DEVELOPMENTAL VALIDATION OF InnoTyper® 21, A NUCLEAR DNA TYPING SYSTEM BASED ON RETROTRANSPOSABLE ELEMENT POLYMORPHISMS FOR DEGRADED FORENSIC SAMPLES

Hiromi Brown, Ph.D., Gina Murphy, M.S., Anne Montgomery, M.S., Jonathan Tabak, B.A., Sudhir K. Sinha, Ph.D., InnoGenomics

The InnoTyper® 21 kit, developed for use in human identification, is a small amplicon DNA typing system for challenging forensic samples and is compatible with currently used PCR/CE instrument platforms. It contains 20 bi-allelic Alu retrotransposon markers and Amelogenin for gender identification. This is the first human DNA typing system that has been developed utilizing di-morphic Alu-insertion polymorphisms. Due to the insertion and null/no-insertion alleles we have named them INNUL markers. INNUL markers are suitable for human identification since they are stable inheritance dependent polymorphisms that are not deleted, and that the likelihood of parallel independent insertions is essentially zero. Our primer designing strategy allows for the size differences between insertion and no-insertion alleles less than 10 bp despite the fact *Alu* elements are ~300 bp long, and also all the 21 markers are between 60 bp to 124 bp, which gives an advantage for typing degraded DNA samples.

Results from the developmental validation study composed of multiplex optimization, species, sensitivity, precision/reproducibility, mixture, inhibition, degradation, non-probative sample studies. The population frequencies and discrimination power of the system will also be presented.

Our results demonstrate that the InnoTyper® 21 kit is highly sensitive and can provide a full 21 marker profile from as low as 100 pg of total DNA. Usable partial profiles can be obtained from 30-40 pg of highly degraded DNA samples. For highly degraded low level DNA samples InnoTper 21 produced results with higher power of discrimination that would typically require mtDNA sequencing. Extracted DNA samples were quantified and assessed for the extent of degradation as Degradation Index (DI) using InnoQuant® Human DNA Quantification & Degradation Assessment Kit prior to DNA typing. Higher DI indicates more DNA degradation. DNA extract from 2 cm hair shaft without any root, which had ~7 DI showed 88 % profile recovery with 100 pg DNA. For highly degraded DNA samples, the InnoTyper® 21 kit produced >95 % profile recovery up to 75 DI at which AmpFlSTR® Identifiler® Plus provided about 15 % allele recovery with 200 pg DNA.

The InnoTyper® 21 kit is a useful and robust 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 roots, paraffin embedded tissues and other sample types where STR testing has failed to generate a useful profile.