Materials and Methods

The software requires only the rs# of the target SNV to create an assay. Public data for uDesign is queried via the user’s input rs#. The rs# retrieves the variant information as well as the surrounding sequence from NCBI through E-utils. The resulting information is parsed from the query response and the primer design algorithm places the primers one base pair away from the target SNV, continually adding nucleotides until the melt temperature ($T_m$) reaches the user’s desired $T_m$. If $\Delta T_m$ of homoduplex products is less than 0.3°C, a snapback probe is designed and displayed to the user.

Results

Once the user has submitted a rs# and the design algorithm is completed, the full amplicon, individual primers, and melting curve predictions are displayed. Melting curves are plotted and labeled for both genotypes and duplexes. In addition, melting temperatures of both primers and the product, GC content, the alleles present, and the frequency data retrieved from NCBI are displayed. Users may change the default primer melting temperature, adjust laboratory conditions, move primers or place constraints on primer length. A set of optimized PCR cycling conditions is also listed.

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Contact Information

zach.dwight@path.utah.edu

Website

DNA.UTAH.EDU