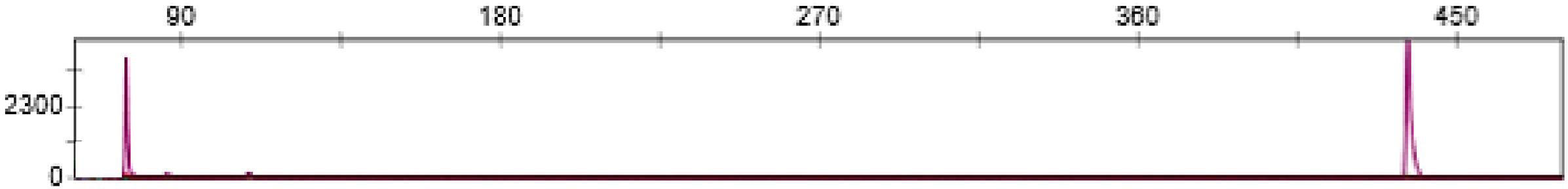
7th QIAGEN Investigator Forum, May 2018;
San Antonio, TX USA

In-field Collection of DNA from Decomposing Remains for More Direct Analysis:

Experiences with Investigator Quality Sensors



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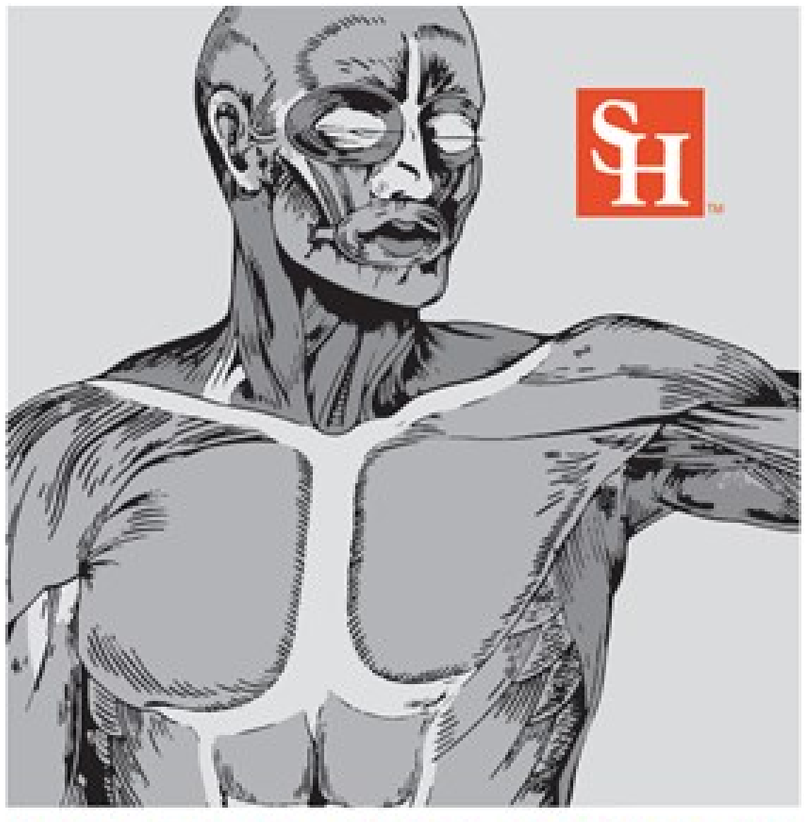
Huntsville, TX USA

ROLE OF FUNDING

This project was partially funded by a Graduate Research Fellowship Award #2015-R2-CX-0029 (National Institute of Justice, Office of Justice Programs, U.S. Department of Justice).

NIJ National Institute of Justice

Please do NOT take any photographs of slides depicting human remains



APPLIED ANATOMICAL RESEARCH CENTER

SAM HOUSTON STATE UNIVERSITY

Disaster Victim Identification (DVI)

- Mass fatalities
- Remote locations
- High temperatures & humidity
- Limited/lack of facilities
- Loss of electricity
- Inability to perform DNA analysis for weeks/month
- Skeletal samples

2004 SE Asian Tsunami >220,000 deceased

Goals

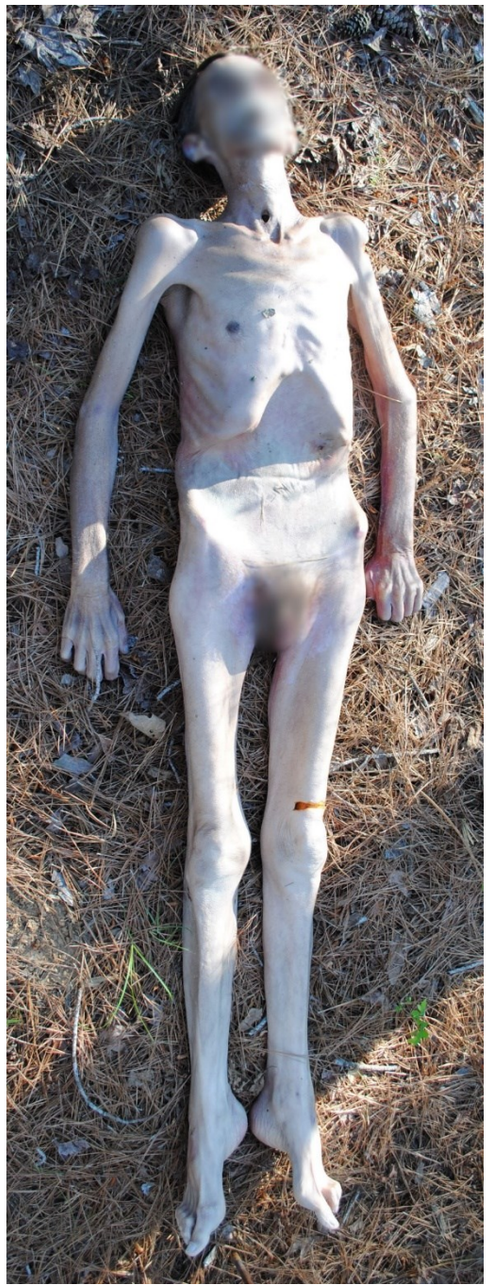
- Fast, simple DNA collection (in field)
  - First 2 weeks (or prior to skeletonization)
- RT storage of samples
- Faster sample processing for STR typing

APPLIED ANATOMICAL RESEARCH CENTER
SAM HOUSTON STATE UNIVERSITY

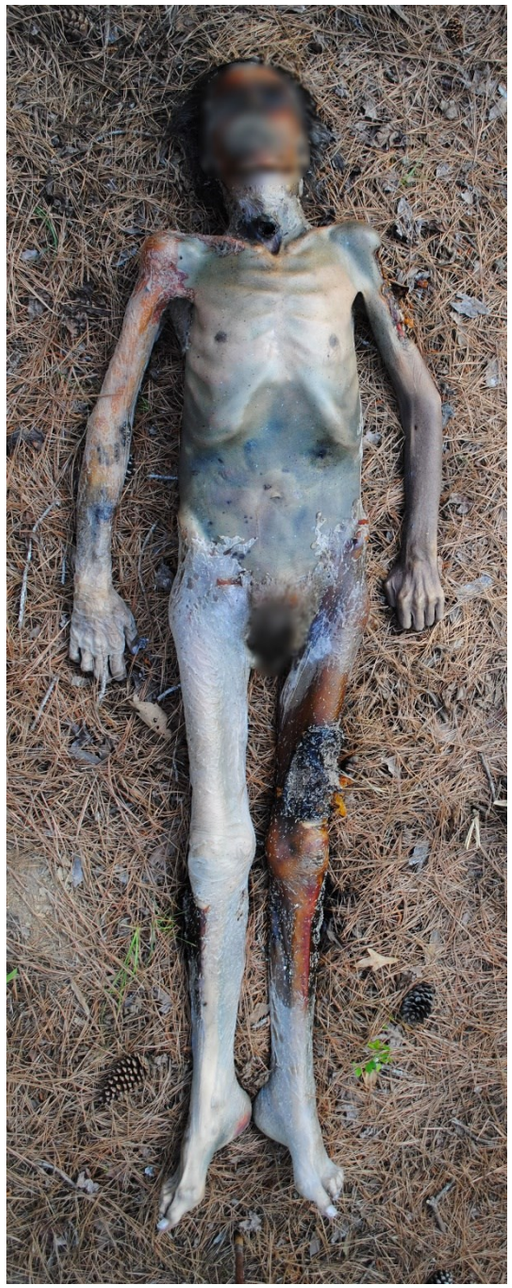
Days of Decomposition

April – Average Temp 15 – 25ºC

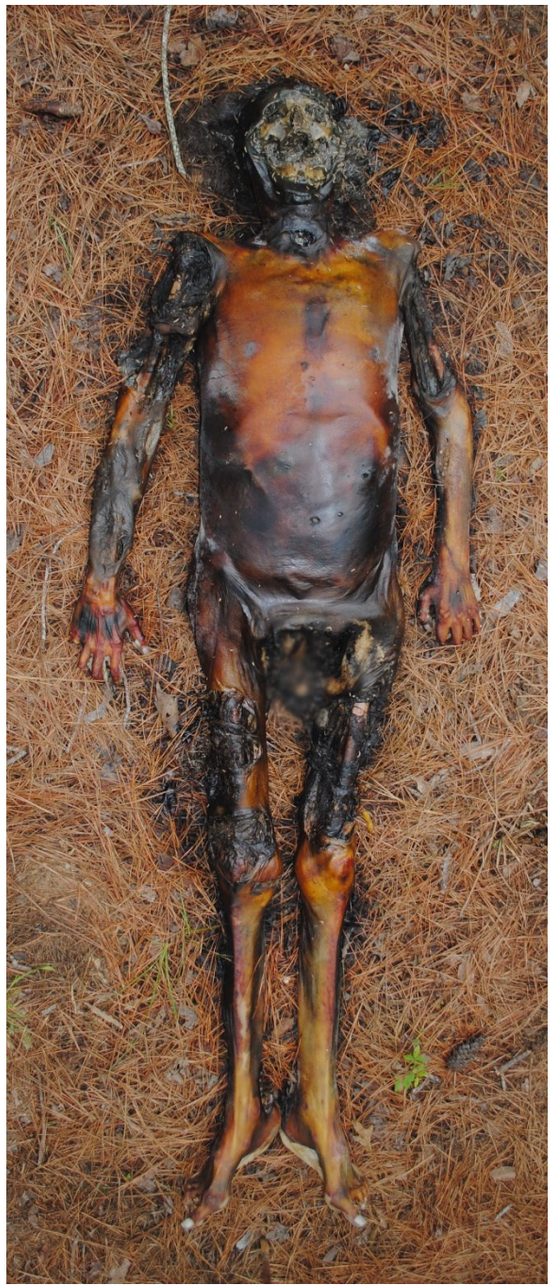
Day 1



Day 7



Day 14



Investigator 24plex QS – 100% Success

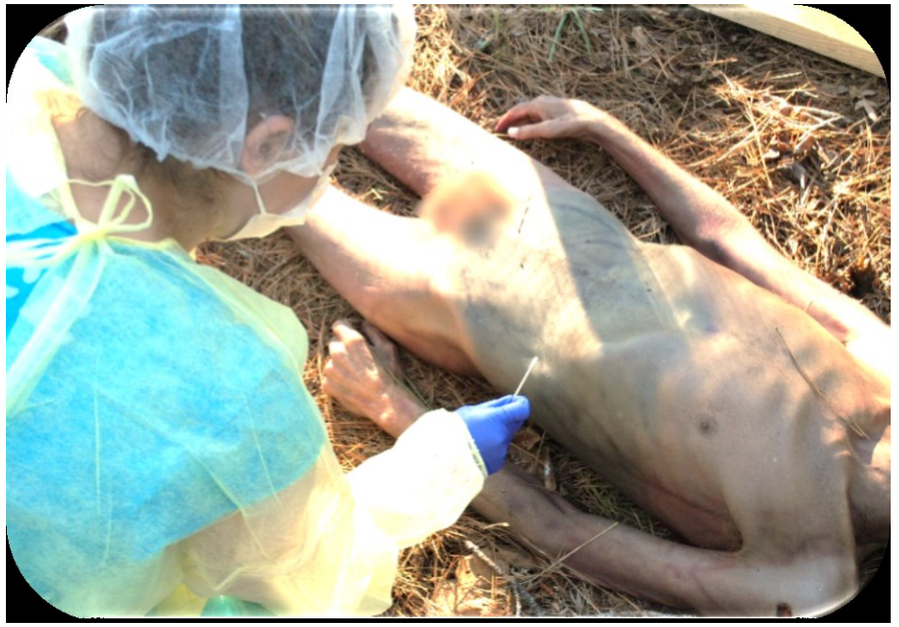
STR analysis fails when samples are stored at RT with no form of preservation

Sampling Approach - Swabs

Whatman® EasiCollect™ Foam

Cotton

Nylon



Skin

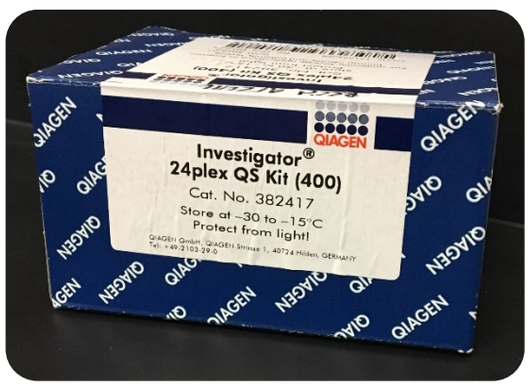
Muscle

Processed immediately or stored for 1 month

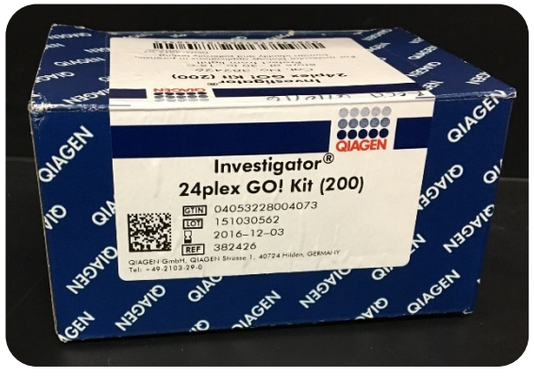
Traditional

QIAamp® DNA Investigator kit

Investigator Quantiplex® Pro



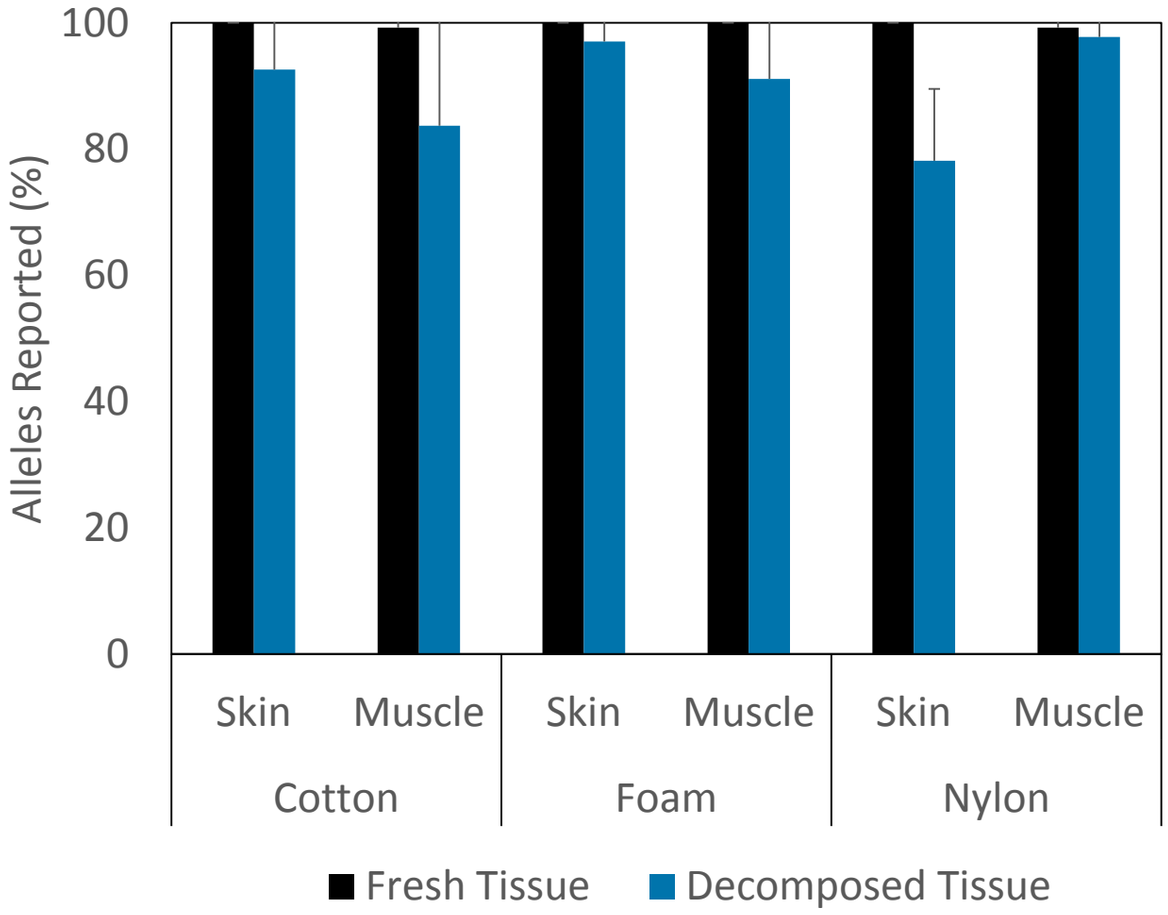
Direct



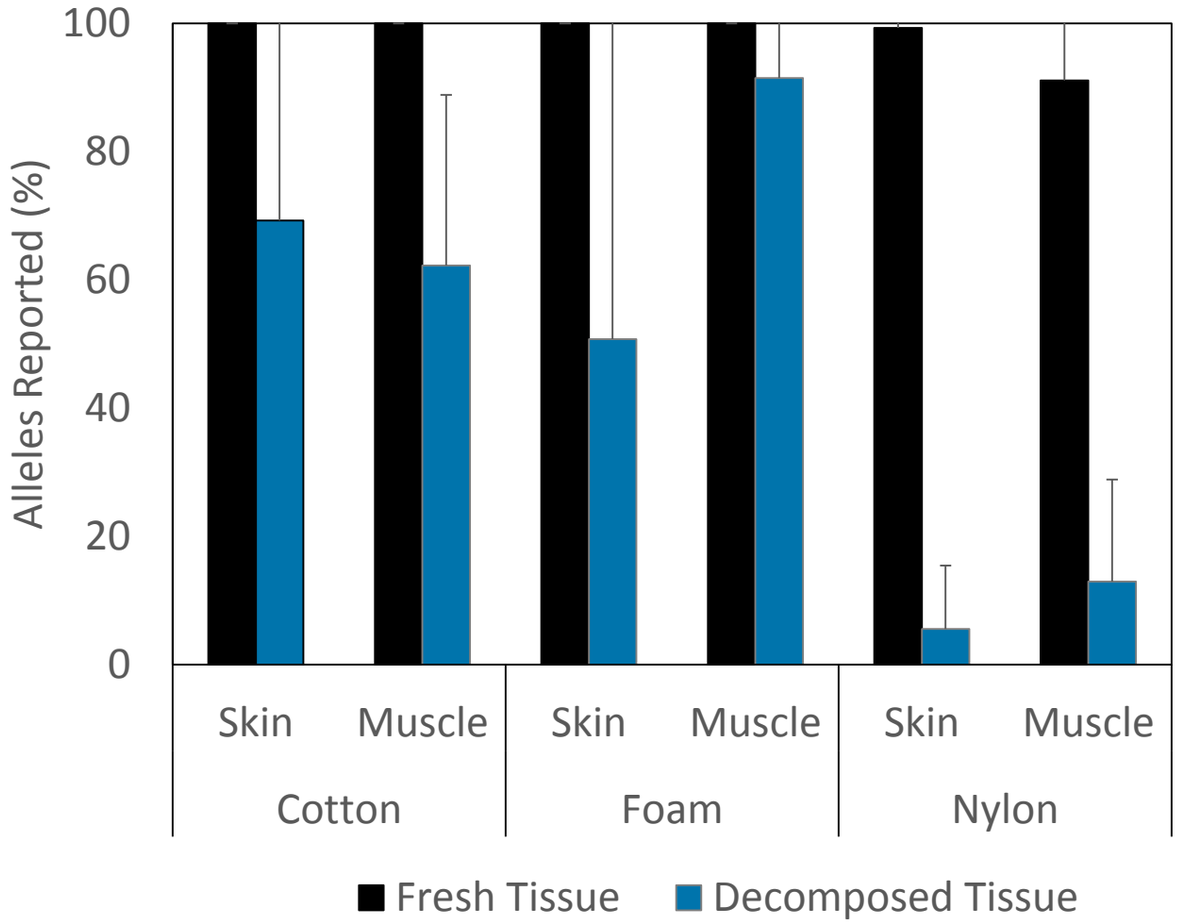
Investigator® STR GO! Lysis Buffer

STR Results - Swabs

Traditional Analysis – QS



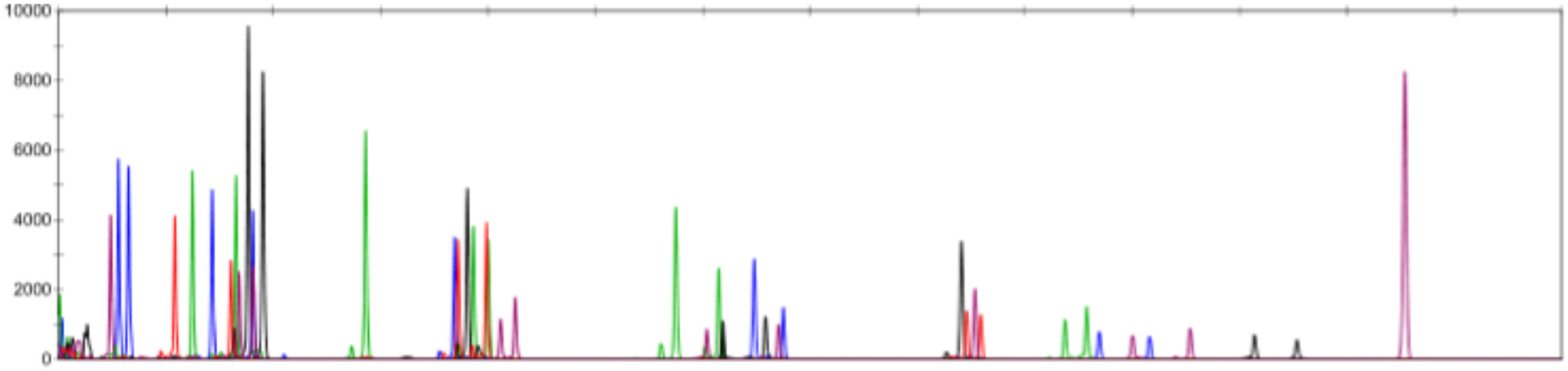
Direct PCR – GO!



- Full profiles with fresh tissues (skin & muscle)
- Cotton swabs & the foam heads from Whatman® EasiCollect™ Devices seemed to generate the most consistent results regardless of the tissue type sampled
- Direct PCR of swabs less successful from decomposed remains; Nylon – PCR inhibition, dilution required

Inhibition – Nylon Swabs

QS

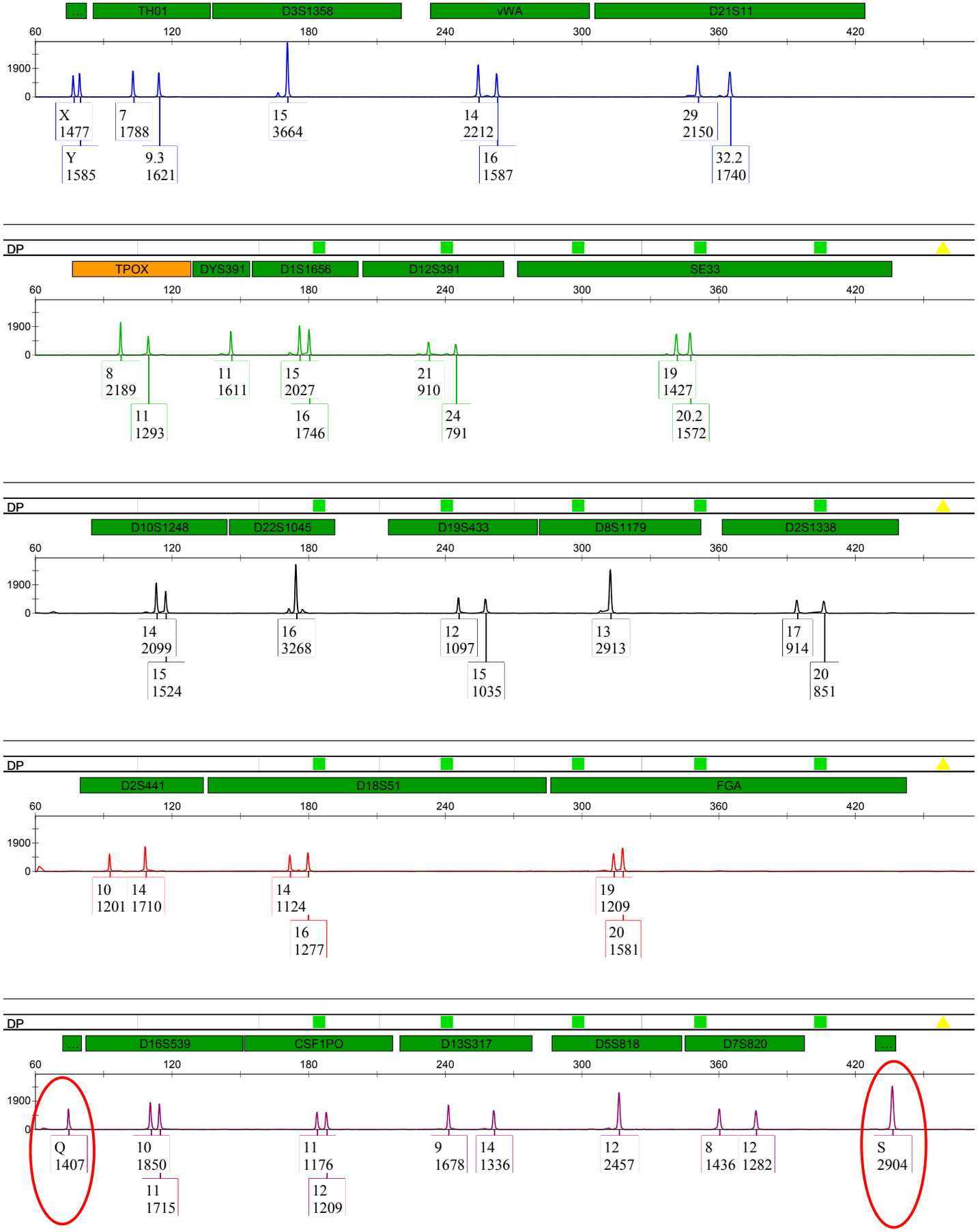


Small Amplicon	0.0487
Large Amplicon	0.0000
Degradation I	n/a
IPC	-0.31
Allele %	100

GO!

Small Amplicon	0.0084
Large Amplicon	0.0000
Degradation I	n/a
IPC	-13.87
Allele %	0

- Other direct STR kits?
- No DNA, PCR failure, and/or complete inhibition?

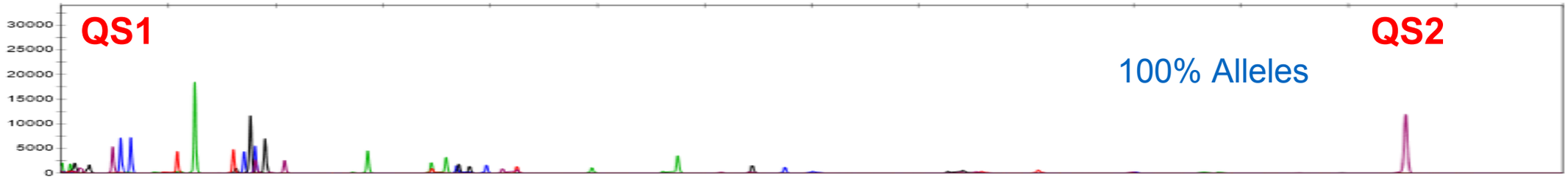

...
TH01
D3S1358
vWA
D21S11
60
120
180
240
300
360
420
1900
0
X 1477
Y 1585
7 1788
9.3 1621
15 3664
14 2212
16 1587
29 2150
32.2 1740
DP
TPOX
DYS391
D1S1656
D12S391
SE33
8 2189
11 1293
11 1611
15 2027
16 1746
21 910
24 791
19 1427
20.2 1572
DP
D10S1248
D22S1045
D19S433
D8S1179
D2S1338
14 2099
15 1524
16 3268
12 1097
15 1035
13 2913
17 914
20 851
DP
D2S441
D18S51
FGA
10 1201
14 1710
14 1124
16 1277
19 1209
20 1581
DP
...
D16S539
CSF1PO
D13S317
D5S818
D7S820
...
Q 1407
10 1850
11 1715
11 1176
12 1209
9 1678
14 1336
12 2457
8 1436
12 1282
S 2904

Dilution

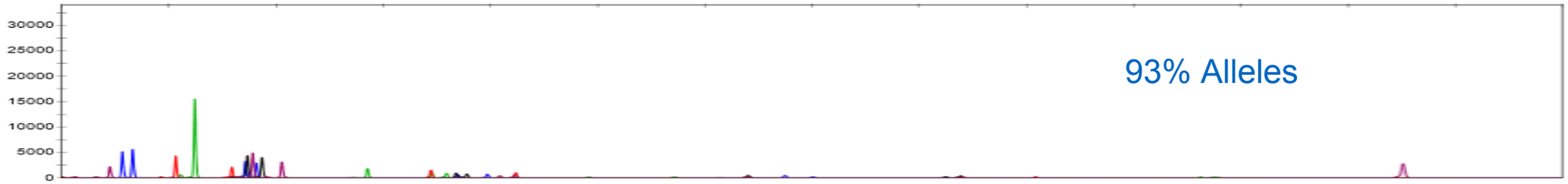
DECOMPOSED

Cotton

QS

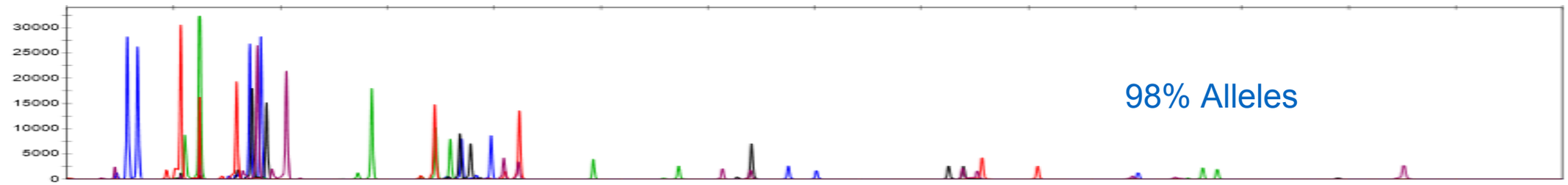


GO!

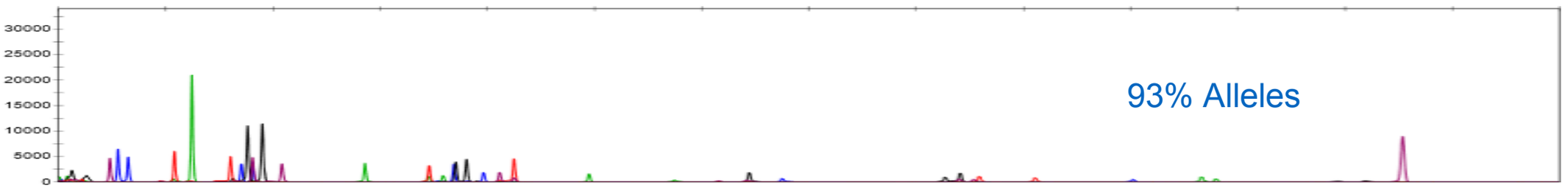


Foam

QS

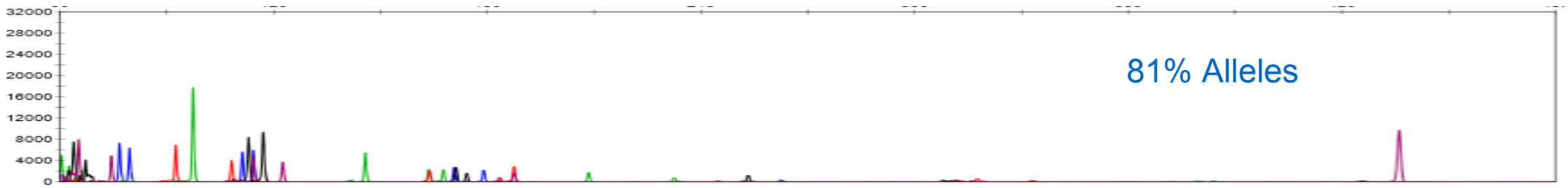


GO!

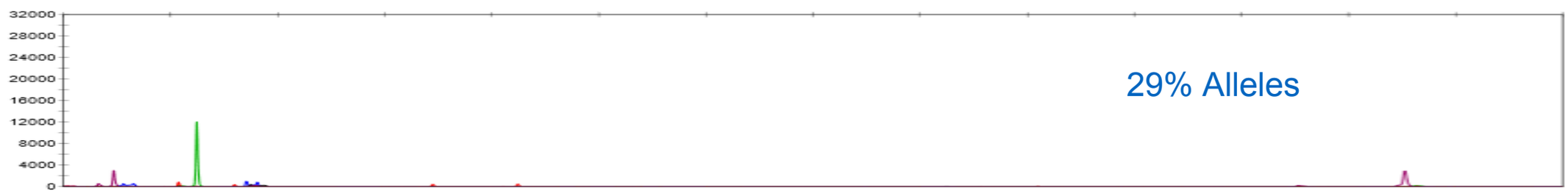


Nylon

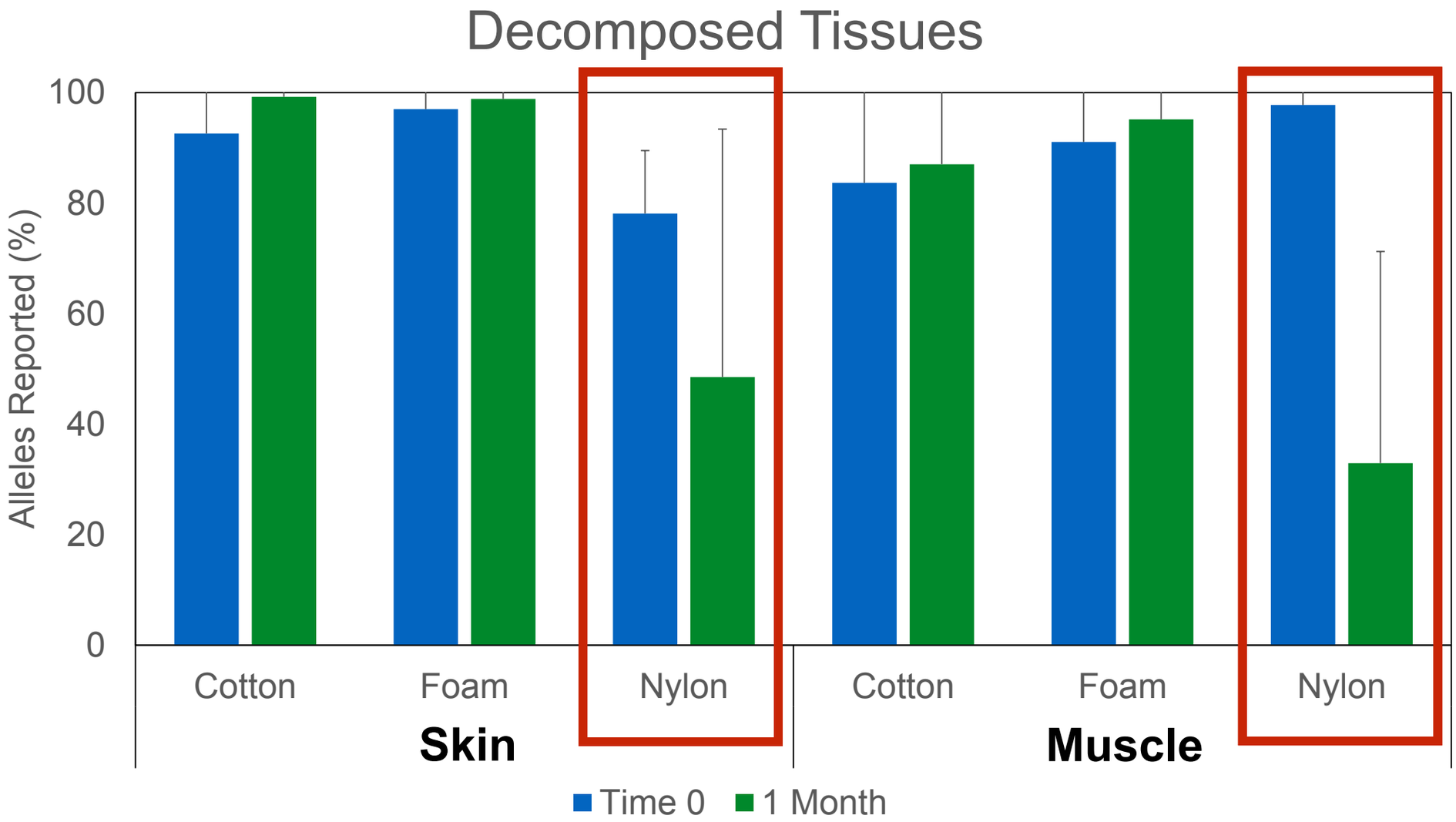
QS



GO!



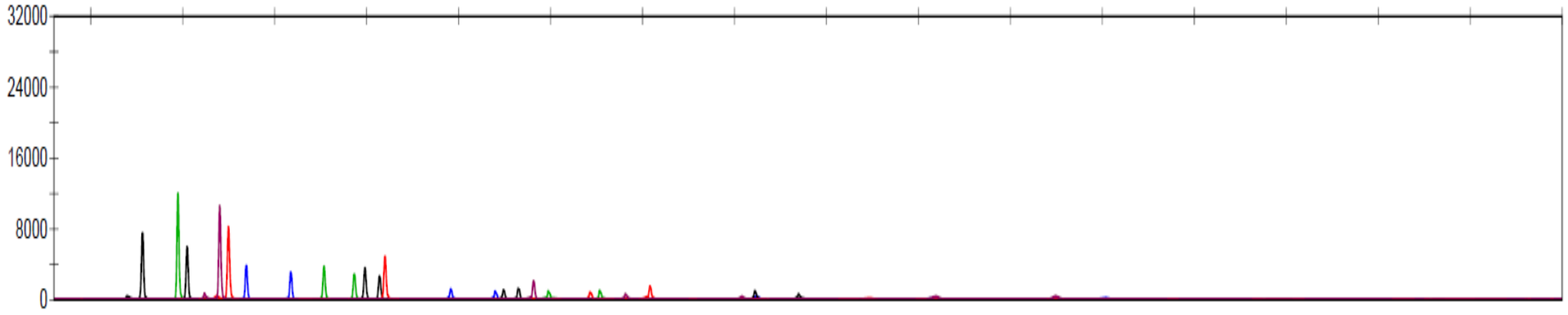
Preservation – 1 month



- No significant difference between skin & muscle tissue
- Significantly fewer alleles were recovered from samples collected & stored on Nylon 4N6FLOQSwabs for 1 month

Examples of Reworked Samples

Quantifiler Trio & GlobalFiler

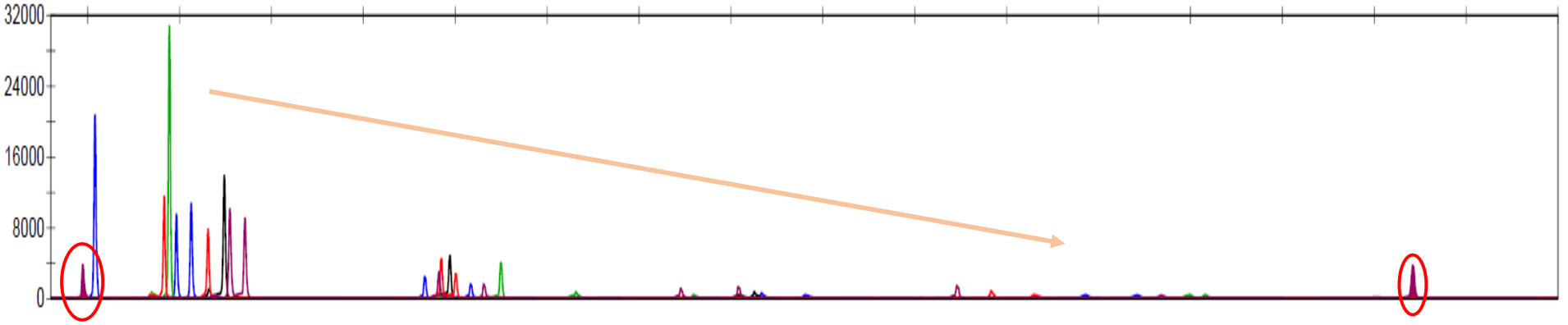


ST: 0.18
LT: 0.054
DI: 3.26
IPC: -0.57
98% Alleles

- Quantifiler Trio indicates low to moderate degradation, no inhibition

- Quantiplex Pro indicates high degradation and no inhibition

Quantiplex Pro & 24plex QS

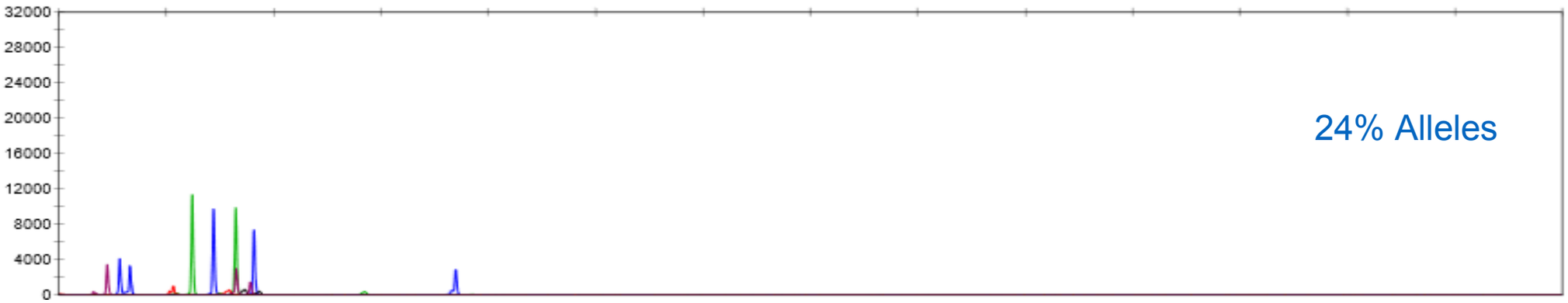


ST: 0.24
LT: 0.011
DI: 21.6
IPC: -0.34
98% Alleles

Confirmed by QS

Examples of Reworked Samples

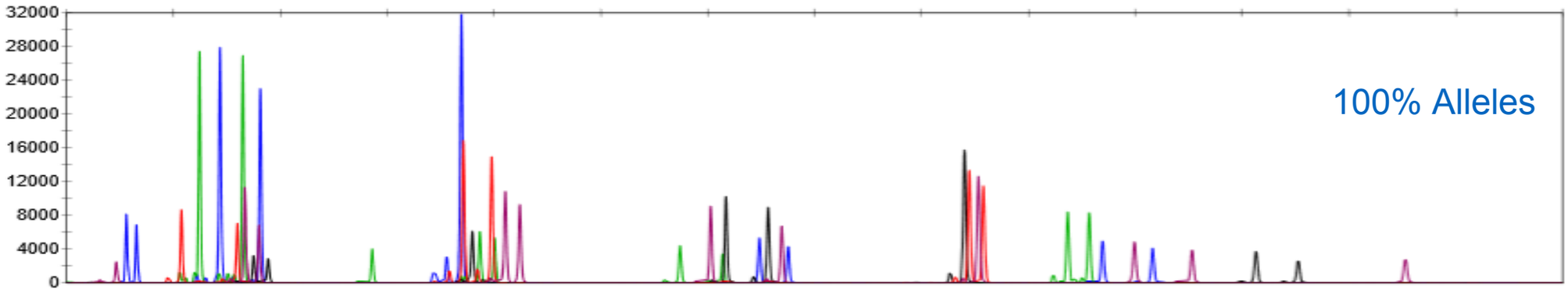
GO!
Partial Profile



24% Alleles

Other direct PCR kits - Extreme degradation, inhibition?

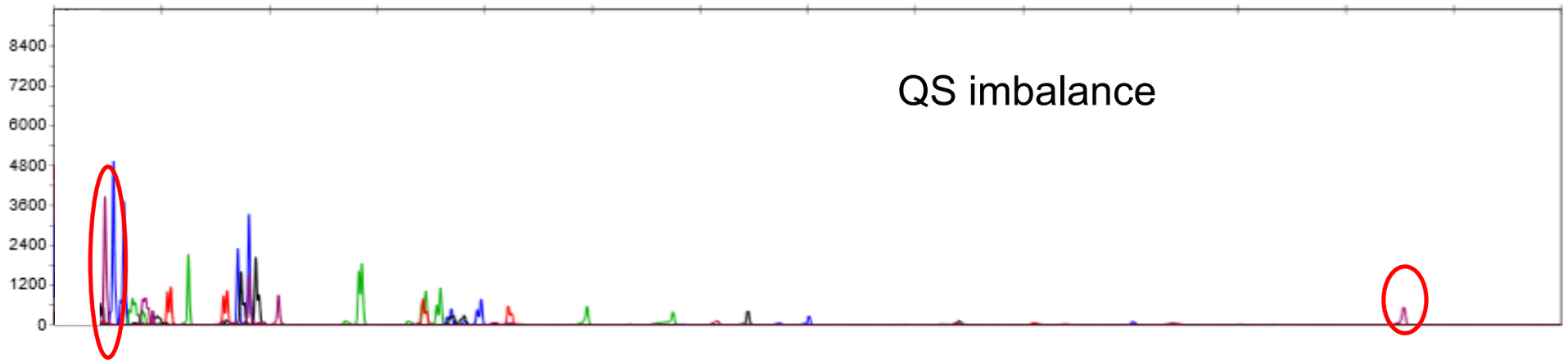
Dilution



100% Alleles

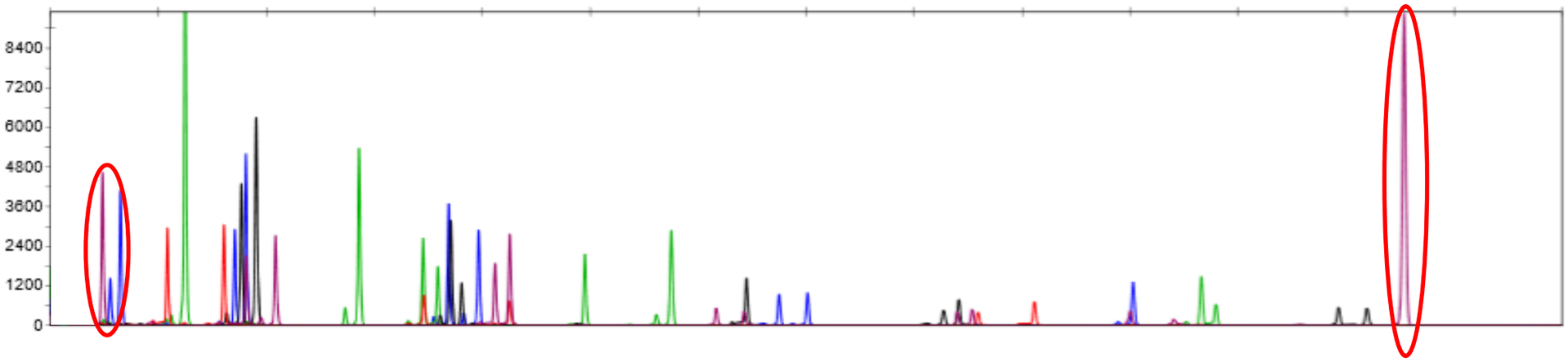
Examples of Reworked Samples

GO!
Partial Profile



69% Alleles

Dilution



100% Alleles

Sampling Approach - Biopsy

Modified **TENT Buffer**
10mM Tris, 10mM EDTA, 1M NaCl, 2% Tween 20

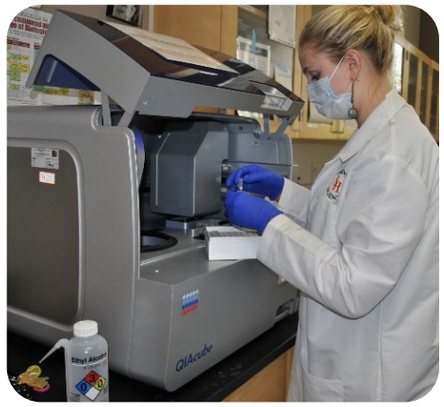
(DNA leaches into solution)

Stored for up to 1, 3, & 6 months

PDQeX (ZyGEM)

<20 min

QIAquick



<30 min

1:10 Dilution ***(no purification)***

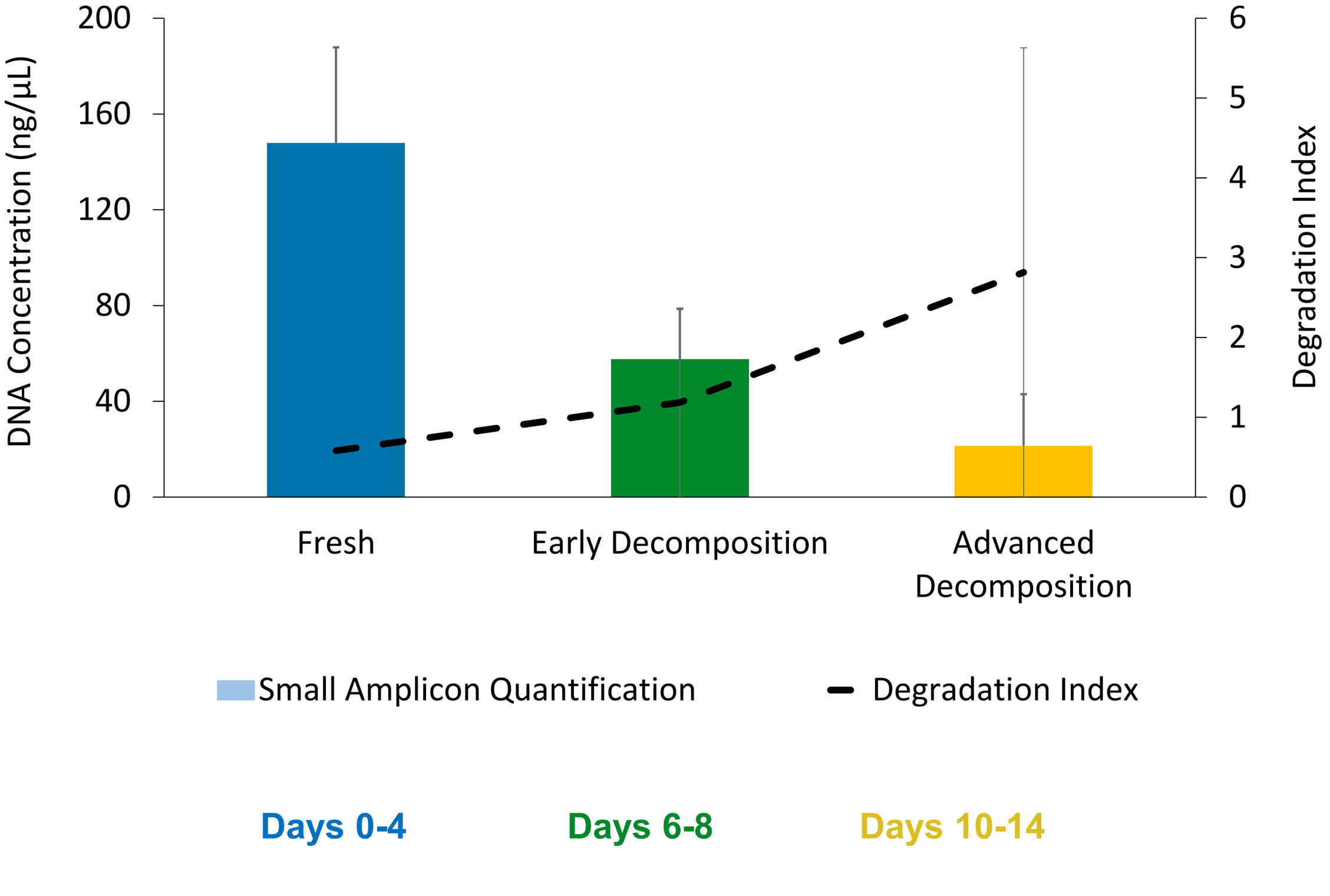
2µL

Quantifiler Trio

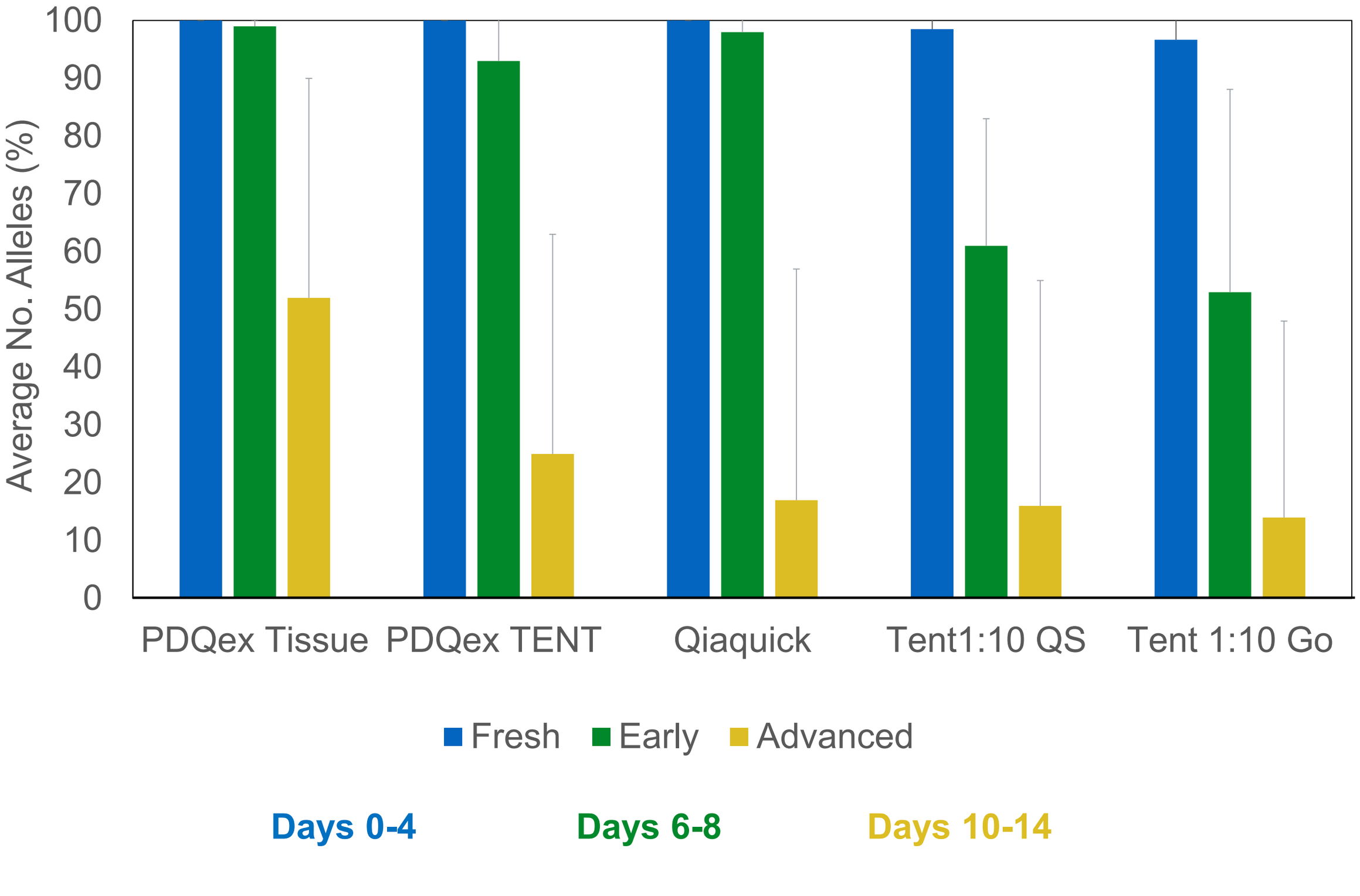
24plex QS

24plex GO!

Quantification Results

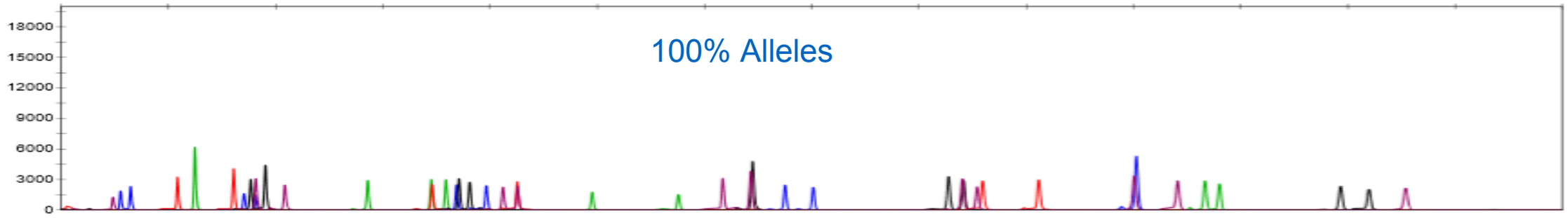


STR Results

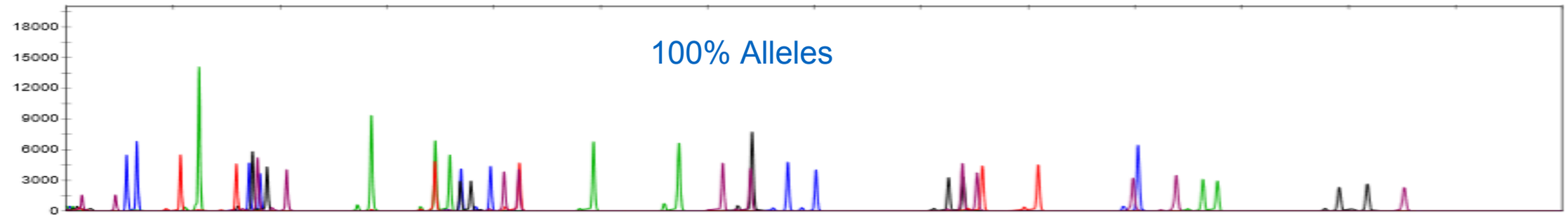

Average No. Alleles (%)
100
90
80
70
60
50
40
30
20
10
0
PDQex Tissue
PDQex TENT
Qiaquick
Tent1:10 QS
Tent 1:10 Go
Fresh
Early
Advanced
Days 0-4
Days 6-8
Days 10-14

FRESH TISSUES – 6 month storage

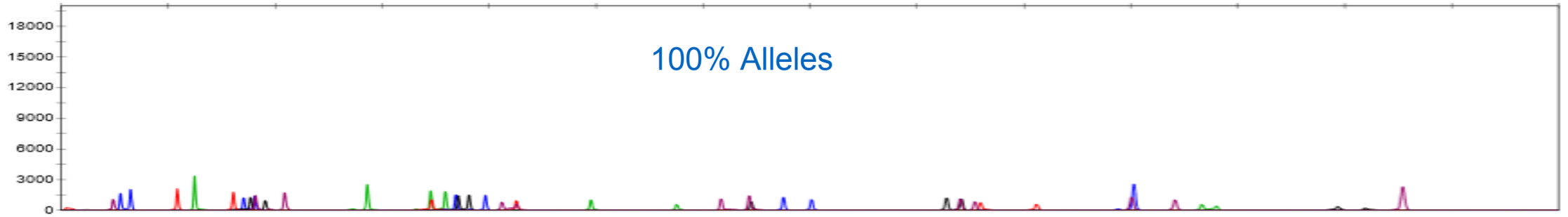
QIAquick
TENT



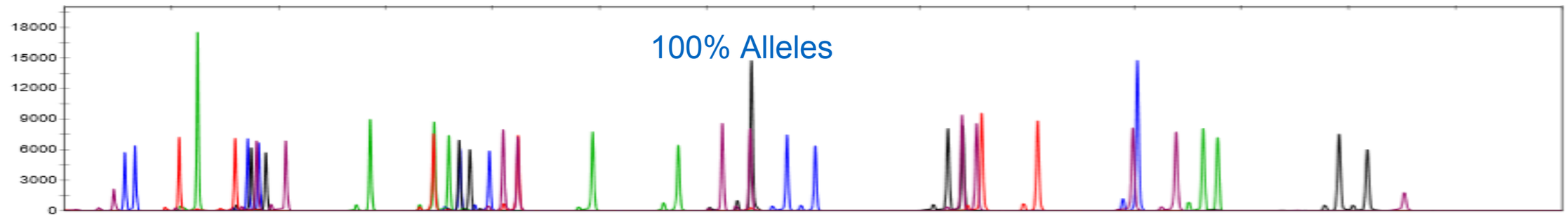
PDQeX
TENT



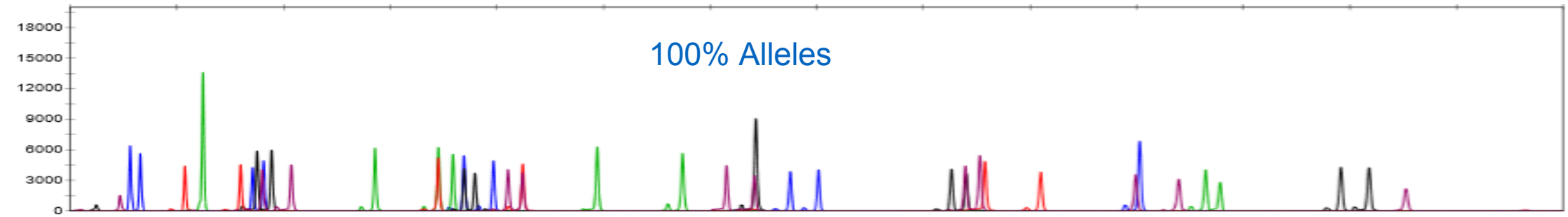
TENT 1:10
QS



TENT 1:10
GO!



PDQeX
Tissue

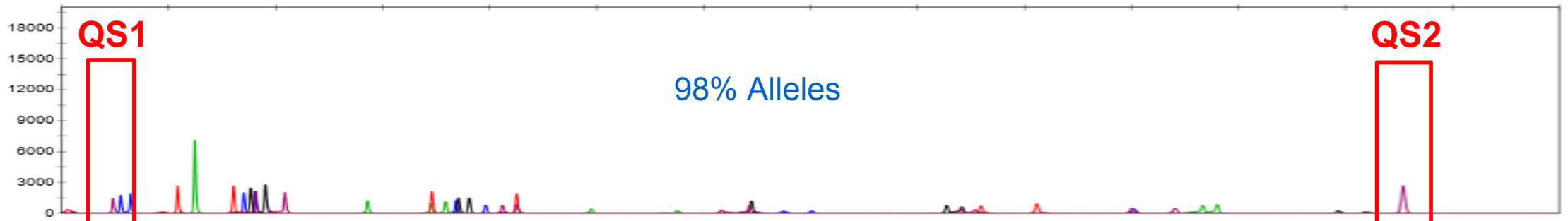


Differences in Decomposition

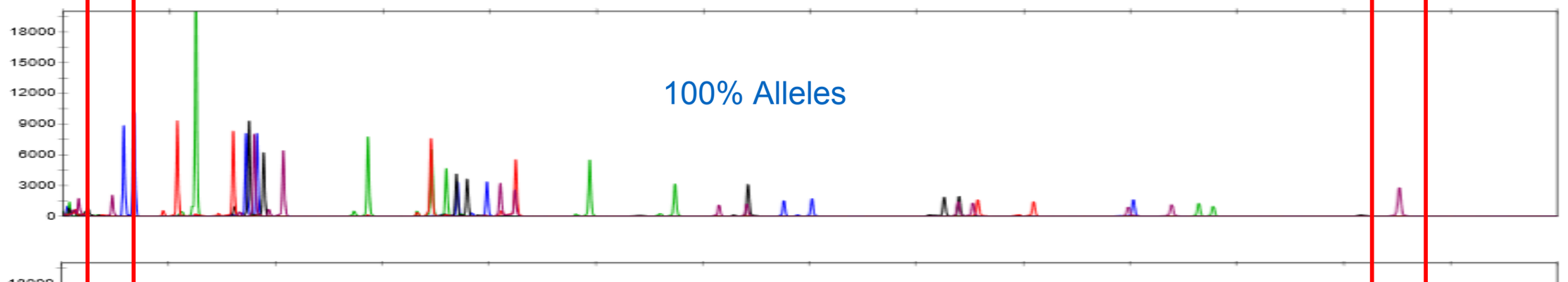
Early Decomposition (days 6 – 8)
Before/onset of Bloat

DECOMPOSED – Before/Onset of Bloat

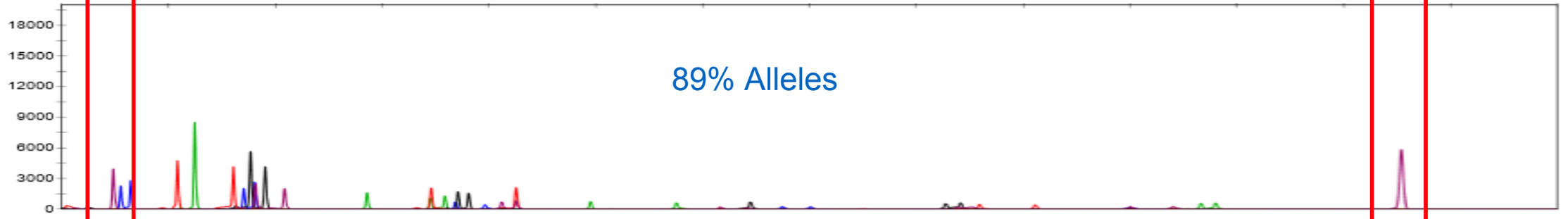
QIAquick
TENT



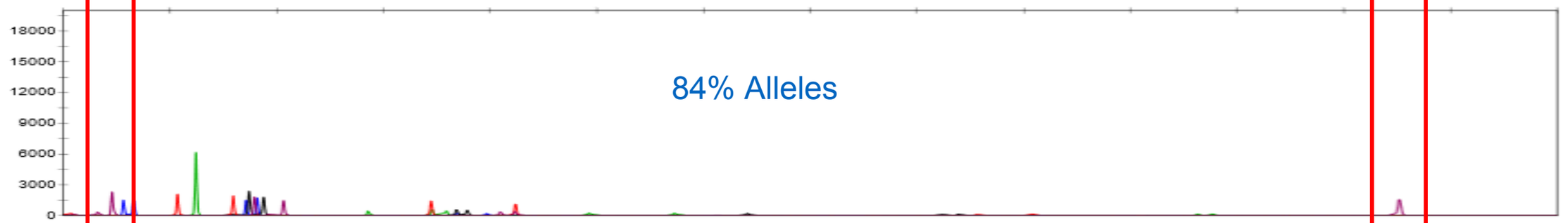
PDQeX
TENT



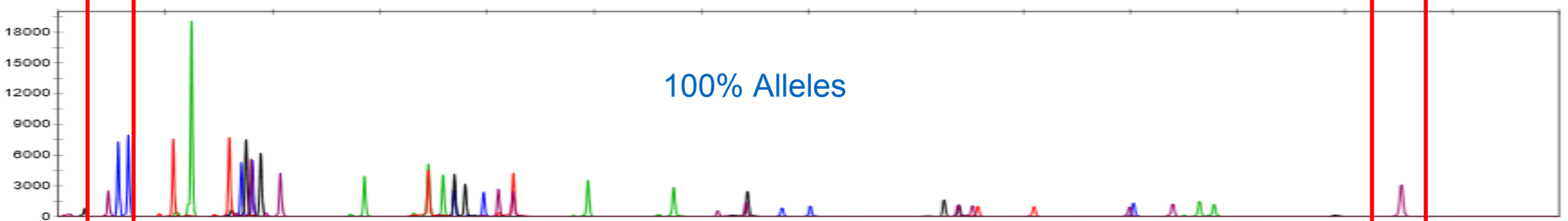
TENT 1:10
QS



TENT 1:10
GO!

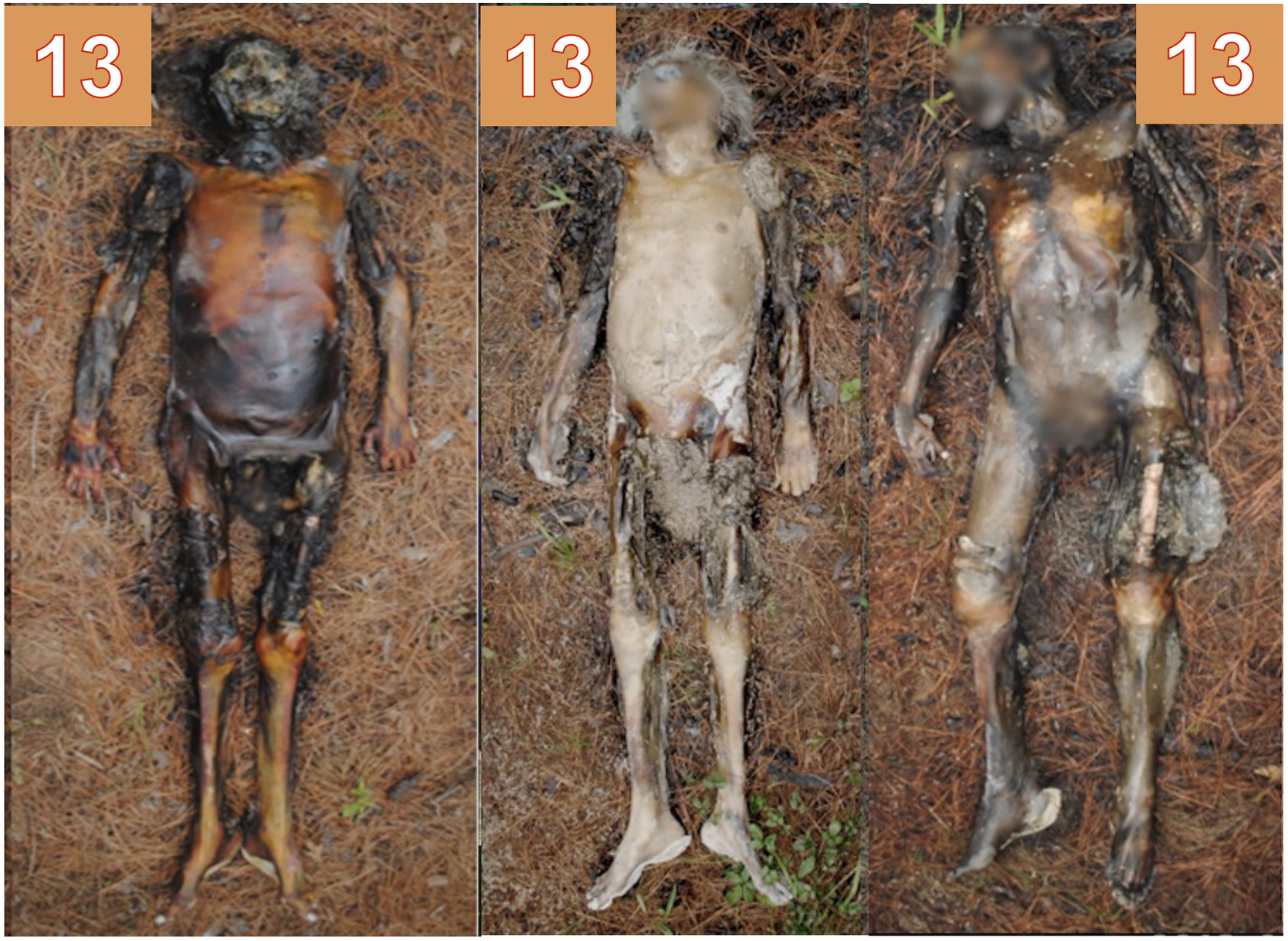


PDQeX
Tissue



Difference in Decomposition

Advanced Decomposition (days 10 - 14)



ADVANCED DECOMPOSITION

QIAquick
TENT

98% Alleles

PDQeX
TENT

99% Alleles

TENT 1:10
QS

80% Alleles

TENT 1:10
GO!

82% Alleles

PDQeX
Tissue

100% Alleles

ADVANCED DECOMPOSITION

QIAquick
TENT

60% Alleles

PDQeX
TENT

42% Alleles

TENT 1:10
QS

22% Alleles

TENT 1:10
GO!

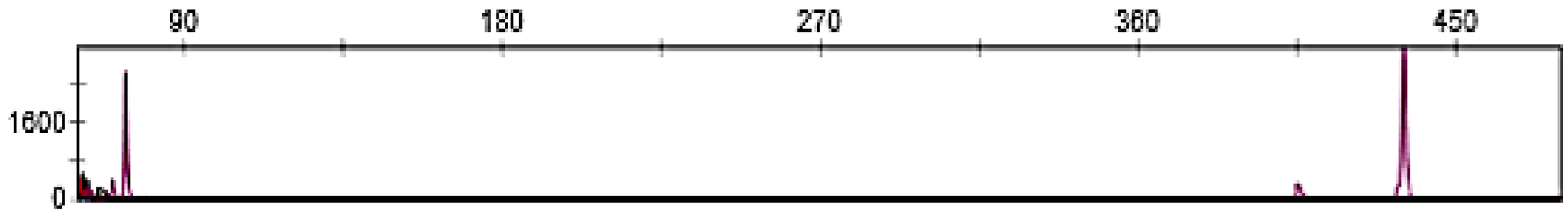
20% Alleles

PDQeX
Tissue

87% Alleles

Low/No DNA present

GO!

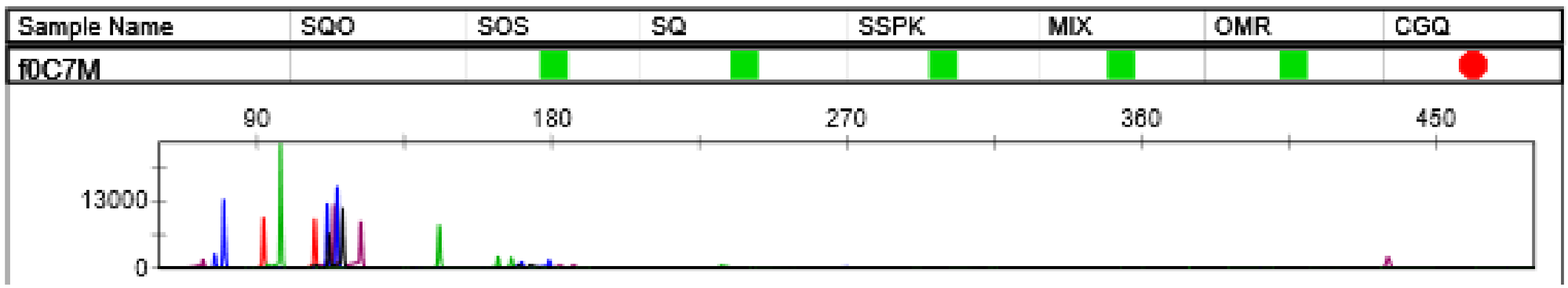


Small Amplicon	0.0001
Large Amplicon	0.0000
Degradation	N/A
IPC	0.049

No/little benefit in re-running sample in attempt to improve profile
Re-extract?

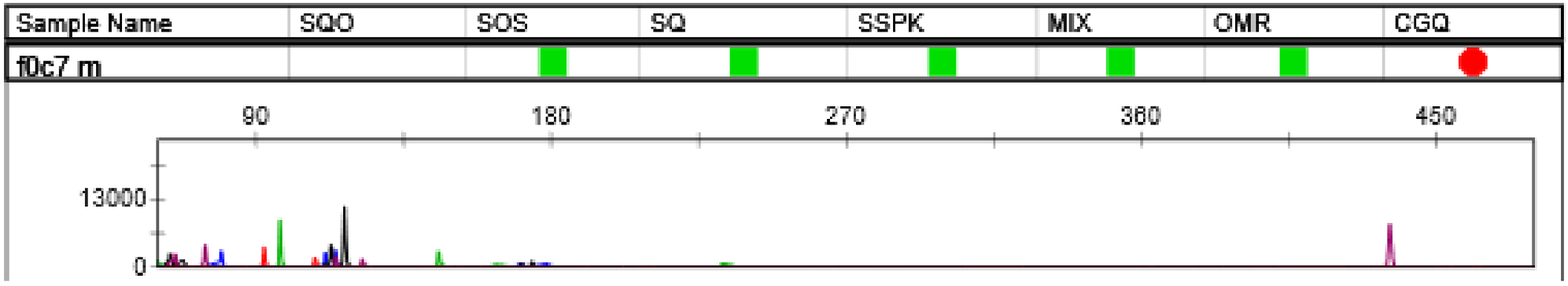
Highly Degraded

GO!



84% Alleles

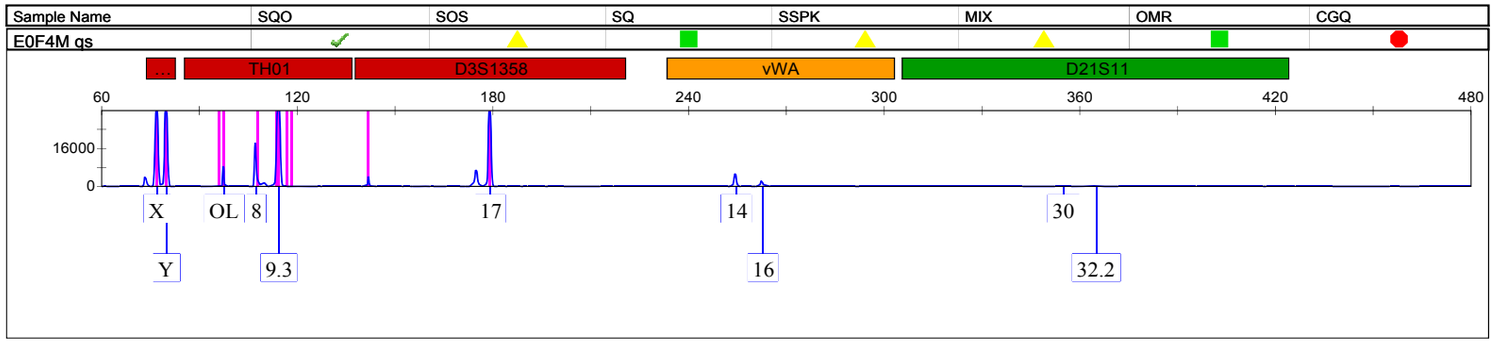
QS

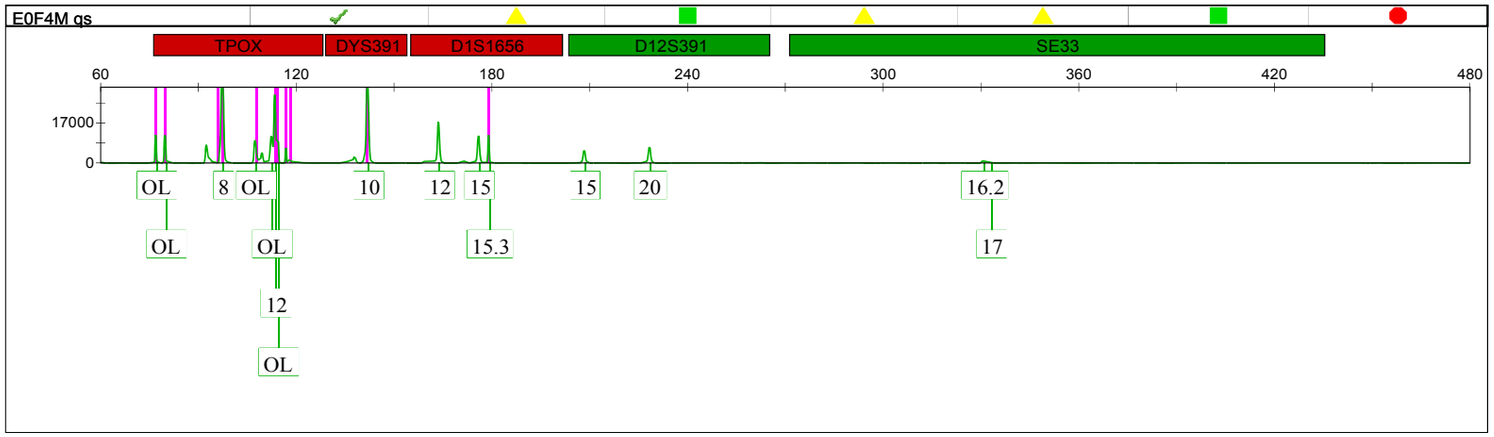


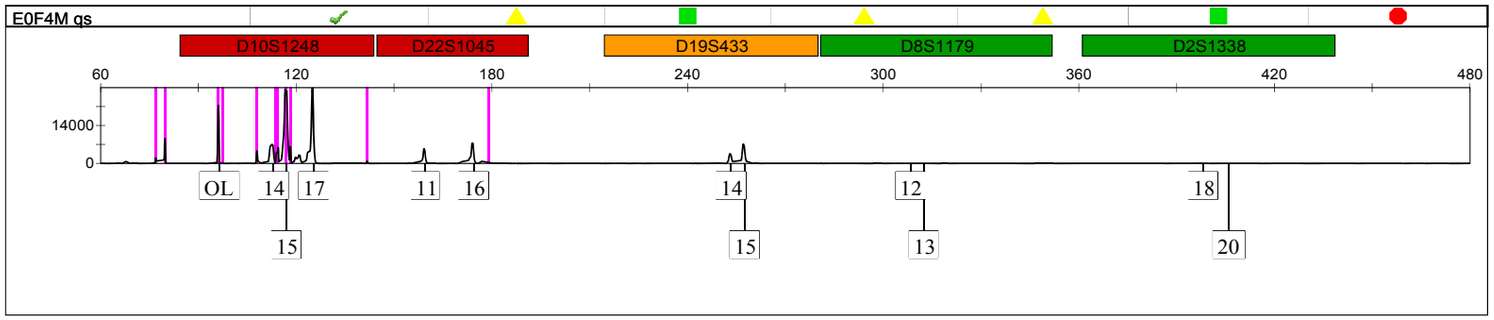
Small Amplicon	8.2841
Large Amplicon	0.0066
Degradation I	1255.17
IPC	0.12
Allele %	75.56

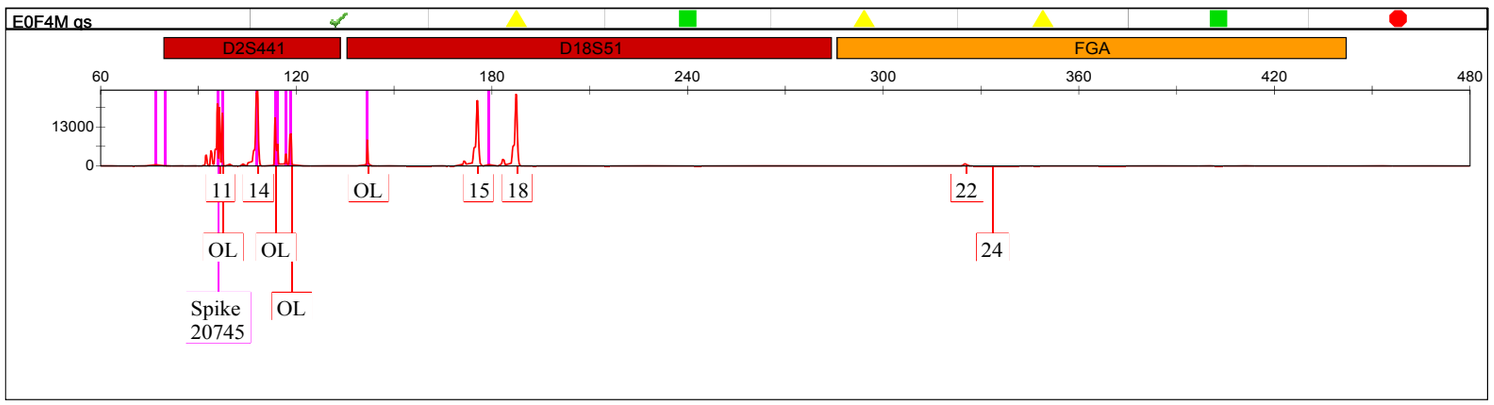
Highly degraded, No inhibition detected

Re-run based on LA or another approach?

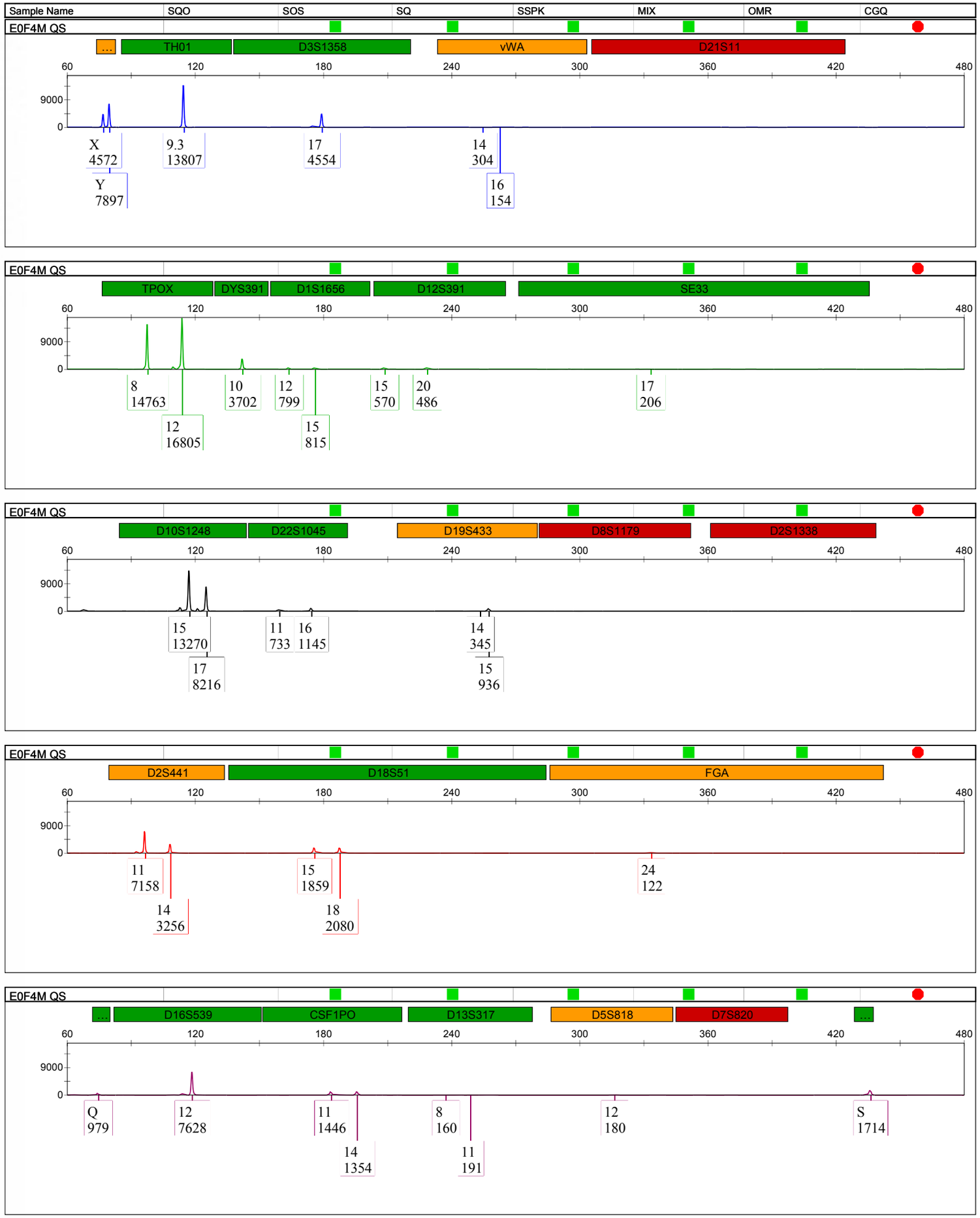








Large Target



Average of Small & Large Targets

Conclusions

- All methods preserved DNA well for relatively fresh tissues *(up to 4 days in this study)*
- Most promising methods for collecting & storing DNA at RT from decomposed remains:
    - Cotton or foam swabs via incision in underlying muscle tissue
    - Tissue biopsy in TENT buffer rapidly purified with PDQeX 2400 System
- Direct PCR approaches show some potential but require optimization
- Quality indicators in the Quantiplex Pro kit can increase first-pass success rates, and Quality Sensors in the Investigator 24plex STR kits can be used to confirm best route for sample rework (or not)

Acknowledgements

- Department of Forensic Science, Sam Houston State University
- PhD Advisor: Dr. Sheree Hughes-Stamm
- Graduate Students: Carrie Mayes, Kyleen Elwick, Michelle Harrel, Esiri “Ace” Tasker, Ryan Gutierrez, Maddie Roman

Acknowledgements

- QIAGEN® for support. Special thanks to Keith Elliot, Dr. Meredith Turnbough, Bryan Davis, Angela Cacioppo, Inga Gerard

- NIJ GRF Funding #NIJ 2015-R2-CX-0029

- Applied Anatomical Research Center (AARC)

  - Donors and families of donors

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References

- Kraemer M, Prochnow A, Bussmann M, Scherer M, Peist R, Steffen C. Developmental validation of QIAGEN Investigator 24plex QS kit and Investigator 24plex GO! Kit: Two 6-dye multiplex assays for the extended CODIS core loci. FSI Genetics (2017) Mar 12;29:9-20

- Sorensen A, Berry C, Bruce D, Gahan ME, Hughes-Stamm S, Mcnevin D. Direct-to-PCR tissue preservation for DNA profiling. Int J Legal Med (2016) 130: 607-13.

- Sorensen A, Rahman E, Canela C, Gangitano D, Hughes-Stamm S. Preservation and rapid purification of DNA from decomposing human tissue samples. FSI Genetics 25 (2016) 182-90.

- Holt A, Wootton SC, Mulero JJ, Brzoska PM, Langit E, Green RL. Developmental validation of the Quantifiler HP and Trio kits for human DNA quantification in forensic samples. FSI: Genetics 21 (2016) 145-57.

- Investigator Quantiplex Pro Field Test Handbook, Sept. 2016

- Ewing MM, Thompson JM, McLaren RS, Purpero VM, Thomas KJ, Dobrowski PA, DeGroot GA, Romsos EL, Storts DR. Human DNA quantification and sample quality assessment: Developmental validation of the PowerQuant system. FSI: Genetics 23 (2016) 166-77.

- Scherer M, Konig M, Breitbach M, Cornelius S, Bussman M, Prochnow A, Peist R. Improving sample analysis and interpretation using QIAGEN's latest Investigator® STR multiplex PCR assays with a novel quality sensor. FSI: Genetics Supplement Series 5 (2015) e308-9.

- Brownlow RJ, Dagnall KE, Ames CE. A comparison of DNA collection and retrieval from two swab types (cotton and nylon flocked swab) when processed using three QIAGEN extraction methods. J Forensic Sci 57:3 (2012) 713-17.

- http://www.med.navy.mil/sites/nepmu2/Documents/lab/FTA-Elute-Applications-Whatman.pdf

- Caputo M, Bobillo MC, Sala A, Corach D. Optimizing direct amplification of forensic commercial kits for STR determination. Journal of Forensic and Legal Medicine 47 (2017) 17-23.