Methylation Sensitive Hi-Res Melting™ using Calibration

TECHNICAL
::: NOTE

Method Background

Bisulfite conversion of genomic DNA enables amplification-based detection of 5-methylcytosines. Methylation-sensitive (MS) Hi-Res Melting is a fast and simple detection method that compares melting temperature (Tm) and melting curve shape to assess methylation in a region of interest. Individual or multiple CpGs can be analyzed. Using the High Sensitivity Master Mix, with internal calibration, this technique is sensitive enough to show a single methylation event within a group of CpGs. High discrimination is possible because of the exquisite sensitivity of Hi-Res Melting combined with the internal calibration made possible with HSMM.

High Sensitive Mastermix

High Sensitivity Master Mix includes LCGreen® Plus and specially designed oligonucleotide calibrators. Ultimate temperature precision results from this calibration. Amplicon Tm is a function of average methylation because methylation protects C's during bisulfite conversion. Hypermethylated samples are differentiable by Tm. Internal calibration also enables volume and buffer discrepancies to be overcome. High-precision melting curve alignment and low cost make Hi-Res Melting ideal for demanding applications such as epigenetic studies.

Assay Design

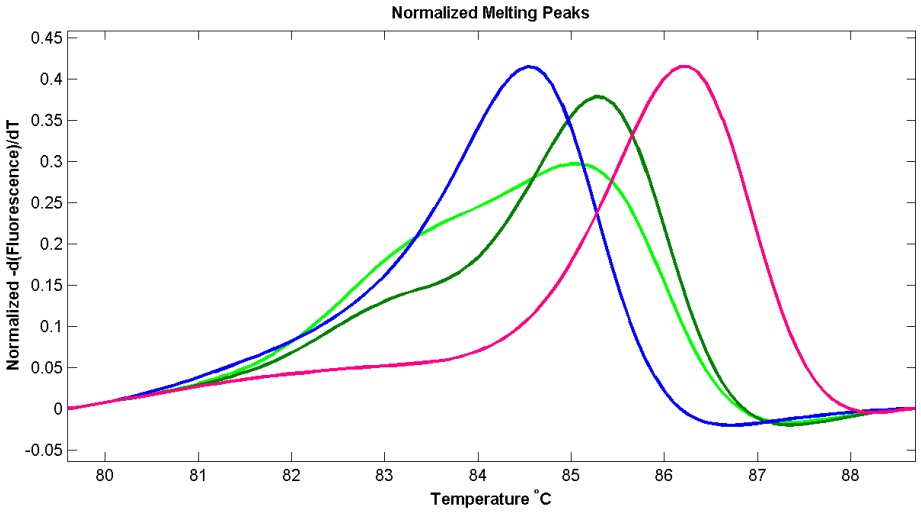
Use the following guidelines for optimal methylation sensitive Hi-Res Melting assays.

- ❑ Consider a commercial kit for bisulfite conversion. To assess melting curve variability, perform duplicate conversions.
- ❑ For maximum coverage of CpGs and minimal loss of sensitivity, 100–150 bp amplicons are excellent. Smaller amplicons can be designed to assess fewer CpGs. Larger amplicon design enables analysis of more CpGs serving as a homogeneous solution for overall methylation in a promoter hot spot.
- ❑ Amplicon Tm should be 70–88°C. This can be estimated using the Tm tool available for customers at http://www.idahotech.com/Support/
- ❑ Use tools such as Idaho Technology's LightScanner® Primer Design software to minimize complementarity, hairpins, etc.
- ❑ Make sure that forward and reverse primers are within about 1°C predicted Tm for efficient amplification. Design the primers with a Tm of 55–65°C.
- ❑ Use online tools such as BLAST or University of California Santa

Cruz's in-silico PCR predictor to ensure primer specificity within the genome of interest.

- ❑ No probe is needed; although, if you are interrogating a single CpG, small amplicons (~ 50 bp) or LunaProbes™ assays may be used.
- ❑ Typical PCR cycling involves a 94°C/2 min. initial denaturation followed by 40–45 cycles of 94°C/30 sec. and 55–65°C/30 sec. Program a final heteroduplex generation step immediately after PCR by denaturing to 94°C/30 sec. and annealing at room temperature (~ 28°C) for 30 sec. or indefinitely. For the final heteroduplex generation, set temperature transition rates to maximum for best results.

Methylation analysis results



	Average	Site #1	Site #2	Site #3	Site #4	Site #5
Sample 1	40%	11%	82%	85%	10%	14%
Sample 2	40%	11%	88%	85%	7%	11%
Hypermethy Control	99%	99%	100%	99%	100%	99%
Hypomethy Control	8%	10%	9%	13%	0%	9%

References

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Gundry CN, Dobrowolski SF, Martin YR, Robbins TC, Nay LM, Boyd N, Coyne T, Wall MD, Wittwer CT, Teng DH (2008) Base-pair neutral homozygotes can be discriminated by calibrated high-resolution melting of small amplicons. Nucleic Acids Res. 2008 Apr 29. [Epub ahead of print] PMID: 18448472 [PubMed - as supplied by publisher]