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.
What are SINEs?

1. Short INterspersed Elements
2. 70 - 300 Base Pairs
3. High Copy Number
   (>100,000 Copies/Genome)
A multi-copy, Alu based approach, to quantify human specific DNA in forensic samples, has been used previously with high sensitivity.

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)
Internal Synthetic DNA Control

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

Short Target

Long Target
AB 7500 Amplification Plots

Long Target

Short Target
AB 7500 Standard Curves

Long Target
Long: efficiency = 94.9%, R²=0.992

Short Target
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 =
  - $(1-[\text{Long Qty}/\text{Short Qty}]) \times 100$
SPECIES STUDY

Average DNA Quantity of Species

Species

- Human Control...
- Human Control...
- 163 cat - 1
- 164 cat - 2
- 165 dog - 1
- 166 dog - 2
- 170 deer
- 171 rat
- 172 orangutan
- 173 chimpanzee
- 167 e. coli
- 169 bacteria

Quantity [ng]

- Short
- Long
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)
SENSITIVITY STUDY

Expected Concentration of Standards vs. Average CT
REPRODUCIBILITY STUDY

Average Quantity vs. Fold Change Standards

Fold Change

1.70
1.60
1.50
1.40
1.30
1.20
1.10
1.00

Expected Quantity (ng)

100  50  20  6.7  2.2  0.74  0.25  0.08  0.027  0.009  0.0045  0.0023

Short
Long
INHIBITION STUDY
HEMATIN

![Graph showing the average Ct of Hematin against Hematin concentration (uM). The graph compares different conditions: Short, Long, and IPC.](image)
INHIBITION STUDY
HUMIC ACID

Average Ct of Humic Acid

Humic Acid Concentration (uM)

Average Ct

0.0 5.0 10.0 15.0 20.0 25.0 30.0 35.0

0 2 4 8 16 32 64

Short
Long
IPC
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
Degradation Study: Sonication
1 ng Input [DNA]

Control - 13% DR

1 hour - 85% DR

3 hour - 99% DR
# Degradation Study: Sonication

## Degradation - Sonication

<table>
<thead>
<tr>
<th>DNA Concentration</th>
<th>Average RFU (Loci Present)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1ng 0hr, 13% DR</td>
<td>(16/16)</td>
</tr>
<tr>
<td>0.5ng 1hr, 85% DR</td>
<td>(16/16)</td>
</tr>
<tr>
<td>0.2ng 3hr, 99%</td>
<td>(16/16)</td>
</tr>
<tr>
<td>1ng</td>
<td>(13/16)</td>
</tr>
<tr>
<td>0.5ng</td>
<td>(6/16)</td>
</tr>
<tr>
<td>0.2ng</td>
<td>(1/16)</td>
</tr>
<tr>
<td>1ng</td>
<td>(3/16)</td>
</tr>
<tr>
<td>0.5ng</td>
<td>(0/16)</td>
</tr>
<tr>
<td>0.2ng</td>
<td>(0/16)</td>
</tr>
</tbody>
</table>

**Average RFU (Loci Present)**

- 4000
- 3500
- 3000
- 2500
- 2000
- 1500
- 1000
- 500
- 0

**DNA Concentration**

- 1ng
- 0.5ng
- 0.2ng
- 1ng
- 0.5ng
- 0.2ng
- 1ng
- 0.5ng
- 0.2ng

**Degradation Levels**

- 0hr, 13% DR
- 1hr, 85% DR
- 3hr, 99%
**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 - DNase I
Average RFU (Loci Present)

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<td>(15/16)</td>
<td>(6/16)</td>
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DEGRADATION STUDY: ENVIRONMENTAL DEGRADATION: 200 pg input [DNA]
Environmental Degradation

Environmental Degradation*
Average RFU (Loci Present)

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</tr>
<tr>
<td>0.5ng</td>
<td>(14/16)</td>
</tr>
<tr>
<td>0.2ng</td>
<td>(10/16)</td>
</tr>
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<td>1ng</td>
<td>(16/16)</td>
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*Degradation ratios > 99%
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