Design Software for Small Amplicon Genotyping by High-Resolution Melting

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Introduction

High-resolution melting is a straightforward technique for genotyping that relies on thermodynamic differences of PCR products and distinguishable melting profiles. Often, researchers use a variety of sources to design, validate, and assess PCR primers without the context of designing the final PCR product. To help aid researchers in designing assays with minimal input, we developed web accessible software, uDesign, to streamline the small amplicon design process. uDesign automates sequence retrieval, primer design, melting curve predictions for all products, and hairpin design if nearest-neighbor symmetry exists.

Figure 1: Guidelines of Design

1. Create 1bp buffer around SNV
2. Place 3' end of both primers next to buffer region
3. Sequentially add nucleotides until melt temperature (T_m) reaches user's desired primer T_m
4. If ΔT_m of homoduplex products is less than 0.3°C, a snapback probe is designed and displayed to user

rs#733164 >>

GCCATCCCACTTGGAAA[G/A]TTTGCCTGACATTCCTGAG

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-utilities. The resulting information is parsed from the query response and the primer design algorithm places the primers one base pair from the target SNV, continually adding bases until both primers have melting temperatures (T_m) close to 60 °C (default value can be changed by user). Once primers are selected, the amplicon is assembled and predicted melting curves are calculated and displayed for all genotypes and duplexes using the uMelt algorithm. Product melting temperatures are derived from the curves along with the ΔT of the homozygous products. Primer melting temperatures are calculated using nearest-neighbor thermodynamics. If the ΔT of the homozygous products is less than or equal to 0.3 °C, the algorithm appends a snapback tail to the forward primer. A snapback tail on the forward primer allows genotyping by melting the hairpin formed after dilution.
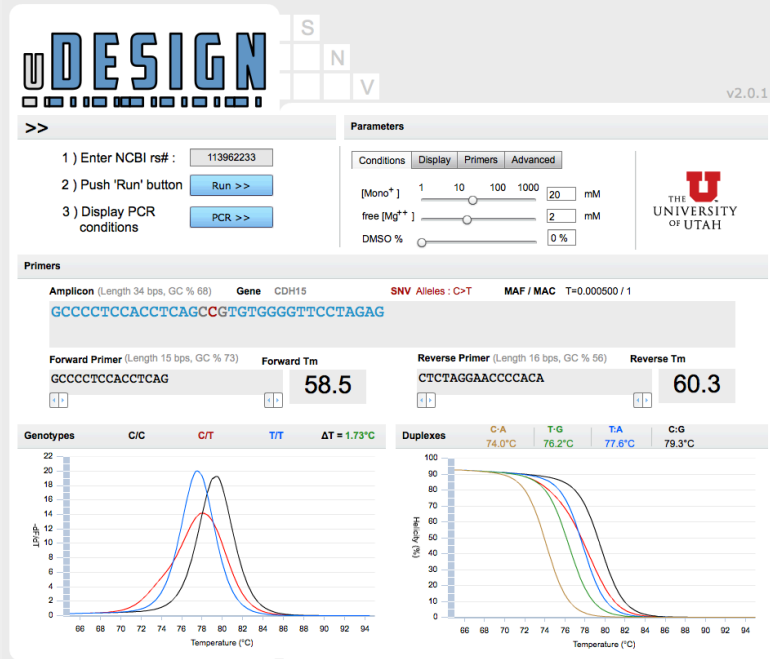
Results

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.

Figure 2: Experimental Validation
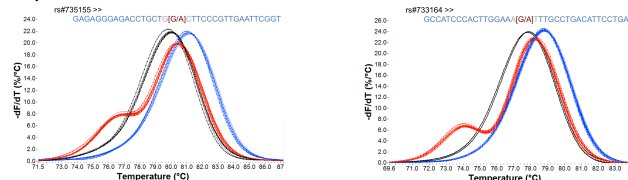
Discussion

By combining algorithms for primer design, nearest-neighbor thermodynamics, and melting curve prediction with NCBI web services, uDesign provides a singular, automated interface for design of small amplicon assays. The software was built with Adobe Flex 4.5 and is freely available without registration in a web application at 'https://www.dna.utah.edu/udesign/index.html'.

Figure 3: Computational Workflow
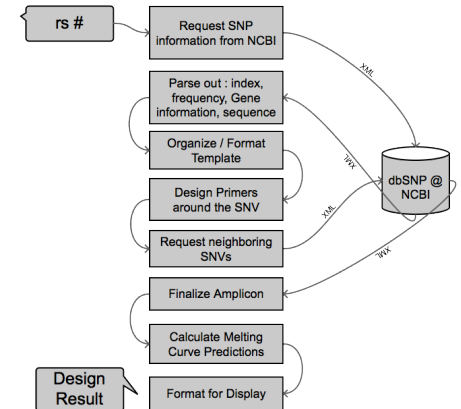
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