Quantitative digital PCR analysis of cancer gene promoter methylation using low amounts of input DNA

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The Beauty of Science is to Make Things Simple

Abstract

Silencing of tumor suppressor and tumor-related gene transcription by hypermethylation at promoter CpG motifs is a significant mechanism at work in human tumorigenesis. Various methods have been developed to analyze DNA methylation levels, both across the genome and at specific loci, in order to discover and interrogate disease relevance loci for methylation-based transcriptional control. Methods able to quantitatively measure differences in DNA methylation between normal and cancer cells provide promising sources for biomarker identification and assessment.

Zymo Research’s OneStep qMethyl™ Kit is used for the detection of region-specific DNA methylation via the selective amplification of methylated cytosines after digestion with methylation-sensitive restriction enzymes. RainDance Technologies RainDrop™ qPCR system is a highly sensitive tool for precise quantification of nucleic acids using probe-based qPCR reagents. RainDrop offers unique analytical advantages for clinical diagnostics due to its exceptionally high sensitivity for absolute quantification and capability to multiplex assays, using a wide dynamic range of input DNA in a contamination-free and simple workflow. The combination of both technologies enables a novel, fast, and robust method for quantitative methylation analysis of small amounts of DNA.

Here we report on the use of Zymo’s methylation assay and RainDrop digital PCR counting to enable quantitative measurements of DNA methylation at specific genomic loci using small amounts of non-isothermal converted DNA. MGMT promoter methylation measurements are used to illustrate the method’s robust quantification of small percent methylation changes using low amounts of input DNA in singleplex and multiplex assay formats (duplex RAB25-VIC and MGMT-FAM probes). Duplex assays using a methylation-independent reference assay and either RARB or CCND2 are used to assess promoter methylation in breast cancer tumor and adjacent normal tissue samples showing stage-specific differential methylation.

RainStorm Droplet-Based Microfluidics

Nanoparticle and micrometer-sized droplets of reagents or reagents are enabled by RainDrop’s droplet array plates and automated systems with microscopic droplets and disposable fluidic chips. Aqueous samples (beads, cells, enzymes, antibodies, DNA) can be encapsulated within each droplet, surrounded by an immiscible organic sol. The droplets are stabilized with biocompatible surfactants, allowing for robust manipulations both in and off chip. Droplet fluorescence can be measured by viewing the droplets through a laser spot positioned in the microfluidic channel.

RainDrop Droplet Digital PCR

Divide and Count: Single volume divided into countable volume elements

Digital Multiplex Analysis With Endpoint PCR

Multiplex with color

Multiplex with intensity

Fluorescence Endpoint Defined by Probe Concentration

Standard One Step qMethyl Methylation Kit

The sample DNA is divided in two parts, a Test Reaction and a Reference Reaction. Test Reaction samples are digested with Methylation Sensitive Restriction Enzymes (MSREs) while the Reference Reaction samples are not (mock digest). Following digestion, DNA from both samples is used for real-time PCR.

Digital One Step qMethyl: Duplex Assay

Blends of Methylated and Non-Methylated Control DNA Used to Test Digital Quantification

Counts

• Linear quantified counting of differences of 5-10% methylation across entire methylation range
• 20ng (or less) can be used directly without bisulfite treatment or additional purification steps
• Multiplex analysis works well for methylation determination using digital One Step qMethyl kit

Digital One Step qMethyl: Single-plex Assay

Combining RainDrop digital PCR and One Step qMethyl Kit enables digital methylation analysis using low DNA input

Methylation Assay: Cluster Plot

Methylation Assay: Counts

• DNA treated using One Step qMethyl kit is able to be digitally quantified “right out of the box”

RainDrop dPCR Platform Methylation Analysis with Zymo Research OneStep qMethyl Kit

• High sensitivity
• Multiplex analysis
• Single pipetting step
• Contamination-free design
• Simple and flexible workflow
• Robust open reagent platform