Taqman® Vs Molecular Beacon®

Design and optimisation of an improved probe

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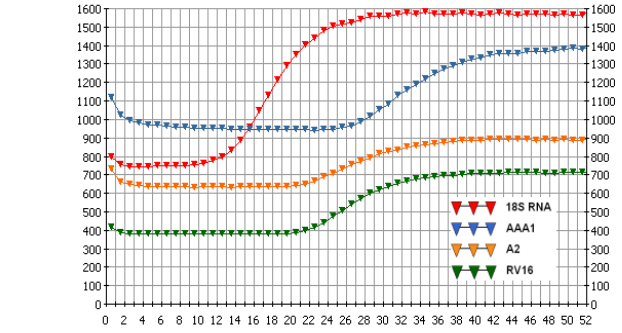
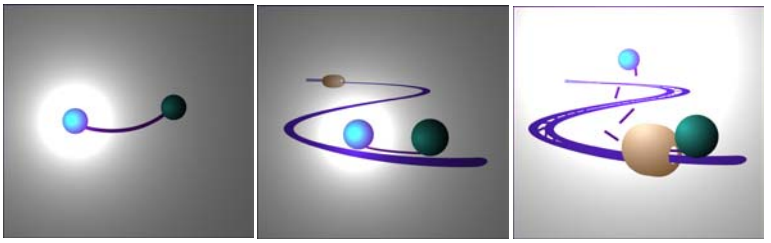
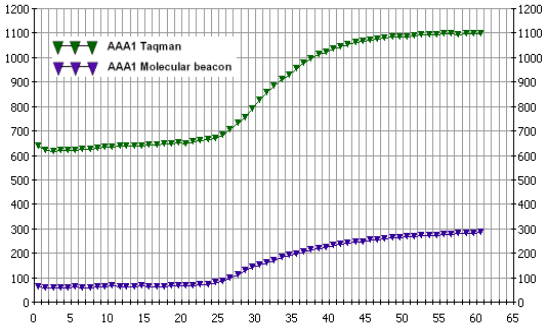
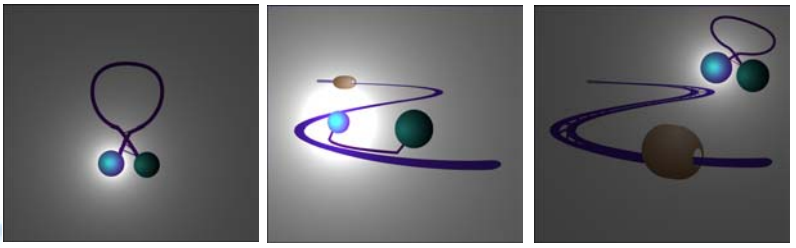
Taqman® Probe



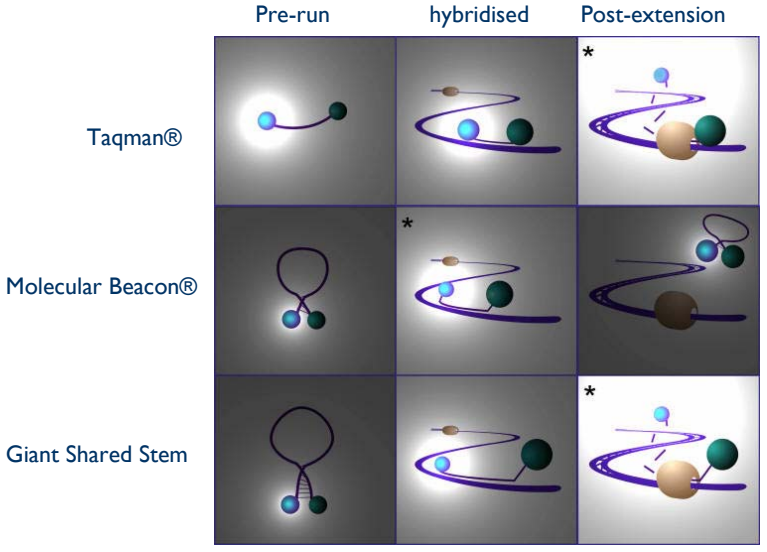


Molecular Beacon® Probe

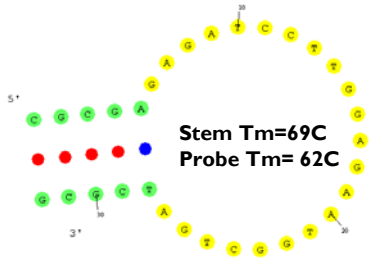




Hypothetical "best of Both"

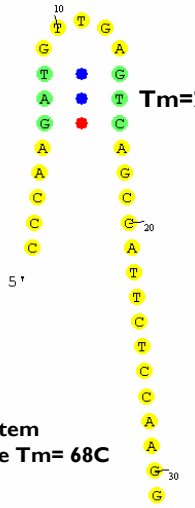


Molecular Beacon



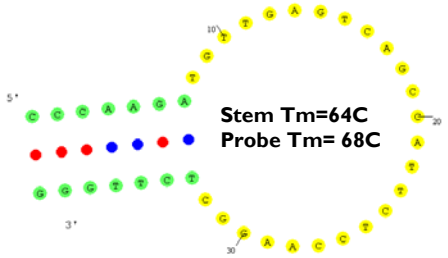
Stem Tm=69C
Probe Tm= 62C

Taqman



Tm=23C

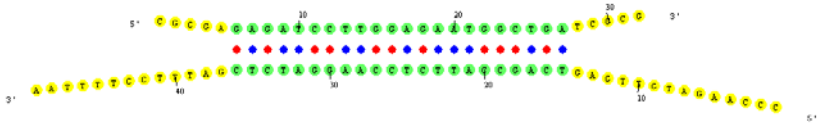
Shared Stem Giant Beacon



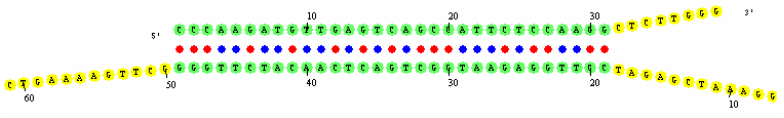
Stem Tm=64C
Probe Tm= 68C

No Stem
Probe Tm= 68C

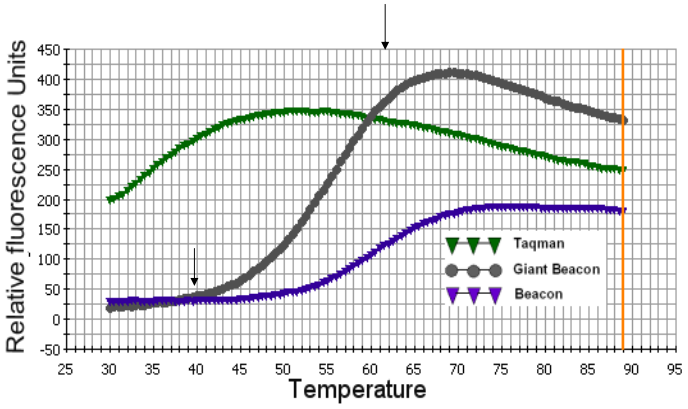
Molecular Beacon



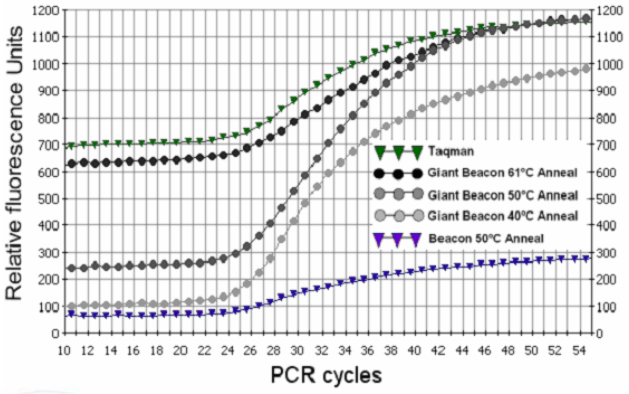
Shared Stem Giant Beacon

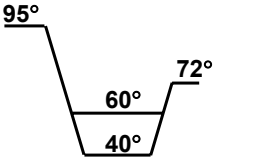


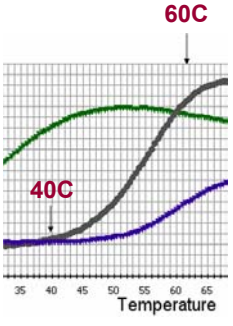
Pre-amplification melt curve



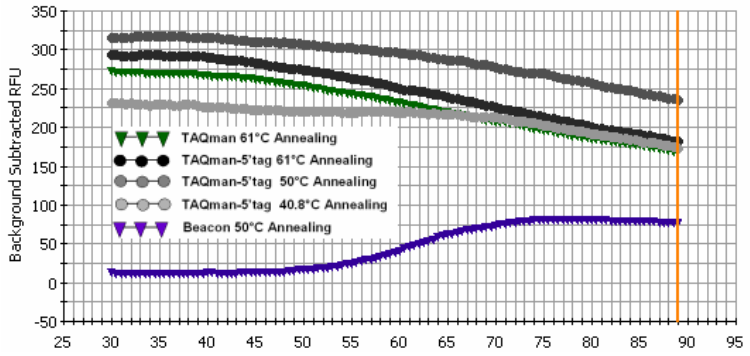
Gradient plate



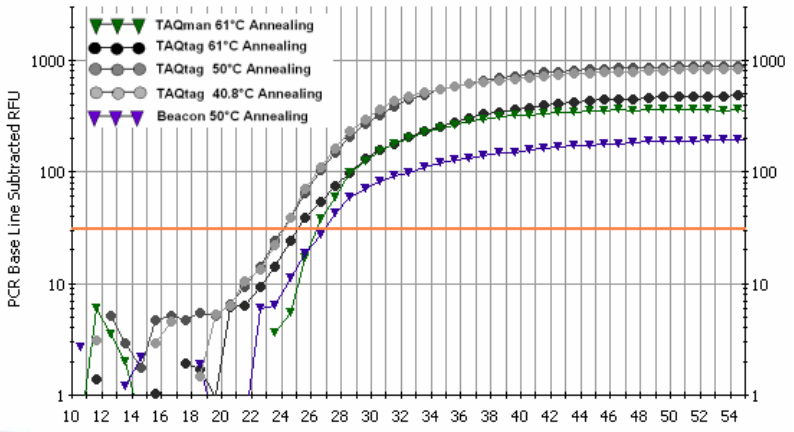




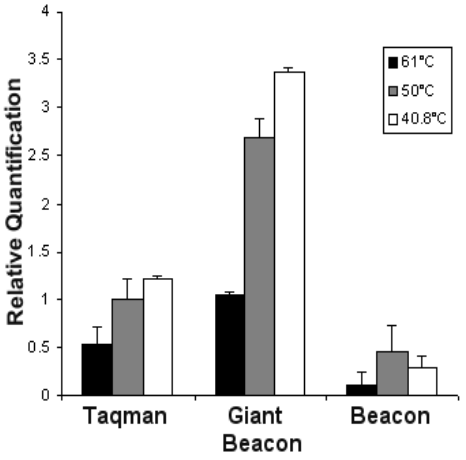
Post-run melt curve



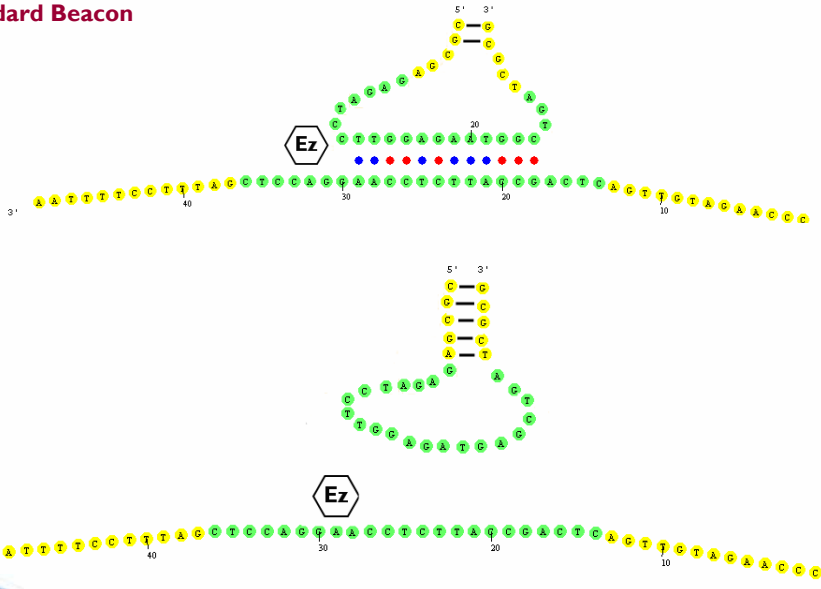
Baseline corrected data



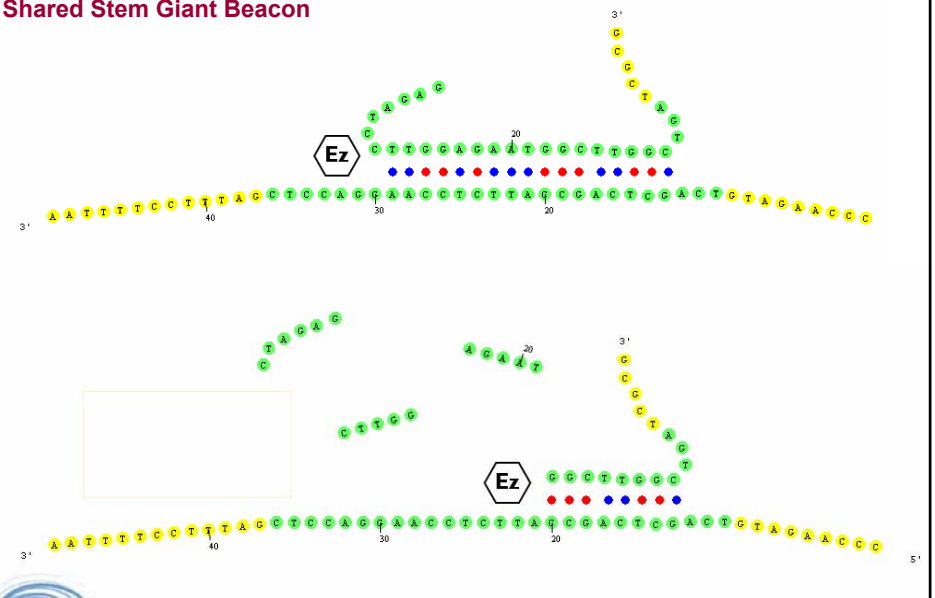
Relative Quantification



Standard Beacon



Shared Stem Giant Beacon



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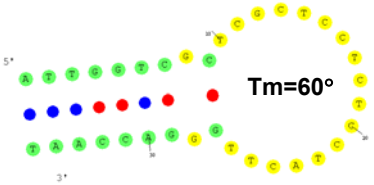
Conclusions

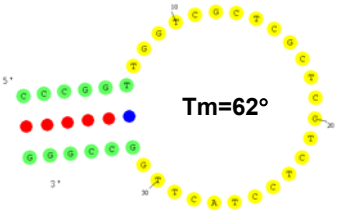
- Hairpin melt profile is predictive of quenching
- A fully quenched probe that is also cleave is more sensitive (earlier CT values)

18S Probes

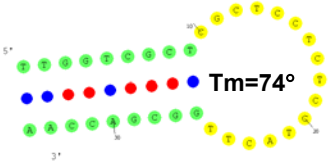
Shared Stem Giant Beacon

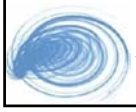


Giant Beacon



High Tm Shared Stem Giant Beacon





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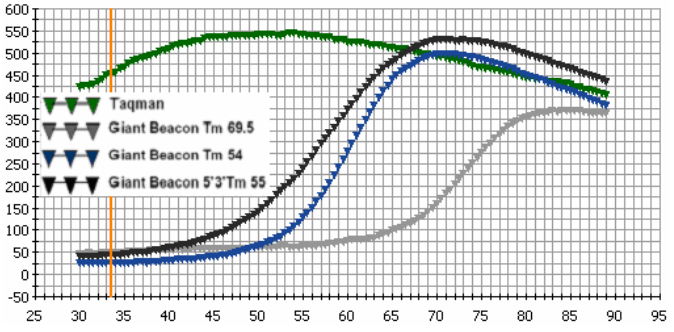
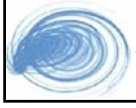
Pre-amplification melt curve





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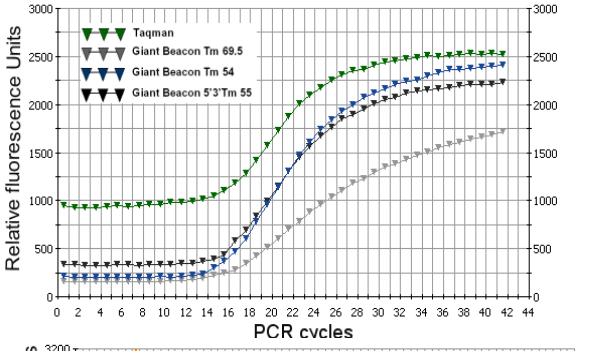
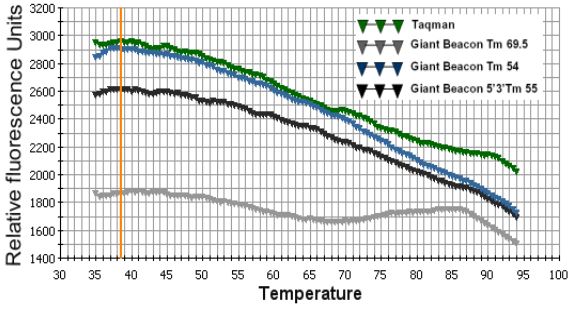
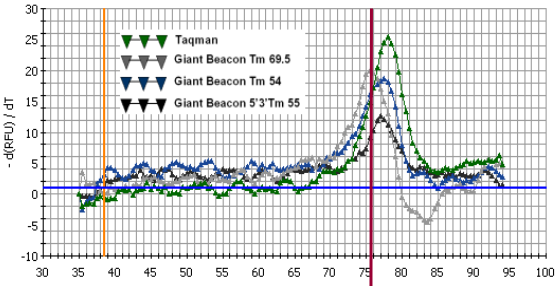
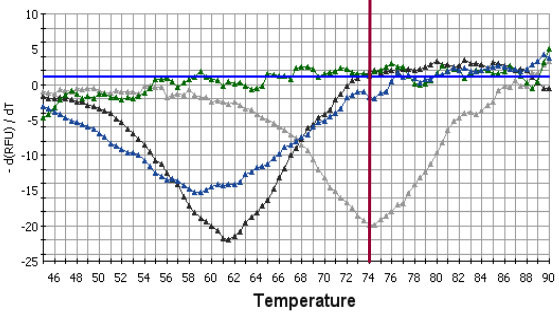




Probe to Template melt curve



Probe Hairpin melt curve



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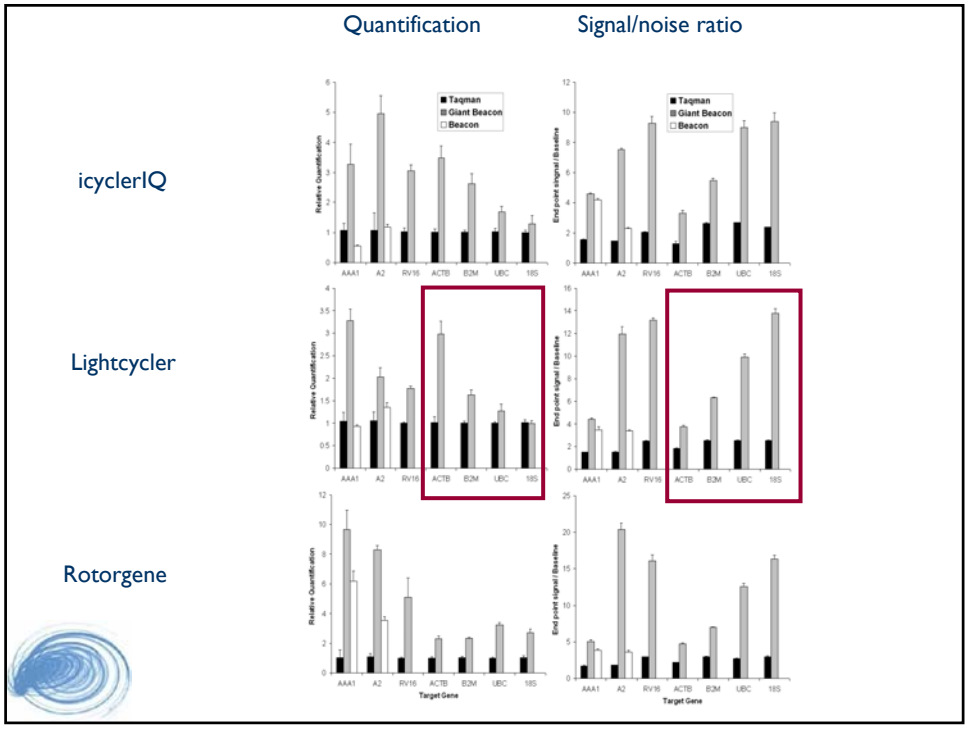
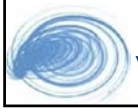


Conclusions

- Shared Stem Giant Beacons exhibit improved performance over Taqman and Molecular Beacons.
    - Improved signal/background ratio
    - Improved sensitivity (earlier CT)
    - More flourogenic (signal – background)
- Balance between optimal quenching and optimal cleavage
- Probes require precise design criteria and a modified cycling protocols.

Design Criteria

- Probe to Template Tm >68
- Probe length 26-34bp
- No Secondary structure in Probe backbone.
- Shared Stem is better than independent Stem.
- Stem Melt point 50°C-54 using MFold.
    - Na^{+} 0.05M Mg^{++} 0.003M
- Read temp of 50°C



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