uDESIGN

User Guide 1.0b

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Query

Enter the rs# of interest

Parameters

Adjust parameters if necessary on the corresponding tabs such as lab conditions.

Display parameters control the melting curve graph density and temperature range. Users can also output nearest neighbor Tm values rather than recursive Tms.

Primer constraints may be placed on the design algorithm such as desired Tm and length of primers.
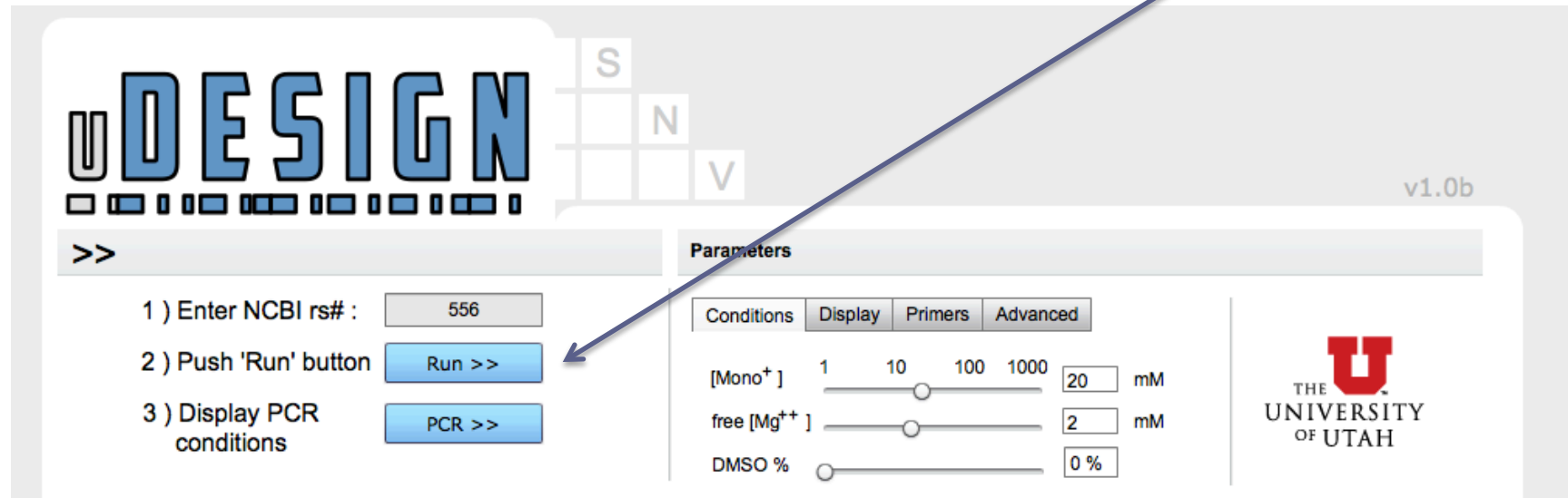
Analyze

Push 'Run' button
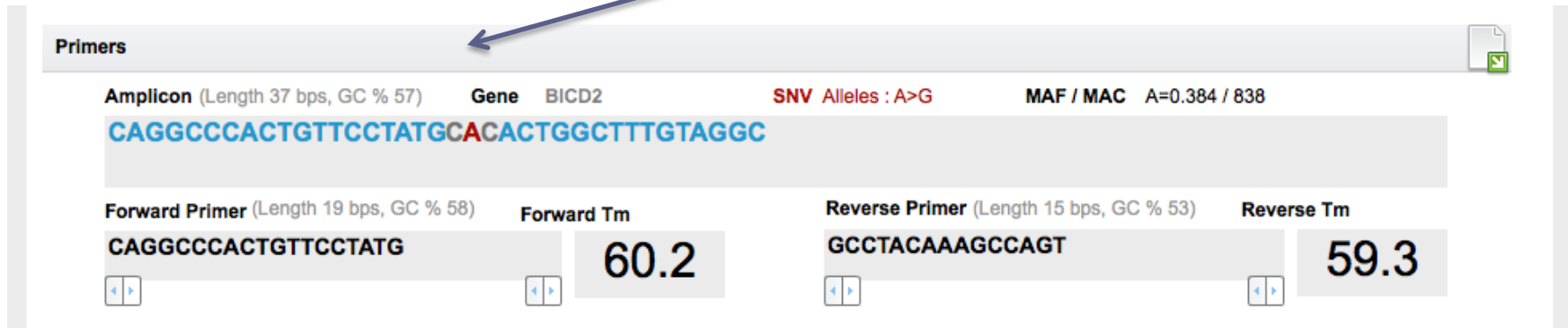
Design

Primers and full amplicon design is displayed on run complete.

Primers

Amplicon (Length 37 bps, GC % 57)   **Gene** BICD2   **SNV** Alleles : A>G   **MAF / MAC** A=0.384 / 838

CAGGCCCACTGTTCCTATGCACACTGGCTTTGTAGGC

Forward Primer (Length 19 bps, GC % 58)   **Forward Tm**

CAGGCCCACTGTTCCTATG   60.2

Reverse Primer (Length 15 bps, GC % 53)   **Reverse Tm**

GCCTACAAAGCCAGT   59.3

Information queried and calculated :
  Amplicon : Length, GC%, Gene, SNV Alleles, and Minor
  Allele Frequency and Count (MAF / MAC)
  Primer : Length, GC%, Tm
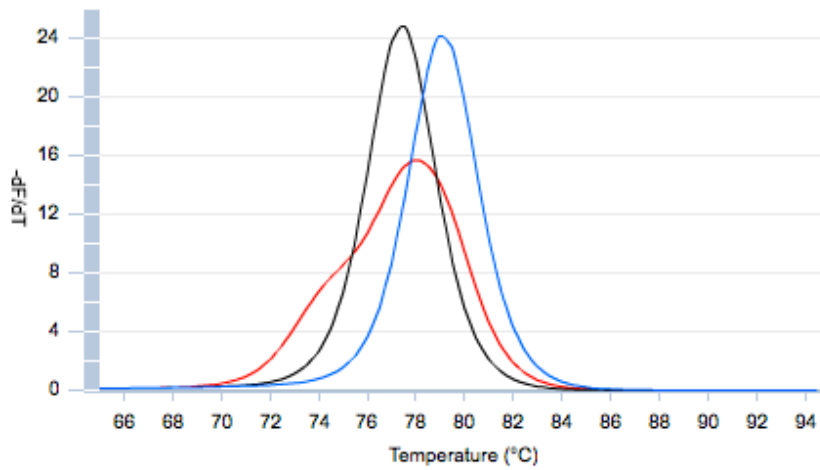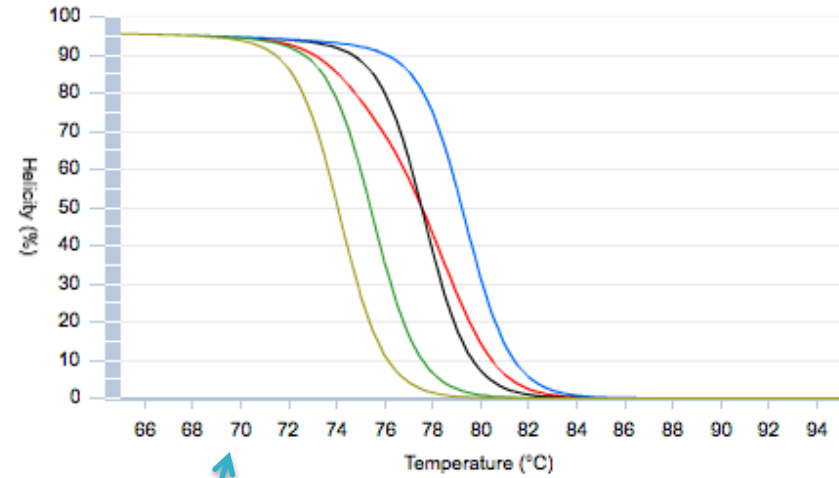
Melt Curves

Melting curves with Tms are displayed as genotypes and duplexes below the design.

>>

Parameters

1) Enter NCBI rs# : 556

2) Push 'Run' button Run >>

3) Display Tabs Input >>

Suggested PCR Cycling Conditions (2mM [MgCl2])
Initial Denaturation 95 C for 30 s.
Ten cycles of Touchdown (85 C for 5 s, 73 C for 5 s, decreasing 1C each cycle to 64 C).
Thirty cycles of 85 C for 5 s, 63 C for 5 s.
Heteroduplex formation 95 C for 5s, cooling to 40 C.
Melting : 0.1 C/s for 60-90 C.

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Primers

Amplicon (Length 41 bps, GC % 54)   **Gene** BICD2   **SNV** Alleles : A>G

CAGGCCCACTGTTCCTATGCACACTGGCTTTGTAGGCATTC

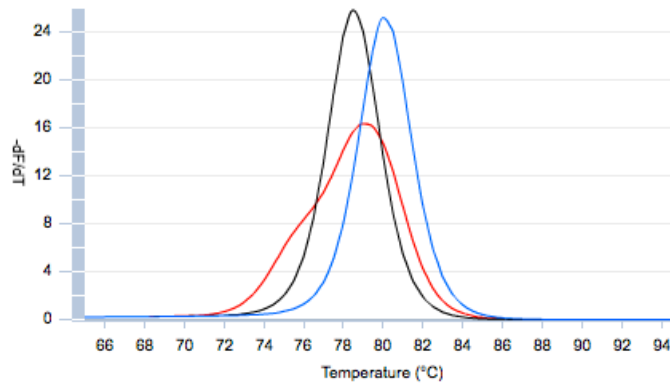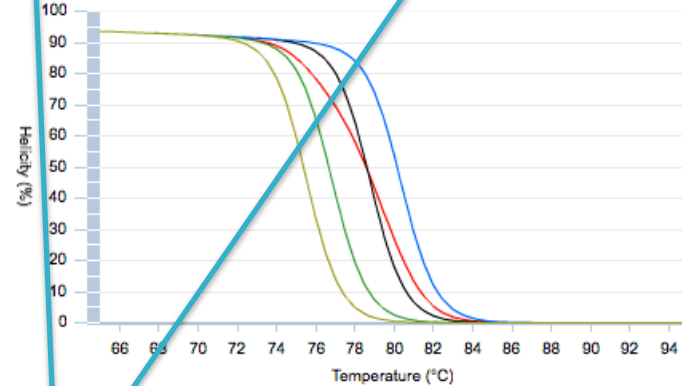**Forward Primer** (Length 19 bps, GC % 58)   **Forward Tm**
CAGGCCCACTGTTCCTATG   60.2

Reverse Primer (Length 19 bps, GC % 47)   **Reverse Tm**
GAATGCCTACAAAGCCAGT   63.3

Gentotypes   A/A   A/G   G/G   ΔT = 1.55
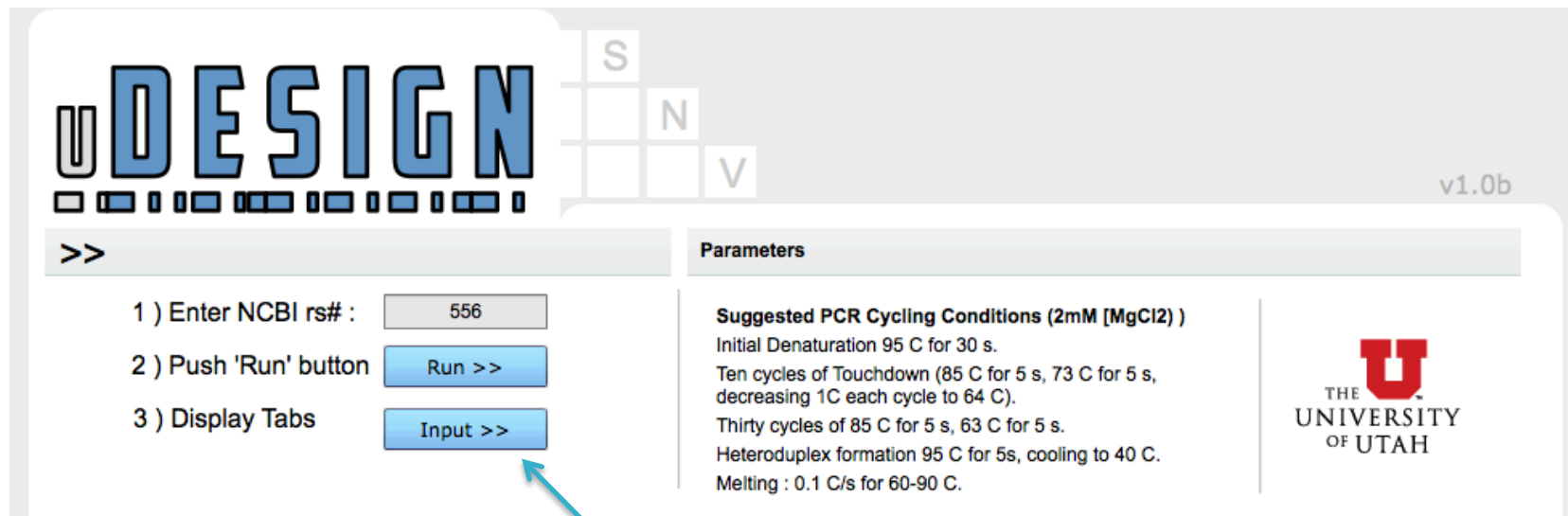
Duplexes   A·C 75.4   G·T 76.7   A:T 78.6   G:C 80.2

Primers

Designed primers can be adjusted for Tm or to improve melting curve shape in real time with the primer controls.

Protocol

A protocol designed by the Wittwer Lab was used and the suggested PCR conditions are available within the application.

Future Development

uDesign is currently in beta : meaning it works well and has most (75%) of the features we'd like to provide users. However, we are always open to feedback, improvements and features that have value to our users. Our software group can be contacted through : zach.dwight@path.utah.edu.

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