Methylation Profiling of FFPE Samples

The widely-used GoldenGate® Assay for Methylation supports accurate single-site DNA methylation analysis from partially degraded FFPE samples.

Introduction

Formalin-fixed, paraffin-embedded (FFPE) human tissue samples represent a vast resource for molecular analyses and retrospective clinical studies. Illumina recognizes the value of these samples and the potential they have to contribute to studies of the epigenome and the role of DNA Methylation in numerous biological processes and diseases, including cancer. Although the number of epigenetic cancer studies continues to grow, the wealth of FFPE samples available remains largely untapped.

This technical note describes research done—and lessons learned—at the National Cancer Institute and Illumina demonstrating the ability of the GoldenGate Assay for Methylation[1] to detect differential methylation in FFPE-treated DNA. The Illumina Methylation Cancer Panel I used for these studies supports assaying 96 samples at a time for the methylation status at 1,505 CpG loci simultaneously in over 800 cancer-related genes.

The GoldenGate Assay for Methylation with FFPE DNA

Killian et al. have adapted the GoldenGate Assay for Methylation to analyze DNA obtained from samples originally processed with an FFPE tissue preparation step[2]. Genomic DNA was extracted from FFPE tissues in quantities sufficient for bisulfite treatment using the EZ-96 DNA Methylation-Gold kit (Zymo Research). The resulting bisulfite-converted DNA was quantified with OliGreen (Invitrogen) and used as input for the standard GoldenGate Assay for Methylation.

The authors tested matched pairs of FFPE and fresh-frozen samples from a set of 20 lymph node tissue samples. Samples from both archiving methods performed nearly identically. The GoldenGate Assay for Methylation identified the same set of more than 200 loci that were significantly ($p < 0.001$) differentially methylated in benign hyperplasia versus malignant lymphoma for either FFPE or fresh-frozen samples. Overall, the median correlation (r^2) between fresh-frozen and FFPE tissue pairs was 0.973.

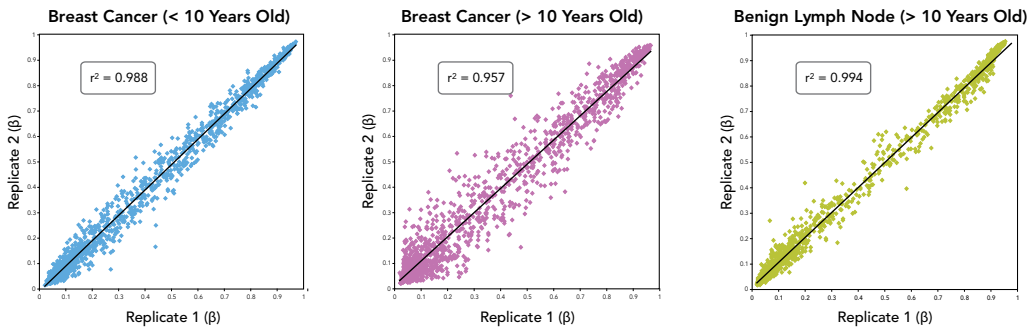
Recommendations For Using FFPE DNA

Findings from the Killian study and research at Illumina have provided significant insights into how to achieve optimal results from GoldenGate methylation analyses of FFPE samples. By following these guidelines in addition to the standard protocol, robust results are easily achievable.

Accurately quantify your bisulfite-converted DNA

Certain DNA quantification methods, such as spectrophotometry, can be influenced by the presence of free nucleotides and RNA in the DNA sample. The resulting measured DNA concentrations are thus overestimates, leading to suboptimal inputs for the GoldenGate Assay for Methylation and lower data quality. The fixation and embedding processes may also contribute contaminants that further confound absorbance-based quantification. Therefore, OliGreen is recommended for sample quantification, because it is specific to single-stranded DNA. However, Killian et al. note that a single bisulfite-converted oligonucleotide standard may not reflect the base composition of the experimental sample, and suggest using a pool of oligo standards to overcome this limitation.

Figure 1: Highly Reproducible Methylations Results From FFPE Tissue



Reproducibility experiments from diverse samples showed high quality GoldenGate Assay for Methylation results from FFPE samples. Younger samples (left) show very high correlations, whereas older samples (middle and right) were more variable.

Use sufficient amounts of input DNA

The GoldenGate Assay for Methylation protocol recommends an input of 250 ng DNA per sample, but not all samples yield enough DNA to meet this requirement. We found that samples with a minimum input of 200 ng DNA (quantified after bisulfite conversion) gave replicate correlations of $r^2 \geq 0.98$ in approximately 80% of cases (Figure 1). Samples with less than 200 ng of input gave replicate correlations of $r^2 \geq 0.98$ in only 30% of cases. Although not formally tested, data quality from low-yield samples may be improved if DNA is re-extracted or concentrated to allow for an input of more than 200 ng.

Know your tissue sample

DNA yield and resulting data quality are directly dependent on both the tissue type and age of the sample being studied. Less-cellular tissues tend to be more sensitive to long-term (> 10 years) FFPE storage and are more likely to have lower DNA yields and replicate correlation (r^2) values. For example, among a set of 96 samples, the seven samples with the lowest replicate correlations ($r^2 < 0.96$) were all breast tissues over 10 years old with low DNA yields. Conversely, lymph node tissues are highly cellular, and nearly all of these samples had high DNA yields and $r^2 \geq 0.98$, even those older than 10 years.

Test your samples

Although these guidelines should support successful use of FFPE samples in the GoldenGate Assay for Methylation, not all sample sets will behave identically. Tissue processing and fixation protocols vary over time, between institutions, and between technicians at the same institution. Examining a cross-sectional subset of samples to be analyzed in any given study may provide a useful representation of the data quality one might expect from the study set as a whole. By evaluating groups of samples that tend to produce less reliable data, and excluding samples if necessary, investigators may improve the overall quality of the data and its subsequent interpretation.

Frequently Asked Questions

Can I use real-time PCR to quantify my samples?

Real-time PCR quantification has not been specifically tested with FFPE DNA samples used in the GoldenGate Assay for Methylation. Nonetheless, evidence from other studies[3,4] indicates this method is effective in ascertaining the amount of amplifiable DNA present in FFPE samples, and it may be particularly useful in helping to determine the likelihood that older FFPE samples will provide reliable data with the GoldenGate Assay for Methylation.

Can I study loci of interest not included in the Cancer Panel using the GoldenGate Assay for Methylation?

Yes, custom panels of up to 1,536 loci are regularly designed by customers. For information about designing custom methylation analysis panels, please contact Illumina Customer Solutions5 or read the Technical Note, Designing Custom GoldenGate Methylation Profiling Panels6.

Can I use FFPE DNA for the Infinium® Methylation Assay?

Analysis of a limited number of samples that performed well in the GoldenGate Assay for Methylation indicates that the Infinium system is much more sensitive to the quality of DNA obtained from FFPE tissue. Preliminary data suggest that a subset of samples less than five years old may yield $r^2 \geq 0.95$, but that the majority of samples will have lower correlations between replicates.

References

1. Bibikova M, Lin Z, Zhou L, Chudin E, Garcia EW et al. (2006) High-throughput DNA methylation profiling using universal bead arrays. Genome Res 16: 383-393.
2. Killian K, Bilke S, Davis S, Walker RL, Hipp J, Pittaluga S, Raffeld M, Smith W, Bibikova M, Fan JB, Buck M, Jaffe E, and Meltzer P (2008). Genome-wide methylation profile of follicular lymphoma reveals distinct on- and off-island epigenotype. AACR Annual Meeting, Permanent Abstract Number 32.
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5. Illumina Customer Solutions contact information below.

Illumina, Inc. • 9885 Towne Centre Drive, San Diego, CA 92121 USA • 1.800.809.4566 toll-free • 1.858.202.4566 tel • techsupport@illumina.com • illumina.com

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