Developmental validation of InnoTyper® 21, a nuclear DNA typing system based on retrotransposable element polymorphisms for degraded forensic samples

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Introduction

The InnoTyper® 21 kit, developed for use in human identification, is a small amplicon DNA typing system for challenging forensic samples that is compatible with currently used PCR/CE instrument platforms. It contains 20 bi-allelic Alu retrotransposon markers and Ancelogens for gender identification. It is the first DNA typing system that has been developed utilizing di-allelic Alu insertion polymorphisms. Due to the insertion and null/no-insertion allele states, the markers have been termed PONI®. NMI® markers are suitable for human identification because they are stable inheritance dependent polymorphisms that are not deleted, and the likelihood of parallel independent insertions is essentially zero. A novel “smartprimer” design strategy (Fig. 1) allows the size differences between insertion and no-insertion alleles to be less than 10 bp, despite the fact that Alu elements are ~500 bp long; also, all 21 markers are between 60 bp to 174 bp, which provides suitable use with STR typing samples.

Figure 1: Primary design strategy to target two insert and null/no alleles with similar but not exact amplicon size differences between the two allele states. This strategy employs a common forward primer with two “smartprimers” labeled "1" and "2" and two specific primers to amplify either insertion allele or null allele or both in all cases of a letterside insert.

The kit is a 5-dye system with 20 Alu markers and Ancelogens labeled in FAM, JOE, TAMRA, and ROX, with T2 QOrange used for the size standard (Fig. 2). The sizes of the resultant allele ranges between 61 and 121 bp, making their small size useful for degraded DNA typing. Comparison with currently other available systems reveals the InnoTyper kit contains a much smaller overall size range (Fig. 3).

Results

Sensitivity Study

Table 1 shows sensitivity of InnoTyper® 21. This table shows a profile recovery, peak height, and peak height ratio. The results show that 100% of the DNA samples were amplified by the InnoTyper® 21 kit.

Non Probative Samples Study

Table 2 shows the results of samples that were degraded with a Covaris S2 Sonicator into 75, 100, 150, 200, and 250 bp. The InnoTyper® 21 kit was able to produce a profile in all 75, 100, 150, 200, and 250 bp samples.

Degradation Study: Effects of Fragmentation Level

Table 3 shows the degradation study results. It shows the mean profile recovery, peak height, and peak height ratio for each sample. The InnoTyper® 21 kit was able to produce a profile in all 75, 100, 150, 200, and 250 bp samples. The results show that 100% of the DNA samples were amplified by the InnoTyper® 21 kit.

Degradation Study: Effects of Input DNA Amount

Table 4 shows the results of degradation study. It shows the mean profile recovery, peak height, and peak height ratio for each sample. The InnoTyper® 21 kit was able to produce a profile in all 75, 100, 150, 200, and 250 bp samples. The results show that 100% of the DNA samples were amplified by the InnoTyper® 21 kit.

Population Database Study

Table 5 shows the results of population database study. The table shows the mean profile recovery, peak height, and peak height ratio for each sample. The InnoTyper® 21 kit was able to produce a profile in all 75, 100, 150, 200, and 250 bp samples. The results show that 100% of the DNA samples were amplified by the InnoTyper® 21 kit.

Materials and Methods

- qPCR quantitation: The InnoQuant® kit was used for all DNA quantitations. The kit contains 2 amosvalent targets: a “short” Alu based target of 80 bp in size, and a “long” target from a separate retrotransposon of 207 bp in size. Real-time PCR reactions were performed on the AB 7500 Real-Time PCR System using Applied Technologies Brilliant Multiplex QPCR Master Mix as follows: 10 min at 95°C, and 12 cycles of 30 sec at 95°C, and 60 min at 63°C. Degradation Index (DI) was determined by the ratio of the short quantity value and the long quantity value (DI = short/long).
- Capillary electrophoresis: All STR Genetic Analyzer was utilized with POPES. Data analysis was performed with GeneMarker® and/or GeneMarker ID-X.

Other Results

- Accuracy, Precision, and Reproducibility: The determinations of the sample allele sizes from the allelic ladder sites were all less than ±0.1 bp. The genotypes of the DNA samples amplified in triplicate produced the same profiles.
- Species Specificity: Three nucleic acids produced with DNA from non-primate species. Chimpanzee and a lesser extent orangutan and green monkey yielded partial profiles.
- Idiomatic Study: A full profile was obtained in the presence of humans ≤ 45 µM, melanin up to 10 ng/µL (the highest amount tested), or hematin ≤ 50 ng/µL with 600 µg DNA input. A partial profile was observed with humans up to 60 µg and melanin up to 600 ng.

Concluding Summary

The InnoTyper® 21 kit is a robust and reliable complement to conventional STR kits and is an appropriate alternative when attempting to profile challenging single source samples, such as degraded human remains, hair shafts with no root, paraffin-embedded bones and other sample types where STR testing has failed to generate a useful profile.

Additionally, the InnoTyper® 21 kit is more discriminating than mtDNA sequencing, which is one of the few options for characterizing extremely degraded single source samples. Thus, a forensic analyst has alternatives in deciding which typing system to use, especially when confronted with a compromised DNA sample that yields sufficient DNA for only a single analysis.

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