










Internal Validation of the
Promega® PowerPlex® Fusion System
with the
Applied Biosystems® 3130xl
Genetic Analyzer

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Marshall University Forensic Science Center DNA Laboratory

Overview

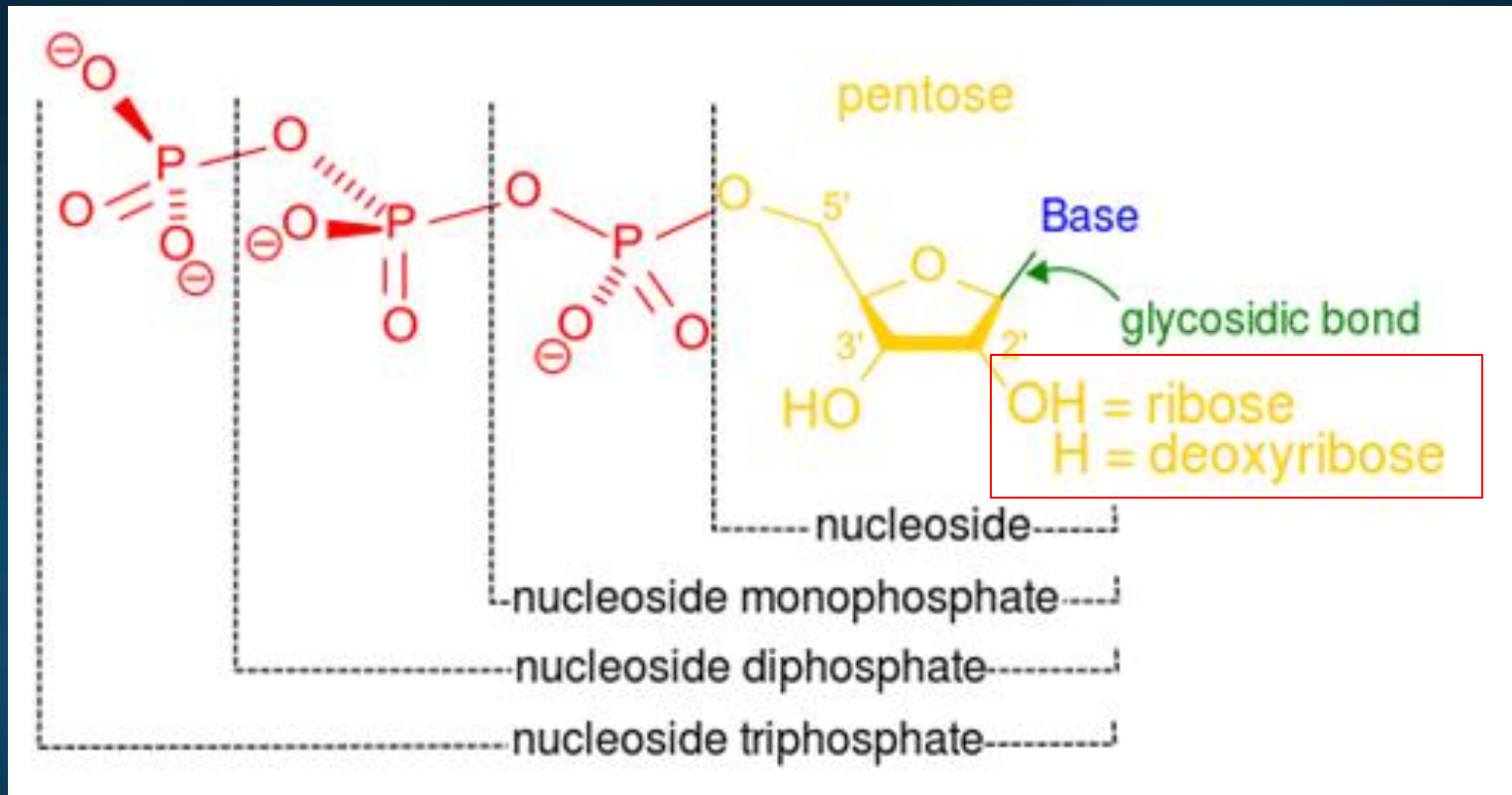
-  What is DNA?
-  Processing
-  Extraction
-  Quantification
-  Amplification
-  Capillary Electrophoresis
-  Analysis

What is DNA?



Deoxyribo **N**ucleic **A**cid

Nucleoside triphosphate



Nucleotide

Purines

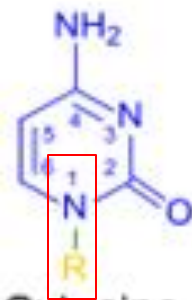


Adenine

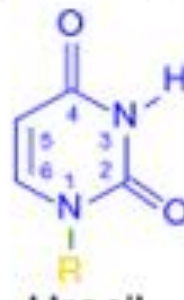


Guanine

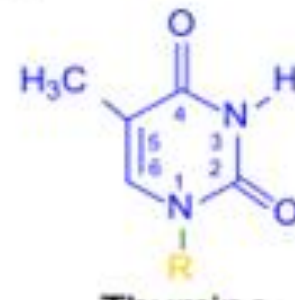
Pyrimidines



Cytosine

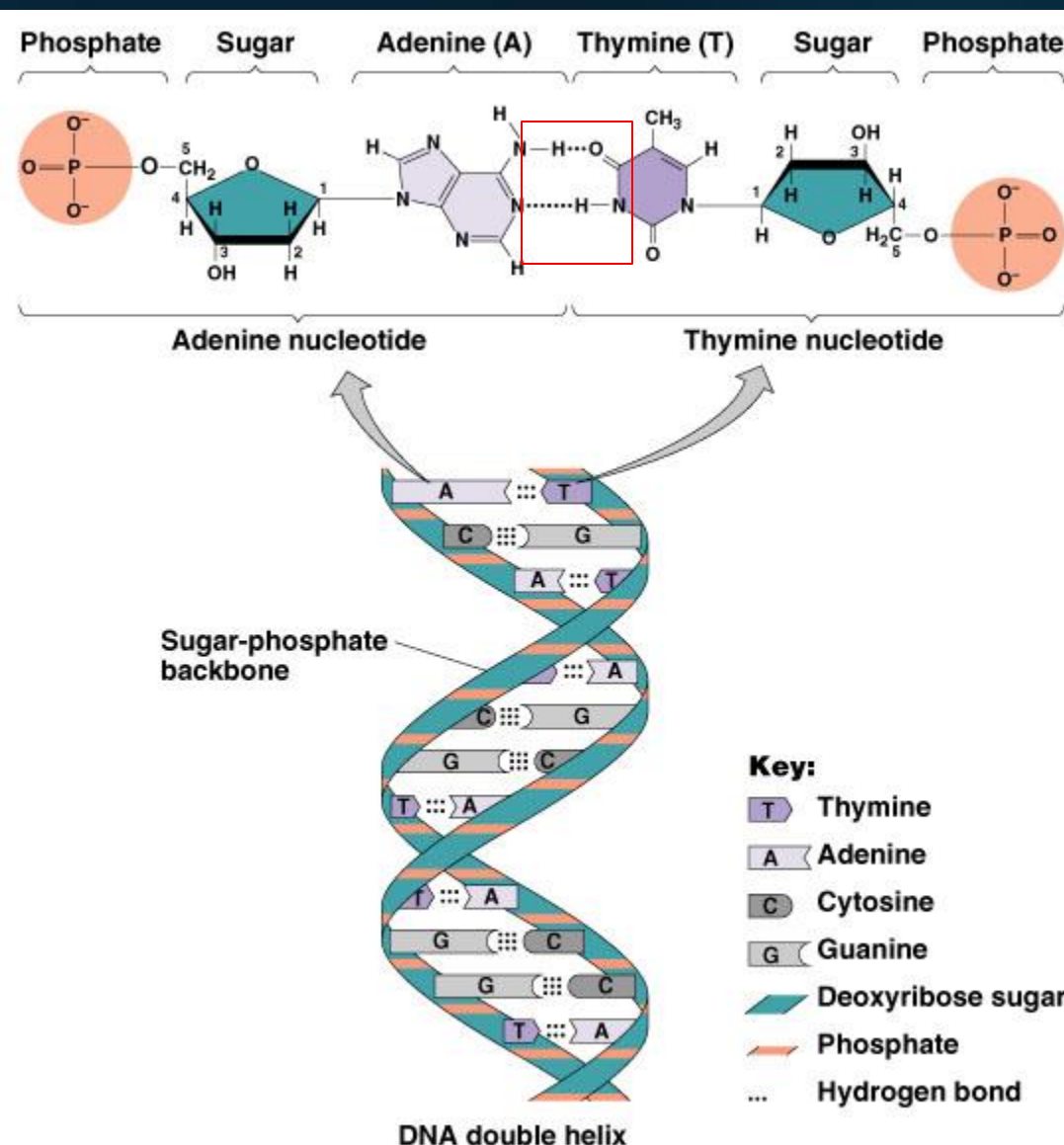


Uracil



Thymine

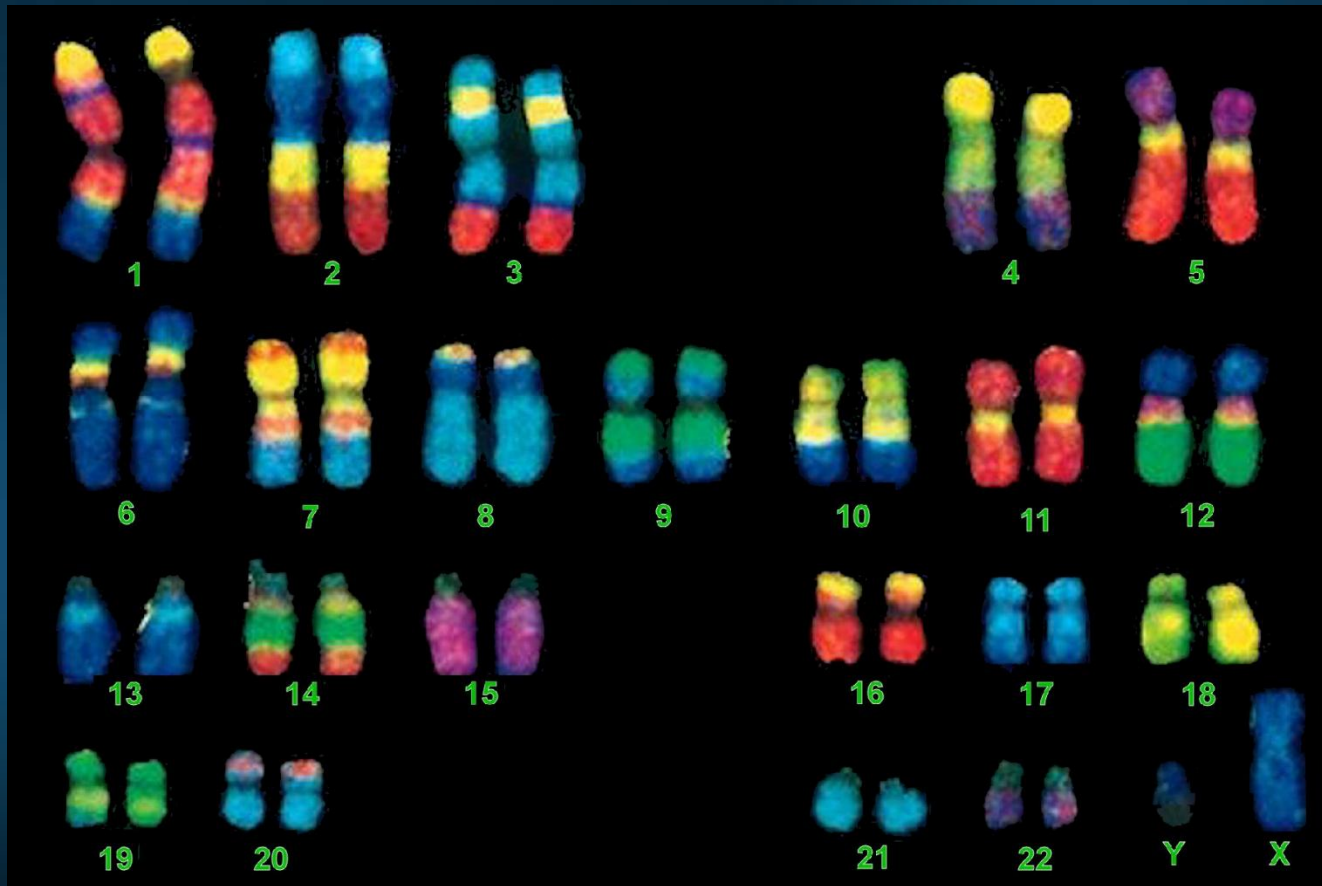
2D Structure



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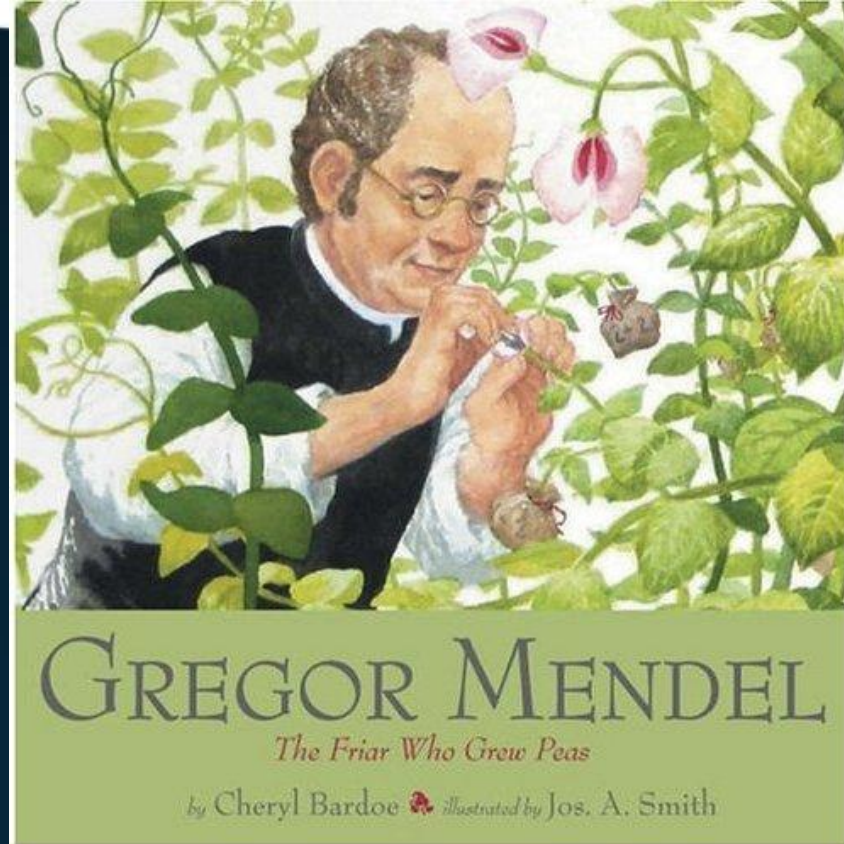
3D Structure

- Chromosomes -44 XY/XX



Alleles and Traits

- Chromosomes carry traits in forms of alleles



So.....What is DNA????

- Blueprint
- User's Manual

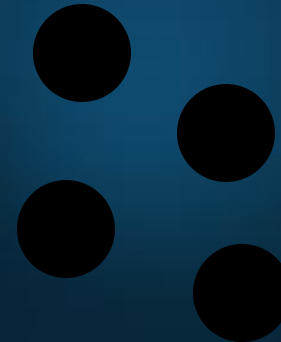
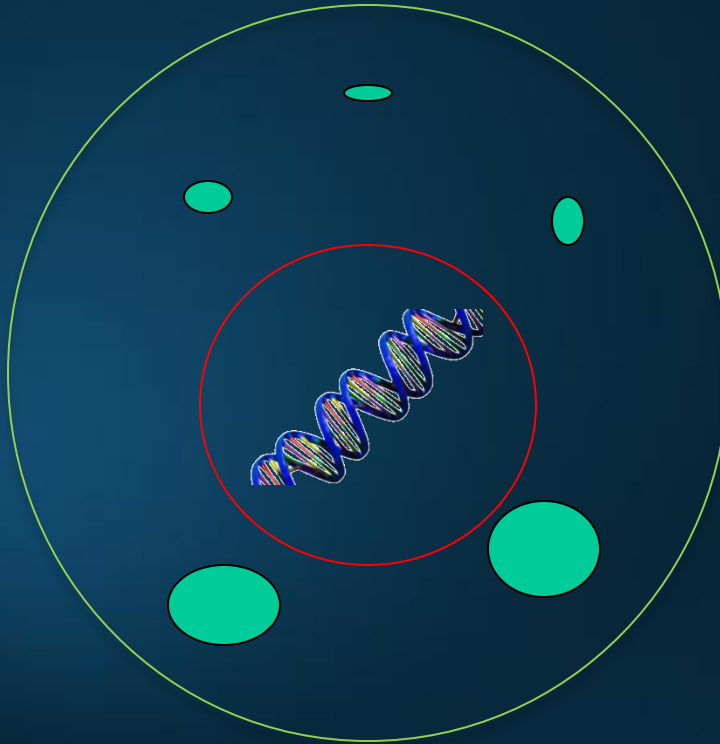




DNA in Forensics

- STR- Short Tandem Repeats
- Alleles identified by number of STR repeats

Extraction

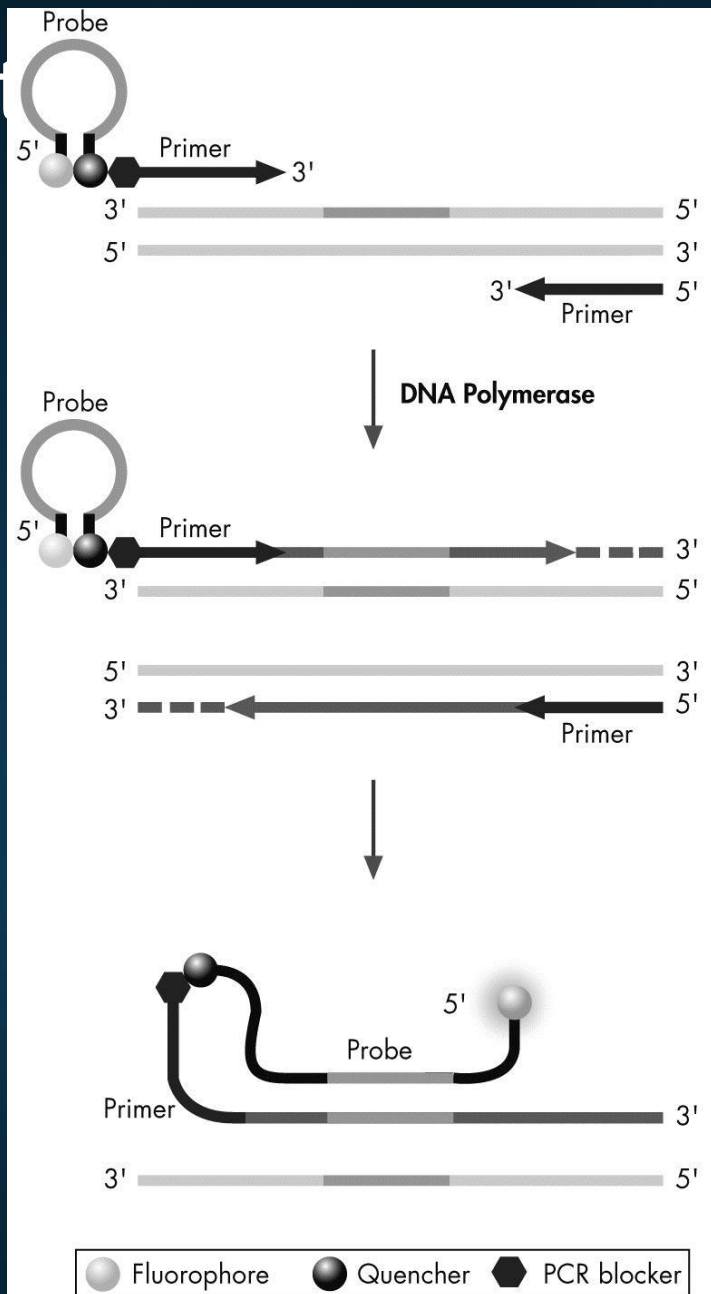


Quant

DAB Standards Want **You** to **Quant**



Quantification

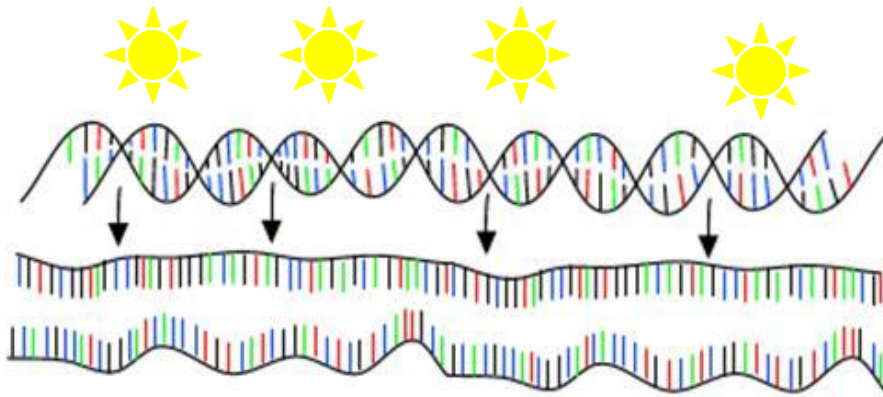


PCR : Polymerase Chain Reaction

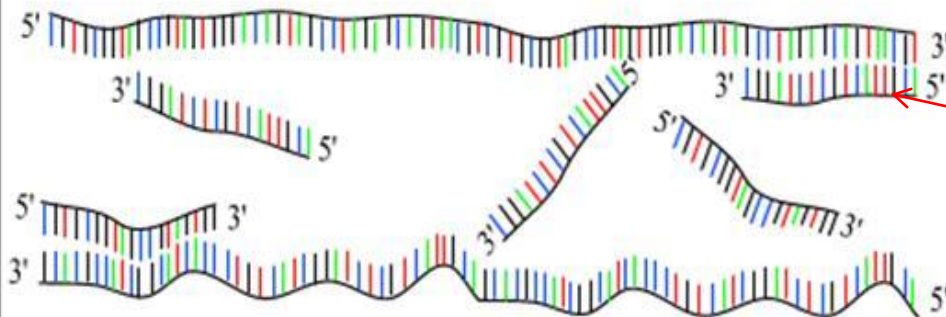
A

30 cycles of 3 steps :

Step 1 : denaturation



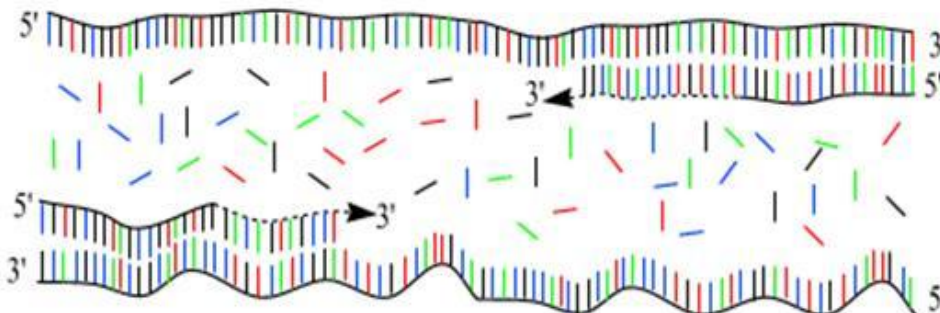
Step 2 : annealing



Primers

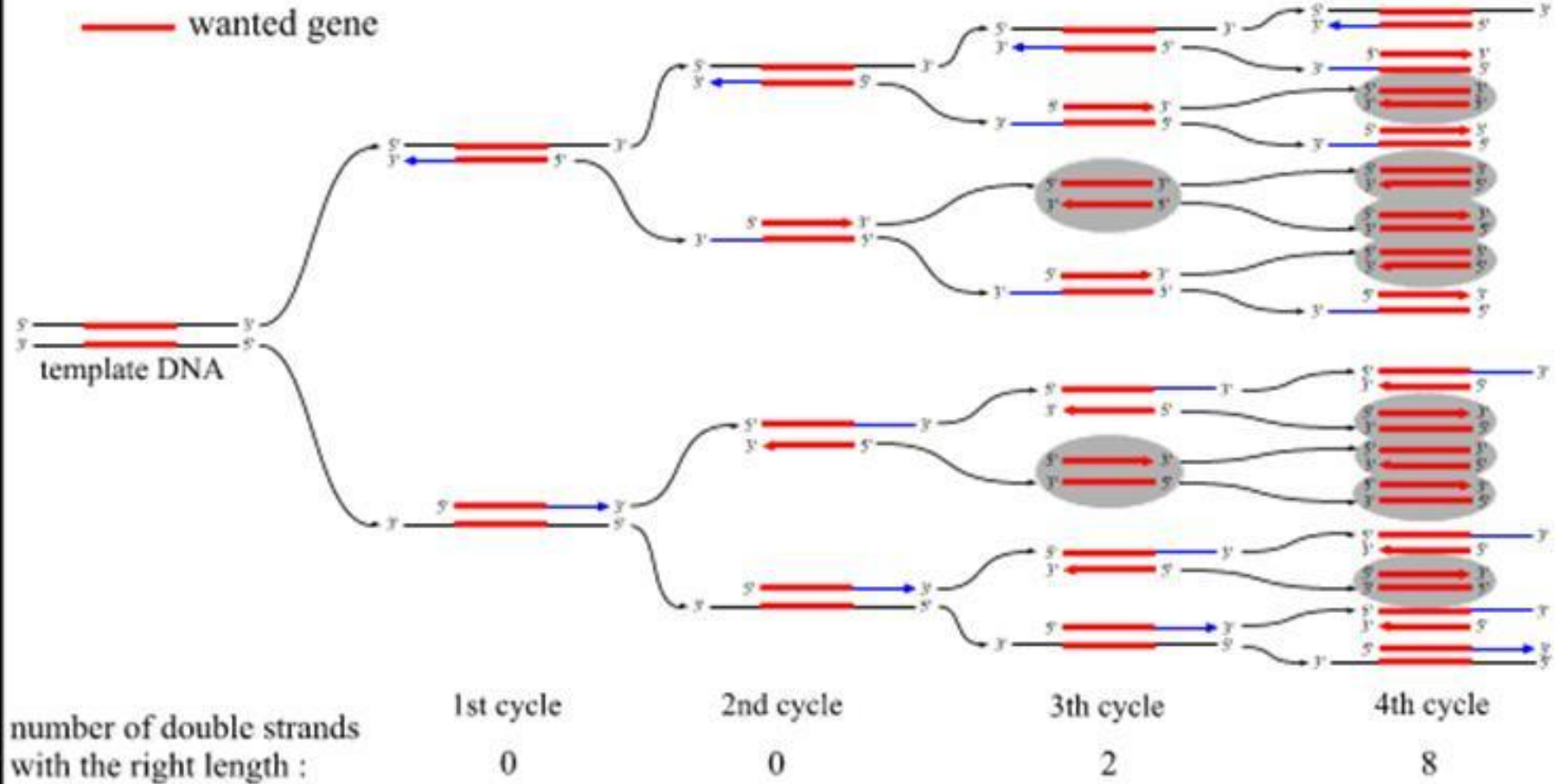
forward and reverse primers !!!

Step 3 : extension



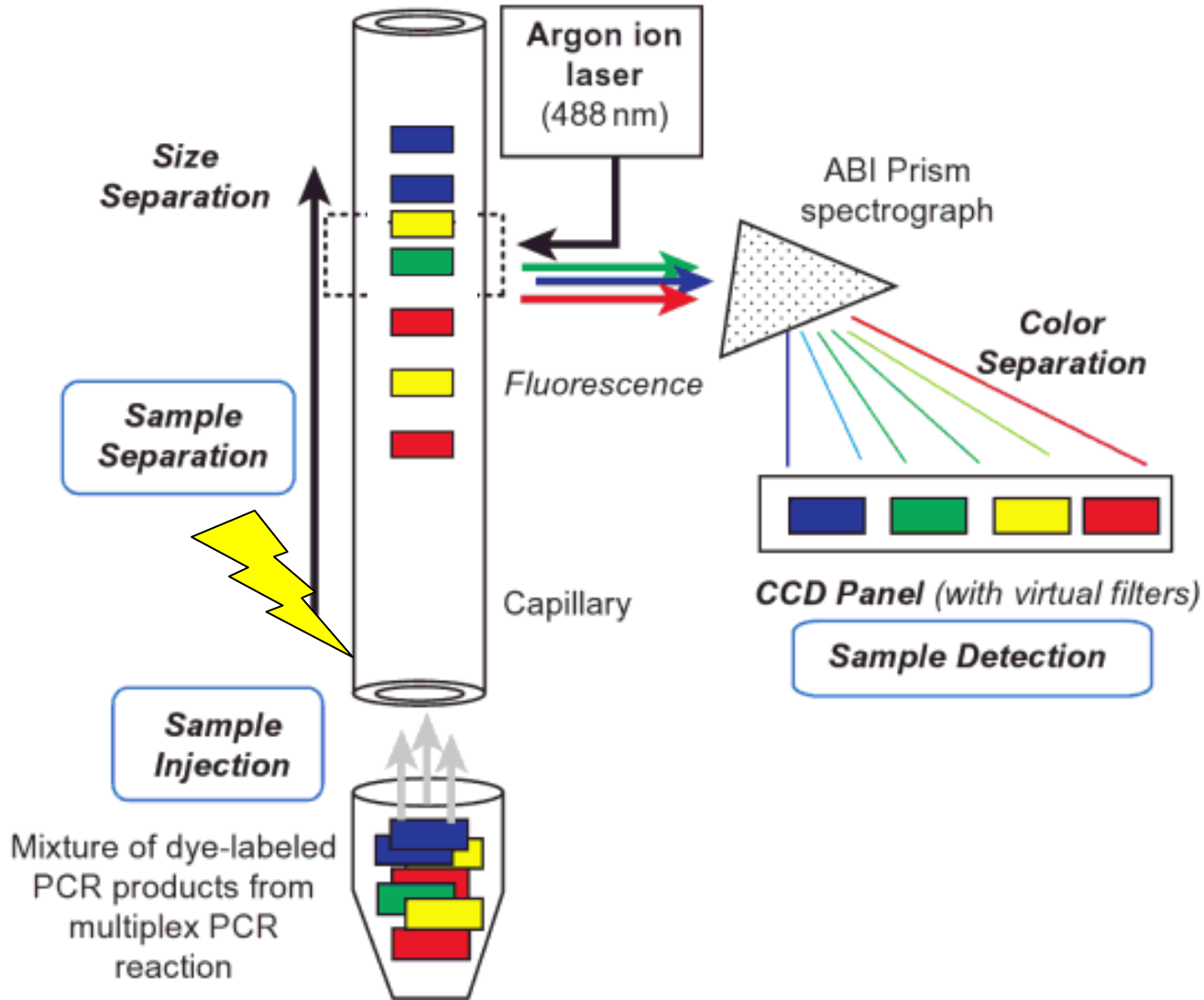
only dNTP's

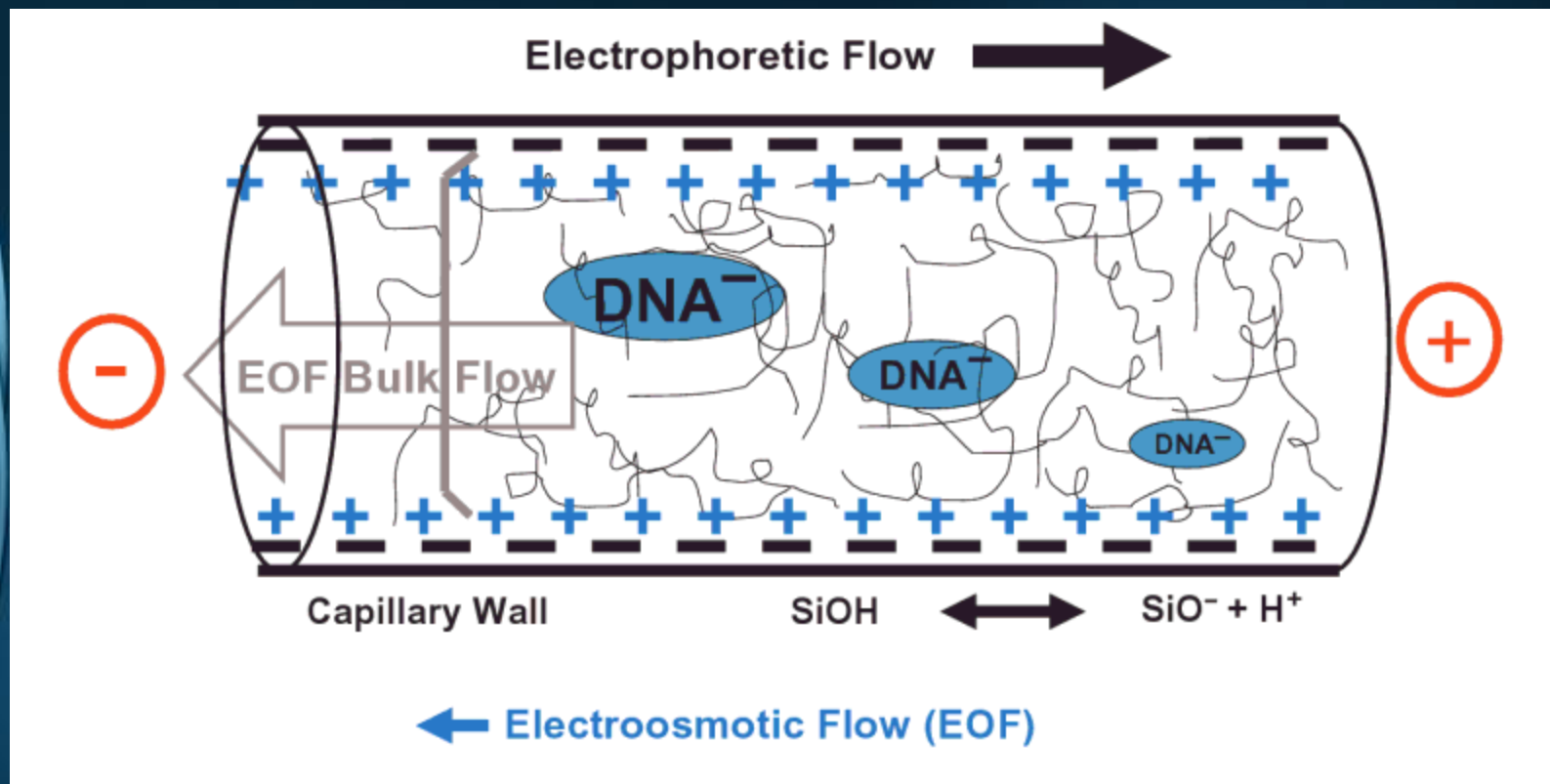
The first 4 cycles of PCR in detail

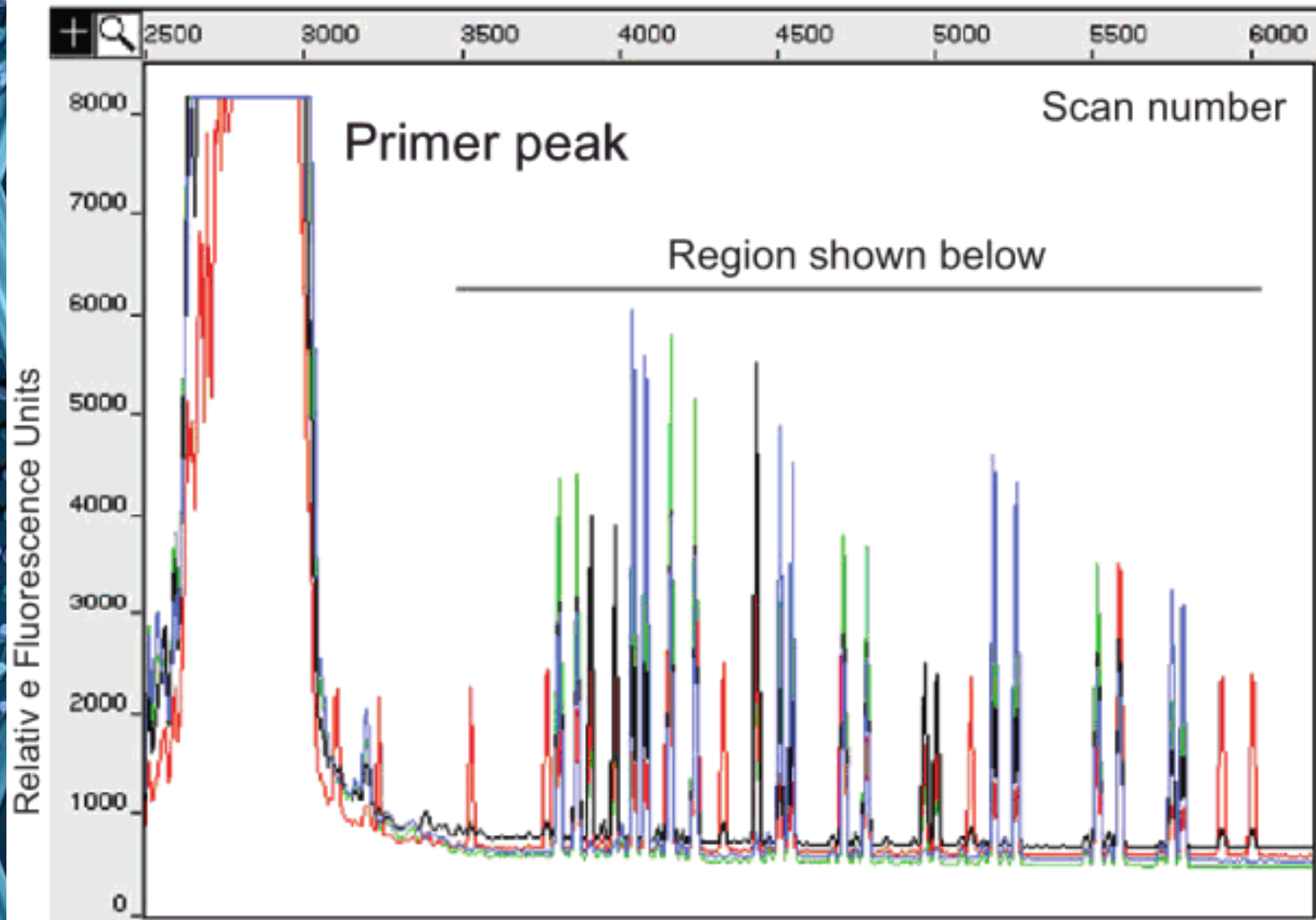


(Andy Vierstraete 2001)

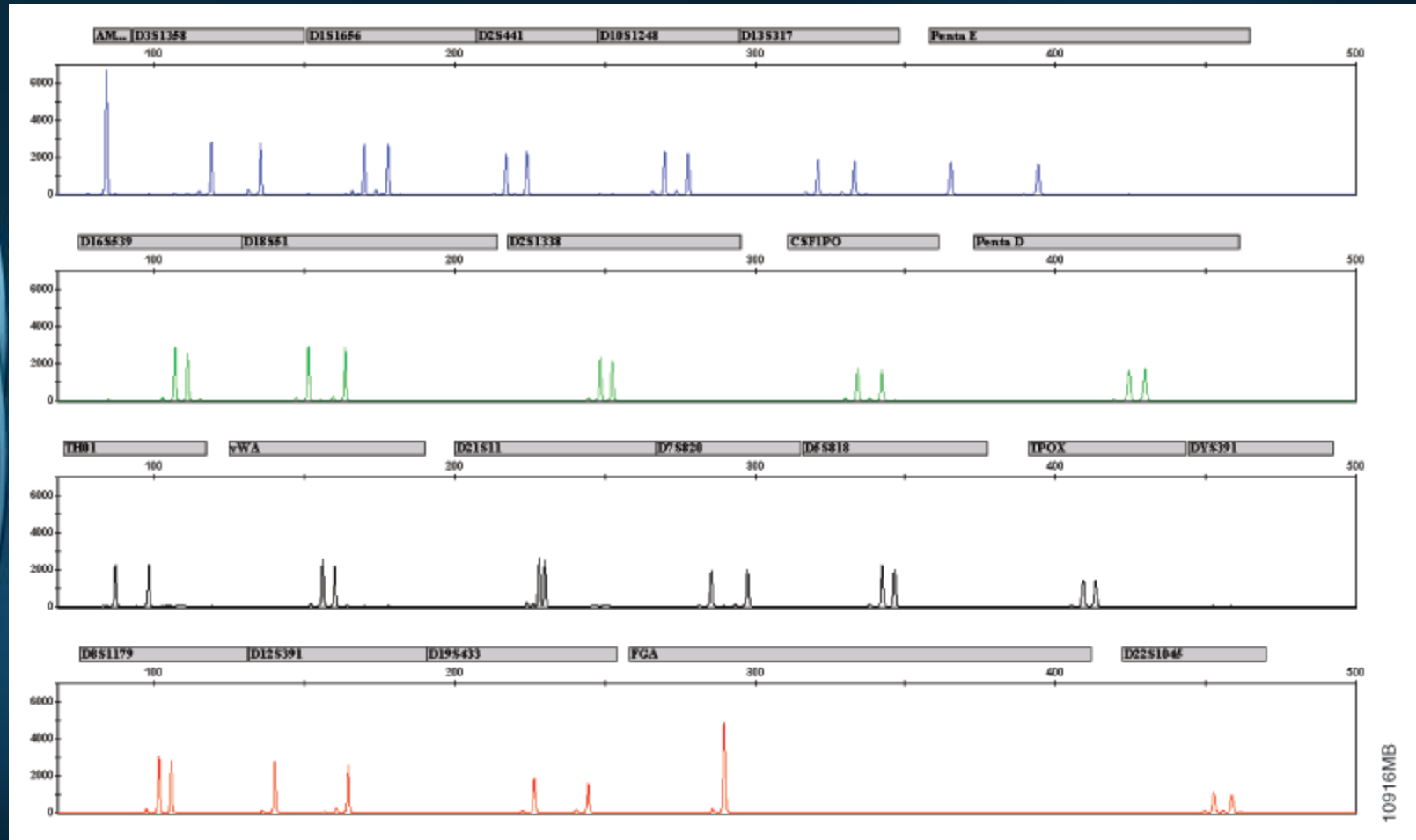
Capillary Electrophoresis







Analysis



Overview Validation

 Overview of the Chemistry

 Validation Work

 Troubleshooting

 Future Studies

 Conclusion



Promega® PowerPlex® Fusion System


- 24 loci – 5 Dye Chemistry
- Core 13 CODIS loci

(CSF1PO, FGA, TH01, TPOX, vWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, and D21S11)

- 12 European Standard Set loci

(TH01, vWA, GA, D21S11, D3S1358, D8S1179, D18S51, D10S1248, D22S1045, D2S441, D1S1656, and D12S391)

- Penta E, Penta D, D2S1338, D19S433 and Amelogenin and a Y-STR loci (DYS391)

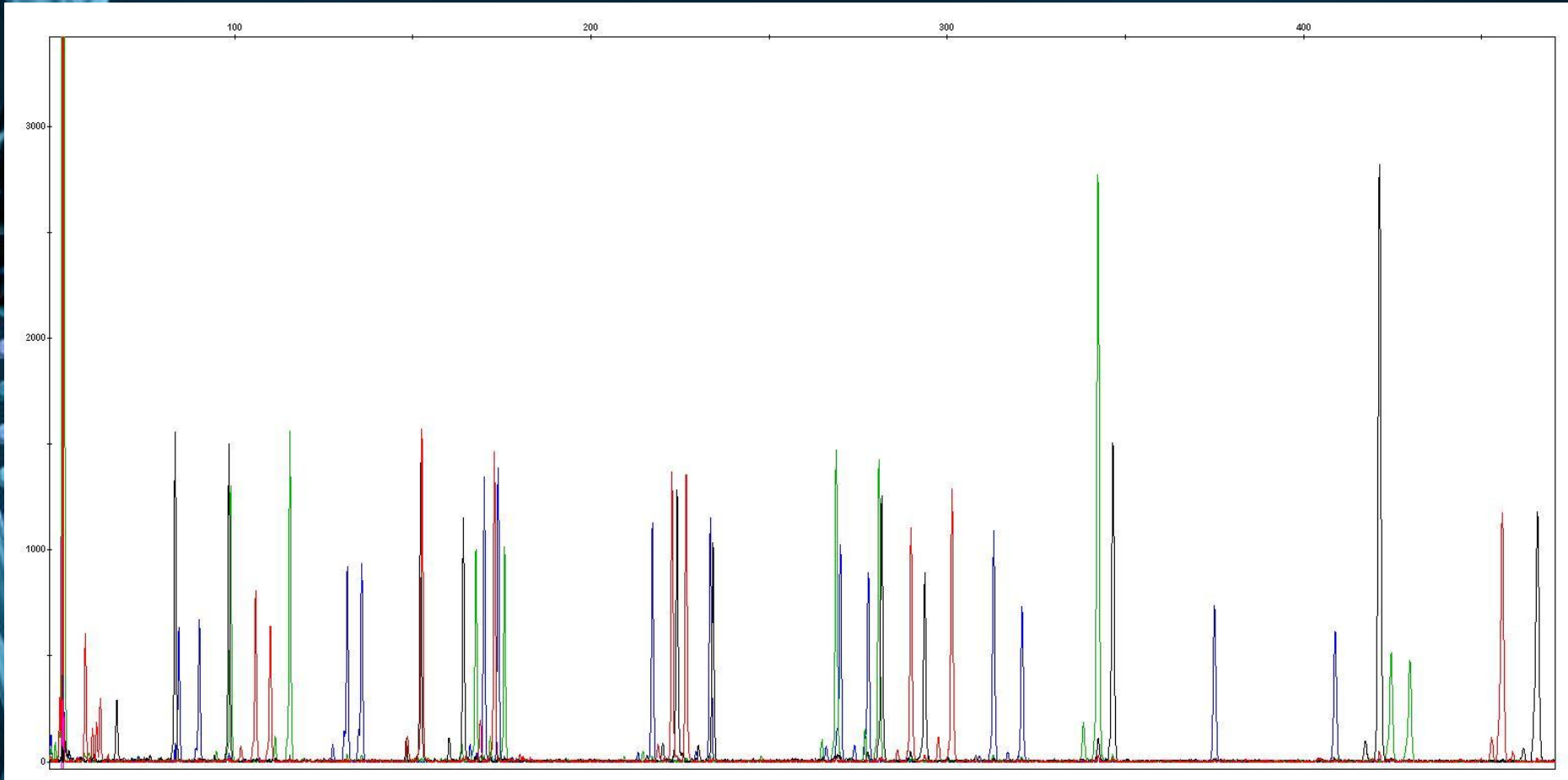


Promega® PowerPlex® Fusion System

(cont'd)

- Amplifies on 9700 at Max Ramp Speed
- Works on ABI® 310, 3130, 3130xl , 3500, 3500xl
- No upgrades necessary
- Only simple software upgrades for GeneMapper®
ID v3.2.1 present free online

Preliminary Testing – 2800M Raw Data






Validation Work

1. Cycle Number, DNA Target, Injection Time Study
2. Threshold Study
3. Precision Study
4. Peak Height Ratio Study
5. Concordance Study
6. Contamination Study
7. Stutter Study
7. Mixture Study
9. Inhibition Study
10. Non-Probativ Samples Study

Cycle Number, DNA Target, Injection Time

- 30 cycles vs. 31 cycles
- Serial Dilution in triplicates

10 ng
5 ng
2.5 ng
1 ng
0.5 ng
0.25 ng
0.125 ng
0.0625 ng
0.0313 ng
0.0156 ng



Cycle Number, DNA Target, Injection Time (*cont'd*)

- Injection at 3, 5, 10, 15 seconds at 3kV
- Total Injection Time changed from 1500 seconds to 1700 seconds



Threshold Study

- Reagent Blanks ran in 5 replicates and injected 5 times
- Results analyzed at 1 rfu

Analytical Threshold

- Method 1: IUPAC

$$AT = Y_{bl} + kS_{bl}$$

AT= Analytical Threshold

Y_{bl} = Average blank RFU signal

$k = 3$

S_{bl} = Standard Deviation of the blank signal



Analytical Threshold

- Method 2: SWGDAM

$$AT = 2(Y_{max} - Y_{min})$$

AT = Analytical Threshold

Y_{max} = Highest peak within instrumental noise

Y_{min} = signal of the lowest trough

Results

Table 1: Analytical Threshold- Method 1

Dye	Average Height	Standard Deviation Height	Minimum Height	Maximum Height	Analytical Threshold
Blue	4.63	1.56	1	18	9.31
Green	6.03	1.87	1	20	11.63
Yellow	8.37	2.46	2	27	15.75
Red	6.34	1.86	2	35	11.93

Table 2: Analytical Threshold- Method 2

Dye	Average Height	Standard Deviation Height	Minimum Height	Maximum Height	Analytical Threshold
Blue	4.63	1.56	1	18	34
Green	6.03	1.87	1	20	38
Yellow	8.37	2.46	2	27	52
Red	6.34	1.86	2	35	68



Troubleshooting

- Artifact present in Reagent Blanks but not in samples
- Consistent with Qiagen® *EZ1 DNA Investigator* kit contaminant profile
- Re-amplified- not replicated
- Eliminated because not reproducible.

LOD and LOQ

- $LOD = \text{Average noise signal} + 3 * \text{Std}$
- $LOQ = \text{Average noise signal} + 10 * \text{Std}$

Results

Dye	Average Height	Standard Deviation Height	Minimum Height	Maximum Height	LOD	LOQ
Blue	4.63	1.56	1	18	9.31	20.21
Green	6.03	1.87	1	20	11.63	24.72
Yellow	8.37	2.46	2	27	15.75	32.96
Red	6.34	1.86	2	35	11.93	24.96

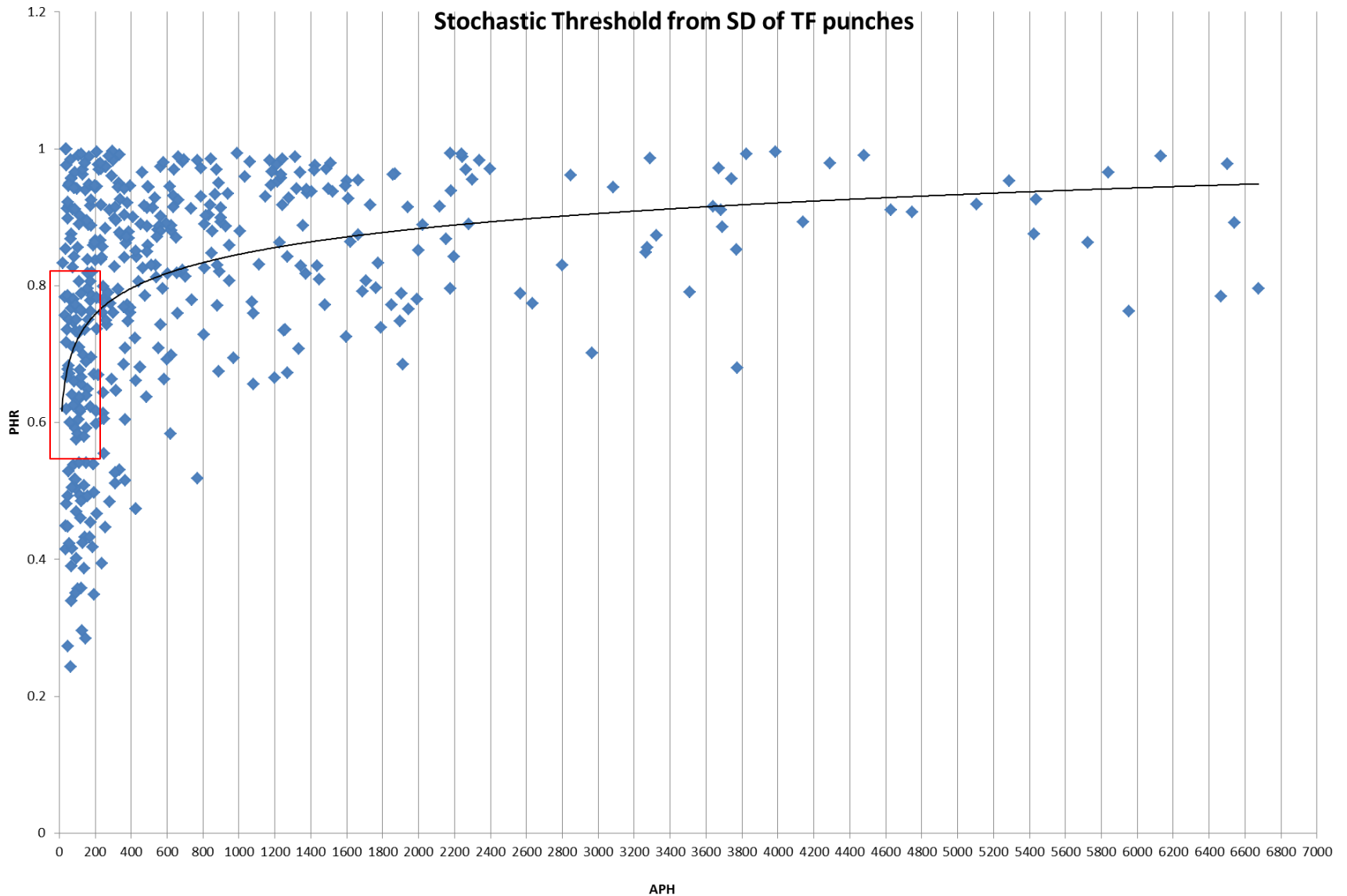
Stochastic Threshold

$$ST = [1 / (\text{Average PHR} - 3x \text{ STD})] \times AT$$

Results

Dye	AVG PHR	STD PHR	AT-M1	AT-M2	ST-M1	ST-M2
Blue	0.8191	0.0883	9.31	34	16.8044	61.3696
Green	0.8023	0.0666	11.63	38	19.3093	63.0915
Yellow	0.7874	0.1107	15.75	52	34.603	114.245
Red	0.7611	0.1234	11.93	68	30.517	173.944

Results





Precision Study

- 16 ladders injected 5 times
- TF punches ran previously
- 3 Standard Deviation must be less than 0.5bp



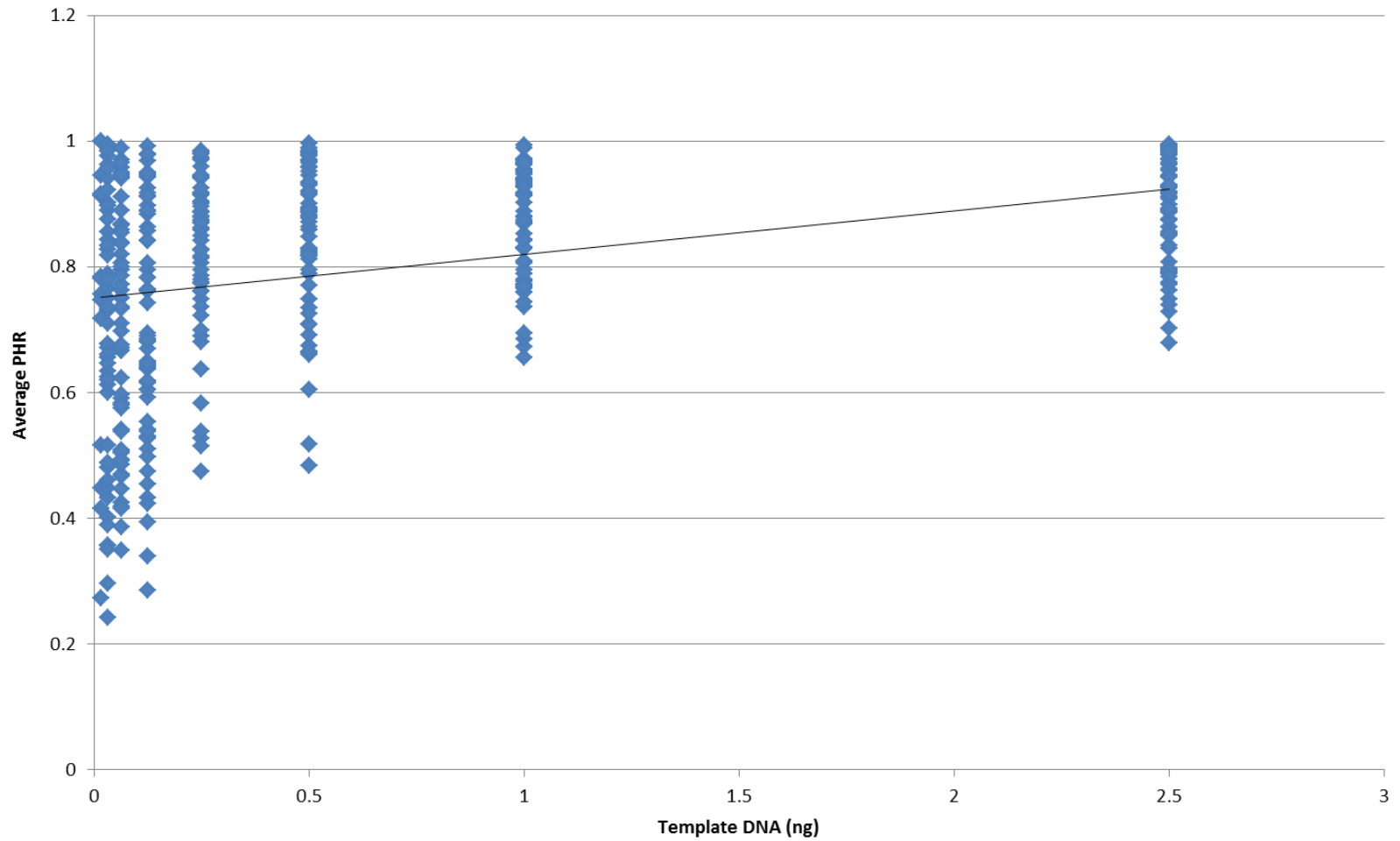
Results

- Highest $3*STD = 0.284$ at allele 15 for D12S391 at 31 cycles and 5 second injection
- Lowest $3*STD = 0.069$ at allele 12 for D16S539 at 31 cycles and 5 second injection

Peak Height Ratio

- 5 second injection of the TF punches was used
- Average PHR versus template DNA calculated

Results





Concordance

- 35 convicted offenders samples used
- Compared to the PowerPlex® 16 results



Results

- All samples were in concordance with the previous results except for 1 sample that had drop out

Contamination

- Ladder and Run Negative checkerboard plate was ran and injected with the initial plate at 3, 5, 10, 15 seconds



Results

- No cross contamination was present in the run negatives.
- One injection did show some peaks in the Run Negative, yet those peaks were not present in the other injections so they were not considered since they were not reproducible.

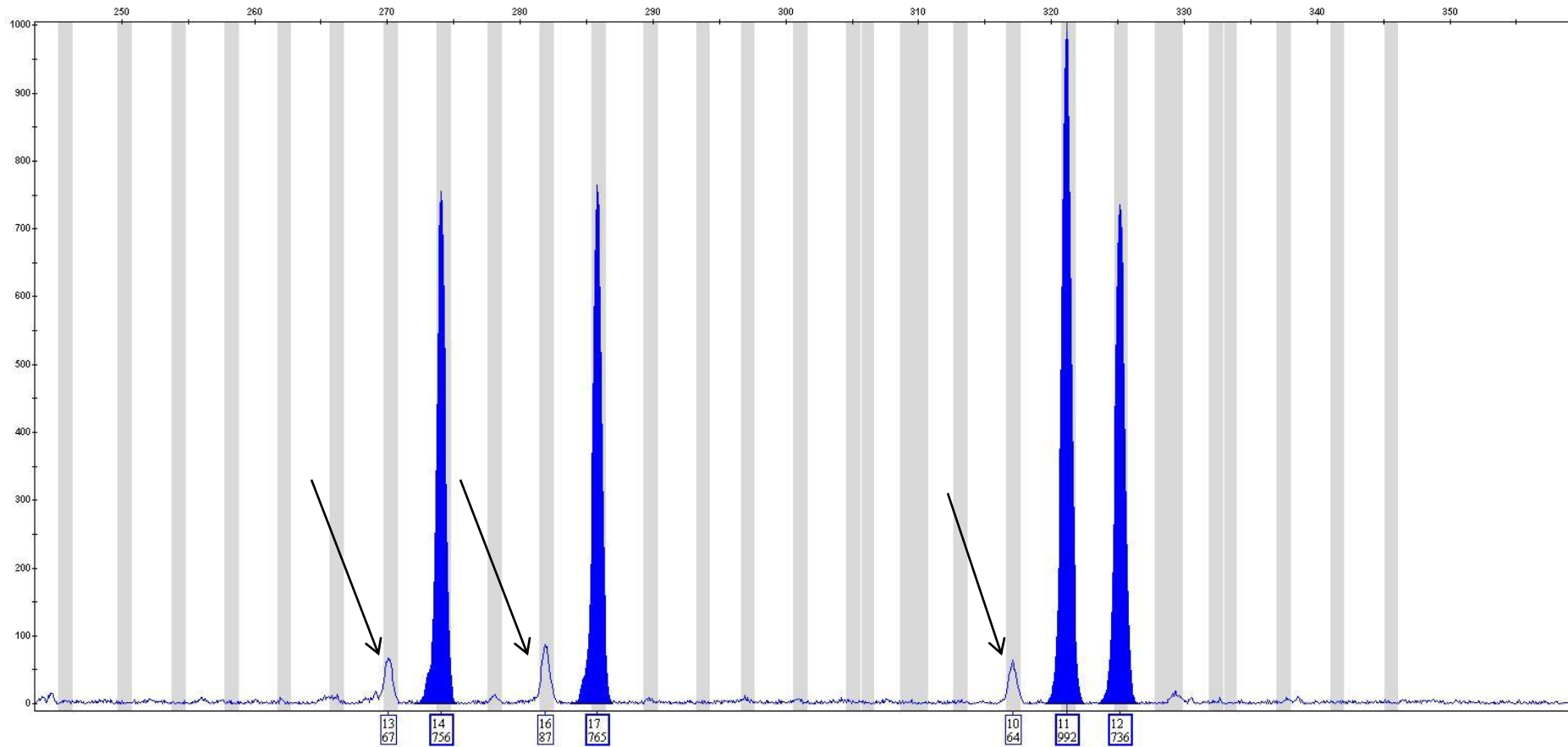
Stutter Study

- 35 Convicted offenders samples amplified at 0.125ng, 0.5 ng , 1.875 ng
- Macro from strbase.com
- Formula:

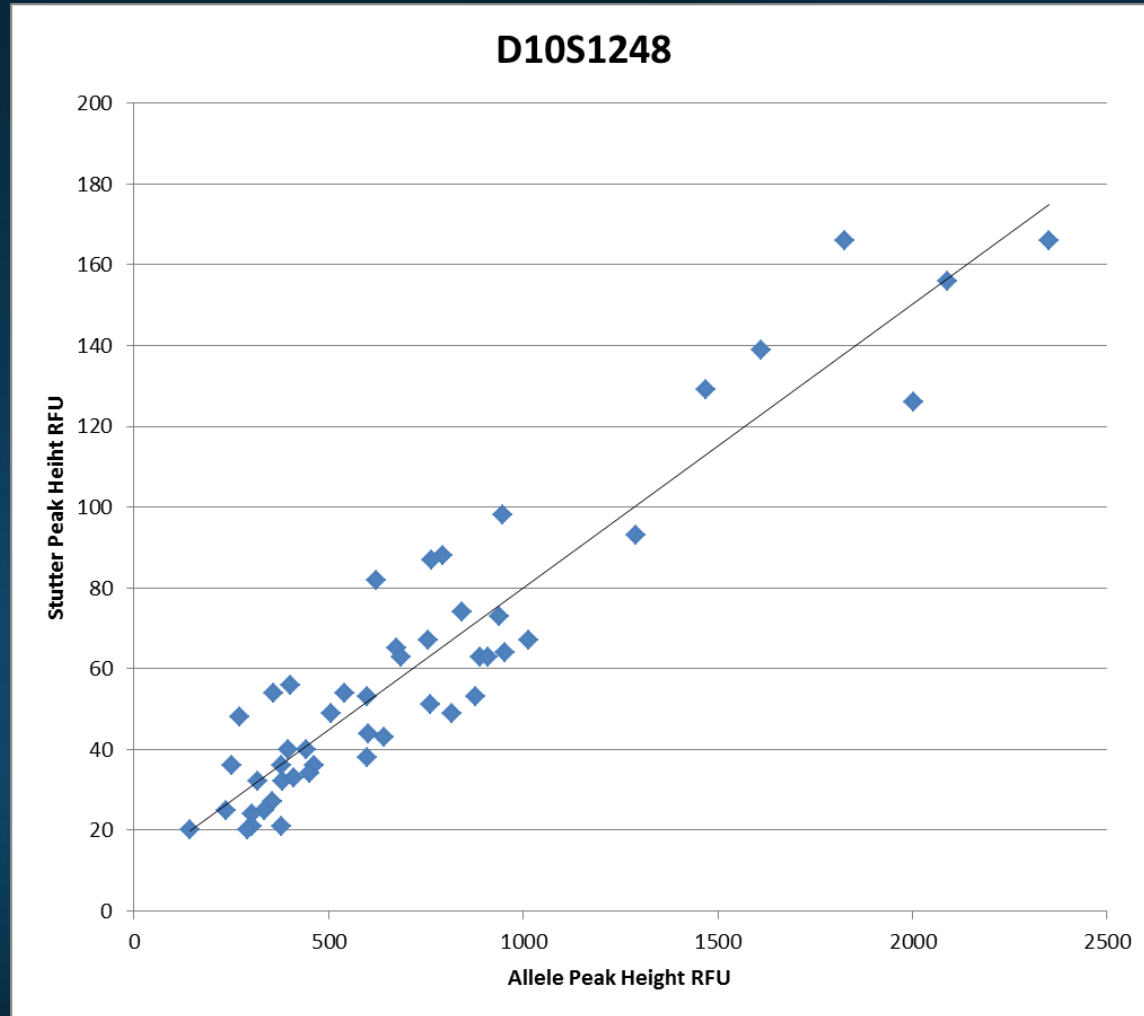
$$\textit{Stutter} = \textit{Avg Stutter Ratio} + 3 * \textit{Stutter Ratio Std}$$

Stutter example at *D10S1248* – *D13S317*

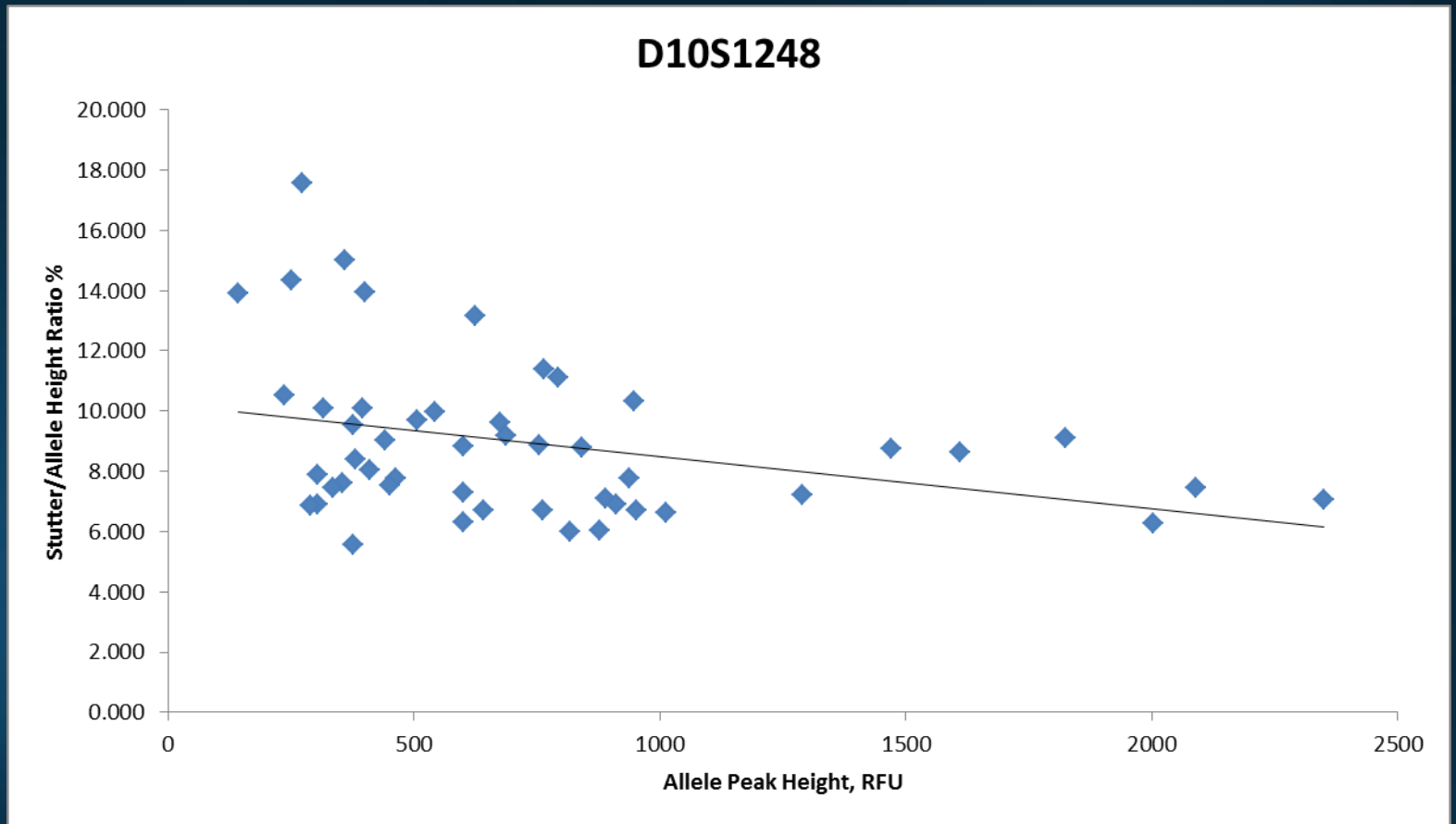
BC0000013988.G04.Ludwig.fsa BC0000013988 PowerPlex_Pusion_Fands ■ ■ *D10S1248* *D13S317* Penta E



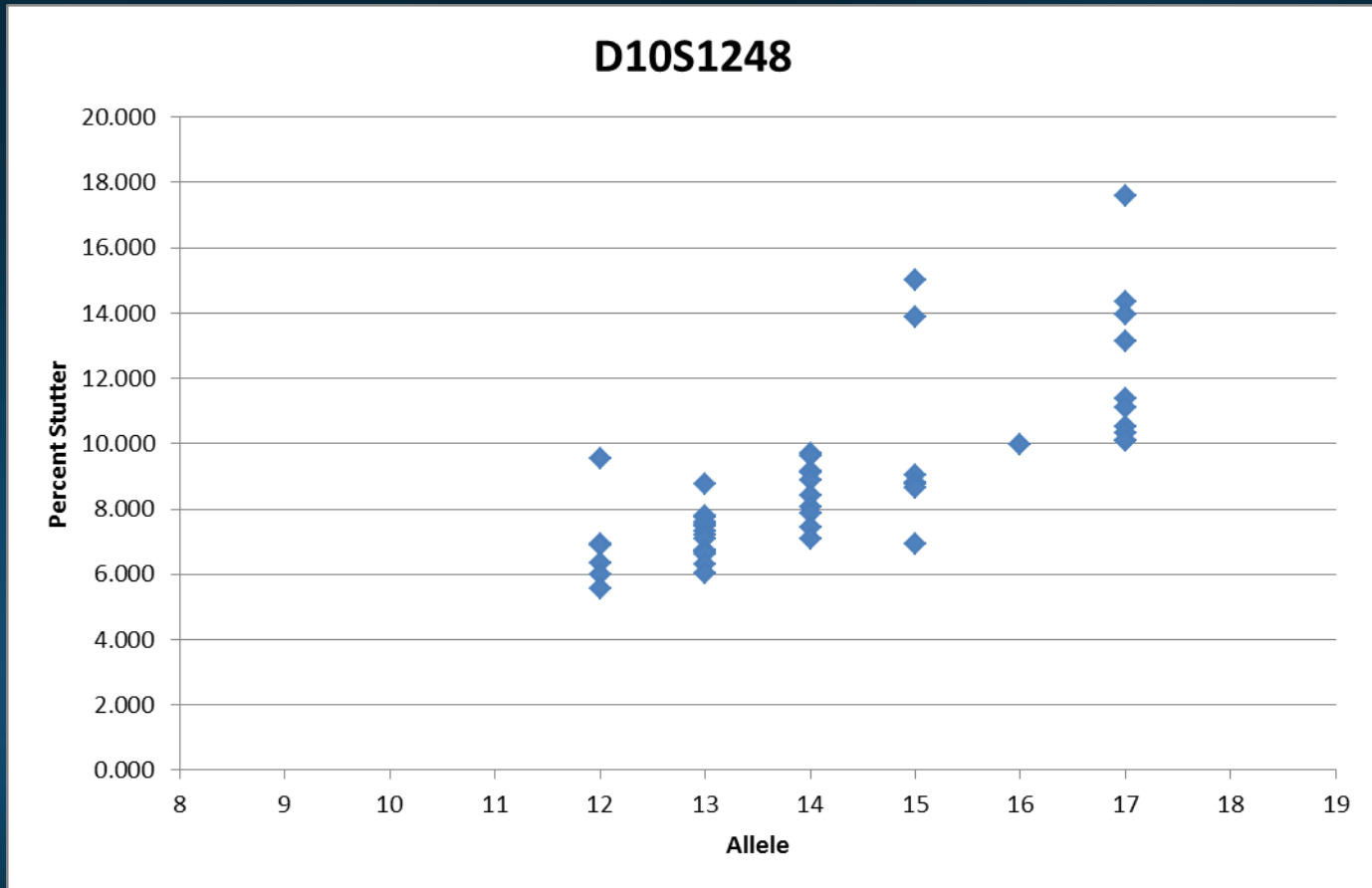
Results



Results



Results



Results

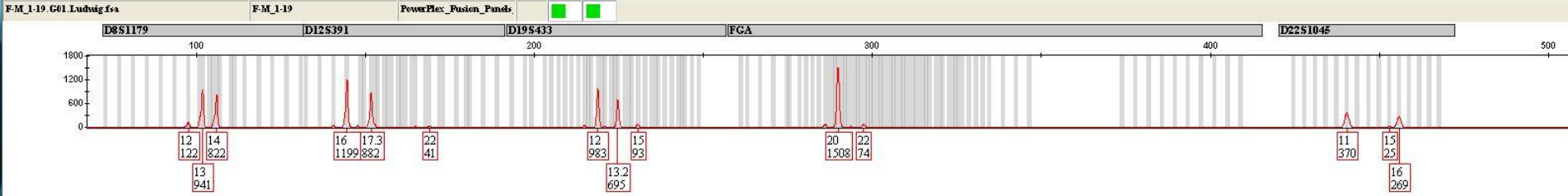
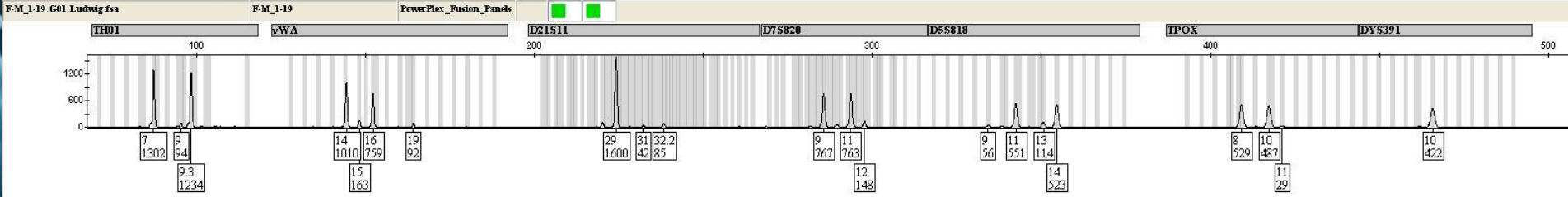
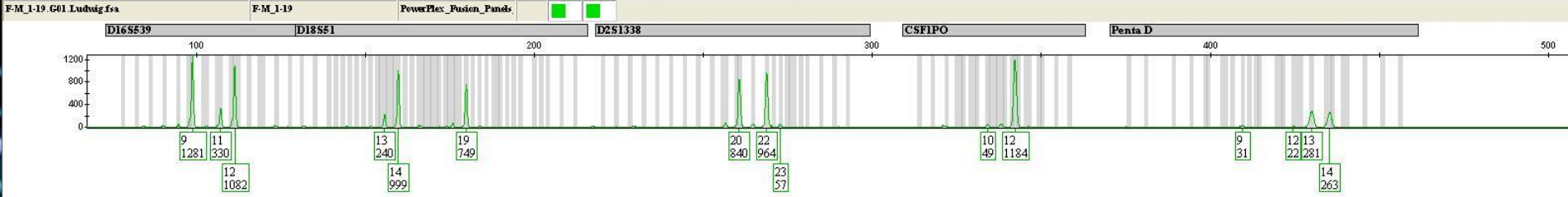
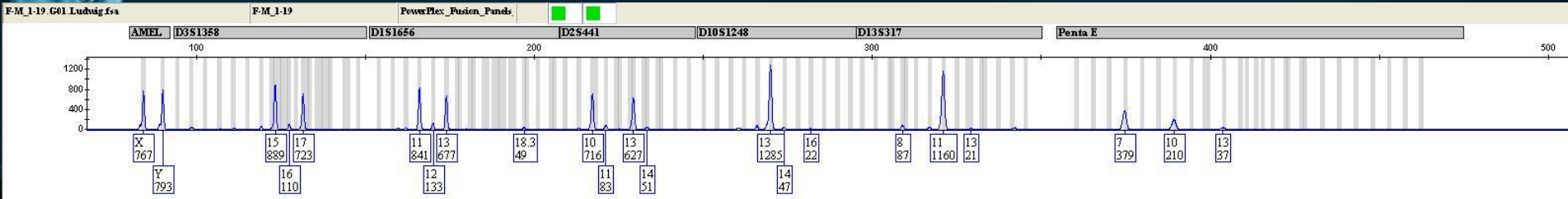
Locus	Min	Max	Avg PHR	STD PHR	Stutter (fx)	Stutter
D10S1248	5.556	17.582	8.930	2.620	16.78936	17%
D12S391	4.178	20.619	10.120	3.455	20.48612	20%
D13S317	1.573	10.345/28.125	6.431	4.045	18.56583	28%
D16S539	3.287	9.050/14.925	5.986	1.950	11.83729	15%
D18S51	3.752	22.472	9.281	3.408	19.50568	22%
D19S433	3.241	13.359	7.479	2.171	13.99052	14%
D1S1656	4.247	19.802	9.093	2.813	17.5322	20%
D21S11	5.395	15.301/22.115	9.191	2.533	16.7903	22%
D22S1045	2.581	17.857	10.922	2.992	19.89792	20%
D2S1338	5.040	14.043	8.781	2.061	14.96469	15%
D2S441	2.164	10.313	5.514	1.661	10.49599	10%
D3S1358	5.726	13.043	8.681	2.007	14.70219	15%
D5S818	2.257	15.347/21.359	7.176	2.971	16.08873	21%
D7S820	2.379	18.537/24.528	6.600	3.578	17.33509	24%
D8S1179	3.414	13.873	7.656	2.020	13.71633	14%
DYS391	5.157	15.302	8.069	2.056	14.23779	15%
FGA	3.994	14.220/17.021	8.017	2.473	15.435	17%
TH01	1.266	7.014	2.851	1.519	7.407025	7%
CSF1PO	2.558	11.607	7.018	1.747	12.25882	12%
TPOX	1.808	7.962	3.570	1.353	7.628283	7%
vWA	4.895	21.622/28.986	9.306	4.040	21.42701	29%



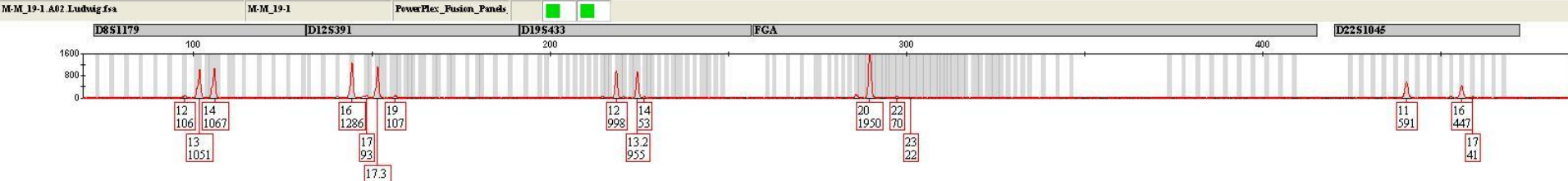
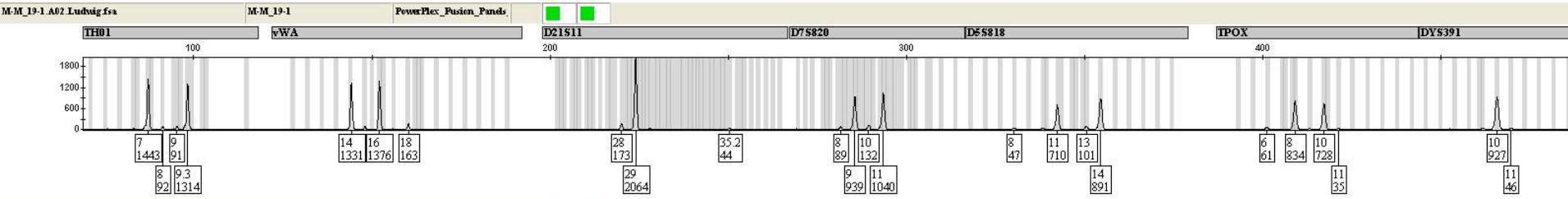
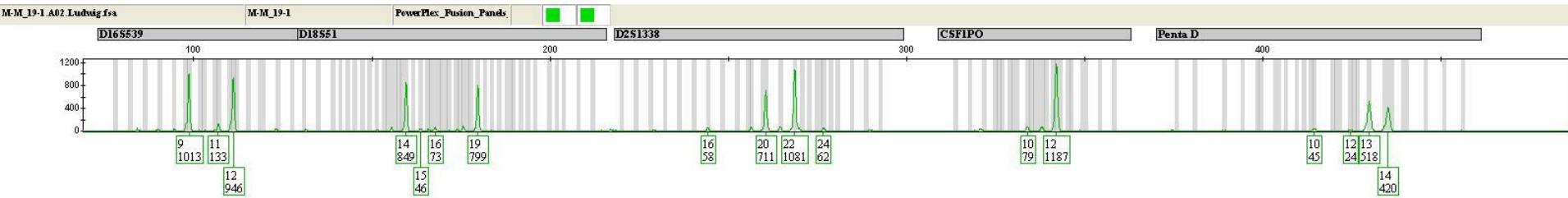
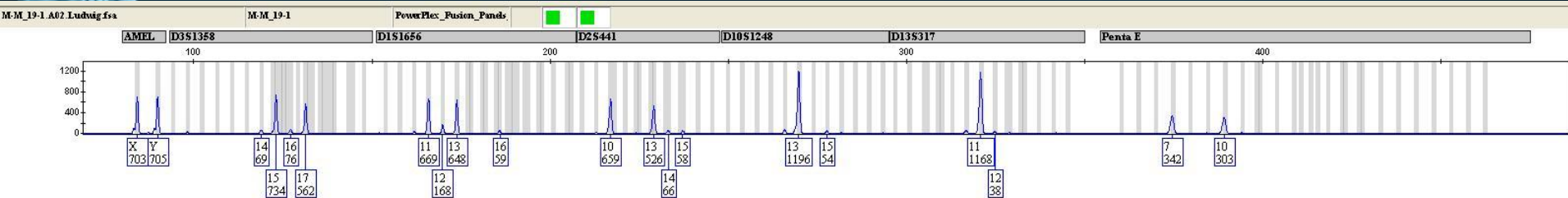
Mixtures

- Female:Male (19:1, 9:1, 4:1, 1:1, 1:4, 1:9, 1:19)
- Male:Male (19:1, 9:1, 4:1, 1:1, 1:4, 1:9, 1:19)
- Male:Male:Male (1:1:1)

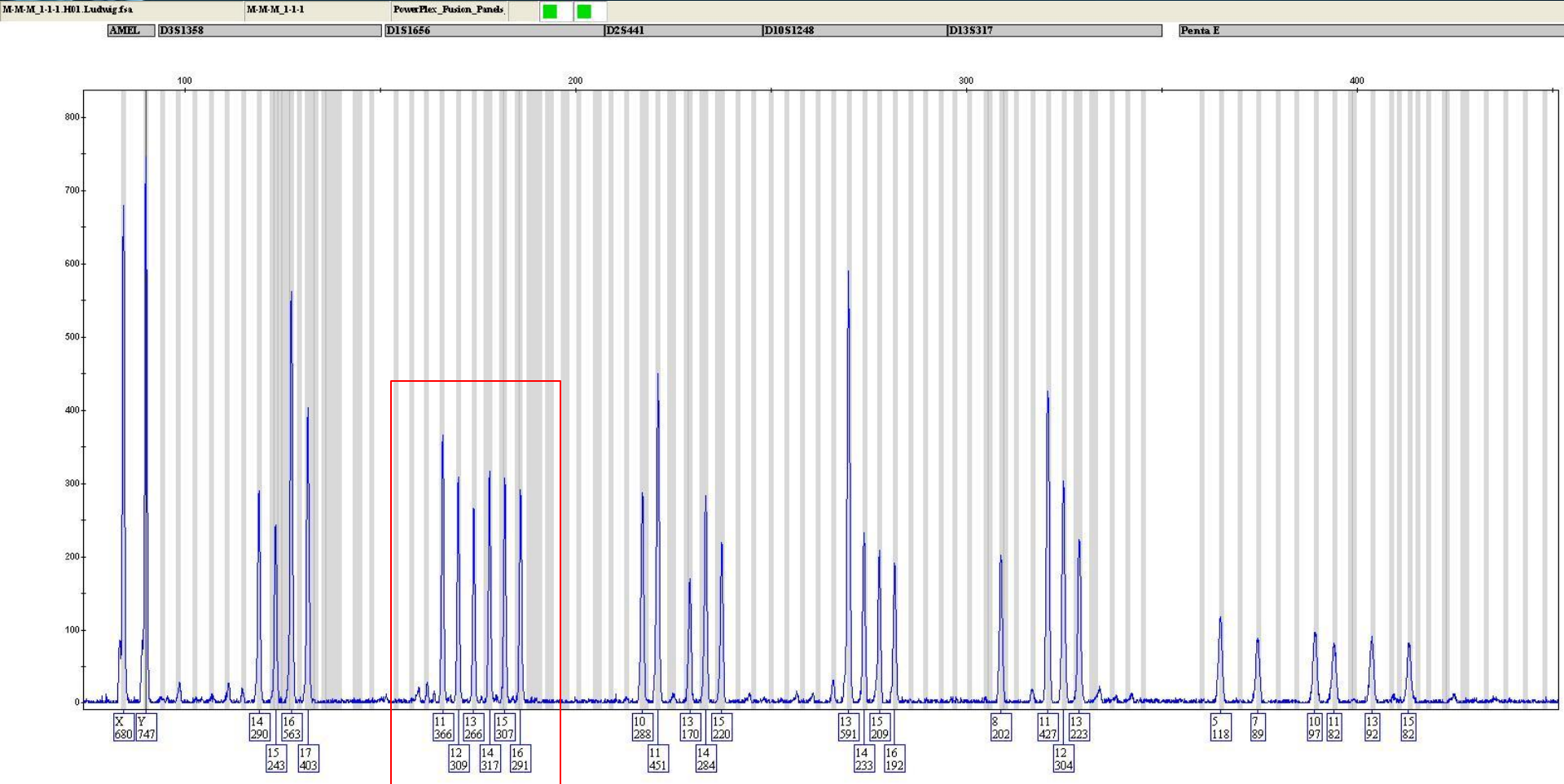
Results- Female:Male 19:1



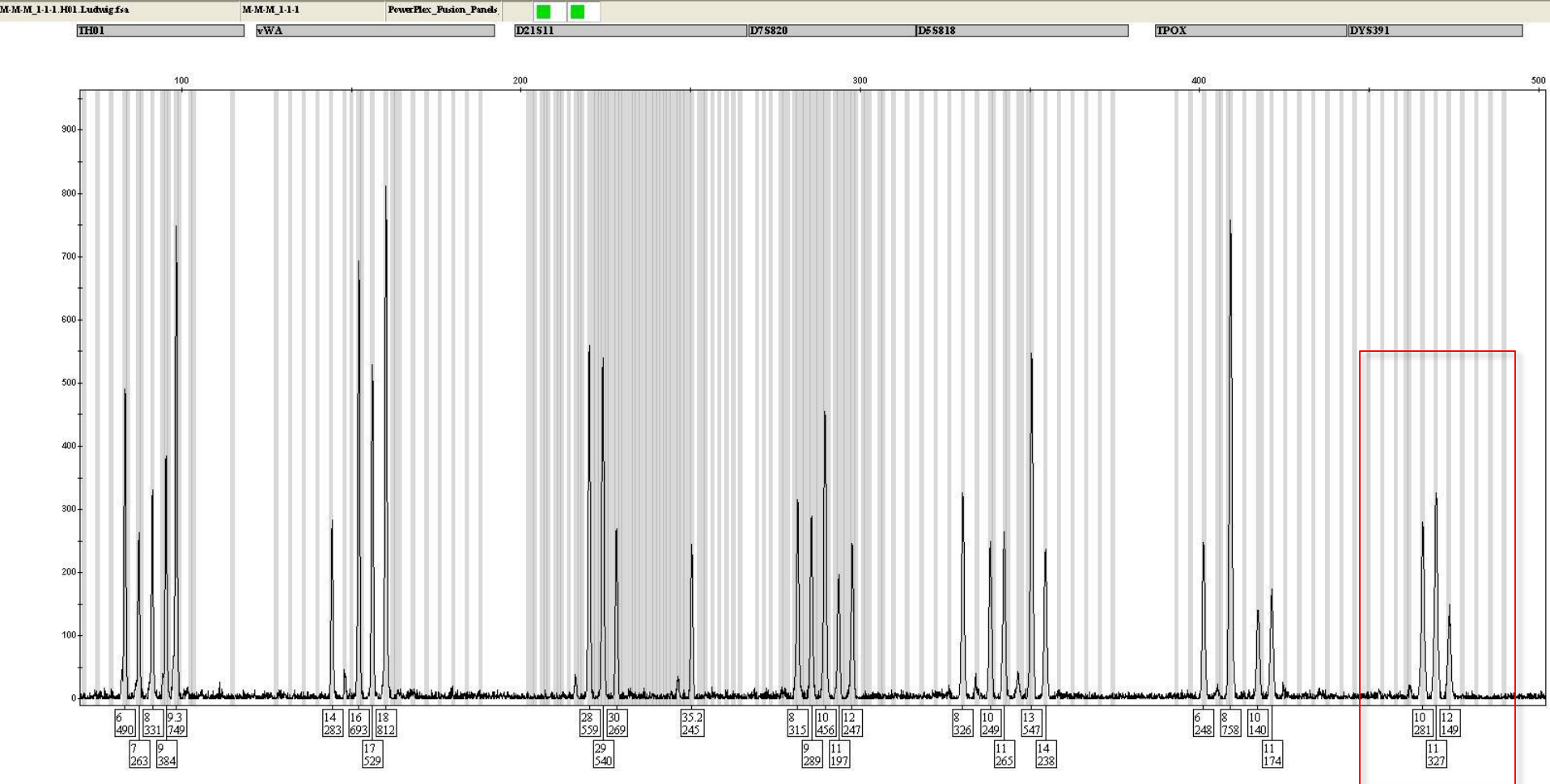
Results- Male:Male 19:1



Results Male:Male:Male 1:1:1

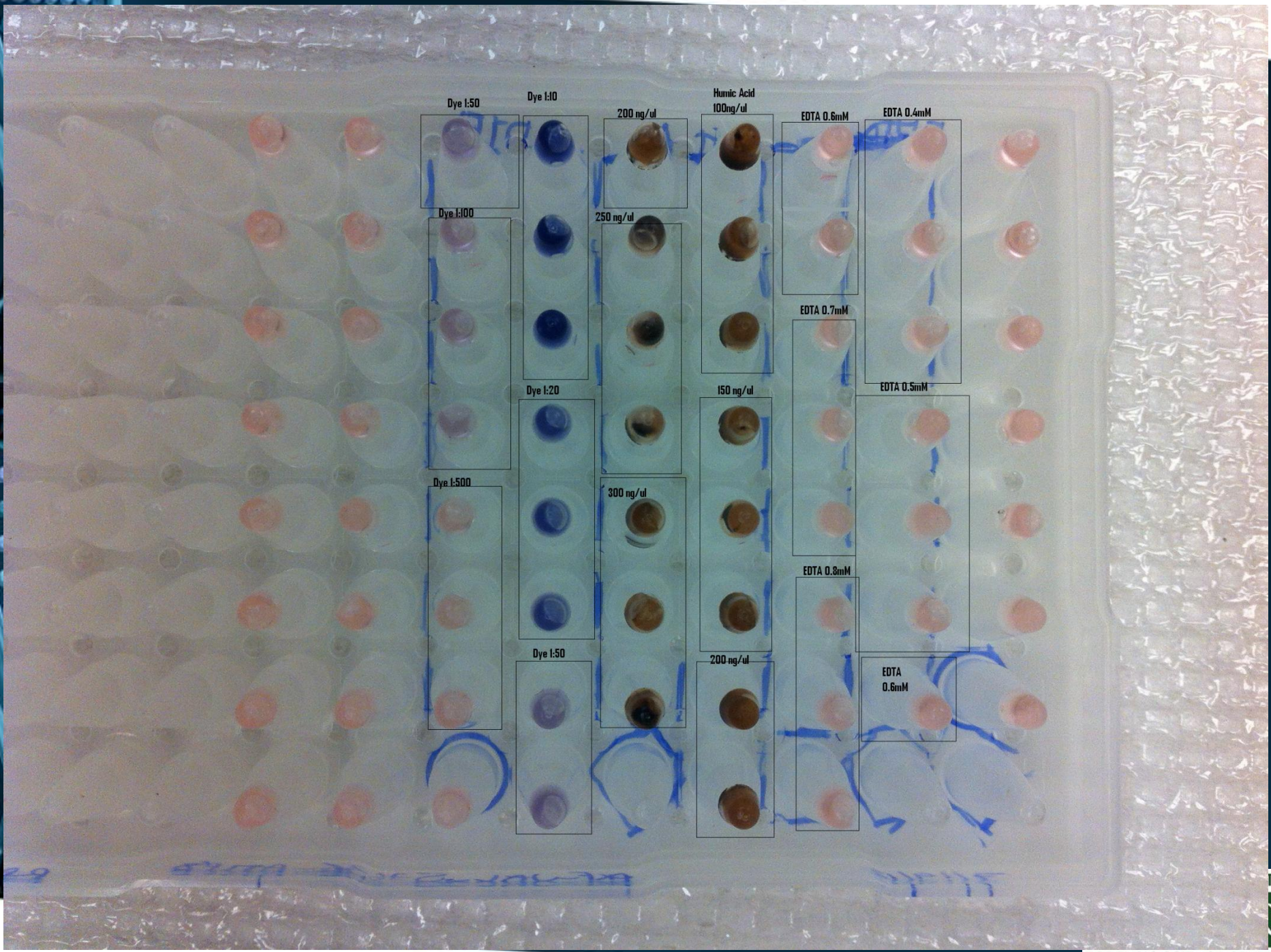


Results Male:Male:Male 1:1:1



Inhibition

- Humic acid
(100 ng/μl, 150 ng/μl, 200 ng/μl, 250 ng/μl, 300 ng/μl)
- EDTA
(0.4 mM, 0.5 mM, 0.6 mM, 0.7 mM, 0.8 mM)
- Blue Denim Dye
(1:10, 1:20, 1:50, 1:100, 1:500)
- 0.5 ng DNA target



Dye 1:50

Dye 1:10

200 ng/ul

Humic Acid
100ng/ul

EDTA 0.6mM

EDTA 0.4mM

Dye 1:100

250 ng/ul

EDTA 0.7mM

Dye 1:20

150 ng/ul

EDTA 0.5mM

Dye 1:500

300 ng/ul

EDTA 0.8mM

Dye 1:50

200 ng/ul

EDTA
0.6mM

Results

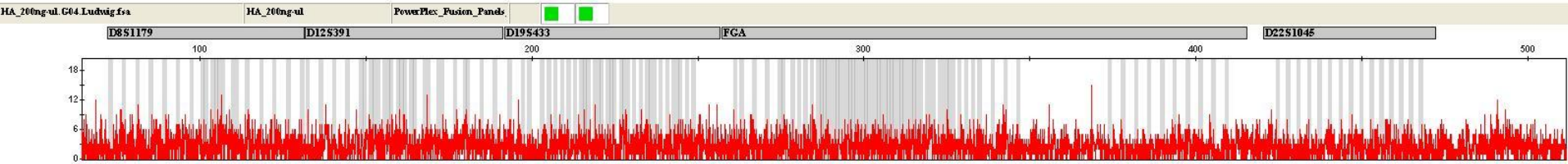
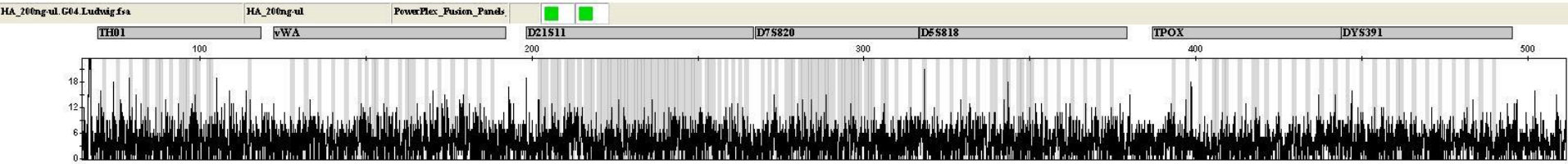
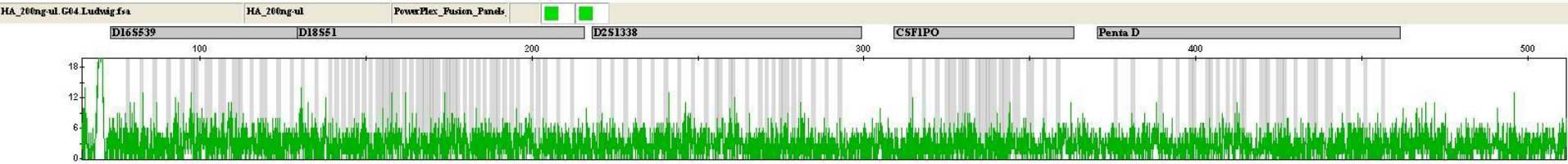
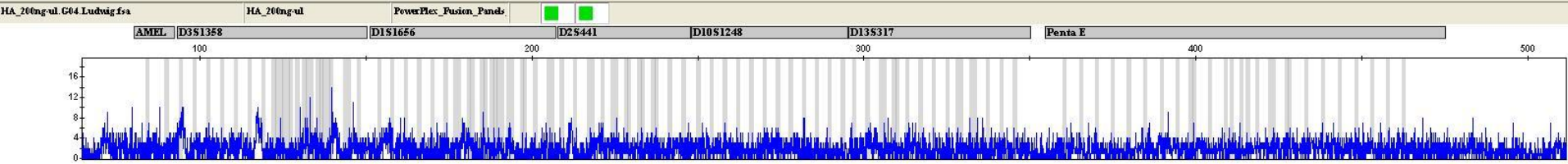
- *Humic Acid*- complete inhibition
- *EDTA* -no difference the total peak heights ranging from 26819 rfu at 0.7mM to 25,134 rfu at 0.8mM
(minus A artifacts in red channel below 250bp)



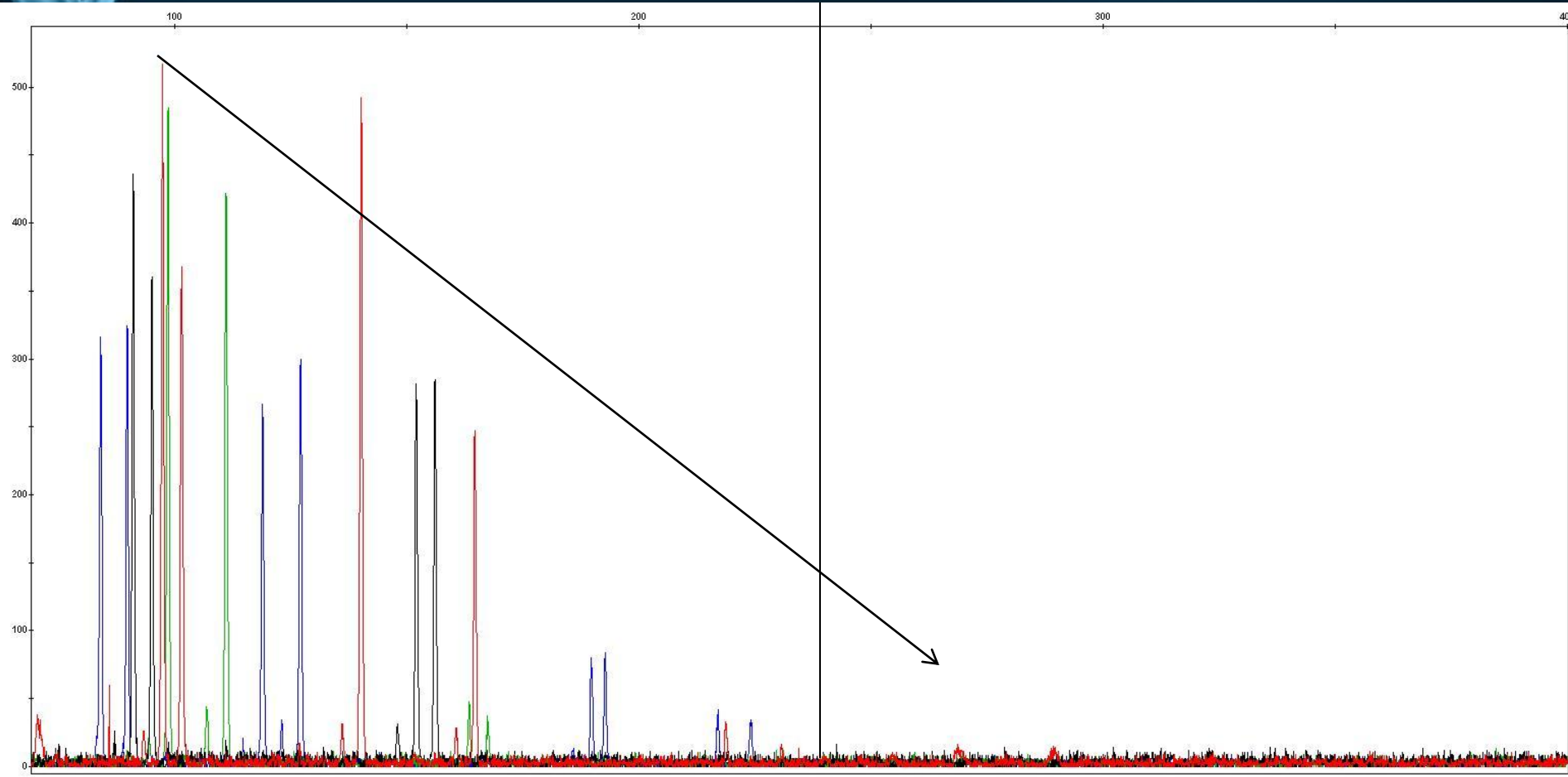
Results

- *Denim Dye:*
- *1:10 – complete drop out*
- *1:20- drop out in loci greater than 250bp- (Ski Slope seen)*
- *1:50, 1:100, 1:500 – complete profiles with TPH of 20,564, 26,498, 27,572 rfu respectively.*

Results- Humic Acid 200ng/ul.



Results- Dye 1:20

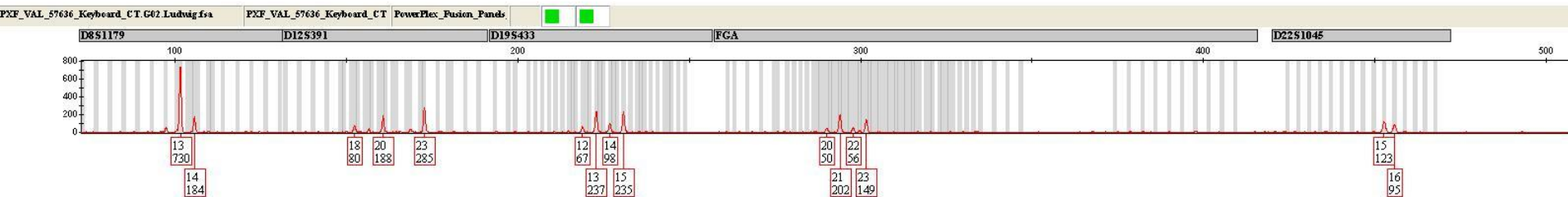
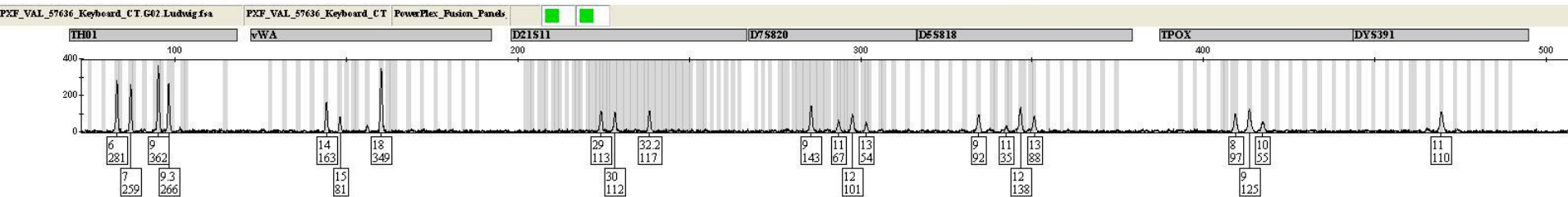
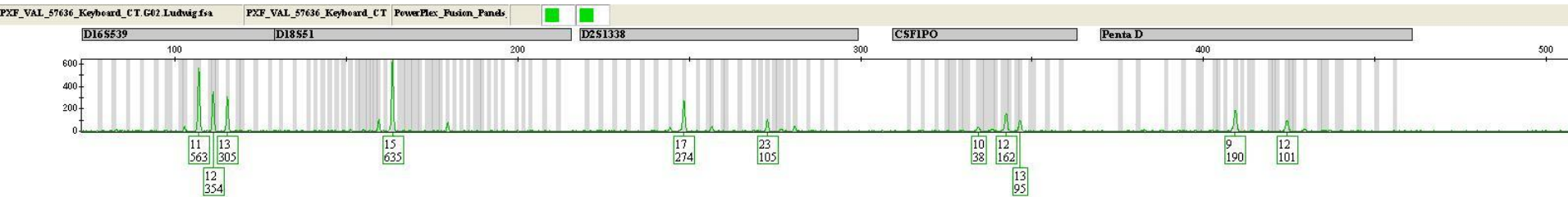
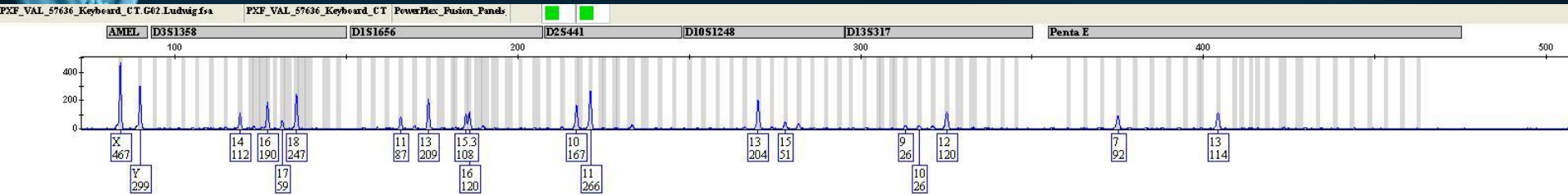




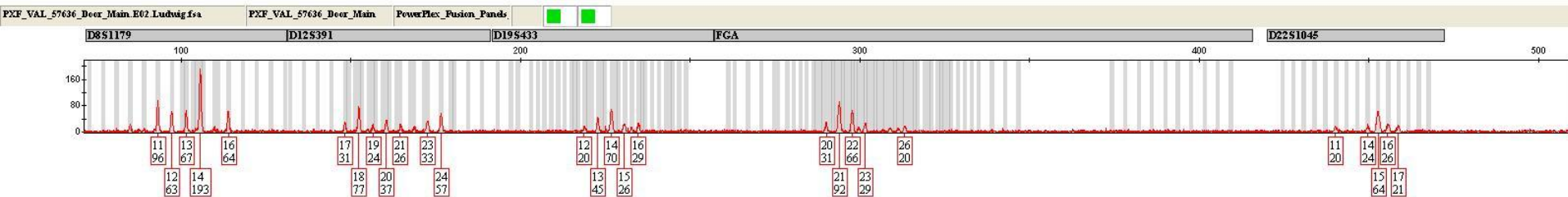
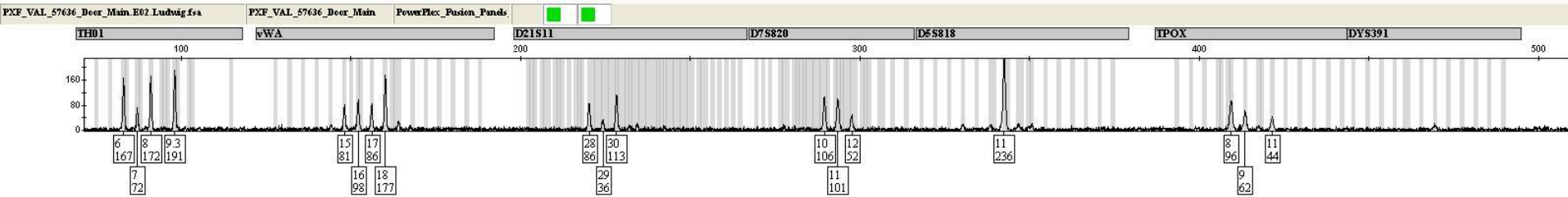
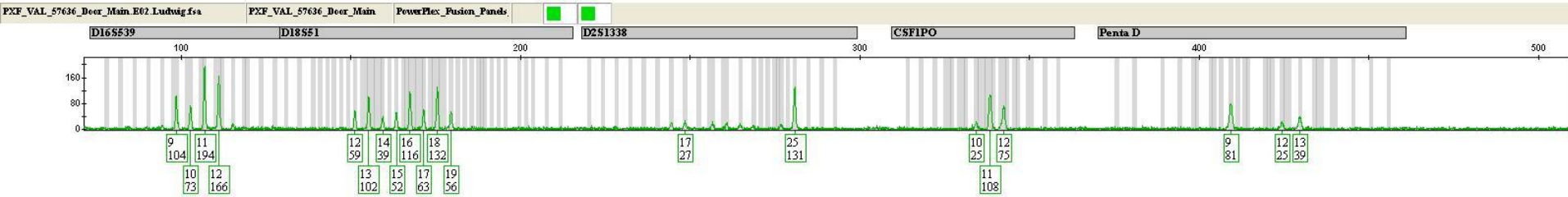
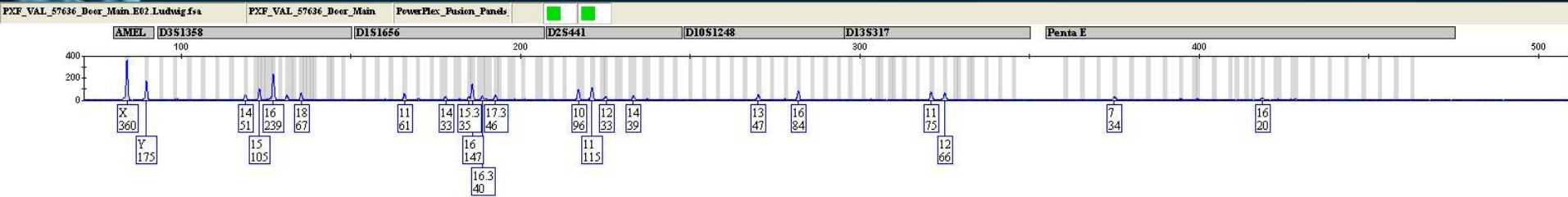
Non-probative Samples

- 25 samples:
 1. 2 Buccal Swabs
 2. 5 Touch evidence
 3. 5 Differentials
 4. 2 Gum
 5. 5 Cigarette Butts
 6. 2 Hair Samples
 7. 4 Phone Swabs

Results



Results

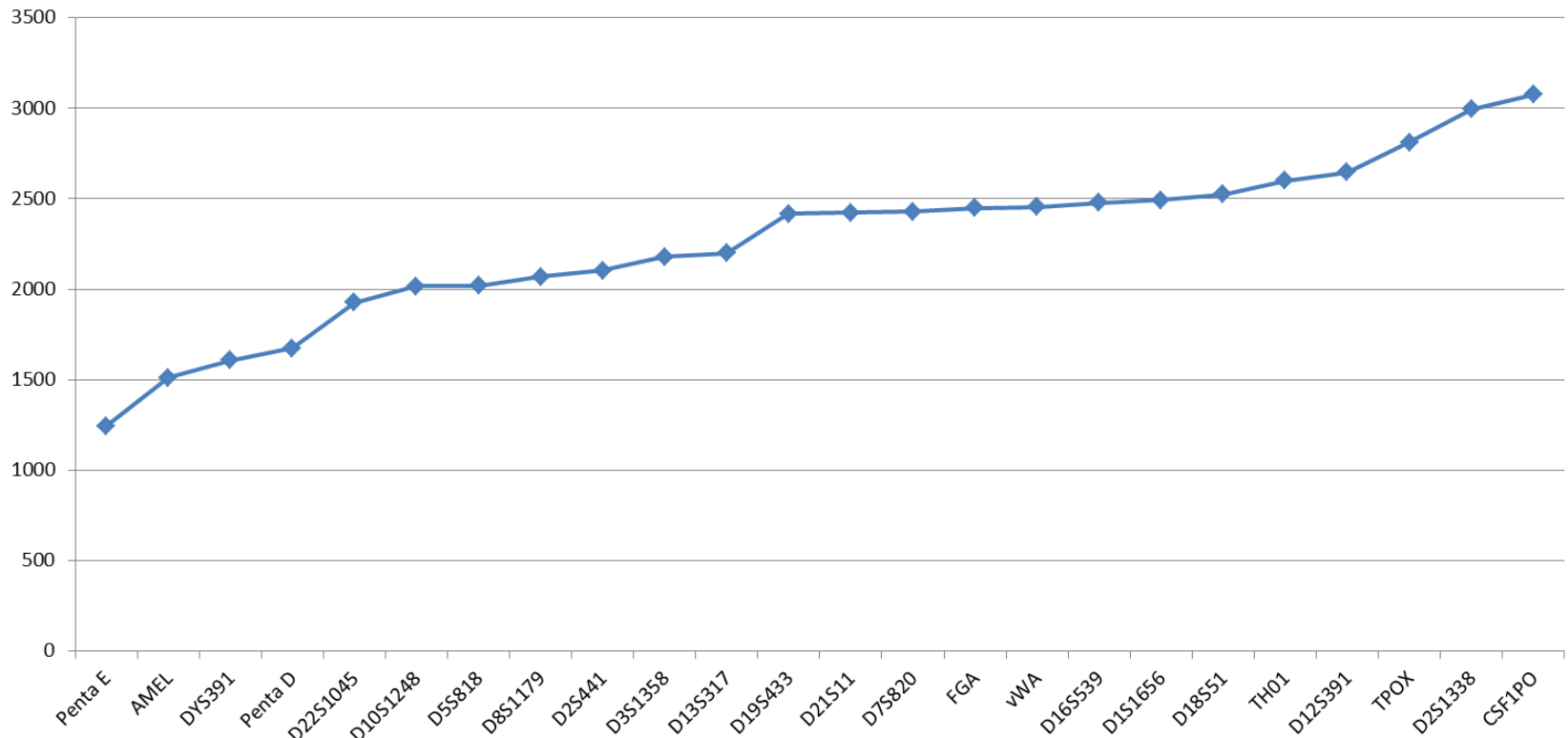


Troubleshooting

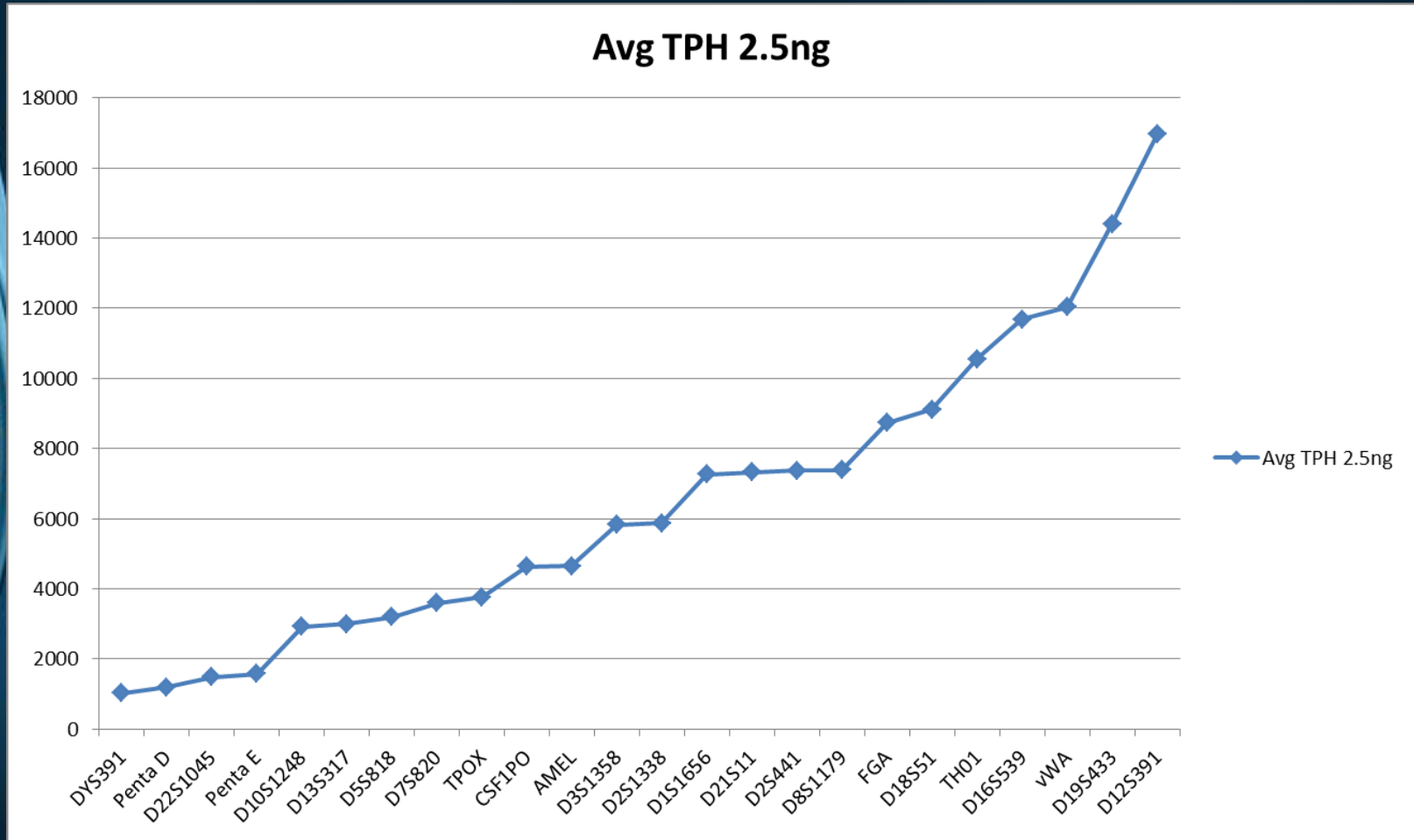
- Total Peak height was showing slope similar to degradation with the lowest ratio of smallest to largest peak being 6% and the highest ratio was 40%.
- Tried 29 cycles
- Tried 0.25, 0.5 and 1 ng loads
- 2800M positive control showed better ratios than TF
- Study of TF versus Buccal Swab with 3 different Extraction Methods (*EZ1* water elution, *EZ1* TE elution, Organic Extraction)

Results of TPH at 40%

TPH 2800M 0.5ng- 30cycles MAX



Results of TPH at 6%





Future Studies

- Mixture Study
- Inhibition Study
- Cross Loci Calling
- Finish the Total Peak Height Sloping Issue
- Non-specific STR calling especially from microbial DNA
- Quantification through the Amplification Kit



Conclusion

- PowerPlex® Fusion provided accurate profiles and a wide range of input target DNA with low amount of artifacts.
- The use of the Promega® PowerPlex® Fusion amplification kit is recommended for the use in future casework samples



Acknowledgement

- *Marshall University Forensic Science Center*
- Jason Chute
- Josh Stewart
- Christopher William Thatch
- Jennifer Hayden
- Season Seferyn
- Heather Harrah-Lea
- Amanda Hoffman
- Promega®'s Tech Services

References

- Butler, J.M. (2009). *Advanced Topics in Forensic DNA Typing Methodology*. Burlington, MA: Elsevier.
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Thank You

Questions?

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