

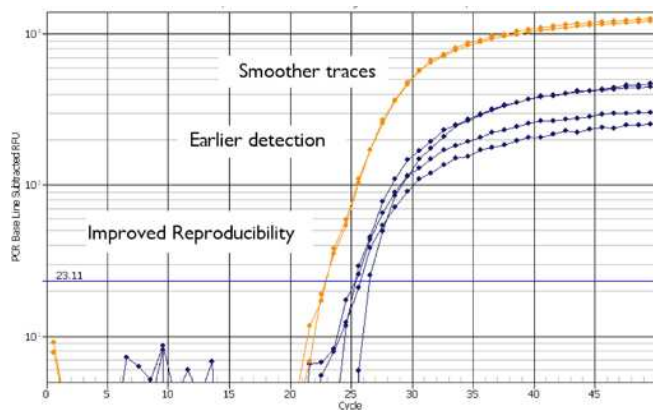
PerfectProbe

An improved Real-Time PCR Probe

PerfectProbe (Orange) Characteristics compared to Taqman (Blue)

- More sensitive detection (earlier CT values)
- Better reproducibility (especially at the limits of detection)
- More fluorogenic than Taqman and Molecular Beacon Probes
- Higher signal to noise ratio than Taqman
- Custom real-time PCR assays based on the PerfectProbe are available on demand for any human, mouse or rat genes

Fig.1 Primers for the human YWHAZ gene were tested with a PerfectProbe (Orange) or Taqman probe (Blue)



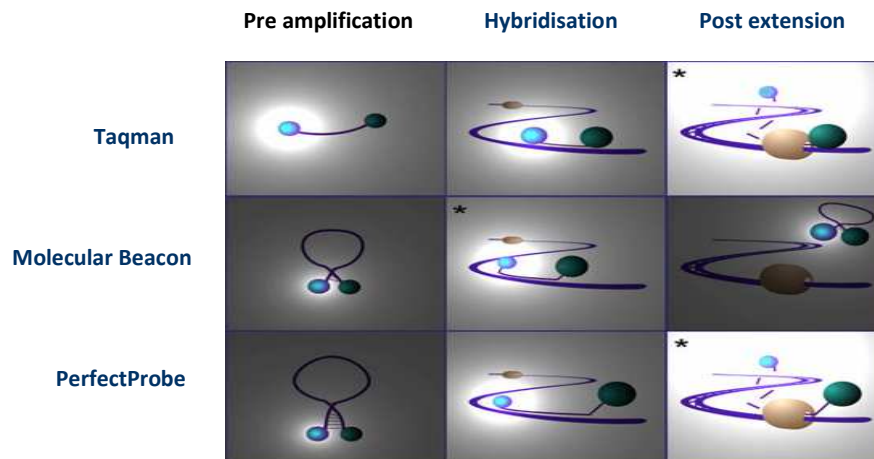
What do our customers say?

"...The PerfectProbe technology has greatly improved both replicate reproducibility and the quality of the traces. After working with PerfectProbes I wouldn't go back to using Taqman Probes..." Dr C Boxall. Synairgen Research Plc. Drug discovery company UK

How do PerfectProbes work?

What are the characteristics of the perfect reporter probe for real-time PCR? Clearly it would have optimal quenching properties and also be very fluorogenic. The hairpin structure of Molecular beacon probes leads to very efficient quenching, but this probe is not cleaved and hence the single strength is low. By contrast, the Taqman probe is cleaved but it has a high background because the dye and quencher are spatially far apart from each other at either end of the probe.

The perfect real-time PCR probe would incorporate the best feature of both systems. It would have a secondary structure that reduced the spatial separation of quencher and dye leading to a lower background fluorescence. Furthermore, it would have the high fluorogenic potential of a hydrolysis probe (Fig.1). We have designed and validated a probe that is based on this concept. The result is a reporter system with greatly improved signal to noise ratios and detection of amplification at earlier cycle numbers. We have called our probe the PerfectProbe.



*Fluorescence is measured during this stage

Supporting Data

Fig.2a Identical forward and reverse primers for the asthma-associated alternatively spliced gene 1 were used in conjunction with different reporter probes. The Taqman® and PerfectProbe had identical target annealing sequences whilst the Molecular Beacon® was shorter but fell within the same target region. Consistent with the diagrams above, the PerfectProbe gave a background level similar to the Molecular Beacon®, but had an endpoint fluorescence similar to the Taqman® probe

Fig.2a Asthma associated gene (AAA1), primary data

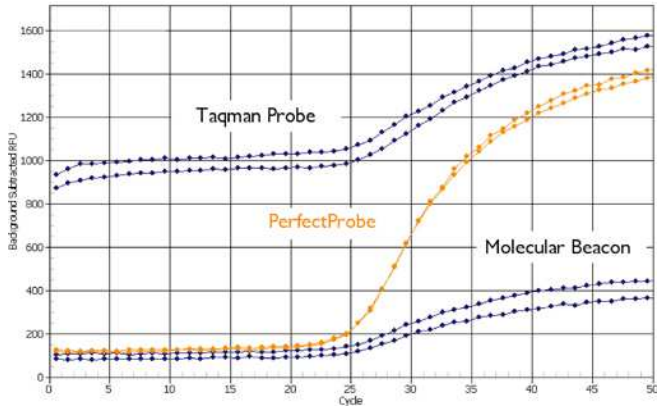


Fig.2b The iCycler performs a simple arithmetic baseline subtraction
Asthma associated gene (AAA1), baseline corrected data, linear plot

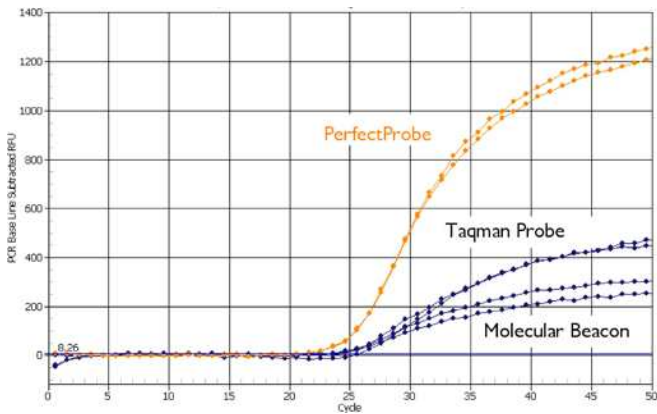


Fig.3 Relative quantification using the above assays was performed on a range of hardware platforms. In general the PerfectProbes were between 3 and 5 fold more sensitive. The Rotorgene is atypical in that it derives amplification plots from the signal to noise ratio. Probes with a low background noise are therefore much more sensitive on this system than the Taqman® probe

