Deterministic Design and Implementation of Single Nucleotide Variant Genotyping by High Resolution Melting

Adam L. Millington1; Jana O. Kent, PhD2; Carl T. Wittwer, MD, PhD2
1Department of Pathology, Univ. of Utah, Salt Lake City, UT

Introduction/Background

PCR and high resolution melting is potentially the simplest and fastest method for genotyping single nucleotide variants (SNVs). PCR primer design is a first and crucial step of assay development. Overwhelming amount of available software with numerous criteria commonly yield disappointing primer pairs requiring re-design. We propose a simple method for both primer design and optimal PCR conditions development so that the process works well the first time.

Materials/Methods

The 52 forensic SNVs were selected as the target for our study. PCR primers were designed by Adam™ software (Figure 1). Universal PCR protocol for simultaneous amplification of all 52 SNVs was developed, consisting of a 2-step touch down (Figure 5.).

Results

Using Adam™ primer design and universal protocol, we successfully amplified and produced melting curves for all 52 SNVs. In 7 out of 52 cases, the melting curves of the wild-type (WT) and mutant homozygote (HOM) SNVs were indistinguishable due to sequence thermodynamics. All 7 cases were correctly predicted by uMELT (https://dna.utah.edu/umelt/umelt.html). The reliability of this prediction allows us to incorporate automatically designed snapback genotyping probes on the 5’ end of one of the PCR primers in order to type 100% of WTs and HOMs (Figure 3, 4).

Conclusions

A simplified primer design algorithm provided clear PCR results in 100% of the targets tested when amplified according to the universally optimized PCR protocol. The algorithm did not discriminate against primers with sequence runs or repeat, or sequences producing primer-dimers. This method reduces the complicated task of assay design to a user-friendly and effective algorithm requiring only knowledge of the target sequence and a basic thermodynamic calculation.

References:

Contact Information: adam.millington@path.utah.edu carl.wittwer@path.utah.edu