Web Software for Design of Snapback Genotyping Assays

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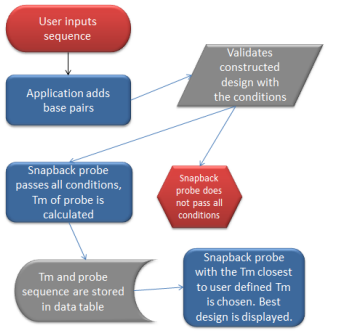
Introduction

DNA hairpins are useful tools in molecular diagnostics. PCR products amplified with a snapback primer show both hairpin melting at lower temperatures and full-length amplification melting at higher temperatures. The Tm of the hairpin duplex depends on the sequence of the target, thereby allowing genotyping. Snapback primer genotyping with saturating dyes provides the specificity of a probe using only 2 standard primers in a closed-tube system. Special covalent modifications of the primers are not required.[1]

Materials and Methods

Keeping the targeted base in the middle, different hairpin duplexes were constructed by sequentially adding bases to each end of the duplex. Additional criteria for hairpins included confirming a mismatched pair just inside the loop and incorporating 3'- mismatches to prevent extension (see Results). Melting temperatures (Tms) were obtained from nearest neighbor thermodynamic parameters and the loop size. The hairpin with the Tm closest to the user specification was then selected.

Probe Design Flowchart



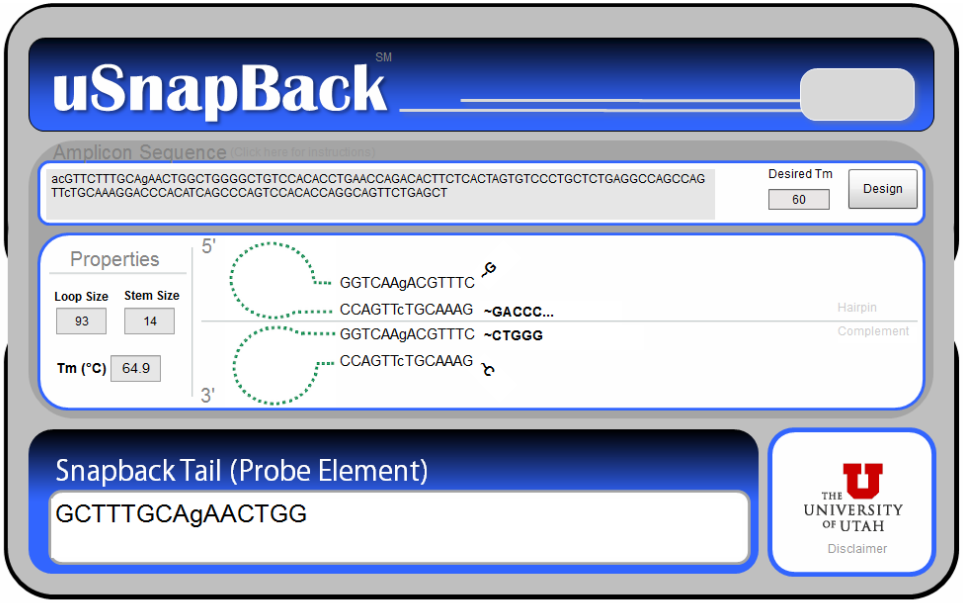
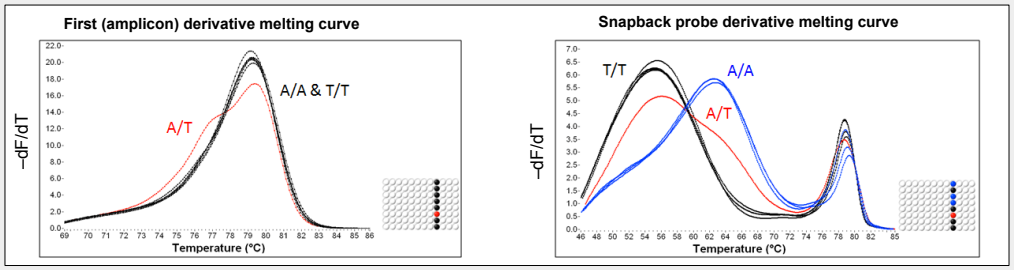


Figure 1: uSnapback screenshot of user interface found at: dna.utah.edu/usb/snap.php



Conclusion

Using simple criteria to check loop size and by adding 3'-mismatches to prevent extension, optimal snapback probe elements can be designed for the user based on preferred Tm and snapback thermodynamics. This prediction model is compiled in Flex and is freely available as the user web application, uSnapback (http://www.dna.utah.edu/usb/snap.php).

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Results

The following criteria allow the online tool to provide an optimal design for snapback primer genotyping.

Criteria

- **Loop inspection:** Loop inspection checks the loop length by looking for possible complementarity continuing into the loop region.

- **Extension Prevention**: To best prevent snapback probe extension from an extendable 3'-end, the most debilitating mismatch is incorporated (A·G, C·C, and G·A) when the next base on the template is an A, C or G.

- **Avoidance of T Mismatches.** When the next base on the template is a T, it is difficult to prevent 3'-end extension. Therefore, such duplexes are not considered.

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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. *Clinical Chemistry, (2008)* 1648-56.