

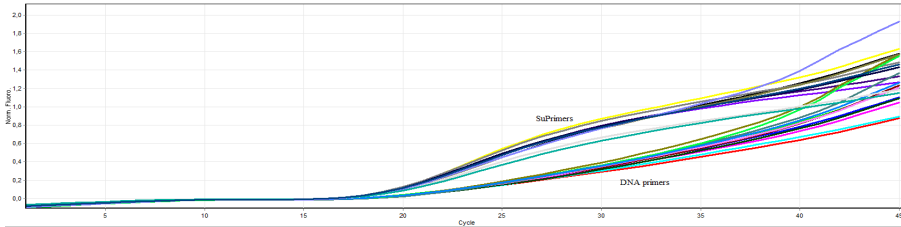


3rd generation Real-Time PCR primers and probes.

PentaBase's technologies can and have been used in many applications. Below are listed a few examples. The data are collected in-house and from external laboratories.

DNA primers versus SuPrimers™

PentaBase™ has developed a primer technology completely compatible with all applications of your standard primers. In the examples below different primer designs for a specific assay have been tested in SYBR Green. All the primers are made in a DNA and a SuPrimer™ version and tested in various combinations.

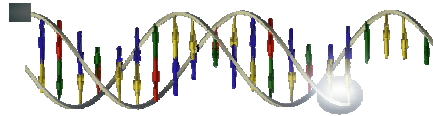


The highest signal and sensitivity was obtained when both forward and reverse primers were designed as a SuPrimer™. Also a few combinations of one DNA primer together with one SuPrimer™ gave signal. Standard DNA primers gave no signal.
95°C 2 min; 45 x 95°C 10 sec.; 50°C 60 sec.

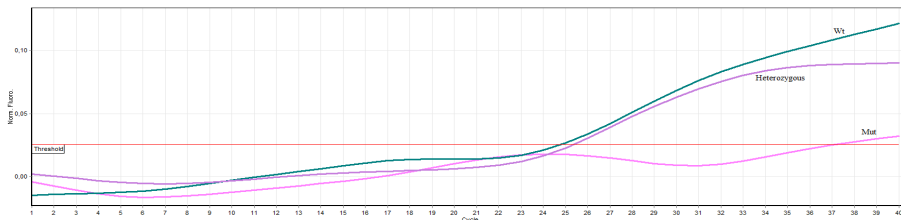
EasyBeacons™ used in SNP detection (Real-time data)

A unnamed customer wanted to develop a prognostic assay for discriminating between almost similar genetic sequences (SNPs). Due to EasyBeacon's nuclease resistance, they can be used for End-point detection and verification of Real-time signal. This is a valuable feature especially for SNP detection. In this case a wild type probe labeled with a green color and a mutant probe labeled with a yellow color have been used.

No complementary target.
Background from an unbound EasyBeacon™ is quenched efficiently as the pentabases keep the fluorophore and quencher in close proximity.

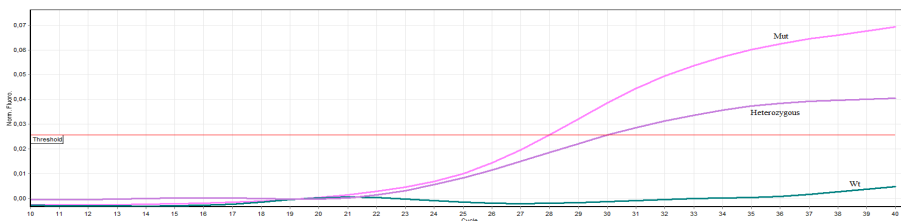


EasyBeacon™ bound to complementary target.
Binding to the target is stabilized by the pentabases in the probe.



Wild type probe (green color)

There is only generated a signal if at least one allele is wild type. If both alleles are wild types a higher signal is generated.

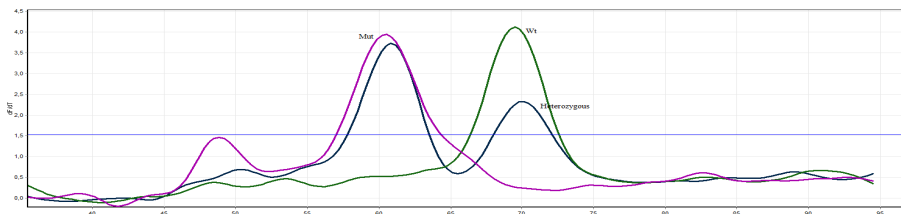


Mutant probe (yellow color)

The is only generated a signal if at least one allele is mutant. If both alleles are mutant a higher signal is generated.

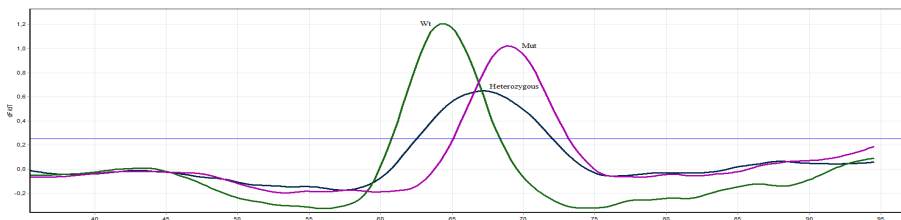
EasyBeacons™ used in SNP detection (End-point data)

After a (Real-time) PCR has been conducted, melting curves can be used to distinguish between amplicons. The presence of a SNP will cause a shift in the melting peak of several degrees. The End-point measurement can either be used as stand alone data or in combination with Real-time PCR data.



Wild type probe (green color)

Homozygous for the wild type allele (green curve) has a high melting temperature, heterozygous (black curve) has a dual melting temperature and homozygous for the mutant allele (purple curve) has a low melting temperature.



Mutant probe (yellow color)

Homozygous for the wild type allele (green curve) has a low melting temperatures, heterozygous (black curve) has a broad melting peak and homozygous for the mutant allele (purple curve) has a high melting temperature.