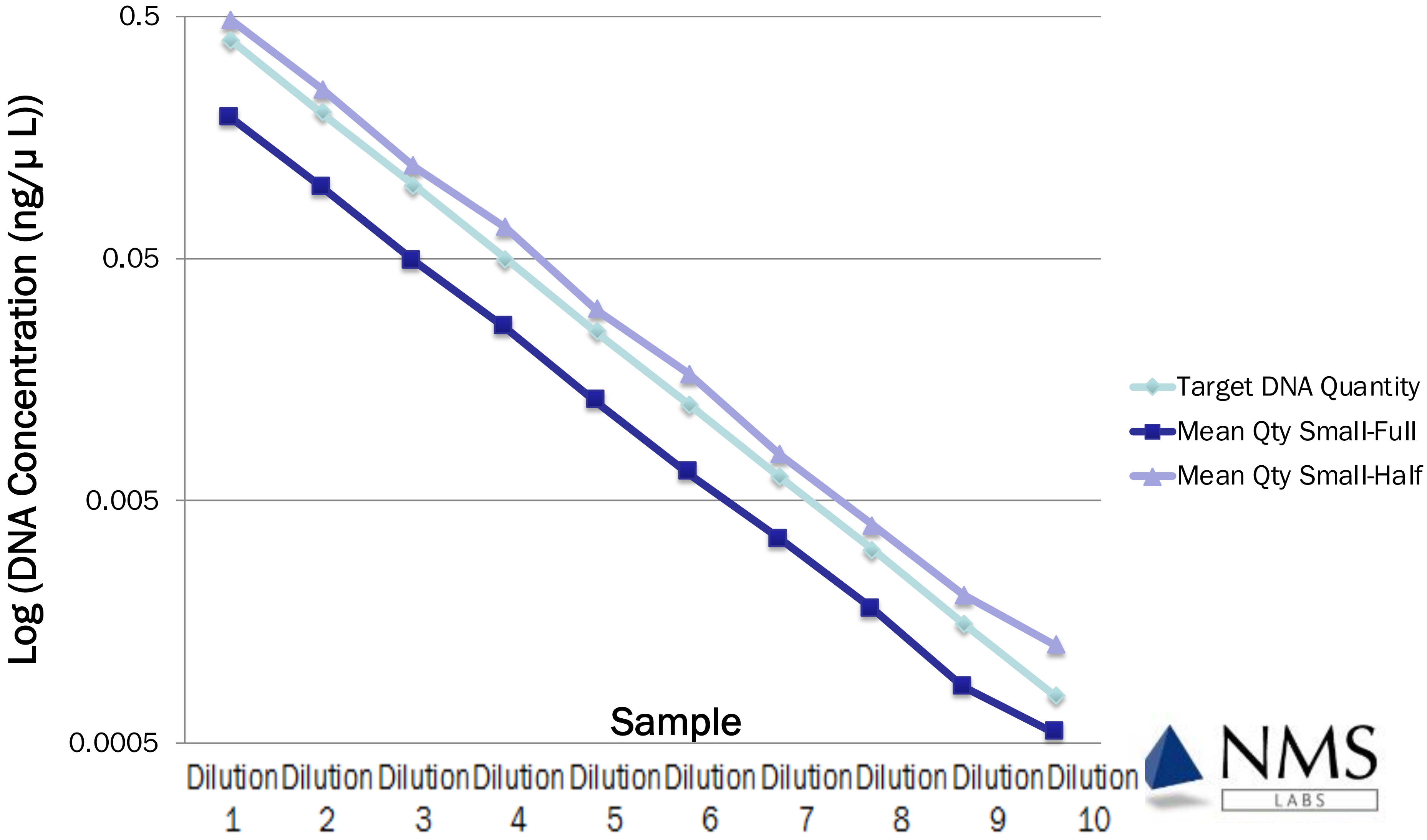
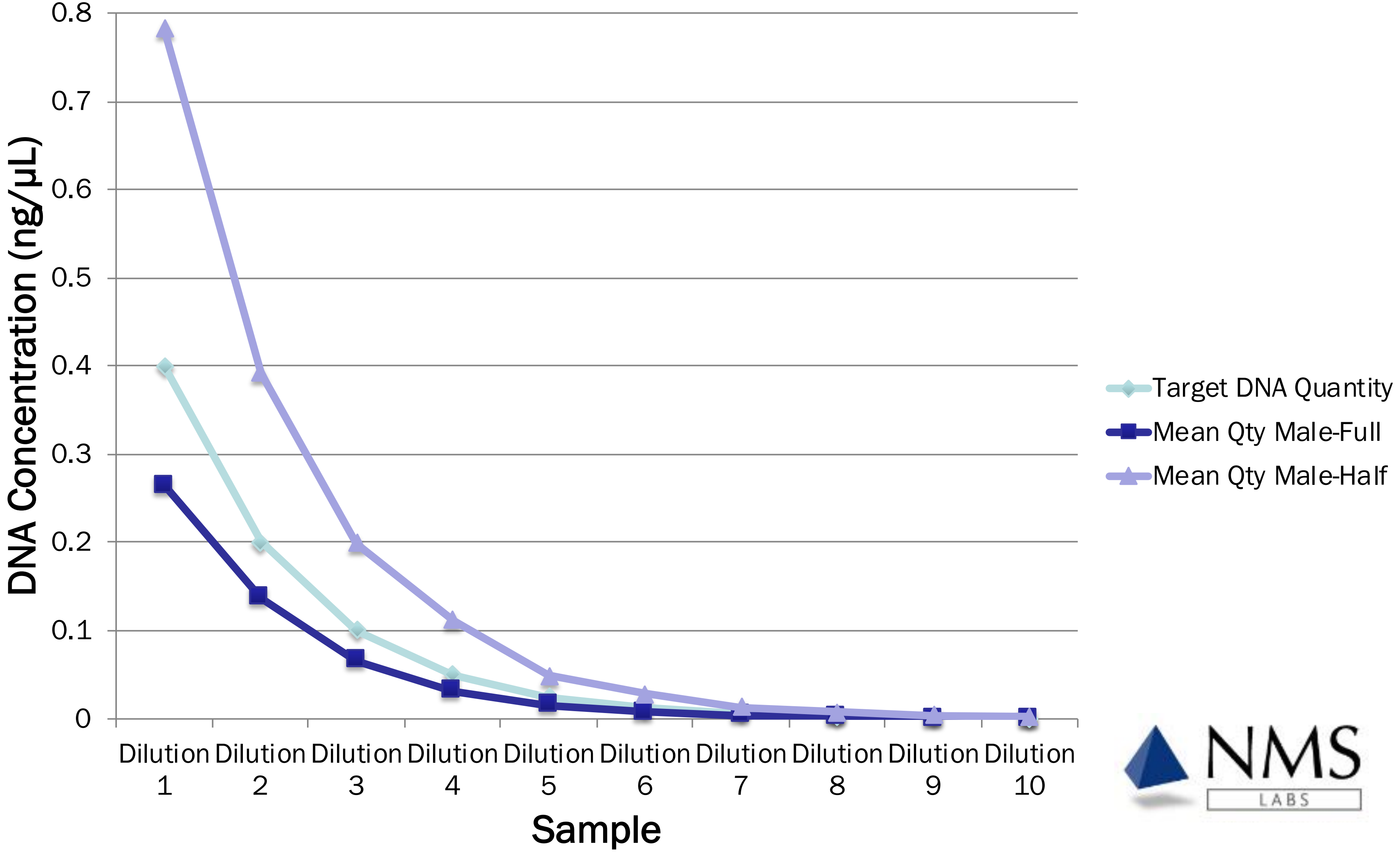
Small Autosomal Target



Small Autosomal Target



Male Target



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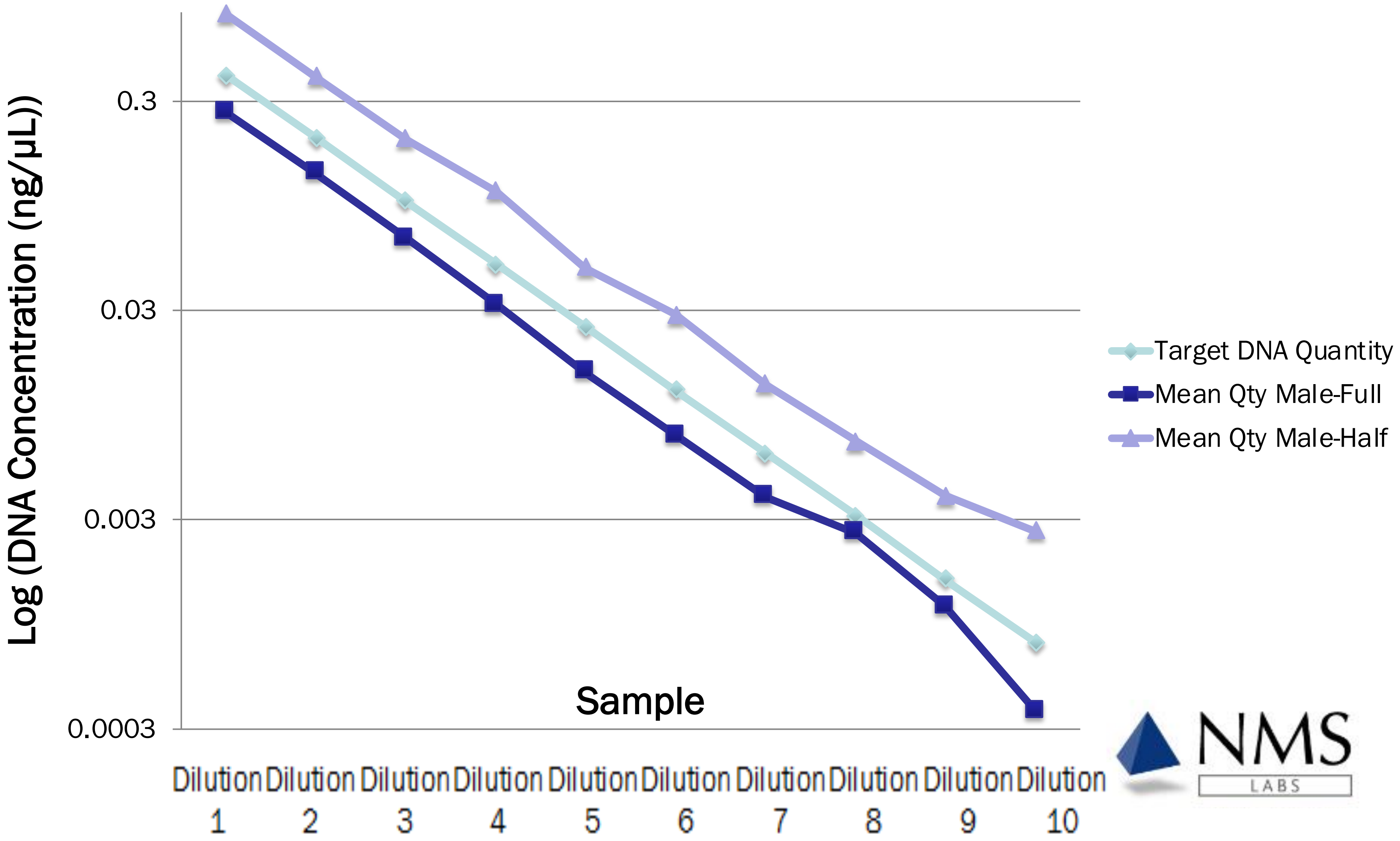
Male Target



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Sensitivity/Accuracy-Full Scale PowerPlex 16 HS

Expected DNA Concentration	Expected amount of Total DNA (ng) Amplified	Total DNA (ng) Amplified	Alleles Counted
0.4 ng/µL	0.500	0.500	30
0.2 ng/µL	0.500	0.500	30
0.1 ng/µL	0.500	0.500	30
0.05 ng/µL	0.500	0.462	30
0.025 ng/µL	0.438	0.205	30
0.0125 ng/µL	0.219	0.101	30
0.00625 ng/µL	0.109	0.042	29
0.00325 ng/µL	0.057	0.019	21
0.001563 ng/µL	0.027	0.015	3
0.000782 ng/µL	0.014	0.006	7

Sensitivity/Accuracy - Full Scale YFiler

Expected DNA Concentration	Expected amount of Total DNA (ng) Amplified	Total DNA (ng) Amplified	Alleles Counted
0.4 ng/µL	0.500	0.500	17
0.2 ng/µL	0.500	0.500	17
0.1 ng/µL	0.500	0.500	17
0.05 ng/µL	0.500	0.321	17
0.025 ng/µL	0.250	0.152	17
0.0125 ng/µL	0.125	0.076	17
0.00625 ng/µL	0.062	0.039	14
0.00325 ng/µL	0.033	0.025	7
0.001563 ng/µL	0.016	0.011	2
0.000782 ng/µL	0.008	0.003	1

Sensitivity/Accuracy - Half Scale PowerPlex 16 HS

Expected DNA Concentration	Expected amount of Total DNA (ng) Amplified	Total DNA (ng) Amplified	Alleles Counted
0.4 ng/µL	0.500	0.500	30
0.2 ng/µL	0.500	0.500	30
0.1 ng/µL	0.500	0.500	30
0.05 ng/µL	0.500	0.500	30
0.025 ng/µL	0.438	0.500	30
0.0125 ng/µL	0.219	0.283	30
0.00625 ng/µL	0.109	0.136	30
0.00325 ng/µL	0.057	0.061	30
0.001563 ng/µL	0.027	0.023	22
0.000782 ng/µL	0.014	0.009	7

Sensitivity/Accuracy-Half Scale YFiler

Expected DNA Concentration	Expected amount of Total DNA (ng) Amplified	Total DNA (ng) Amplified	Alleles Counted - YFiler
0.4 ng/µL	0.500	0.500	17
0.2 ng/µL	0.500	0.500	17
0.1 ng/µL	0.500	0.500	17
0.05 ng/µL	0.500	0.500	17
0.025 ng/µL	0.250	0.483	17
0.0125 ng/µL	0.125	0.287	17
0.0625 ng/µL	0.062	0.133	17
0.00325 ng/µL	0.033	0.071	15
0.001563 ng/µL	0.016	0.039	11
0.000782 ng/µL	0.008	0.026	3

Mixtures: Male Female Ratio

- Extracted Male DNA and Extracted Female DNA
- Male remained at 0.025 ng/uL
- Female increased with each sample
- Quantified in Triplicate

Mixtures-Male Female Ratio

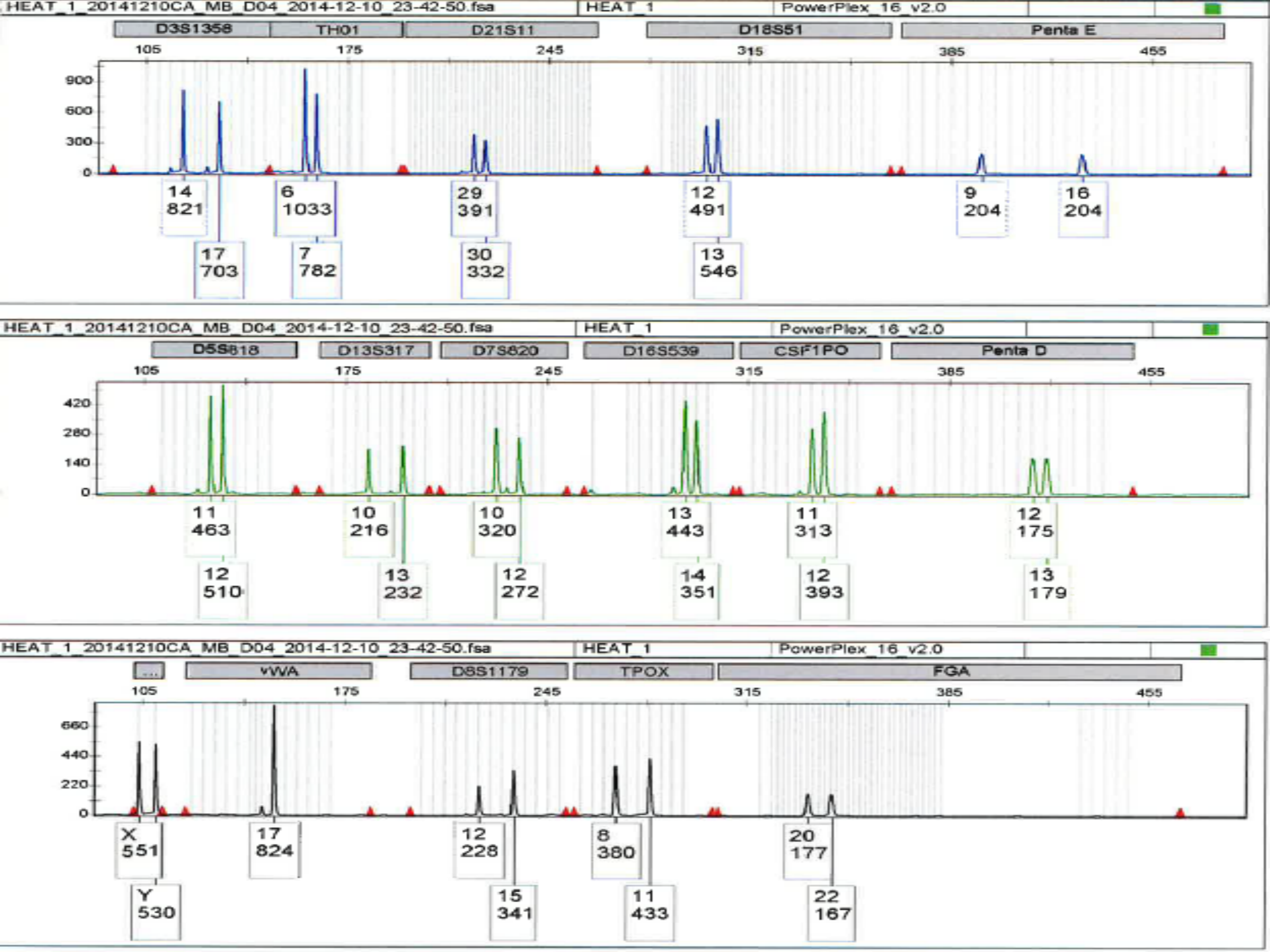
Sample	Male Qty	Male Std	Large Qty	Large Std	Small Qty	Small Std	M:F Ratio
0.025ng M/μL	0.038263	0.00209	0.029131	0.003005	0.022422	0.001571	--
0.025ng M to 0.025 ng F/μL	0.040825	0.012567	0.052315	0.009645	0.033599	0.004446	--
0.025ng M to 1.25ng F/μL	0.049582	0.005047	1.194988	0.085374	0.914332	0.04756	1:17.56
0.025ng M to 2.5ng F/μL	0.046342	0.004169	2.524319	0.158221	1.800461	0.136149	1:37.949
0.025ng M to 5ng F/μL	0.04812	0.011106	5.230286	0.235769	4.157054	0.369275	1:89.219
0.025ng M to 12.5ng F/μL	0.053886	0.001813	12.20116	0.448795	10.25819	0.670396	1:189.601
0.025ng M to 20 ng F/μL	0.04753	0.004141	19.34236	1.634037	16.45045	0.619248	1:347.566
0.025ng M to 25ng F/μL	0.043533	0.00767	23.08518	4.357499	18.08087	3.693459	1:413.077

Degradation Study

- Heated Whole Blood
  - At 99^{o}C for One Hour
- Aged Dried Whole Blood
  - Age Unknown
  - Dried in Blood Tube
- Three Samples Taken from Each

Degradation Study

SAMPLE		Extraction Control	Heat 1	Heat 2	Heat 3	Old Blood 1	Old Blood 2	Old Blood 3
Mean (ng/µL)	Large Autosomal	0.3307	17.5583	28.1139	30.3021	0.0382	0.0148	0.0088
	Small Autosomal	0.3175	16.7173	28.8214	28.7658	0.2595	0.0777	0.1022
	Male	0.3221	17.9538	30.7071	30.4917	0.2334	0.0672	0.1042
Standard Deviation	Large Autosomal	0.0247	1.1373	1.3602	1.2732	0.0052	0.0004	0.0029
	Small Autosomal	0.0054	0.7086	1.0487	1.1340	0.0155	0.0043	0.0046
	Male	0.0198	0.5110	0.9083	0.5037	0.0027	0.0040	0.0014
Degradation Indication		0.9601	0.9521	1.0252	0.9493	6.7932	5.2500	11.6136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PowerPlex_16_v2.0
D3S1358
TH01
D21S11
D18S51
Penta E
105
175
245
315
385
455
900
600
300
0
14
821
17
703
6
1033
7
782
29
391
30
332
12
491
13
546
9
204
16
204
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D13S317
D7S820
D16S539
CSF1PO
Penta D
105
175
245
315
385
455
420
280
140
0
11
463
12
510
10
216
13
232
10
320
12
272
13
443
14
351
11
313
12
393
12
175
13
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PowerPlex_16_v2.0
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vWA
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TPOX
FGA
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175
245
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455
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11
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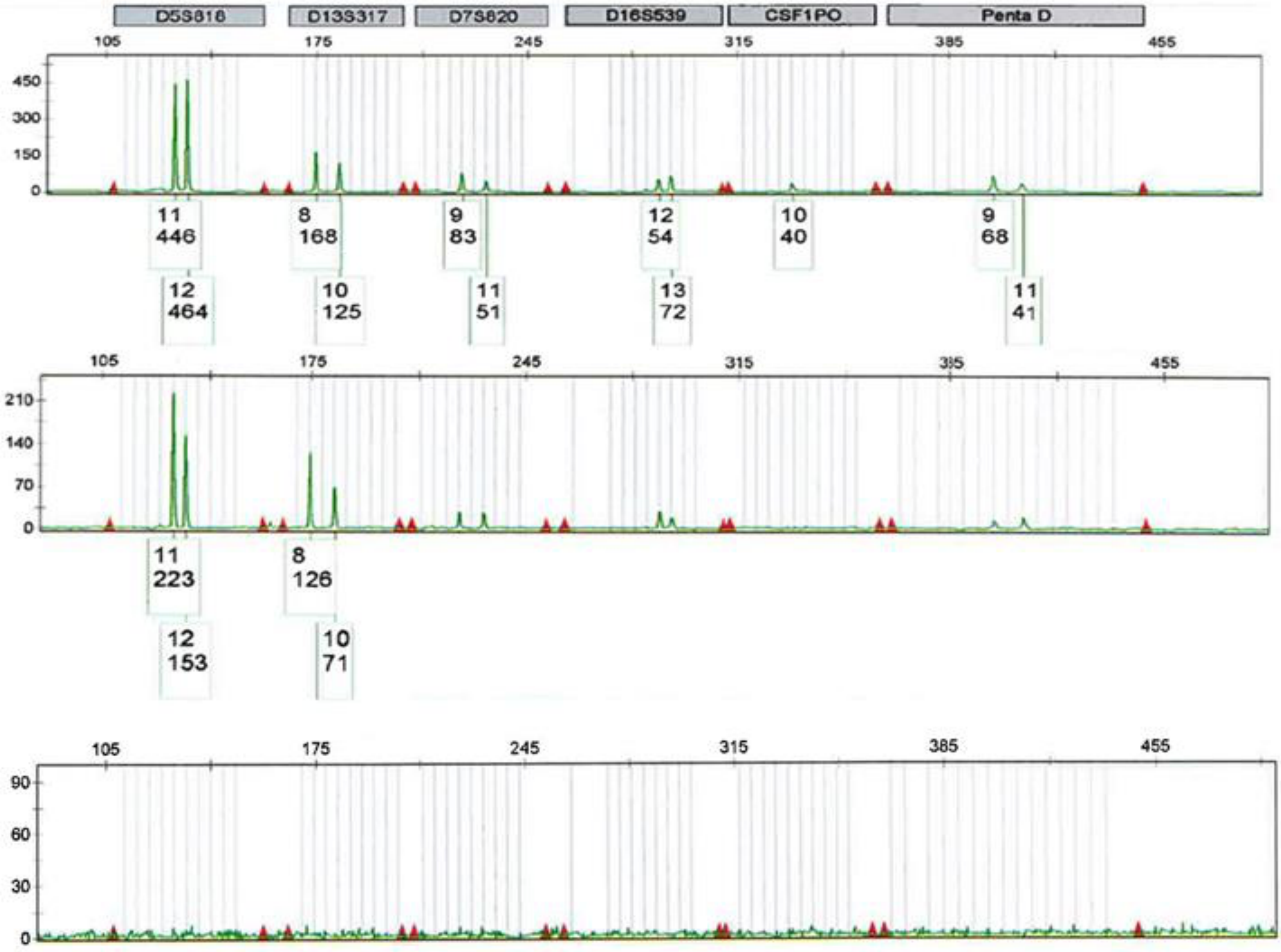
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125
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51
12
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72
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40
9
68
11
41
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153
8
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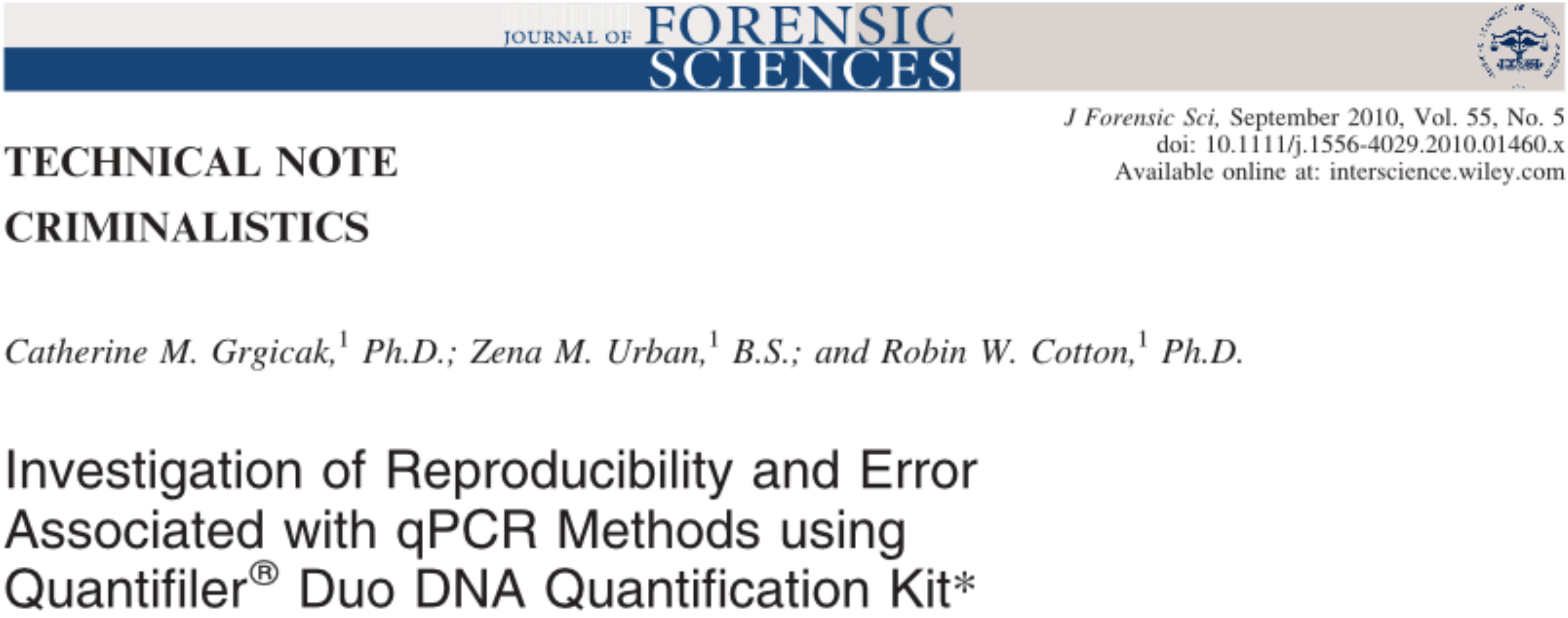
10
71
90
60
30
0

Use of an External Standard Curve for Quantification



JOURNAL OF FORENSIC SCIENCES

J Forensic Sci, September 2010, Vol. 55, No. 5
doi: 10.1111/j.1556-4029.2010.01460.x
Available online at: interscience.wiley.com

TECHNICAL NOTE

CRIMINALISTICS

Catherine M. Grgicak,[1] *Ph.D.; Zena M. Urban,*[1] *B.S.; and Robin W. Cotton,*[1] *Ph.D.*

Investigation of Reproducibility and Error Associated with qPCR Methods using Quantifiler® Duo DNA Quantification Kit*

Generation of the ESC

- Run 10 assay specific standard curves following manufacturer’s guidelines
  - Each STD run in triplicate
  - Run by multiple analysts
  - Must pass all criteria
- Use Excel to perform Linear Regression
  - Perform separately for each target
- Create Excel Conversion Worksheet for casework samples

Data Organization

T.Large Autosomal

PLATE	STD	Concentration	Log (Conc)	Cycle Threshold
051215_MT	A	50	1.698970004	19.71039009
	A	50	1.698970004	19.70301437
	A	50	1.698970004	19.64899254
	B	5	0.698970004	22.90184784
	B	5	0.698970004	22.90602684
	B	5	0.698970004	22.83069992
	C	0.5	-0.301029996	26.49364281
	C	0.5	-0.301029996	26.38239479
	C	0.5	-0.301029996	26.3770504
	D	0.05	-1.301029996	30.23571205
	D	0.05	-1.301029996	29.83619118
	D	0.05	-1.301029996	30.29860497
	E	0.00		
	E	0.00		
	E	0.00		

T.Small Autosomal

PLATE	STD	Concentration	Log (Conc)	Cycle Threshold
051215_MT	A	50	1.698970004	21.10110092
	A	50	1.698970004	21.13996124
	A	50	1.698970004	21.15828323
	B	5	0.698970004	24.39478302
	B	5	0.698970004	24.51132774
	B	5	0.698970004	24.57847786
	C	0.5	-0.301029996	27.95670319
	C	0.5	-0.301029996	28.02471161
	C	0.5	-0.301029996	28.0610218
	D	0.05	-1.301029996	31.3233757
	D	0.05	-1.301029996	31.57466698
	D	0.05	-1.301029996	31.59591866
		.005	-2.301029996	34.38751984
		.005	-2.301029996	34.40555191
		.005	-2.301029996	35.29163361

T.Y

PLATE	STD	Concentration	Log (Conc)	Cycle Threshold
051215_MT	A	50	1.698970004	20.36420822
	A	50	1.698970004	20.33000374
	A	50	1.698970004	20.37609673
	B	5	0.698970004	23.67354393
	B	5	0.698970004	23.72281456
	B	5	0.698970004	23.73950958
	C	0.5	-0.301029996	27.18520355
	C	0.5	-0.301029996	27.22159767
	C	0.5	-0.301029996	27.14003563
	D	0.05	-1.301029996	30.51333237
	D	0.05	-1.301029996	30.61066437
	D	0.05	-1.301029996	30.5756321
	E	0.005	-2.301029996	32.85361862
	E	0.005	-2.301029996	33.96843719
	E	0.005	-2.301029996	33.90359497

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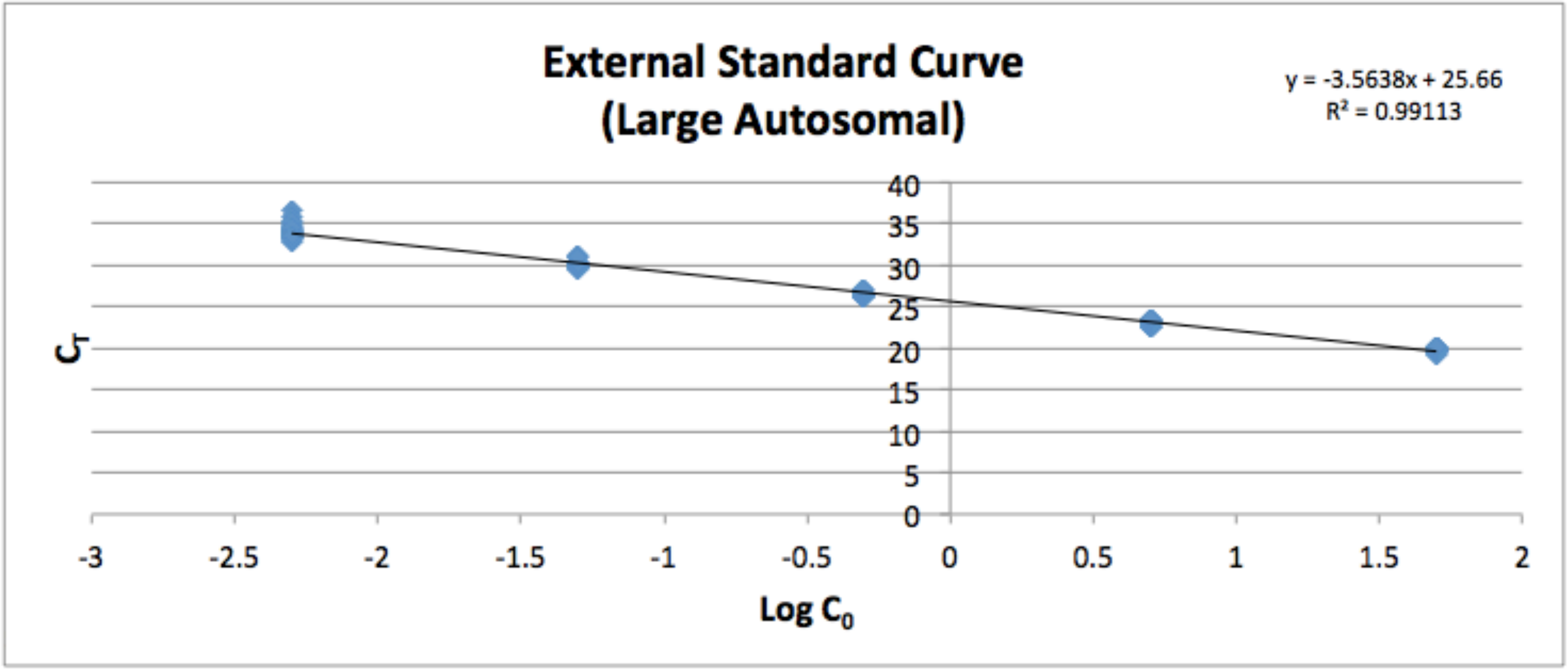
Large Autosomal Target



Perform Linear Regression

- DataPack tool in Excel – Linear Regression

- Output is three tables
  - Log(Concentration) – X-Values
  - C_T –Y-Values

Regression Statistics

Regression Statistics	
Multiple R	**0.995555336**
R Square	**0.991130428**
Adjusted R Square	**0.991070498**
Standard Error	**0.479988386**
Observations	**150**

Anova

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	**1**	**3810.231131**	**3810.231131**	**16538.26181**	**9.1787E-154**
Residual	**148**	**34.09754991**	**0.230388851**		
Total	**149**	**3844.328681**			

Regression Coefficients

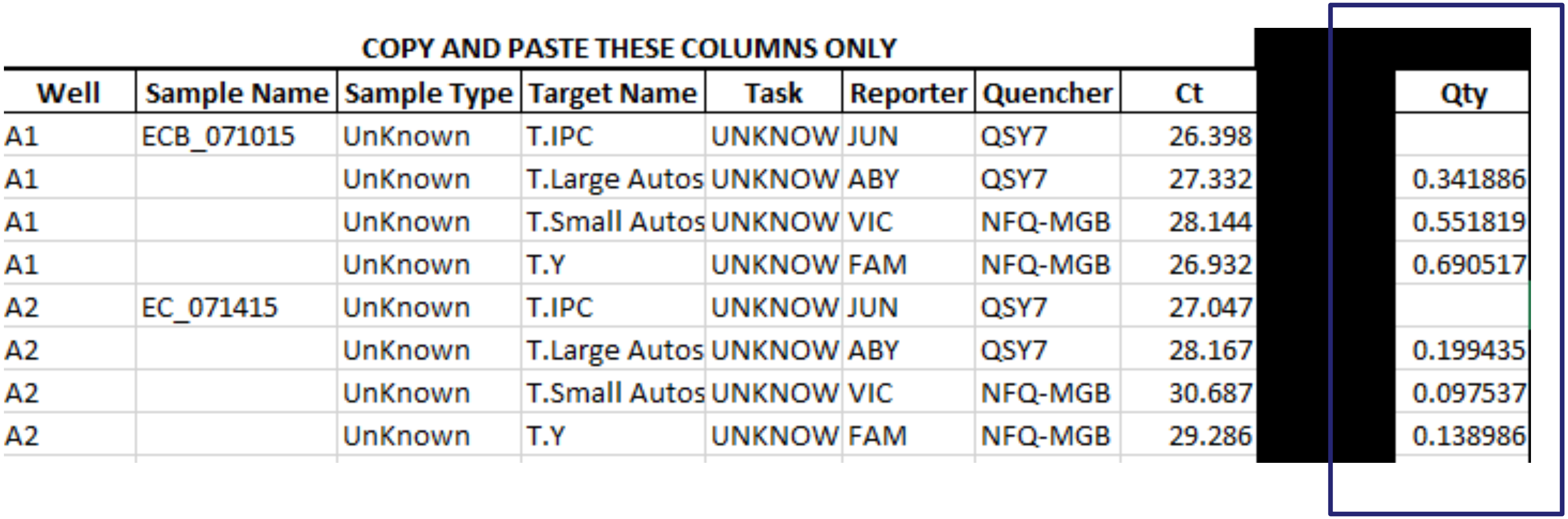
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	**25.66045705**	**0.040068912**	**640.4081247**	**1.1177E-256**	**25.58127597**	**25.73963812**	**25.58127597**	**25.73963812**
X Variable 1	**-3.56381403**	**0.027712142**	**-128.6011734**	**9.1787E-154**	**-3.618576618**	**-3.509051442**	**-3.618576618**	**-3.509051442**

Formulas for External Standard Curve

Detector	Formula for DNA Concentration
ABY	Qty = 10^[(C_T -25.669281507164)/-3.5671597353617]
VIC	Qty = 10^[(C_T -27.2715758345178/-3.378825302124]
FAM	Qty = 10^[(C_T - 26.388215293048/-3.381204064687

$$DNA\ Concentration\ \left(\frac{ng}{\mu L}\right) = 10^{\frac{Cycle\ Threshold\ - Intercept}{X - Variable}}$$

Conversion Excel



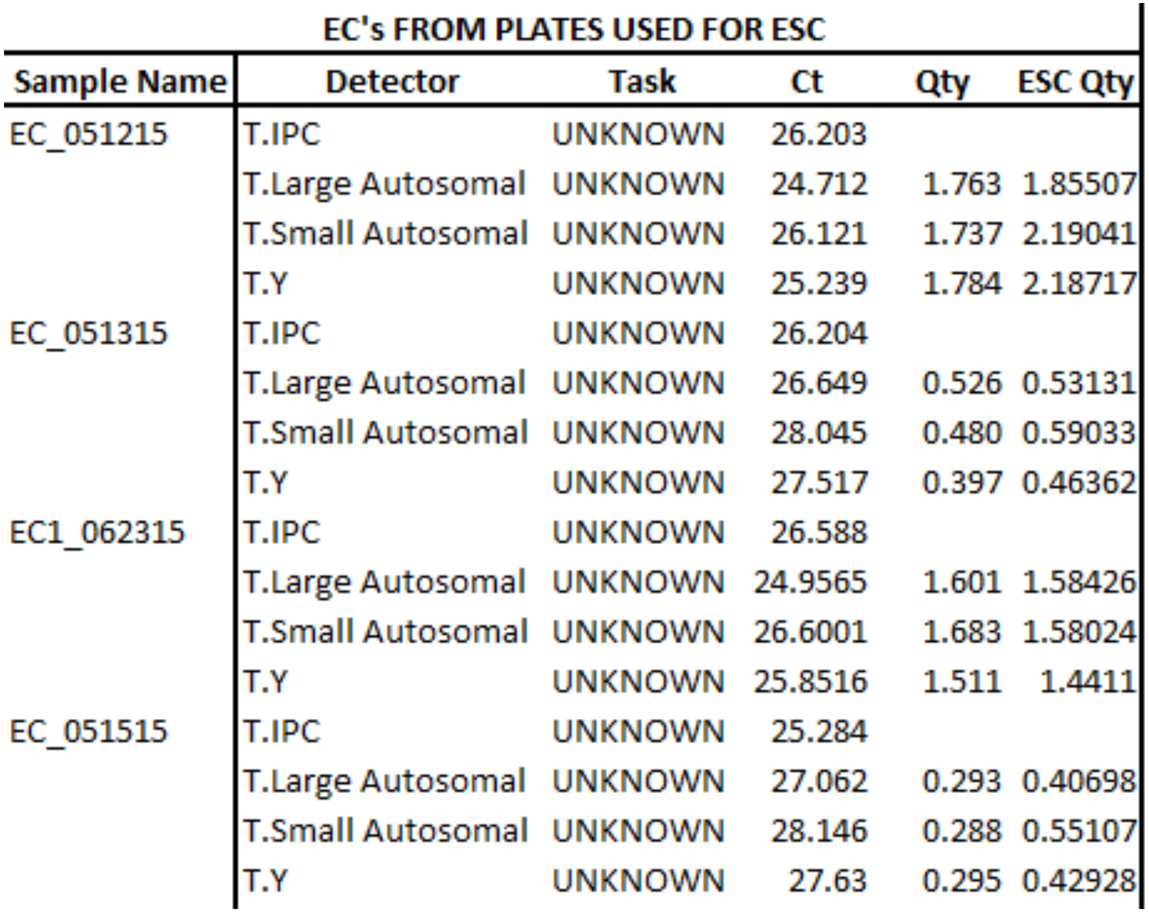
COPY AND PASTE THESE COLUMNS ONLY

Well	Sample Name	Sample Type	Target Name	Task	Reporter	Quencher	Ct	[redacted]	Qty
A1	ECB_071015	UnKnown	T.IPC	UNKNOW	JUN	QSY7	26.398	[redacted]	
A1		UnKnown	T.Large Autos	UNKNOW	ABY	QSY7	27.332	[redacted]	0.341886
A1		UnKnown	T.Small Autos	UNKNOW	VIC	NFQ-MGB	28.144	[redacted]	0.551819
A1		UnKnown	T.Y	UNKNOW	FAM	NFQ-MGB	26.932	[redacted]	0.690517
A2	EC_071415	UnKnown	T.IPC	UNKNOW	JUN	QSY7	27.047	[redacted]	
A2		UnKnown	T.Large Autos	UNKNOW	ABY	QSY7	28.167	[redacted]	0.199435
A2		UnKnown	T.Small Autos	UNKNOW	VIC	NFQ-MGB	30.687	[redacted]	0.097537
A2		UnKnown	T.Y	UNKNOW	FAM	NFQ-MGB	29.286	[redacted]	0.138986

Validation of an ESC

- Three Data Sets of Extraction Controls
- Used ESC worksheet to calculate quantitation value and compared to quantitation value calculated using plate specific standard curve
- Perform Student T-Test for statistical comparison

Set 1 – 12 Samples

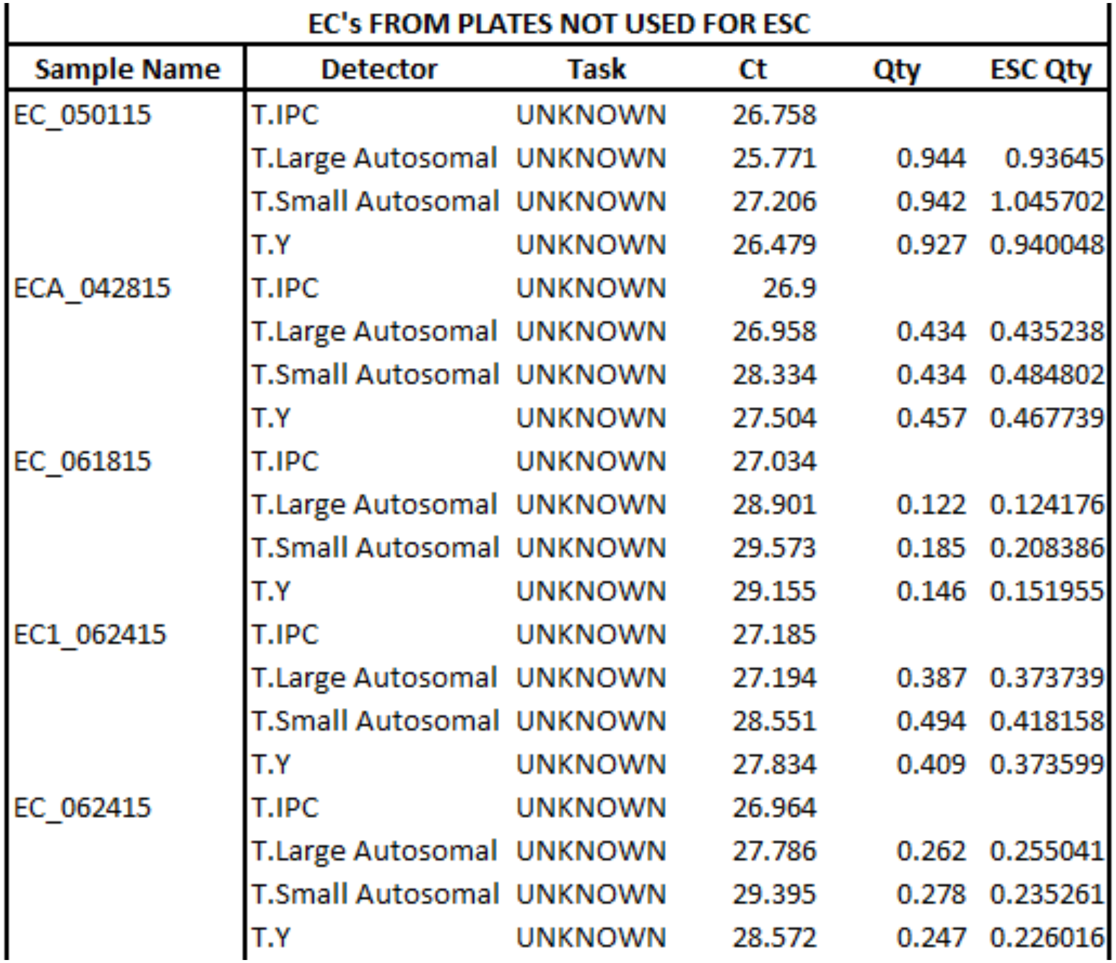


EC's FROM PLATES USED FOR ESC

Sample Name	Detector	Task	Ct	Qty	ESC Qty
EC_051215	T.IPC	UNKNOWN	26.203		
	T.Large Autosomal	UNKNOWN	24.712	1.763	1.85507
	T.Small Autosomal	UNKNOWN	26.121	1.737	2.19041
	T.Y	UNKNOWN	25.239	1.784	2.18717
EC_051315	T.IPC	UNKNOWN	26.204		
	T.Large Autosomal	UNKNOWN	26.649	0.526	0.53131
	T.Small Autosomal	UNKNOWN	28.045	0.480	0.59033
	T.Y	UNKNOWN	27.517	0.397	0.46362
EC1_062315	T.IPC	UNKNOWN	26.588		
	T.Large Autosomal	UNKNOWN	24.9565	1.601	1.58426
	T.Small Autosomal	UNKNOWN	26.6001	1.683	1.58024
	T.Y	UNKNOWN	25.8516	1.511	1.4411
EC_051515	T.IPC	UNKNOWN	25.284		
	T.Large Autosomal	UNKNOWN	27.062	0.293	0.40698
	T.Small Autosomal	UNKNOWN	28.146	0.288	0.55107
	T.Y	UNKNOWN	27.63	0.295	0.42928

Student T-test: $\alpha = 0.05$

Set 2 – 19 Samples



EC's FROM PLATES NOT USED FOR ESC

Sample Name	Detector	Task	Ct	Qty	ESC Qty
EC_050115	T.IPC	UNKNOWN	26.758		
	T.Large Autosomal	UNKNOWN	25.771	0.944	0.93645
	T.Small Autosomal	UNKNOWN	27.206	0.942	1.045702
	T.Y	UNKNOWN	26.479	0.927	0.940048
ECA_042815	T.IPC	UNKNOWN	26.9		
	T.Large Autosomal	UNKNOWN	26.958	0.434	0.435238
	T.Small Autosomal	UNKNOWN	28.334	0.434	0.484802
	T.Y	UNKNOWN	27.504	0.457	0.467739
EC_061815	T.IPC	UNKNOWN	27.034		
	T.Large Autosomal	UNKNOWN	28.901	0.122	0.124176
	T.Small Autosomal	UNKNOWN	29.573	0.185	0.208386
	T.Y	UNKNOWN	29.155	0.146	0.151955
EC1_062415	T.IPC	UNKNOWN	27.185		
	T.Large Autosomal	UNKNOWN	27.194	0.387	0.373739
	T.Small Autosomal	UNKNOWN	28.551	0.494	0.418158
	T.Y	UNKNOWN	27.834	0.409	0.373599
EC_062415	T.IPC	UNKNOWN	26.964		
	T.Large Autosomal	UNKNOWN	27.786	0.262	0.255041
	T.Small Autosomal	UNKNOWN	29.395	0.278	0.235261
	T.Y	UNKNOWN	28.572	0.247	0.226016

Student T-test: α = 0.05

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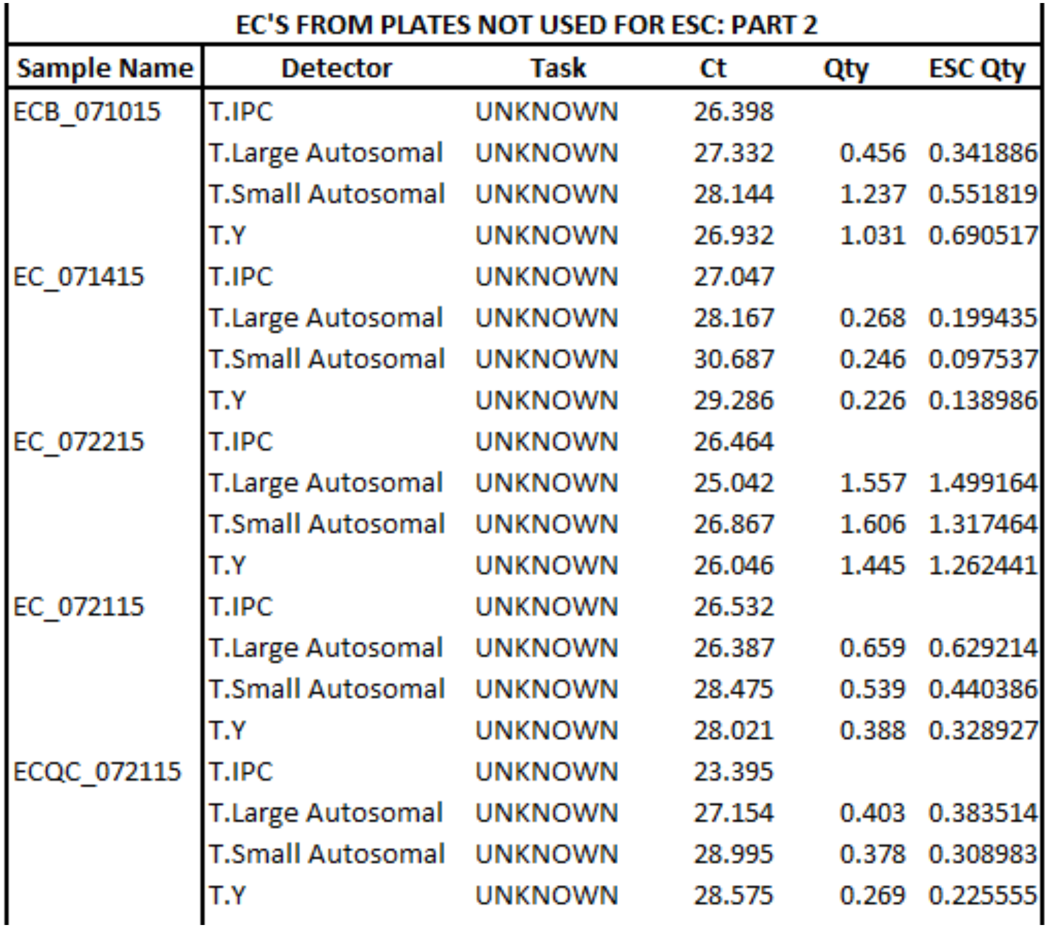
Set 3 – 19 Samples



EC'S FROM PLATES NOT USED FOR ESC: PART 2

Sample Name	Detector	Task	Ct	Qty	ESC Qty
ECB_071015	T.IPC	UNKNOWN	26.398		
	T.Large Autosomal	UNKNOWN	27.332	0.456	0.341886
	T.Small Autosomal	UNKNOWN	28.144	1.237	0.551819
	T.Y	UNKNOWN	26.932	1.031	0.690517
EC_071415	T.IPC	UNKNOWN	27.047		
	T.Large Autosomal	UNKNOWN	28.167	0.268	0.199435
	T.Small Autosomal	UNKNOWN	30.687	0.246	0.097537
	T.Y	UNKNOWN	29.286	0.226	0.138986
EC_072215	T.IPC	UNKNOWN	26.464		
	T.Large Autosomal	UNKNOWN	25.042	1.557	1.499164
	T.Small Autosomal	UNKNOWN	26.867	1.606	1.317464
	T.Y	UNKNOWN	26.046	1.445	1.262441
EC_072115	T.IPC	UNKNOWN	26.532		
	T.Large Autosomal	UNKNOWN	26.387	0.659	0.629214
	T.Small Autosomal	UNKNOWN	28.475	0.539	0.440386
	T.Y	UNKNOWN	28.021	0.388	0.328927
ECQC_072115	T.IPC	UNKNOWN	23.395		
	T.Large Autosomal	UNKNOWN	27.154	0.403	0.383514
	T.Small Autosomal	UNKNOWN	28.995	0.378	0.308983
	T.Y	UNKNOWN	28.575	0.269	0.225555

Student T-test: $\alpha = 0.05$

Use of ESC on Casework Samples

- New ESC generated after maintenance
  - (PMs, dye calibrations)
  - Validated before use on Casework
    - All curves passed, passing criteria from linear regression statistics, new worksheet generation
- Run Positive Control on every plate
  - In duplicate
  - 0.5 ng/µL standard

Y-Screen Procedure at NMS Labs

- Two Services Provided
  - Y-Screen on SAEK, DNA Testing
  - Y-Screen on SAEK, Serology and DNA Testing

- Contracts with Clients
  - Police agencies, laboratories, etc.

Current Procedure - Examination

- Cut ½ of one swab from samples in kit
  - Number of samples tested per case dependent on contract
  - Also take case notes into consideration

- Cut reference sample – hold on DNA

Current Procedure - Extraction

- Qiagen’s EZ1 Advanced XL
  - Robotic extraction
  - ~20 minute run time after incubation
  - 14 samples per run
  - Currently looking at procedure for standardization of a high-throughput workflow
- All question samples (besides male on male assault)

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Current Procedure – Quant 1

- Quantify using Quantifiler® Trio and an external standard curve
- Determination if sample should be re-cut for differential extraction
  - Quant Value
  - Male:Female Ratio
  - Case Information

Current Procedure – Differential Extraction

- Cut other ½ of swab from sample (if sufficient material left)
- Manual Separation including a wash step
- Extract both fractions with an organic procedure

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https://thumbs.dreamstime.com/z/sperm-character-9335815.jpg

Current Procedure – Quant 2

- Quantify differential samples
- Assess each case separately
  - Send best sample(s) forward to amplification (number of samples dependent on contract)
    - Quant values
    - Male:female ratio
    - Degradation index
    - Case information

Current Procedure – Typing & Analysis

- Amplify best sample(s)
- Extract and type victim reference sample if available
- Perform comparison/analysis and report out
- Send results back to contracted lab or request upload for CODIS
  - Either through the state or national database

Current Procedure – Re-testing

- May go back and test additional swabs or evidence (panties) from kit
- May go back and amplify samples in Y-STR amplification kit

Backlog Projects at NMS - 1

- 1324 Kits
- State law did not require audit or tracking of untested kits. Now laws require testing of all backlogged kits, victims to be notified
- Grant received

Backlog Projects at NMS - 1

- 256 kits tested as of July 2017
- 47% - Negative Cases based on Quant Results
- 16% - Negative Cases based on Typing Results
- 9% - Profile matched victim (no male detected, produced inconclusive mixture, produced too partial of data)
- 26%-Unknown Profile generated

Backlog Projects at NMS - 1

- 33 CODIS Hits
- 58% were individuals associated with the case
- 42% new leads
- Link a serial rapist to additional case
- Link one individual to two separate cases

Backlog Projects at NMS - 2

- 85 cases tested to date
- 22% - Negative Cases based on Quant Results
- 20% - Negative Cases based on Typing Results
- 39% - Profile matched victim (no male detected, produced inconclusive mixture, produced too partial of data)
- 19% (16 cases) – sent for CODIS evaluation

Acknowledgements

- NMS Labs Forensic Biology Team
- ThermoFisher

Questions?

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http://projects.nfstc.org/otc/images/module1/DNACD_mod01-1-04.jpg