Development of a Highly Sensitive Quantification System for Assessing DNA Quality in Forensic Samples

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QUANTIFICATION

- Several different fluorescence-based quantification assays are currently available.
- Reduced amplicon size has enabled STR analysis of highly compromised samples.
- A system to assess the amount of DNA degradation in forensic samples would be useful in determining which test kit to use.

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What are SINEs?

1. Short INterspersed Elements
2. 70 - 300 Base Pairs
3. High Copy Number
   (>100,000 Copies/Genome)

QUANTIFICATION

- A multi-copy, *Alu* based approach, to quantify human specific DNA in forensic samples, has been used previously with high sensitivity.
- Walker et al 2005; Shewale et al 2007; Opel et al 2008; Nicklas JA, 2012

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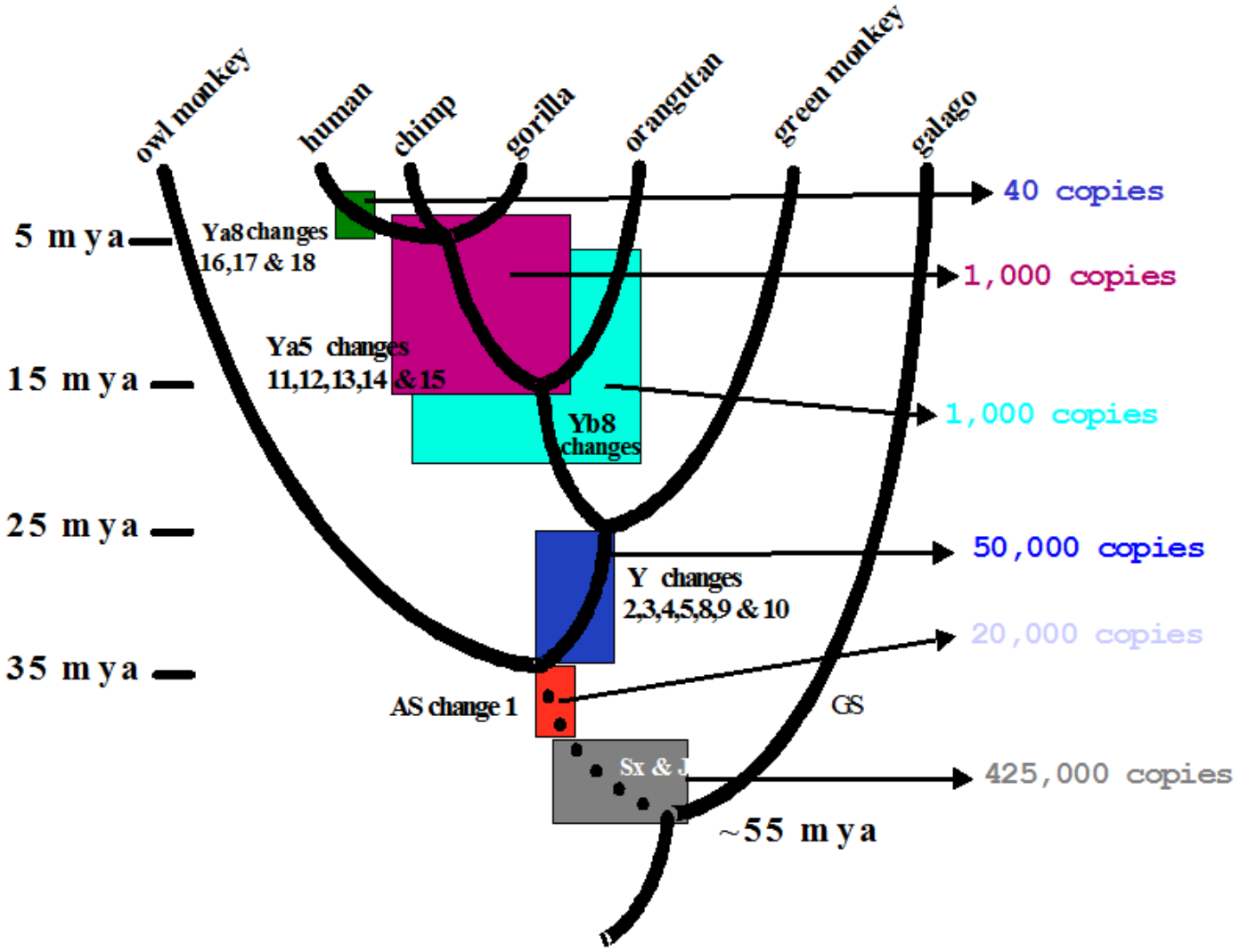
owl monkey
human
chimp
gorilla
orangutan
green monkey
galago
5 mya
15 mya
25 mya
35 mya
Ya8 changes
16,17 & 18
Ya5 changes
11,12,13,14 & 15
Yb8
changes
Y changes
2,3,4,5,8,9 & 10
AS change 1
GS
Sx & J
~55 mya
40 copies
1,000 copies
1,000 copies
50,000 copies
20,000 copies
425,000 copies

QUANTIFICATION SYSTEM

Primers and TaqMan® probes for 2 independent *interspersed elements*:

- ~80 bp target sequence labeled with FAM ("short" target)
- ~290 bp target sequence labeled with Cy5 ("long" target)

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Internal Synthetic DNA Control

- Cy3 labeled ~90 bp fragment for an Internal Positive Control (IPC)
- IPC assessment for PCR inhibitors

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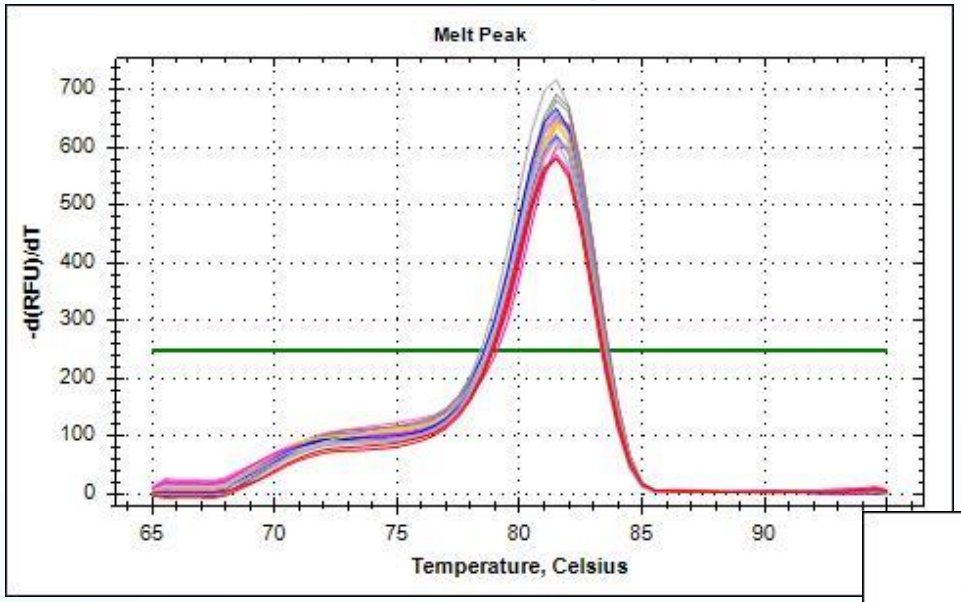
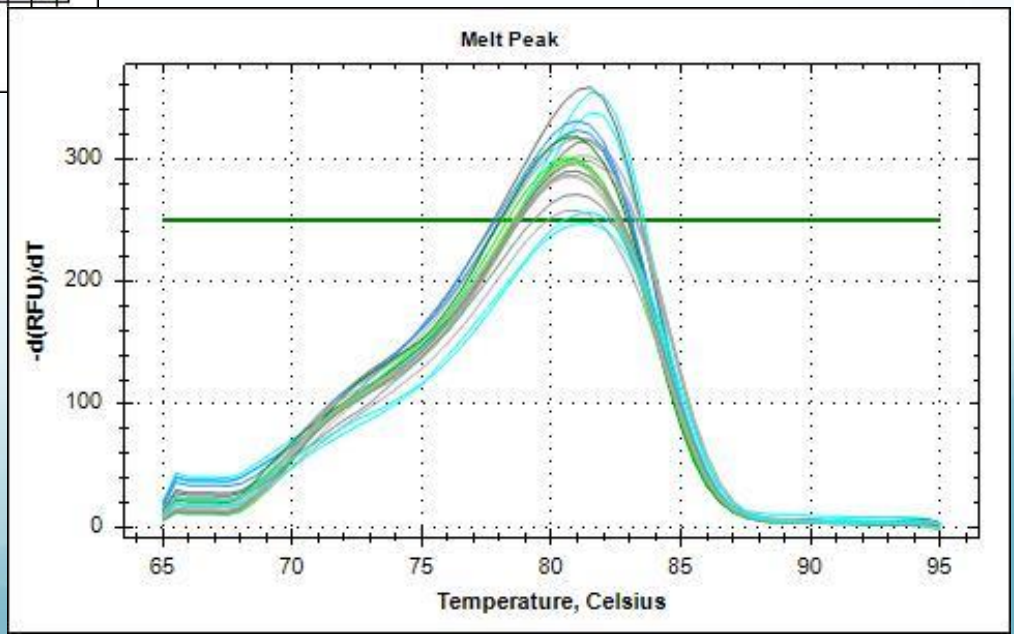
Melt Curve Analysis

Short Target

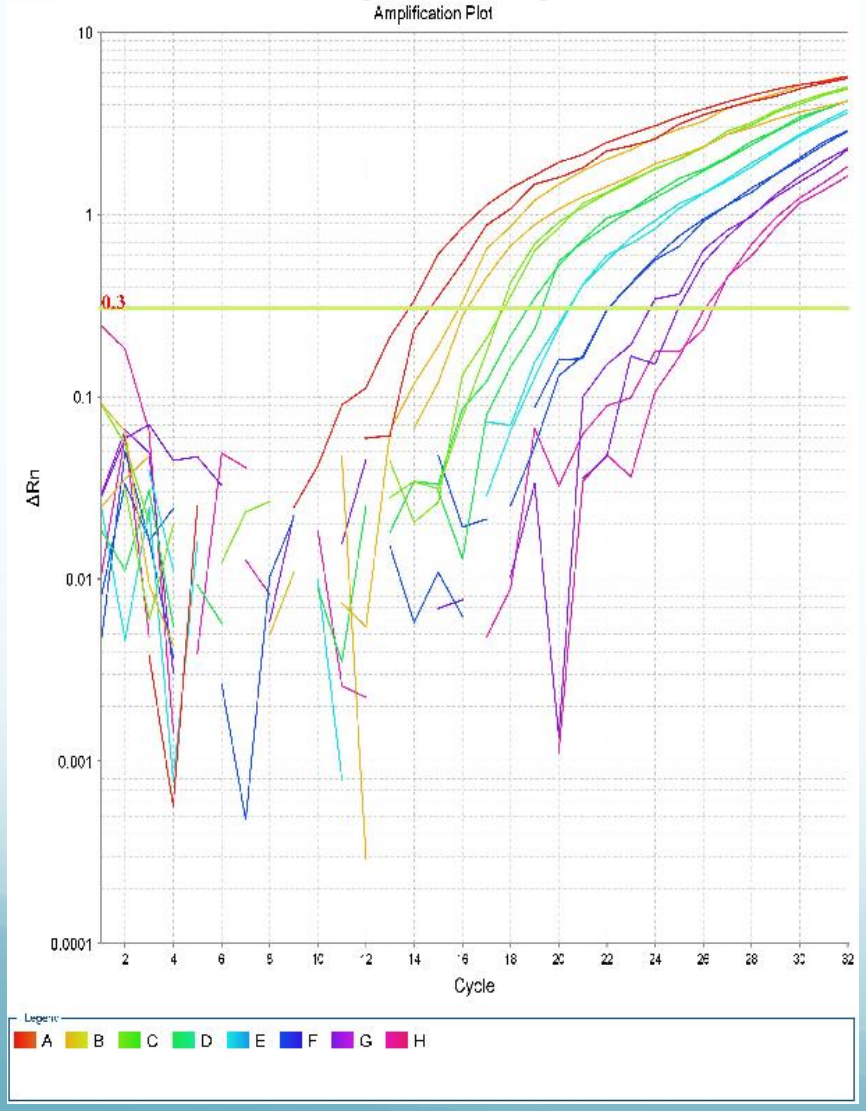


Long Target

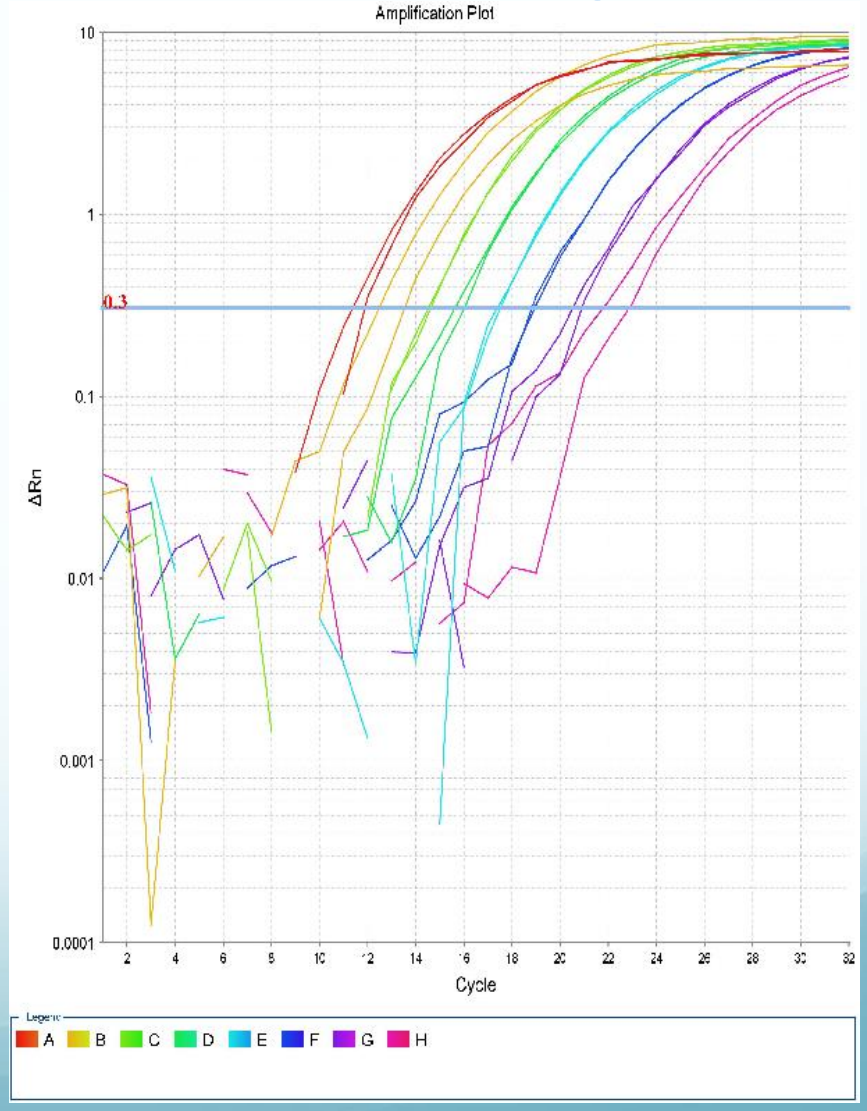


AB 7500 Amplification Plots

Long Target



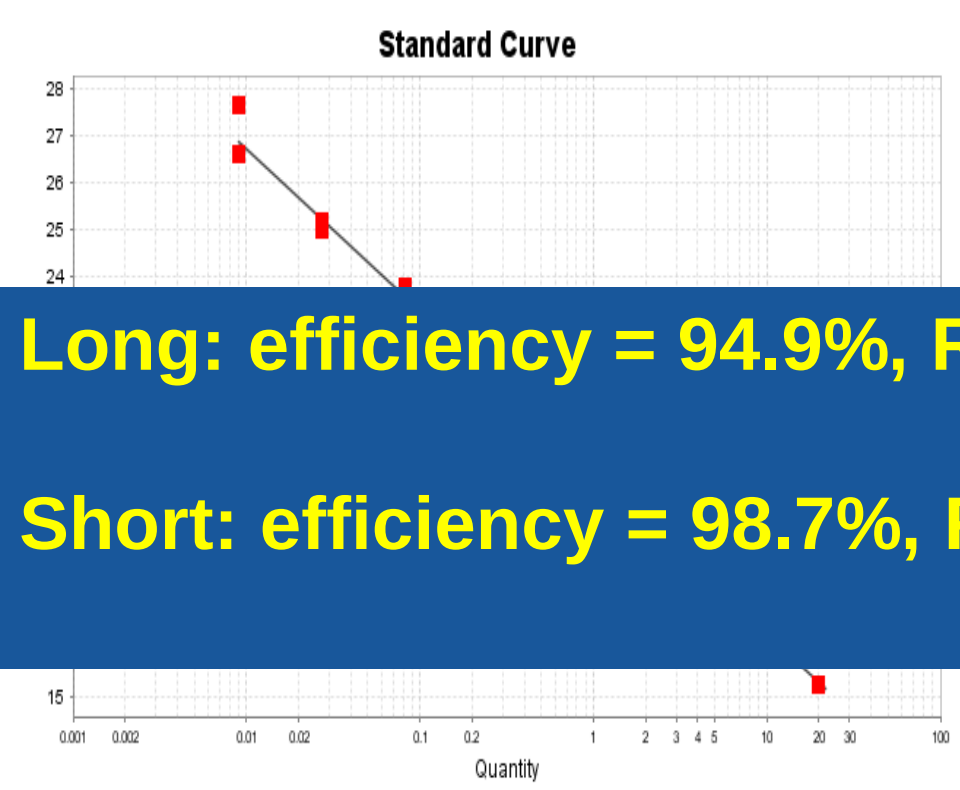
Short Target

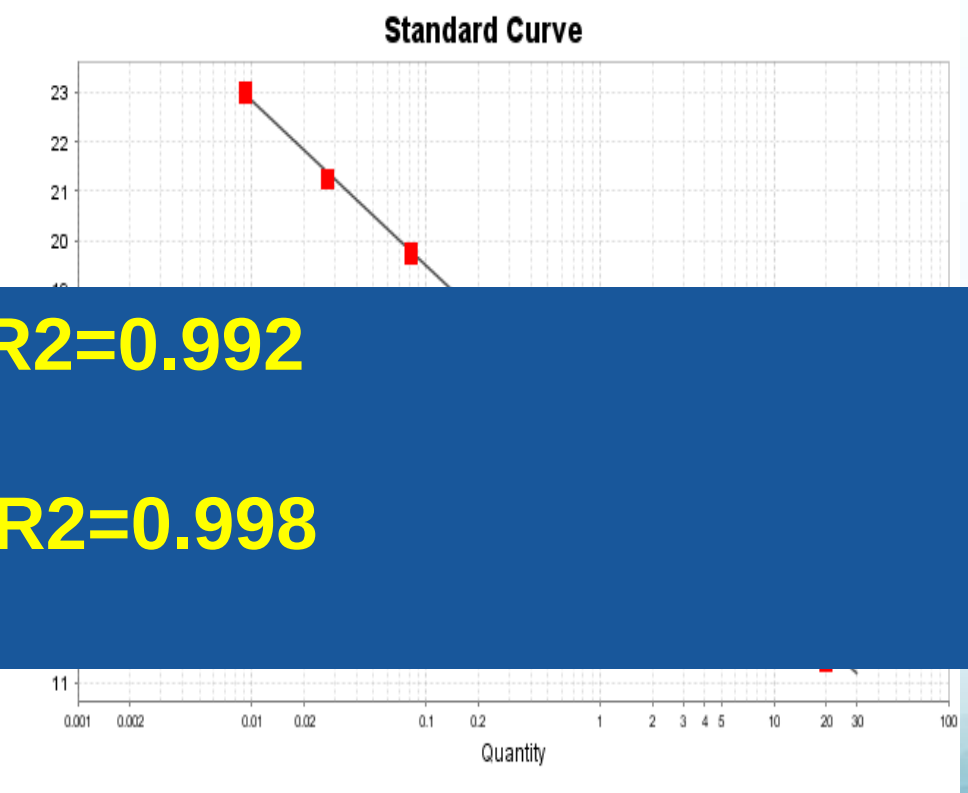


AB 7500 Standard Curves

Long Target

Short Target





Long: efficiency = 94.9%, R2=0.992

Short: efficiency = 98.7%, R2=0.998

Real time PCR Metrics

- Observations from 35 runs
  - Short target:
    - Average efficiency: **95%**, Average R^2 value: **0.994**
  - Long target:
    - Average efficiency: **91%**, Average R^2 value: **0.993**
- Standards dilution scheme ranges from:
  - 20 ng/ul to 0.009 ng/ul.
- Degradation Ratio expressed as a percentage =
  - (1-[Long Qty/Short Qty]) * 100

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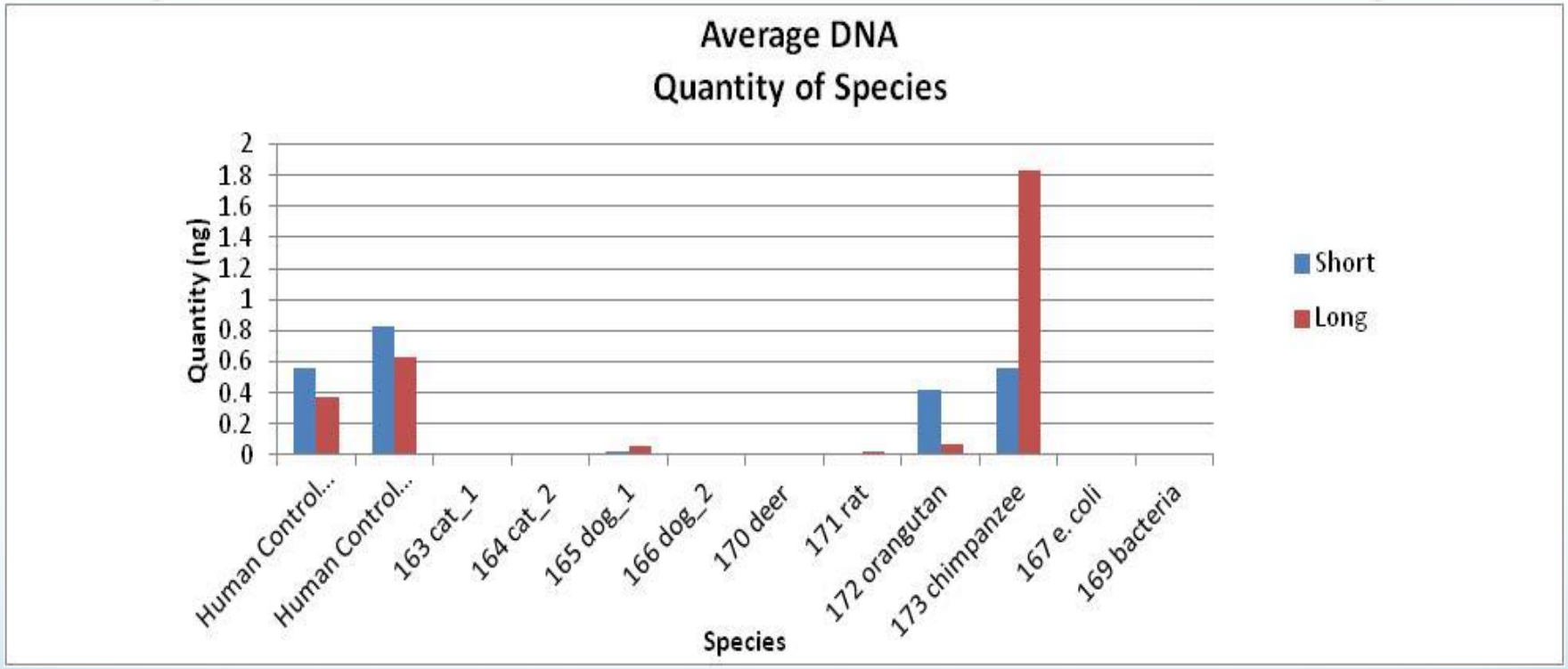
SPECIES STUDY



CONCORDANCE STUDY

- 19 samples quantified using Degradation Assay and Quantifiler® Human
- Quantifiler® human DNA concentrations averaged 140% of those calculated using the short target of this dual target assay
- If differences were observed, in all instances, Quantifiler® human values were higher than dual target assay values
- Differences are attributed to differences in the DNA standards and differences in amplicon length (62 bp vs. 80 bp)

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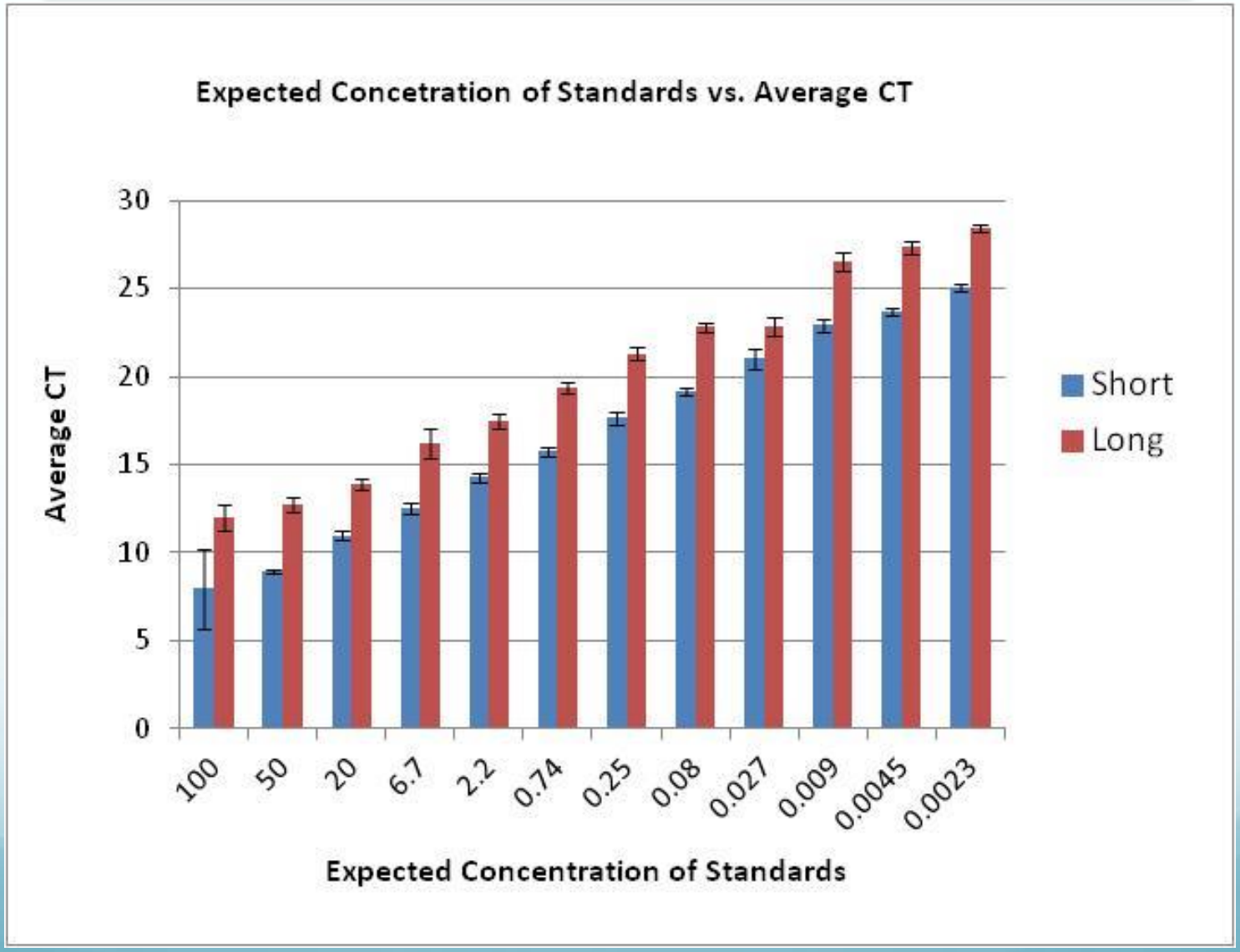
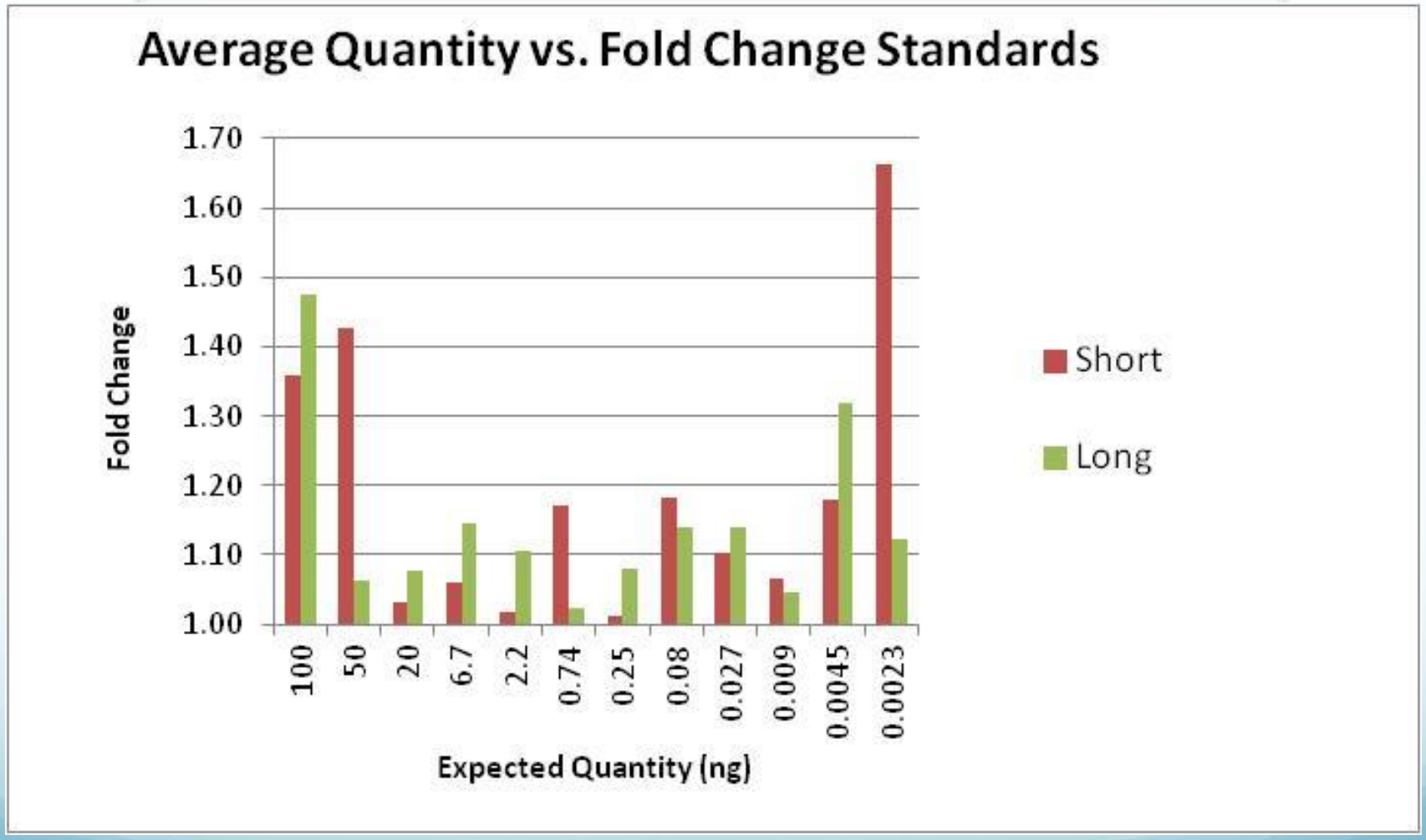
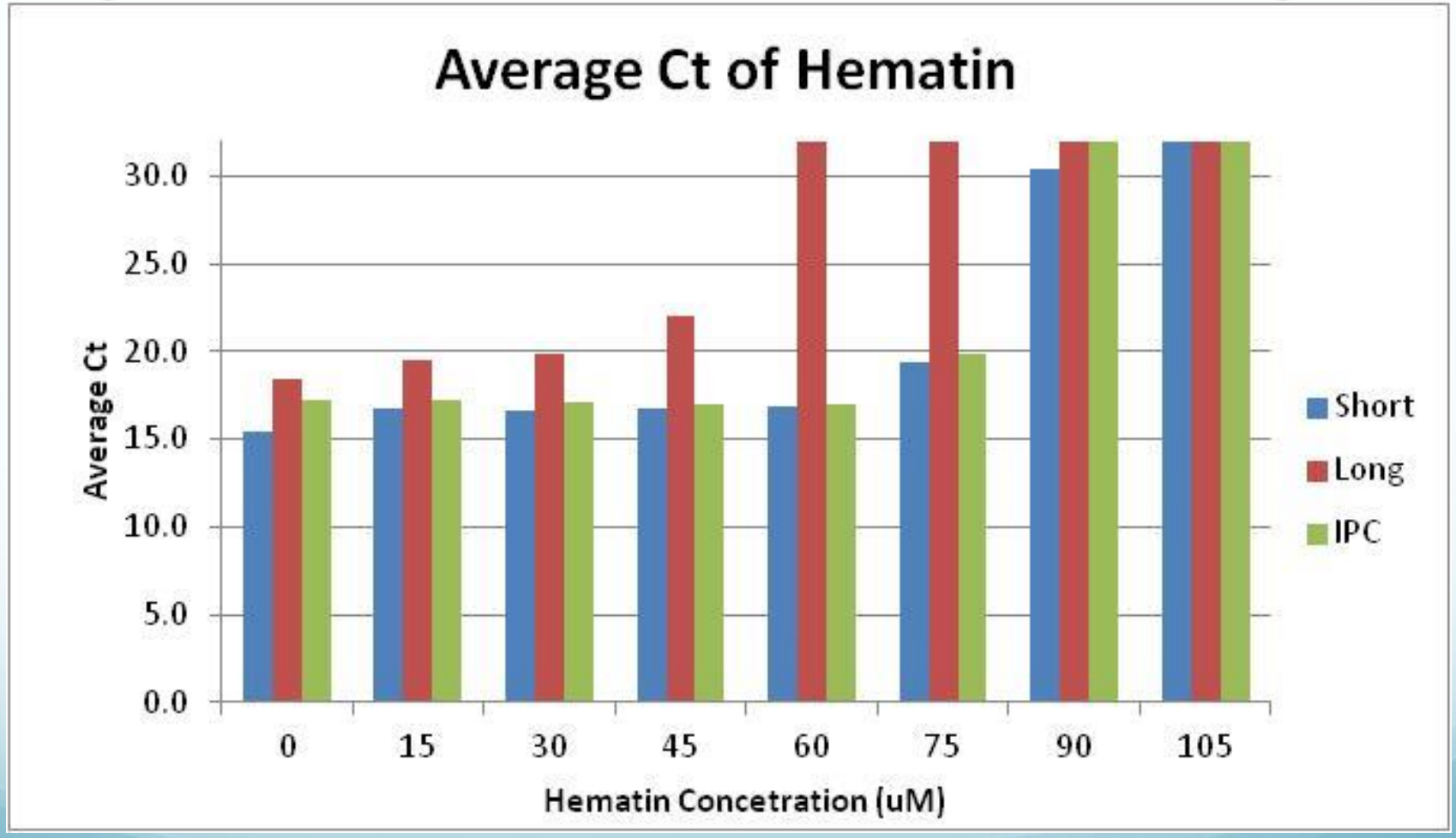
SENSITIVITY STUDY



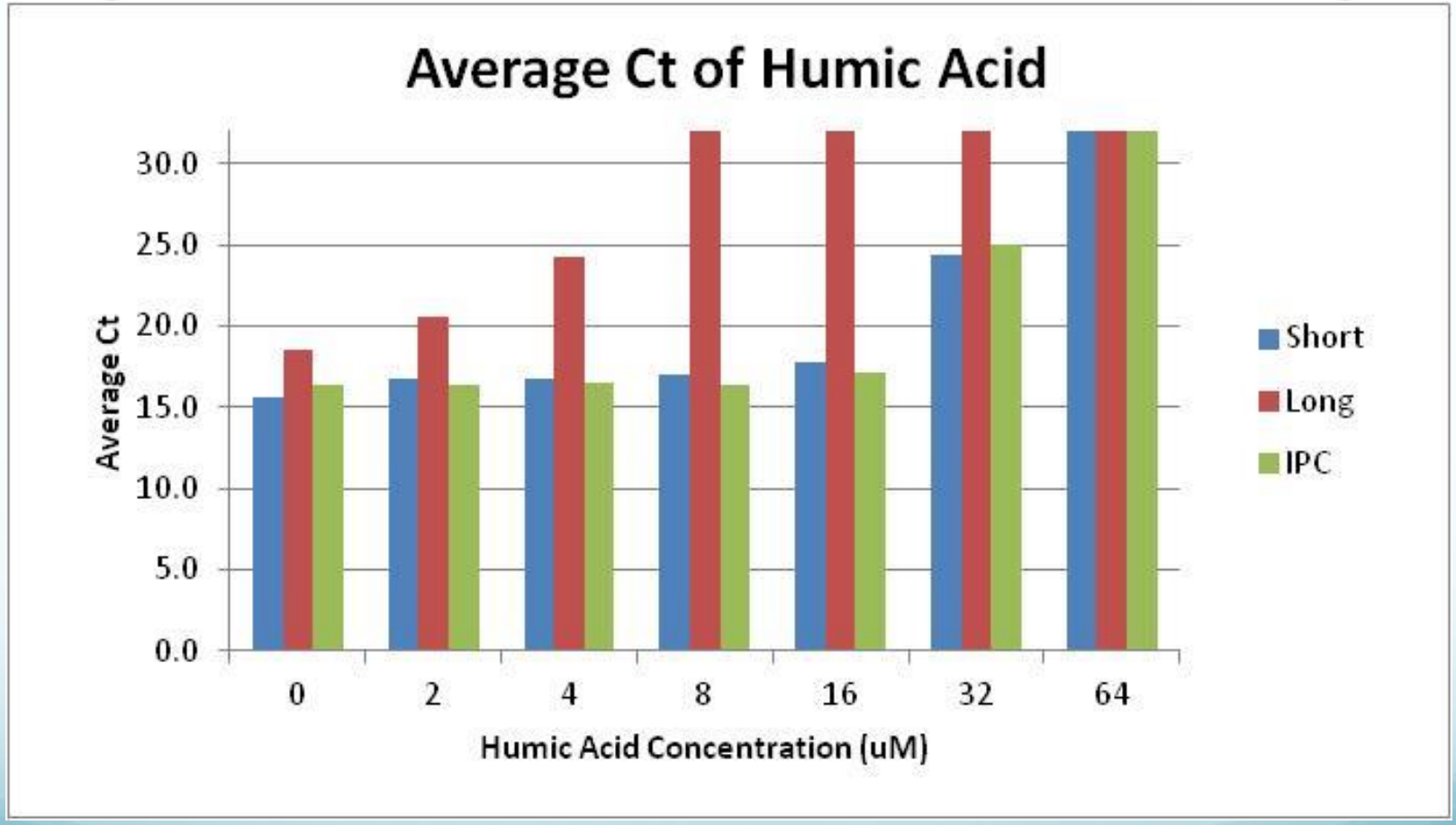
REPRODUCIBILITY STUDY



INHIBITION STUDY HEMATIN



INHIBITION STUDY HUMIC ACID



Degradation Studies

- Sonication
  - Mixture sample
  - Single source sample
- DNase-I
  - Mixture sample
  - Single source sample
- Environmental Degradation
- Targeted 3 concentrations of total DNA for Identifiler Plus Amplifications: 1 ng, 500 pg, and 200 pg
  - 28 cycles for IDP

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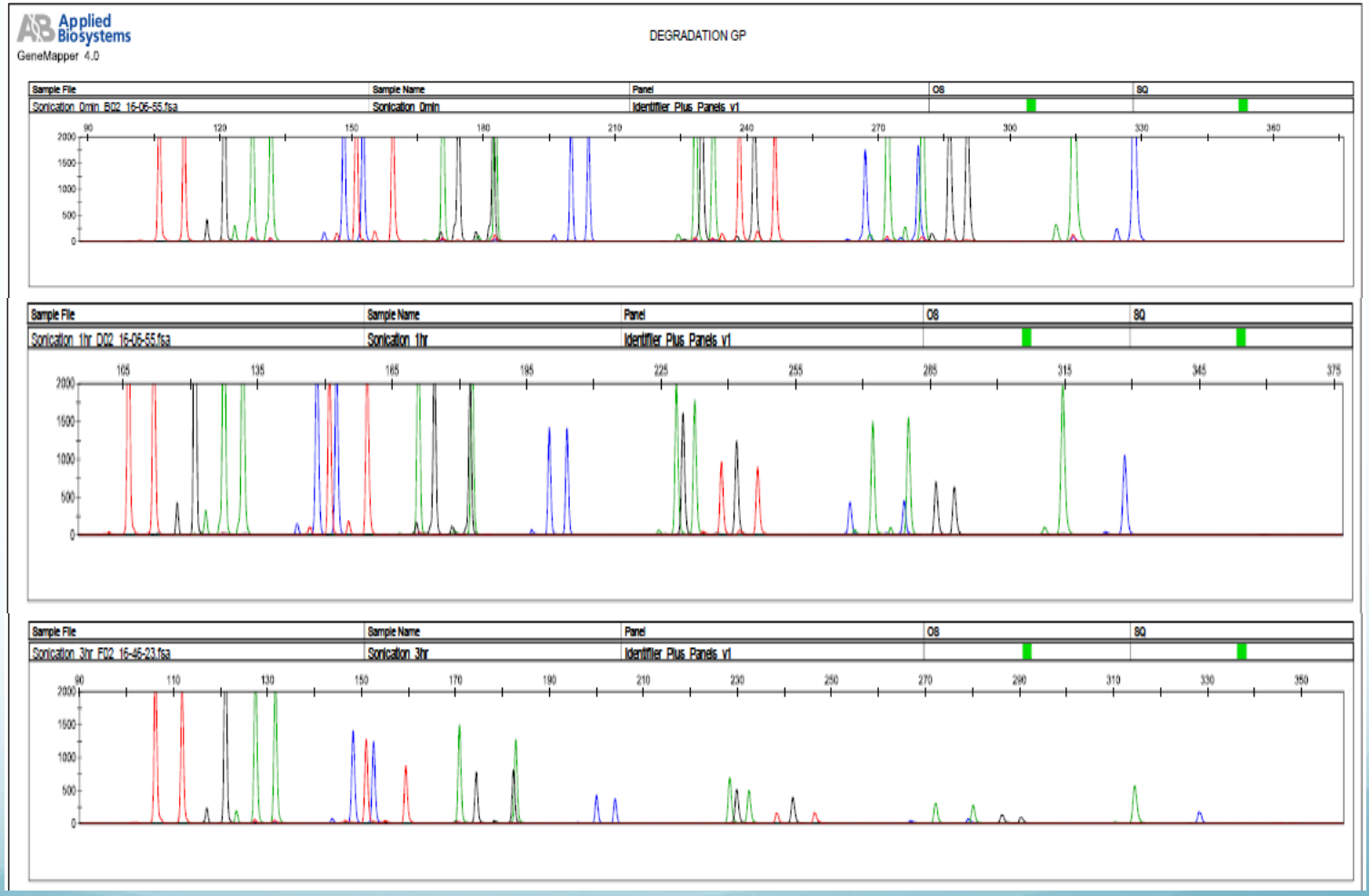
Degradation Study: Sonication 1 ng Input [DNA]

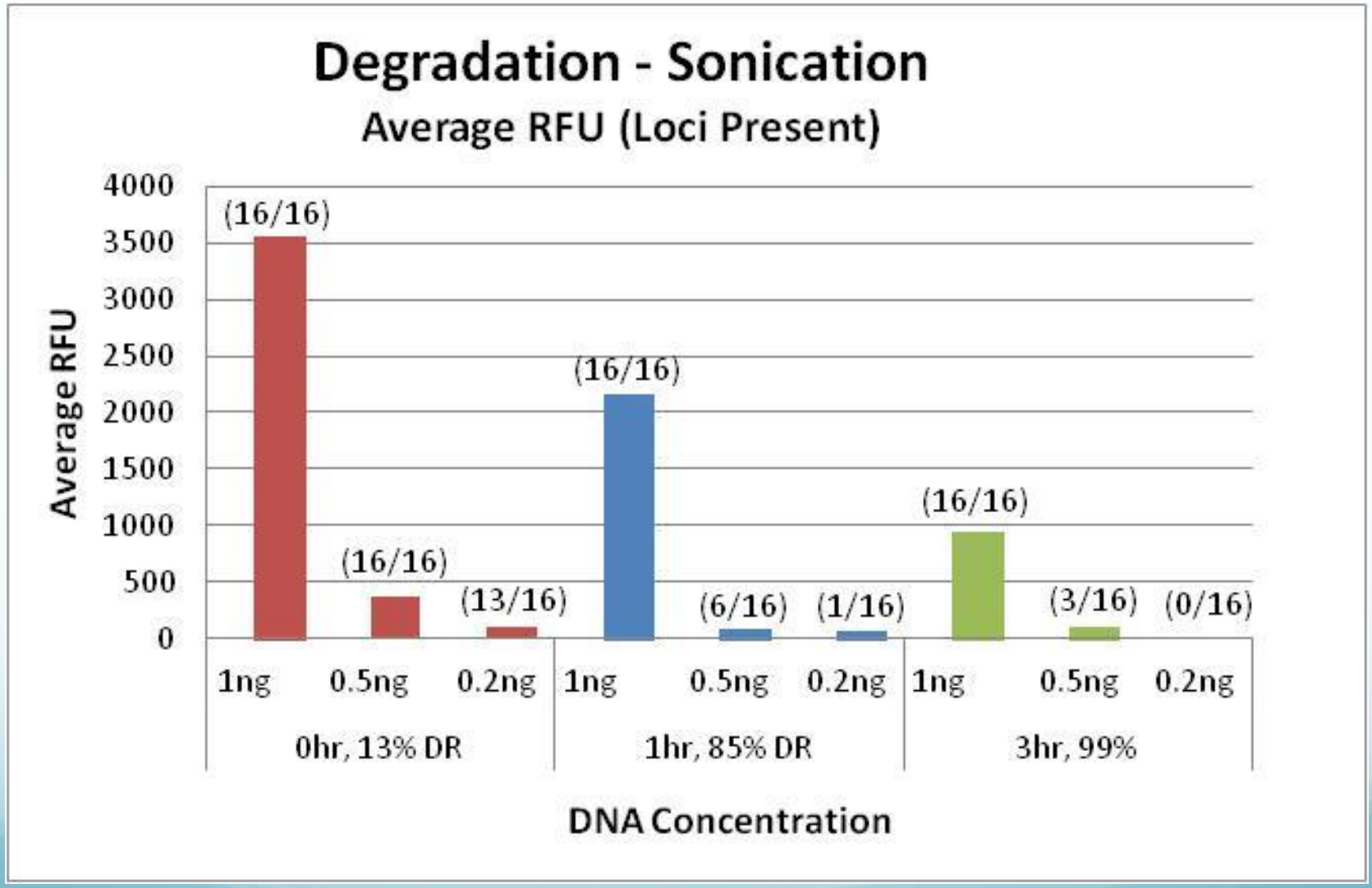
Control-
13% DR

1 hour-
85% DR

3 hour-
99% DR



Degradation Study: Sonication



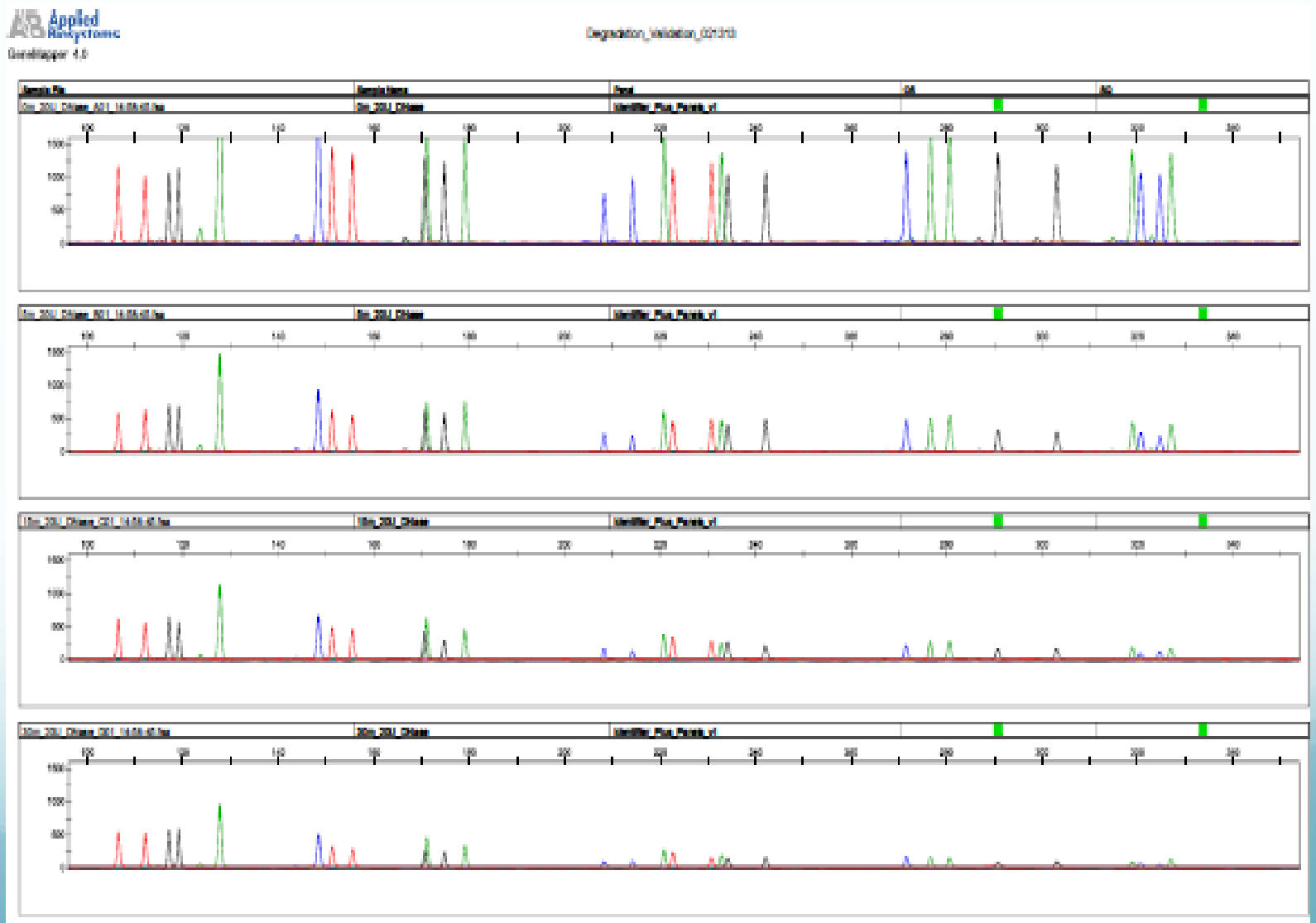
Degradation Study: DNase I 1 ng Input [DNA]

Control-
13% DR

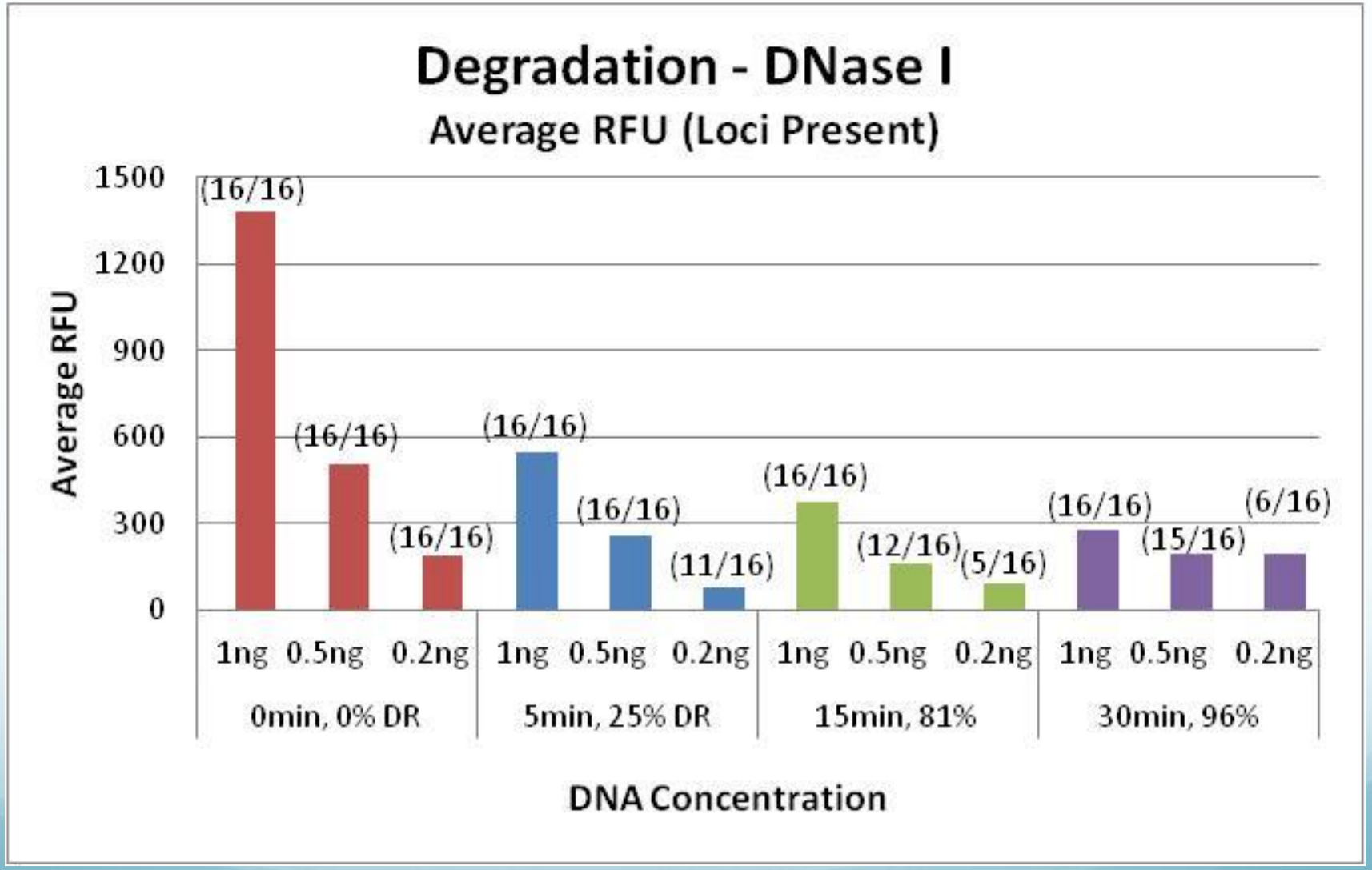
5 min
25% DR

15 min
81% DR

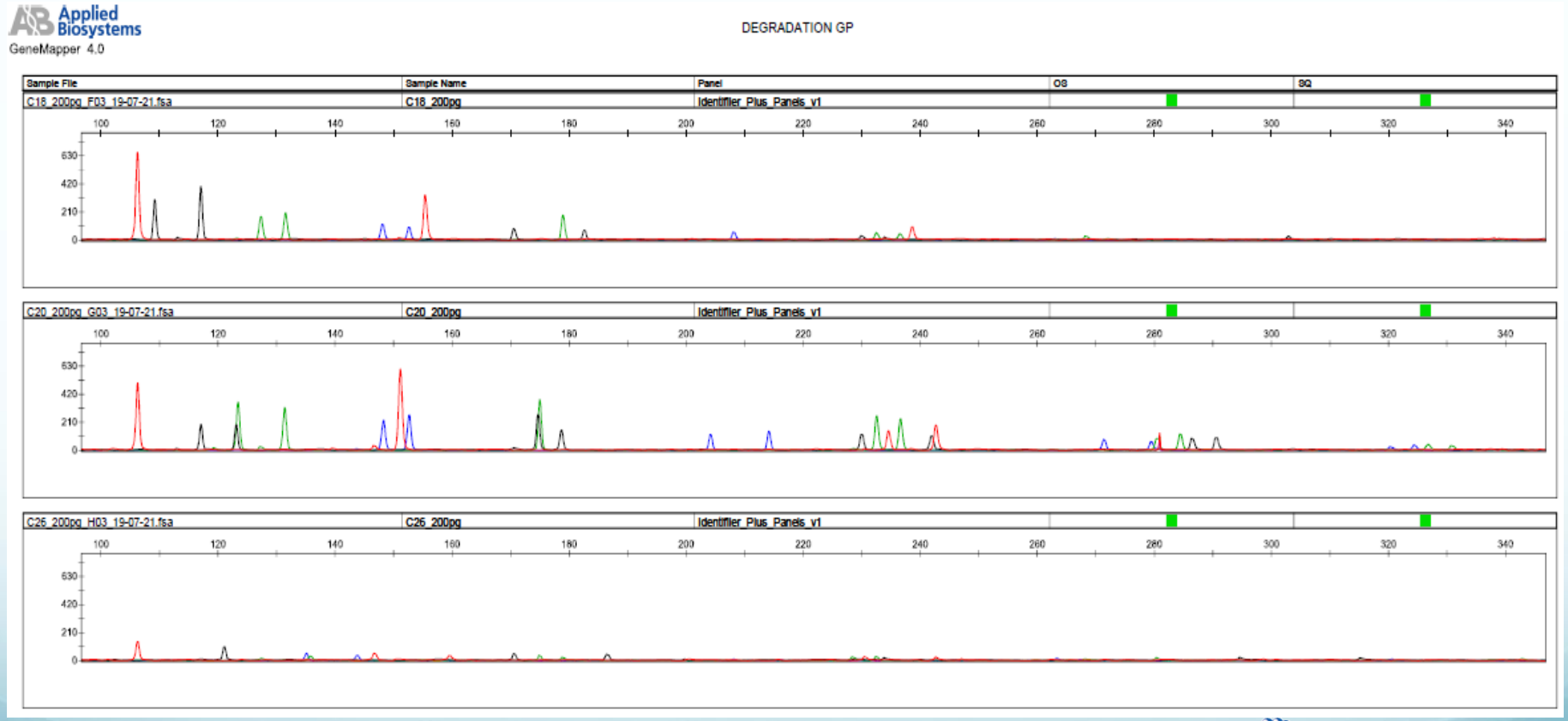
30 min
96% DR



Degradation Study: DNase I



Degradation Study: Environmental Degradation: 200 pg input [DNA]



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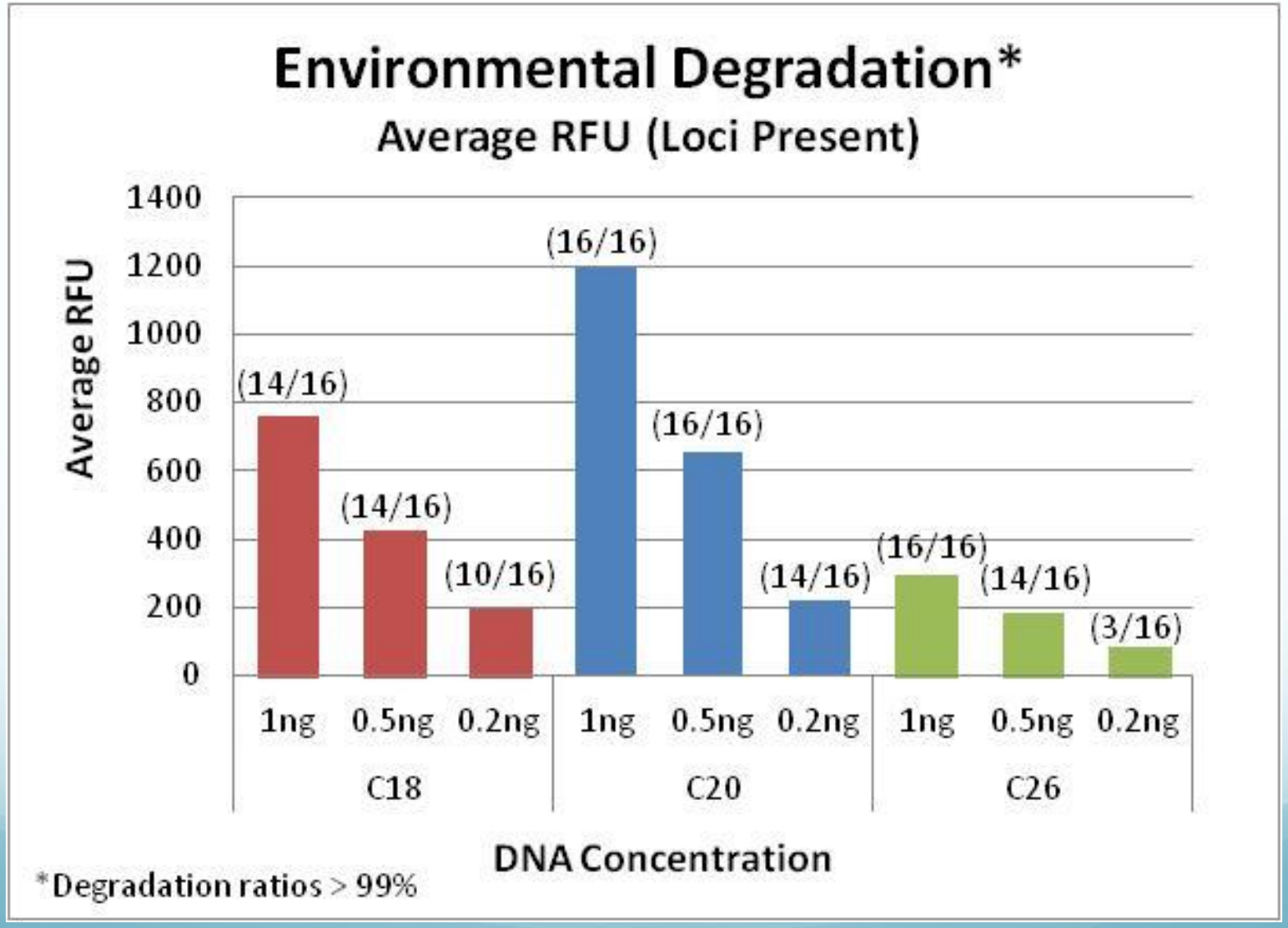
Environmental Degradation



Conclusion

- A dual target human qualitative / quantitative / inhibition assessment system has been developed
- Extremely sensitive: ~9 picograms/µl
- Accurately predicts degradation ratio of a biological sample
- Valuable tool for deciding which DNA test kit to utilize and how much input DNA to use when processing forensically compromised samples

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Q&A

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Thank you for your time

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