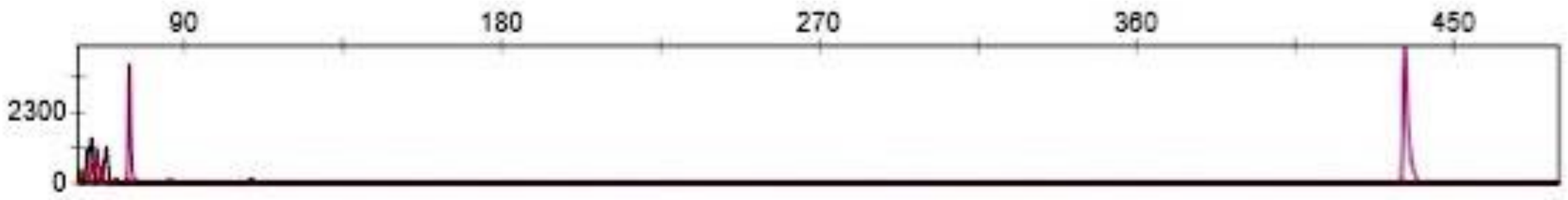
Application of QIAGEN Workflow with Quality Sensors and Interpretation:

Database and Casework Samples



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Disclaimer

This project was supported and funded by QIAGEN

Speaker Biosketch

See separate document

Introduction

- Forensic database and/or casework labs process 100,000s samples for the criminal justice community each year.
- Results must be accurate and reliable. Techniques and methods need to be robust, reproducible, validated, and *efficient.*
- Triage samples to generate most probative results and employ the most economical workflows.
  - Highest first-pass rates, less (and more effective) rework strategies
- Use as much information as possible about every sample to make the most informed decisions.

- Quality flags during DNA quantitation
- Quality Sensors in STR profiles
- Can they better guide rework strategies? Can we avoid unnecessary work?

Materials - QIAGEN's workflow

1. DNA Extraction
   - *QIAamp DNA Investigator kit*
   - *EZ1xL*

2. Liquid handling
   - *QIAgility*

3. DNA Quantitation
   - *Investigator® Quantiplex® Pro RGQ (Rotor-Gene Q)*

4. STR Amplification
   - *Investigator 24plex QS*
   - *Investigator 24plex GO!*

This Study

Assess the effectiveness of the QIAGEN Quality Sensor system with reference and forensic casework type samples.

Concordance between:

- Quality flags during DNA quantification
- Quality Sensors in STR profile
- STR profile quality

Investigator® Quantiplex® Pro RGQ

- For use on the Rotor-Gene Q real-time instrument

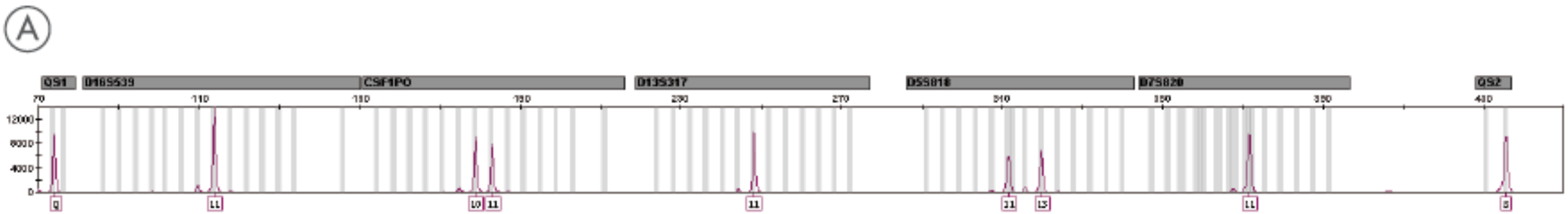
Target	Amplicon length	Channel	Copy number
Human target, small autosomal	91 bp	Yellow	Multi-copy
Human target, large autosomal	353 bp	Red	Multi-copy
Human male target, small gonosomal	81 bp	Green	Multi-copy
Human male target, large gonosomal	359 bp	Orange	Multi-copy
Internal PCR control (IC)	434 bp	Crimson	Synthetic fragment

IC of the Investigator Quantiplex Pro RGQ Kit reflects the Quality Sensor of the Investigator 24plex STR kit

Investigator 24plex QS & GO!

- 21 autosomal and 2 sex markers (amelogenin and DYS391)
- Fast cycling technology (~ 60 min. QS, ~ 45 min GO!)
- Quality Sensors
- Direct amplification kit for reference samples

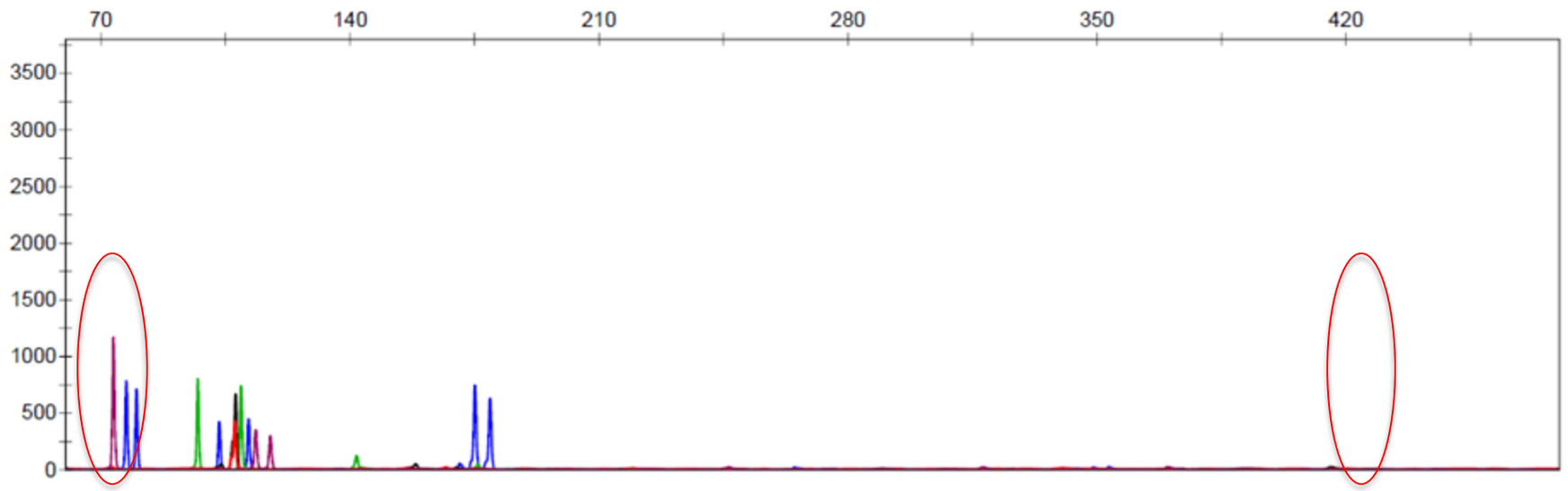
QIAGEN – Investigator 24plex QS & GO! Kits



Good quality

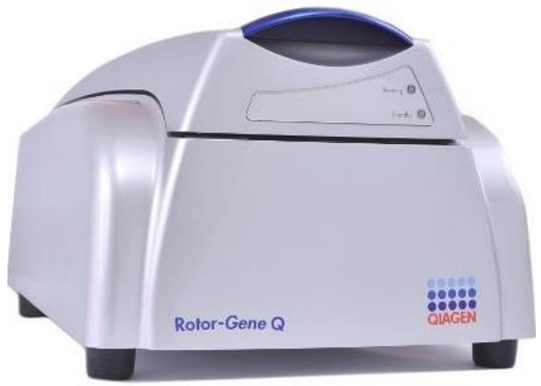
Quality Sensors

- Significant levels of PCR Inhibition when S/Q ratio <20%
- Manufacturer recommendation
  - Requires in-house testing & validation
- May be used as a guide, or a threshold for reworks



Casework Workflow - Overview

Quant Setup



QIAGEN Data Handling Tool

Sample Screening

Data Handling Tool – Screen Quants

QIAGEN Quantification Assay Data Handling and STR Setup Tool

Result Summary		Human	Human Degradation	Male	Male Degradation	Quality Assessment							
Well	Sample Name	Quantity	Quantity	Quantity	Quantity	Mixture Index	Mixture Threshold	Degradation Index	Degradation Threshold	Male Degradation Index	Male Degradation Threshold	Inhibition Index	Inhibition Threshold
10	9 E3T14 ZY	0.1065	--	0.2136	--	0.50	Below Threshold	Not Applicable	Possible Degradation	Not Applicable	Possible Degradation	-15.87	Possible Inhibition
11	10 D3T10 TD	0.0854	0.0003	0.0388	0.0012	2.20	Possible Mixture	269.07	Possible Degradation	32.74	Possible Degradation	-6.64	Possible Inhibition
12	11 D3T14 TD	0.0346	0.2469	0.0210	0.1555	1.65	Below Threshold	0.14	Below Threshold	0.14	Below Threshold	-7.28	Possible Inhibition
13	12 E3T10 TD	0.0021	0.0238	0.0013	0.0096	1.67	Below Threshold	0.09	Below Threshold	0.13	Below Threshold	-8.98	Possible Inhibition
14	13 E1T14 TD	--	--	--	0.0055		Below Threshold	Not Applicable	Possible Degradation	Not Applicable	Possible Degradation	-7.55	Possible Inhibition
15	14 E0N4 SGO	0.0000	--	0.0000	--		Below Threshold	Not Applicable	Possible Degradation	Not Applicable	Possible Degradation	-5.68	Possible Inhibition
16	15 E0N4 MGO	0.0005	--	0.0002	--	2.68	Possible Mixture	Not Applicable	Possible Degradation	Not Applicable	Possible Degradation	-4.07	Possible Inhibition
17	16 D4BC S	18.6439	5.6390	15.0175	3.2179	1.24	Below Threshold	3.31	Below Threshold	4.67	Below Threshold	0.16	Below Threshold
18	17 D7BC M	3.4076	0.3550	1.2376	0.0555	2.75	Possible Mixture	9.60	Below Threshold	22.29	Possible Degradation	0.13	Below Threshold
19	18 E4BC S	15.9483	2.8568	6.0260	1.0973	2.65	Possible Mixture	5.58	Below Threshold	5.49	Below Threshold	0.13	Below Threshold

Possible Inhibition

Customize threshold values based on validation data

Quality Assessment	Threshold
Mixture Index (Human/Male)	2
Human Degradation Index (Human/Human Degradation)	10
Inhibition Index (IC Shift)	1
Male Degradation Index (Male/Male Degradation)	10

Casework Workflow - Overview

Dilutions & PCR Setup

QIAGEN Data Handling Tool

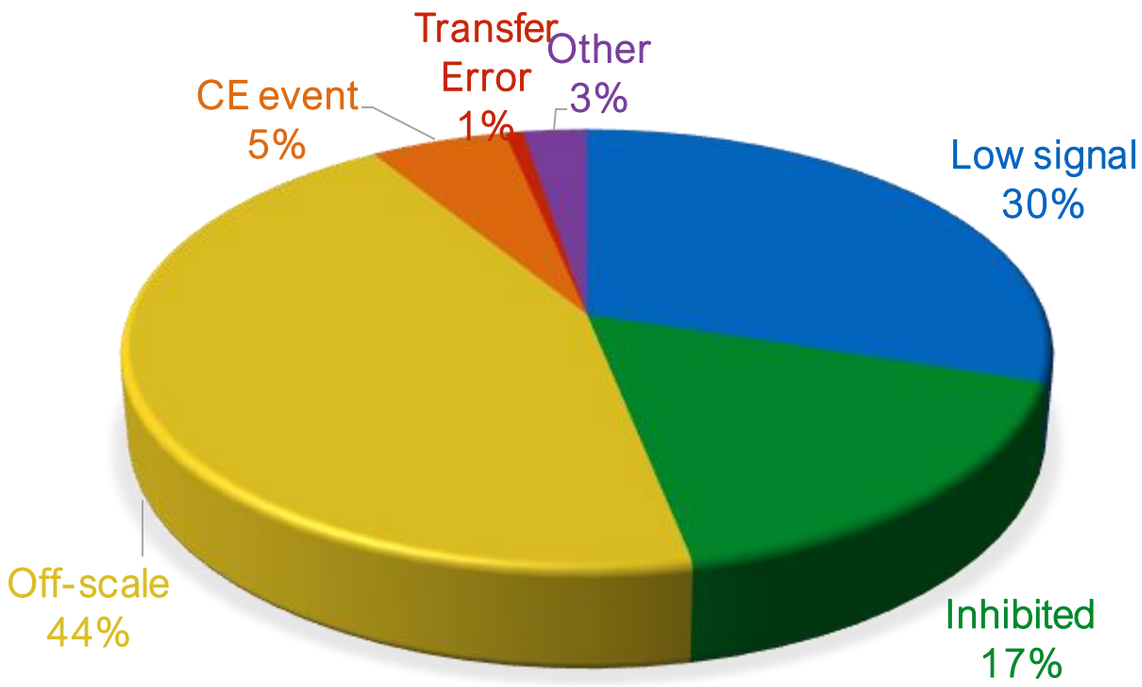
Databasing Samples

Blood and Saliva on FTA cards, Buccal swabs (BODE Buccal DNA Collector and Cotton) with Investigator® 24plex GO! Kit

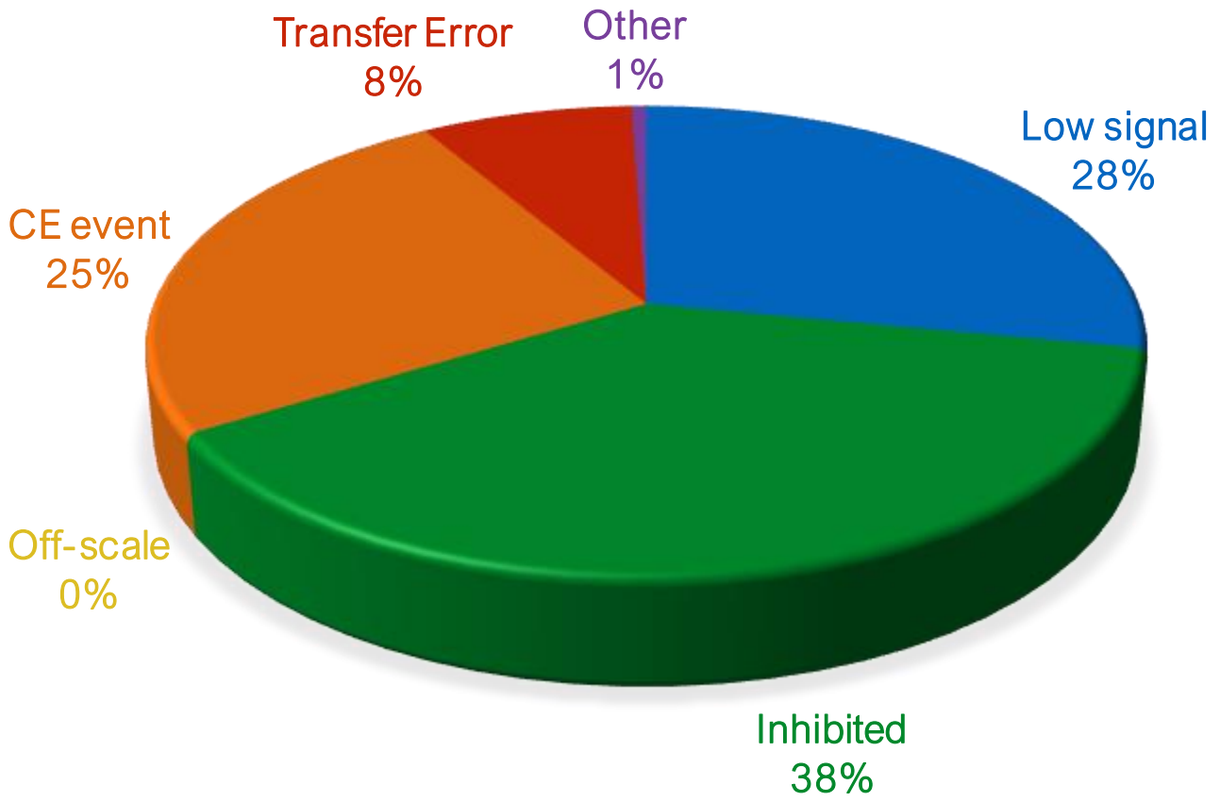
US State Databasing Lab

4 months data (2018)

- Buccal swabs (N = 6480)
  - 5.1% samples reworked



- Blood FTA (N = 6370)
  - 4.3% samples reworked



Cotton (N=87) & Bode (N=97) Swabs

Room Temp. | Hot & Humid | Poor/Dirty Collection | UV

Entire swab in Investigator® Lyse & Spin Basket, 500µL Investigator® STR GO! Lysis Buffer

Incubate at 95°C for 5min and shaking at 1200rpm, Centrifuge and discard swab and basket

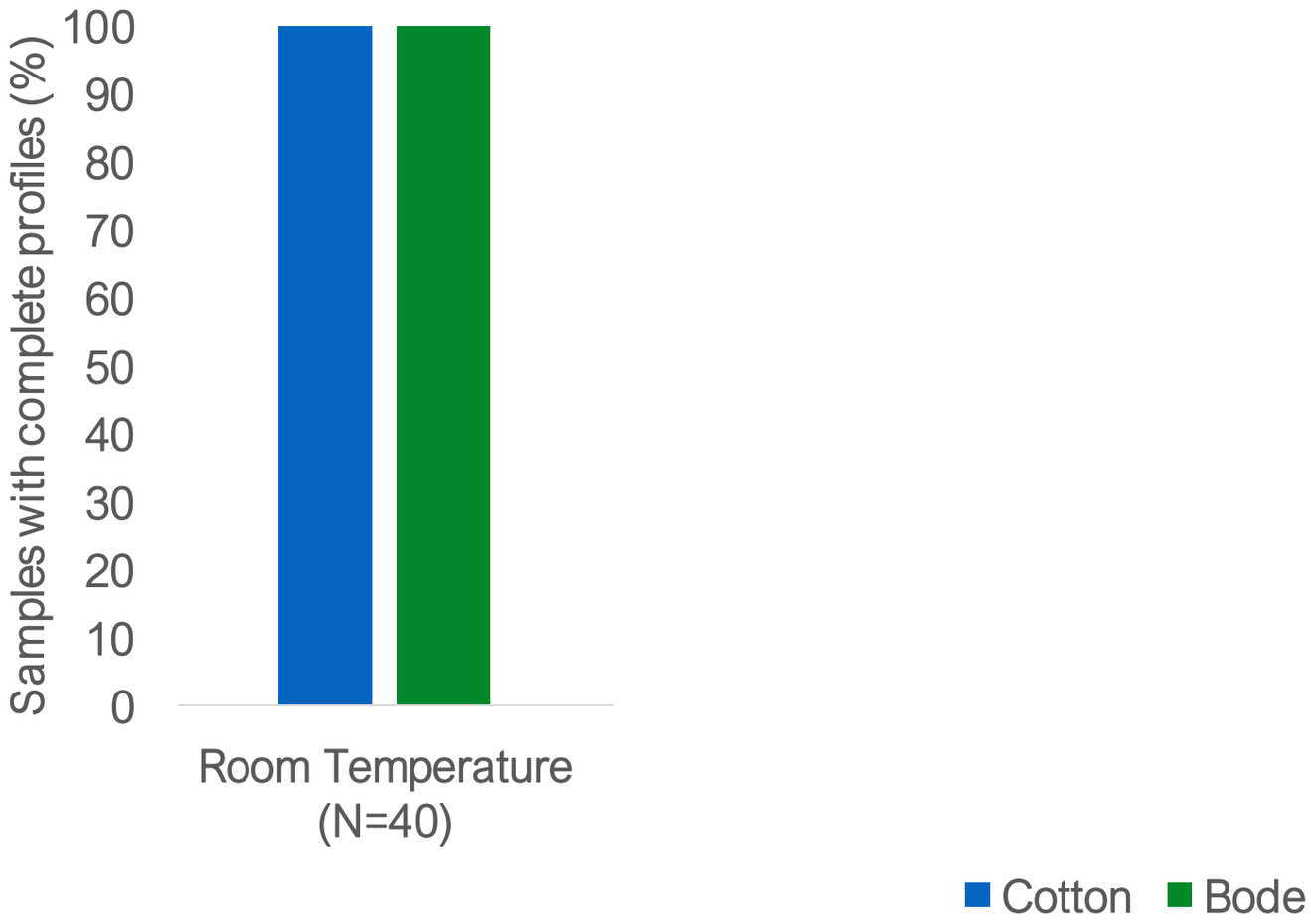
QIAgility adds GO! mastermix and 2µL lysate to reaction plate

1 x 1.2mm 2µL Investigator® STR GO! Lysis Buffer

Incubate at 95°C for 5min

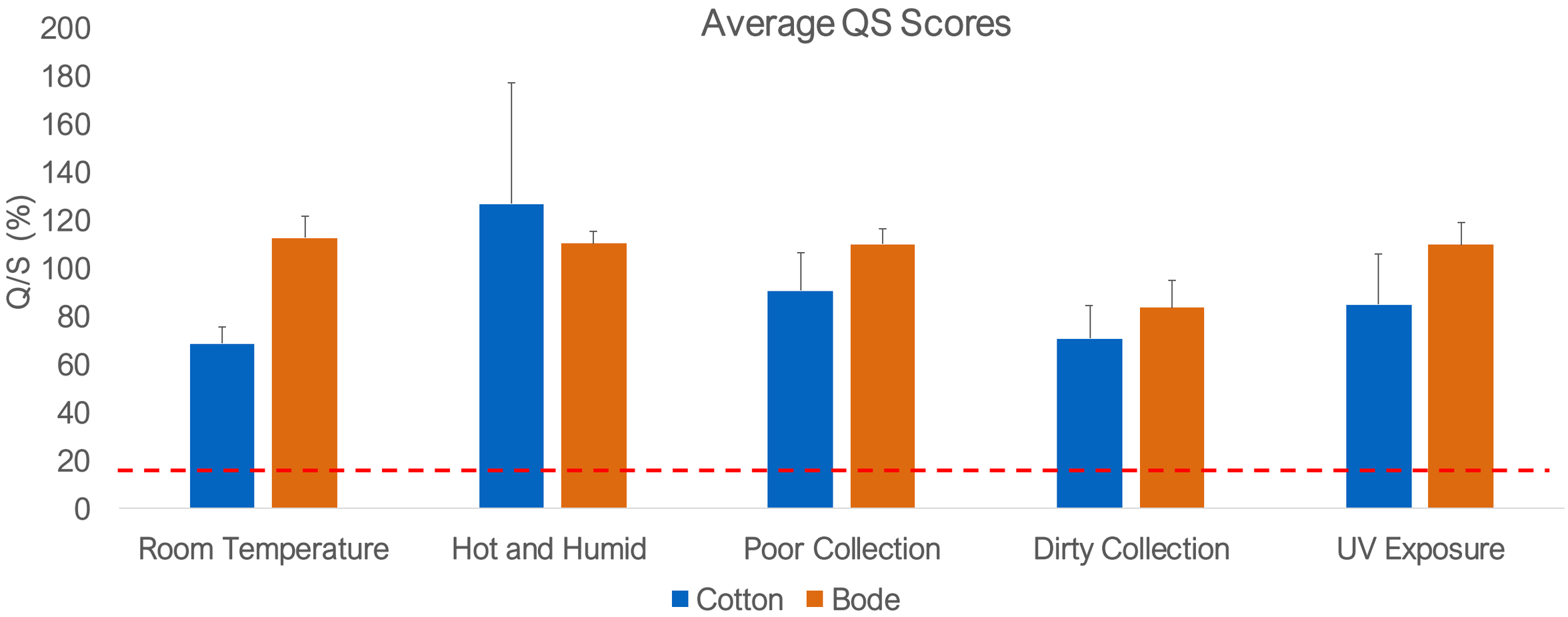
GO! mastermix added to punch in reaction plate

First Pass Rates - Swabs



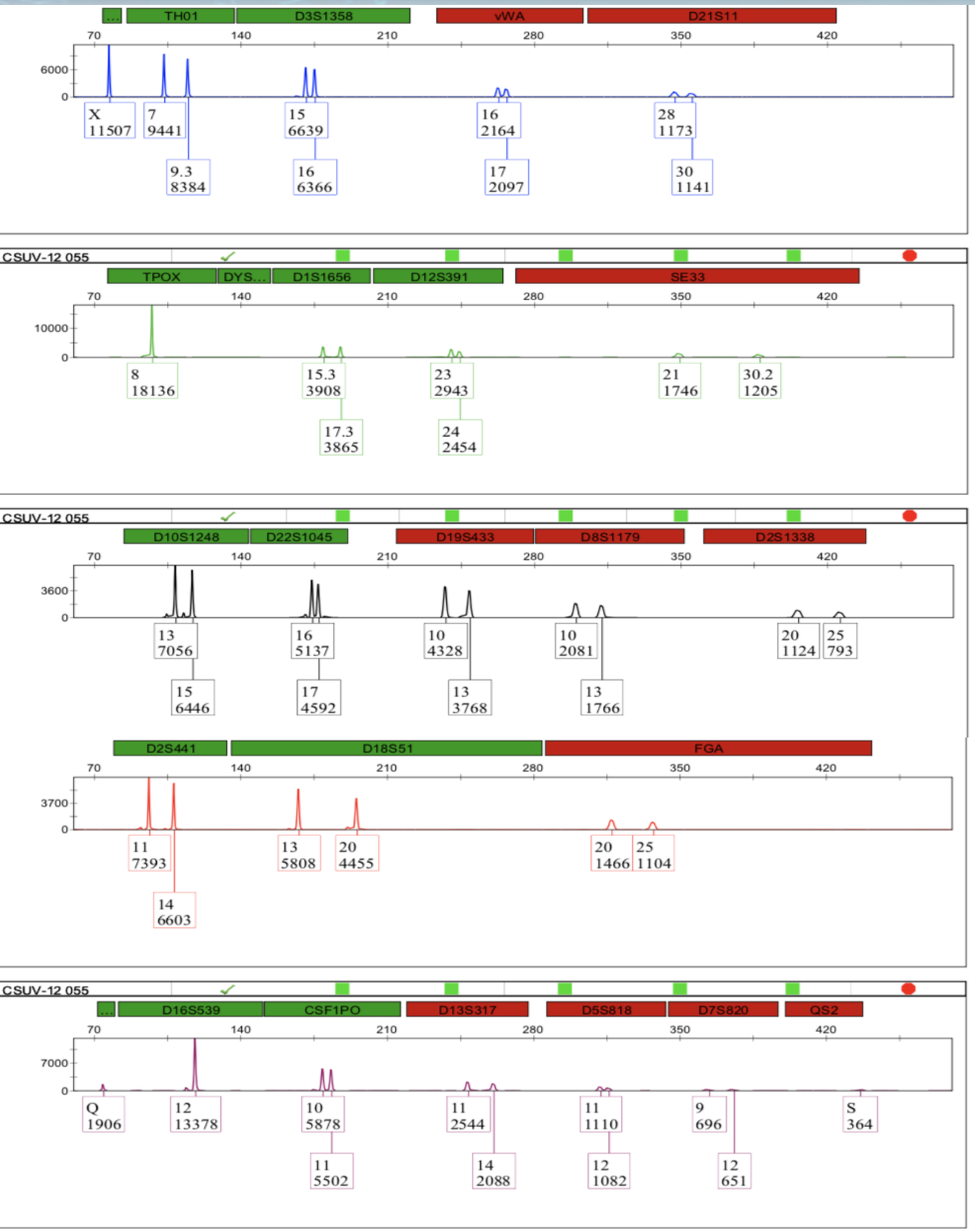
- 100% complete profiles for Room Temp.
- 64% of challenged swabs yielded complete profile

QS Score - Swabs



- QS markers confirmed no significant inhibition

Were the QS Markers right?



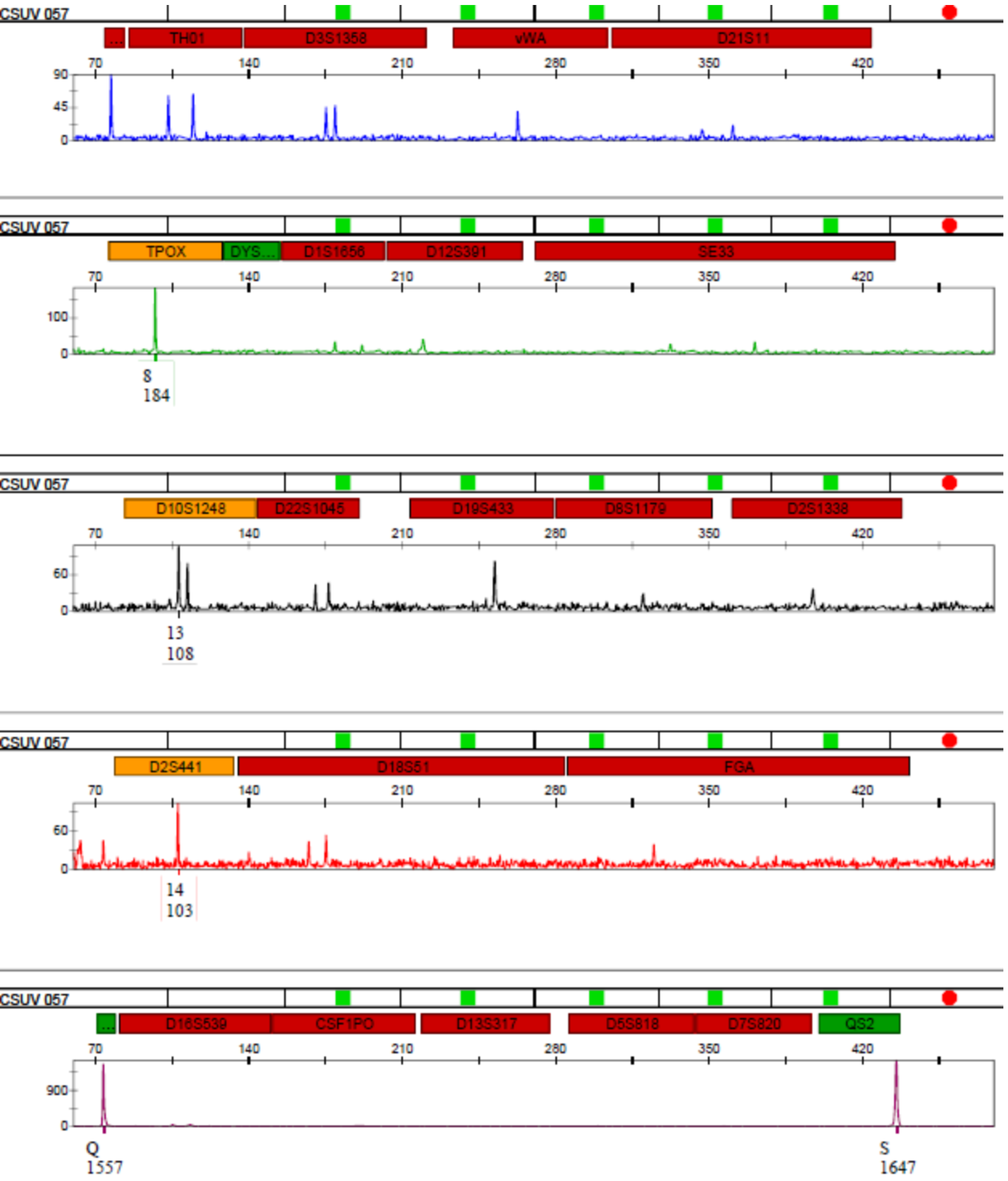
Only <u>one</u> sample in the 184 flagged inhibition

100% Alleles

QS = 19%

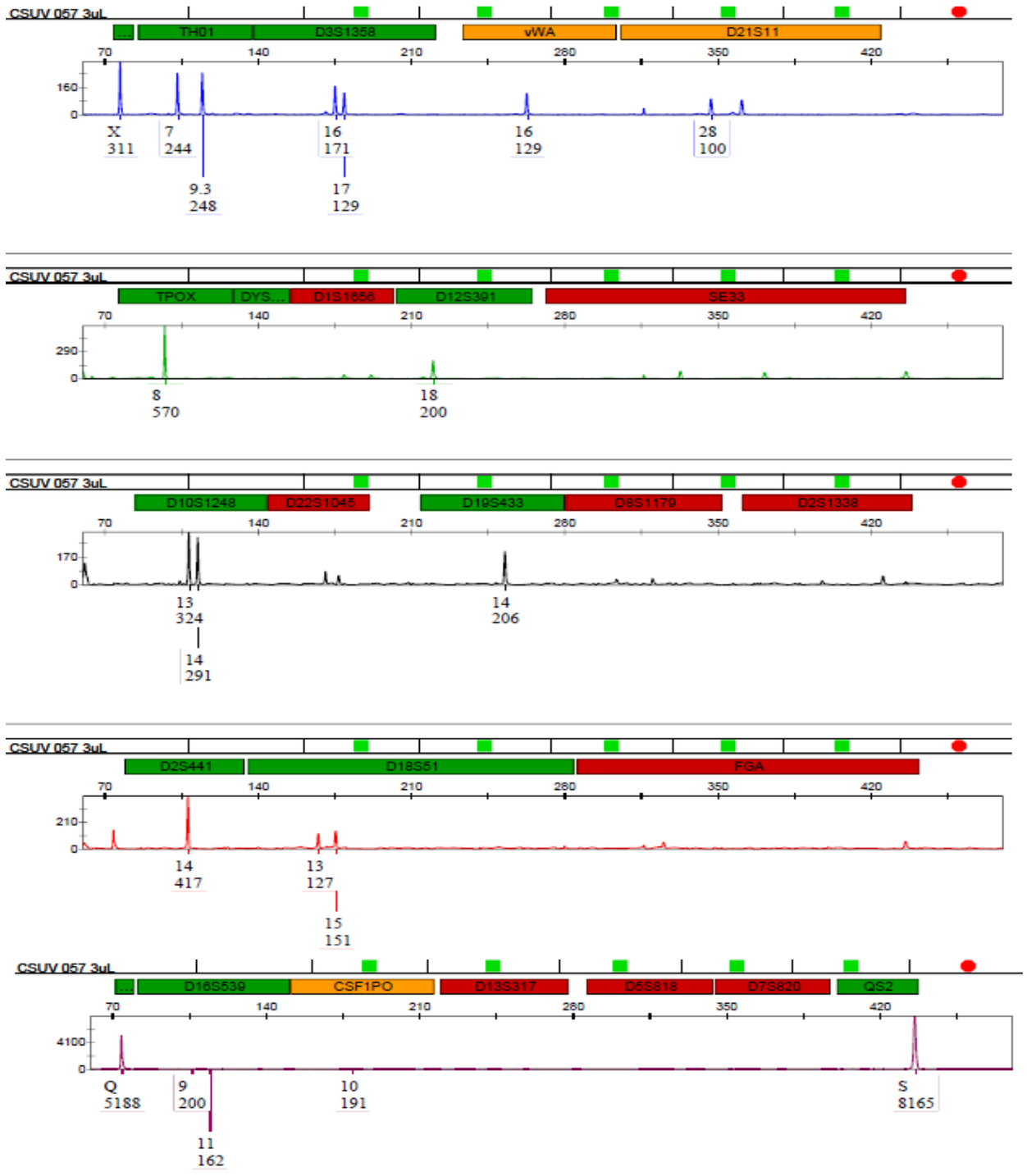
CE issues/inhibition ?

Example – Rework Cotton Swab



7% alleles
S/Q 105%

No inhibition
confirms LT/deg



Rework with 3µL

52% alleles
S/Q 156%

FTA Cards

Saliva (N=350) & Blood (N=370)

Room Temp. | Hot & Humid | UV | Room Temp. | Hot & Humid | Low Blood Volume | Direct Punch

1 x 1.2mm
20µL Investigator® STR GO! Lysis Buffer added with QIAgility

↓

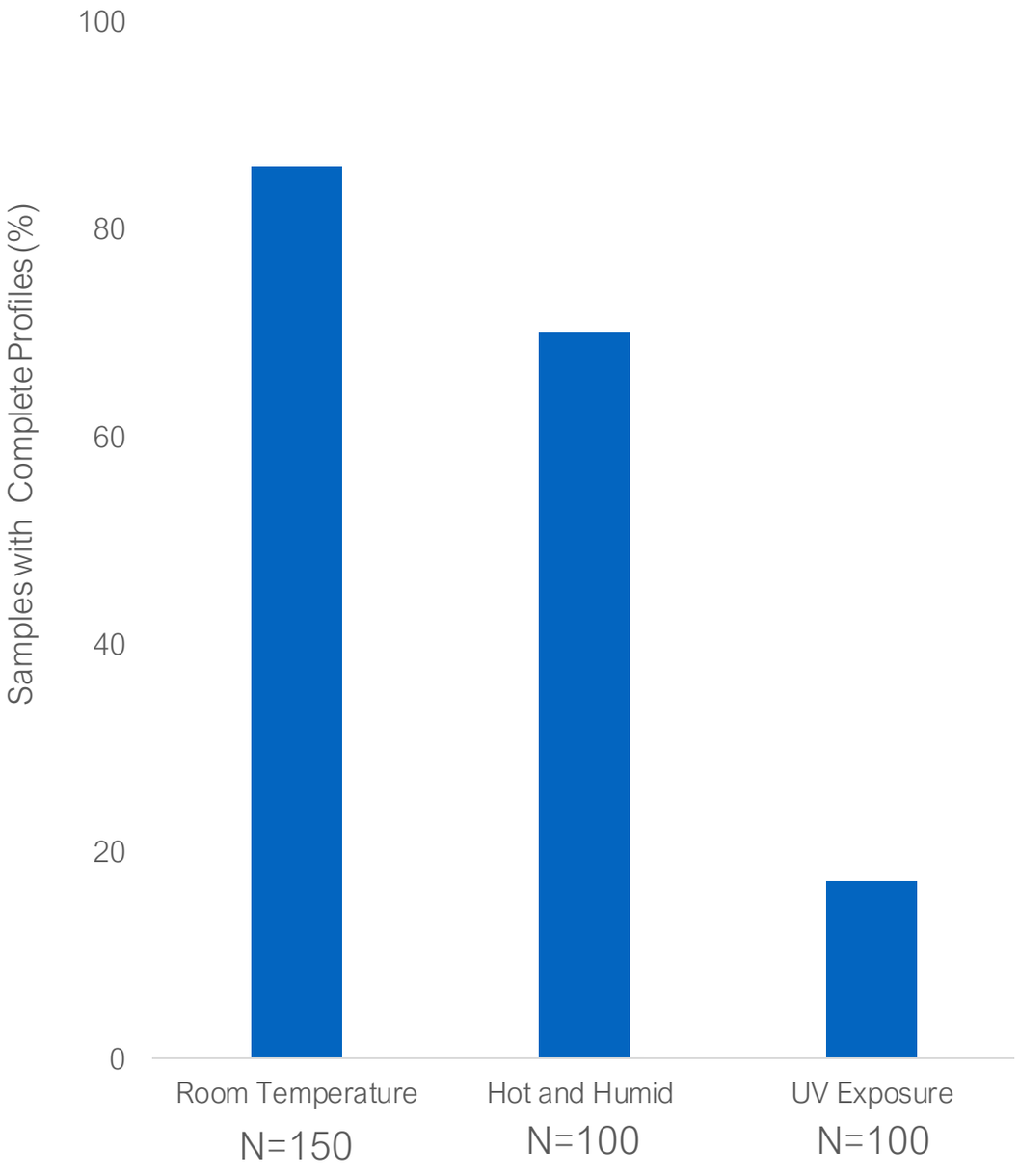
Incubate at 95°C for 5min

↓ 2µL

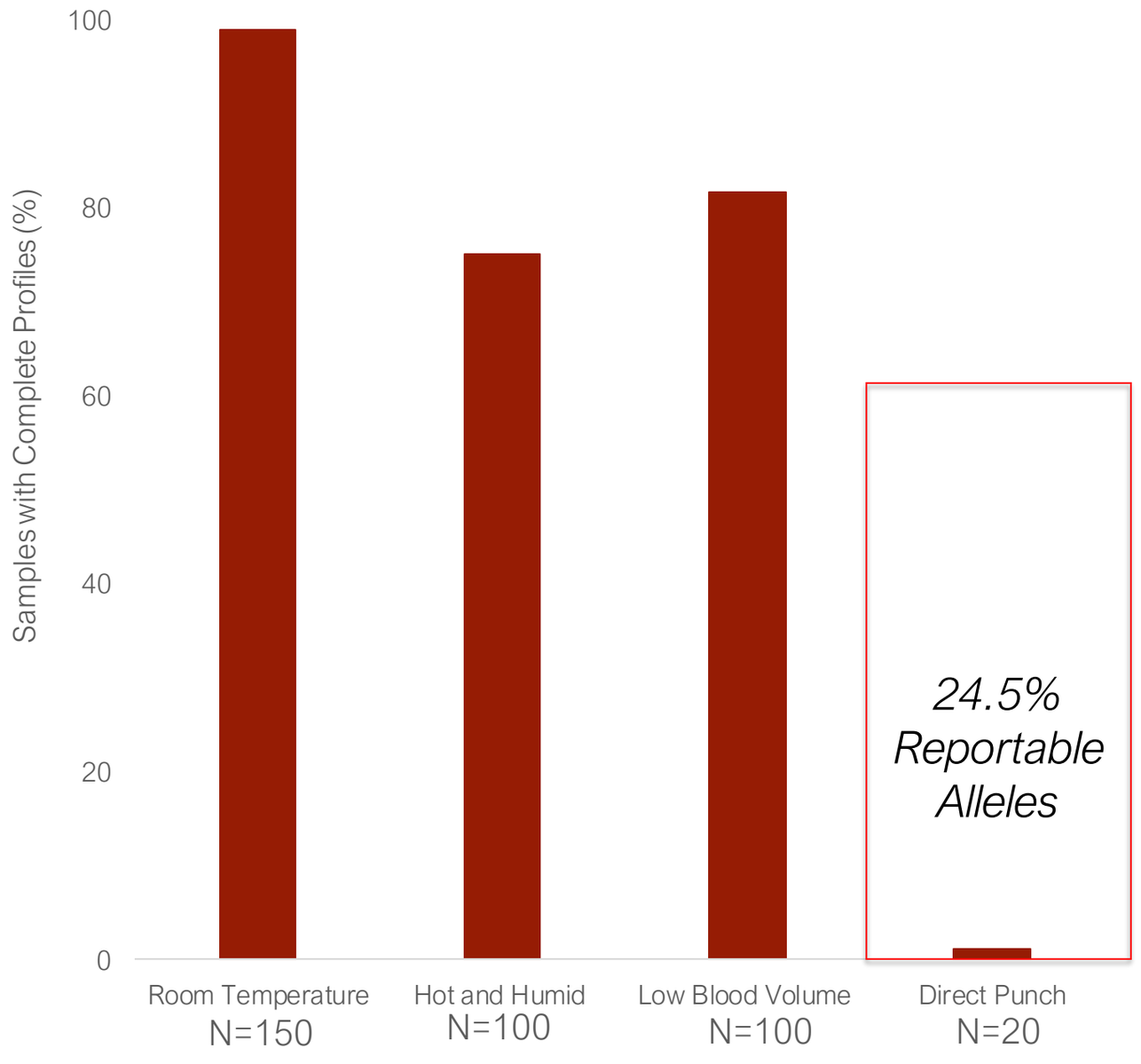
QIAgility adds GO! mastermix

First Pass Rates – FTA

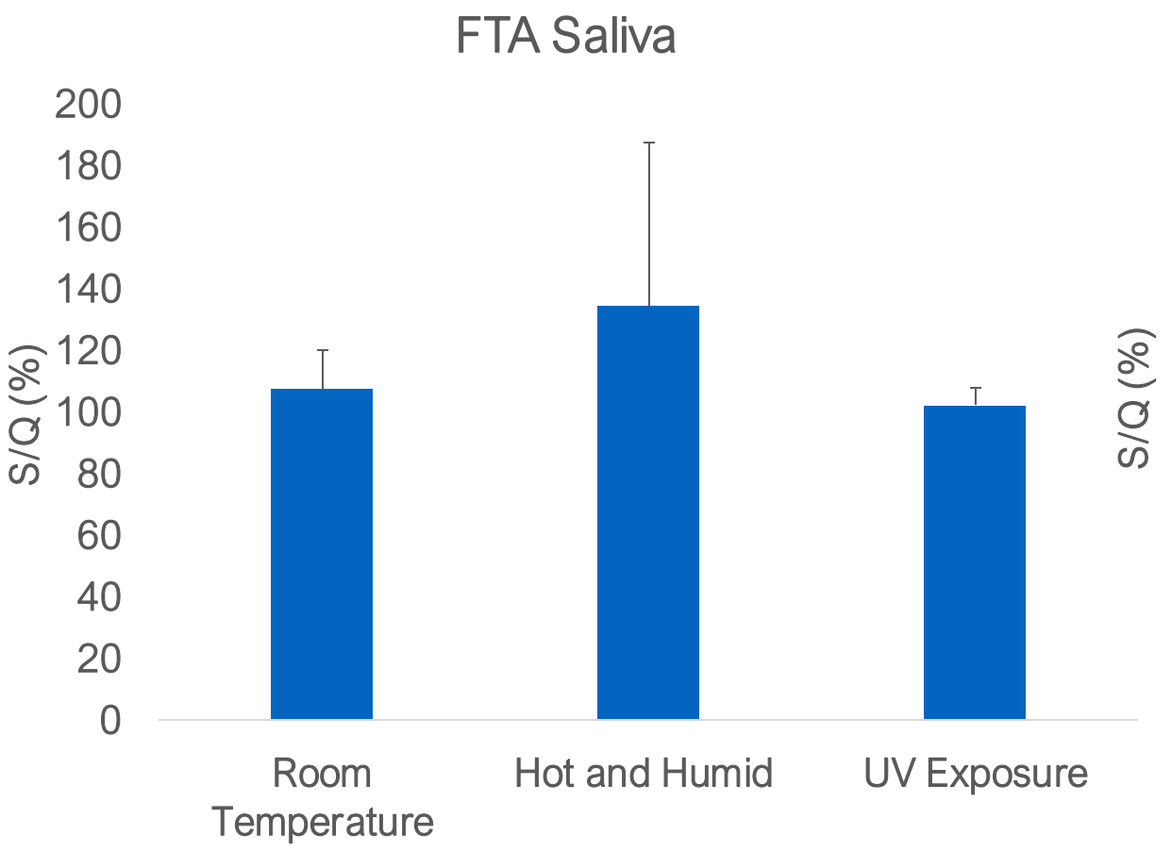
Saliva

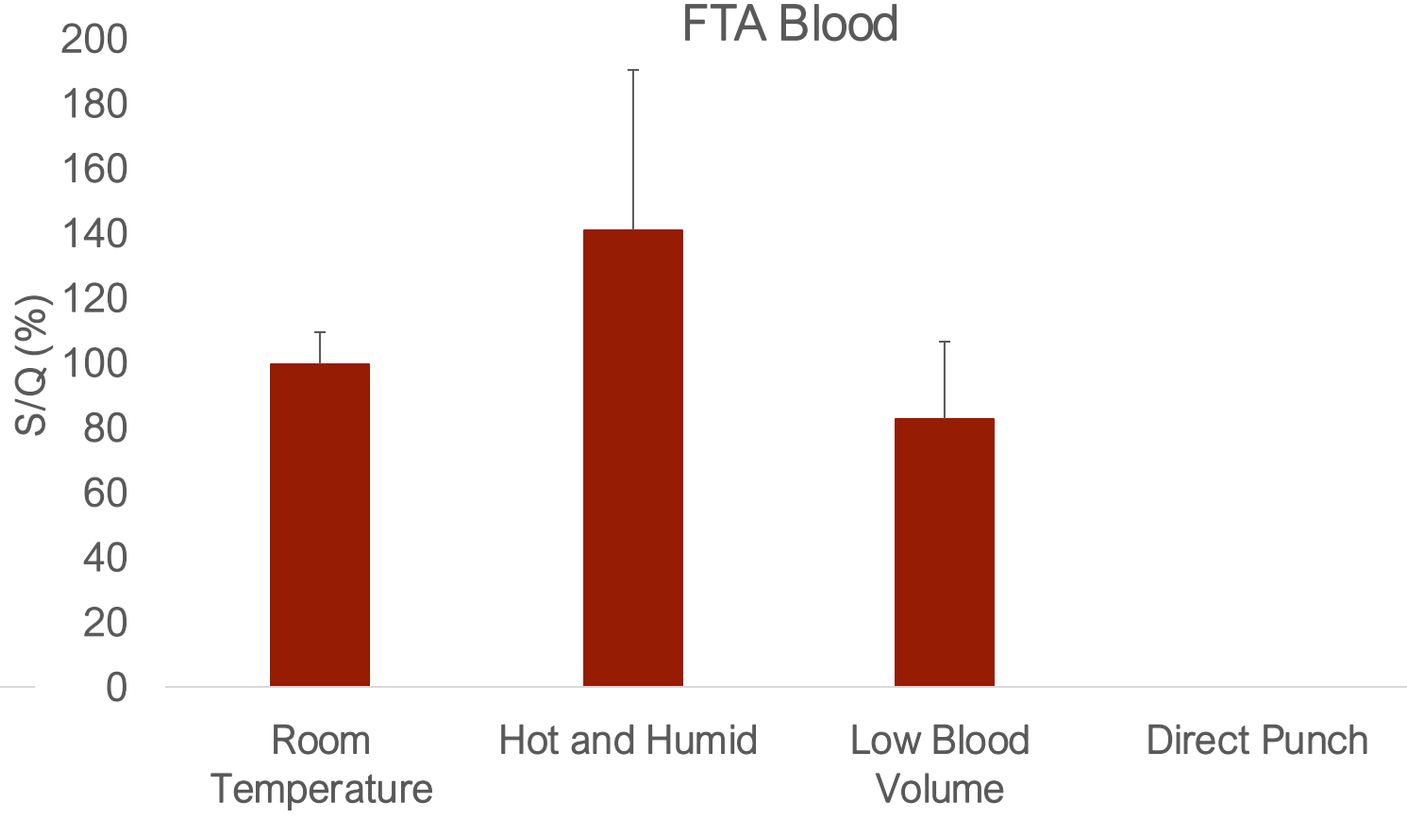


Blood



Were the QS Markers right?





100% concordance

- All saliva samples (no inhibition)
- Blood: RT and H&H samples (no inhibition)
- Direct punch samples (severe inhibition/failed amp detected)

Low Blood Volume Sample

- QS Balanced — 70/100 (2 punches with 0 alleles – confirmed no DNA)
- QS < 20% — 2/100 (100% alleles)
- QS imbalance 20 – 70% — 28/100 (100% alleles)

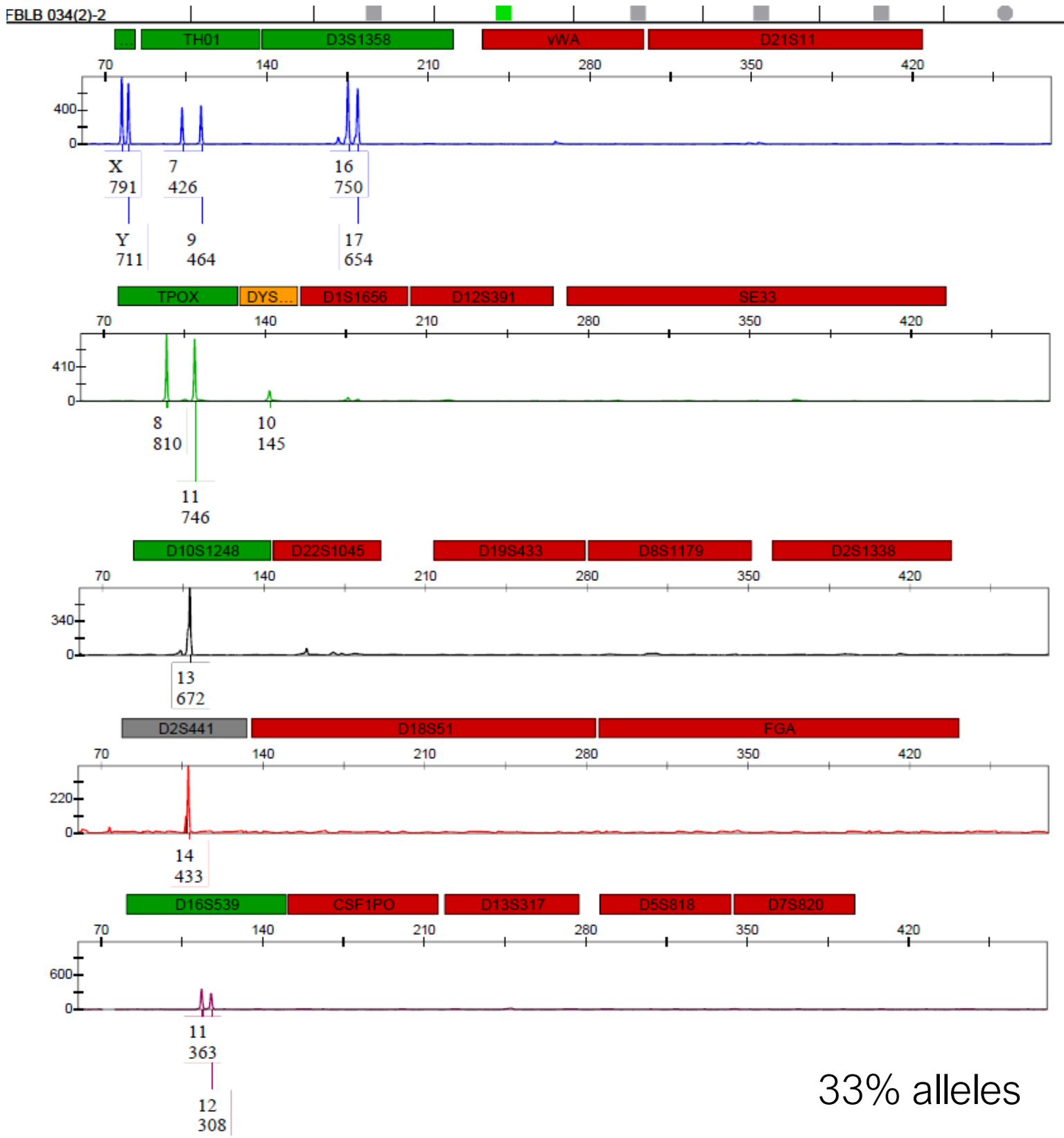
Examples – Degradation or Inhibition?

Collaboration with local laboratory for blind STR assessment

Blood on FTA

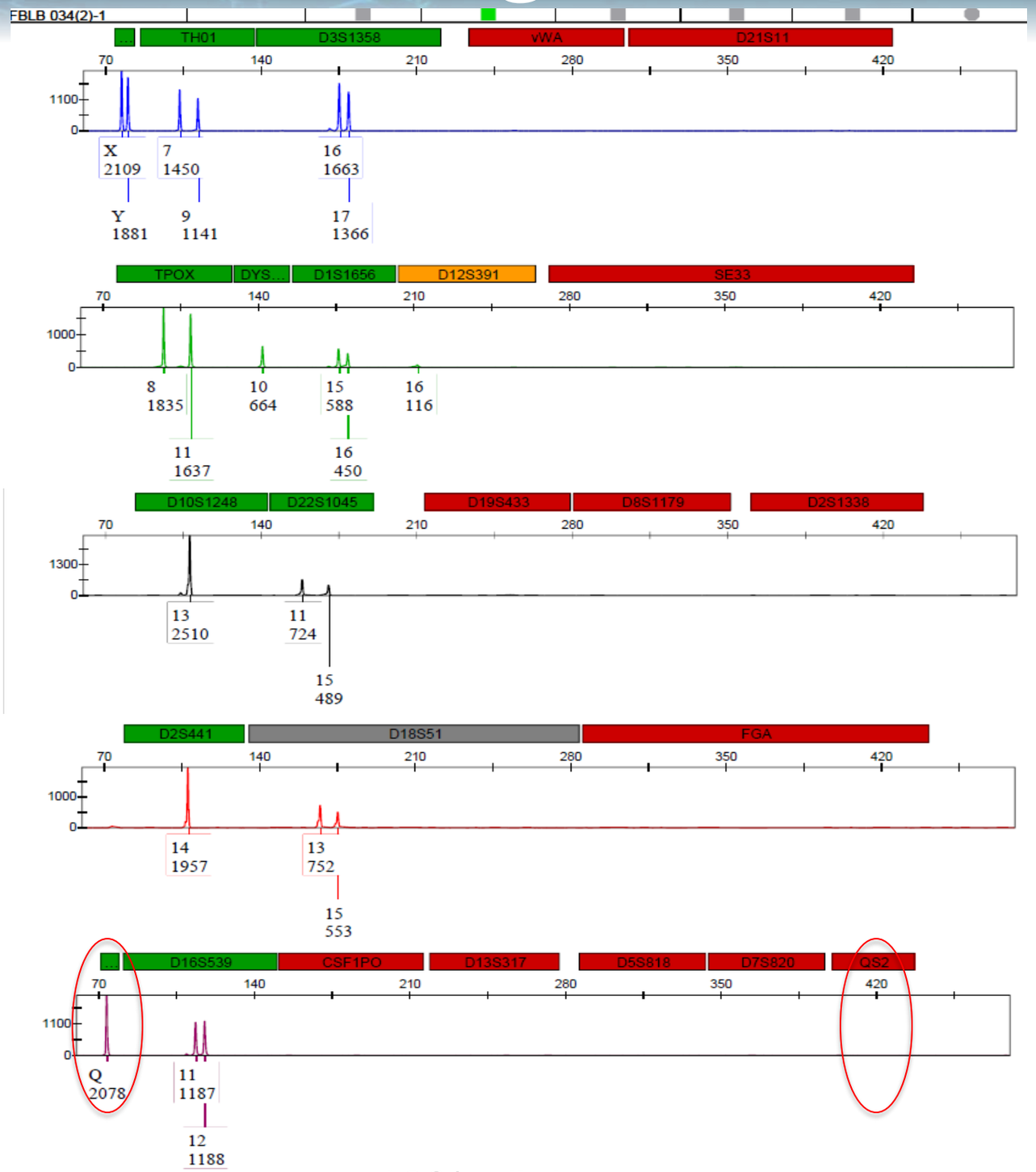
Without QS, extreme degradation was assumed

Rework = New punch



33% alleles

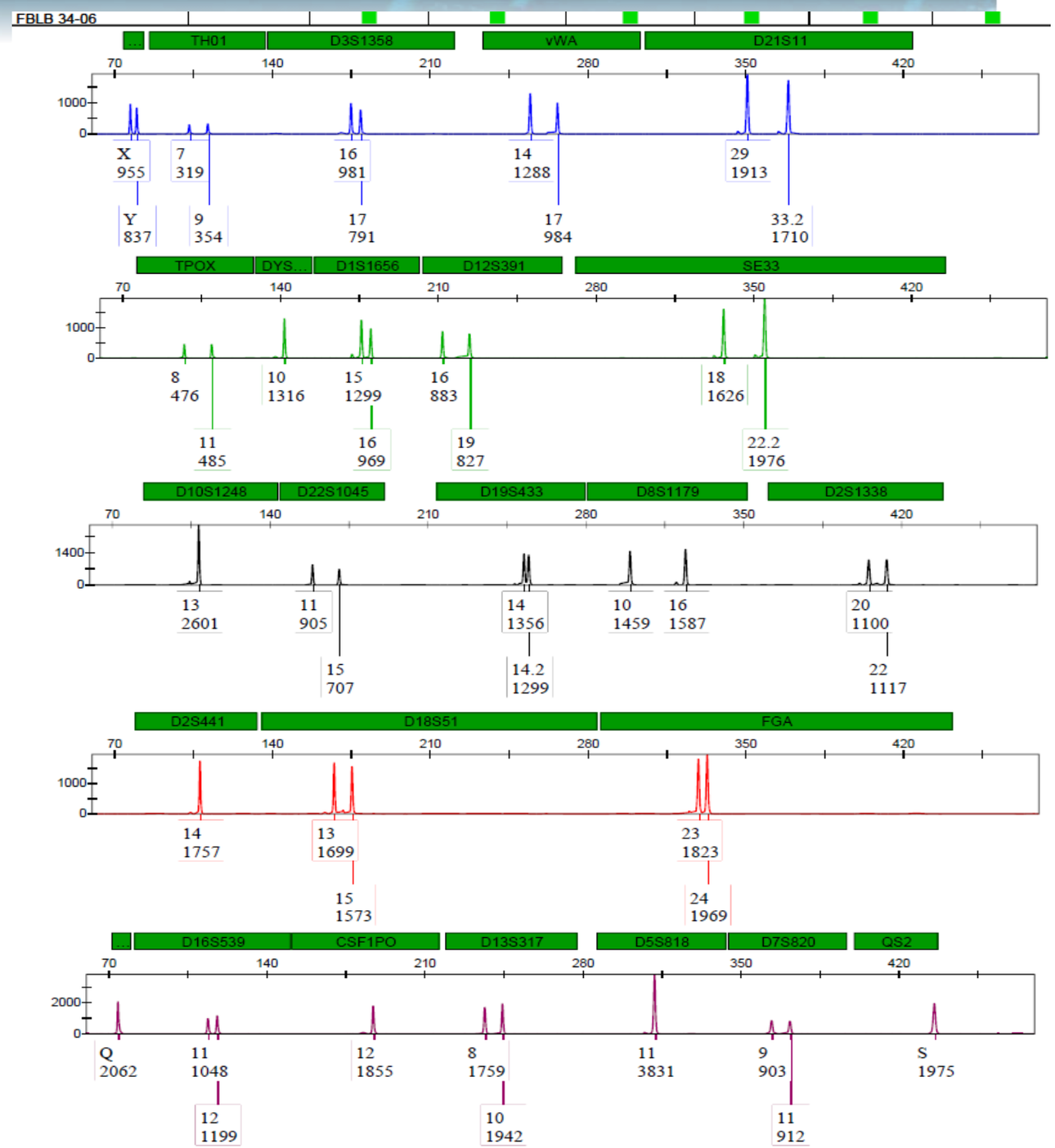
Degradation or Inhibition?



49% alleles

New punch = similar result

QS shows inhibition



100% alleles

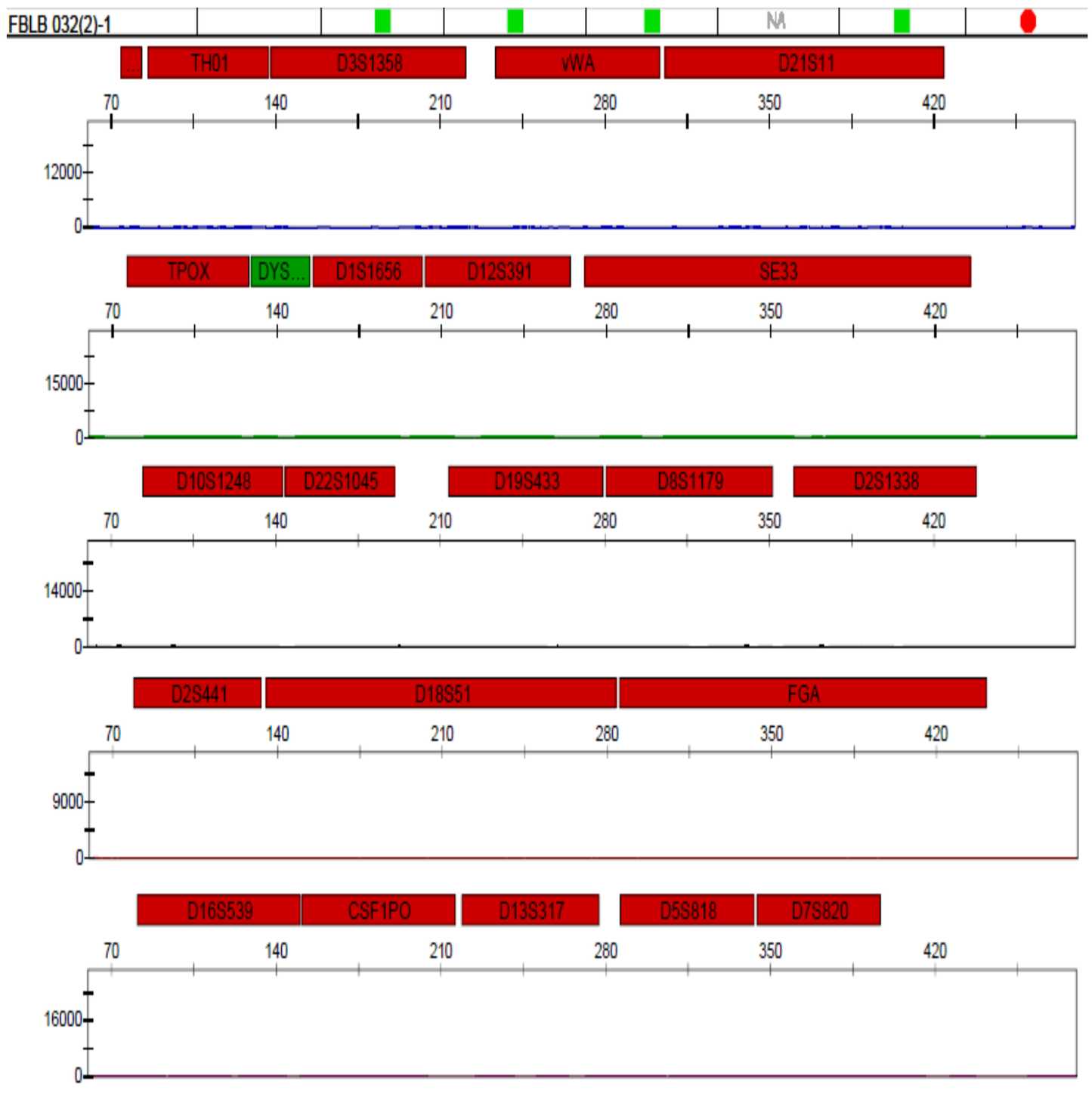
2nd Rework = new punch lysed in 20 µL GO! Lysis buffer and reamp. with 2 µL lysate (or water wash)

Failed Amp or Severe Inhibition?

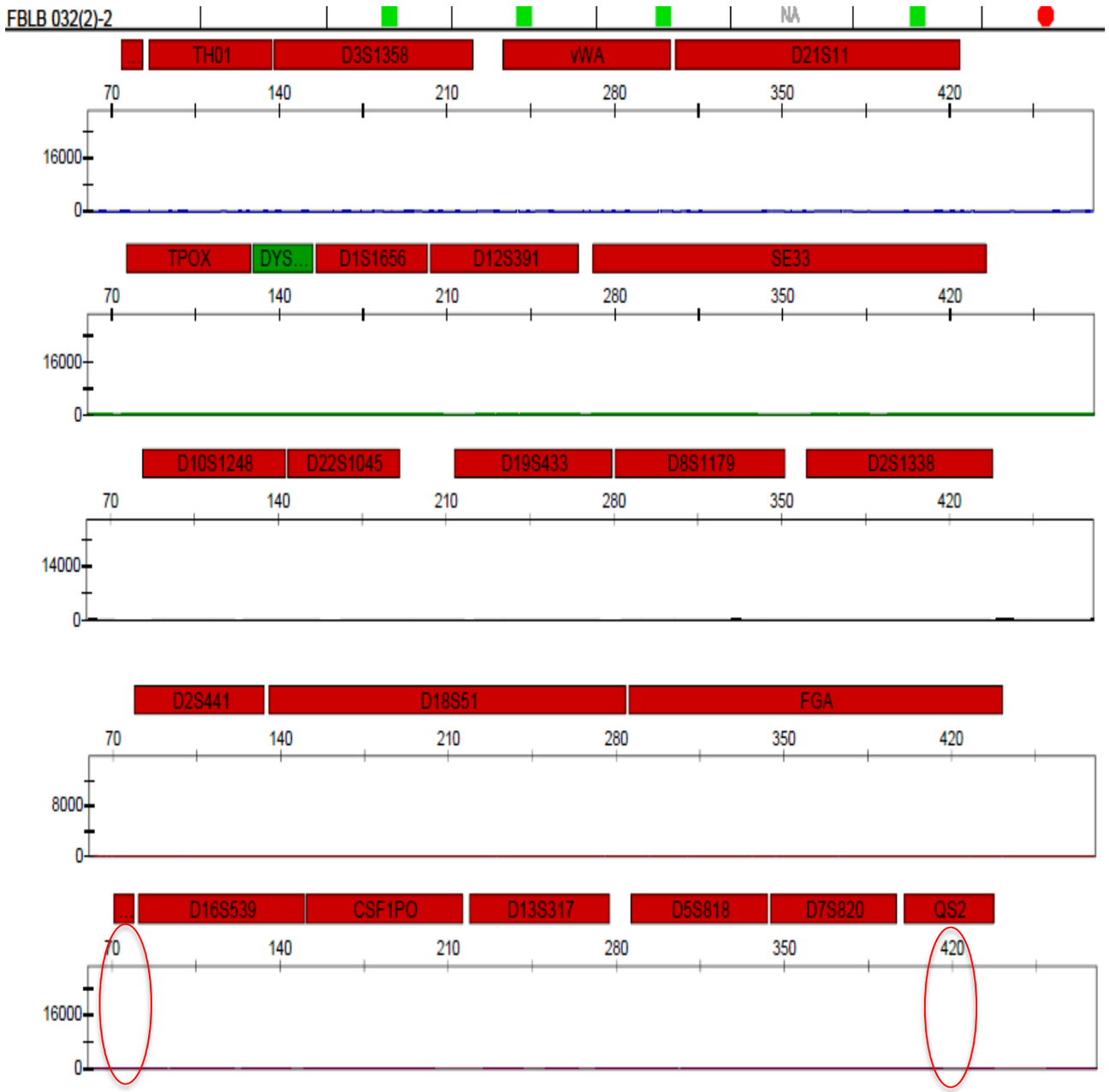
Blood on FTA

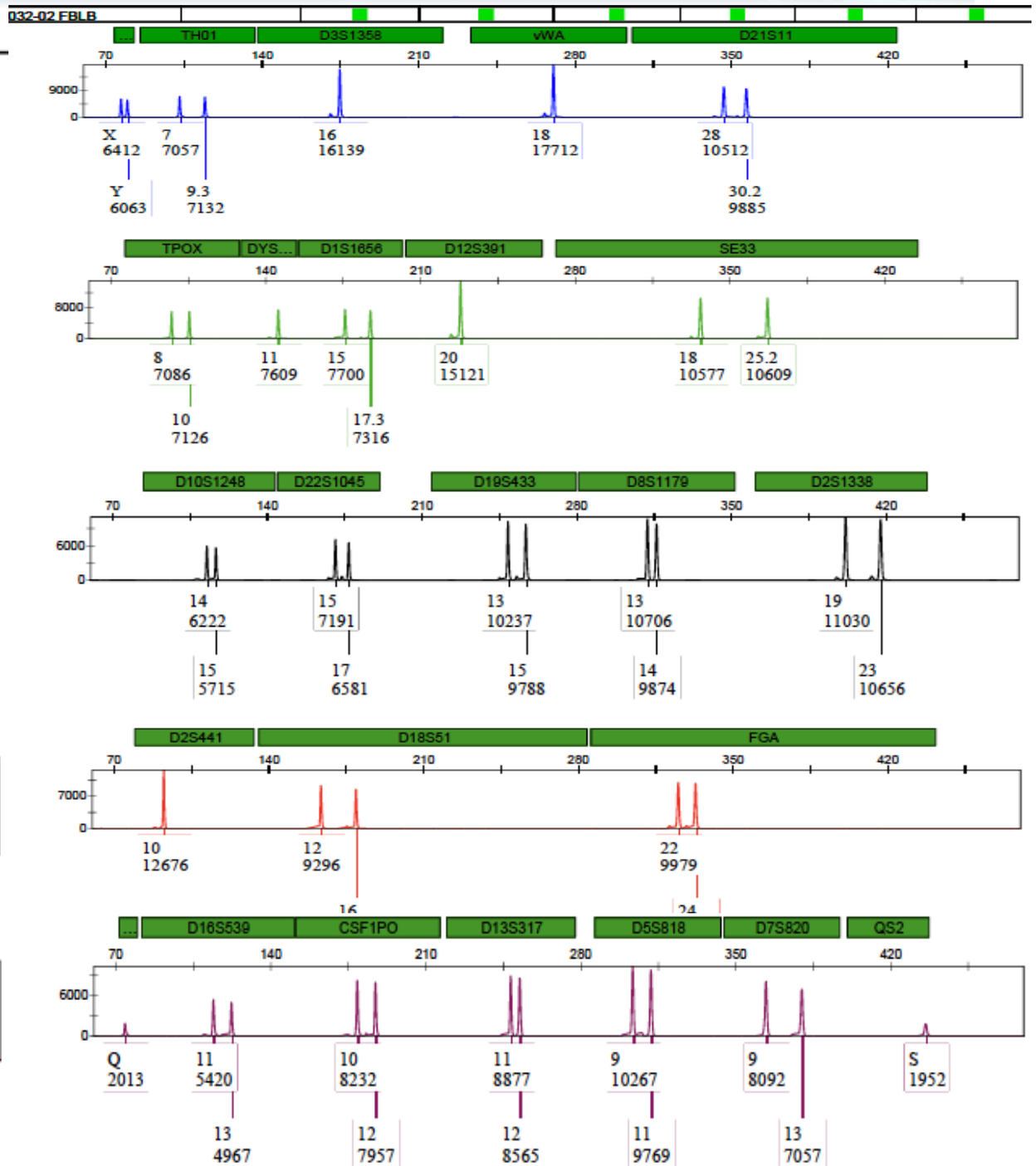
Without QS markers analyst was unsure whether there was no DNA present or a failed amplification

Rework = New punch



Failed Amp or Inhibition?





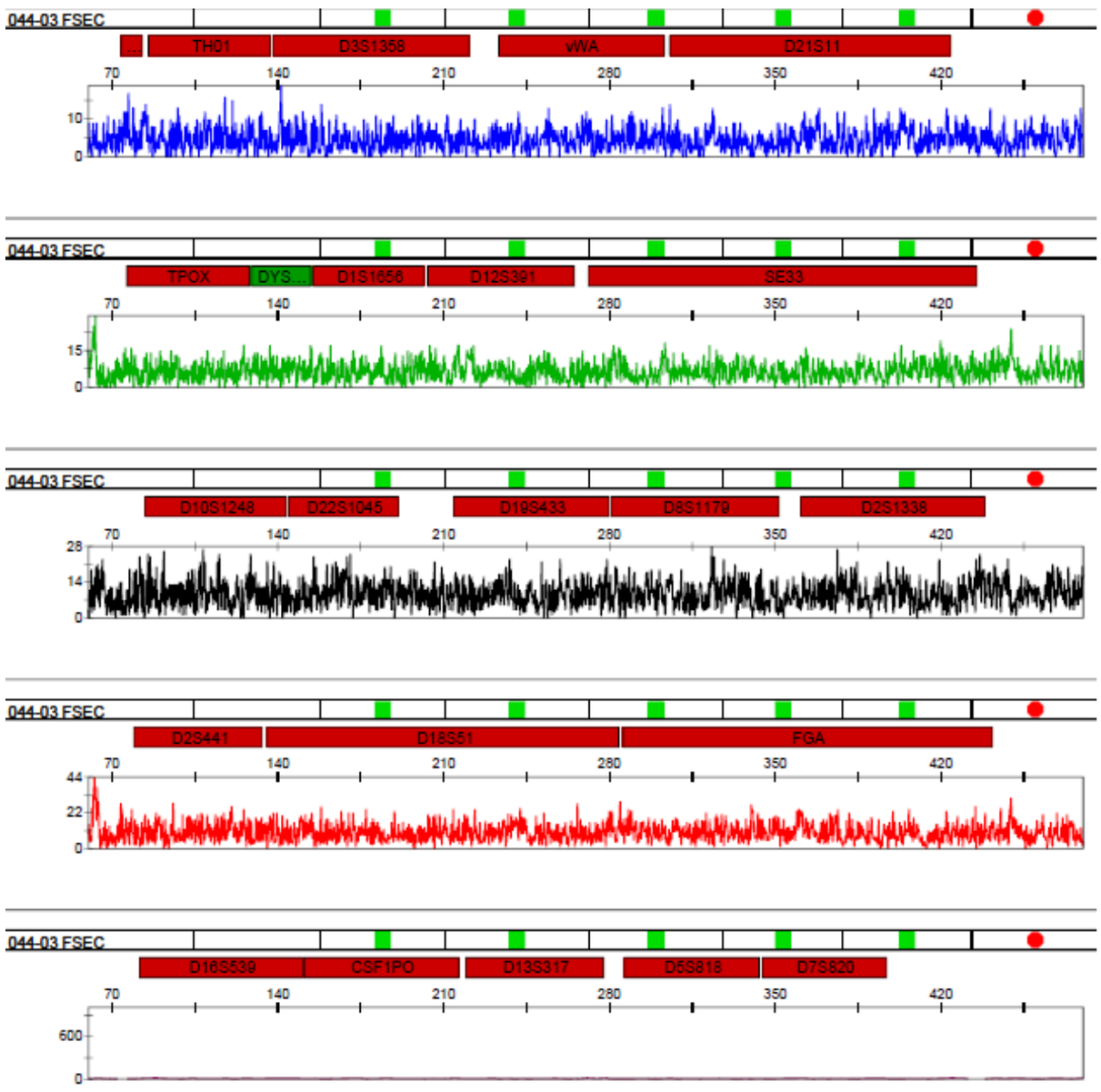
New punch = looks same

Amp with QS shows inhibition

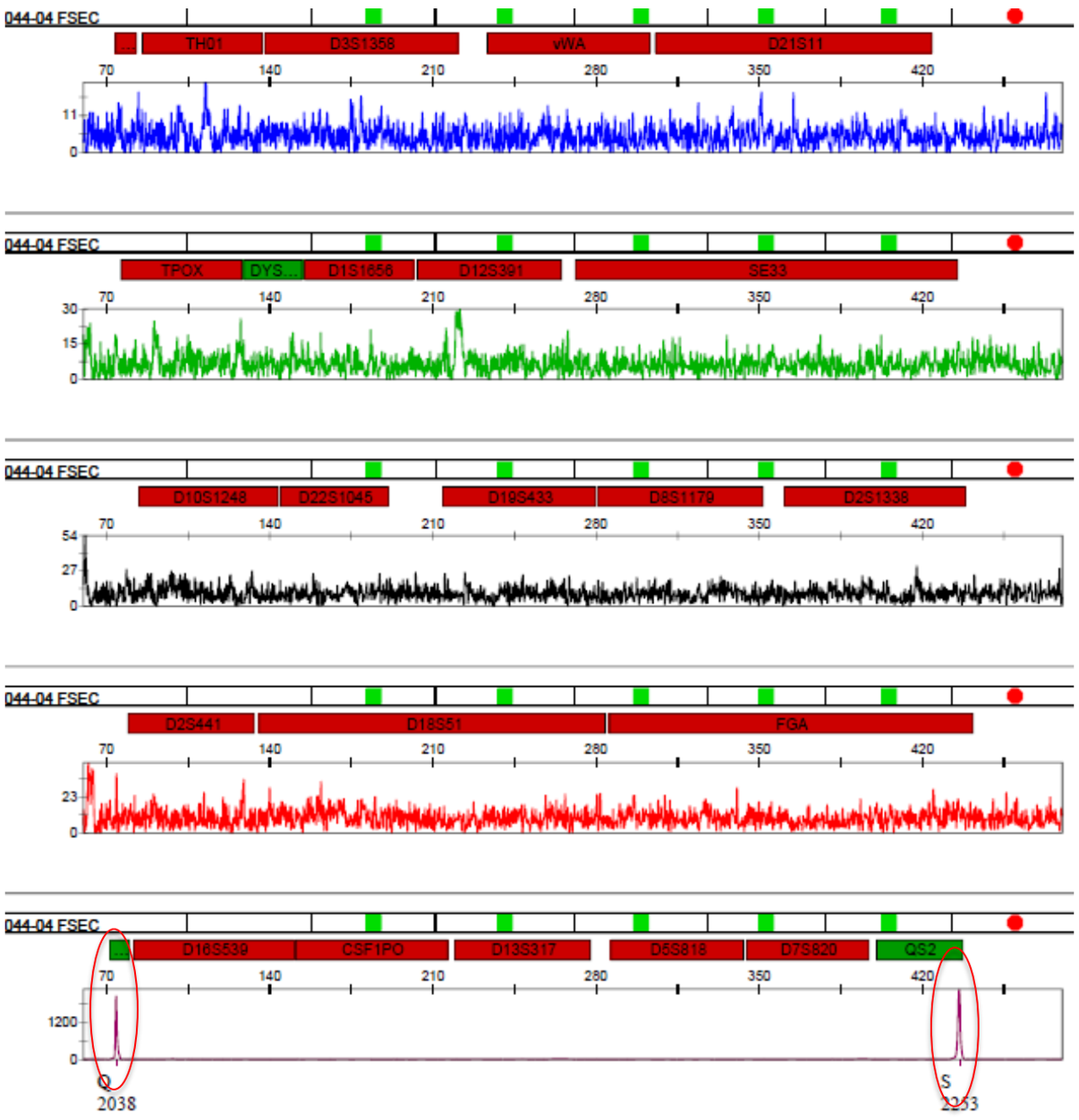
Rework = lysed in 20 µL GO! Lysis buffer and reamp with 2 µL lysate (or water wash)

No DNA?

Saliva on FTA



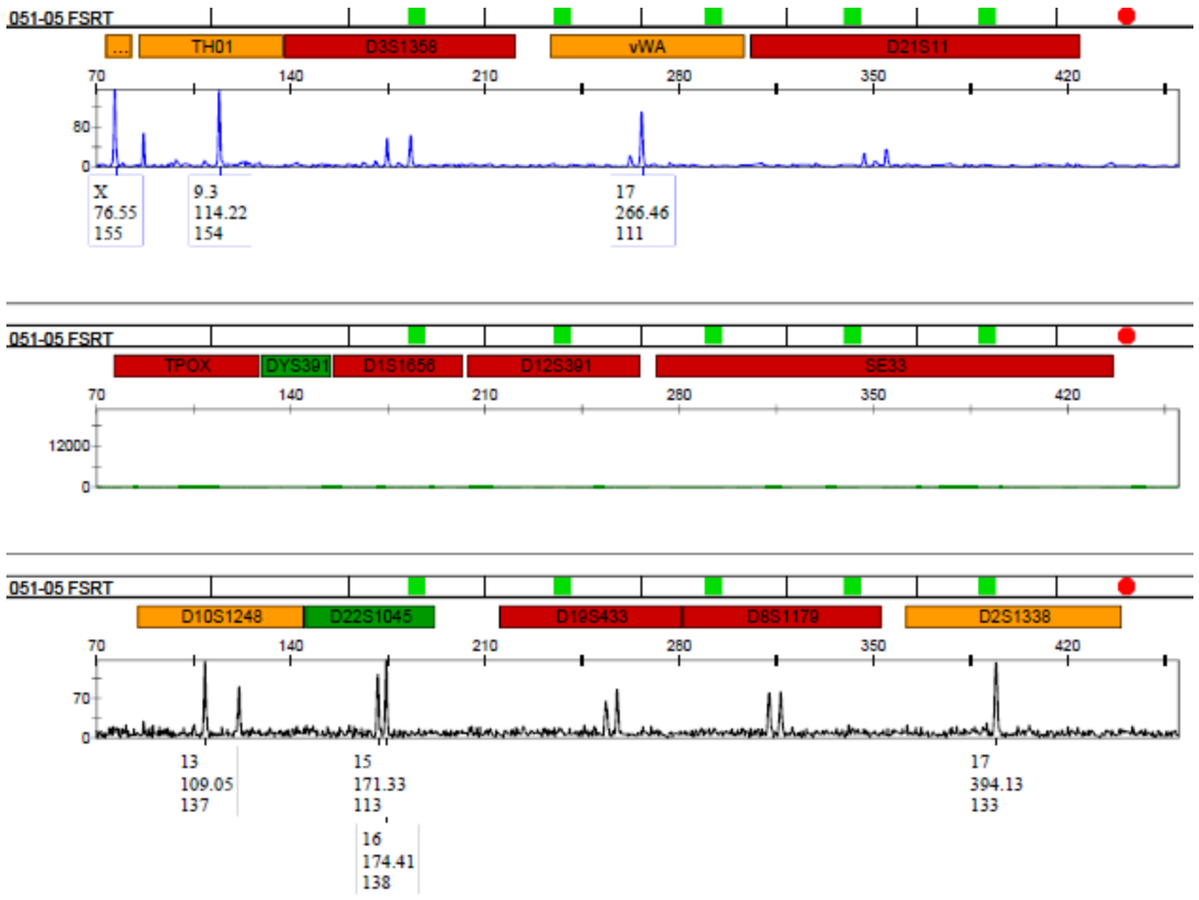
Rework = New punch

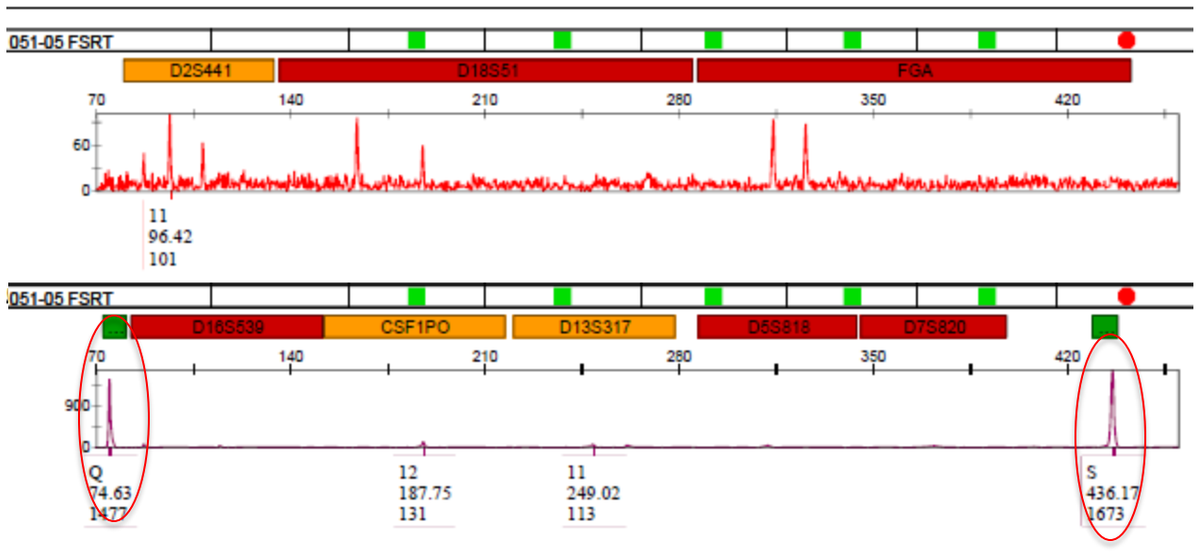


Confirmed no DNA

Inhibition and/or Low Template

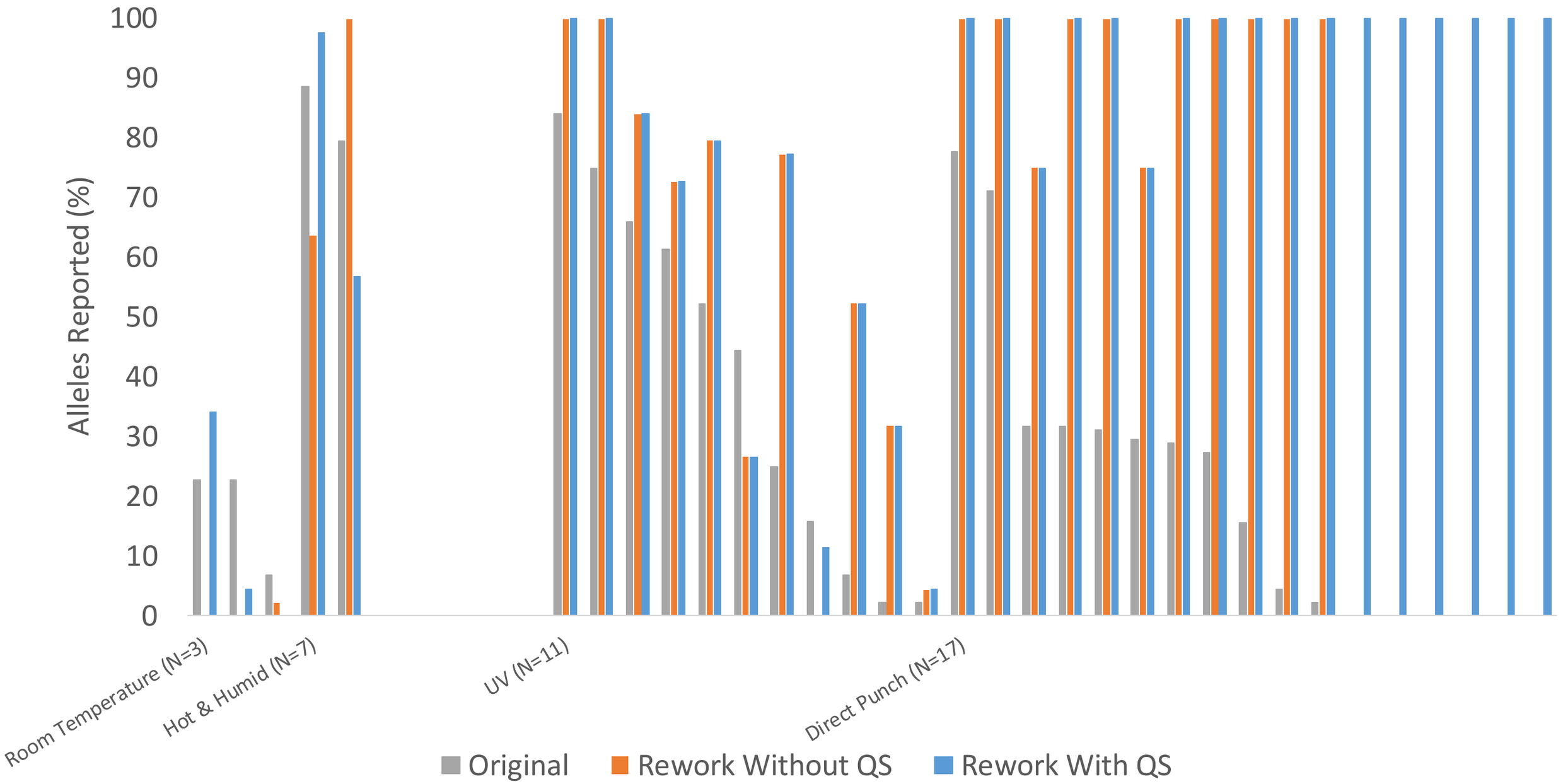
Thought to be inhibition and LT – Rework?





Confirmed low template and deg – no inhibition
Dilution/water wash (for suspected inhibition) no additional benefit

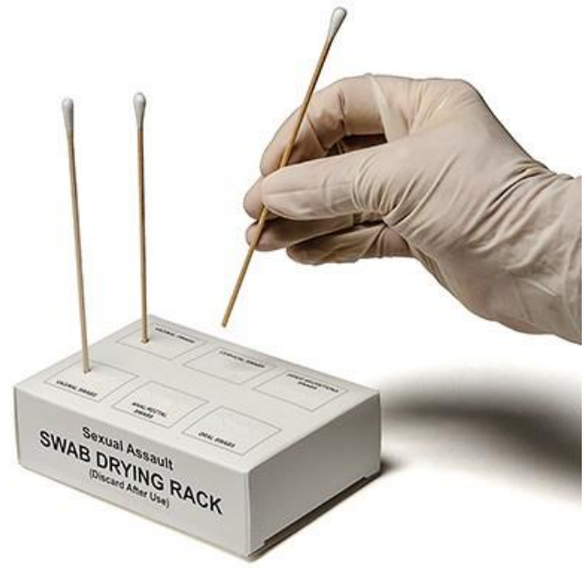
Reworked Databasing Samples



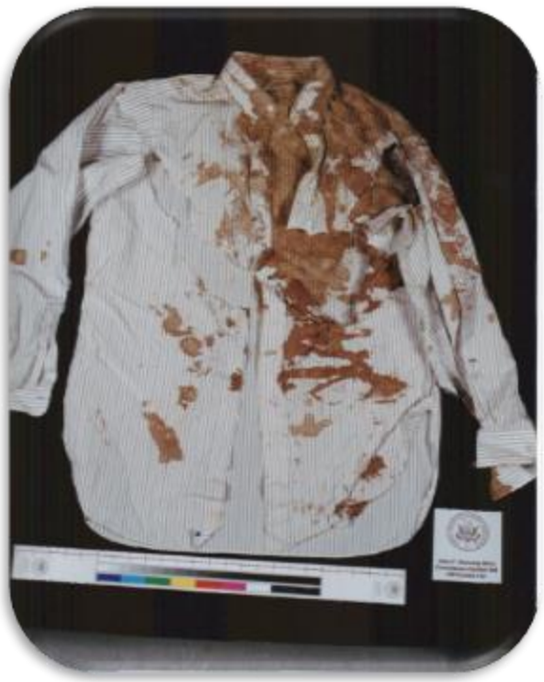
- 21 out of 38 samples improved based on STR profile alone
- With the QS markers, more alleles were recovered in 10 additional samples compared to reworks without QS

Casework Samples

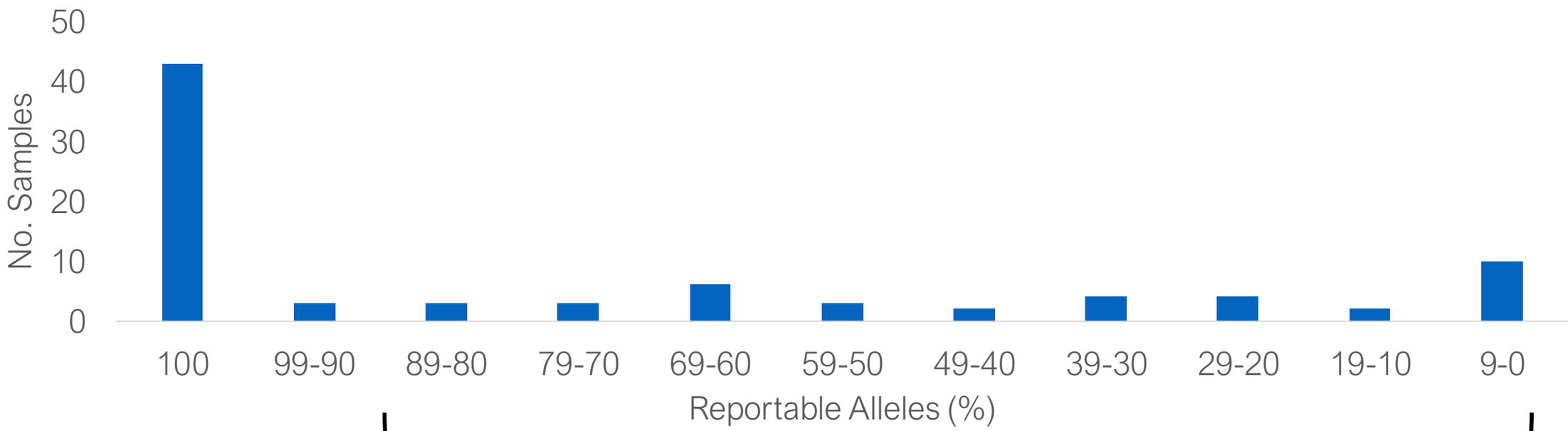
Investigator® 24plex QS Kit

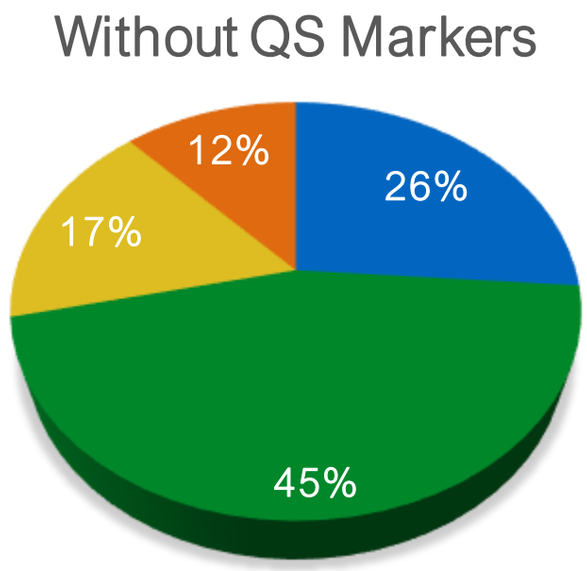


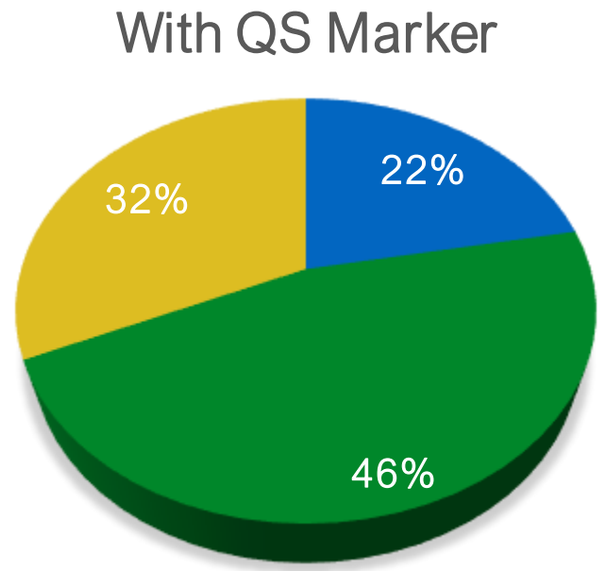
Skeletal (N = 20)
Touch samples (weapons) (N = 24)
Decomposed human tissues (N = 10)
Aged Blood and Saliva Stains (N = 10)
Mixture (N = 5)
Inhibited (N = 19)
Mock Sexual Assault & Post-Coital (N = 32)



Casework Samples





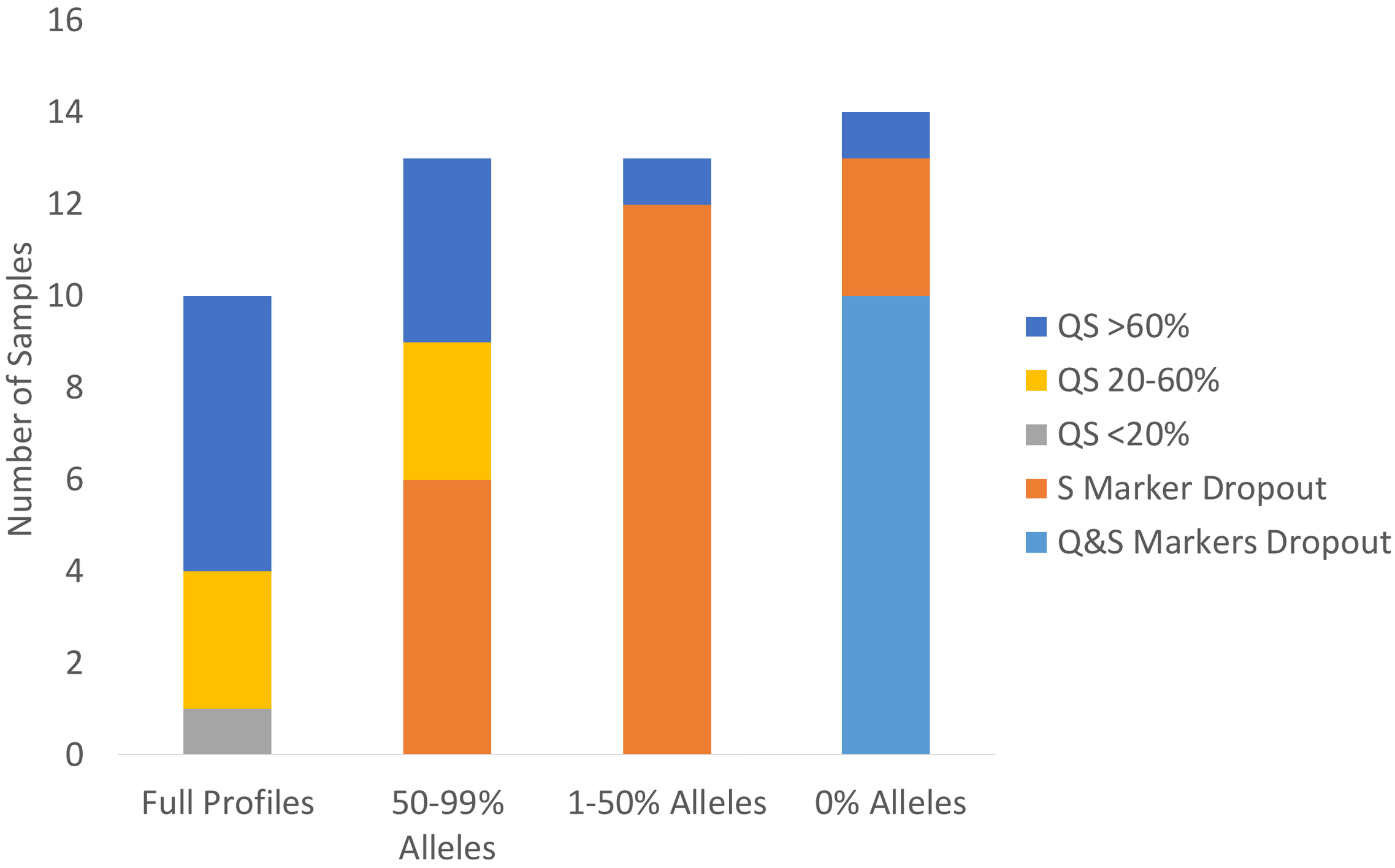


Degraded Low Template Inhibition No Template/Failed Amp

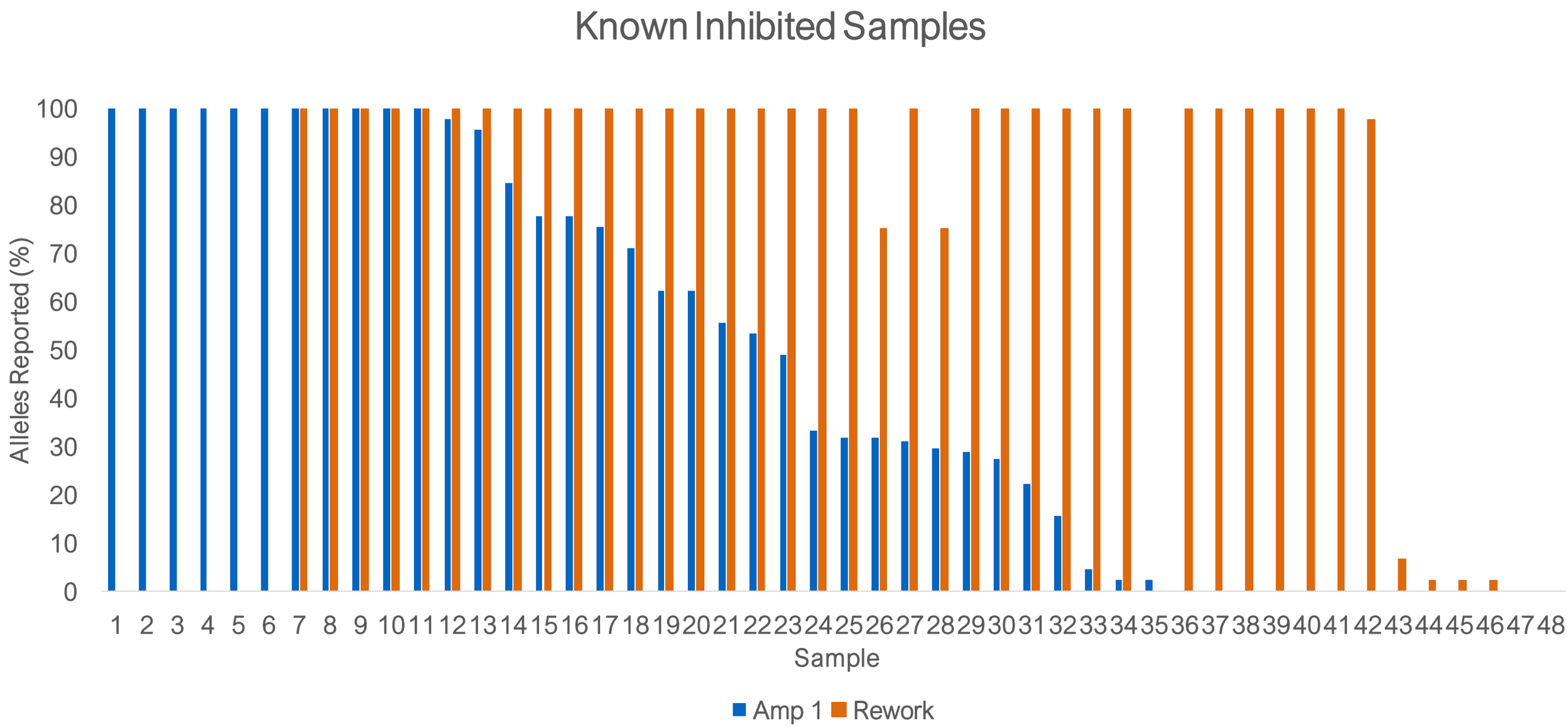
Degraded Low Template Inhibition

- Able to resolve failed profiles (as inhibited) for targeted reworking
- Able to confirm ambiguous low quality profiles as low template and/or highly degraded samples (targeted rework, or avoid reworking)

Known Inhibited Samples (N=50)

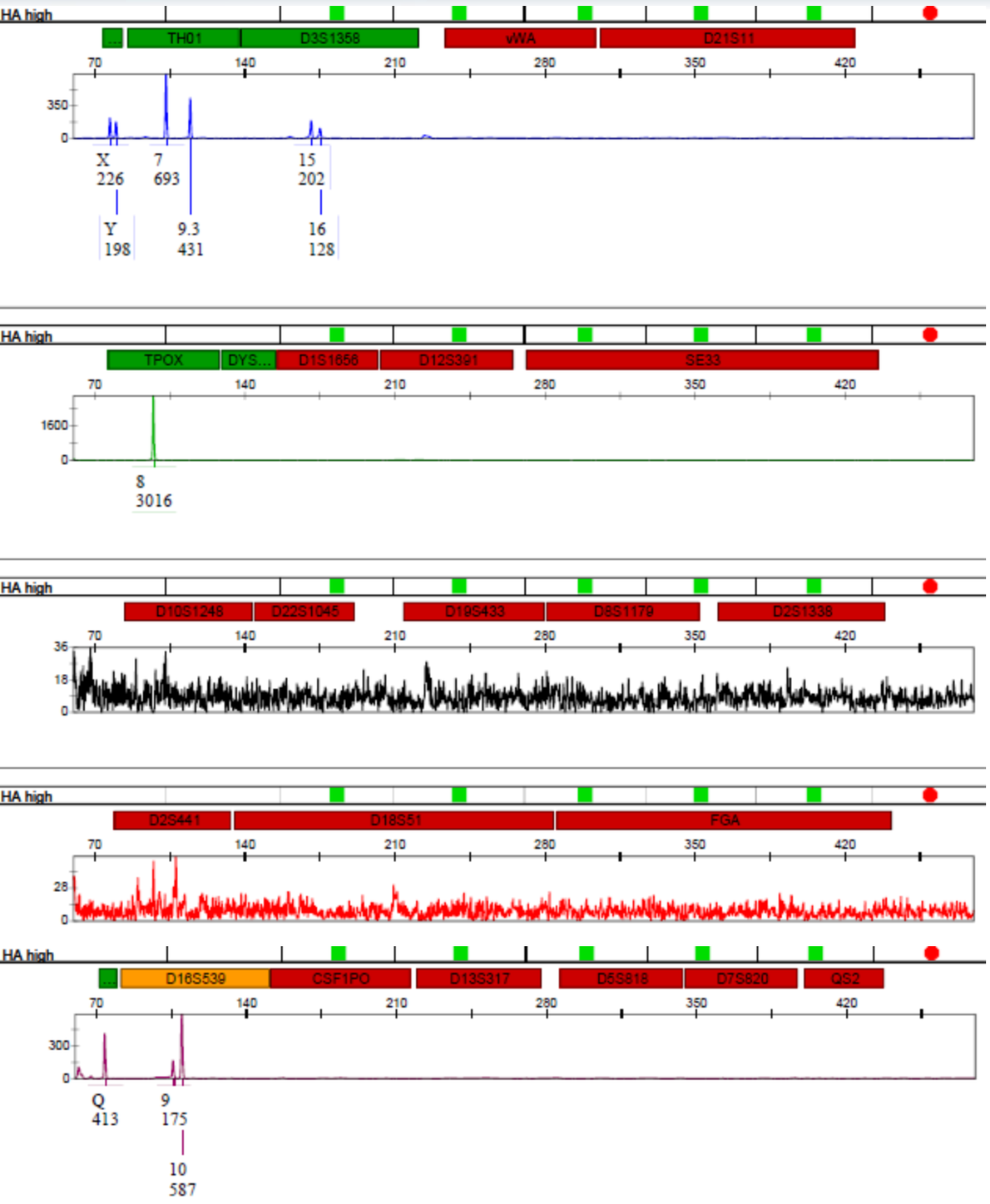


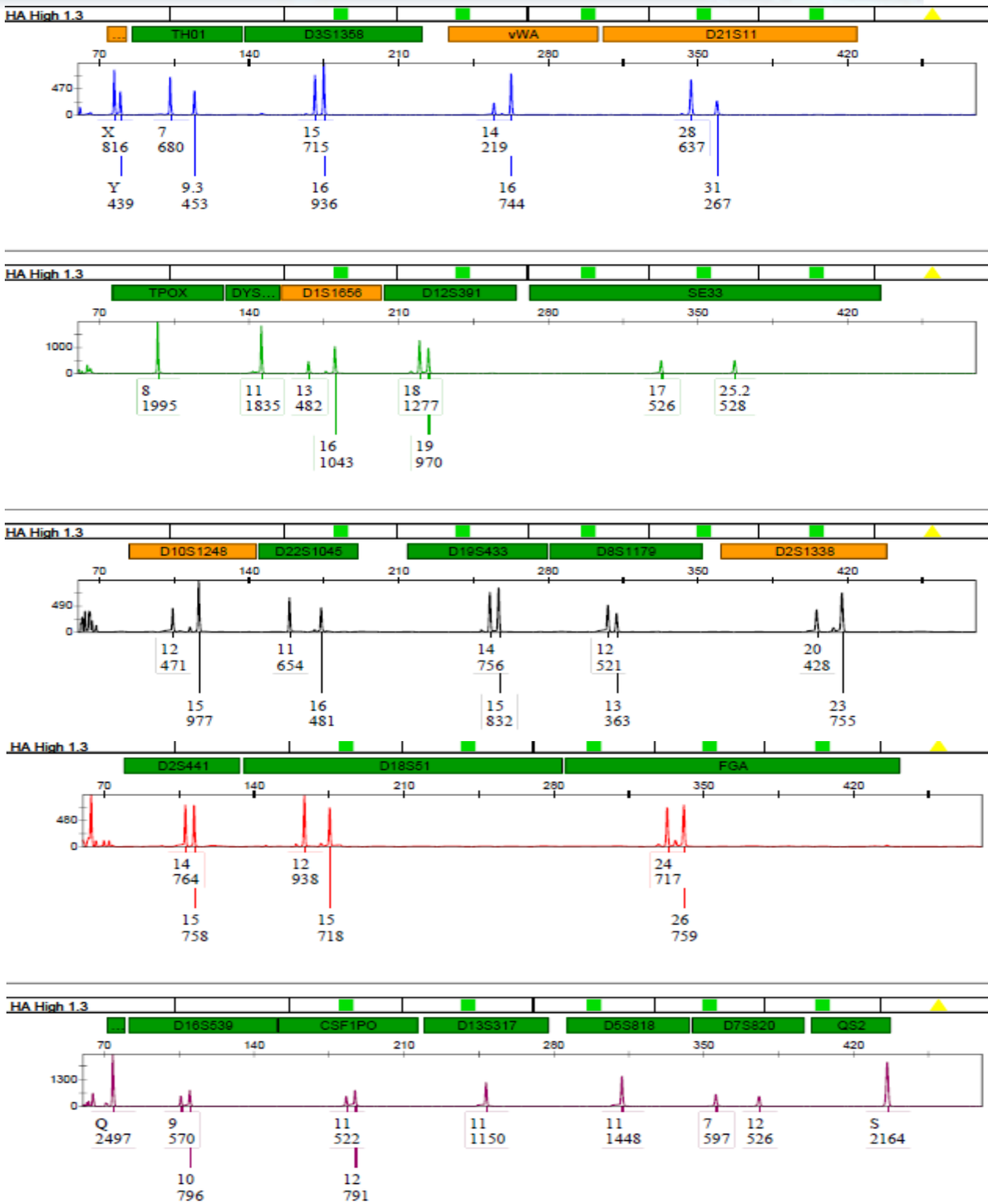
Reworks based on QS



Rework = 1:3 dilution and re-amp

Humic Acid Inhibition



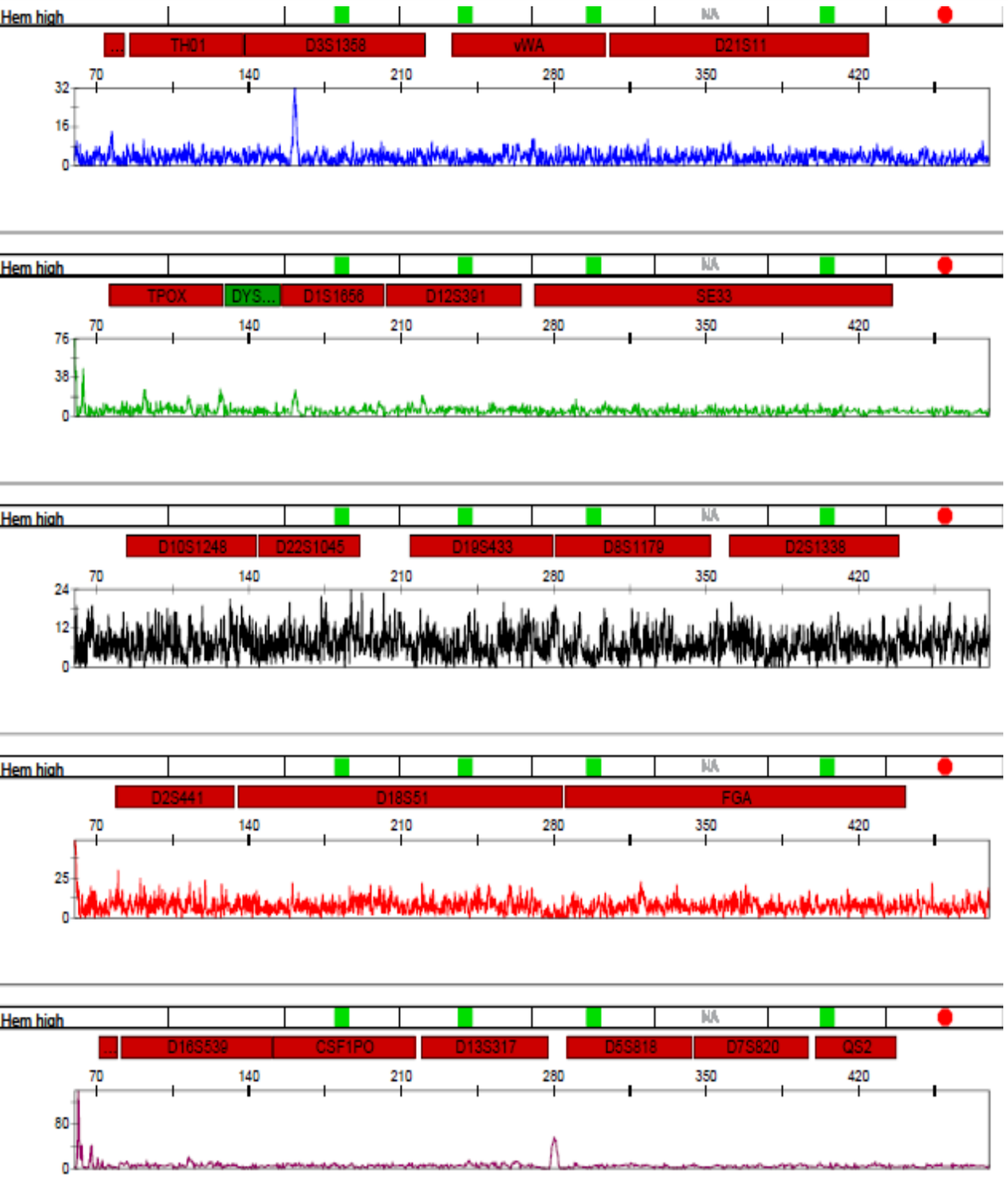


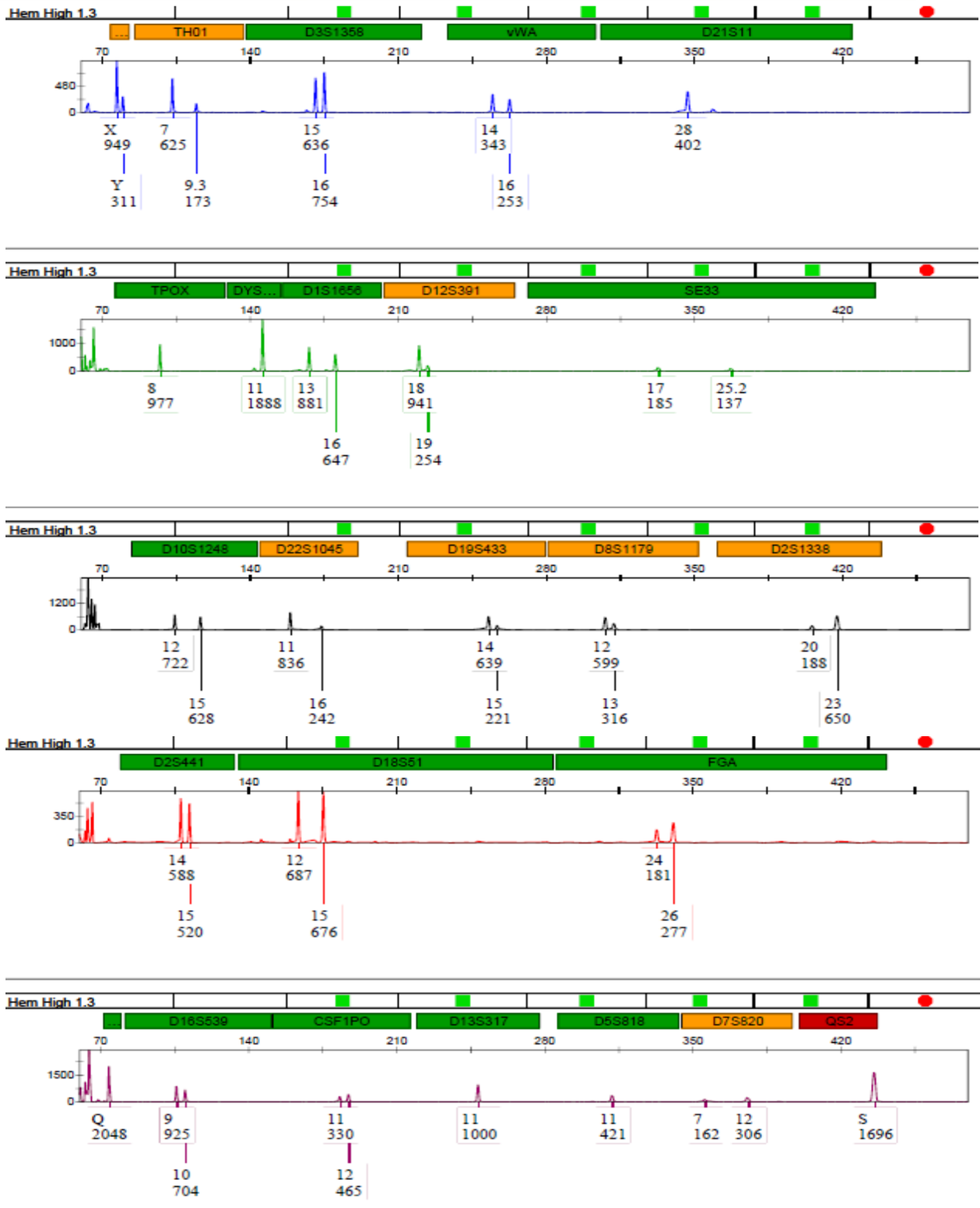
22% alleles
S Dropout

Re-amp
1:3 dilution

100% alleles
87% S/Q

Hematin Inhibition





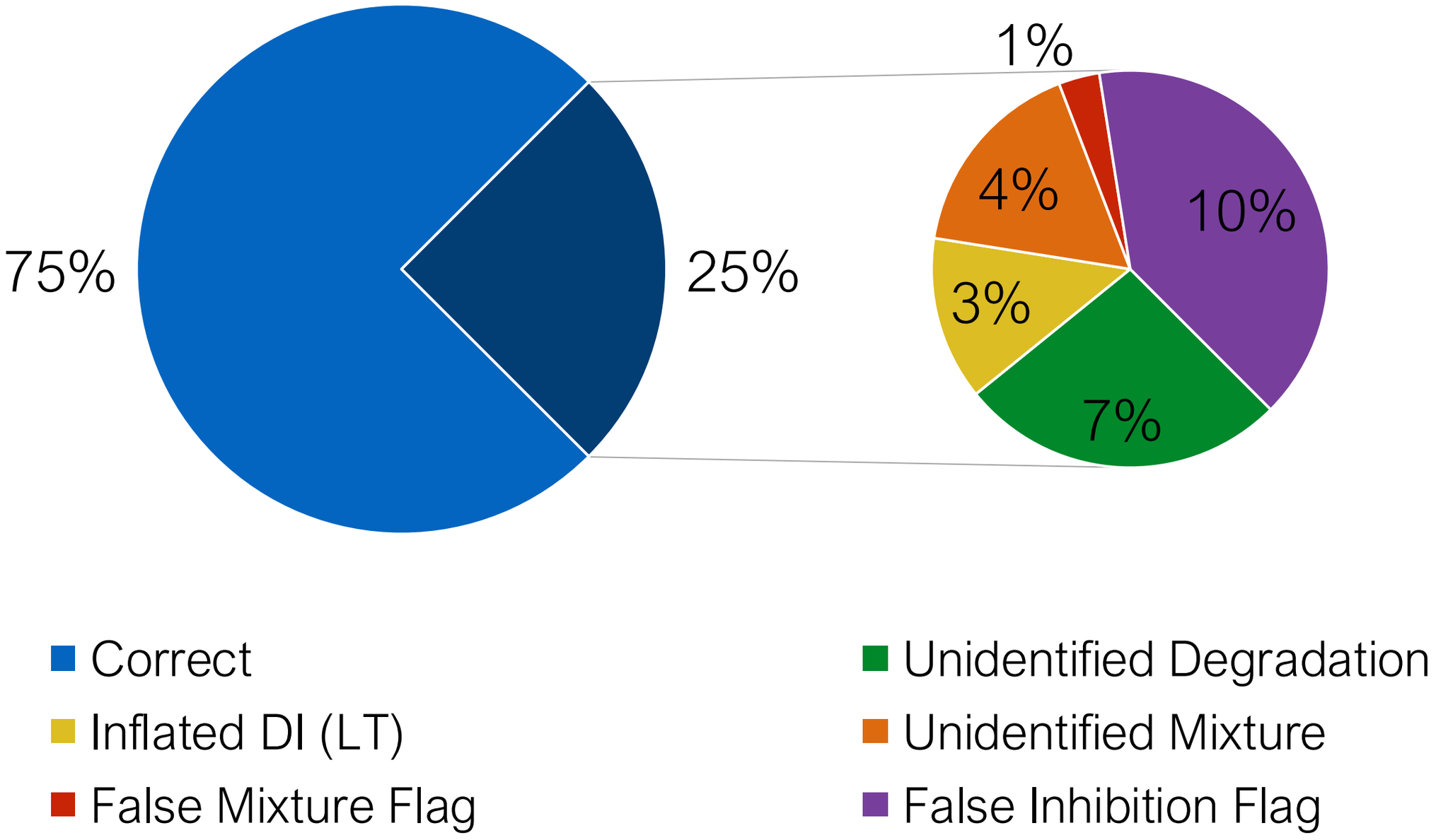
0% alleles
No Q/S – failed amp

Re-amp
1:3 dilution

98% alleles
83% S/Q

How predictable was qPCR?

Concordance between Quantiplex Pro RGQ Quant Flags and STR Profiles

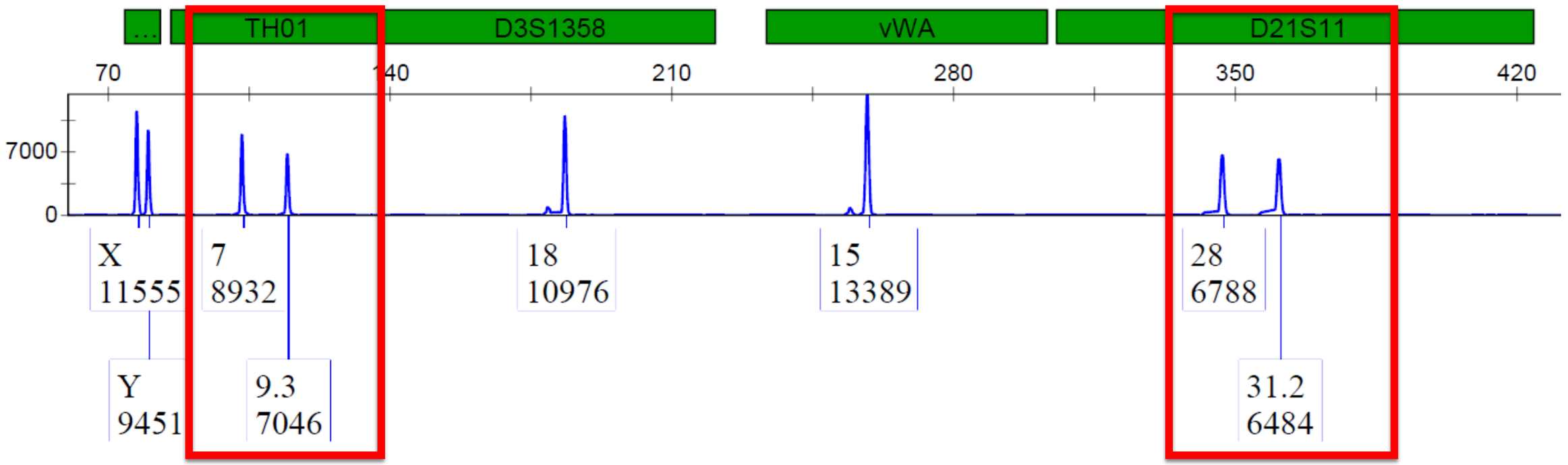


(N=120)

Degradation – Correlation between qPCR & STR "DI"

Input DNA – 0.8 ng (blood stain)

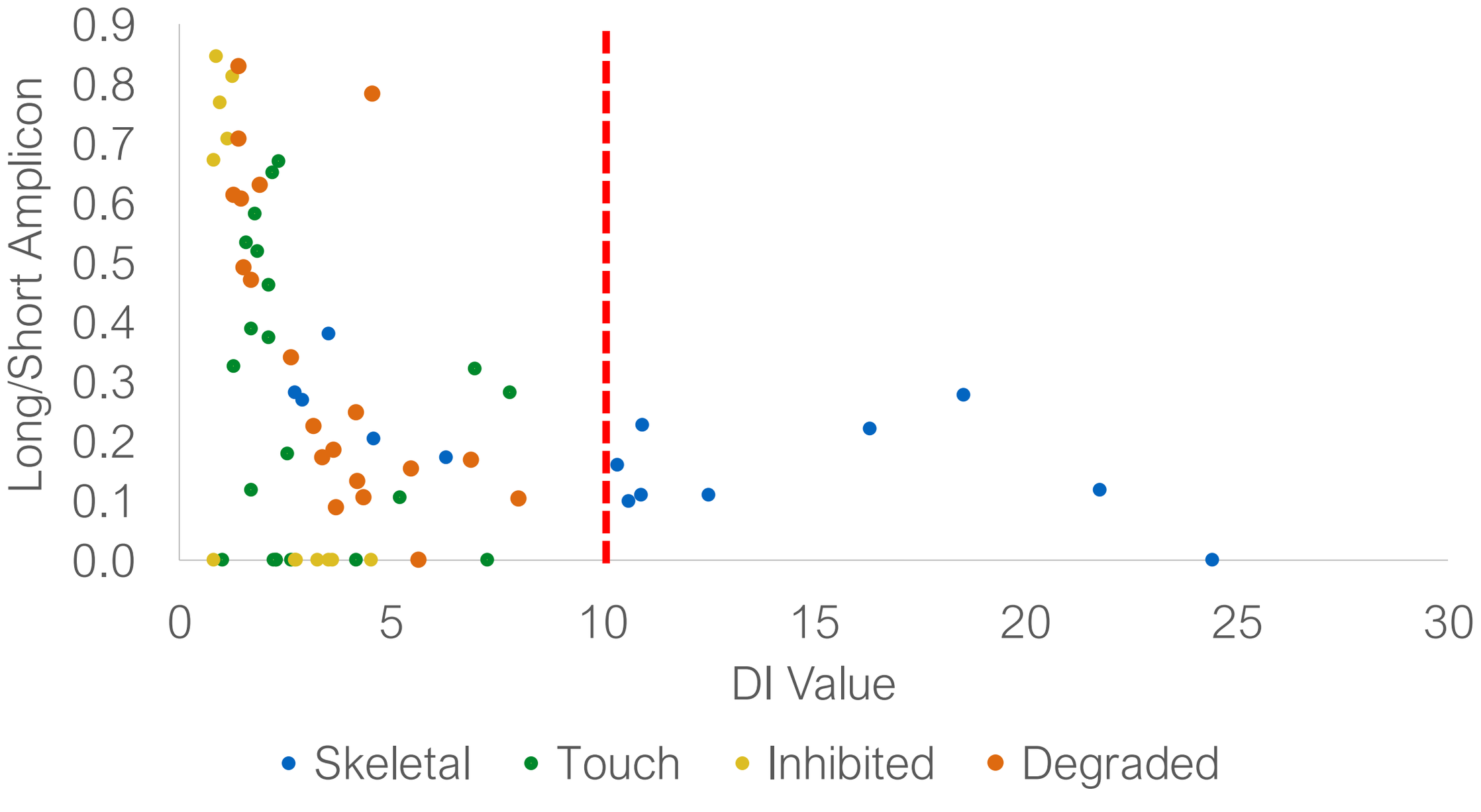
Quality Assessment							
Mixture Index	Mixture Threshold	Degradation Index	Degradation Threshold	Male Degradation Index	Male Degradation Threshold	Inhibition Index	Inhibition Threshold
1.04	Below Threshold	1.36	Below Threshold	1.59	Below Threshold	0.02	Below Threshold



(8932+7046) / (6788+6484) = 1.2

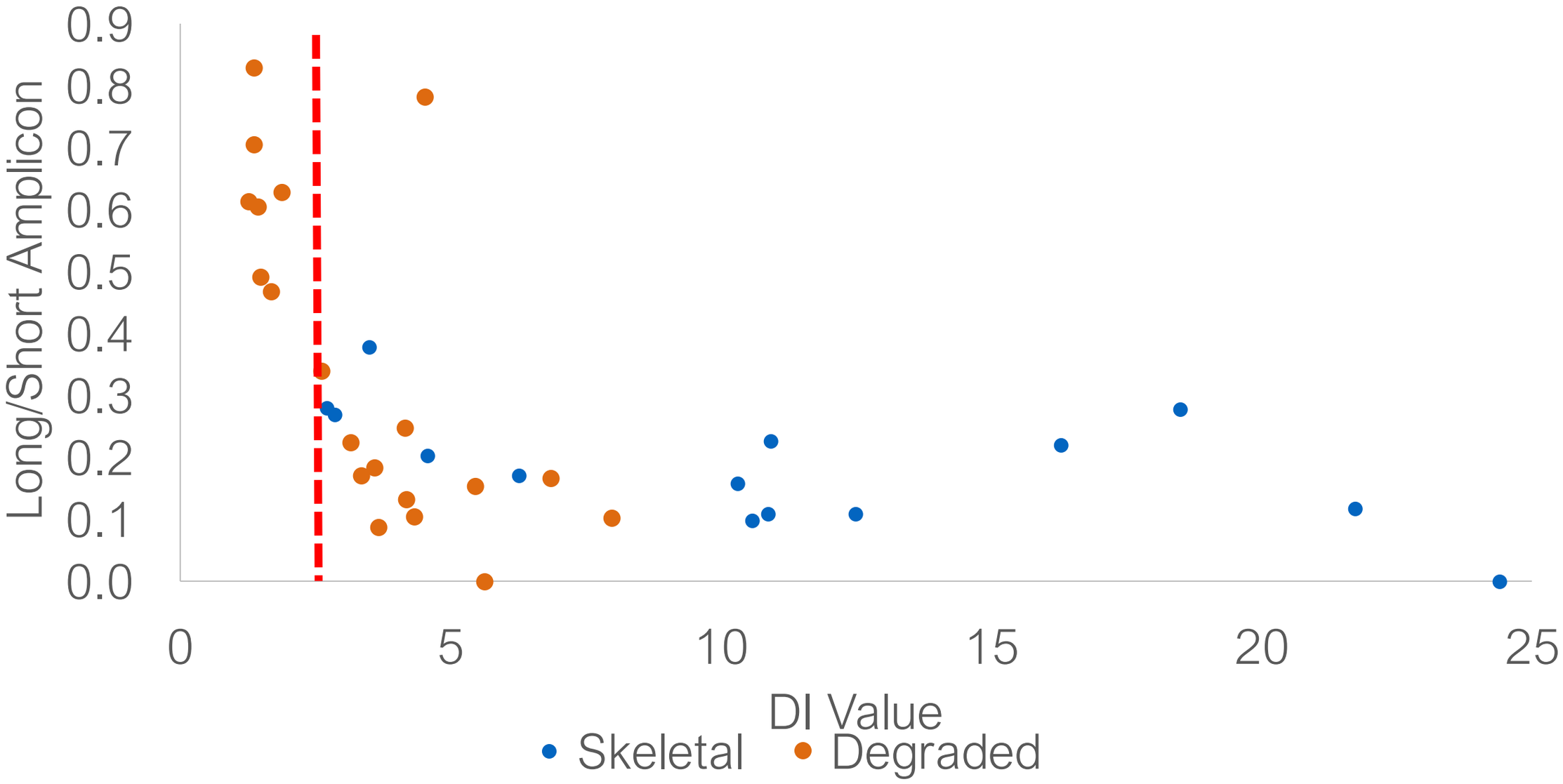
Degradation – Correlation between qPCR & STR "DI"

Flag Threshold = 10 (human and male)
We see marked DNA degradation much earlier



Degradation – Correlation between qPCR & STR "DI"

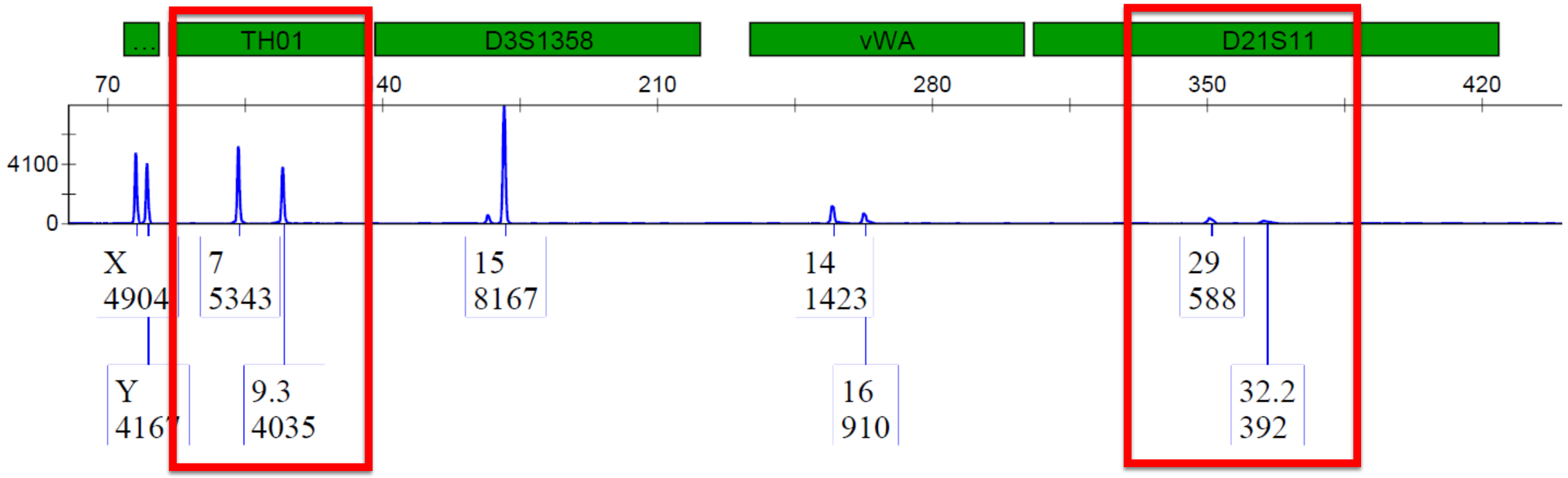
Observable degradation in STR profiles with DI value of >2.5



Decomposed Tissue

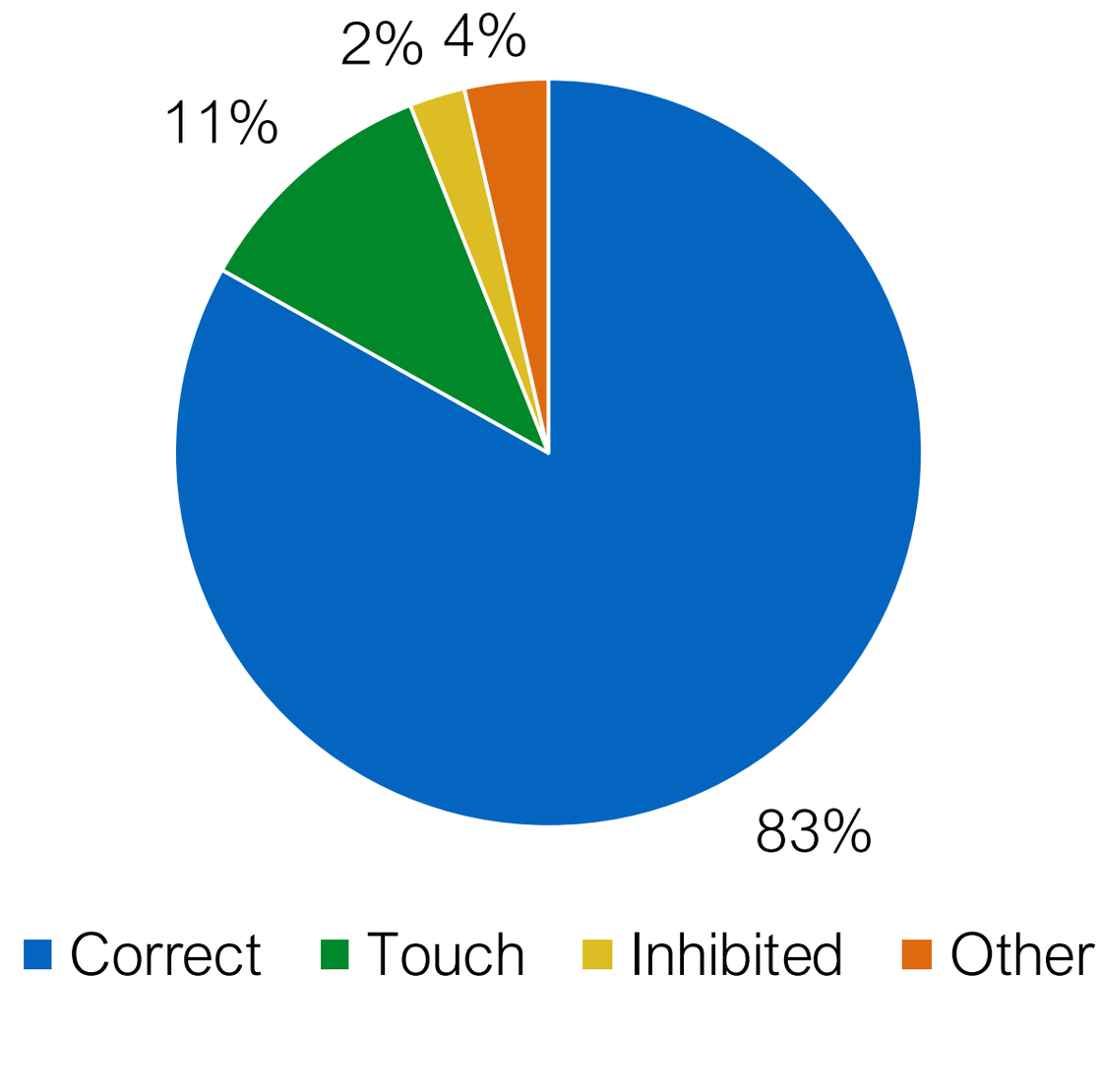
Input DNA – 0.8 ng

Quality Assessment							
Mixture Index	Mixture Threshold	Degradation Index	Degradation Threshold	Male Degradation Index	Male Degradation Threshold	Inhibition Index	Inhibition Threshold
2.65	Possible Mixture	7.98	Possible Degradation	18.18	Possible Degradation	-0.15	Below Threshold



(5343+4035) / (588+392) = 10

DI of 2.5 predicting degradation



(N=83)

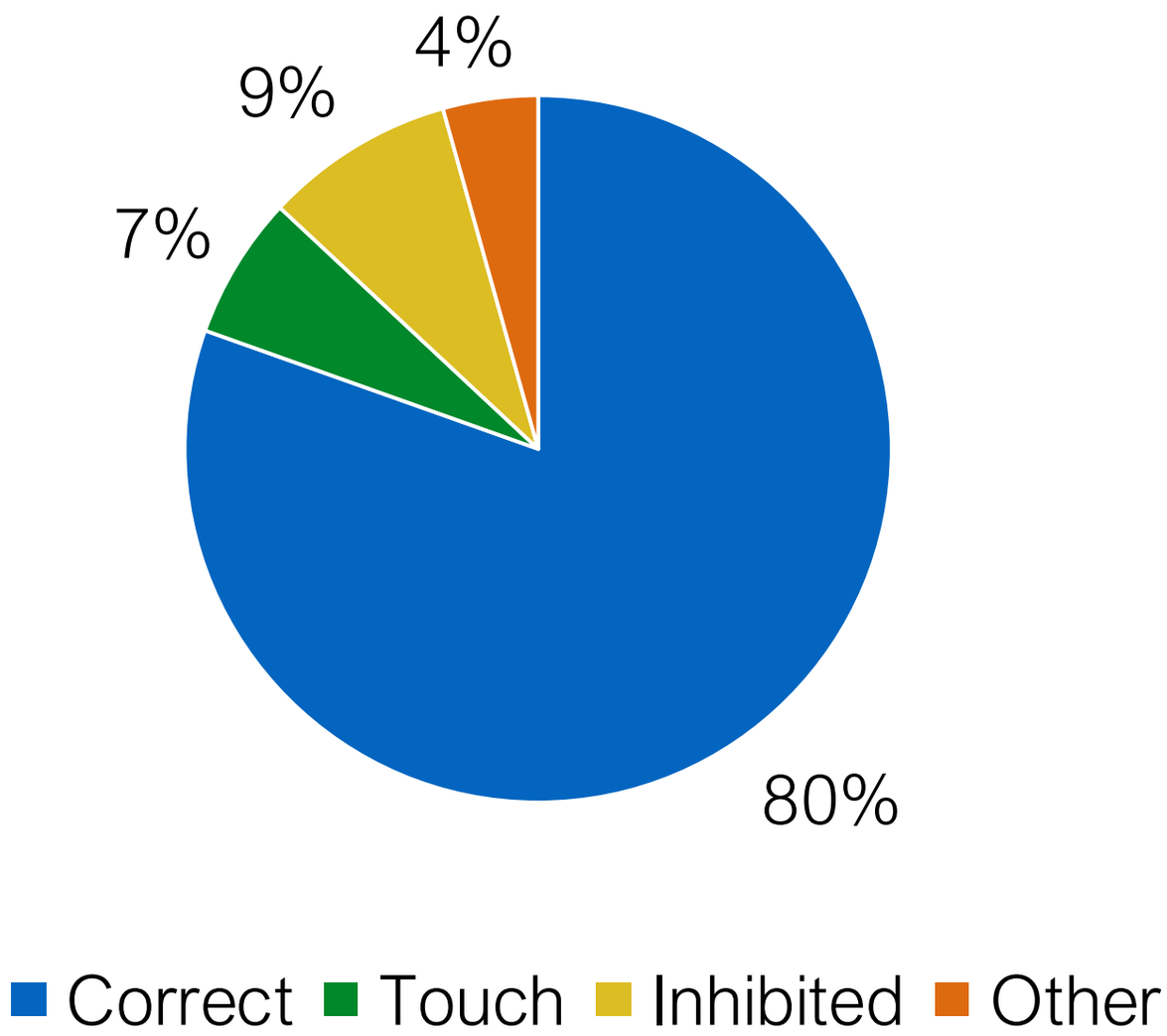
RGQ DI threshold of ≥2.5, correctly predicted degradation in 83% of the samples tested

*Degradation defined as D21/TH01 <0.5

However, allelic DO due to degradation was not observed until DI >10-20

Was male degradation predicted?

Single Source Male Profiles



RGQ Male DI threshold of ≥2.5, correctly predicted degradation in 80% of the samples tested

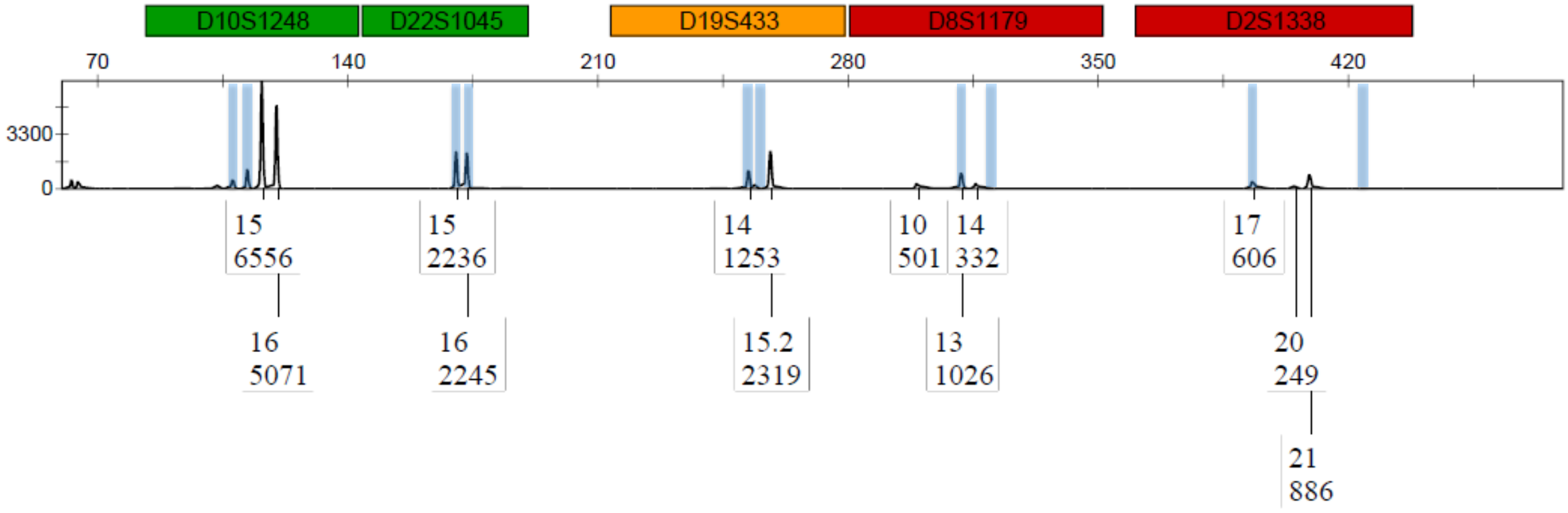
(N=46)

*Degradation defined as D21/TH01 <0.5

Male Degradation - RGQ

Touch sample

- Flags:
  - Mixture, Male Degradation
- Item handled by female; male contamination (confirmed)

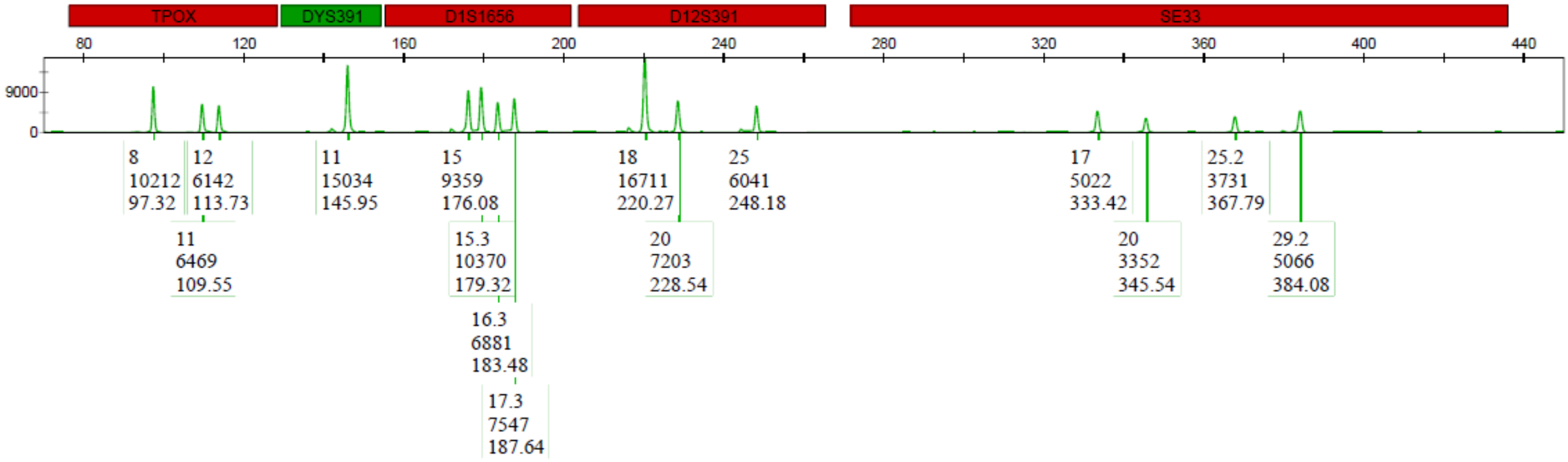


Human (ng/µL)	Human Deg.	Human DI	Male	Male Deg.	Male DI	IPC ΔCT	Mixture Index	Flags
0.024	0.003	7.78	0.002	--	N/A	-0.03	15.78	Mixture, Male Deg.

Mixture not flagged

Differential Extraction

- Sperm fraction
- No flags

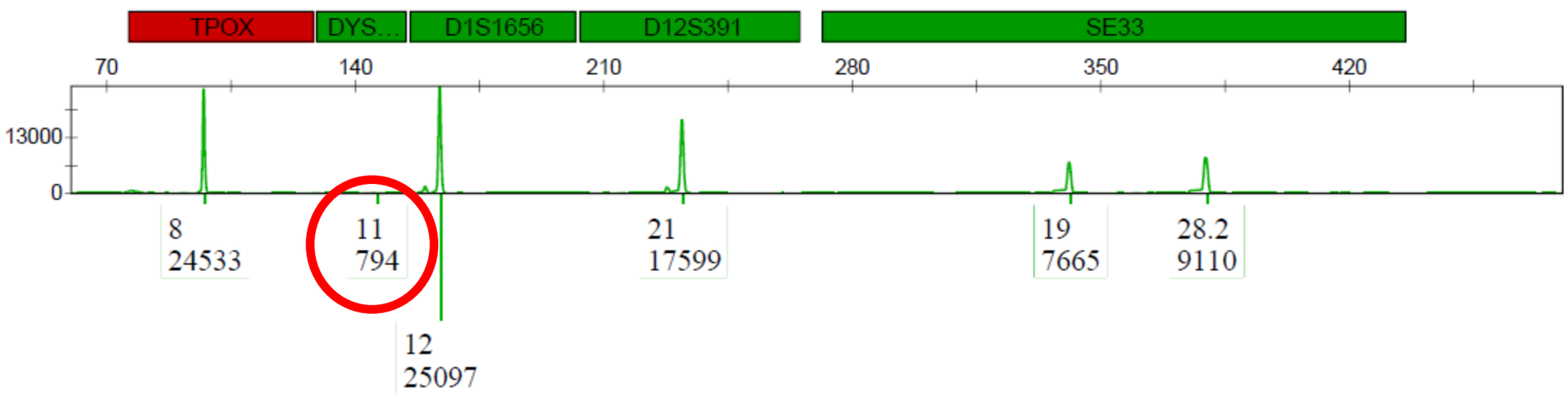


Human (ng/µL)	Human Deg.	Human DI	Male	Male Deg.	Male DI	IPC ΔCT	Mixture Index	Flags
0.09	0.08	1.14	0.06	0.07	0.75	2.56	1.58	--

Male Degradation - RGQ

Low level mixture

- Flags:
  - Mixture, Male Degradation
- Male diluted out in STR profile
- Potential male degradation
- Y-STRs

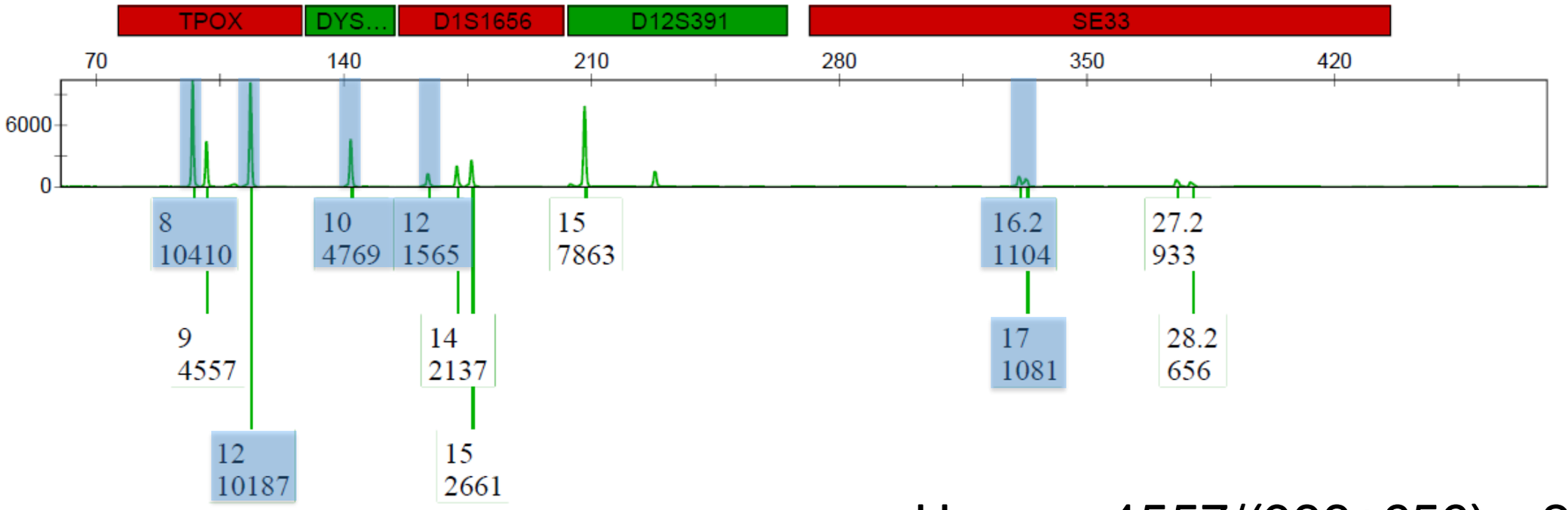


Human (ng/µL)	Human Deg.	Human DI	Male	Male Deg.	Male DI	IPC ΔCT	Flags
4.48	3.12	1.44	0.57	0.04	15.98	-0.10	Mixture, Male Deg.

Male Degradation - RGQ

Degraded mixture

- Flags:
  - Mixture, Human Degradation, Male Degradation



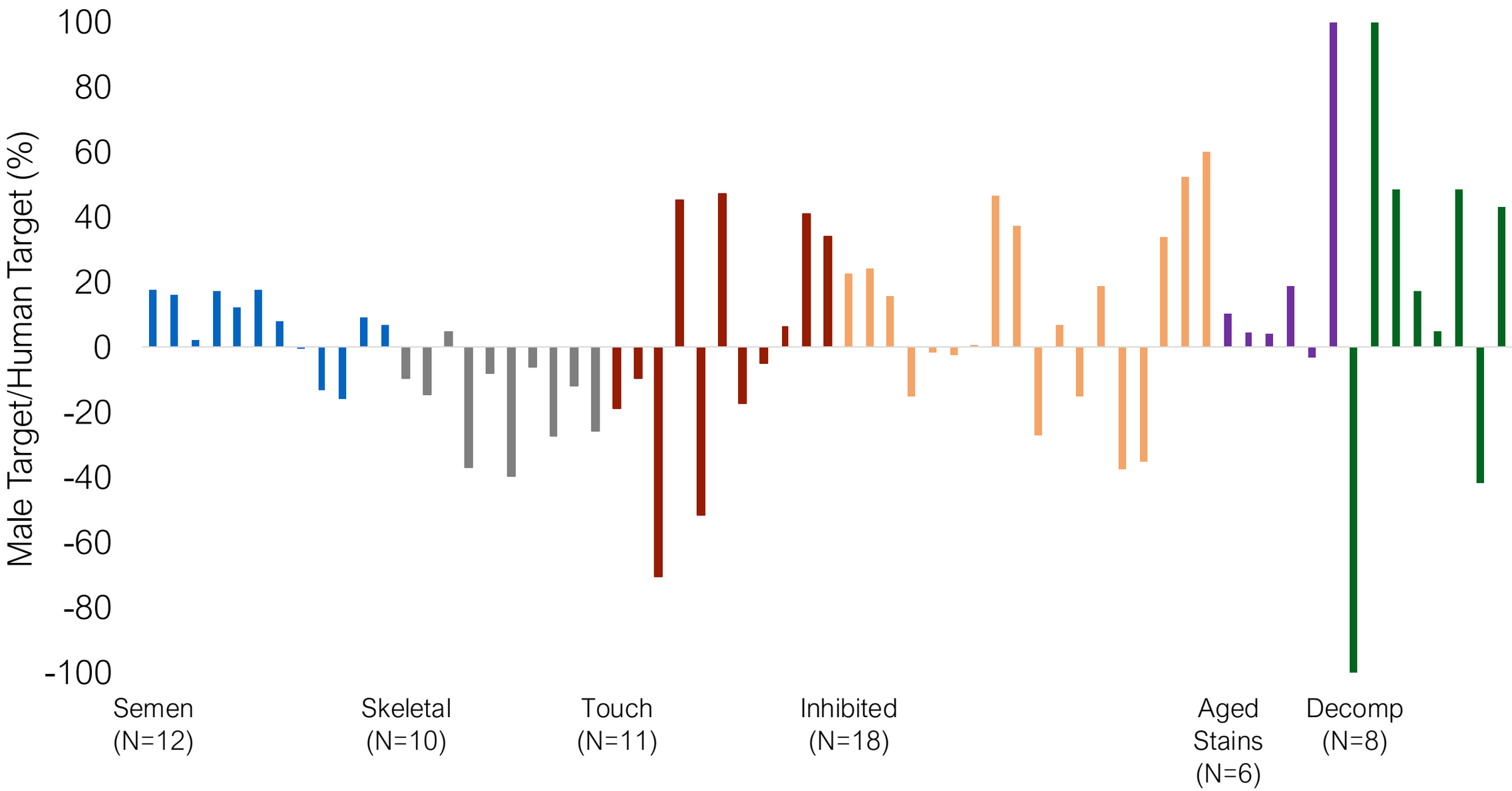
Human: 4557/(933+656) = 2.9

Male: (10410+10187)/(1104+1081) = 9.4

Human (ng/µL)	Human Deg.	Human DI	Male	Male Deg.	Male DI	IPC ΔCT	Mixture Index	Flags
1.18	0.23	5.16	0.44	0.03	12.75	2.56	2.67	Mixture, Male and Human Deg.

Male Target - RGQ

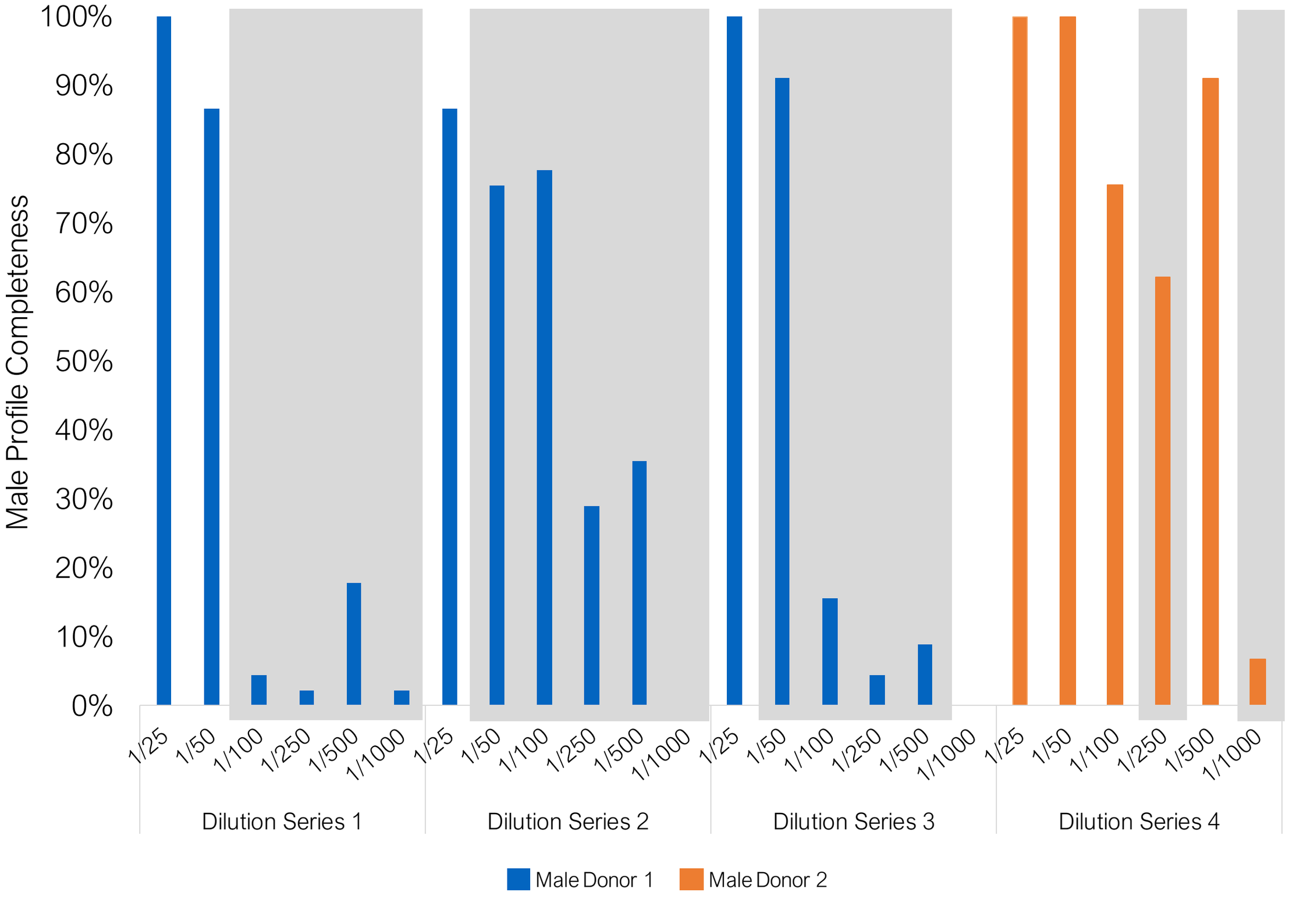
- Single source male profiles and % difference between the human and male targets



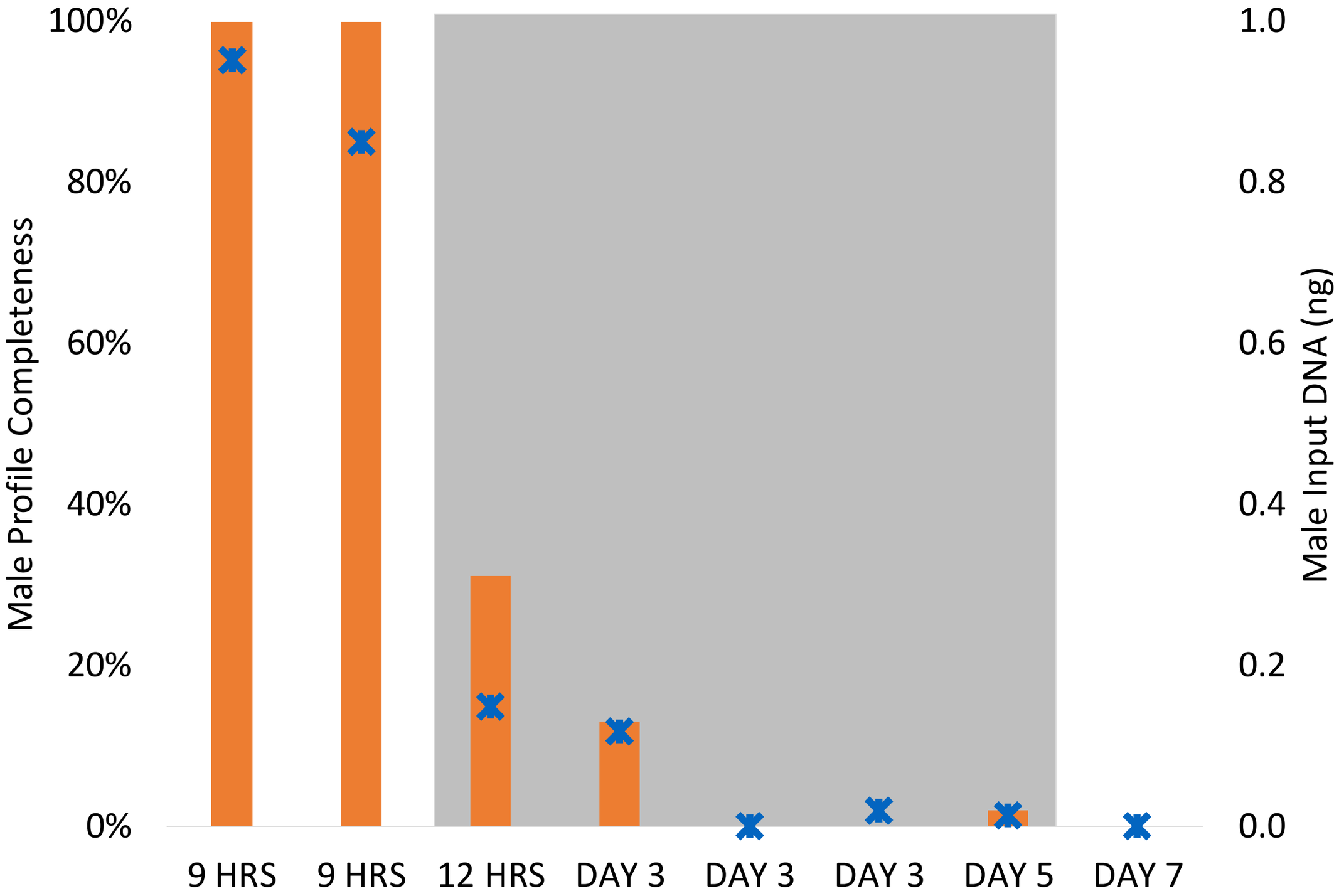
Mock Sexual Assault (N=32)

- Semen dilution series (N=24)
  - 20µL diluted semen (1/25 to 1/1000) added to half a vaginal swab
  - 4 different vaginal swab sources; 2 semen sources
- Post-coital samples (N=8)
  - Collected at various time periods (9 hrs to 7 days)
- Manually separated – purified with EZ1xL

Mock Sexual Assault (N=24)



Post-Coital (N=8)



- Male DI < 2; no degradation

Summary

- Quality flags in the *Investigator® Quantiplex® Pro RGQ accurately* predicted STR quality in majority of samples (~ 80%)

- The QS markers in the *Investigator 24plex QS & GO! Kits* correctly confirmed sample/STR quality in almost all samples tested
  - 99.9% reference samples, and 91.7% casework samples
  - More complete profiles were obtained when samples were reworked based on the QS markers in conjunction with STR quality compared to the EPG alone

- In-house testing to define user thresholds/guides for DIs
  - Human and male degradation was accurately predicted ~ 80% of the time (reduced to threshold of 2.5)

- Quality Sensors enabled analysts to more accurately detect sample quality and triage samples for more efficient rework strategies and avoid unnecessary reworks

Acknowledgements

- Esiri Tasker, Dr Kyleen Elwick
- **QIAGEN:** Keith Elliot, Bryan Davis, Richard Newton, Dr Meredith Turnbough