

## HAIRPIN CAPTURE PROBES

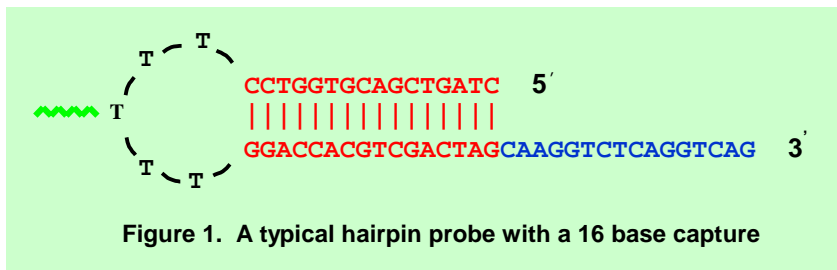
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### Introduction

Single-stranded linear oligonucleotide probes are commonly used in hybridization reactions. However, it has been shown that a hairpin capture probe, which contains a duplex region adjacent to a single-stranded target capture region, hybridizes to its target with a thermodynamic advantage over a similar linear probe. This target capturing advantage of the hairpin system can be attributed, at least in part, to the stacking interaction between the 5' terminal base of the hairpin probe and 3' terminal base of the single-stranded target. Moreover, recent studies have shown that the duplex region of a partly double-stranded DNA capture molecule can enhance its target capture ability (Lane et. al.). Tm Bioscience has put these observations into practise by developing hairpin (HP) capture probes and shown their potential utility in various nucleic acid detection assays.

### Hairpin Capture Probes

Hairpin capture probes typically consist of three major parts: 1) a duplex stem region; 2) a penta-thymidine loop, and; 3) a single-stranded DNA capture region. The central nucleotide in the loop is derivatized with an appropriate attachment moiety (ie. biotin, amino-link, etc.) to allow for coupling to a variety of solid support media including microwell plates, glass microscope slides and latex microspheres.



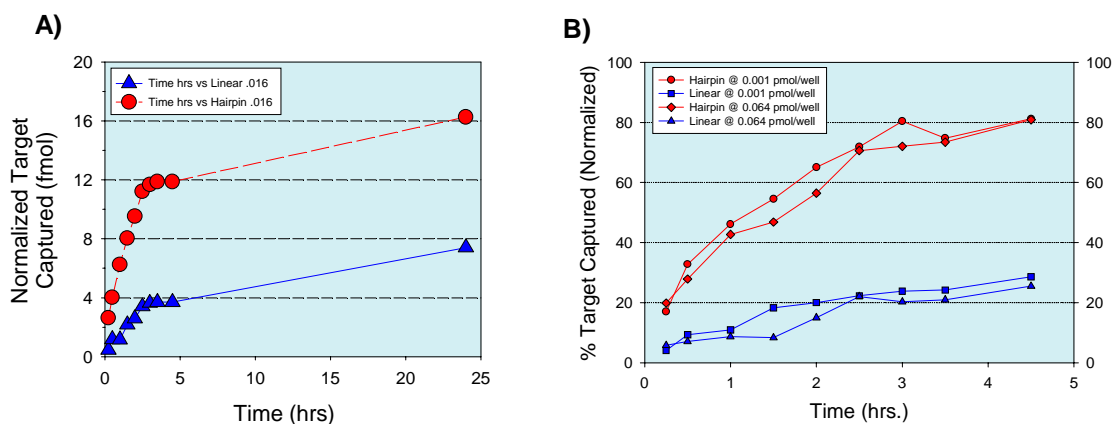
The target capture region consists of single-stranded DNA with a 3' dangling end of appropriate length which is identical to a typical linear probe. As with linear probes capture lengths can range from 10–30 bases depending on specific applications.

### Experimental Approach

To determine the precise benefit of HP probes, an experimental system was devised to directly compare HP to linear capture probes with identical target capture regions (Riccelli et. al.). Using this experimental system, the kinetics of target capture by the two types of probes as a function of target concentration and temperature under standard DNA assay conditions were investigated and compared. Capture assays were conducted in tandem under identical conditions for the hairpin and linear capture probes.

### Results

The target capture profile of a 32 mer HP and linear probe is shown in Figure 2. Under all conditions examined, hairpin probes consistently displayed higher rates of hybridization and greater total amounts of captured target. Rates of hybridization were at least four times greater for the HP relative to the linear capture probes at all target concentrations examined (see Figure 2A for results using 16 fmol. target). Ultimately, a greater concentration of target was captured at conditions near equilibrium (see Figure 2B). These characteristics are maintained irrespective of the input target concentrations tested (1-64 fmol).



**Figure 2. A. Rate of target capture for 32-mer HP and linear probes at 16 femtomole input target over a range of temperatures B. Comparison of percent target capture by 32-mer HP and linear probes.**

The 32 mer HP probes were able to capture approximately 80% of the input target in approximately 4 hours, whereas linear probes captured only approximately 20-30% of the input target.

To address the possibility that steric influences may limit the efficiency of target capture by linear probes as compared to HP probes, the spacer region between the attachment moiety and the capture region on the linear probe was investigated. Linear probes whose distance off the surface was increased, to mimic the surface-to-capture-region distance of HP probes, were *unable* to attain the HP's hybridization efficiency. This indicated that the distance from the surface was not the determining factor in improving capture kinetics by HP probes.

## Conclusions

Our results clearly demonstrate that HP probes exhibit significantly **higher rates of target capture** relative to linear capture probes (up to 4x better performance). Moreover, HP probes are capable of capturing greater quantities (up to 4x more) across a large target concentration range and perform significantly better at low target concentrations relative to linear probes. This results in **greater assay sensitivity**.

Hence, HP probes can **significantly enhance the performance** of nucleic acid assays, such as high throughput diagnostics, single nucleotide polymorphism (SNP) detection and microarray based gene expression profiling, where the current challenge is to develop more rapid and sensitive detection methods.

## HP Technology Status

This technology has been patented in the United States (Patent No. 5,770,365). Additional International patent filings are pending. 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](mailto:jbridgecook@tmbioscience.com).

## References

Lane MJ, Paner T, Kashin I, Faldasz BD, Li B, Gallo FJ, Benight AS, The thermodynamic advantage of DNA oligonucleotide 'stacking hybridization' reactions: energetics of a DNA nick. *Nucleic Acids Res* 1997 Feb 1; 25(3):611-617.

Riccelli PV, Merante F, Leung KT, Bortolin S, Zastawny RL, Janeczko R, and Albert S. Benight, Hybridization of single-stranded DNA targets to immobilized complementary DNA probes: comparison of hairpin versus linear capture probes, *Nucleic Acids Research* 2001. Feb 15; 29(4): 996-1004.