# illumina®

# Infinium<sup>®</sup> FFPE DNA Restoration Solution

A complete workflow for genotyping, CNV, and methylation analysis, from sample prep through data analysis.

### Highlights

- Integrated Quality Control Kit Provides primers for a real-time PCR assay to identify recoverable samples
- Novel Restoration Kit
  Includes reagents and a simple protocol to restore
  degraded FFPE samples
- FFPE Sample-Enabled BeadChips Choose from several arrays providing custom content, whole-genome genotyping, or methylation sites
- High-Quality Data Robust data for reliable genotyping, copy number variation, and methylation analysis

### Introduction

Millions of formalin-fixed paraffin-embedded (FFPE) archival cancer tissue samples provide an enormous and invaluable repository of information for cancer research. The samples hold a wealth of data for the discovery of biomarkers, drug development, and diagnosis and treatment of diseases. However, the fixation process and storage of FFPE samples frequently leads to nucleic acid degradation and base modification. Because it is inefficiently amplified in the whole-genome amplification step, degraded DNA from FFPE samples generally results in poor performance for array-based studies. Such samples have been traditionally unusable with the Infinium HD Assay. To overcome this problem, the Infinium FFPE DNA Restore Kit provides a simple workflow to repair damaged DNA and achieve high-quality genotyping, copy number variation (CNV), and methylation data using Infinium HD Assays.

### Simple Restoration Workflow

The FFPE DNA restoration workflow allows researchers to easily evaluate sample quality and repair DNA from FFPE samples in preparation for the Infinium HD Assay (Figure 1). After DNA extraction using one of many commercially available kits, the Illumina FFPE QC Kit is used to evaluate the quality of prospective DNA samples and determine if they are usable. The kit provides primers for a real-time PCR assay that can be carried out using standard instrumentation available in most biological laboratories.

Samples that pass the quality control evaluation are moved on to the DNA restoration step. Using two straightforward enzymatic reactions, the Infinium HD DNA Restore Kit restores degraded FFPE DNA in preparation for the re-optimized whole-genome amplification step in the Infinium HD Assay protocol for FFPE samples\*. In general, over 80% of FFPE samples that passed the QC assay metric are successfully restored (Figure 2).



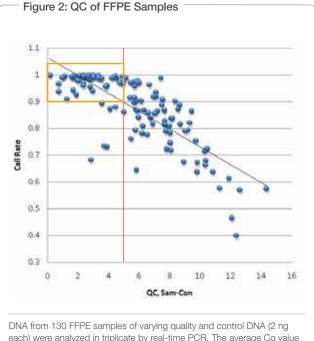
Figure 1: FFPE QC and Restoration Workflow

A streamlined workflow to QC FFPE samples and restore viable DNA for whole-genome amplification using a modified protocol. Restored samples can be used in the proven Infinium HD Assay. The assay results can be easily analyzed using the highly intuitive, powerful DNA analysis tools in GenomeStudio® and KaryoStudio software.

# Proven Infinium Technology

Illumina offers several microarray options for gaining valuable genetic insight from FFPE samples:

- The HumanOmniExpress-FFPE (693,000 markers) and CytoSNP-FFPE (262,000 markers) are 12-sample genotyping BeadChips that are ideally suited for whole-genome CNV analysis and provide coverage of regions associated with cancer.
- The HumanMethylation450 BeadChip allows researchers to interrogate > 485,000 methylation sites per sample at single-nucleotide resolution. The content covers 99% of RefSeq genes and 96% of CpG islands.
- Infinium iSelect<sup>®</sup> BeadChips allow researchers to create a completely custom BeadChip, by selecting from 3,072 to 1,000,000 markers, including content that is highly relevant to cancer studies in FFPE samples such as known somatic mutations.



each) were analyzed in triplicate by real-time PCR. The average Cq value for the control DNA was subtracted from Ct values for FFPE DNA. The resulting Delta Ct values were plotted against the Infinium HD Assay call rates for the same samples after restoration. Greater than 80% of samples with a QC value below 5 were successfully genotyped (orange box). Depending on sample origin and age (1–35 years), 35% to 95% of samples passed QC.

Each of these BeadChips runs on the proven Infinium HD Assay, consistently producing high call rates (> 95%), very high reproducibility, and extremely low error rates<sup>†</sup>. This assay technology has enabled many revolutionary discoveries in disease research, resulting in a vast publication record.

## **Structural Variation Analysis**

Structural variation is thought to be a significant contributor to the genetic basis of many human diseases. Dense marker spacing on the whole-genome BeadChips coupled with the sensitive

Table 1:	<b>FFPE-Enabled</b>	BeadChip §	Specifications -
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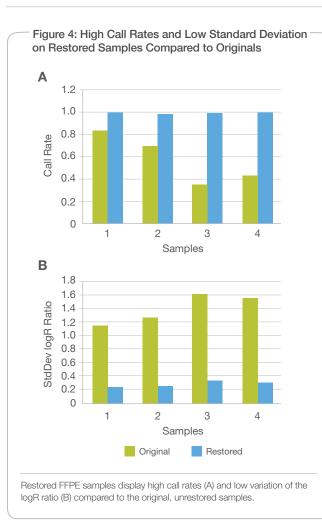
# Figure 3: Infinium HD Twelve-Sample BeadChip Image: State of the state

Infinium HD Assay offers a powerful tool for structural variation analysis. Researchers can profile virtually any chromosomal aberration such as amplifications, deletions, rearrangements, point mutations, copy number changes, and copy-neutral loss of heterozygosity (LOH) events. The assay delivers very high signal-to-noise ratios and low overall noise levels, which are ideal for precise structural variation analysis.

# **Exceptional Data Quality**

To evaluate the data quality produced from degraded DNA, a series of FFPE samples were treated with the Infinium HD DNA Restore Kit and evaluated on the HumanCytoSNP-FFPE-12 BeadChip. The assay signal intensities were referenced against canonical genotype cluster files. The clusters were generated from artificially degraded Coriell DNA samples that were processed with the Infinium HD FFPE DNA Restore Kit and evaluated using the Infinium HD Assay.

	HumanOmniExpress FFPE BeadChip	HumanCytoSNP-FFPE BeadChip	HumanMethylation450 BeadChip	Infinium iSelect BeadChips
Number of Markers	> 693,000	> 262,000	450,000	3,072-1,000,000
Number of Samples/BeadChip	12	12	12	4-24
DNA Requirement (ng)	100	100	250	100
Assay	Infinium HD	Infinium HD	Infinium HD	Infinium HD
Instrument Support	HiScanSQ™ or iScan	HiScanSQ, iScan, BeadArray™ Reader	HiScanSQ, iScan	HiScanSQ, iScan, BeadArray Reader
Applications	Whole-Genome Genotyping and CNV Analysis	Whole-Genome Genotyping and CNV Analysis	Epigenetic Studies	Custom Genotyping



As shown in Figure 4A, the highly degraded FFPE samples showed initial call rates of 40-70%. After restoration, these same samples produced call rates of > 90%. The associated standard deviation of log intensity (logR Dev) is < 0.4, indicating high reproducibility and high signal-to-noise ratios (Figure 4B). The HapMap concordance for these samples was > 99.0%, indicating that the assay is capable of accurate genotype calls (data not shown).

# Data Analysis

Assay data is fed directly into Illumina GenomeStudio DNA analysis software. GenomeStudio, along with the integrated CNVPartition program, provide simplified graphical analysis of SNPs and copy number variants across many data points.

An open plug-in interface easily integrates third-party applications for more downstream data analysis options. The Illumina•Connect program leverages this open architecture and has made numerous plug-ins available to support genotyping and copy number analysis.

Product	Catalog No.
Illumina FFPE QC Kit	WG-321-1001
Infinium HD FFPE DNA Restore Kit (24 samples)	WG-321-1002
HumanCytoSNP-FFPE (24 samples)	WG-321-1003
HumanCytoSNP-FFPE-12 (48 samples)	WG-321-1004
HumanOmniExpress-FFPE BeadChip (24 samples)	WG-321-1005
HumanOmniExpress-FFPE BeadChip (48 samples)	WG-321-1006
HumanMethylation450 BeadChip (24 samples)	WG-314-1003
HumanMethylation450 BeadChip (48 samples)	WG-314-1001
HumanMethylation450 BeadChip (96 samples)	WG-314-1002
Human iSelect 3–90K BeadChip (24 samples)	WG-401-1004
Human iSelect 3–90K BeadChip (48 samples)	WG-401-1001
Human iSelect 3–90K BeadChip (288 samples)	WG-401-1002
Human iSelect 3–90K BeadChip (1152 samples)	WG-401-1003
Human iSelect 90–250K BeadChip (24 samples)	WG-400-1004
Human iSelect 90–250K BeadChip (48 samples)	WG-400-1001
Human iSelect 90–250K BeadChip (288 samples)	WG-400-1002
Human iSelect 90–250K BeadChip (1152 samples)	WG-400-1003
Human iSelect 250–1M BeadChip (24 samples)	WG-404-1004
Human iSelect 250–1M BeadChip (48 samples)	WG-404-1001
Human iSelect 250–1M BeadChip (288 samples)	WG-404-1002
Human iSelect 250–1M BeadChip (1152 samples)	WG-404-1003

## Summary

Archival FFPE samples hold an abundance of invaluable information for human cancer studies. Because these samples generally yield highly degraded DNA, they perform poorly in most array-based studies. The Infinium HD FFPE DNA Restore Kit can repair these degraded DNA samples in preparation for use with the Infinium HD Assay. Analysis of a series of FFPE samples demonstrated that after restoration, these samples produce high-quality genotyping, CNV, and methylation data.

### References

 Pokholok DK, Le JM, Steemers FJ, Ronaghi M, Gunderson K. Analysis of restored FFPE samples on high density SNP arrays. Presented at American Association of Cancer Research Meeting, May 2010.

\*When using FFPE samples with the HumanMethylation450 BeadChip, a bisulfite conversion step is required between the QC and restoration steps. Please refer to the Infinium HD FFPE QC Protocol for more information on workflows.

<sup>†</sup> Due to the variability of FFPE samples, specifications for these BeadChips are calculated at > 80% of the samples passing QC will achieve call rates of > 95% of CpG sites detected for the HumanMethylation450 BeadChip and logR Dev < 0.4. Specifications are for normal samples only.

### RESEARCH FIELD OF USE

ILLUMINA's Infinium HD FFPE DNA Restore Kit (Kit) uses a thermostable ssDNA ligase (Ligase) covered by U.S. or international patents or patent applications (IP) owned by EPICENTRE Biotechnologies, LLC, Madison, WI (EPIBIO). Purchase of this Kit from ILLUMINA or an Affiliate or distributor of ILLUMINA gives the purchaser a limited, non-exclusive license under the IP to use the Ligase in the purchased Kit solely for performing methods for restoration of degraded DNA from FFPE or other samples containing degraded DNA for subsequent analysis of that restored DNA using an ILLUMINA array-based platform for research and educational purposes, including commercial research-use-only services, but excluding array-based analysis or sequencing of RNA or array-based analysis or sequencing of DNA that is made by reverse transcription of RNA. No license is granted to use the Ligase for manufacturing a product, for medical, therapeutic or diagnostic use (including as an analyte-specific reagent) in humans or non-humans, or for any other use other than research and educational purposes. For questions related to licensing, please contact the Intellectual Property Manager at EPIBIO, 726 Post Road, Madison, WI 53713, USA.

Illumina • 1.800.809.4566 toll-free (U.S.) • +1.858.202.4566 tel • techsupport@illumina.com • www.illumina.com

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