TRIPLE PRIMED PCR OF SE33: A NOVEL METHOD FOR ALLELE SEQUENCE DETERMINATION

Kerry Mullaney[1], MFS, Carolyn Hill[2], MS, and Daniele Podini[1], PhD
[1]The George Washington University, Department of Forensic Sciences, 2100 Foxhall Rd NW, Washington DC, 20007, USA
[2]National Institute of Standard and Technology, 100 Bureau Drive, MS8314, Gaithersburg, MD, 20899, USA

The SE33 locus is currently the most polymorphic Short Tandem Repeat (STR) locus and is included in many of the next generation forensic STR kits. The SE33 alleles are characterized by having a typical 4bp (AAAG) length variation and an additional sequence polymorphism, a single hexanucleotide (AAAAAG), that occurs once at different locations within the repeat region but doesn't affect the overall length. Because of this complex repeat pattern, the 70 different length alleles have multiple sequence variations (up to 13). This increases the total number of alleles to 171. The conventional Polymerase Chain Reaction (PCR) method can only distinguish length variation, limiting the discrimination power of this locus. The conventional allele sequencing method is to isolate the allele from an agarose gel run of a singleplex PCR amplification of the locus followed by Sanger sequencing. This is an expensive, cumbersome and time consuming process. The purpose of this study is to evaluate the use of Tripled Primed PCR (TP-PCR) for allele sequence determination of SE33. TP-PCR uses a triad of primers: a locus specific and fluorescently labeled flanking primer (P2), a primer with the repeat unit on the 3' ($[AAAG]_6$) end and a non-binding non-human DNA sequence on the 5' end (P4), and a paired primer of the same nonhuman tail sequence (P3). In the first amplification cycles, the repeat specific primer P4 anneals at multiple sites along the repeat region, generating several PCR products differing by one repeat. When P4 encounters the hexanucleotide unit AAAAAG it cannot anneal effectively resulting in a gap in the fragment sizes produced. This gap is subordinated to the location of the hexanucleotide repeat within the sequence. In later amplification cycles, P2 and P3 amplify the fragments produced by P2/P4, preventing the gradual shortening of the average PCR product due to P4 priming at sites internal to the PCR products of earlier rounds. Conventional capillary electrophoresis is then performed allowing alleles of the same length, but different sequence, to be differentiated by the mixture of PCR fragments produced during amplification. During the interpretation phase, for correct sequence determination of the two alleles at a locus the conventional allele call must be known.

A pilot study using samples with allele sequence determined *via* Sanger sequencing resulted in 100% concordance between the inferred allele sequence, based on the observed TP-PCR profile, and the known sequence on the sample set tested. Additional samples are being analyzed in a blind study.

This study demonstrates that TP-PCR could be used to infer SE33 allele sequence without the need for Sanger sequencing, utilizing a process very similar to conventional STR typing and the same technology. This would allow crime labs, for which conventional sequencing is simply impractical, to be able to significantly increase the discrimination power of locus SE33. Finally this approach could be applied to other loci with common internal sequence variation, like D21S11 and FGA, providing additional information that could help, for example, in complex paternity testing or wherever the power of discrimination needs to be maximized. ⌘