

EasyBeacons™ and HydroEasy™ probes



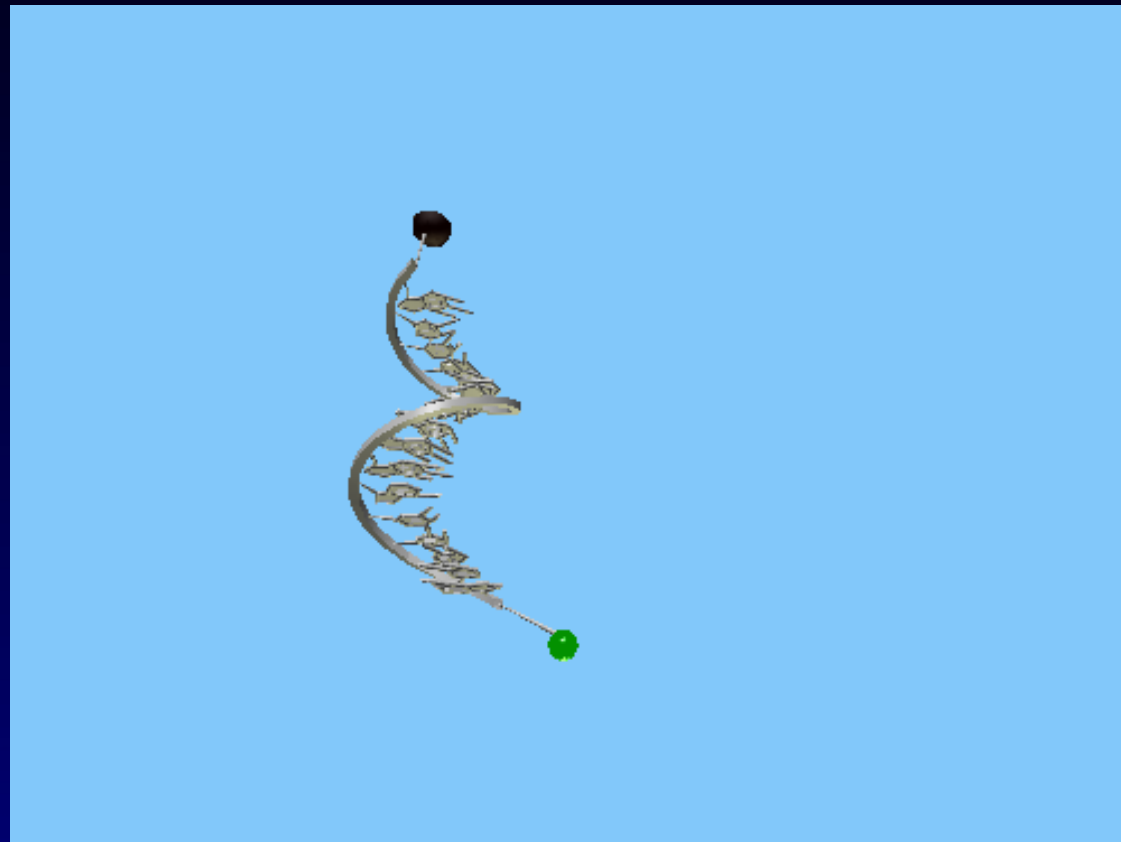
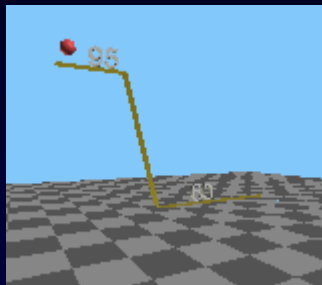
- Two new probe systems
from PentaBase ApS

by

Ulf Bech Christensen

28-03-2007

Hydrolysis assay (TaqMan[®])



Hydrolysis summary



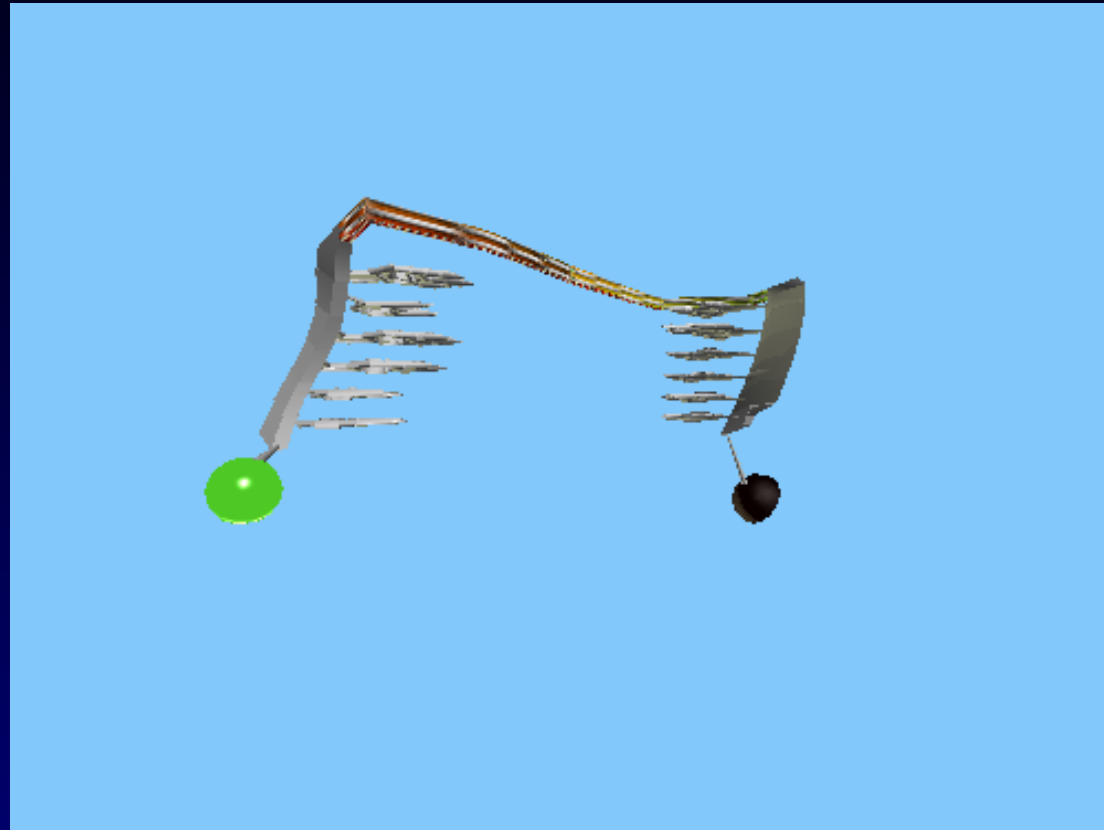
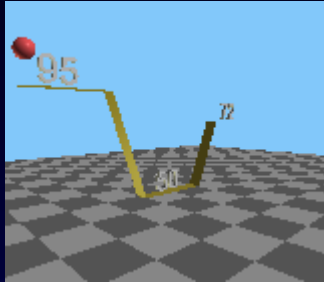
Advantages

- Highly fluorogenic
- Easy setup
- Assays-on-demand

Disadvantages

- High background
- Same conditions for primers and probes
- Probe positioning limited
- Probe degraded - no end point measurement
- SNP detection challenging

Molecular Beacon assay



Molecular Beacons summary



Advantages

- Low Background
- Specific annealing
- Different temperatures for hybridization and elongation

Disadvantages

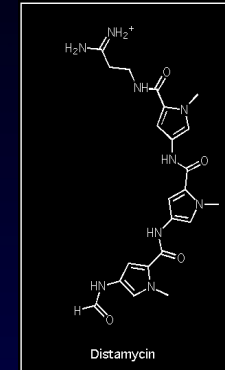
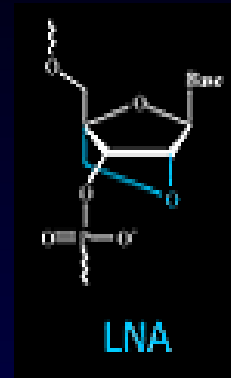
- Challenging design
- Long probes – less yield
- Intramolecular competitive binding
- Optimization required for individual probes
- Often low signal levels

Advances in technology



Locked Nucleic Acid,
LNA modifications

Minor groove binders



Advantages:

- Higher affinity
- Increased specificity

Disadvantages:

- Restrictions in design and flexibility

LNA-DNA ΔT_m 1-8°C

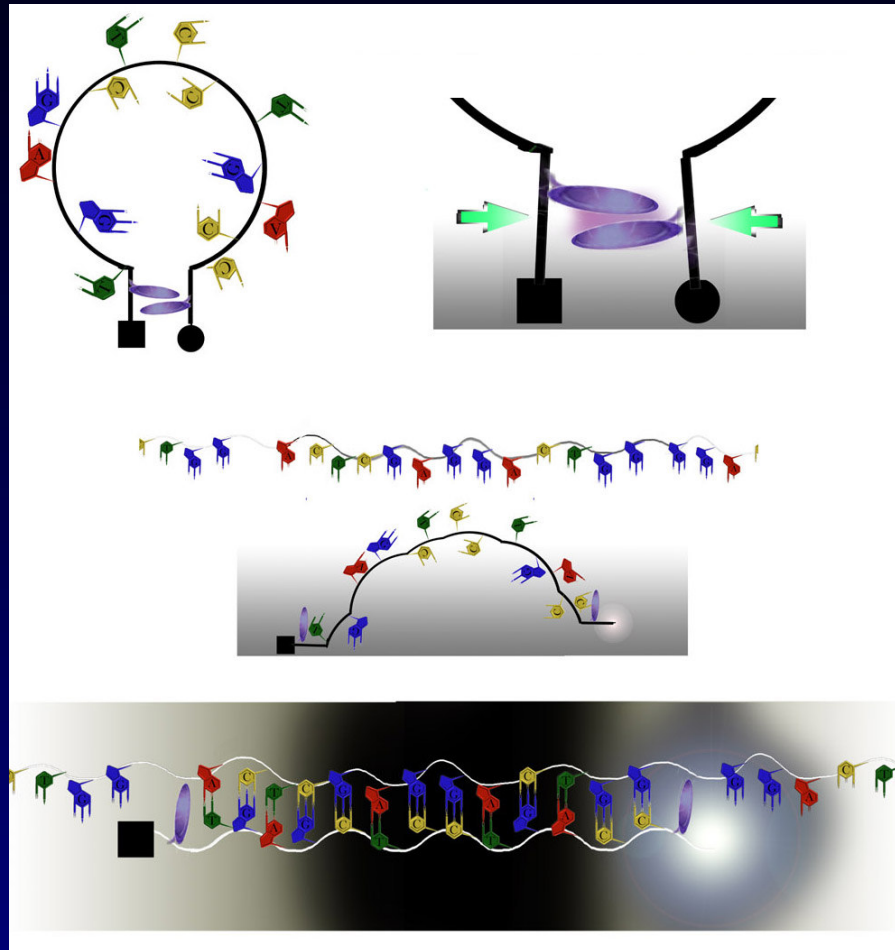
LNA-LNA ΔT_m 4-20°C



EasyBeacons™ HydroEasy™ probes

*- Solid science is not
necessarily hard!*

EasyBeacons™ & HydroEasy™ Probes mechanism

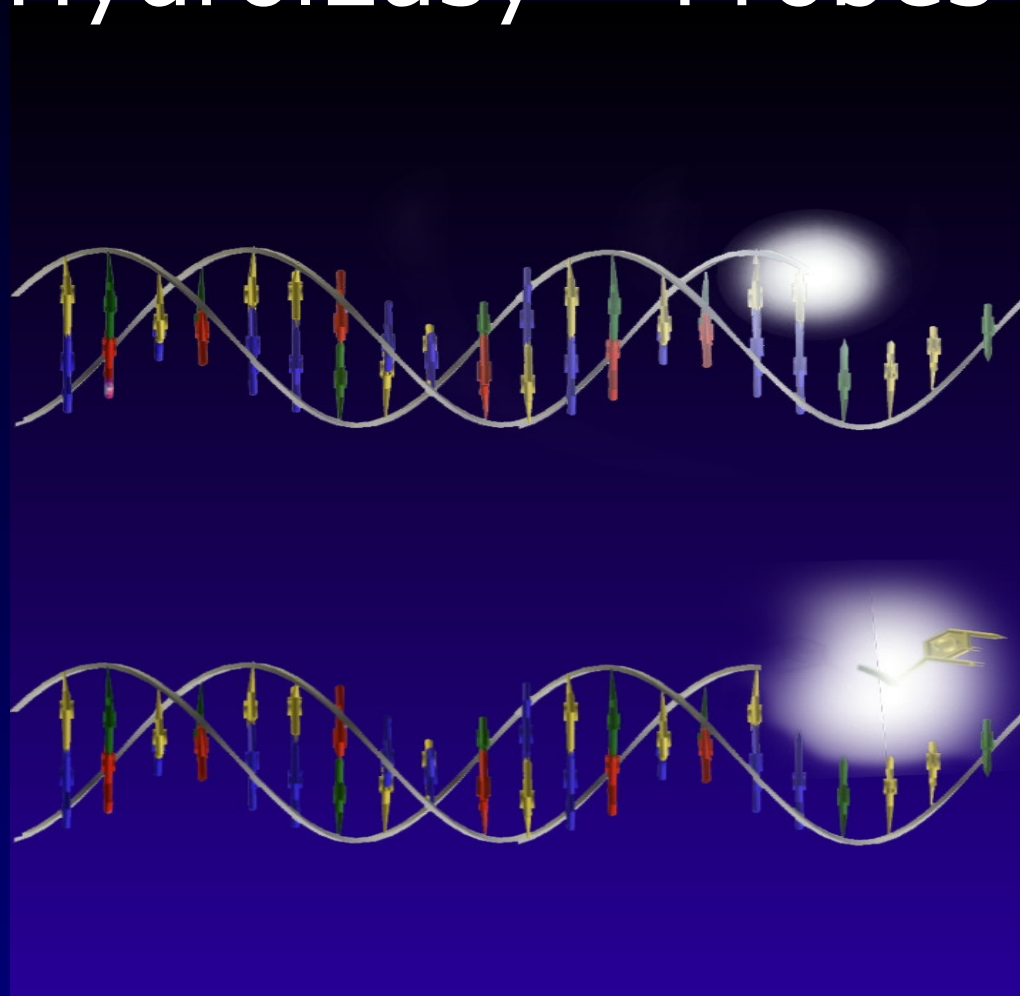


Hydrophobic forces



- Hydrophobic molecules - easy dissolved in non-polar solvents, but only sparingly soluble in water
- The hydrophobic effect is a consequence of the special properties of liquid water
- Water molecules in the vicinity of apolar moieties reorient themselves to maximize hydrogen bonding amongst them - decrease in entropy of the system
- Hydrophobic interactions can be viewed as a process of exclusion from aqueous phase and maximization of entropy
- The hydrophobic interactions are the main driving force for protein folding. This decreases 3-4-fold on folding.
- As the temperature decreases, the strength of the hydrophobic interaction decreases.

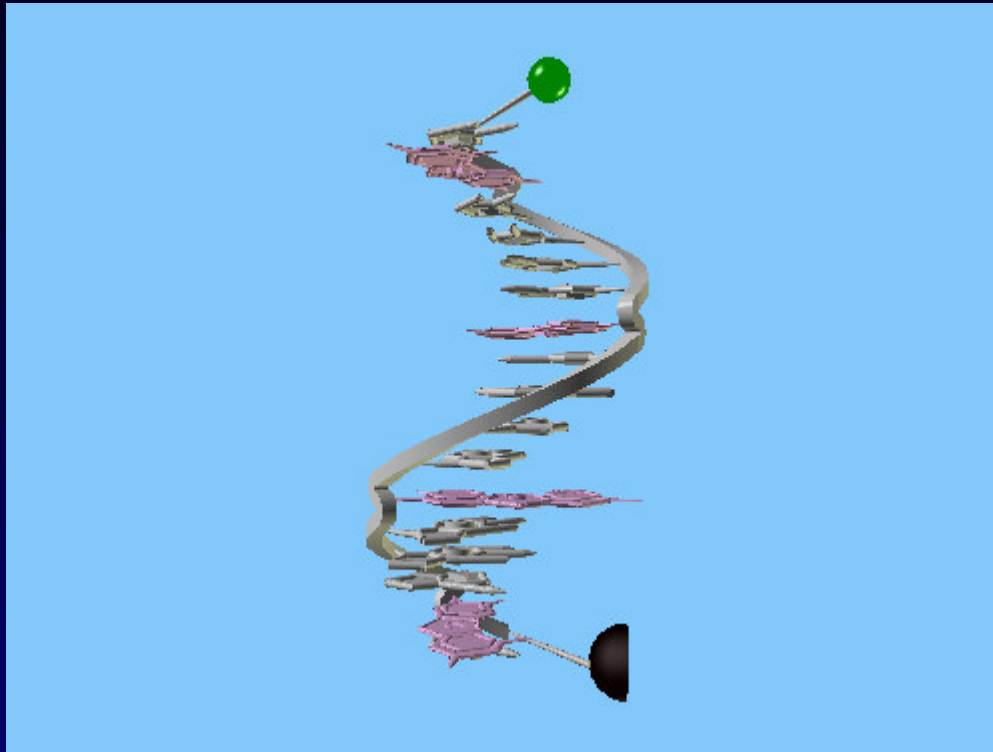
EasyBeacons™ vs. HydroEasy™ Probes



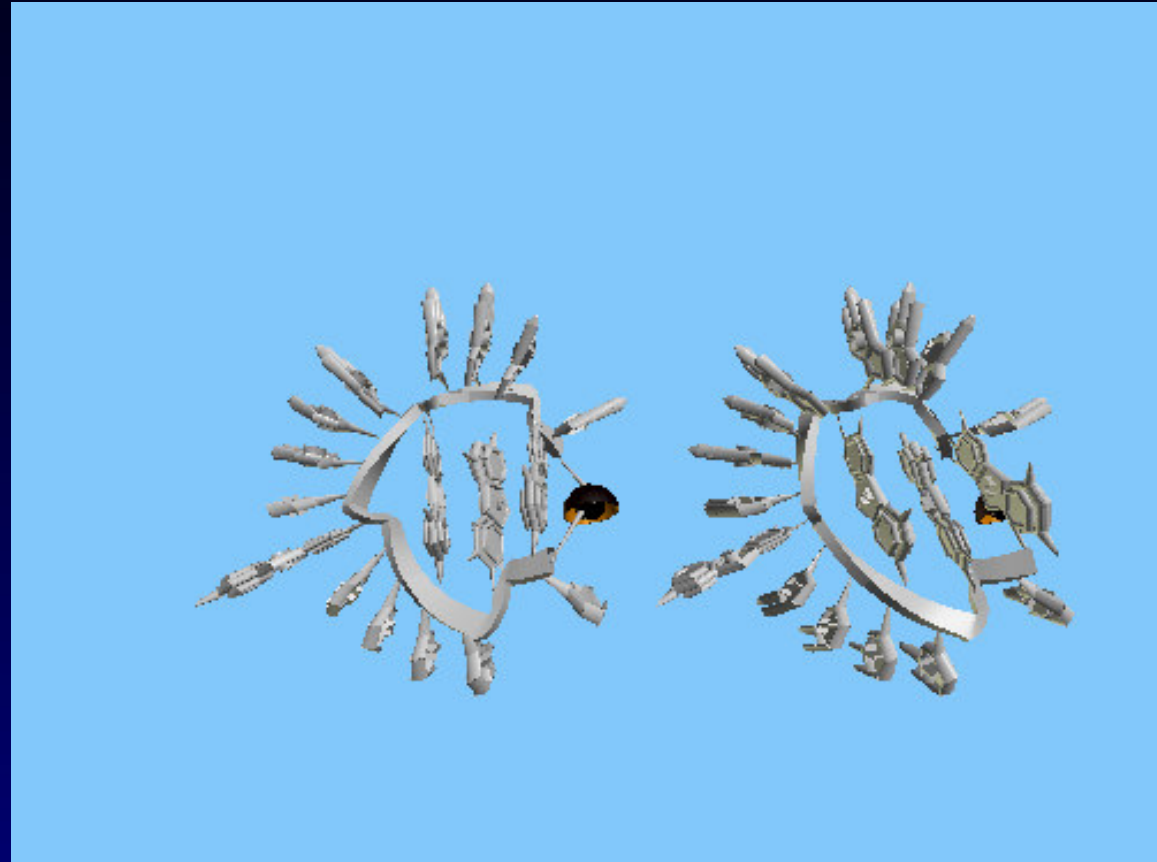
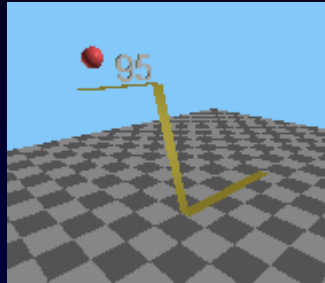
3rd qPCR event, Munich
Ulf Bech Christensen

www.Pentabase.com
ubc@PentaBase.com

EasyBeacon™/HydroEasy™ probe structure



EasyBeacon™ / HydroEasy™ probe assay



3rd qPCR event, Munich
Ulf Bech Christensen

Graphic by www.scigraph.com

www.Pentabase.com
ubc@PentaBase.com

EasyBeacons™ vs. HydroEasy™ probes



EasyBeacons™

- Nuclease resistant
- Three step PCR
- Easy to design
- Low background over the entire temperature range
- No stem needed
- QC after amplification
- Multiplexing

HydroEasy™ probes

- Hydrolysed by nucleases
- Two step PCR
- Easy to design
- Low background over the entire temperature range
- High fluorescence
- Multiplexing

Additional features of the hydrophobic nucleotides



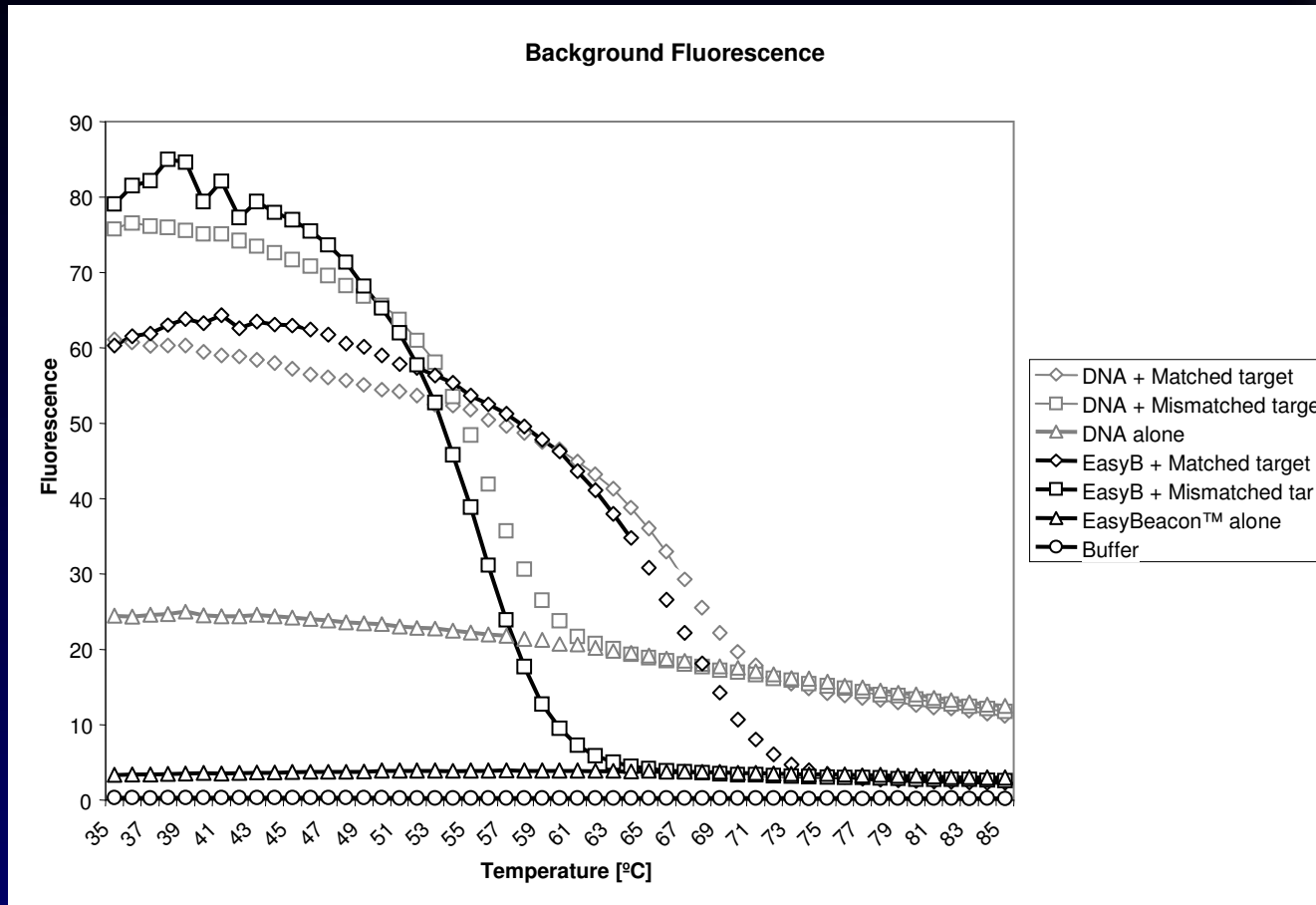
- Hydrophobic part
 - Higher affinity
 - Higher specificity
- Unnatural backbone
 - Nuclease stability
 - Less “primer dimer”



Examples of features

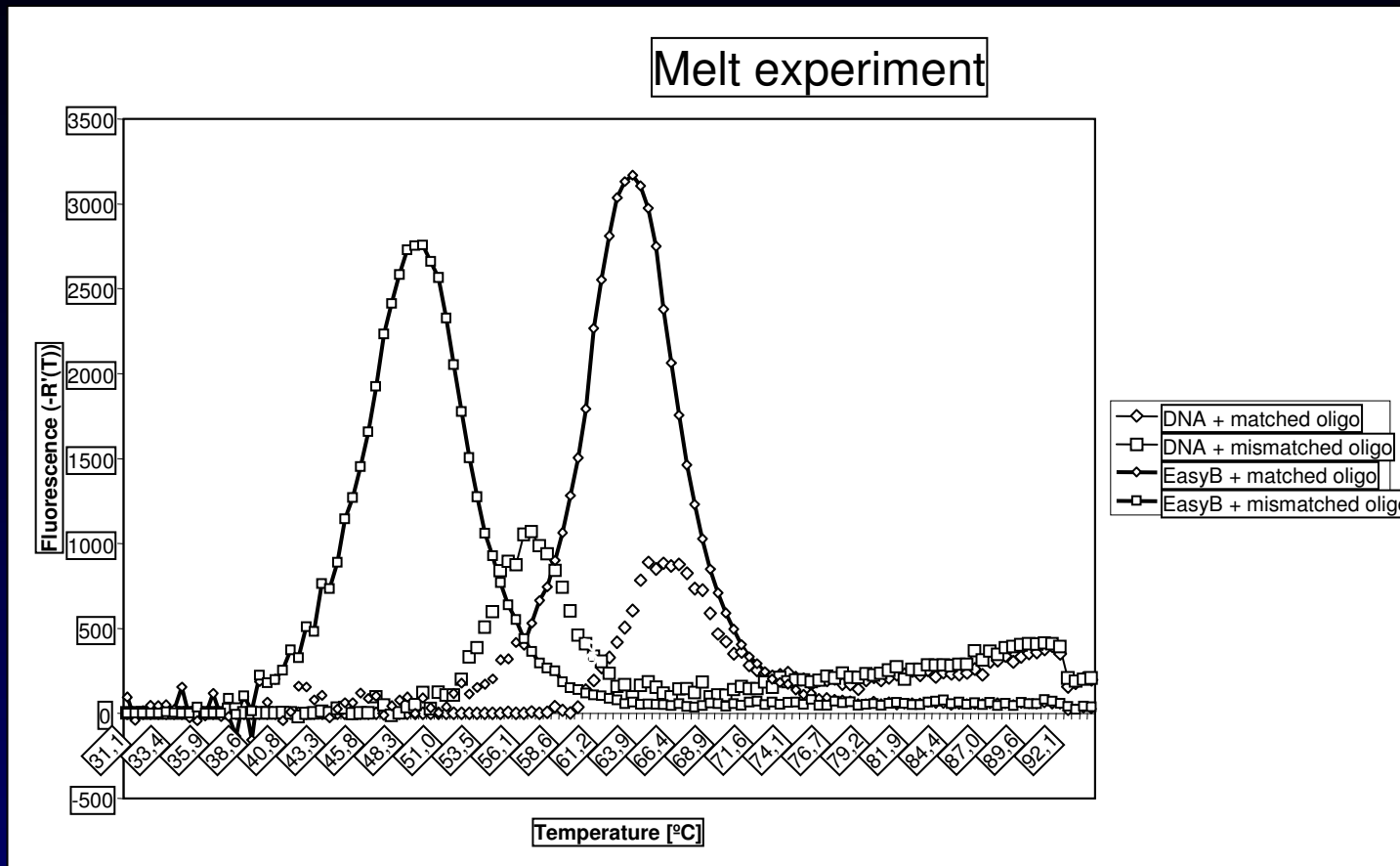
Including fast optimisation
and reliable SNP's and ^{Me}C
determination

Example of background



matched target (diamonds, GCGGGAGTTCGCGGGATTTTTAG) and mismatched target (squares, GTGGGAGTTIGIGGGATTTTTAG)

Example of specificity



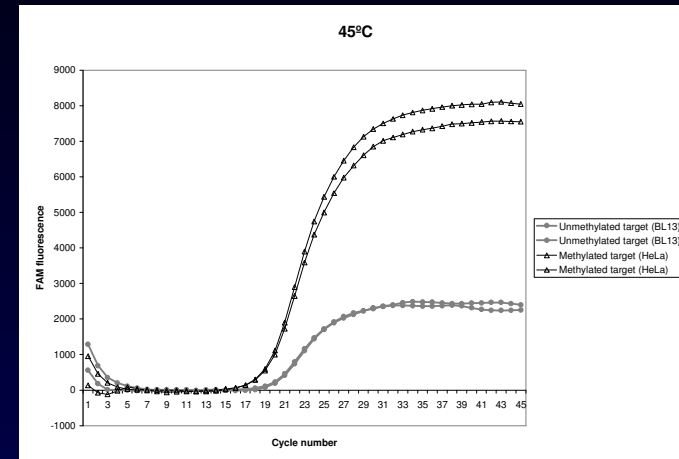
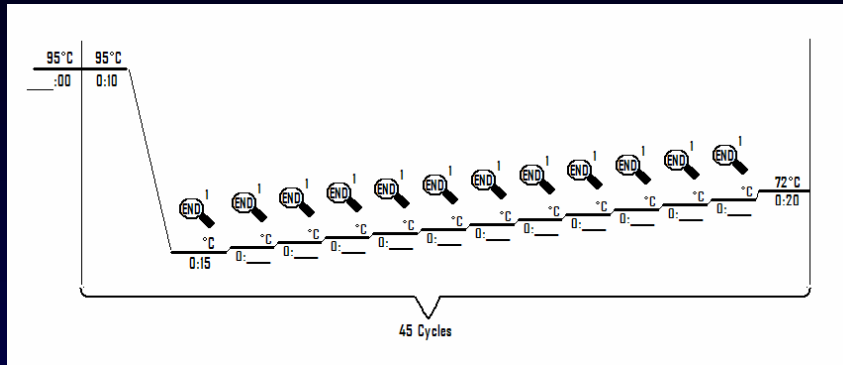
Androgen Receptor – Prostate cancer
matched target (diamonds, CCGCAAAAAAACGCCCTAAATCCC) and
mismatched target (squares, CCGCAAAAAAACAACCTAAATCCC)



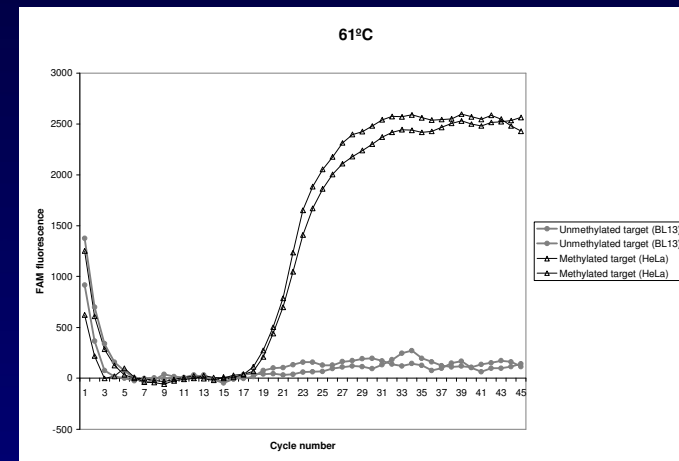
Novel features EasyBeacons™

Nuclease resistance =
fast optimization
and
end-point detection

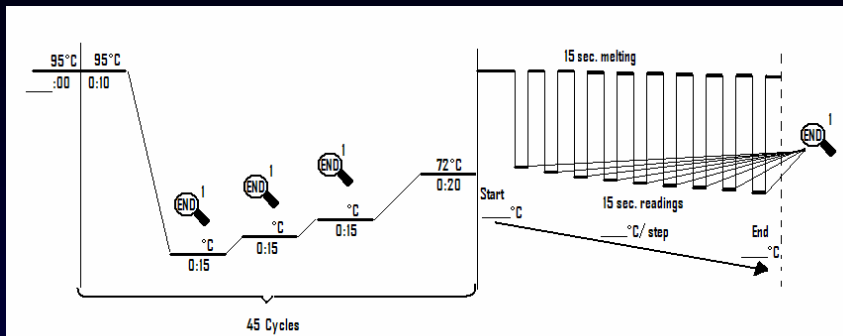
Fast optimisation type 1



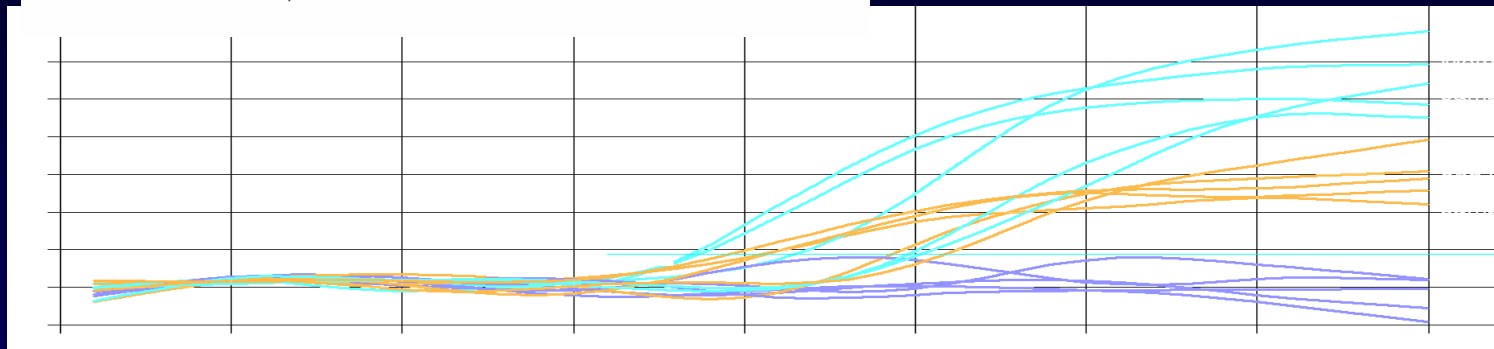
Machines with option for many reading points e.g. Mx3000 (Stratagene)



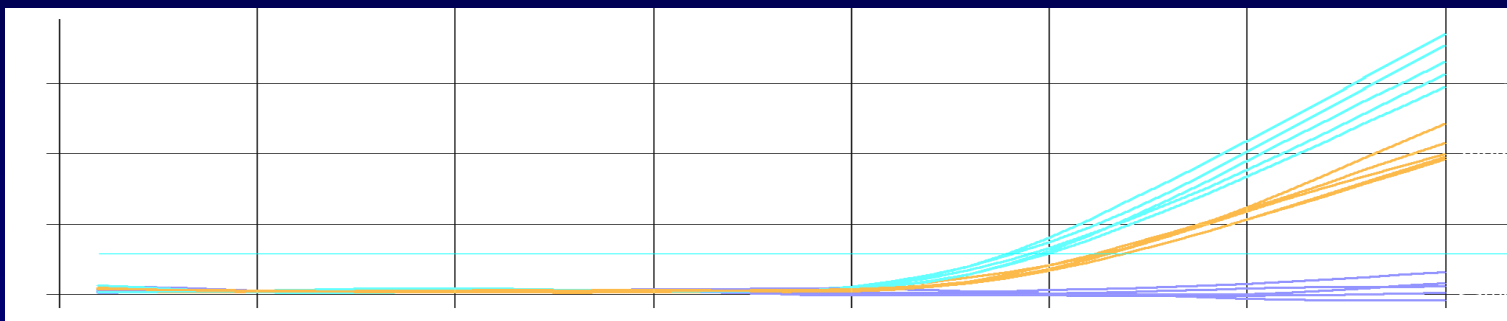
Fast optimisation type 2



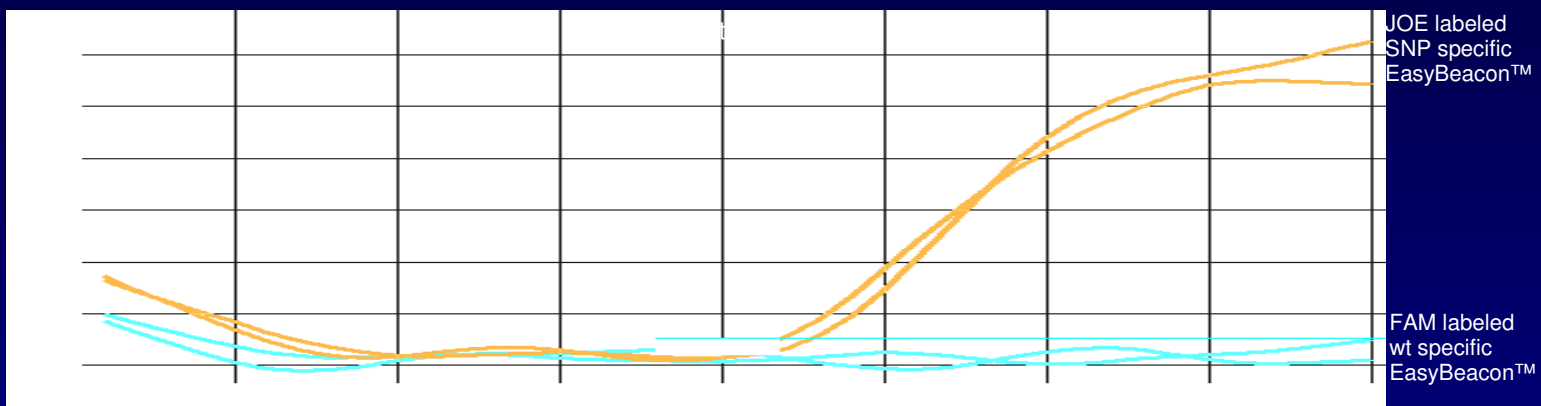
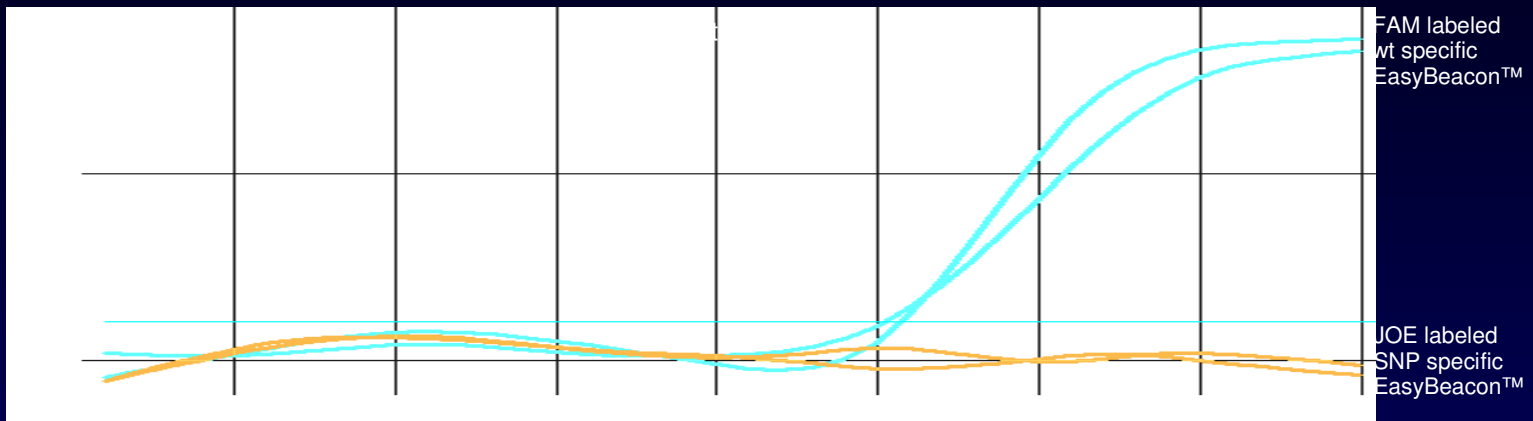
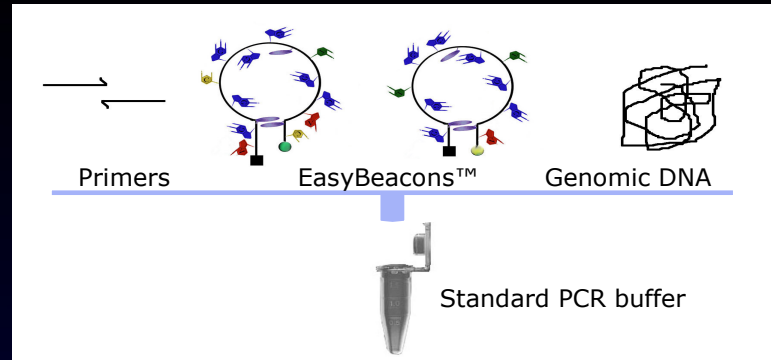
Fast ramping machines like:
Rotorgene™, MasterCycler™,
Lightcycler™, ABI 7500™, ...



...methylated
...bles
...ure of methylated
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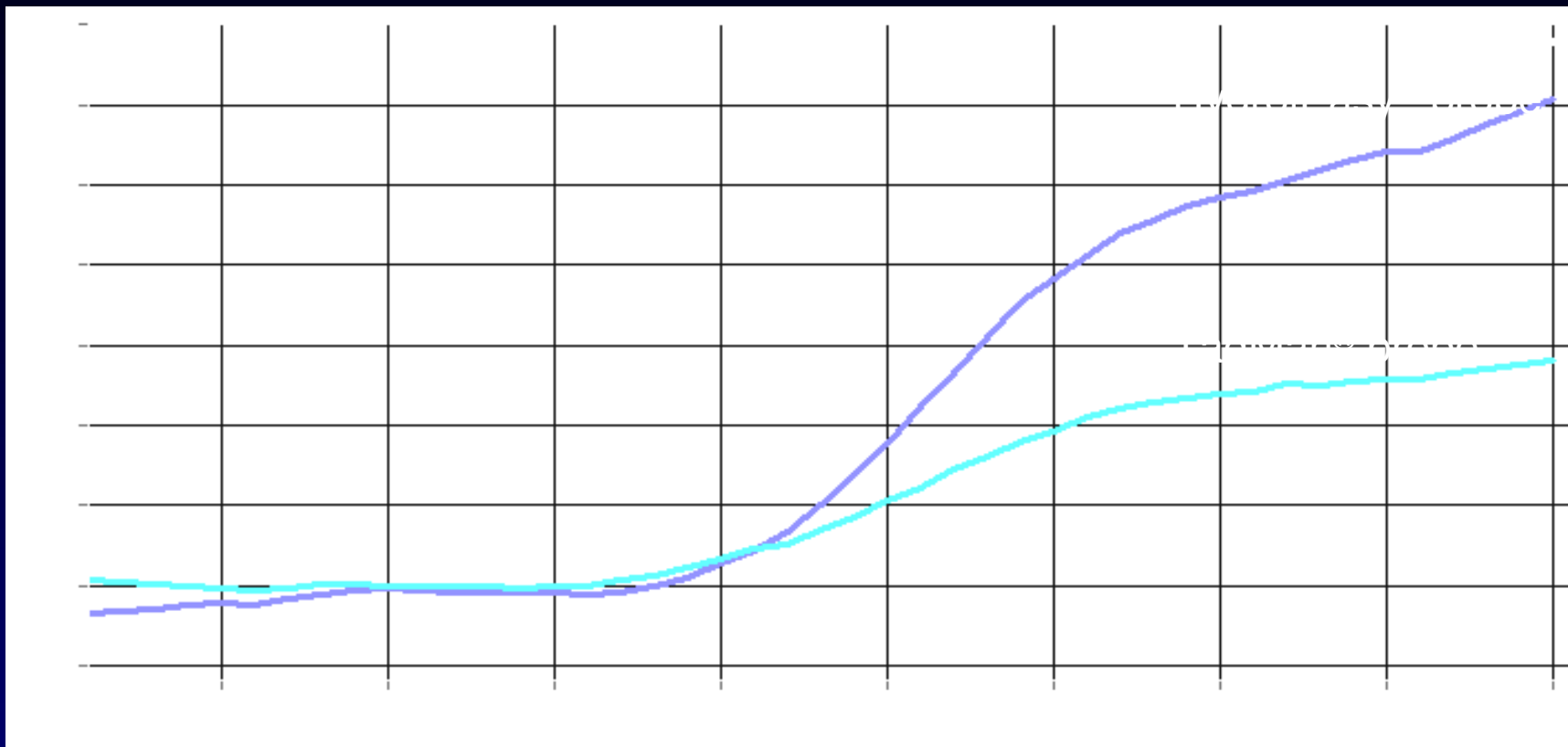




HydroEasy™ probes

Example of the improved
signal-to-noise ratio

Example of HydrolyEasy™ probe FAM-BHQ1



Hutchinson-Gilford Progeria Syndrome



Male patient, 7 years



Female patient, 14 years



Male patient, 15 years

www.progeriaresearch.com

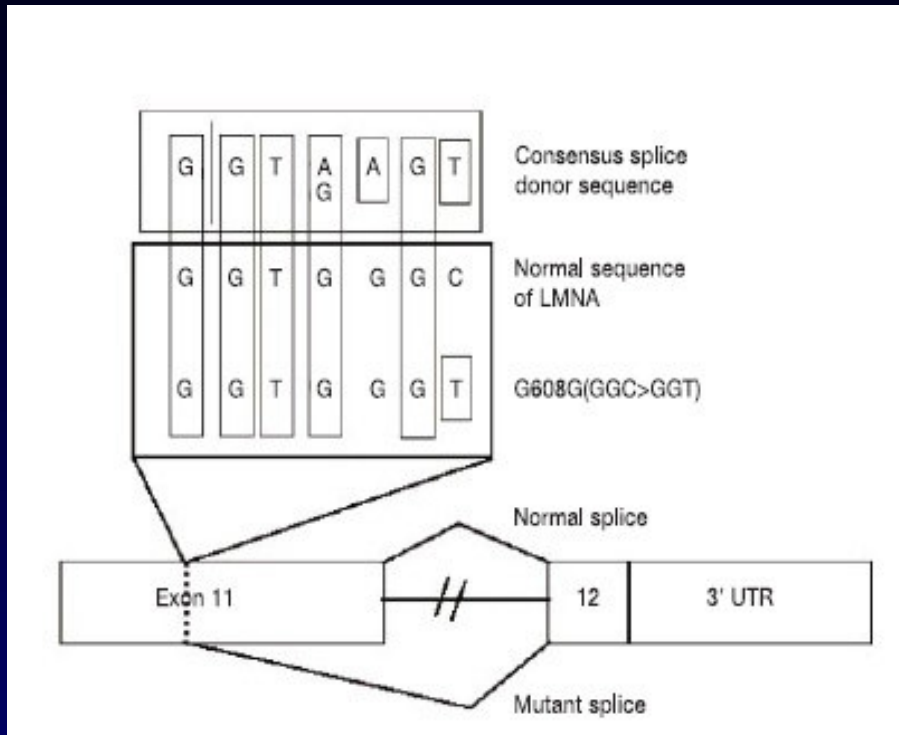
Statistics:

- 1 in 4 million births
- Death at approximately 13 years

Symptoms:

- Growth retardation
- Deformation of bones, joint stiffness, hip dislocations
- Loss of subcutaneous fat
- Osteoporosis
- Cardiovascular abnormalities, atherosclerosis
- Dentition abnormalities, alopecia
- Sclerodermatosis

A single spontaneous mutation



Adapted from Eriksson *et al.* 2003

Normal LMNA sequence

Potential hairpin formation :

5' GAGCCCAGGTGGGCGGACCCATCTCCTCTG 3'

All potential self-annealing sites are marked red

5' GAGCCCAGGTGGGCGGACCCATCTCCTCTG 3'
 3' GTCTCCTCTACCCAGGCGGGTGGACCCGAG 5'

Progeria LMNA sequence

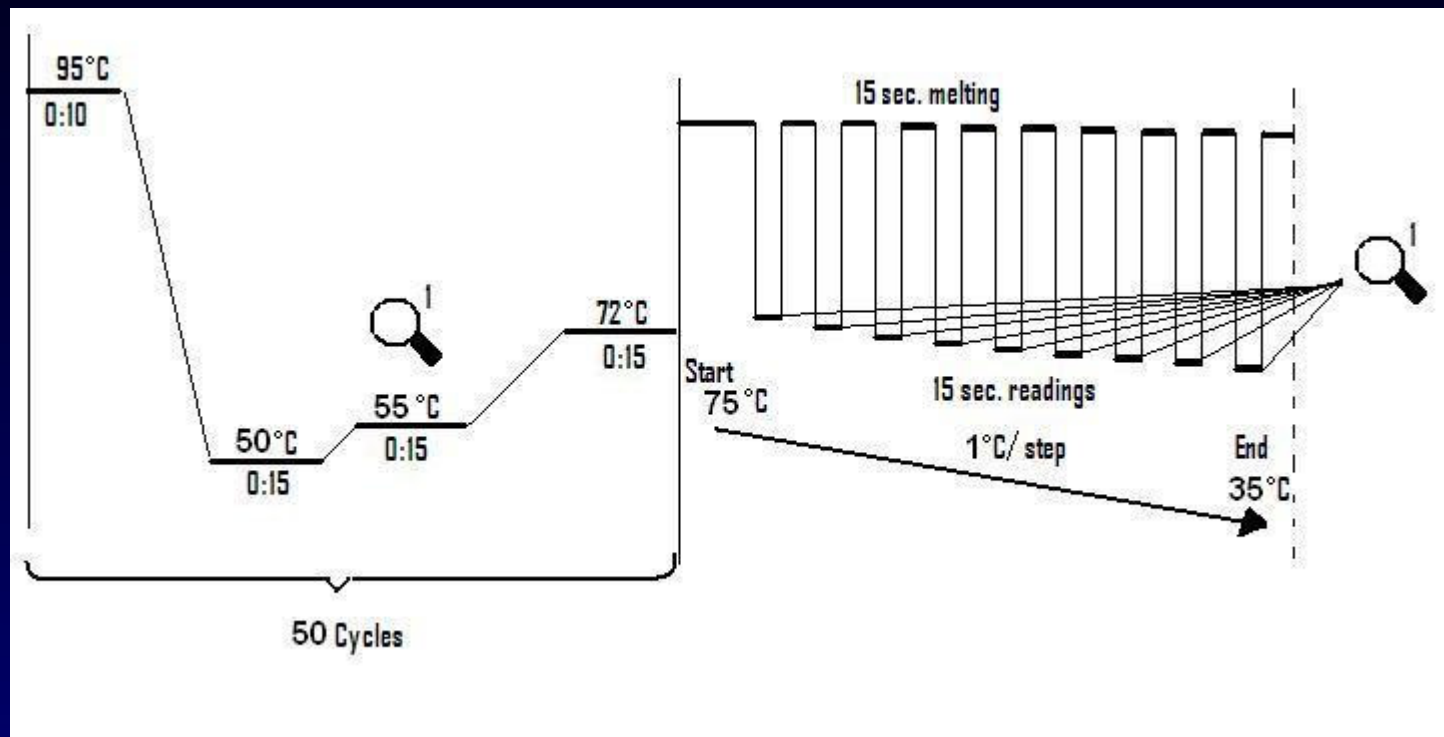
Potential hairpin formation :

5' GAGCCCAGGTGGGTGGACCCATCTCCTCTG 3'

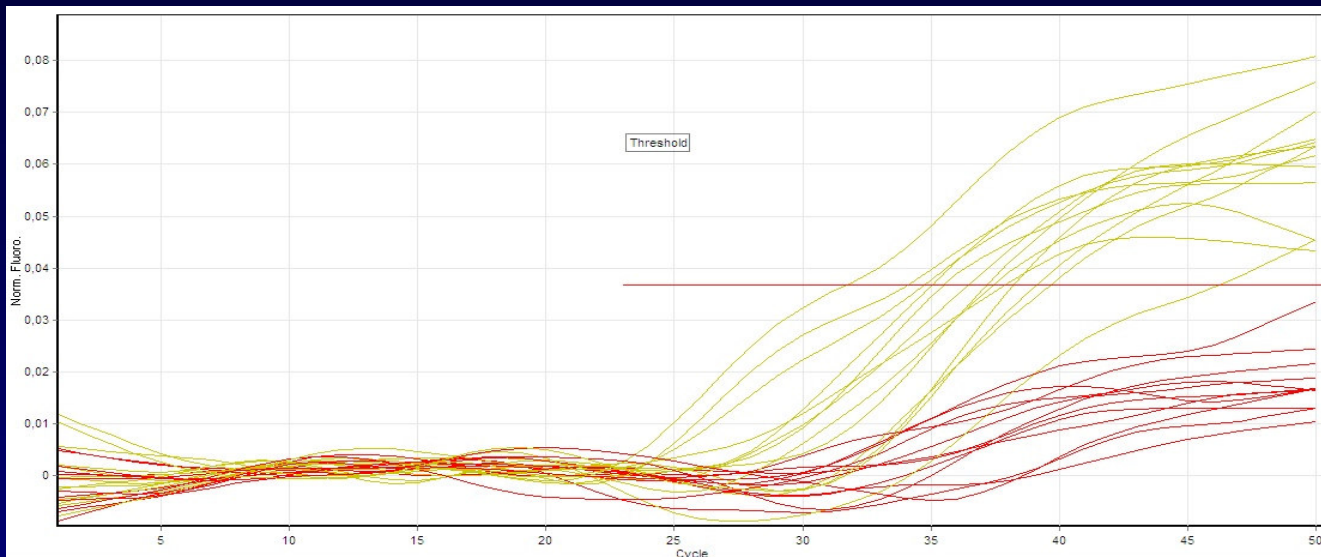
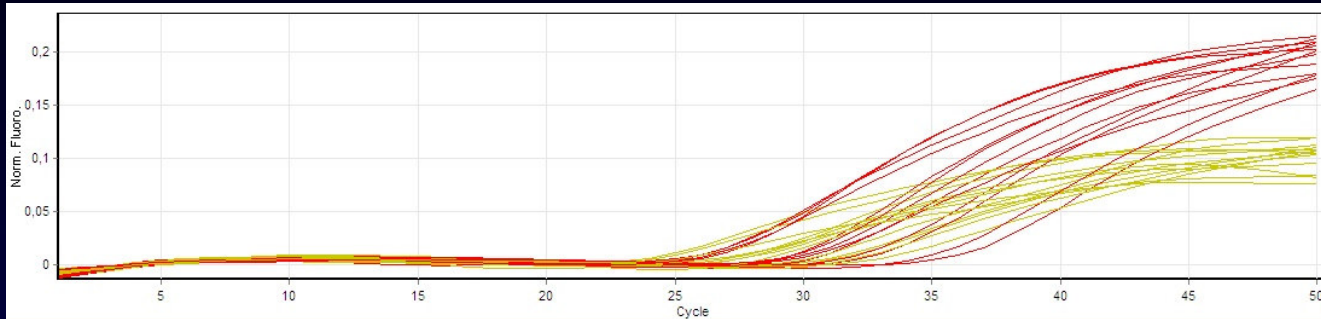
All potential self-annealing sites are marked red

5' GAGCCCAGGTGGGTGGACCCATCTCCTCTG 3'
 3' GTCTCCTCTACCCAGGTGGGTGGACCCGAG 5'

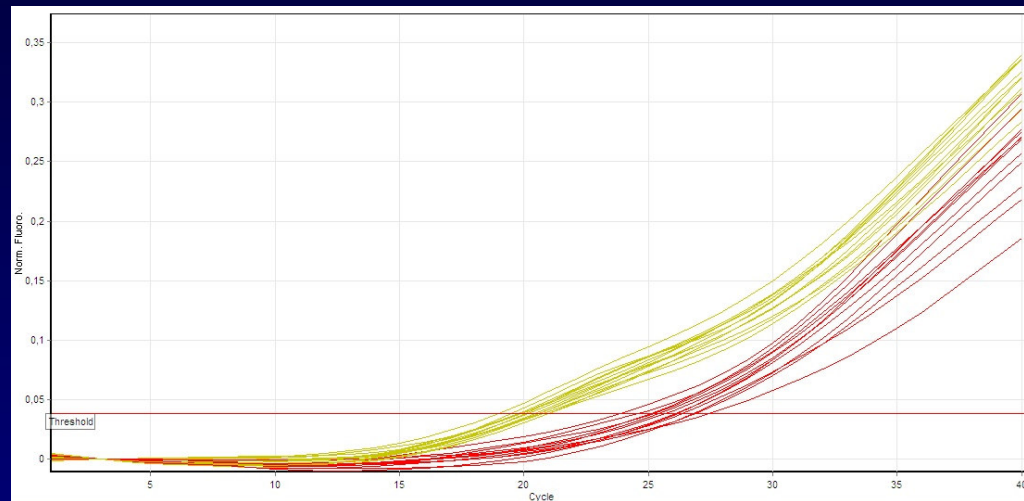
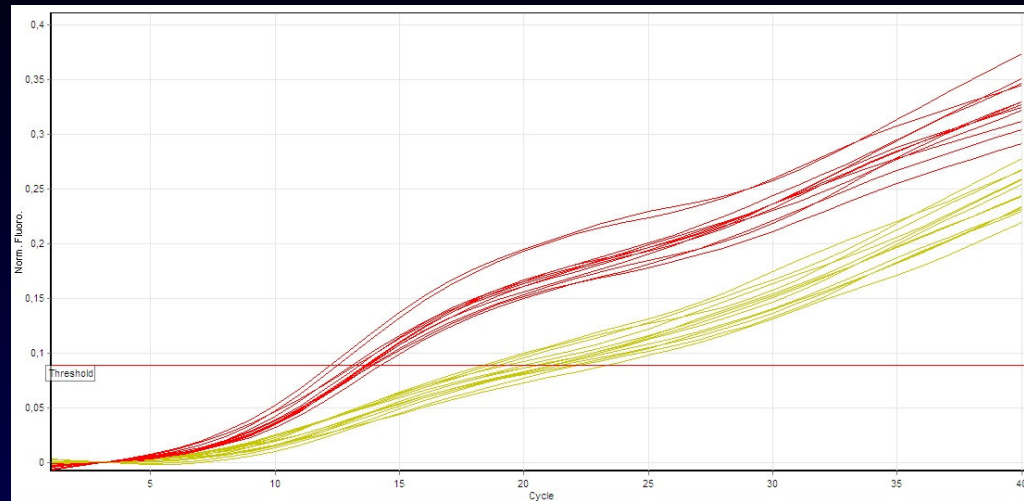
PCR profile



55 Degrees



End-point affinity



Acknowledgements



Special thanks to:

Christina Nielsen (manufacturing of INA)

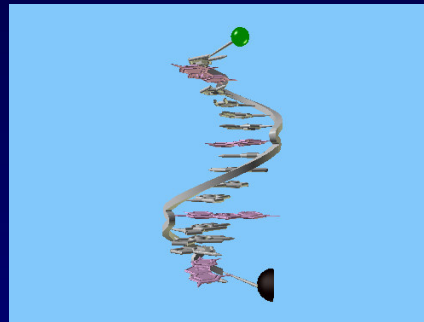
Kirstine Skadhauge (molecular biology)

Esben N. Flindt (molecular biology)

Preben Firkløver (graphic design)

Eva C Arnspang (contributing to LMNA work)

Tony Wiess (contributing to LMNA work)





Thank you for your attention

More Info

Poster #77

or

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