EasyBeacons™
New probes ideal for Realtime PCR detection of methylation status of single CpG duplets and SNPs

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Abstract

The invention of real time PCR has revolutionized the way of determining the expression status of a gene in a fast and precise manner. Detection and quantification of a target sequence can be done by using different technologies, among others molecular probes. One of the major challenges for the molecular probes is to be able to discriminate between very similar targets varying in as little as one nucleotide, e.g detection of the methylation status of a single CpG duplet and SNP detection. The EasyBeacons™ presented here are based on the novel technology Intercalating Nucleic Acid, INA®, linked to a fluorophore and a quencher. INA® is composed of normal DNA nucleotides and Intercalating Pseudo Nucleotides (IPNs). The fact that the EasyBeacons™ are mostly composed of normal DNA nucleotides means, that in many respects EasyBeacons™ behave like DNA based probes, allowing use of standard buffers, primers and enzymes and hence reduces the optimisation efforts. The IPNs of an EasyBeacon™ comprise hydrophobic moeities that will bring the fluorophore and the quencher of the unbound probe in close proximity of each other. This leads to an improved quenching of unbound probes and hence an improved signal-to-noise ratio. This mechanism is independent of the sequence of the DNA nucleotides. Due to the high affinity of INA® towards complementary DNA, EasyBeacons™ can be made shorter and hence more specific than their DNA counterparts. The non-temperature dependent quenching of EasyBeacons™ is also very suitable for multiplex PCR, and compatible with most, if not all the commercially available real time PCR instruments. The results presented here show the use of EasyBeacons™, generating a better signal-to-noise ratio, a higher affinity and specificity in detection of the methylation status of single CpG duplets and SNPs. Furthermore the possibility to include an end point verification measurement, since the probes are not degraded during PCR amplification, is proven to be valuable. The ease of design and optimization of the real time PCR assays using the EasyBeacons™ are also discussed.

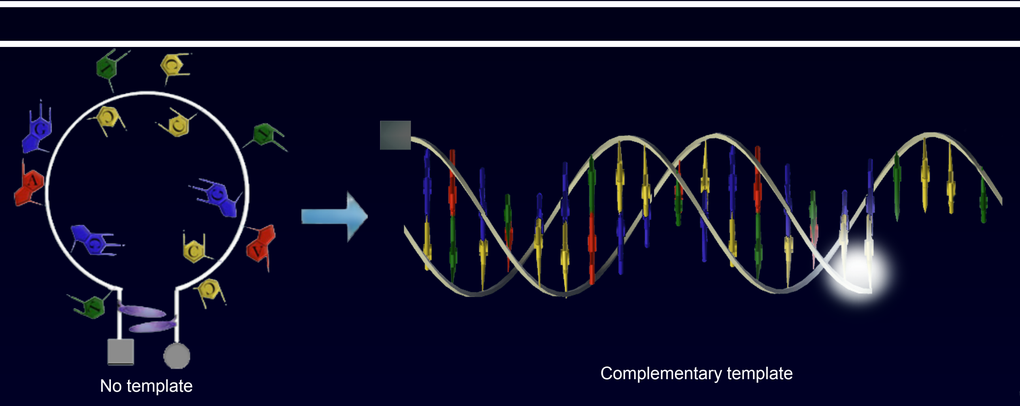
EasyBeacons™

An EasyBeacon™ is composed of a fluorophore like FAM, HEX, TET, VIC or others and a quencher such as Tamra, Dabcyl, Black Hole Quencher® or others normally linked respectively to the 5' and 3' end of an INA®.

EasyBeacons™ combine some of the advantageous features associated with conventional probes in one product:

- Low background fluorescence yielding a very good signal-to-noise ratio
- High stability
- High specificity
- Reduced self affinity
- Fast optimization and endpoint verification
- Ease of design

In addition, as EasyBeacons™ are not degraded they can be used at optimal temperatures for the individual probes.



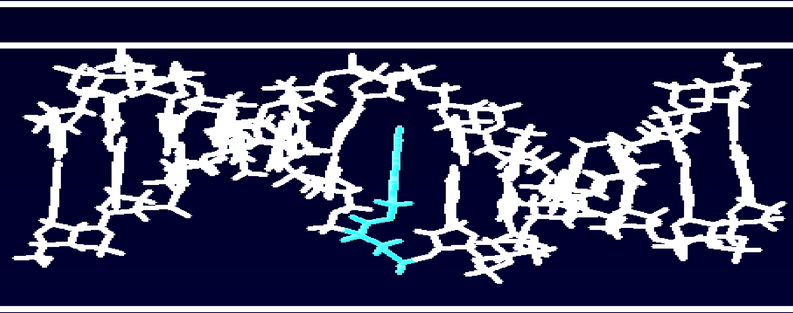
INA® and EasyBeacons™

The novel features of EasyBeacons™ are introduced by the relatively new DNA analouge – Intercalating Nucleic Acids, INA®.

DNA + IPN = INA®.

INA® is composed of normal DNA nucleotides and Intercalating Pseudo Nucleotides, IPN.

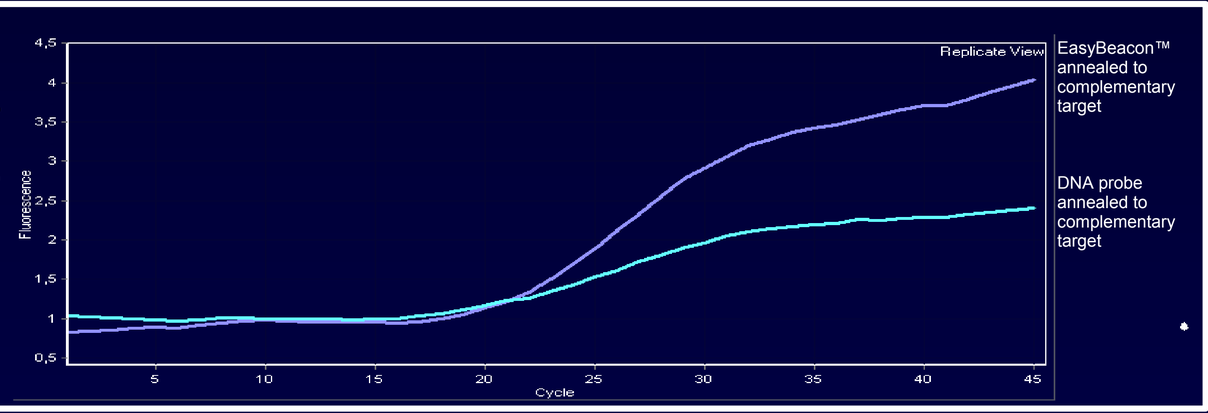
The fact that INA® is mostly composed of normal DNA nucleotides means, that in many respects INA® behave like DNA, making it easier to adapt INA® to assays where DNA has previously been used. This includes the use of standard buffers and reagents and hence reduces costs and optimisation efforts.



Improved signal-to-noise ratio with EasyBeacons™

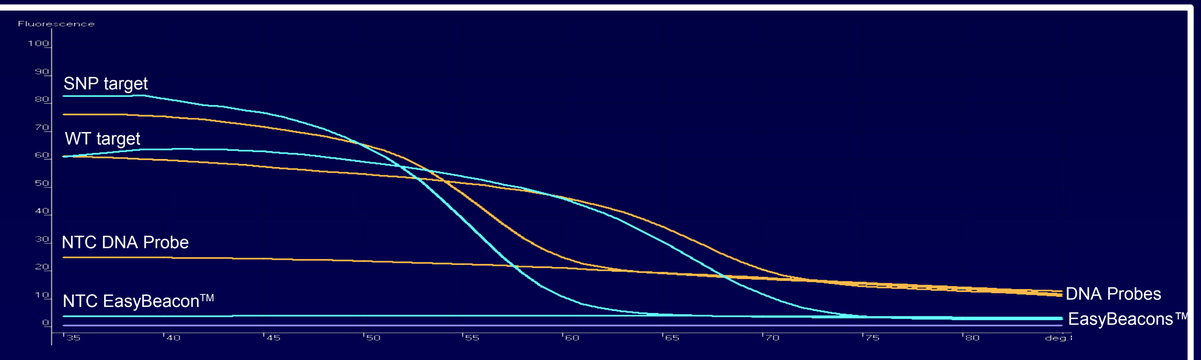
When hybridised to a complementary target, the fluorophore and the quencher of EasyBeacons™ are separated in spatial distance allowing the fluorophoreto fluoresce.The IPNs of an INA® comprise flat (hetero)aromatic and hydrophobic molecules, which, when they cannot intercalate between base pairs in acomplementary duplex, tends to stack on top of each other making hydrophobic interactions and in this way minimising the hydrophobic surface exposure to water. Hence if there are no or fewer complementary targets than EasyBeacons™ in a mixture, the IPNs of the unbound probes will facilitate contact between the two ends of the probe and thereby induce quenching of the fluorophores.

This folding back mechanism is *independent of the sequence of the probe*, and it is therefore not necessary to design a self complementary stem structure to obtain a proper quenching of the fluorescence in contrast to conventional DNA based molecular beacons.



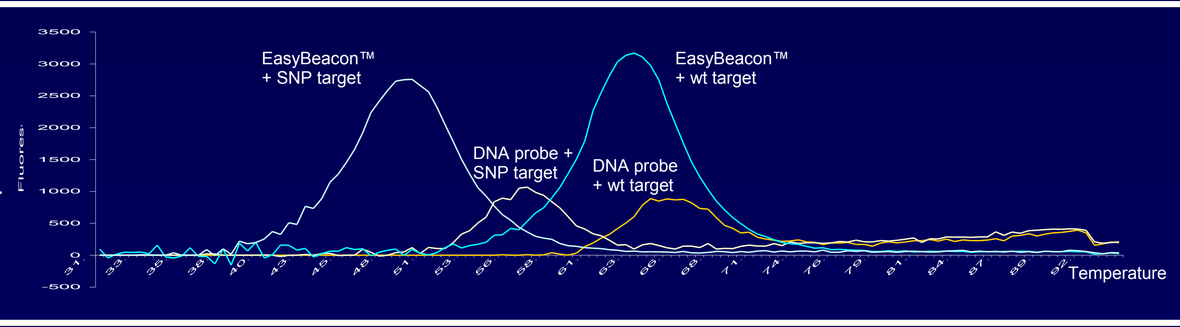
Example of low background flourescens with EasyBeacons™

EasyBeacons™ have significantly reduced background fluorescence compared to corresponding DNA probes (fluorescence of unbound probe). In the example shown here two probes, a DNA based probe and a corresponding EasyBeacon™ have been designed to distinguish between a methylated and unmethylated CpG doublet in genomic DNA. The genomic sequence is first treated with sodium bisulphite to convert non-methylated CpG's in the DNA to UpG's while leaving methylated CpG's intact. The two types of probes have the same maximum fluorescence (at 35ºC),but the background fluorescence of the unbound EasyBeacon™ is significantly lower (approximately 7 times) than the background fluorescence of the corresponding DNA probe.

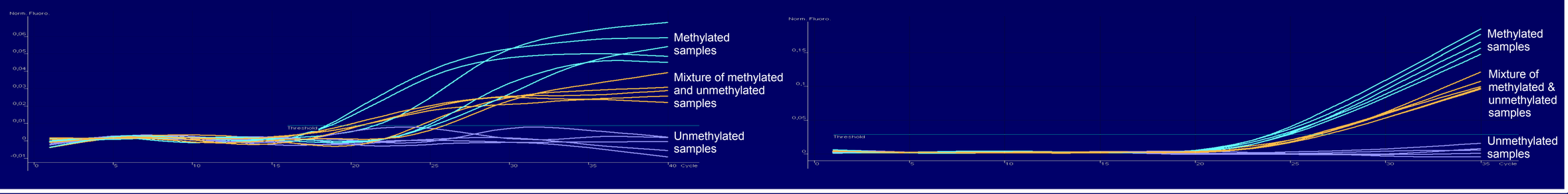


High Affinity & High Specificity with EasyBeacons™

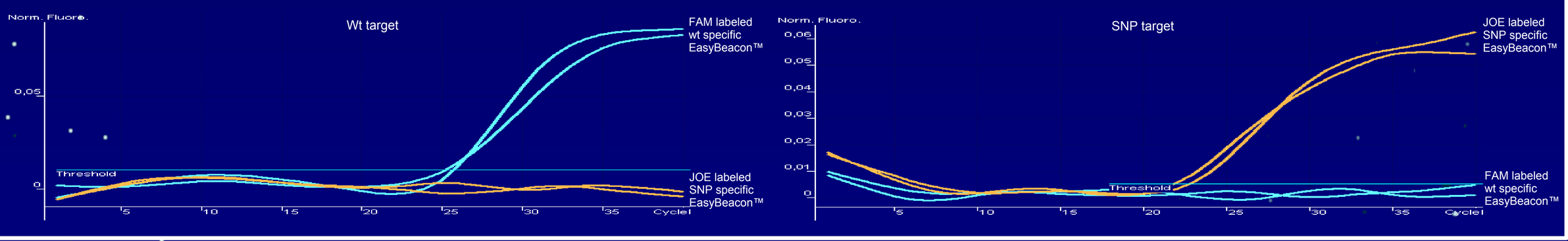
Due to the high affinity of INA® towards complementary DNA, EasyBeacons™ can be made shorter than their DNA counterparts. Generally speaking the shorter a probe is, the better it is to discriminate between single point mismatches. A typical EasyBeacon™ comprises between 7 and 18 DNA nucleotides, 1 fluorophore, 1 quencher and 2 to 4 IPNs. The figure demonstrates that EasyBeacons™ can be designed to achieve a higher specificity than their DNA counterparts. In the example shown here the specificity is 77% higher for the EasyBeacon™ than for the dual labelled DNA probe, measured as the difference in affinity to a matched versus a mismatched target.



Detection of methylation status of single CpG duplets and Endpoint verification with EasyBeacons™



Multiplexing and SNP detection with EasyBeacons™



For further information please contact ubc@pentabase.com, www.PentaBase.com