A SYSTEMATIC APPROACH FOR REDUCTION OF STR STUTTER PEAK HEIGHTS FROM LCN SAMPLES

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When small amounts of DNA are extracted from forensic evidence, methods of increasing PCR product yield are attempted to obtain more complete STR profiles. An increase of the number of PCR cycles is one of several approaches to increase the sensitivity of detection. However, stochastic problems, e.g., increased stutter and heterozygous peak height imbalance, can occur. An increase in the peak height of stutter alleles can complicate and possibly lead to incorrect interpretation of STR results, especially in mixed low copy number (LCN) samples. In this study, we attempted to reduce stutter height proportions during the PCR and concomitantly increase STR peak heights. For all experiments, 100 pg of DNA template were amplified at 30 or 32 PCR cycles using AmpFlSTR® Identifiler® PCR Amplification Kit (Life Technologies).

The various approaches were:

1) Processivity of DNA polymerase: lower processivity allows a greater opportunity for breathing of the DNA strands during PCR (Walsh et al., 1996). High processivity DNA polymerases were tested compared with AmpliTaq Gold® DNA Polymerase (Life Technologies; processivity - 50-60 bases). The Kod Xtreme™ Hot Start Polymerase with high processivity (Novagen; processivity - 120 bases) did not decrease the stutter proportion, showing a mean stutter proportion of 6.5% higher than stutter generated using AmpliTaq Gold.
2) Strand-displacement activity of DNA polymerase: strand-displacement activity of a polymerase can affect slippage (Viguera et al., 2001). The Deep $Vent_R$® DNA polymerase (both wild and exo- types, New England Biolabs) was evaluated. Deep $Vent_R$ was not compatible with the Identifiler kit format, showing many allele drop-out loci.
3) Single-stranded DNA binding protein (SSB): SSB inhibits slippage of Taq polymerase during primer extension (Viguera et al., 2001). SSB (New England Biolabs) was added to the PCR to attempt to reduce stutter proportion. At 1500 ng of SSB, the stutter ratio decreased an average of 7.7% compared with absence of SSB samples. However, the true allele peak heights decreased as well.
4) Modified PCR conditions using SSB and MinElute PCR purification kit: the annealing and extension portions of the PCR conditions, in conjunction with the addition of SSB and the use of MinElute kit (Qiagen) were evaluated. Lower temperature conditions were tested to determine if slippage could be affected. The modified PCR conditions were 11 min at 95°C hotstart, 32 cycles of 1 min at 94°C denaturation and 3 min at 56°C annealing/extension, with 60 min at 56°C final extension. This protocol decreased stutter ratio 16.1% compared with the standard Identifiler protocol.
5) Modified PCR method and Polyethylene Glycol 8000 (PEG 8K): macromolecular crowding can increase the binding of DNA polymerase to template-primers complex (Zimmerman et al., 1987). It was determined that 2.5 % (w/v) PEG 8K increased peak height of STRs. The combination of the modified PCR method and addition of PEG 8K was tested for its affects on STR results. The combination method increased true allele peak heights on average as 37.5% compared with the standard Identifiler protocol with the MinElute kit and concomitantly decreased stutter proportion approximately 13.3%.

The results of these studies support that LCN typing stochastic effects can be reduced. Indeed sensitivity of detection can be increased and stutter proportions decreased when typing templates of 100 pg of DNA. Further studies are underway to assess the system with smaller template quantities. Such improvements show that LCN typing can be made more robust than possible with current approaches employed.

References:

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