Predicting the Melting Temperature of Snapback Primer Genotyping Assays

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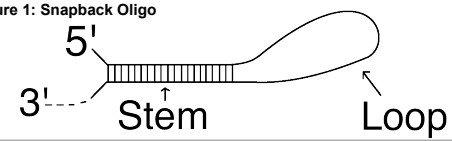
Introduction

If a 5'-tail is added to one PCR primer that is complementary to its own extension product, DNA hairpins form after asymmetric PCR, because the tail 'snaps back' and hybridizes to its own strand. If a single nucleotide variant is within the stem, melting analysis enables simple genotyping[1]. The implementation of such methods, however, requires the ability to accurately predict the melting temperature of the hairpin stem. Standard nearest-neighbor thermodynamics do not adequately account for the stabilizing effect of the hairpin loop. Prior work has shown that snapback Tms are linearly related to the number of base pairs in the stem and inversely related to the log of the loop size. We used multi-dimensional regression to analyze these trends and determine a simple equation that accurately predicts the Tm of single-stranded products.

Figure 1: Snapback Oligo



Materials and Methods

We propose a model equation where loopsize is the number of base pairs within the loop, Stem Tm is the nearest-neighbor predicted Tm of the stem, and A,B,C are coefficients of 'best-fit.' Further adjustment to the concentration term of the calculated StemTm was required as the loopsize increased and the two ends of the single strand behaved more like individual products. Sequences and experimental Tm data from 52 snapback genotyping assays were used as a training set. The values of coefficients were allowed to float until the linear fit algorithm implemented in LabVIEW 2011 returned the least residue.

Figure 2: Model Equation and Linear Algebra

$$T_M = Ax + By + C$$

$$x = \ln(LoopSize)$$

$$y = StemT_M$$

$$\begin{bmatrix} x_1 & y_1 & 1 \\ x_2 & y_2 & 1 \\ x_3 & y_3 & 1 \\ \vdots & \vdots & \vdots \\ x_n & y_n & 1 \end{bmatrix} * \begin{bmatrix} A \\ B \\ C \end{bmatrix} = \begin{bmatrix} T_{M1} \\ T_{M2} \\ T_{M3} \\ \vdots \\ T_{Mn} \end{bmatrix}$$

Figure 3: The Data Plane

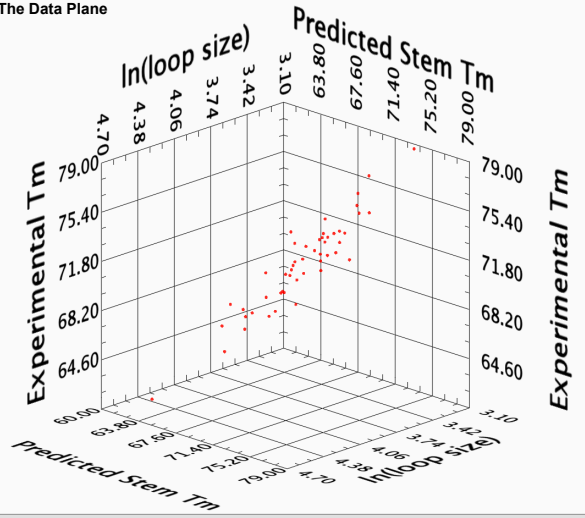
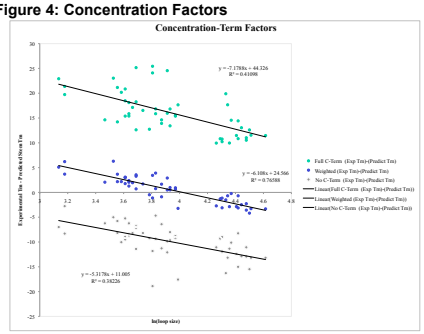


Figure 4: Concentration Factors



The treatment of primer/template concentration for a self-annealing single stranded product is not entirely clear. We proposed a constant, k, as shown in the equation below to affect the weight given to concentration term and found that it increased the bunching of the data points and allowed for a more accurate prediction

$$T_M = \frac{\Delta H}{\Delta S - k * R\ln(c/4)}$$

Results

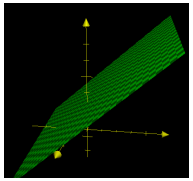
Our computational model produced the 'best-fit' coefficent values shown below. The difference between predicted and experimental Tms had an absolute mean deviation of 1.13˚C and a standard deviation of 1.61˚C. The tightness of this fit suggests that the equation can be used to predict the Tm of other similarly designed snapback genotyping assays to within 3˚C.

Figure 5: Final Coefficients and the Resulting Plane

$$A = -1.31$$

$$B = 0.77$$

$$C = 11.45$$



Conclusion

The simple equation presented in this work predicts the Tm of snapback PCR products within 3˚C. This estimate facilitates the design and interpretation of experimental melting curve data of snapback genotyping assays.

Acknowledgements

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Contact Information

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Citation

[1]Zhou L, Errigo RJ, Lu H, Poritz MA, Seipp MT, Wittwer CT. Snapback primer genotyping with saturating DNA dye and melting analysis. Clin Chem 2008;54:1648 –56.