# Heterozygote PCR Product Melting Curve Prediction

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## Introduction

Melting curve prediction of PCR products is typically limited to perfectly complementary strands. Multiple domain melting curves are predicted using recursive nearest-neighbor thermodynamic calculations. A heterozygote product, however, is the composite of four PCR products: the two matched homoduplex products and the two mismatched heteroduplex products. Our goal was to weight the contribution of heteroduplex products to minimize the difference between predicted and experimental melting curves for each of the 6 different SNV types. Prediction of melting curves after amplification of heterozygotes is helpful in validation of diagnostic assays, primer design, and the study of reaction conditions on both melting curve shape and melting temperature.

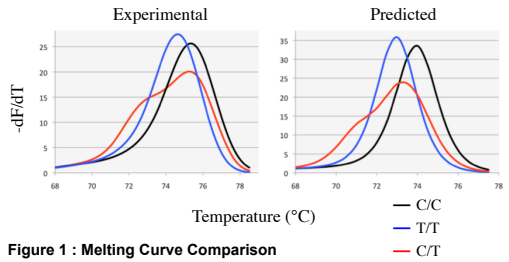


**Figure 1 : Melting Curve Comparison**

## Materials and Methods

Duplex contribution parameters were derived from brute force *in silico* computations of 52 forensic SNPs with uMelt[1] software (http://www.dna.utah.edu/umelt/umelt.html), modified to include mismatched nearest neighbors. The *in silico* predictions were quantitatively compared to experimental curves using the area between the two curves. Minimizing the area by incrementing the contribution of heteroduplex products to the heterozygous products determined the 'best fit'. The average heteroduplex contributions were determined for each variation type: (A/G), (G/C), (C/A), (A/T), (T/G), (C/T). An additional 200 melting curves were simulated to derive mismatch coefficients for nearest neighbor [Na+] and free [Mg++] thermodynamic adjustments. [2,3]

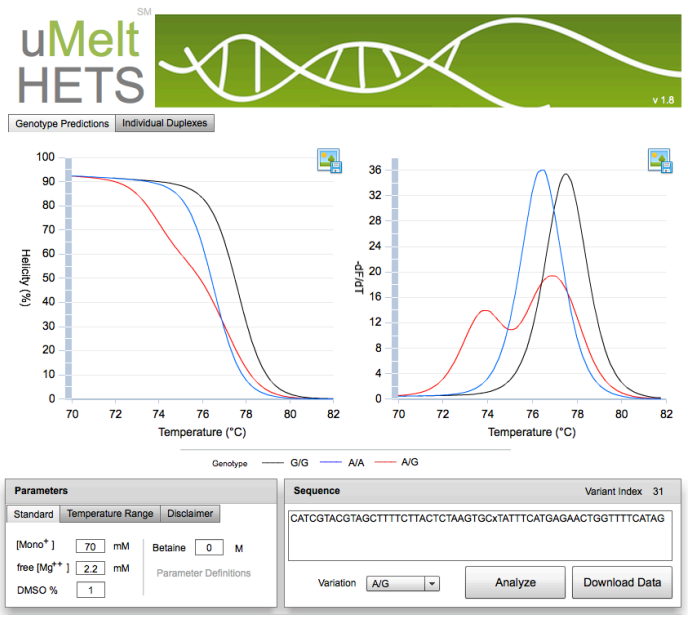


**Figure 2: Screenshot of uMelt HETS Web Interface**

## Results

By minimizing the error between experimental data and thermodynamic predictions, a set of SNV specific parameters was identified, including the heteroduplex contribution to the final composite heterozygote melting curve and [Na+] and free [Mg++] adjustments . Contribution to the final composite product was on average 72.5% (+/- 5.7) homoduplex and 27.4% (+/- 5.7) heteroduplex. Further analysis revealed the highest total heteroduplex contribution was seen with A/T SNVs (34%) followed by A/C (31%), G/T (30%), G/A (27%), C/T (26%), and C/G (20%), although statistical significance was only found between A/T and C/T (p=0.036) and A/T and C/G (p=0.044) using an unpaired t-test. A trend was also observed between A/T and A/G (p=0.10). Results also included adjustment coefficients for the mismatched tetrad thermodynamic calculations. The average [Na+] and free [Mg++] adjustments needed for a mismatched tetrad was ~15% less than a complementary tetrad.

| A/T | A/C | G/T | G/A | C/T | C/G |
|---|---|---|---|---|---|
| 77% | 93% | 99% | 71% | 86% | 84% |

**Table 1: Mismatch Tetrads [Na+] and free [Mg++] coefficients**

## Conclusions

Accounting for the contribution of heteroduplex products and the weakened effect of free magnesium and [Na+] on mismatched tetrads better matches the shape of experimental melting curves that result from the amplification of heterozygotes. The homoduplex contribution is approximately 3 times the heteroduplex contribution. Factors other than SNP type such as sequence content and PCR conditions may contribute to variation in the heteroduplex contribution. The prediction model is freely available in software form, uMelt HETS (http://www.dna.utah.edu/hets).

**Table 2 : Contribution % of PCR Product Melting Curves**

| SNP Type | Contribution % Het | Hom |
|---|---|---|
| A/T | 34% | 66% |
| A/C | 31% | 69% |
| G/T | 30% | 70% |
| G/A | 27% | 73% |
| C/T | 26% | 74% |
| C/G | 20% | 80% |

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## References

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