Working With Challenging Samples: An Independent Assessment of the Relative Performance of the Promega® Fusion™ and InnoGenomics® InnoTyper™ Kit With Probative Samples

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Abstract

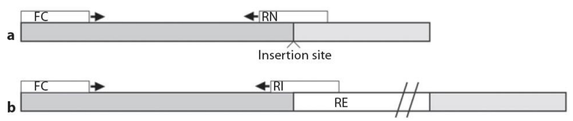
The generation of reliable nuclear profiles from challenging samples has become increasingly important for forensic and relationship testing. Often samples yielding the most severely degraded and lowest quantity of DNA have required mitochondrial sequencing. The increase in sensitivity and robustness of standard genomic marker systems has increased their utility for challenging samples, while the development of unique markers based on Retrotransposable Insertion Polymorphisms (RIPs) has added another tool to the investigators tool box. Promega's PowerPlex® Fusion System interrogates 22 autosomal short tandem repeats (STR), the amelogenin locus for gender identification, and a gender confirmatory marker on the Y chromosome. InnoGenomics' InnoTyper™ 21 kit is a small amplicon (~60-125 bp) DNA typing system containing 20 RIP markers and amelogenin in which each locus is scored for the presence of a stable heritable insertion. This study assessed the relative performance of the Promega Powerplex® Fusion System and the InnoGenomics InnoTyper™ 21 kit on low yield, highly degraded, and challenging forensic samples.

Materials and methods - Brief

DNA extraction - Swabs
The ends of the swabs were excised using a sterile razor and DNA extracted using a Maxwell® magnetic bead extraction system (Promega). The kit used was the Maxwell® 16 Buccal Swab LEV DNA Purification Kit and the extraction was carried out according to the manufacturer's instructions and eluted into 25µl of buffer.
DNA extraction – Bones
Bones were cleaned and sanded to remove surface contamination and then cleaned with 5% bleach and 95% isopropanol. The bones were then drilled at a low speed and between 0.5 and 1.0g of bone shavings were collected. Decalcification was carried out in an EDTA (0.5M) buffer containing SDS for 48 hours at 56˚C on a rocking platform. DNA extraction was then carried out using a Maxwell magnetic bead extraction system (Promega). The Maxwell® 16 Tissue DNA Purification Kit was used and the extraction was carried out according to the manufacturer's instructions. Two cassettes were used to purify DNA from the contemporary bone and eight for the older bone specimens. The extracted DNA was then concentrated using a size exclusion column to a volume of approximately 50µl.
Quantification
Samples were quantified for PowerPlex® Fusion PCR using Investigator Quantiplex HYres from Promega and for InnoTyper™ by InnoQuant™ from InnoGenomics.
PCR
PowerPlex® Fusion – PCR was carried out according to the manufacturer's recommendations with 32 cycles of amplification and ½ volume reactions.
InnoTyper™ – PCR was carried out according to the manufacturer's recommendations with 32 cycles of amplification. PCR clean-up – for samples that produced incomplete profiles, the PCR reaction was cleaned using Zymo Research's DNA clean and concentrator columns.
Genetic Analysis
Analysis was carried out on an Applied Biosytstem 3130xl, running POP7 polymer, according to standard laboratory protocols. Alleles were called using a 75RFU cut-off with full reaction volumes.

InnoGenomics InnoTyper™

- 21 marker system
- Ave. Power of Discrimination – $1*10^{-8}$
- All markers less than 125bp in size (good for compromised samples)



InnoTyper primer design a) A common forward primer (FC) is used for both insertion and null alleles. A fluorescently labeled 'null-specific' reverse primer (RN) straddles the insertion site of the RE and anneals in the absence of the RE. b) In instances where the RE is present, the annealing site of the reverse primer is disrupted, and the 'insertion-specific' reverse primer (RI) anneals at the site that overlaps with the insertion site and the adjacent portion of the RE.

Promega PowerPlex® Fusion

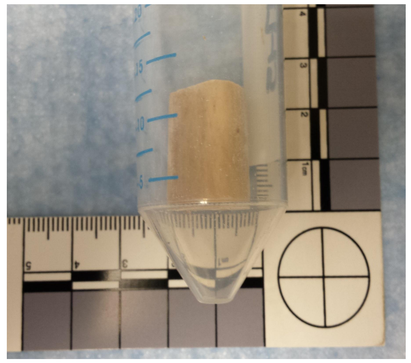
- 24 marker system
- Ave. Power of Discrimination – $1*10^{-28}$
- 8 markers less than 200bp in size (good for compromised samples)

*Amelogenin

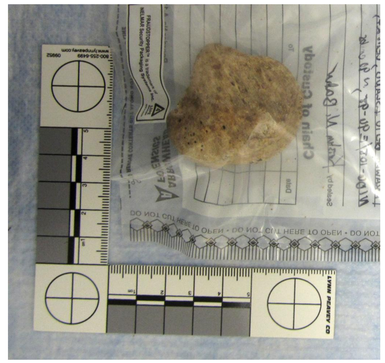
Configuration of the PowerPlex® Fusion System. The PowerPlex® Fusion System allows co-amplification and four-color detection of 24 loci

Introduction

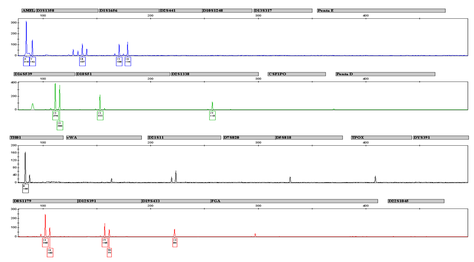
To assess the performance of the Promega PowerPlex® Fusion and InnoGenomics InnoTyper™ kits we selected challenging sample types, which we believed contained relatively small amounts of compromised DNA. We selected two previously adjudicated contemporary (<10 years old) bone samples provided by the University of North Texas and twelve older bones (≈70 years old) provided by History Flight. Profiles from these kinds of samples are used to identify remains either through matching to a known sample or through relationship analysis.



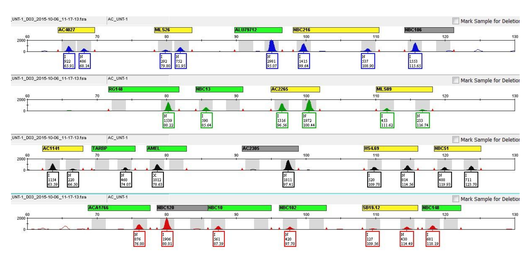
Contemporary bone sample



WWII era bone sample



Profile produced using Promega PowePlex® Fusion genotyping kit for contemporary bone UNT-1



Profile produced using InnoGenomics InnoTyper™ genotyping kit for contemporary bone UNT-1

Acknowledgements – Many thanks to the Centre For Human Identification at the University of North Texas and History Flight for providing samples for this analysis and InnoGenomics™ for providing InnoTyper™ and InnoQuant™ kits along with technical support for their use.

Results

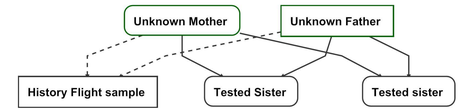
Contemporary Bones (<10 years old)
Both kits produced probative data from the two contemporary bone extractions, for the extract with the highest DNA concentration full profiles were obtained from both kits (UNT-2). The second sample gave a full profile with InnoTyper™, but only a partial profile with PowerPlex® Fusion (shown). Either result would likely provide enough probative data for positive identification or contribute to a relationship match.
Older Bones (≈70 years old)
Both systems also produced probative data for the WWII era bones. The performance of each system was generally in-line with the amount of input DNA available, indicating that this is probably the limiting factor in obtaining results (at least in these samples). The average number of loci producing data was 4 for fusion (with 6 sample producing no data) and 19 for InnoTyper. There was a noticeable homozygote excess in all samples when using PowerPlex® Fusion, indicating the likely presence of "drop-out". Both systems gave some data, even at very low input levels, but InnoTyper™ out-performed PowerPlex Fusion® at this level.

Family reconstructions

System	Tested Sister	Tested Sister	History Flight sample	RI
AC004027	2	2	2	2.3670
MLS26	1,2	1,2	2	0.5132
79712	1,2	1,2	1,2	1.4006
NBC216	1,2	1,2	1	0.4952
NBC106	2	2	2	2.3820
RG148	1,2	2,1	1,2	1.6265
NBC13	1,2	2	2	1.1028
AC2265	1	1	1	1.5511
MLS09	1,2	2,1	1,2	1.4278
AC1141	1,2	1,2	1	0.5317
TARBP	1,2	2,1	1	0.5480
AC2305	2	2	1,2	0.6358
HS4.69	2	1,2	1,2	1.0563
NBC51	2	1,2	1,2	1.0011
ACA1766	2	1,2	2	2.9417
NBC120	1,2	1,2	1	0.6940
NBC10	2	2	2	2.3670
NBC102	1,2	2,1	1,2	1.4505
SB19.12	2	2	2	1.7243
NBC148	1,2	2,1	1,2	2.5941

Shown here is an example of family reconstruction carried out using Innotyper data from a probative bone sample to confirm a putative identification. Analysis was performed using the Brutus pedigree calculator from eDNA.

CRI Pop = 48.9714
Probability = 97.9988%



Conclusions

- Both systems produced probative data for the contemporary bone samples, given the higher power of discrimination of PowerPlex ®Fusion, it would probably be the best choice for samples of this nature.
- Both systems gave probative data for older bone samples, but InnoTyper™ gave easily the most complete profiles and would likely be a better choice of system for generating genomic marker information. This might however, need to be balanced against the higher discriminating power of other systems, depending on the nature of the reference samples available.
- Mixtures may be harder to spot with a bi-allelic system such as InnoTyper™, this should be taken into account when selecting samples and during analysis.

Additional Considerations

- Is the lower discriminating power of a bi-allelic system a drawback for identification which rely on reference samples from more distant relatives.
- Can InnoTyper™ data be statistically combined with other genomic marker data?