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## Introduction

Designing molecular assays for PCR and high-resolution melting often requires convoluted workflows utilizing a variety of software applications and websites. Data sources are cumbersome for novice users and advanced computational expertise is a requirement to consume digital genetic resources. uVariants is a web-based, streamlined assay design and SNP context tool that requires user input of: 1) rs# and 2) the number of flanking bases for investigation. Annotation surrounding the SNP of interest includes the location of neighboring variants, exon/intron casing, minor allele frequencies, and specificity assessment of possible 3' primer placement. The software presents visual, contextual information and allows the user to place their own primers, with real time thermodynamic parameters, and automatically consolidates, saves and sends the results to other tools and formats.

## Materials and Methods

The rs number is the only required input but is accompanied by an option to define a specific number of bases flanking each side of the queried variant. A customized ENSEMBL database of variants is queried with the rs number provided by the user to determine neighboring variant locations and characteristics. A second query is required via web services (NCBI E-utils) for the most up to date minor allele frequency information. Neighboring SNPs are annotated on a frequency-based color gradient to visualize severity to possible primer placement and are linked to their respective dbSNP data at NCBI (<https://www.ncbi.nlm.nih.gov/projects/SNP/>). The user then highlights regions of the sequence to define primers on the annotated sequence. Upon placement, the primer sequence is sent via web service to an internal server script to determine primer melting temperature (nearest-neighbor thermodynamics), length, and GC-content, which is then displayed to the user.

The rs# input creates a one step query to retrieve valuable design context in the target area.

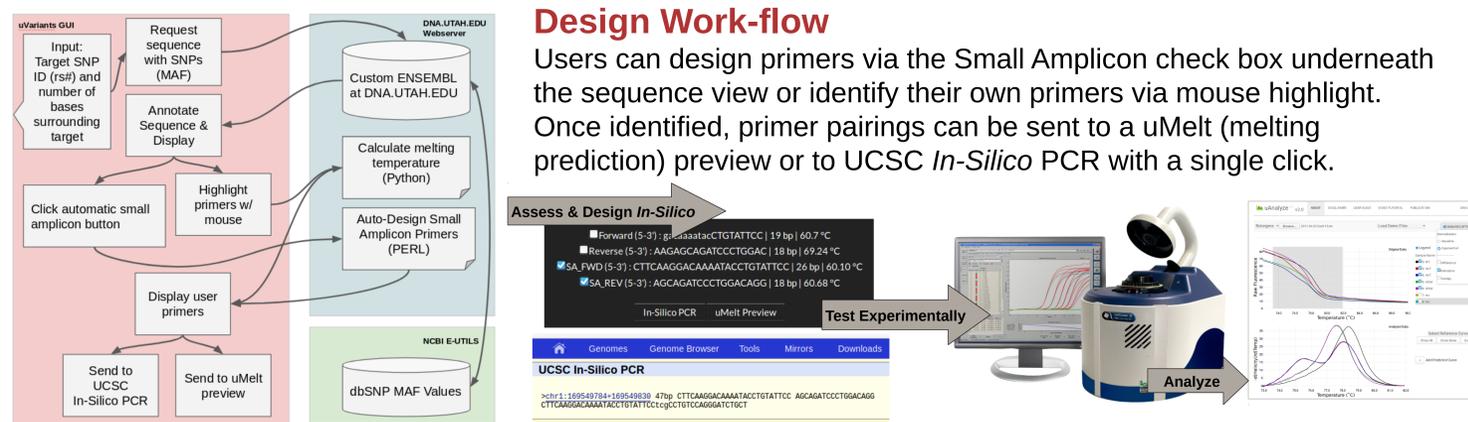
Once sequence is returned, hover over annotated nucleotides for more information:

- (X/Y) SNP of Interest
- (X/Y) Neighboring SNPs with Frequencies Reported
- (X/Y) Neighboring SNPs with No Frequency Reported
- (X) Nucleotide (as 3'end) has high frequency in genome
- (Y) Nucleotide (as 3'end) has low frequency in genome

Users may design primers themselves with simple mouse highlight interaction or click the small amplicon button for automated assay design.

Check for primer dimer or send primer pairings to uMelt or *In-Silico* PCR for product assessment.

Figure 1: uVariants user interface found at: <https://www.dna.utah.edu/variants>

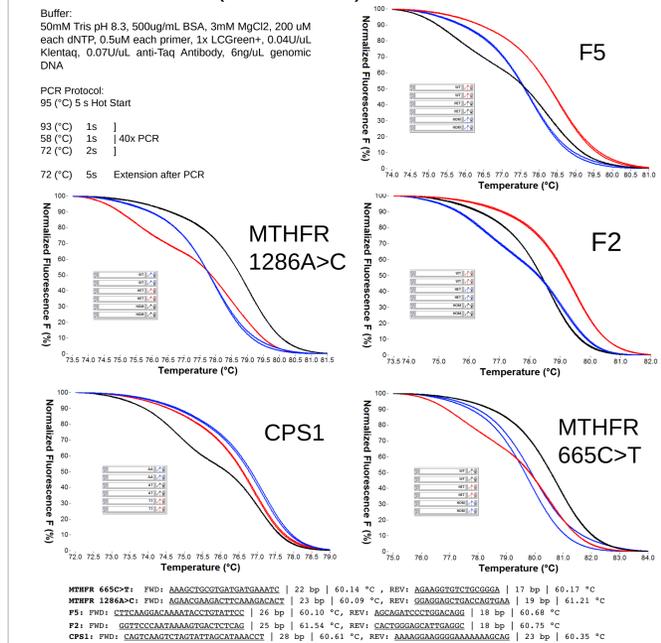


## Results

Once the user has submitted the rs number and the data retrieval / annotation algorithm is completed, all information is displayed. The user can place as many primers as necessary and a running list is maintained. The list of primers is interactive - the user can select any primer pairing to send to other tools such as uMelt (product melting behavior) or UCSC *In-Silico* PCR (specificity). All the resulting SNP, sequence, and primer information may be exported and saved for future use in a non-proprietary format (.csv).

## Experimental Testing

The automated small amplicon design feature was tested using CPS1 (rs#3219784), F5 (rs#6025), F2 (rs#1799963), MTHFR 1286A>C (rs#1801131), MTHFR 665C>T(rs#1801133).



## Conclusion

uVariants provides a quick and easy interface to interrogate a SNP and the surrounding genomic region for the purpose of designing robust high-resolution melting assays. Paired with our web-based melt curve analysis software (uAnalyze), users have access to a variety of tools and web services to help stream line the assay design, assessment, and analysis processes. The software is freely available, without registration in a web application, at :

<https://www.dna.utah.edu/variants/>

## References

- Dwight et al. "uMELT: prediction of high-resolution melting curves and dynamic melting profiles of PCR products in a rich web application." *Bioinformatics*. 2011 Apr 1;27(7):1019-20.
- Liew et al. "Genotyping of single-nucleotide polymorphisms by high-resolution melting of small amplicons." *Clin Chem*. 2004 Jul;50(7):1156-64.
- Owczarzy et al. "Predicting stability of DNA duplexes in solutions containing magnesium and monovalent cations." *Biochemistry*. 2008 May 13;47(19):5336-53.
- SantaLucia Jr and Hicks. "The thermodynamics of DNA structural motifs." *Annu Rev Biophys Biomol Struct*. 2004;33:415-40.
- Yates et al. "Ensembl 2016." *Nucleic Acids Res*. 2016 44 Database issue:D710-6.

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