New probes, same procedure - Improved results.

- Remove the false negatives and positives

PentaBase: “Best Young Biotech Company in Europe”
– Europe Unlimited, December 2008

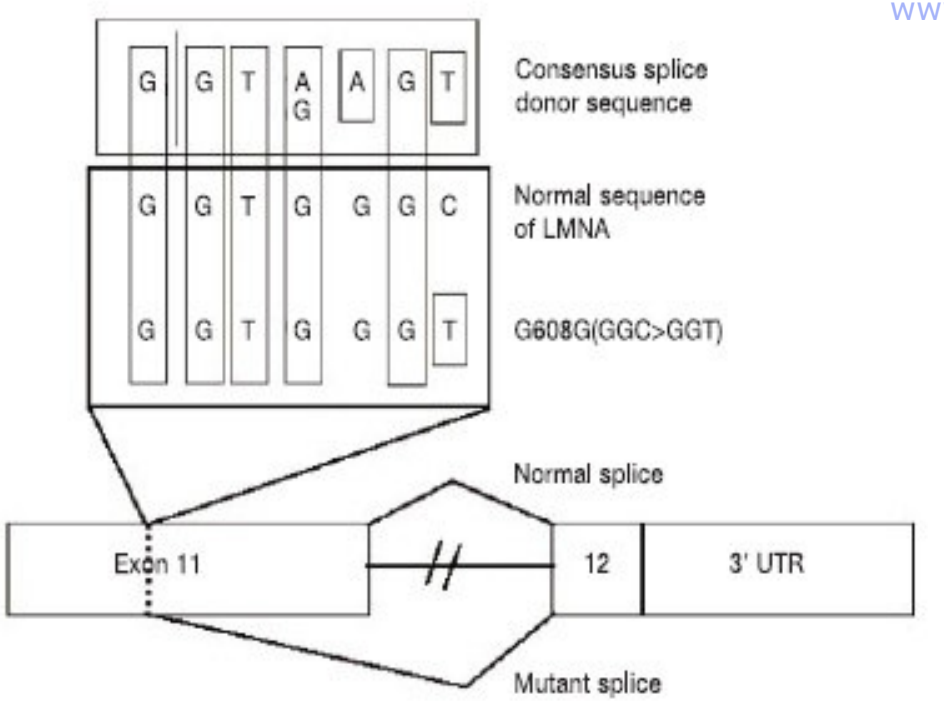
1st Challenge

Male patient age 7

Female patient age 14

Male patient age 15

www.progeriaresearch.com

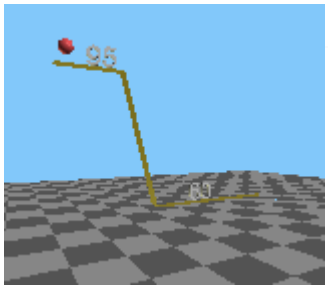


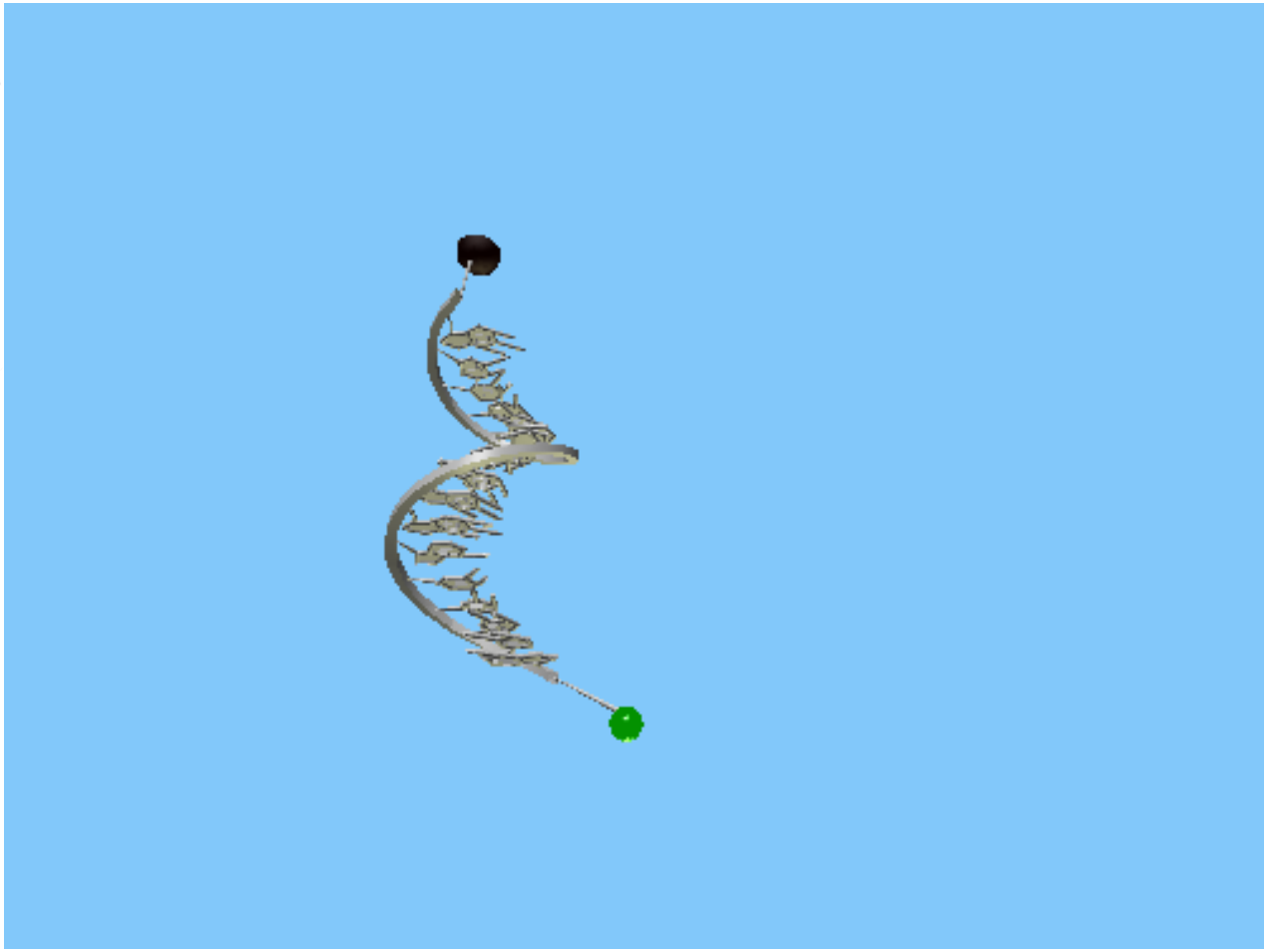
2nd generation probes

Exiqon/ Sigma

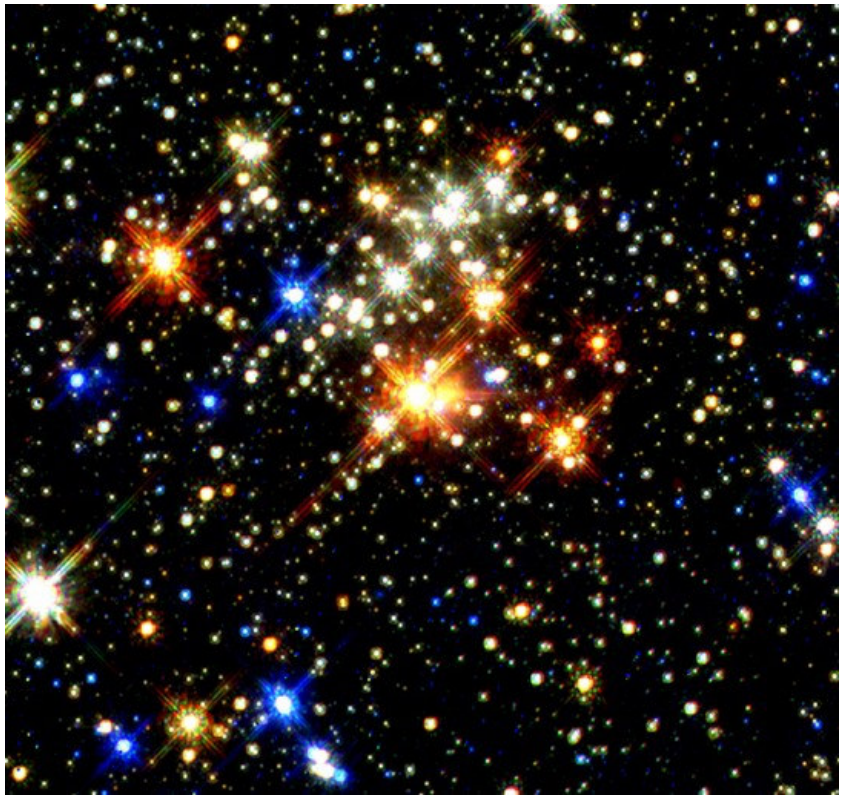
Applied Biosystems (ABI)/ Invitrogen

MGB/LNA TaqMan

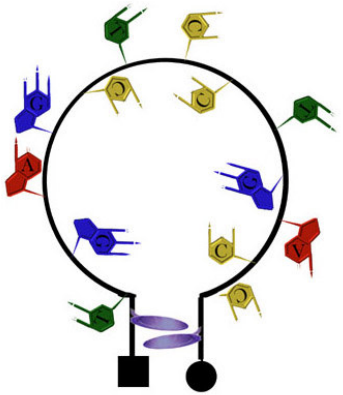


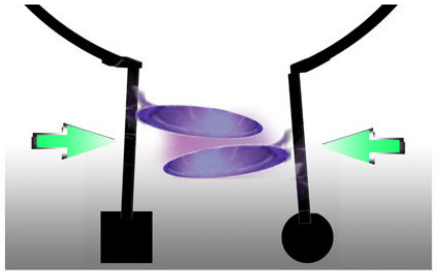


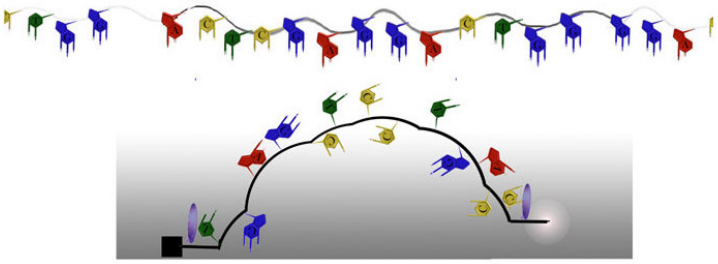
Background effects

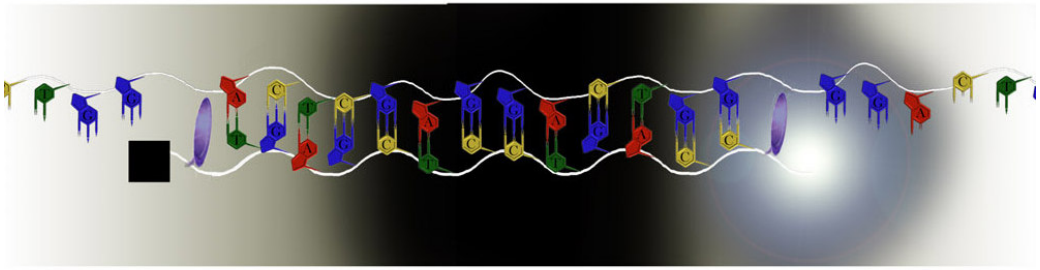


Principle in probes

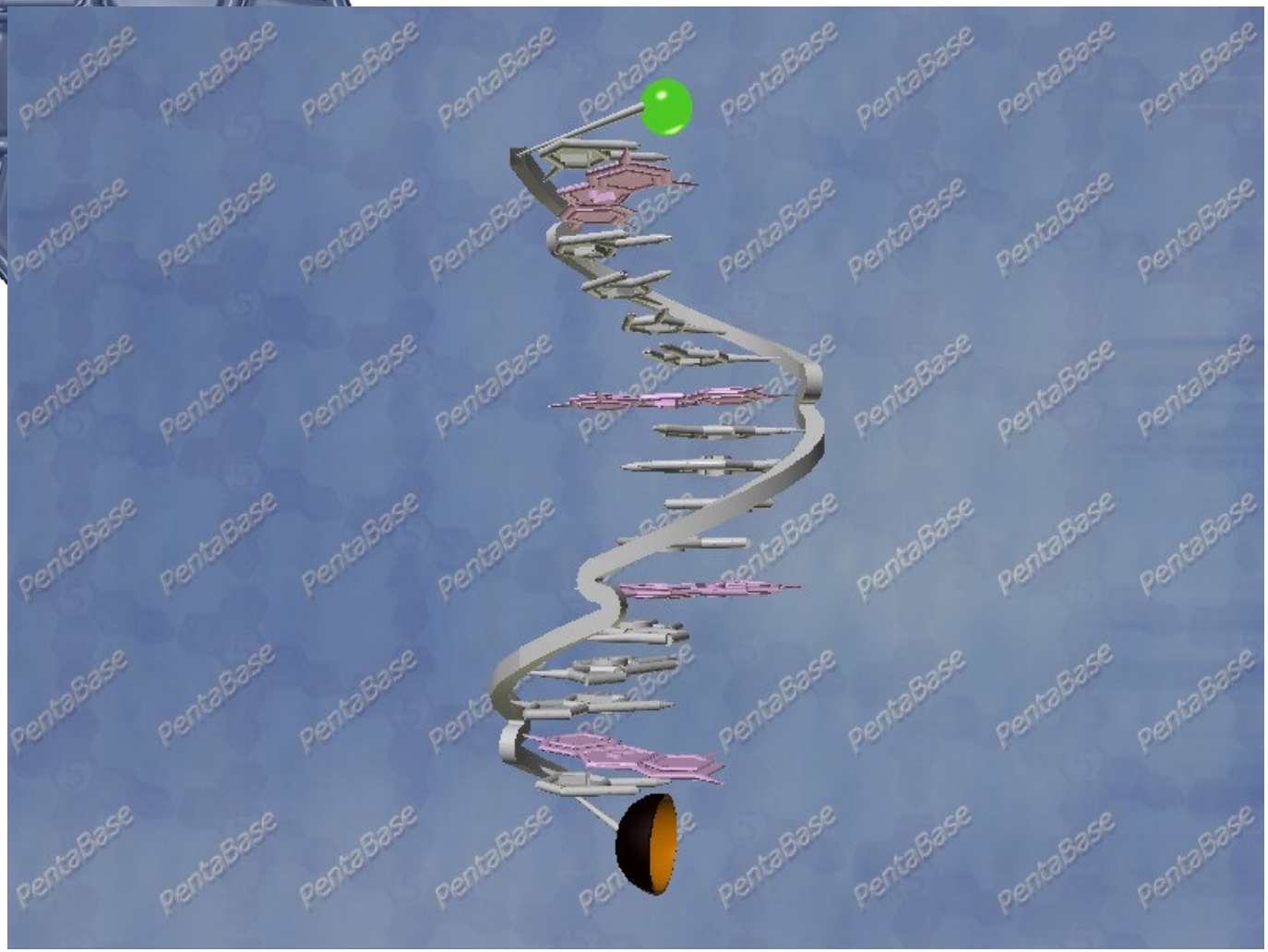




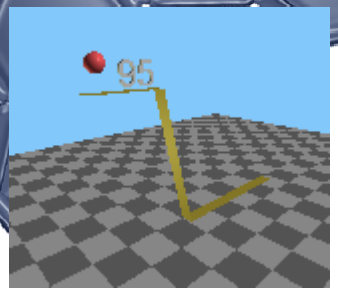


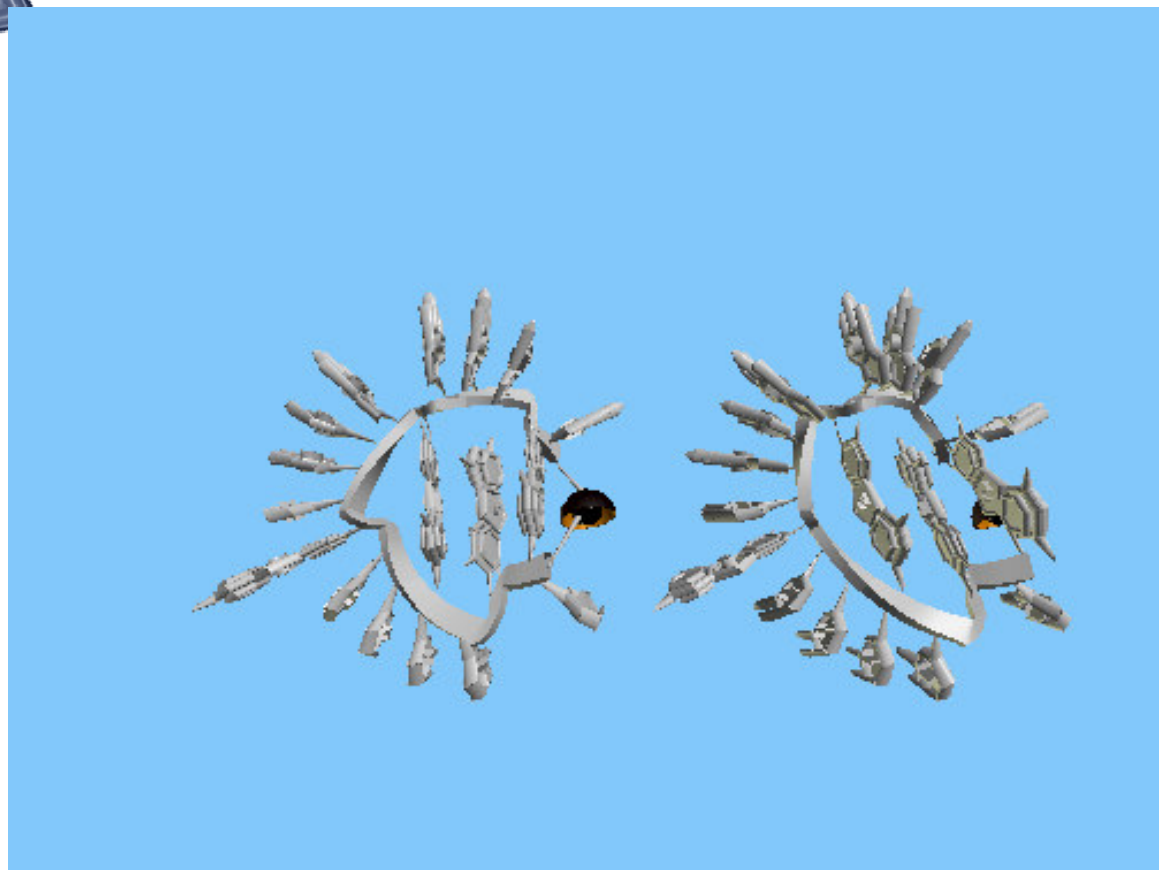


PentaBase's probes



PentaBase's probes





Challenges

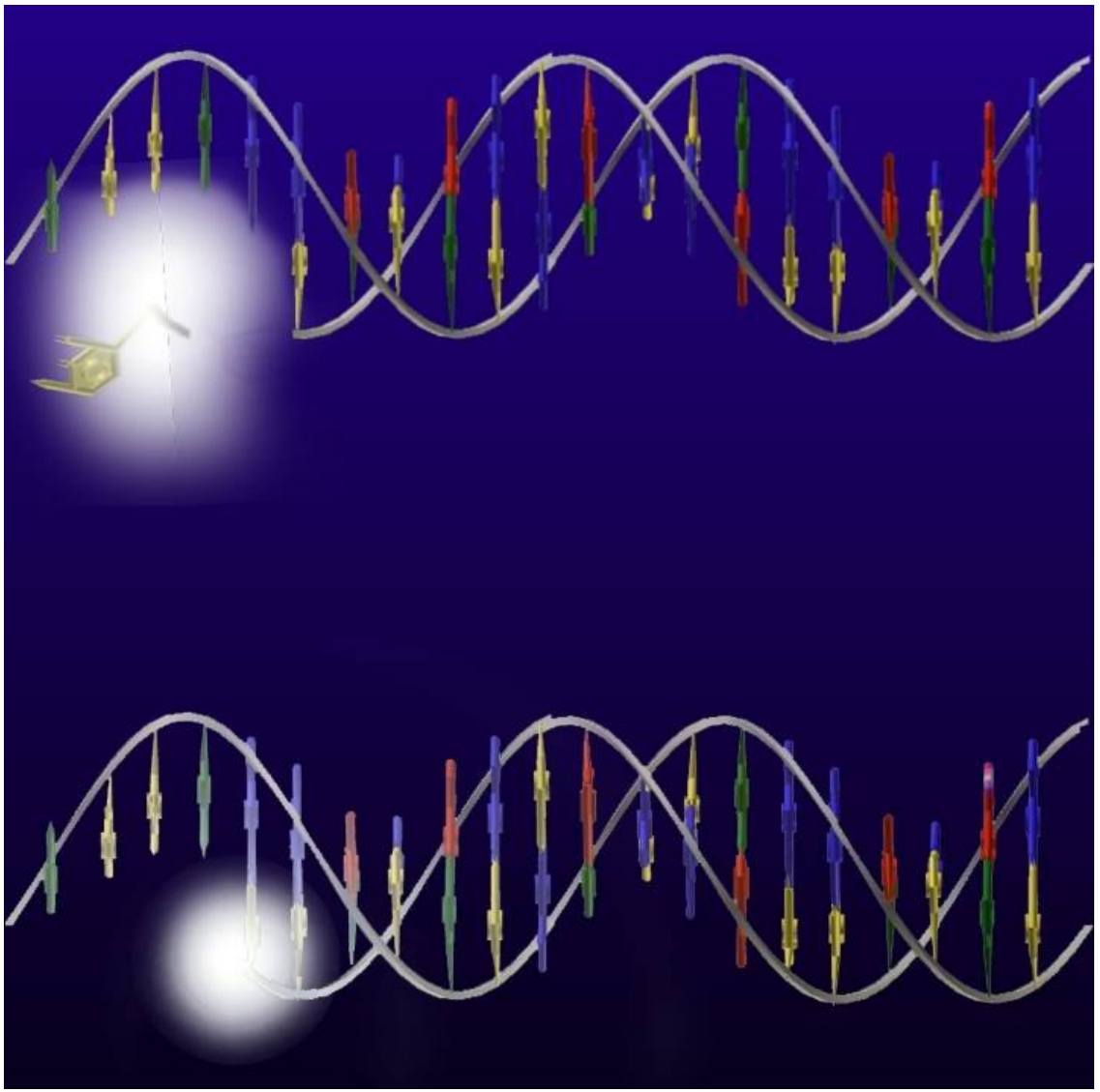
- Seeing the minority (0.5-10%) in a background
- Multiplexing: RSVA, RSVB, INFA, INFB,… H1N1, H1N3, H3N1, H5N1...
- Getting rid of false positives and false negatives

Nuclease resistant +/-

HydrolEasy

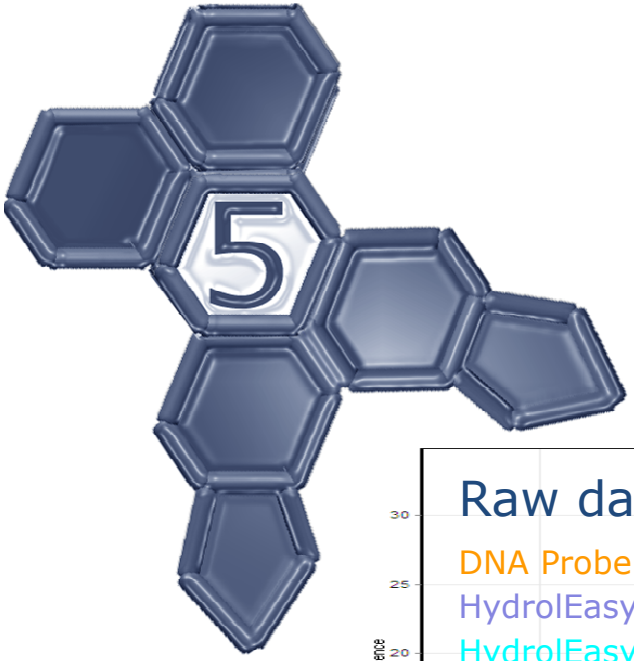
Probes

EasyBeacons



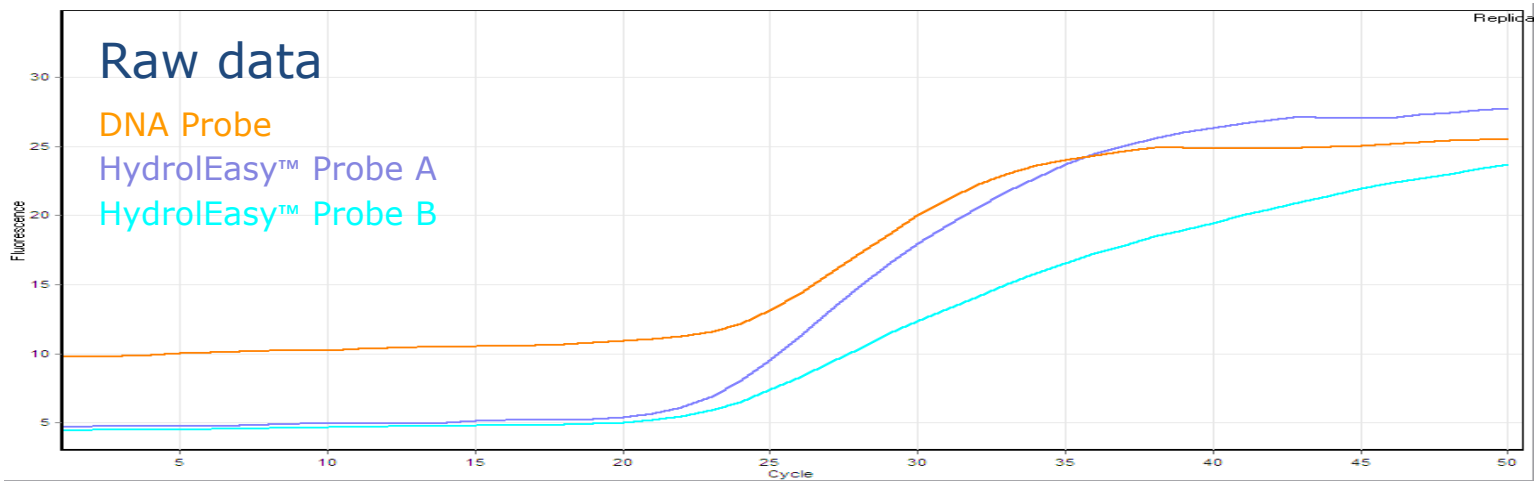
HydrolEasy probes: Data from Challenge 2007

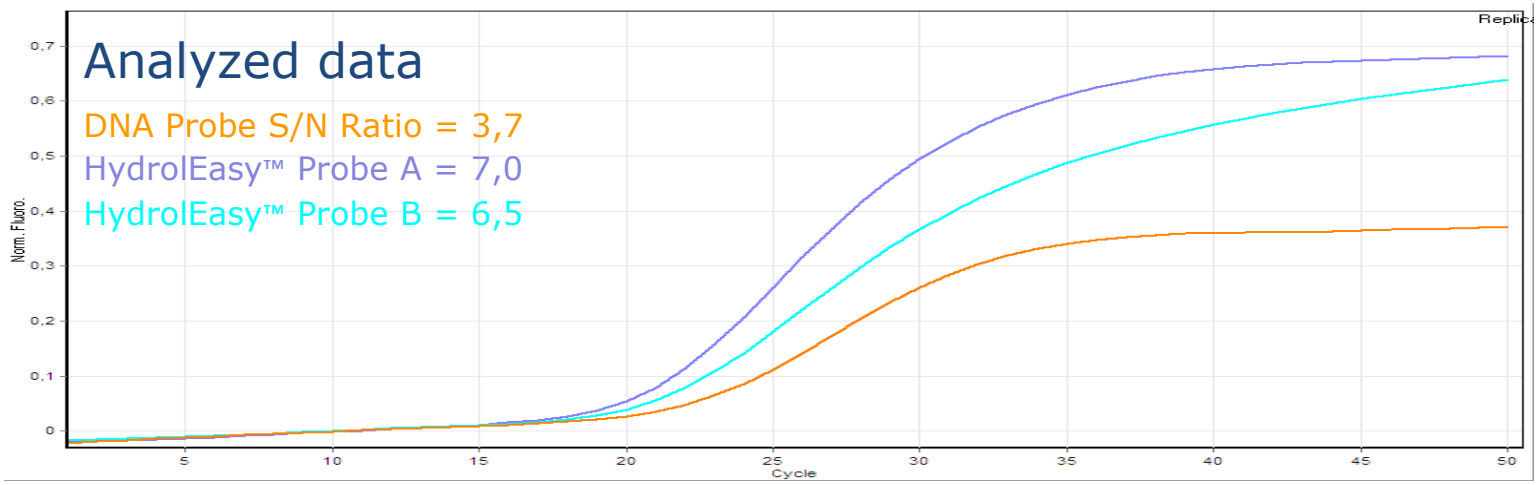
If you have an assay we can improve it!



Signal to noise ratio

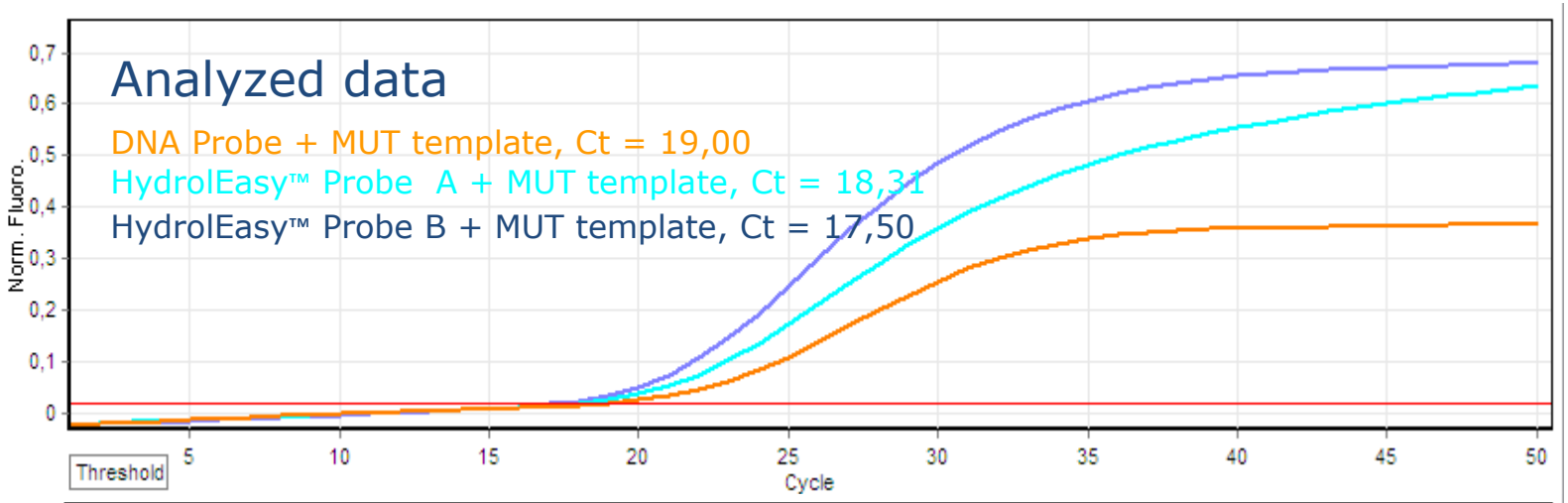
A low backgound gives a high signal-to-noise ratio.

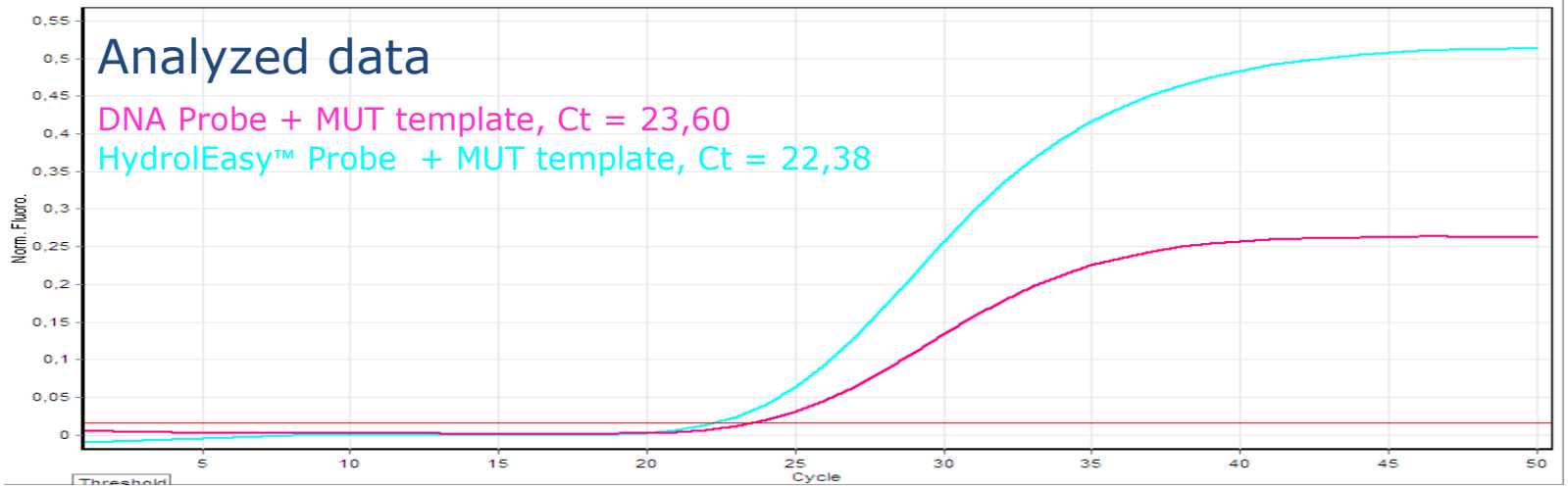




Sensitivity

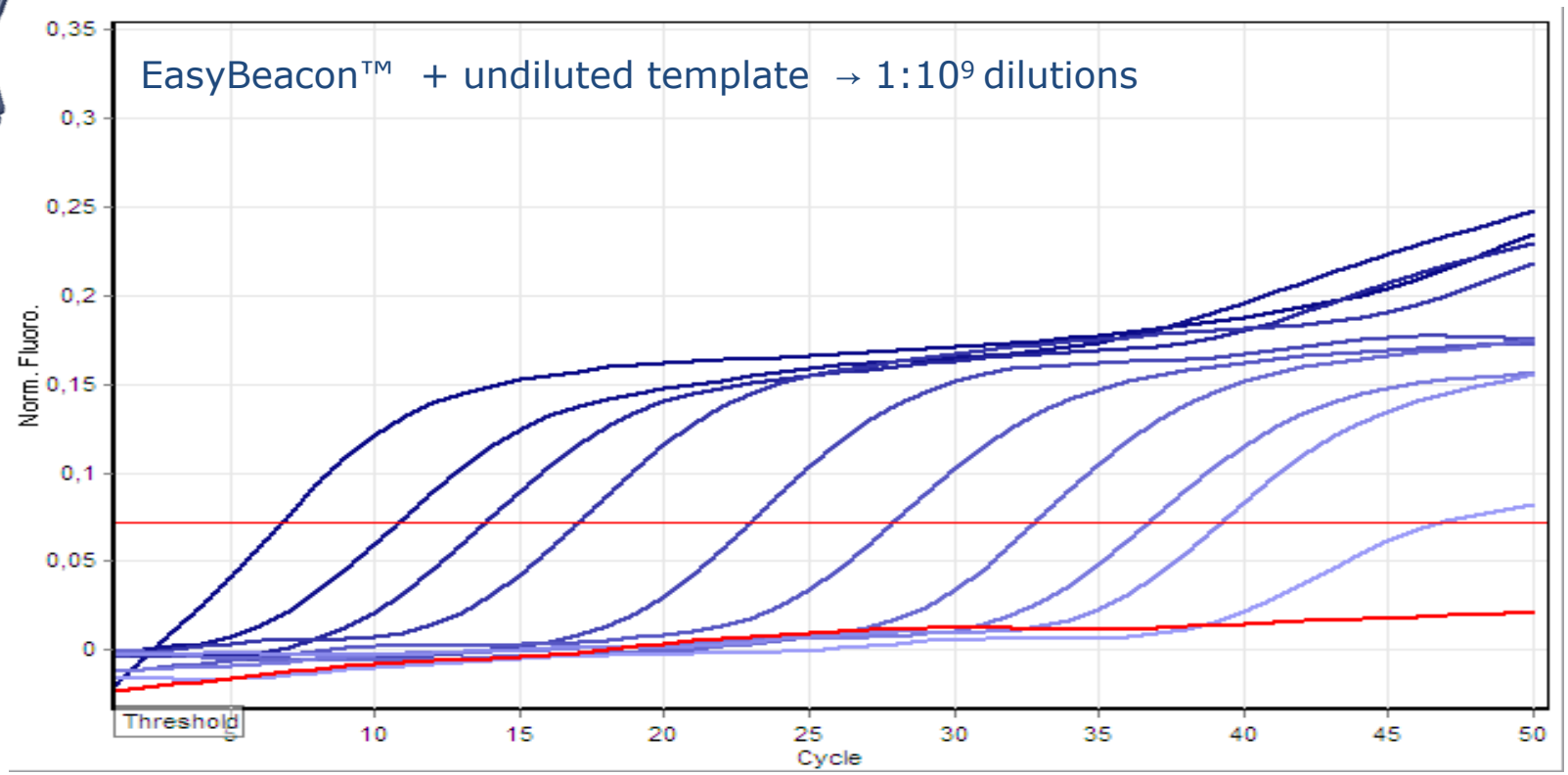
A low background gives a high sensitivity





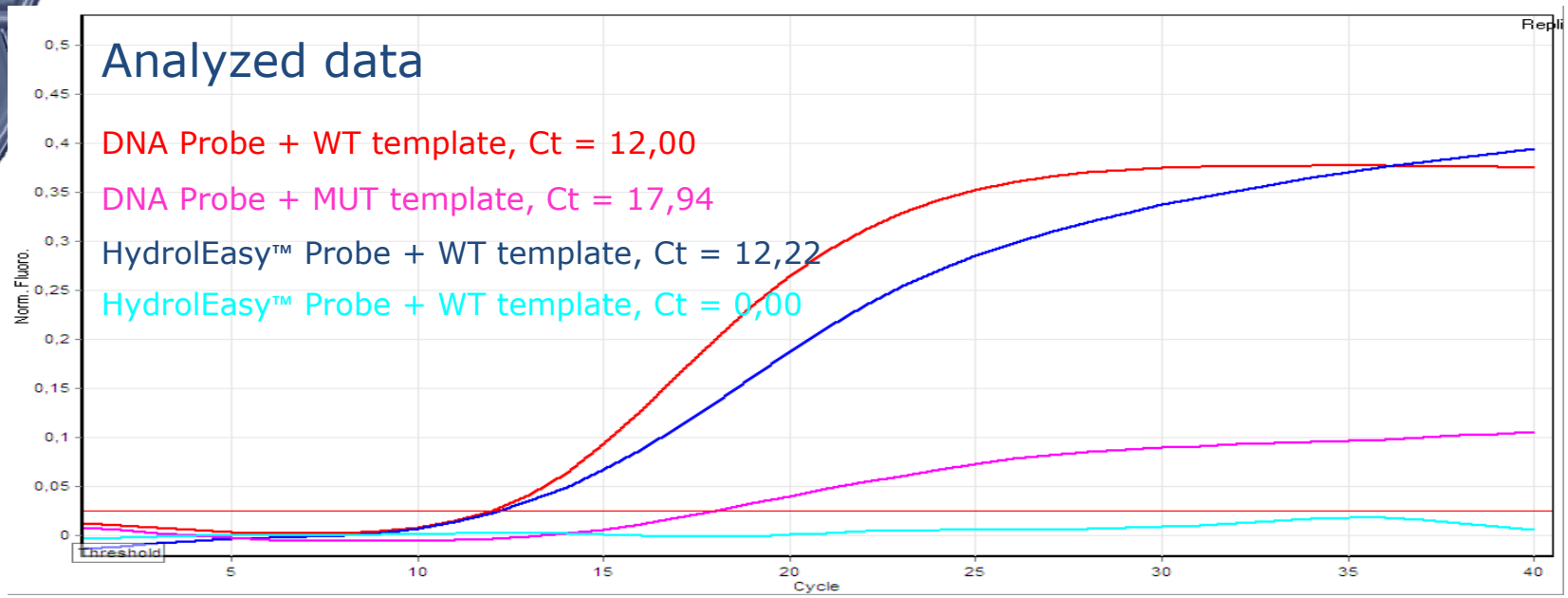
Detection over 10 Logs

PentaBase's probes have a broad working range



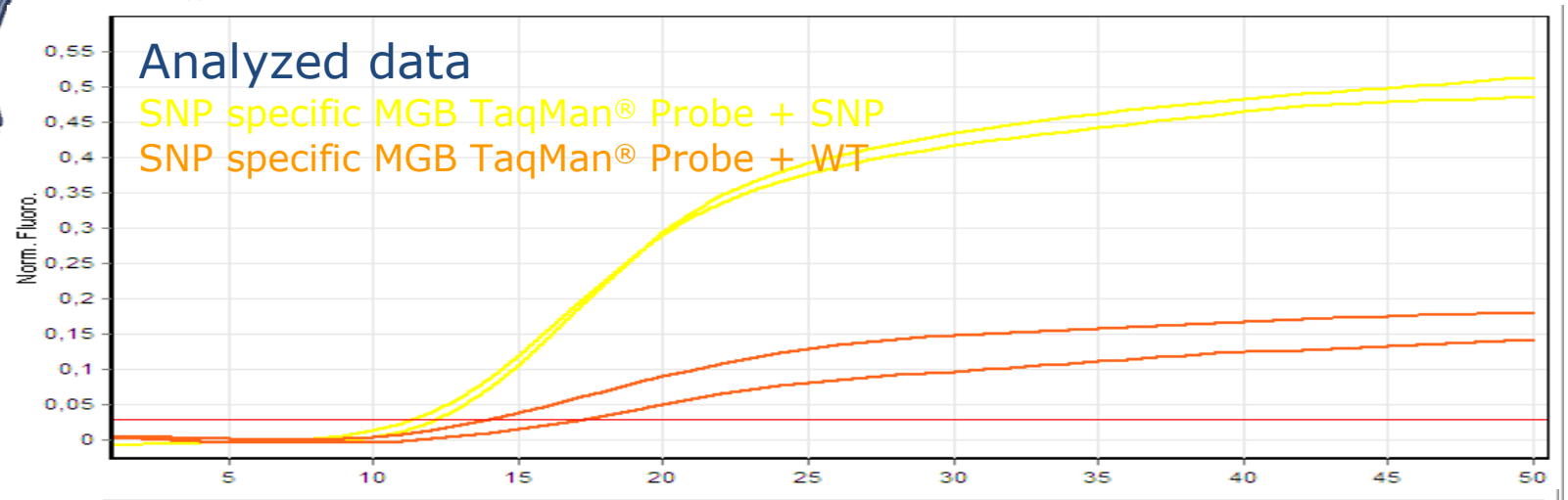
Specificity

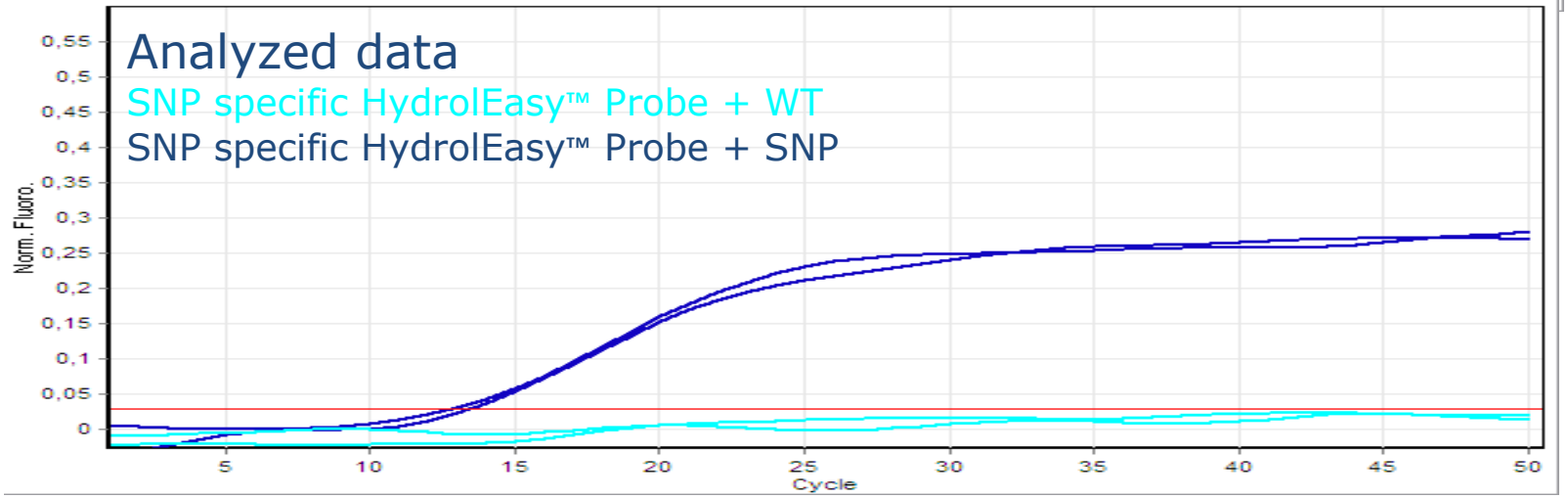
Unspecific signal from DNA probe and WT template



SNP detection
HydrolEasy™ Probe vs. MGB TaqMan® Probe

Higher specificity with HydrolEasy™ probe



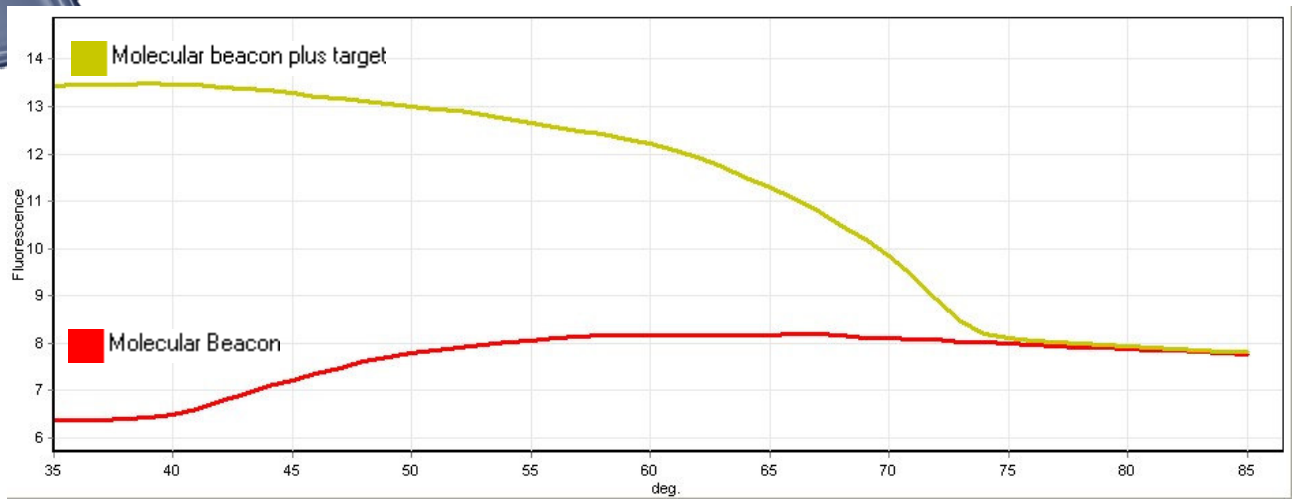


EasyBeacons

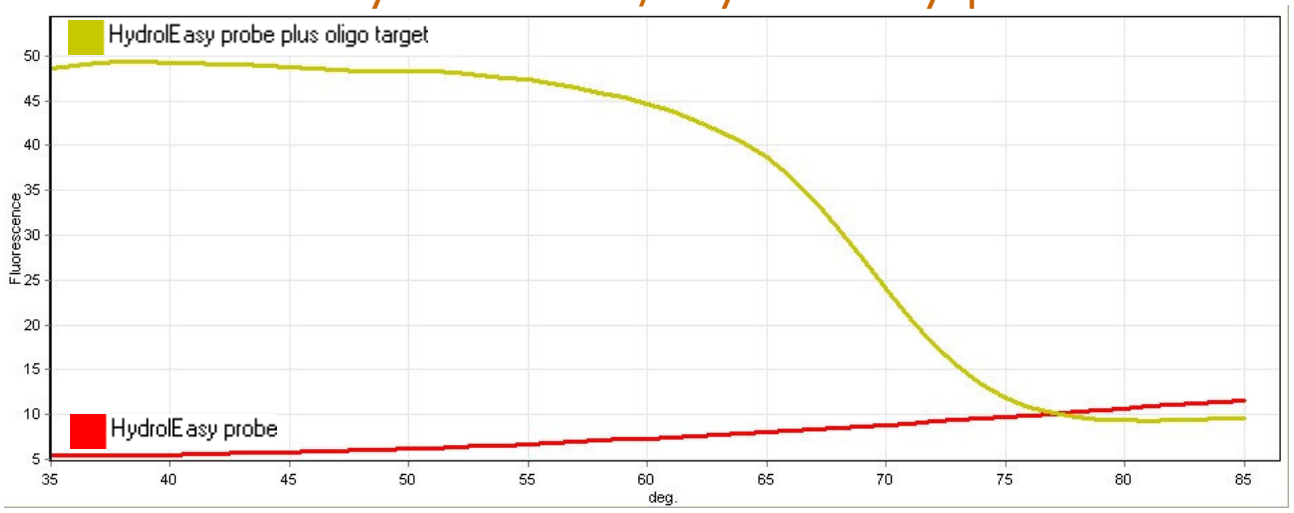
Novel features

Fluorescence +/- target

Molecular Beacons®

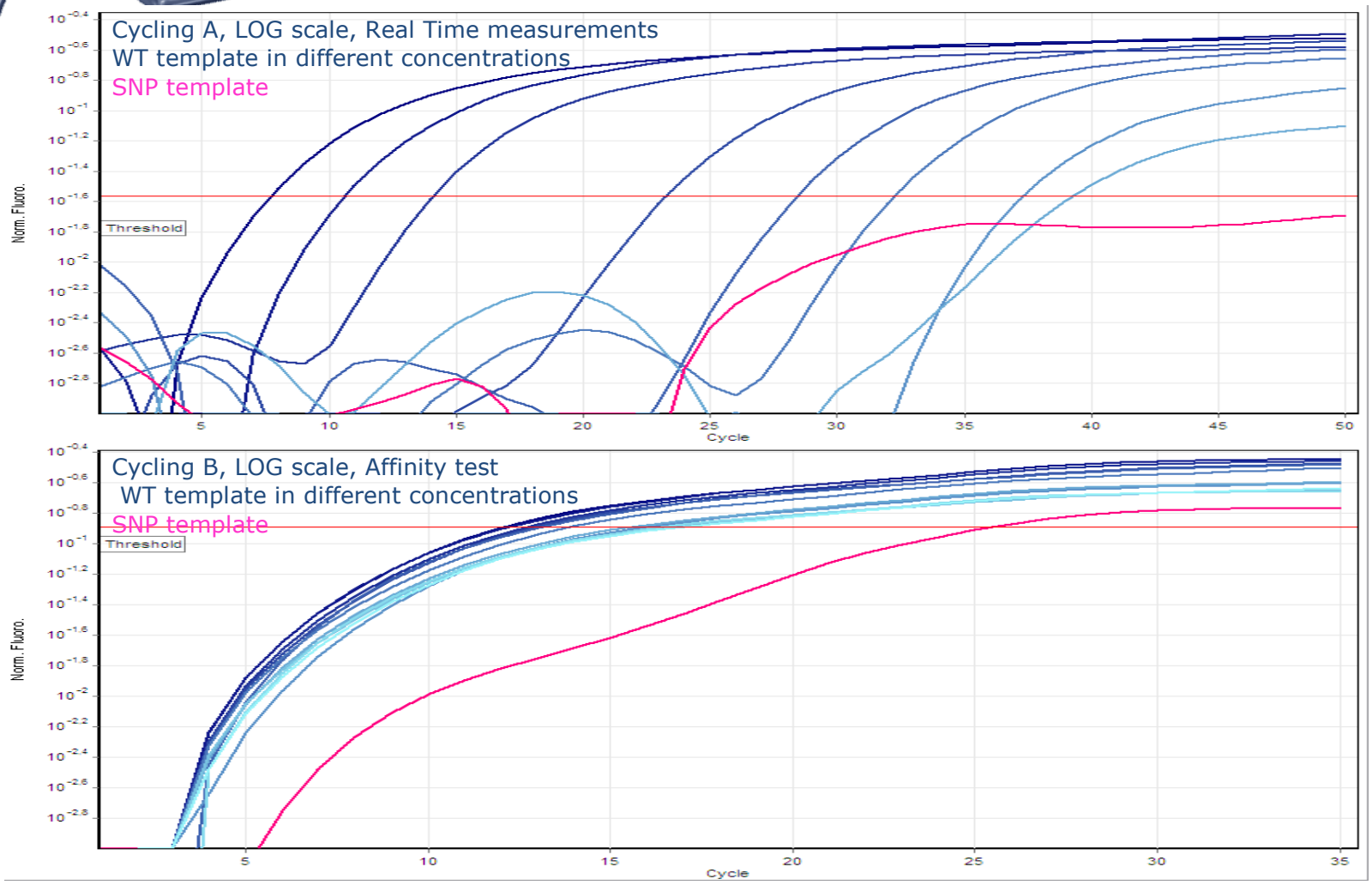


EasyBeacons™/ HydrolEasy probes

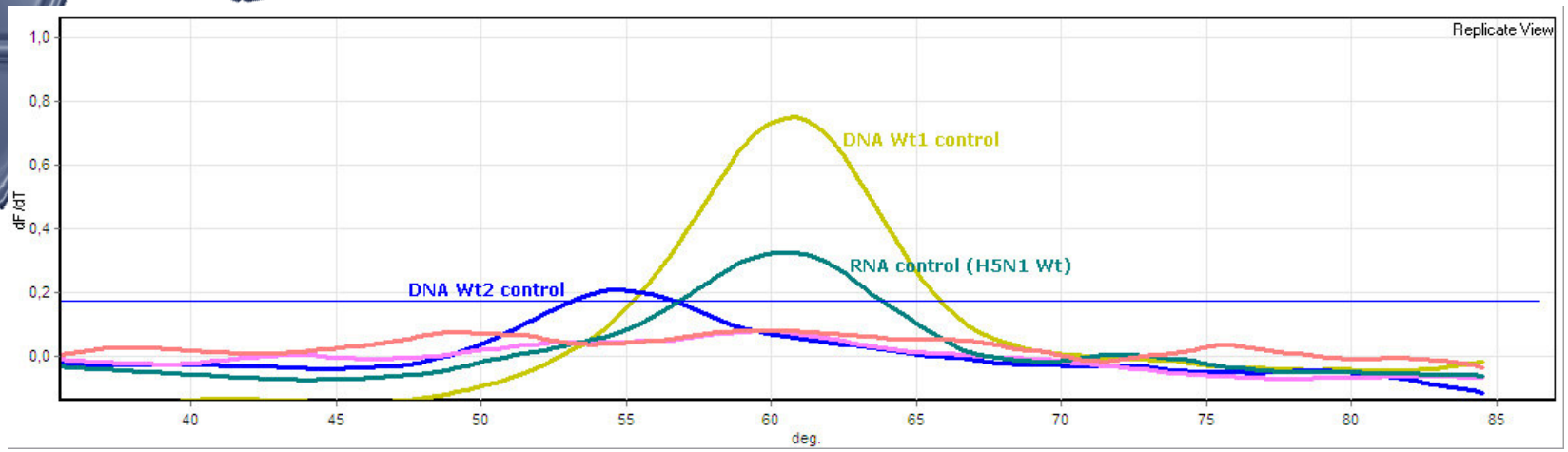


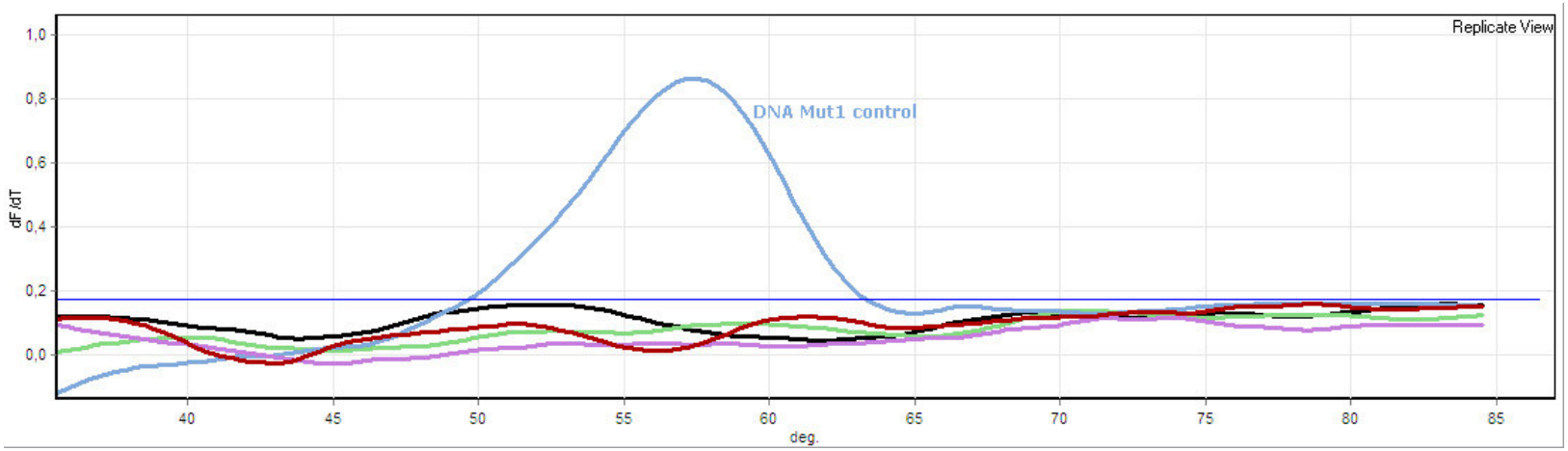
Inherent quality control

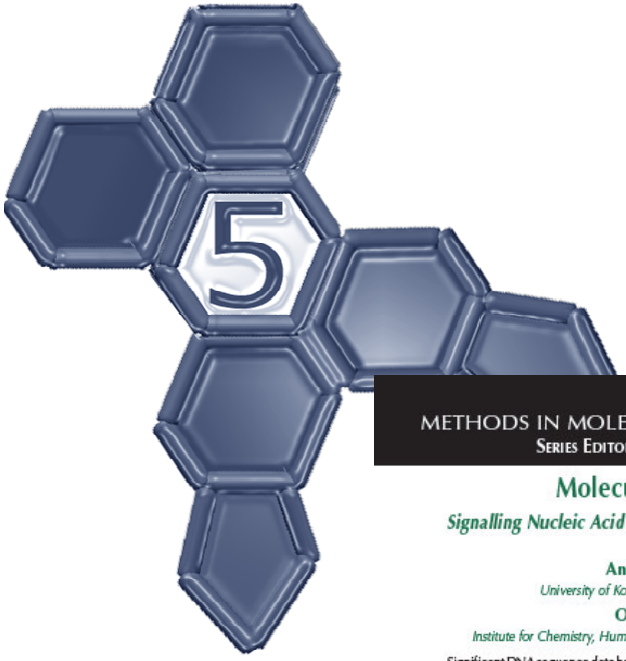
End point quality control
The EasyBeacon™ have a low affinity for the SNP template, and the same affinity for the different concentrations of the WT template.



5 End-point with EasyBeacons







Litterature

METHODS IN MOLECULAR BIOLOGY™ • 429
SERIES EDITOR: *John M. Walker*

Molecular Beacons

Signalling Nucleic Acid Probes, Methods, and Protocols

Edited by

Andreas Marx

University of Konstanz, Konstanz, Germany

Oliver Seitz

Institute for Chemistry, Humboldt University Berlin, Berlin, Germany

Significant DNA sequence data has been made available by several important genome projects. The allocation of functions to a mere string of the four letters in the code of life plays a major if not predominant role of ongoing and future research. Guided by experts in the field, *Molecular Beacons: Signalling Nucleic Acid Probes, Methods, and Protocols*, provides easy-to-follow experiments, commences with an introduction to the basic principles of fluorescence, and shows a diverse set of instructive examples, from probe design to applications in clinical settings, molecular beacon technology, the identification of transgenic mice, along with the potential of realtime PCR. While originally being used for fluorogenic nucleic acid probes with a stem-loop structure, molecular beacon now refers to a whole family of fluorescence-up probes. *Molecular Beacons: Signalling Nucleic Acid Probes, Methods, and Protocols* summarizes all the latest findings and explores powerful techniques designed for geneticist, biotechnologists, molecular and cellular biologists,

FEATURES

- Shows a diverse set of instructive examples from probe design to applications in clinical settings guided by experts in the field who provide easy to follow experiments
- The book commences with an introduction to the basic principles of fluorescence
- Extends the potential of realtime PCR
- Contributions advocate simplifications of the probe technologies
- Presents a new simplified molecular beacon design EasyBeacons and demonstrates the utility in DNA methylation profiling

CONTENTS

I. Introduction. Basic Principles of Fluorescence and Energy Transfer. **II. Applications.** Multiplex Detection of Mutations. Detection of *Salmonella* Species in Foodstuffs. Identification of Homozygous Transgenic Mice by Genomic Real Time PCR. Quantitative Analysis Of Gene Expression Relative to 18S rRNA in Carcinoma Samples Using the LightCycler® Instrument and a SYBR Green I Based Assay: *Determining FAT 10 mRNA Levels in Hepatocellular Carcinoma.* Absolute Quantitation of Feline Leukemia Virus (FeLV) Proviral DNA and Viral RNA Loads by TaqMan® Real-Time PCR and RT-PCR. High Throughput Real-Time PCR. Design and Use of Scorpions Fluorescent Signalling Molecules. Hybridization Probe Pairs and Single Labeled Probes: *An Alternative Approach for Genotyping and Quantification.* **III. New Technologies.** EasyBeacons™ for the Detection of Methylation Status of Single CpG Duplets. Quenched Autoligation (QUAL) Probes. HyBeacon® Probes for Rapid DNA Sequence Detection and Allele Discrimination. FIT-Probes in Real-Time PCR. SNP Genotyping by Unlabeled Probe Melting Analysis. **IV. Non-Nucleic Acid Targets.** Molecular Beacons for Protein–DNA Interaction Studies. DNA Polymerase Profiling. Modular Reporter Hairpin Ribozymes For Analyzing Molecular Interactions. Screening of Molecular Interactions Using Reporter Hammerhead Ribozymes. Index.

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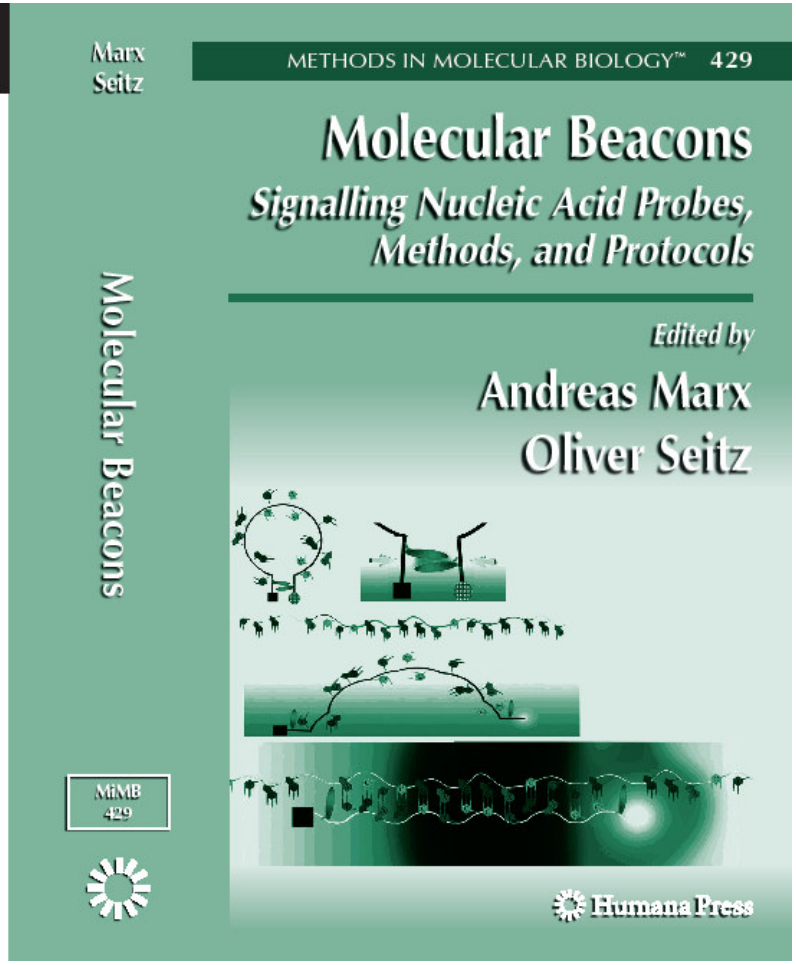
humanapress.com



Poster 111

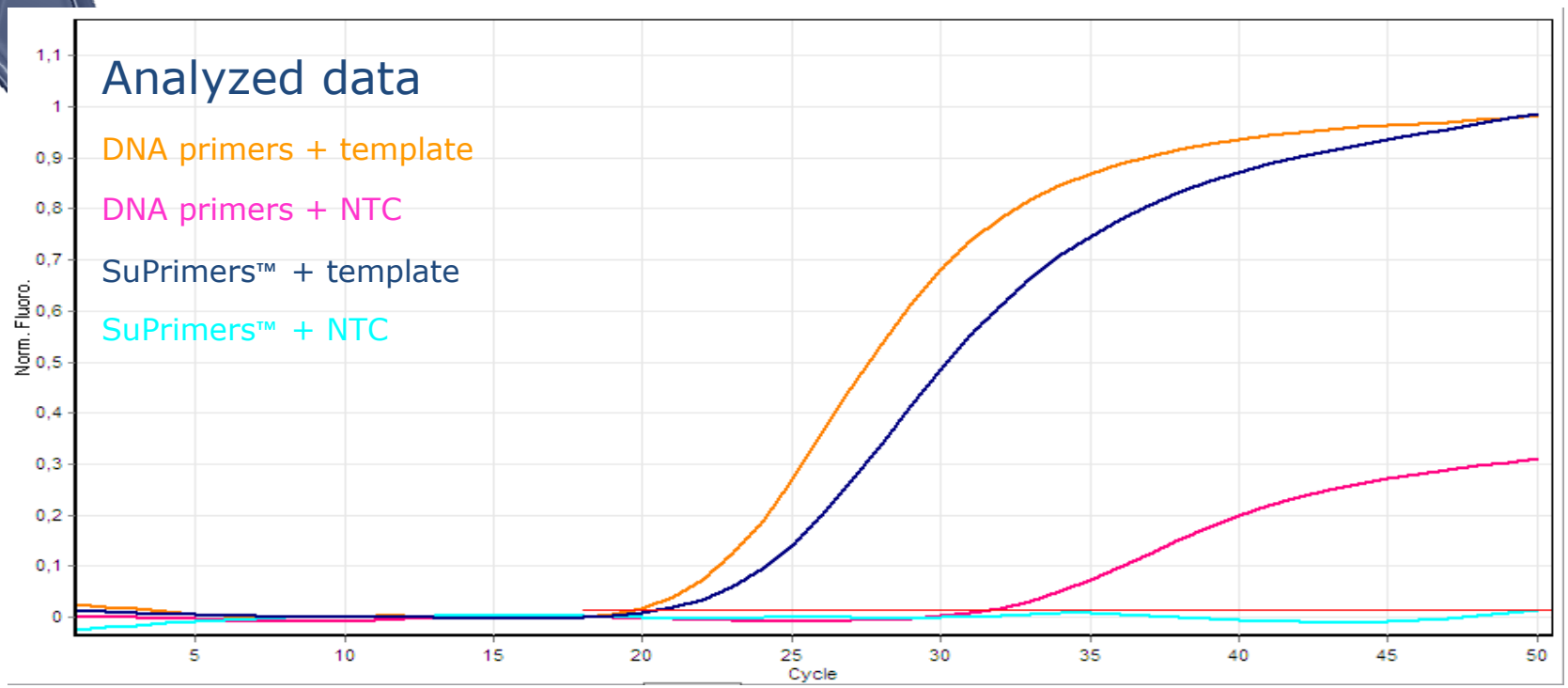
Two novel approaches used to detect single point mutation causing resistance

BEng. Christina Nielsen and M.Sc. Ulf Bech Christensen
PentaBase Aps. www.pentabase.com; For more information on assays and technologies please contact info@pentabase.com

We have developed rapid assays for detection of H274Y in both human influenza (H1N1) and Bird flu (H5N1) viruses using the two novel probe technologies, HydrolEasy® probes and EasyBeacons™ respectively.
HydrolEasy® probes are hydrolysis/Taqman® probes that can be designed to have a specific Tm and thereby be designed to have a better sensitivity and specificity than equivalent assays.
We have also introduced a new type of end-point detection of H274Y in H5N1 based on the novel EasyBeacon™ technology.
The detection of H274Y alters the recommended treatment and it is therefore important to test for the presence of this mutation following a positive H1N1 and H5N1 test.

Primer dimer elimination

Removing problems by using analogues



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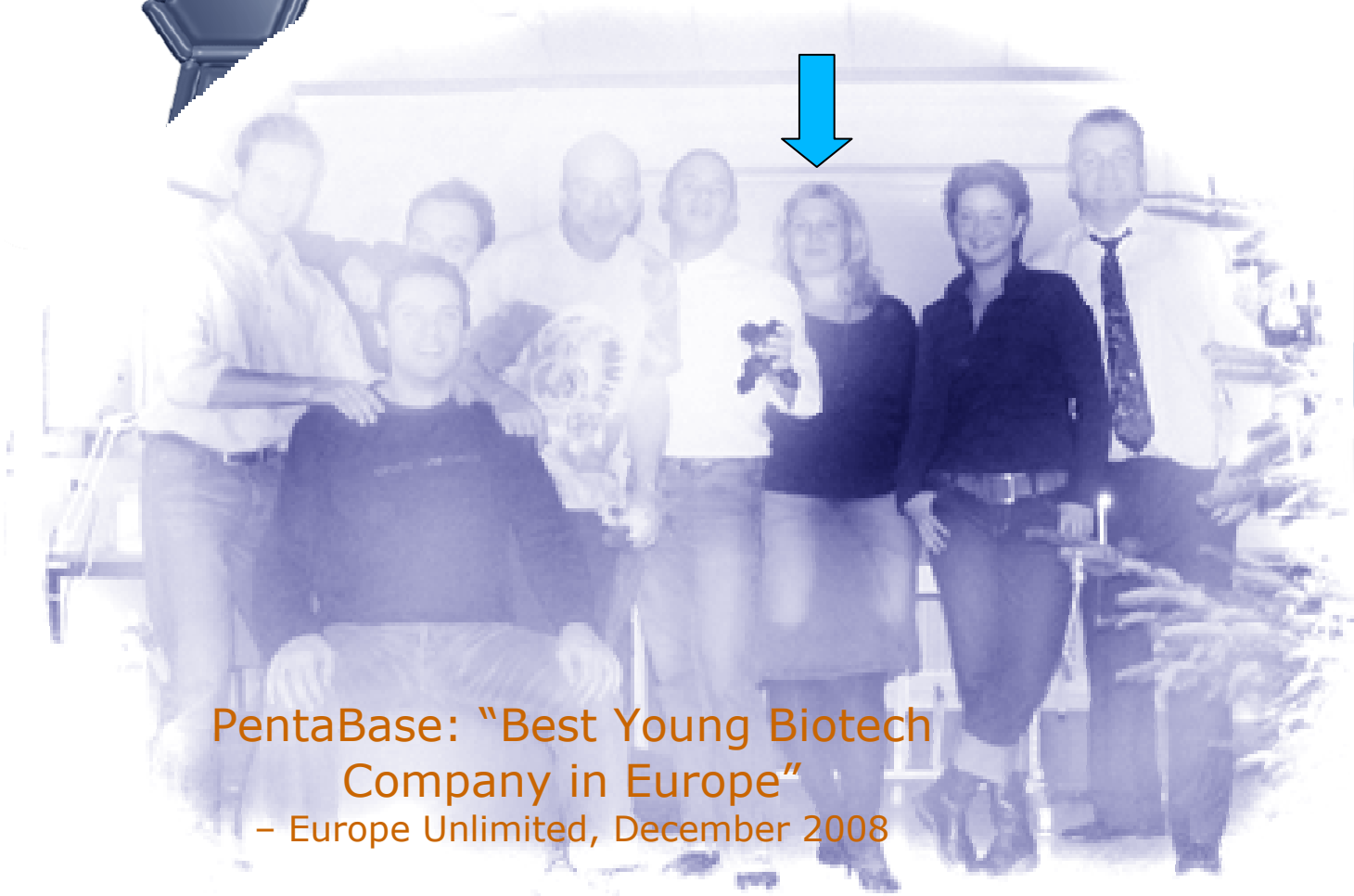
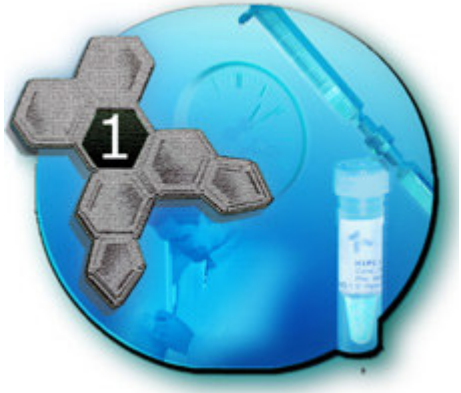


PentaBase: "Best Young Biotech Company in Europe"
– Europe Unlimited, December 2008

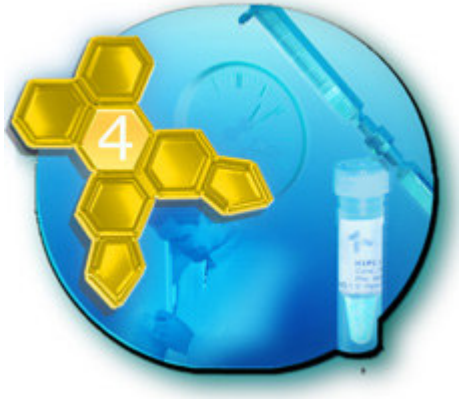
Service levels



Level 1: improve existing assay - change of technology

Level 2: Primer & probe design - one sequence

Level 3: Alignments and multiplex assays



Level 4: Assay optimization - satisfaction guaranteed

Level 5: your external employee