

HP² CAPTURE PROBES

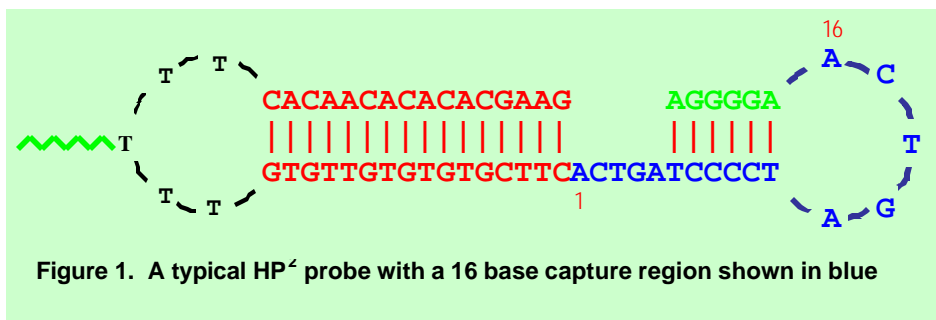
Sensitive · Specific

Introduction

As outlined in Technical Bulletin 101, hairpin (HP) capture probes containing a duplex region adjacent to a single-stranded target capture region hybridize to their respective targets with a thermodynamic advantage over linear probes as evidenced by increased sensitivity. However, in addition to sensitivity, DNA detection systems must be designed so that maximal specificity is also achieved. Tm Bioscience has addressed both of these issues with their novel HP² probes. HP² probes combine the high sensitivity achieved via stacking hybridization with increased specificity achieved through the incorporation of secondary structure within the probe's target capture region. Absolute control of DNA hybridization is the key step in the development of any successful nucleic acid-based testing strategy where the challenge is to uniquely recognize only the intended nucleic acid analyte. HP² probes will prove useful in applications where cross-talk is inherent such as in highly multiplexed systems (i.e. microarrays) or in the detection of single nucleotide polymorphisms (SNPs).

HP² Capture Probes

HP² capture probes are composed of the three major parts typical of an HP probe (i.e. duplex stem region, penta-thymidine loop, and single-stranded DNA capture region) with the added feature that the target capture region is able to fold back onto itself forming a second, less stable hairpin. Hence the name, HP². The secondary structure imposed on the target capture region results in increased specificity since this structure functions as the thermodynamic energy barrier which must be overcome and surpassed in order for hybrid formation to occur. Mismatched targets are unable to displace existing



structure in the target capture region and therefore do not hybridize. Of particular note is the fact that this structure may also be imposed on linear probes to increase specificity.

Experimental Approach

To demonstrate the value of HP² in increasing the specificity of hybridization, an experimental system was devised to directly compare HP to HP². Using this experimental system, we investigated and compared the effect of hybridizing targets resulting in perfectly matched duplexes to targets in which 3 of 16 bases were mismatched (approximately 80% similarity). This degree of similarity is typical of that found in cellular mRNA pools where similar genes or genes from multigene families are simultaneously expressed. Briefly, a perfectly matched target (M) as well as 10 mismatched targets (A to J) 16 bases in

length were hybridized to either HP or HP². The formation of the perfectly matched duplex was monitored using an indirect chemiluminescent assay and compared to the formation of mismatched duplexes where 2 of the mismatched base pairs remained constant at positions 3 and 14 while the third mismatched base pair moved along positions 4 to 13 for targets A to J, respectively.

Results

The ability of HP² to increase the specificity of hybridization as compared to HP is illustrated below in Figure 2. Performance is assessed by determining the signal of mismatched duplexes relative to that of the perfectly matched duplex and expressing this number as % of perfect match (y-axis). If one arbitrarily sets a limit of 10% as the allowable signal generated by a mismatched target relative to a perfectly matched target, then at low concentrations (A), all targets hybridized to HP² meet the criteria (i.e. zero cross-talk) whereas 3 of the 10 targets hybridized to HP generate signals greater than 10% of the perfect match (i.e. 30% cross-talk). At 10-fold higher target concentrations (B), again zero cross-talk is evident using HP² whereas with HP, 80% cross-talk exists (i.e. 8 of the 10 mismatched targets have signals greater than 10% that of the matched target).

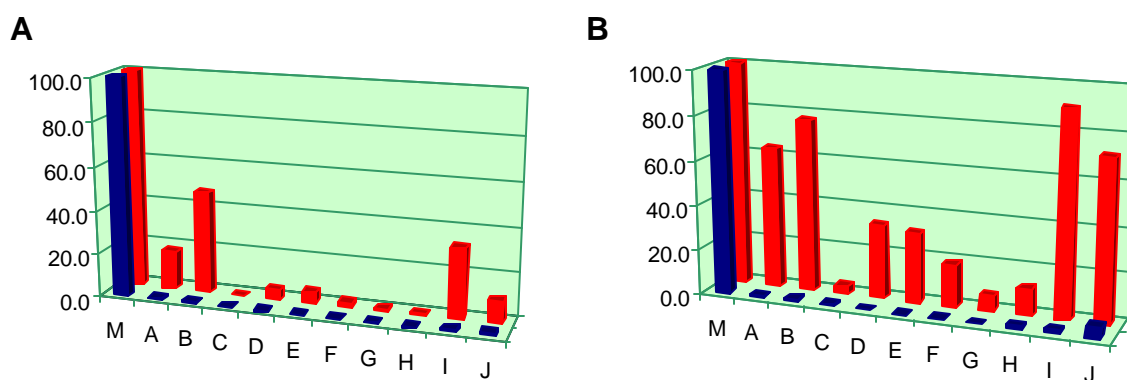


Figure 2. A comparison of HP (red) vs HP² (blue) in discriminating mismatched targets at 2 different target concentrations. (A) Represents a 1x target concentration whereas (B) represents a 10x target concentration. The y-axis, defined as % of perfect match, represents the percentage of signal generated by the mismatched targets relative to the perfectly matched target.

Conclusions

Our results clearly demonstrate the significant advantage of using HP² capture probes in **increasing the specificity** of hybridization. HP² probes were able to discriminate 10 out of 10 mismatched targets (zero cross-talk) whereas 8 of the same 10 mismatched targets were able to effectively hybridize with HP capture probes (80% cross-talk).

HP² probes **enhance the performance** of nucleic acid based assays by combining the **high sensitivity** achieved via stacking hybridization with **superior specificity** achieved through imposed secondary structure within the target capture region. HP² probes will prove especially useful in highly multiplexed systems such as DNA microarrays where multiple hybridization events are occurring simultaneously.

HP² Technology Status

A patent describing this technology and its uses has been filed. Companies interested in licensing this technology should contact: Dr. Jeremy Bridge Cook, VP Business Development at (416)-593-4323 ext. 229 or by e-mail at jbridgecook@tmbioscience.com.