G161 Triple Primed PCR for SE33 Allele Sequence Determination

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After attending this presentation, attendees will understand what the Triple Primed Polymerase Chain Reactiom (TP-PCR) method is, how it works, and how it can be used to sequence alleles of the SE33 locus. Attendees will also have a better understanding of the discrimination power of the SE33 locus.

This presentation will impact the forensic science community by presenting a new technique to determine allele sequences without Sanger sequencing. TP-PCR is an inexpensive, sensitive and fast alternative for allele sequencing. The application of this method will enhance the discrimination power of the most polymorphic marker in current Short Tandem Repeat (STR) kits.

The locus SE33 is currently the most polymorphic STR in forensic DNA kits. The alleles of the SE33 locus are characterized by having a typical four base-pair (AAAG) length variation as well as an additional sequence polymorphism due to a single hexanucleotide (AAAAAG) insert that occurs once within the repeat region. This complex repeat pattern is represented as $[AAAG]_x$ AAAAAG $[AAAG]_y$. Because of this complexity, the 70 different length alleles can have up to 13 different sequence variations for a total of 171 alleles. But, the locus-specific primers (P1, P2) of the conventional Polymerase Chain Reaction (PCR) method utilized in forensic DNA typing can only distinguish length variation, limiting the discrimination power of this locus.

Previously, the only method to determine allele sequence was Sanger sequencing, an expensive and time-consuming process. This study demonstrates a novel method for allele sequence determination of locus SE33 using the TP-PCR. This method uses a triad of primers: a locus specific, fluorescently labeled flanking primer (P2), a primer with the repeat unit on the 3' 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).

During early amplification cycles, the P4 primer anneals directly to the repeat region, producing multiple fragments of varying length. But, the two additional adenine nucleotides of the hexanucleotide unit prevent the P4 primer from binding across this region. This interruption produces a 31 bp gap in the successively longer PCR fragments that correlates directly with the location of the hexanucleotide repeat within the sequence. In later cycles, the paired primer P3 and the fluorescently labeled flanking primer (P2) exponentially amplify the mixture of fragments produced by the P4 primer. This prevents a gradual shortening of the average PCR product due to the P4 primer annealing to sites within the sequences of products of earlier amplification cycles.

Each peak on the resulting electropherogram represents a specific number of repeat units within the SE33 allele. The smallest peak represents the 6 AAAG repeats present in the P4 primer and each successive peak an additional repeat unit. The 31bp gap between peaks separates the fragments produced from the P4 primer binding before and after the hexanucleotide repeat within the sequence. Consequently, by simply counting the peaks on each size of this gap the allele sequence is determined. This study establishes that this technique is powerful enough to differentiate the allele sequences of samples genotyped as homozygous with conventional STR kits. Furthermore, this technique deduced the correct sequence of the previously unpublished 16.2 and 17.2 alleles.

This study demonstrates that TP-PCR is a single reaction method that can be used to maximize the discrimination power of SE33 by allowing allele sequence determination without Sanger sequencing.

SE33, Allele Sequencing, Triple Prime PCR

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