



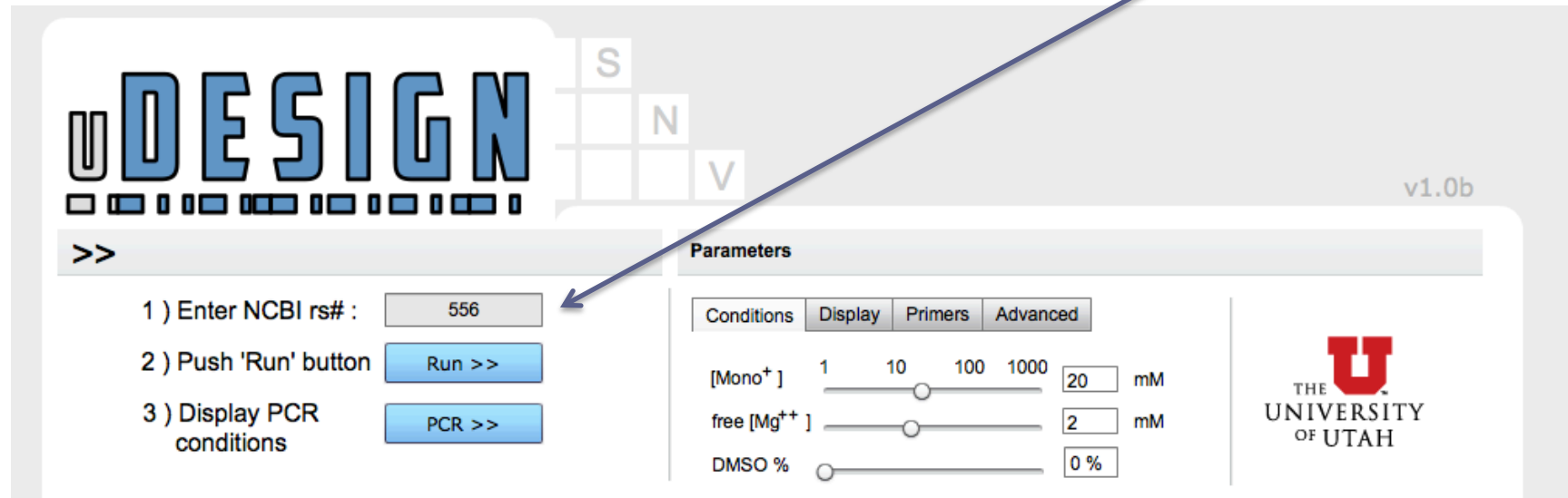
User Guide 1.0b

Available @
DNA.UTAH.EDU



Query

Enter the rs#
of interest



UDESIGN v1.0b

>> Parameters

- 1) Enter NCBI rs# : *(Arrow points to this field)*
- 2) Push 'Run' button
- 3) Display PCR conditions

Parameters

Conditions | Display | Primers | Advanced

[Mono⁺] 1 10 100 1000 mM

free [Mg⁺⁺] mM

DMSO %

THE UNIVERSITY OF UTAH

Parameters

Parameters

Conditions

Display


Primers

Advanced

[Mono⁺] 1 10 100 1000 mM

free [Mg⁺⁺] mM

DMSO %



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 T_m values rather than recursive T_ms.

Conditions

Display

Primers

Advanced

Start Resolution

Finish Nearest Neighbor

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

Conditions

Display

Primers

Advanced

Primer T_m

60

Min Length

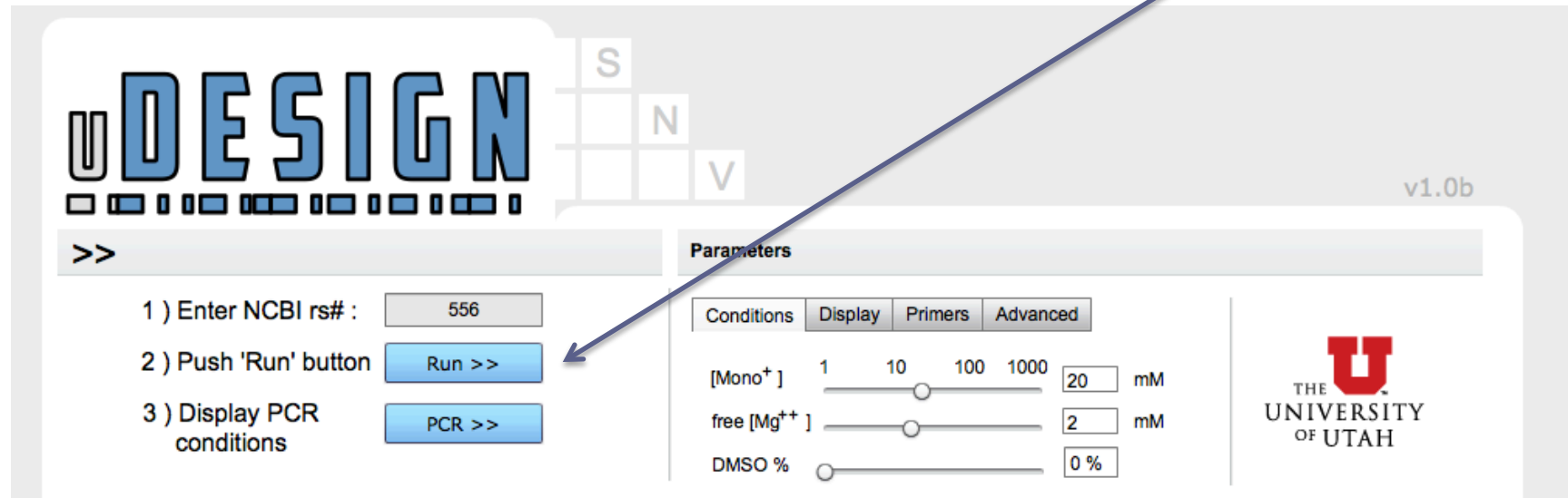
10

Max Length

40

Analyze

Push 'Run' button



The screenshot shows the UDESIGN software interface. On the left, there is a list of instructions:

- 1) Enter NCBI rs# :
- 2) Push 'Run' button
- 3) Display PCR conditions

 An arrow points from the text 'Push 'Run' button' to the 'Run >>' button.

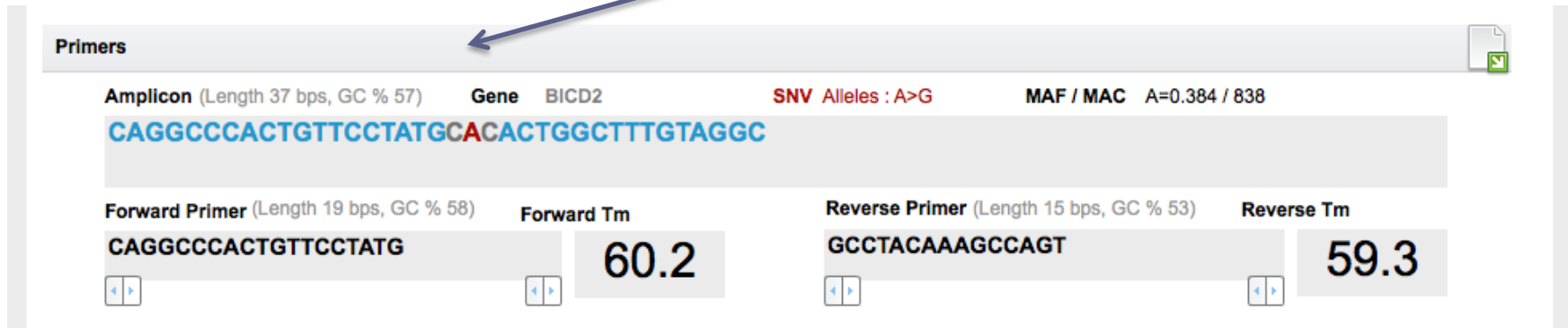
The main interface area is titled 'Parameters' and contains several tabs: 'Conditions', 'Display', 'Primers', and 'Advanced'. The 'Conditions' tab is active, showing sliders and input fields for:

- [Mono⁺] : 1 10 100 1000 mM
- free [Mg⁺⁺] : mM
- DMSO % : %

 The University of Utah logo is visible in the bottom right corner of the interface.

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

CAGGCCCACTGTTCTATGCACACTGGCTTTGTAGGC

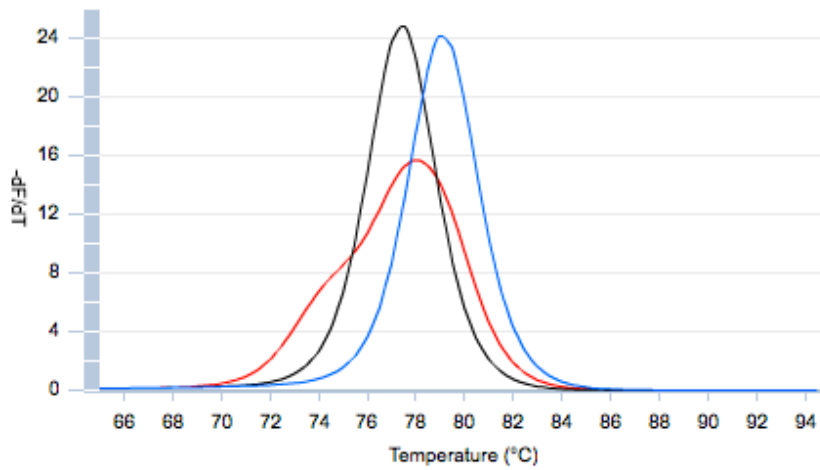
| Forward Primer (Length 19 bps, GC % 58) | Forward Tm | Reverse Primer (Length 15 bps, GC % 53) | Reverse Tm |
|---|-------------|---|-------------|
| CAGGCCCACTGTTCTATG | 60.2 | GCCTACAAAGCCAGT | 59.3 |

Information queried and calculated :

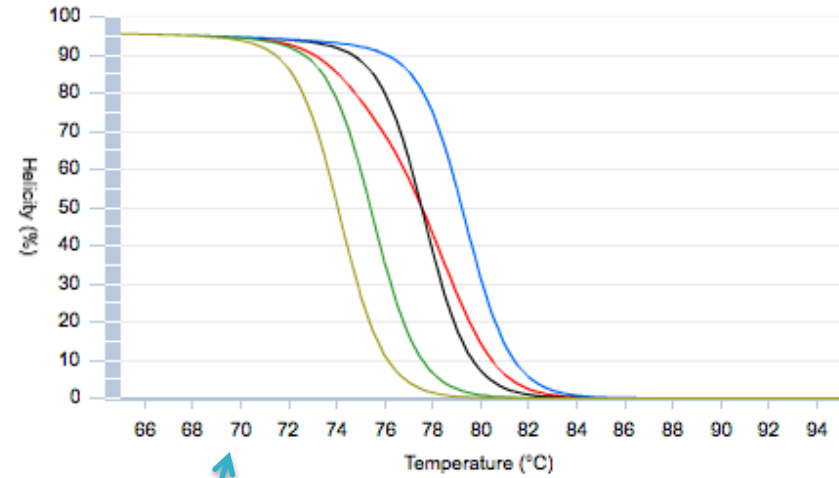
Amplicon : Length, GC%, Gene, SNV Alleles, and Minor Allele Frequency and Count (MAF / MAC)

Primer : Length, GC%, Tm

Genotypes A/A A/G G/G $\Delta T = 1.68$



Duplexes A·C 74.1 G·T 75.4 A·T 77.5 G·C 79.2



Melt Curves

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

>>

Parameters

- 1) Enter NCBI rs# :
- 2) Push 'Run' button
- 3) Display Tabs

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.



Primers

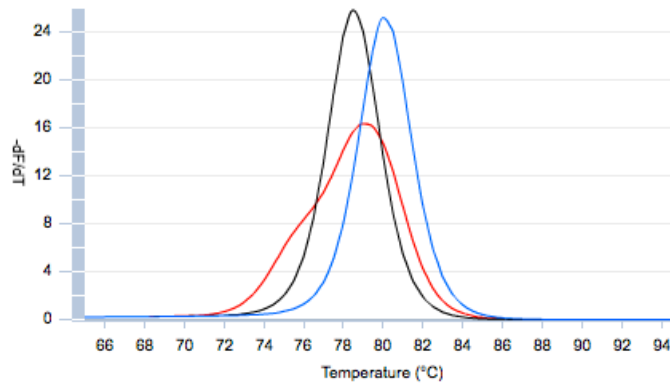
Amplicon (Length 41 bps, GC % 54) Gene BICD2 SNV Alleles : A>G

CAGGCCCACTGTTCCCTATGCACACTGGCTTTGTAGGCATT

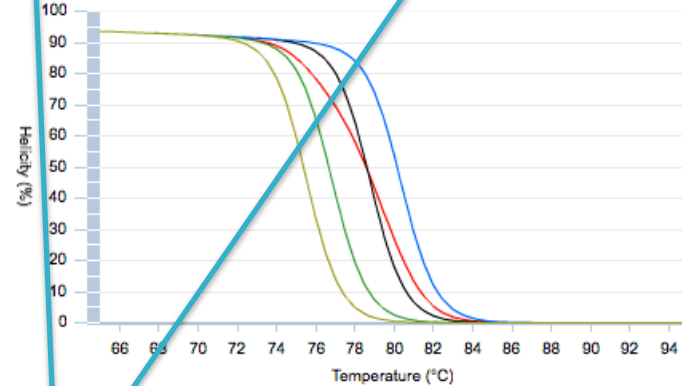
Forward Primer (Length 19 bps, GC % 58) Forward Tm
 CAGGCCCACTGTTCCCTATG

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

Genotypes A/A A/G G/G $\Delta T = 1.55$



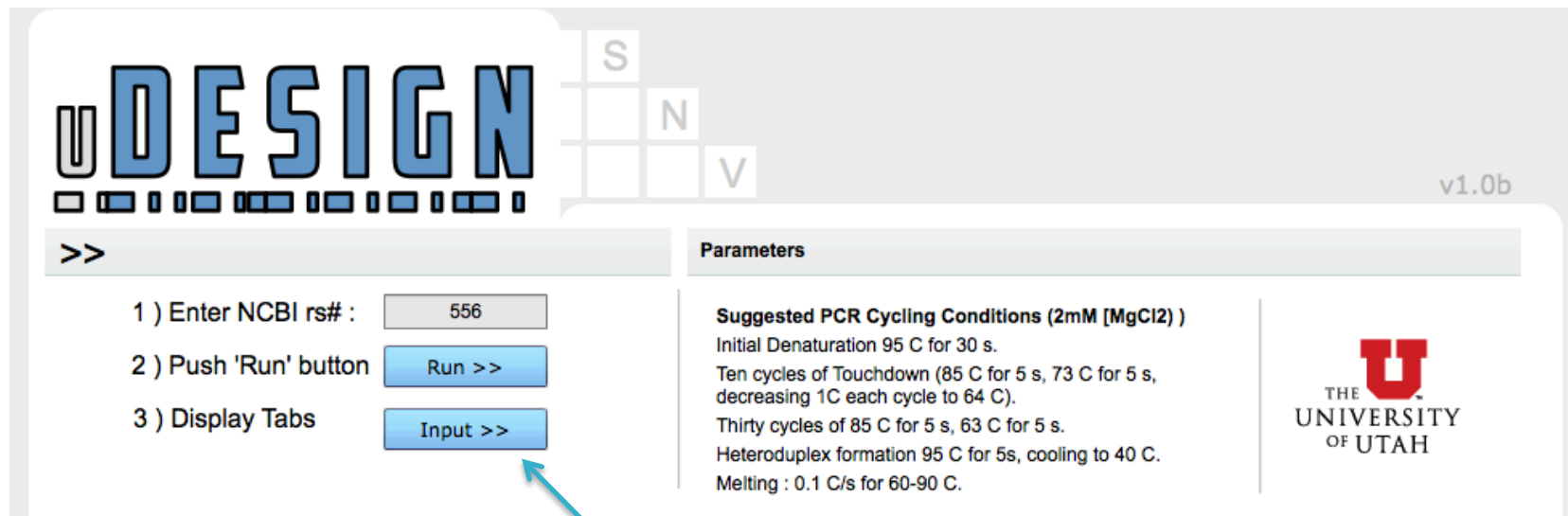
Duplexes A-C 75.4 G-T 76.7 A-T 78.6 G-C 80.2



Primers

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

Protocol



The screenshot shows the UDESIGN application interface. At the top left is the UDESIGN logo. To its right is a grid with letters S, N, and V. Further right is the version number v1.0b. Below the logo is a command prompt area with a double greater-than sign (>>). The main area is divided into two sections. The left section contains a list of instructions: 1) Enter NCBI rs#: 556, 2) Push 'Run' button, and 3) Display Tabs. The right section is titled 'Parameters' and contains 'Suggested PCR Cycling Conditions (2mM [MgCl2])' with the following details: 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, and Melting : 0.1 C/s for 60-90 C. The University of Utah logo is visible in the bottom right corner. A blue arrow points from the 'Input >>' button to the 'Parameters' section.

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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