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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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.

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 investigator’s toolkit. 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 (~90-125 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 kit on low yield, highly degraded, and challenging forensic samples.

Materials and methods - Brief

DNA extraction – Isolation

The ends of the bones were drilled using a sterile razor and DNA extracted using a Maxwell® magnetic bead extraction system (Promega). The kit used was the Maxwell® 16 Multicore (US DNA purification kit) and the extraction was carried out according to the manufacturer’s instructions and entered into 72µl of buffer.

DNA extraction – Biofilm

Both systems gave some data, even at very low input levels, but InnoTyper™ outperformed PowerPlex Fusion™ at this level.

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 (UNIT-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 bone specimens. 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 relation to the amount of DNA sampled. 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™ outperformed PowerPlex Fusion™ at this level.

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 very much 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?