



Fluorescent Molecular Probes

Gene Link Molecular Beacon Melt Curve Protocol

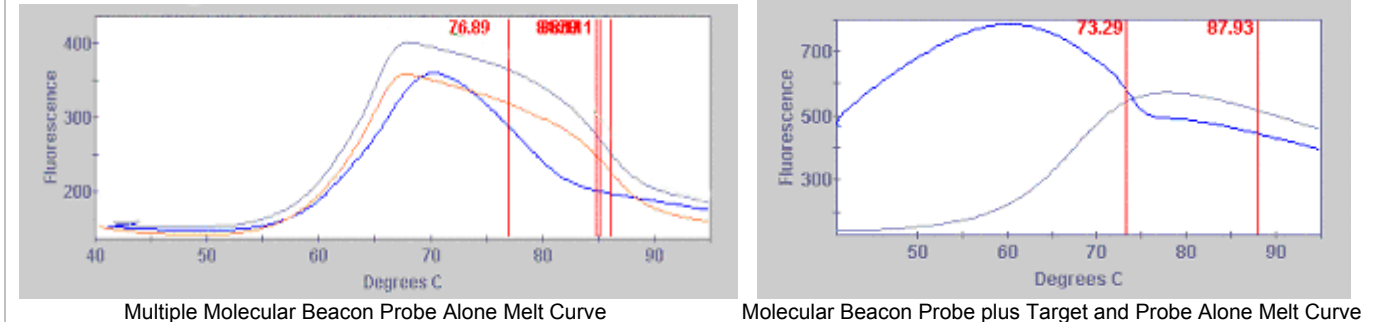
Molecular Beacons*

1. Prepare Molecular Beacon stock solution at 100 pmols/ μ l [100 μ M (micromolar)] in 1 X PCR Buffer. Gene Link provides the exact amount of nmoles of each oligo supplied on the tube and on the Oligo Report. Multiply the 'nmol' amount by 10 to arrive at the volume of solvent to be added.
2. Prepare Molecular Beacon working solution at 5 pmols/ μ l [5 μ M (micromolar)] in 1 X PCR Buffer.
3. Set up two 25 μ l reactions, one with probe alone, one with target + probe as follows.

Molecular Beacon Probe Alone	Molecular Beacon Probe plus Target
2.5 μ l 10X PCR buffer	2.5 μ l 10x PCR buffer
3 μ l 25 mM MgCl ₂	3 μ l 25 mM MgCl ₂
1 μ l 5 pmol/ μ l probe [0.2 pmol/ μ l final or 200 nM]	1 μ l 5 pmol/ μ l probe [0.2 pmol/ μ l final or 200 nM]
	3 μ l 5 pmol/ μ l target [0.6 pmol/ μ l final or 600 nM]
18.5 μ l H ₂ O	15.5 μ l H ₂ O

The protocol for Molecular Beacon Melt Curve ramps from 40 to 95 degrees C at 0.2 degrees/second.

Screen capture of graphs from a Cepheid SmartCycler



Notes:

- MgCl₂ needs to be higher for MB reactions than for regular PCR as it helps to stabilize the stem structure of the probe during the high ramp rate. Final concentration of MgCl₂ should be between 2.5 and 4 mM. Here we use 3 mM final. This is the same range of concentration used in an actual amplification reaction.
- Final concentration of probe should be 200-600 nM. Here we use 200 nM. This is also the same concentration range used for the real time reaction.
- For a melt curve it is important to saturate the probe with target. Use 2-3 X the *Molar* amount of target. Here we use 3X target for a final concentration of 600 nM. For real time monitoring, 500 ng genomic DNA, diluted 10X to various concentrations can be used as a starting point.

QPCR

Once you have your melt curve you want to select an annealing temperature for your real time PCR where the probe alone is completely closed (shows no fluorescence), and the probe+target is completely open (shows maximal fluorescence). This temperature should be about 5-8 degrees below the T_m of the probe/target hybrid (red vertical line on melt curve of probe+target). It is important to test your primers at the annealing T to ensure that you will have strong, clean amplification at this temperature.

For more technical assistance email at support@genelink.com

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Gene Link™

140 Old Saw Mill River Road Hawthorne, NY 10532
Tel: 914.769.1192 www.genelink.com Fax: 914.769.1193