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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.

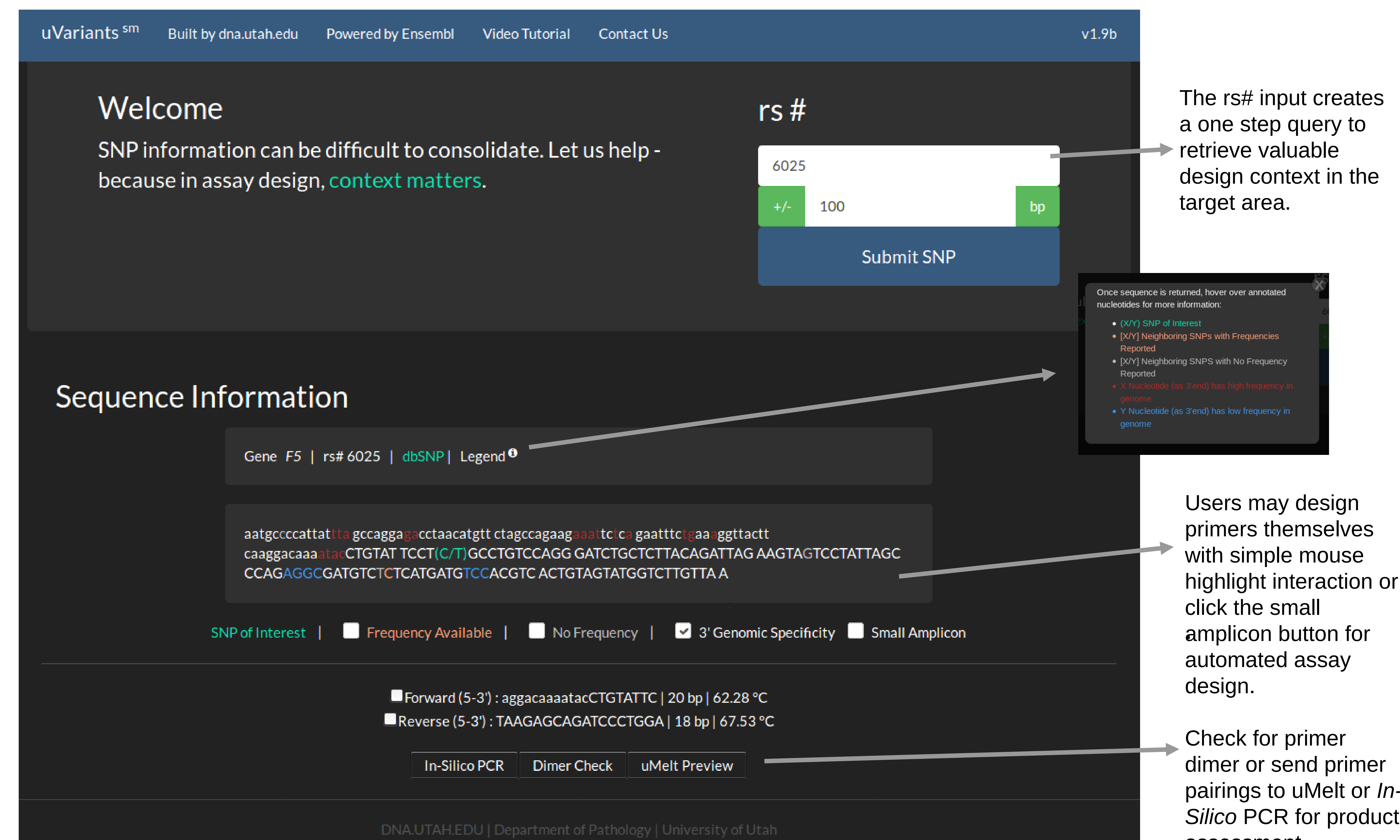
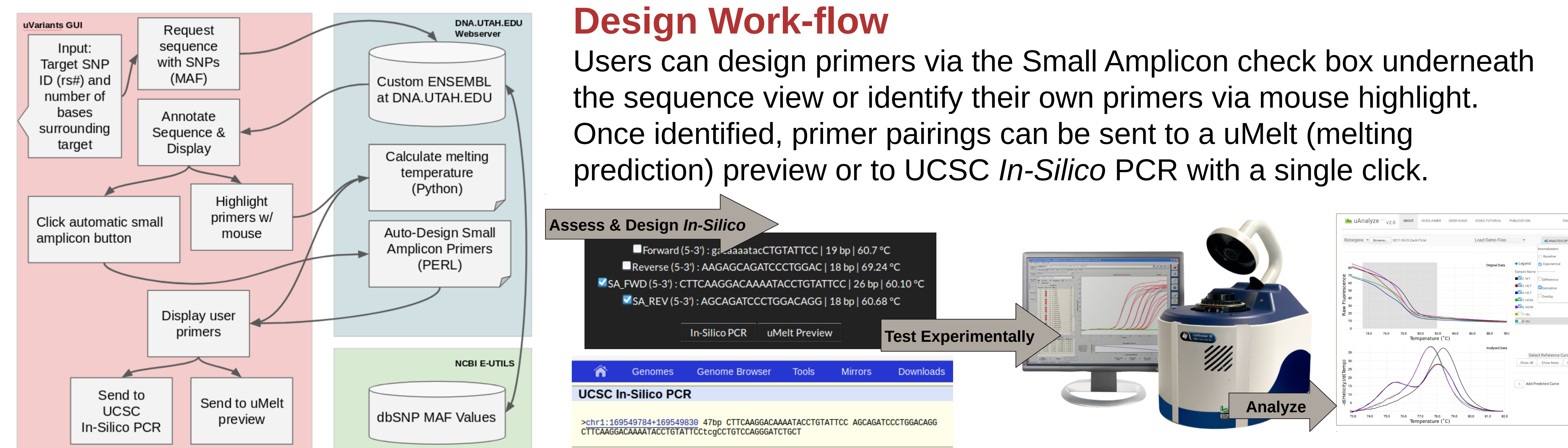


Figure 1: uVariants user interface found at: <https://www.dna.utah.edu/variants>

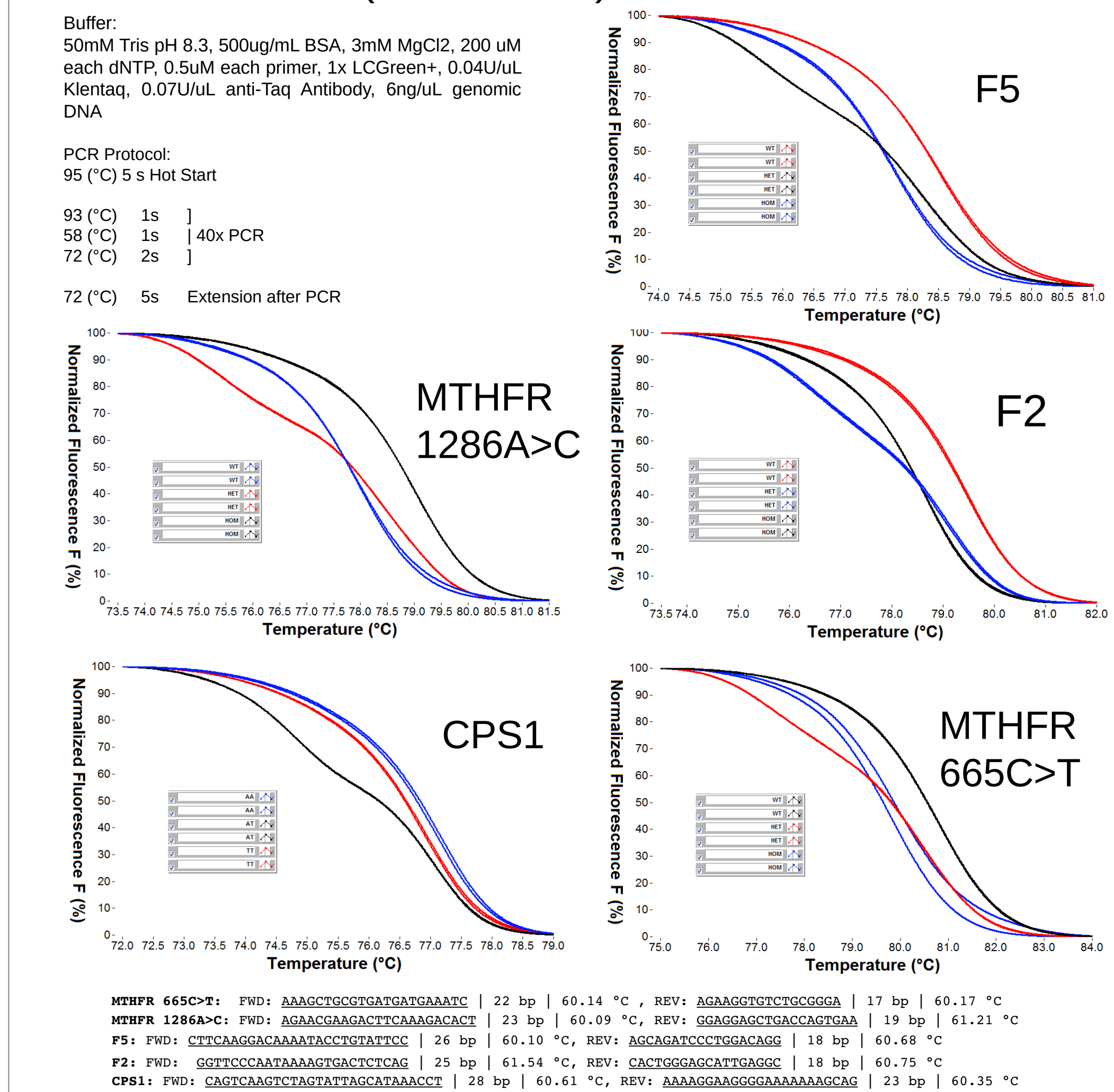


## Results

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

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