Improving Workflow Efficiency for Challenging Samples Utilizing New Technologies

Gina Pineda Murphy, M.S.*, Anne H. Montgomery, M.S., Hiromi Brown, Ph.D., and Sudhir K. Sinha, Ph.D.

InnoGenomics Technologies, LLC; 1441 Canal Street, Suite 307; New Orleans, LA 70112

Introduction

Improved evidence collection procedures and technology advancements using the PCR process have enabled scientists to provide scientific evidence in criminal cases that were previously unimaginable. Next generation platforms and test systems now provide scientists with the tools to evaluate DNA samples that may consist of degraded DNA and/or trace, touch, or contact DNA evidence. There is a need for more robust, highly sensitive, reproducible methods for the assessment (i.e., quality and quantity) of DNA extracts to determine optimal downstream processing methods, as well as improved typing systems for profiling these difficult samples.

We report here utilization of a combination of two recently developed technologies to improve the success rate of obtaining results from highly compromised degraded as well as trace samples:

- A new DNA quantitation kit[1], InnoQuant™, which allows accurate quantitation at picogram levels (~1 pg) of two autosomal targets: a "short" Alu based target of 80 bp in size, and a "long" target from a separate retrotransposon of 207 bp in size.
- A new marker system InnoTyper™, a highly sensitive *Alu* based multiplex[2] which can be utilized for DNA typing of highly degraded DNA samples.

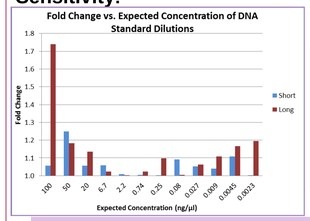
Materials and methods

- DNA extractions were performed using either an organic and Microcon concentration method or Qiagen MinElute method.
- InnoQuant™ qPCR quantitation: Primers and TaqMan probes were designed using two independent intra *retrotransposon* insertions targets and a synthetic target as an IPC. Real-time PCR reactions were processed on the AB 7500 Real-Time PCR System using Agilent Technologies' Brilliant Multiplex QPCR Master Mix as follows: 10 min at 95°C; and 32 cycles of: 15 sec at 95°C, and 2 min at 61°C. Degradation Index was determined by **DI = [short]/[long]**.
- InnoTyper™ 21 amplification: Primers were designed for 20 RIP markers and AMEL in a multiplex assay. PCR was performed on a 9700 as follows: 15 min at 95°C; 32 cycles of: 30 sec at 95°C, 30 sec at 58°C and 1 min at 72°C; one cycle of 60°C for 1 hour. AB 3130 Genetic Analyzer was utilized. Data analysis was performed on GMID with a 50 RFU analytical threshold.

Results

How *InnoQuant*™ Improves Efficiency:

Sensitivity:

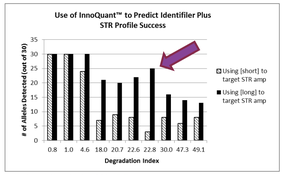


NIST SRM "A" Dilution

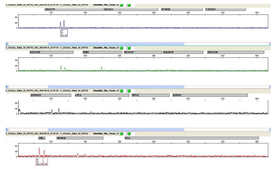
Expected ng/uL	Observed Short Qty	Observed Long Qty	Short Qty Fold change	Long Qty Fold change
5	4.1543	6.8428	1.20	1.37
1.25	1.2454	1.2499	1.00	1.00
0.3125	0.3201	0.2849	1.02	1.10
0.0781	0.0765	0.0608	1.02	1.28
0.0195	0.0202	0.0165	1.04	1.18
0.0049	0.0055	0.0042	1.12	1.17
0.0012	0.0013	0.0010	1.06	1.18
		Average Fold Change	1.07	1.18

Highly sensitive and reproducible: less than 20% variation from expected values as low as 1 picogram of DNA. ZERO means ZERO: can stop at quant

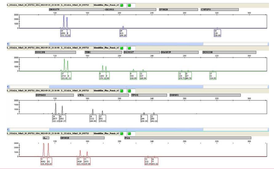
Correlation between DI and STR profile success:



As the DI increases, the [long] value can be used to target the STR amplification and thus obtain more alleles in the first attempt



Using the [short] to target IDP amp:
- 3 out of 30 alleles called in $DI_{80/207}$ = 23 sample



Using the [long] to target IDP amp:
- 25 out of 30 alleles called in $DI_{80/207}$ = 23 sample

InnoQuant™ Results:

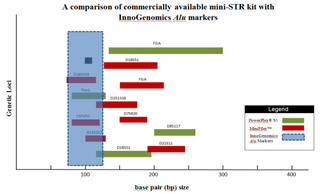
[Short] = 6.68 ng/uL

[Long] = 0.293 ng/uL

DI = 6.68/0.293 = 22.8

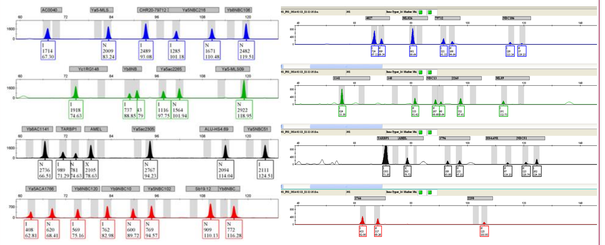
How *InnoTyper™ 21* Improves Efficiency:

A novel primer design allows control of the size of the resultant insertion or null alleles, with amplicon sizes less than 125bp.



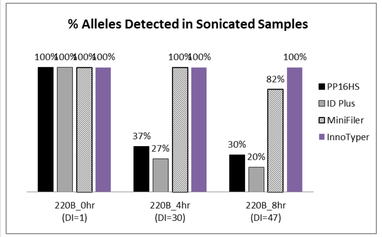
Amplicon size smaller than mini-STR kits

InnoTyper™ 21 | InnoTyper™ prototype on 4 cm hair shaft



Use the InnoQuant™ [short] value to target InnoTyper™ amplifications

Higher Sensitivity than other kits with degraded samples



As the DI increases, InnoTyper™ obtains more alleles from degraded samples than other commonly used typing kits

Conclusions

- A quality/quantity sample assessment assay such as InnoQuant™ is an effective tool in determining:
  - ✓ Which typing system to use
  - ✓ How much DNA to take forward to the typing stage with the highest chances of first pass success rates, eliminating the need for re-works.
- The Degradation Index (ratio of quantity of short target to long target) strongly correlates to STR profiling success.
- InnoTyper™ is a highly sensitive *Alu* marker system which provides high tolerance to degradation and inhibition.
- InnoTyper™ is highly sensitive and has a high discrimination power (approximately 1 in 100 million), thus eliminating the need to resort to mtDNA sequencing for many samples.
- Processing decisions made more intelligibly with the use of the InnoQuant™ and InnoTyper™ next generation systems will undoubtedly improve efficiency in the workflow of challenging samples and conserve the consumption of limited and trace evidence samples.

Literature cited

1. G.M. Pineda et al. *Development and validation of InnoQuantTM, a sensitive human DNA quantitation and degradation assessment method for forensic samples using high copy number mobile elements Alu and SVA.* Forensic Science International: Genetics 13 (2014) 224–235.

2. S.K. Sinha et al. *Development of a Novel and Sensitive DNA Analysis Multiplex Based on INNUL Markers for Highly Degraded Forensic DNA Samples.* Oral presentation at the 66th Annual Meeting of the AAFS. Seattle, WA.

Acknowledgments

This material is based upon work supported by the National Science Foundation under Grant No. 1230352. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation. For additional information, contact: gpineda@innogenomics.com.