Tech Spotlight
dPCR System

DNA Methylation Analysis with dPCR

on the Digital LightCycler® System

DNA methylation is an epigenetic modification characterized by the presence of 5-methylcytosine (5-mC) in specific genomic locations, especially regulatory regions like promoters and enhancers. The methylation status of these regions can affect many biological processes, including development, aging, and disease.

Most approaches for examining DNA methylation rely on the process of sodium bisulfite treatment to distinguish between methylated and unmethylated CpGs. However, many PCR systems—including dPCR systems—are not compatible with bisulfite treatment of DNA due to utilization of uracil DNA glycosylase (UNG) in the reagents; UNG leads to degradation of bisulfite-converted DNA, severely compromising the results.

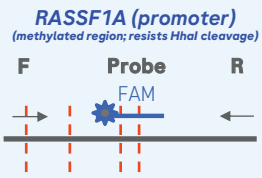
A new dPCR-based method for DNA methylation analysis—which does not rely on bisulfite treatment—has been developed on the Digital LightCycler® dPCR System. This method leverages methylation-sensitive restriction enzymes (MSRE) to reveal the methylation status of specific regions of DNA with a high degree of accuracy, even in CpG-rich regions.

The combination of methylation-sensitive restriction enzymes (MSRE) and dPCR offers accurate, rapid, cost-effective methylation analysis.

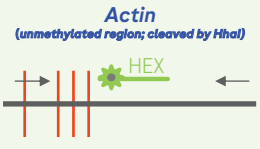
The example below shows how a multiplex dPCR assay has been used to examine the methylation status of the promoter for *RASSF1A*, a tumor suppressor gene whose dysregulated expression is associated with a variety of cancers. Hypermethylation of *RASSF1A* promoter is associated with decreased gene expression.

The two samples used here are (1) a human genomic DNA sample (GDNA), in which the methylation status is typical of a healthy individual, and (2) another DNA sample that has been enzymatically treated to maximize CpG methylation (methylated DNA); this sample is fully resistant to digestion by HhaI, a methylation-resistant restriction enzyme (MSRE).

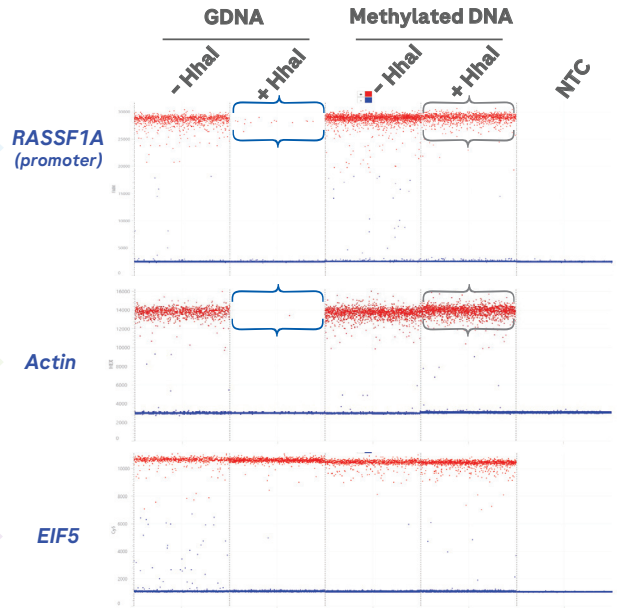
Target region of interest
Dashed lines indicate the location of HhaI cleavage sites. Methylated CpGs are resistant to HhaI cleavage. Thus, following HhaI cleavage, a positive signal in healthy human GDNA (blue brackets) or CpGenome human methylated DNA (gray brackets) indicates the presence of intact PCR amplicons derived from methylated alleles.



Digestion completion control
Solid lines indicate the location of HhaI cleavage sites. This region of the actin gene is *not methylated* in mammalian cells. Thus, efficient HhaI digestion results in an absence of positive signal from the actin region in the test sample input (blue brackets), and a positive signal in the methylated DNA control (gray brackets).



Input correction control
This amplicon lacks HhaI cleavage sites. Thus, all conditions (except for the no-template control) should yield positive signal.



Unmethylated HhaI site

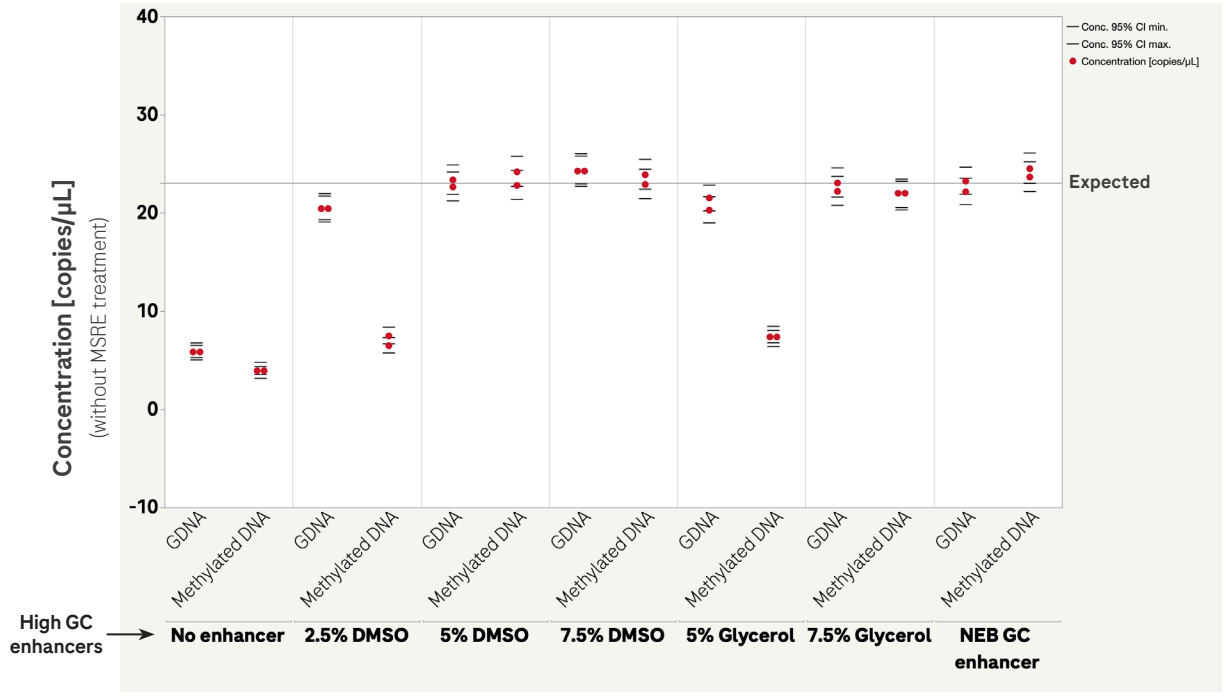
HhaI site with Unknown Me status

Forward and reverse primers for target regions

Probes; each probe is labeled with a different dye to enable multiplexed assays

The challenge of amplifying methylated DNA can be overcome by the addition of “high-GC enhancers”

The amplification of high-GC regions—such as the *RASSF1A* promoter—is often inefficient, even in regions that are not methylated (see the first set of data in the chart below; the GC content of the amplicon tested is 72% GC). This can make it difficult to accurately quantify the amount of template, regardless of methylation status. However, the addition of high-GC enhancers to the amplification reactions can dramatically improve amplification efficiency and quantitative accuracy when using the Digital LightCycler® dPCR System (see below); this increases the sensitivity and accuracy of dPCR-based DNA methylation analysis.



Overall benefits of dPCR	Value of dPCR in DNA methylation analysis
Absolute quantification	No need for standards
High precision	Improved reproducibility and accuracy by detecting very small fold differences
Increased sensitivity	Enhanced target concentration
Reduced competition	
Inhibitor tolerance	Greater tolerance to residual matrix inhibitors

Digital LightCycler® Nanowell plates

HiSens
45µL, ~20k partitions
cf residual DNA testing | Microbial detection | Rare Mutation

Uni
30µL, ~28k partitions
Gene expression
Absolute quantitation

HiRes
15µL, ~100k partitions
Copy number variation

Learn more

about the **Digital LightCycler® dPCR System,** and the applications it supports, at **go.roche.com/dpcr** or by scanning the **QR code**.

Project: DNA methylation analysis on the Digital LightCycler® dPCR System
The Digital LightCycler® is a Class II US IVD instrument.

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