



# HyBeacons<sup>®</sup>: A novel DNA probe chemistry for rapid genetic analysis

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**Abstract.** The analysis of single nucleotide polymorphisms (SNPs) and short tandem repeats (STRs) has proven extremely valuable in both healthcare and forensic sectors. Such analyses have historically been time-consuming, laborious and confined to specialist analytical laboratories. The HyBeacon probe technology presented here provides a rapid method for the detection and identification of specific nucleic acid sequences, which could be employed away from the laboratory in primary healthcare and certain forensic settings. © 2006 Published by Elsevier B.V.

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## 1. HyBeacon probes

The HyBeacon<sup>®</sup> probe technology utilises single-labelled oligonucleotides that do not require a quencher moiety for fluorescence signal generation. Probes comprise specific linear oligonucleotides that possess a fluorescent dye label attached to an internal nucleotide and a 3' phosphate to prevent PCR extension [1–3]. Probes are included in PCR assays and emit greater amounts of fluorescence when hybridised to complementary target sequences than when single-stranded. In the absence of a quencher label, the reported quenching properties of DNA are responsible for the alteration in fluorescence emission upon hybridisation [4].

Following amplification, the presence and identity of target sequences is determined by melting curve analysis. The stability and melting temperature of hybridised probes

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depends on the degree of homology between HyBeacons and their target sequences. Increasing the reaction temperature above the melting temperature ( $T_m$ ) of the HyBeacon causes probe/target duplexes to dissociate and the amount of fluorescence emission to decrease. Sequences differing by as little as a single nucleotide may be detected and differentiated on the basis of melt peak  $T_m$  [1,2]. Fifty cycles of amplification and melt analysis may be completed in as little as 16 min using rapid real-time PCR instruments such as the LightCycler. HyBeacon tests may also be performed in a high throughput format (96 or 384 well) using a LightTyper instrument. The advantage of employing HyBeacons for homogeneous sequence analysis derives from their ability to reliably identify homozygous and heterozygous samples using a single oligonucleotide probe. Furthermore, HyBeacon functionality does not require FRET, enzymatic cleavage or secondary structures, making probe design uncomplicated.

## **2. Rapid DNA analysis**

HyBeacon probes may be employed to detect specific DNA and RNA sequences, for applications such as pathogen detection, and discriminate between closely related sequences. Polymorphisms located within coding and regulatory regions of DNA, such as insertions, deletions and nucleotide substitutions, may influence phenotypic characteristics directly by changing amino acid sequences and affecting protein production respectively. Certain polymorphisms have been identified as possible causative agents of genetic diseases, while others are thought to be closely linked to disease causing factors and are employed as genetic markers. During hybridisation, the interaction that occurs between a HyBeacon probe and target DNA sequence may be fully complementary or contain positions of mismatch, depending on the origin of the polymorphic sequence. Probes that are hybridised to mismatched sequences possess significantly reduced  $T_m$ s compared with fully complementary probe/target duplexes. Since the amount of fluorescent signal that is emitted from hybridised HyBeacons is significantly greater than that emitted from single-stranded probes, polymorphic target sequences differing by as little as a single nucleotide may be differentiated on the basis of hybridisation  $T_m$ . HyBeacon tests may be performed rapidly using crude saliva, buccal or other cell-bearing swabs, urine and blood samples, without the requirement for DNA purification, and could be employed for point-of-care diagnostics in hospitals, pharmacies and doctor's surgeries, or point-of-arrest investigations in police custody suites.

## **3. SNP genotyping**

There has been considerable interest in the use of SNPs to aid forensic investigation over recent years. Panels of bi-allelic SNPs have the potential to offer high discriminating power for individual identification, and other specific markers may provide phenotypic information about an unknown suspect. HyBeacons have proved very effective in the rapid and accurate genotyping of SNPs in a number of applications.

HyBeacon probes may be employed to rapidly genotype samples with respect to SNPs. A HyBeacon was designed to be fully complementary to the G allele of the NAT2\*5C polymorphism and yields melt peaks of 53 °C  $T_m$  when hybridised to these matched targets (Fig. 1A). The A allele of the NAT2\*5C polymorphism exhibits a single (C/A)

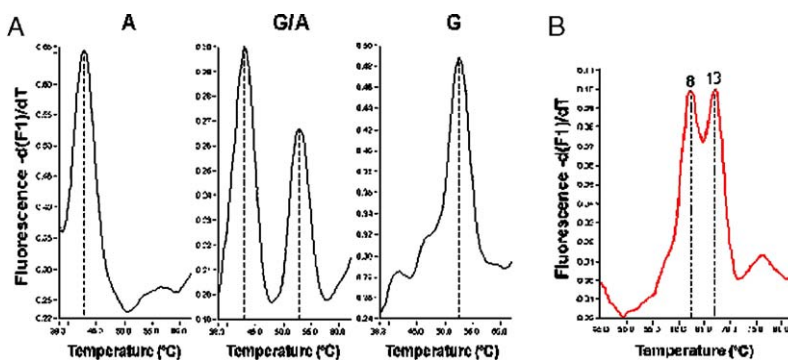


Fig. 1. Analysis of (A) single nucleotide polymorphisms and (B) short tandem repeats using HyBeacon probes.

nucleotide mismatch, which destabilises probe hybridisation and reduces peak  $T_m$  to 42 °C. Heterozygous samples, possessing both G and A alleles, generate both 53 °C and 42 °C melt peaks.

#### 4. Analysis of STRs

HyBeacons also have the potential to rapidly analyse short tandem repeat (STRs), where allele discrimination is performed on the basis of probe stability and target length. The D16S539 STR comprises between 7 and 15 GATA nucleotide repeats and is efficiently detected using HyBeacon probes. HyBeacon assays are capable of discriminating between D16 alleles of different length, amplified directly from saliva samples, within 16 min. The saliva sample presented is heterozygous for 8 and 13 repeat sequences, both of which are detected by melt peak analysis (Fig. 1B). Further work is required to reliably identify repeat number in such long target sequences. Analysis of STRs could be employed to rapidly identify individuals at scenes of crimes and could be used at the point-of-arrest.

#### References

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