CONSENSUS PROFILING: BUILDING CONFIDENCE IN LOW-TEMPLATE DNA CASEWORK SPECIMENS

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Low-template DNA samples are subject to uneven sampling and irregular amplification characterised by the presence of stochastic effects such as heterozygote imbalance, increased stutter, allele dropout and drop-in. The profiles thus obtained are frequently partial, and challenging to interpret. According to interpretational guidelines it is important to define the stochastic threshold value in order to account for these PCR-based stochastic limitations. There are a number of enhanced procedures introduced as alternative methods to setting a threshold for low template DNA samples, such as increasing PCR cycle number and increasing injection time. The consensus method on the other hand does not require an interpretation threshold. This interpretation model relies on deducing a consensus profile using multiple separate PCRs of a sample, where only duplicated alleles are reported. Replication therefore acts as the mechanism to confirm allele validity.

Through previous validation studies, we have validated the PowerPlex ESI 16 kit as most sensitive and have established its analytical and stochastic thresholds at 50 rfu and 200 rfu respectively. The high sensitivity of this amplification kit allows for the retrieval of maximum data from limited input DNA and damaged samples; however the set stochastic threshold is high so that allelic information is lost if interpretation is based upon a single injection. For this reason, in our laboratory we perform a three amplifications consensus to address uncertain profiles (from single or mixed source) that display stochastic effects, requiring an allele to appear in at least two out of three PCR replicates to be included as a true peak in the constructed profile.

In this work we present the results of casework samples for which the consensus method has been applied. In our hands, consensus analysis helped correct for the stochastic effects, as we observed a bettered profile quality in terms of reliability, because consensus provided us with an increase in exploitable data that we could use to confirm peaks.

Description:
This work presents our laboratory's beneficial expertise with the consensus method using a highly sensitive amplification kit (PowerPlex ESI16). Limited information is obtained from low-level DNA samples due to the set stochastic threshold. The consensus approach surpasses the limitations of stochastic data. Replication acts as the mechanism to confirm allele validity. It allows for reliable recovery of maximum exploitable peaks.

Key words: Consensus, replicate PCR amplification, stochastic threshold, low-template DNA.