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

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Introduction
A qPCR quantiﬁcation system which can simultaneously provide human and male quantitative information, as well as assess the extent of degradation in a forensic sample, will be a useful tool for DNA analysis. A multi-copy intra-Alu based approach, to quantify human specific DNA in an evidence sample, has been successfully used to obtain DNA quantiﬁcation with high sensitivity. The advantage of an Alu system is the presence of a large number of ﬁxed insertions (a total of ~1800 copies/ genome of Yb8 and ~1700 full length copies/ genome of SVA).

This system (InnoQuant-HV™) utilizes two independent genomic targets and a male speciﬁc target in a multiplex to simultaneously obtain quantiﬁcation of an 80 bp fragment and a 207 bp fragment, as well as male DNA (See Fig. 1). The 80 bp ”short” target sequence is from the Yb8 lineage Alu insertion whereas the 207 bp ”long” target sequence is from a separate retrotransposon element, SVA.

Materials and methods
Primers and TaqMan probes were designed using two independent intra-retrotransposon insertions targets, and a male target. Use of a synthetic 172 bp target as an IPC provides an additional assessment for the presence of PCR inhibitors in the sample. Real-time PCR reactions were processed on the Applied Biosystems 7300 Real-Time PCR System using Applied Biosystems’ Brilliant Multiplex qPCR Master Mix with the following parameters: 10 min at 95°C, followed by 38 cycles of 15 sec at 95°C, and 2 min at 60°C. The ratio between the DNA quantity of the short target divided by DNA quantity of the long target gives an indication of the degree of DNA degradation for the quantiﬁed sample, and is termed the “Quality Index”, or QI.

<table>
<thead>
<tr>
<th>Quality Index</th>
<th>Long Target</th>
<th>Short Target</th>
<th>Y Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>QI</td>
<td>0.091</td>
<td>0.049</td>
<td>0.060</td>
</tr>
<tr>
<td>Min SD</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Max SD</td>
<td>0.883</td>
<td>0.327</td>
<td>0.325</td>
</tr>
</tbody>
</table>

Highly reproducible

Results
Sample ampliﬁcation plots and PCR efﬁciencies:

Sensitivity:
Low variation from expected values observed in the range of the standards.
Greater variation observed in Y target near the system’s LOD.

Correlation between autosomal and male targets:
The sensitivity dilutions (short DNA and NIST SRM), were analyzed for correlation between the autosomal and Y target quantitation values. The average fold change between the long and Y target quantity values was 1.23. The average fold change between the short and Y target quantity values was 1.23.

Reproducibility:
60 semen samples were run in duplicate and autosomal short/long and male quantitation values were analyzed. The average fold change between the long and Y target quantity values was 1.76 and between the short and Y target quantity values was 1.76.

NIST SRM reproducibility:
Five runs, different days, duplicate dilutions of NIST SRM 2372 Human DNA Quantiﬁcation Standard at 2 ng/ml. Average percent variation from expected (NIST). Short: Y 14% 6% 27% Highly accurate

Conclusions
A DNA based qualitative/quantitative/inhibition assessment system can be a valuable tool when processing forensically compromised samples.

The Quality Index (ratio of quantity of short target to long target) strongly correlates to STR proﬁling success.

The use of retrotransposon targets signiﬁcantly improves both sensitivity and reproducibility compared to currently commercially available kits.

Highly reproducible

<table>
<thead>
<tr>
<th>Long</th>
<th>Short</th>
<th>Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.99</td>
<td>0.943</td>
<td>0.53</td>
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<tr>
<td>Std</td>
<td>0.002</td>
<td>0.002</td>
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<tr>
<td>100</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Literature cited
2. MA, Lower R, and Schumann GG.
3. MA, Lower R, and Schumann GG.
4. 13.

Acknowledgments
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