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Analyzing FFPE Tissue Samples with the Infinium® CytoSNP-850K v1.1 BeadChip

A comprehensive workflow for profiling cytogenetic aberrations in DNA isolated from FFPE tissue samples.

Introduction

Formalin fixation of pathological samples, including tissues biopsied from products of conception (POC) and cancer specimens, preserves their morphology and characteristics for long-term storage and future use in clinical research applications.[1] Many of these samples harbor valuable genetic information that has the potential to further the understanding of structural and numerical genetic variations and how these influence phenotype. Unfortunately, the preservation process frequently leads to nucleic acid degradation and base modification, making genetic analysis of these formalin-fixed paraffin-embedded (FFPE) tissues challenging. Furthermore, analyzing the data generated from FFPE tissue samples using standard cluster-fitting methods can provide a poor representation of the sample, decreasing accuracy of results.

Using the Infinium CytoSNP-850K v1.1 BeadChip, researchers have analyzed cytogenetic aberrations in both tumor[2] and POC[3] FFPE tissue samples. This technical note describes a comprehensive workflow from Illumina using the Infinium FFPE DNA Restoration Solution, the Infinium CytoSNP-850K v1.1 BeadChip, and BlueFuse® Multi Analysis Software (Figure 1) to profile genetic aberrations in FFPE tissue samples.

FFPE Tissue Sample QC and Restoration

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. Samples that pass the quality control evaluation are advanced through the DNA restoration process. Using an optimal starting amount of 100ng recovered DNA and a proprietary enzymatic reaction, the Infinium FFPE DNA Restore Kit repairs degraded FFPE tissue DNA in preparation for the whole-genome amplification step in Infinium Assays.

CytoSNP-850K v1.1 BeadChip Content

The Infinium CytoSNP-850K v1.1 BeadChip offers coverage of nearly 850,000 SNPs with 15× redundancy and enriched coverage for 3262 dosage-sensitive genes. The gene list contains content from the Clinical Genome Resource (ClinGen)[4] and the Cancer Genomics Consortium (CGC)[5], providing a comprehensive view of cytogenetic aberrations. It provides robust performance across a broad range of sample types, including FFPE tissue samples. Processed arrays scanned using the iScan® or NextSeq® 550 Systems produce high-quality results (Table 1).
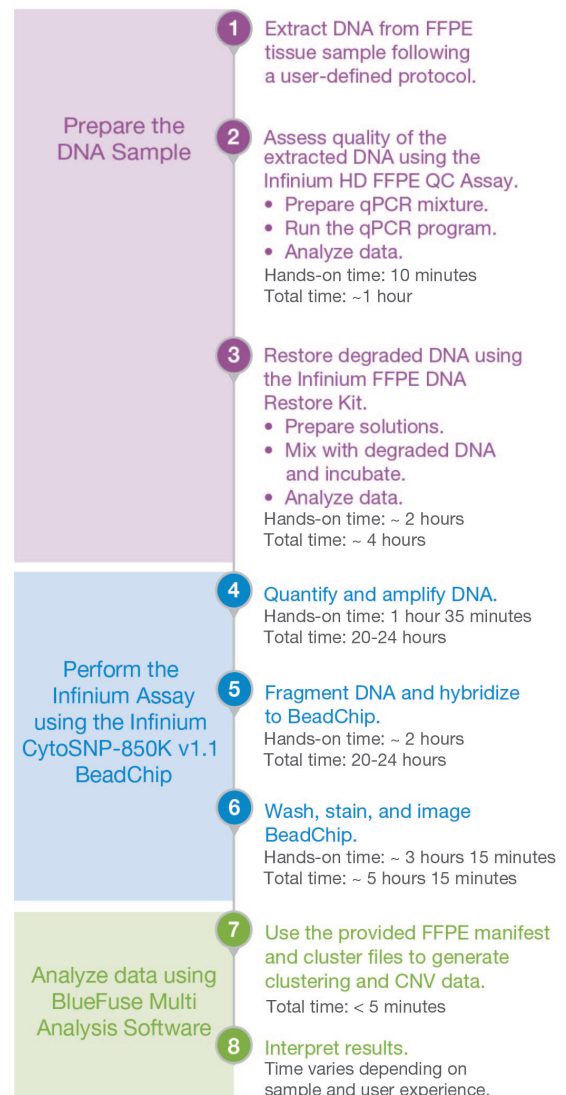
Prepare the DNA Sample

1. Extract DNA from FFPE tissue sample following a user-defined protocol.

2. Assess quality of the extracted DNA using the Infinium HD FFPE QC Assay.
   • Prepare qPCR mixture.
   • Run the qPCR program.
   • Analyze data.
   Hands-on time: 10 minutes
   Total time: ~1 hour

3. Restore degraded DNA using the Infinium FFPE DNA Restore Kit.
   • Prepare solutions.
   • Mix with degraded DNA and incubate.
   • Analyze data.
   Hands-on time: ~ 2 hours
   Total time: ~ 4 hours

Perform the Infinium Assay using the Infinium CytoSNP-850K v1.1 BeadChip

4. Quantify and amplify DNA.
   Hands-on time: 1 hour 35 minutes
   Total time: 20-24 hours

5. Fragment DNA and hybridize to BeadChip.
   Hands-on time: ~ 2 hours
   Total time: 20-24 hours

6. Wash, stain, and image BeadChip.
   Hands-on time: ~ 3 hours 15 minutes
   Total time: ~ 5 hours 15 minutes

Analyze data using BlueFuse Multi Analysis Software

7. Use the provided FFPE manifest and cluster files to generate clustering and CNV data.
   Total time: < 5 minutes

8. Interpret results.
   Time varies depending on sample and user experience.

Figure 1: Workflow for Analyzing FFPE Tissue Samples with the Infinium CytoSNP-850K v1.1 BeadChip

Standard Cluster File
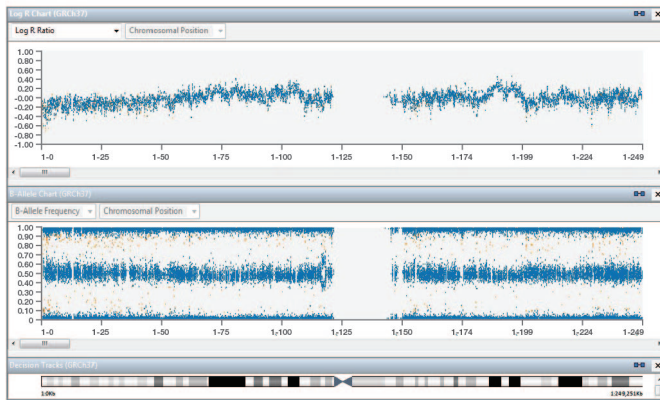
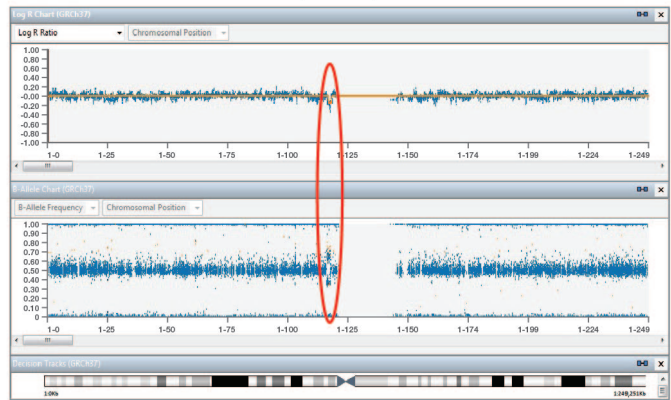FFPE Tissue Sample–Specific Cluster File

Figure 2: FFPE Tissue Sample–Specific Cluster Files Enable Clear Identification of Variants—DNA was restored from an FFPE tissue sample using the Infinium FFPE DNA Restoration Solution, processed on the Infinium CytoSNP-850K v1.1 BeadChip, and analyzed with BlueFuse Multi Analysis Software v4.3 using the standard cluster files and the FFPE tissue sample–specific cluster file. Analysis using the FFPE tissue sample–specific file reduced the noise observed with the standard cluster file and provided tight profiles from the FFPE tissue sample, enabling clear identification of a ~1 MB mosaic deletion (red circle).

Table 1: CytoSNP-850K v1.1 BeadChip Performance with FFPE Tissue Samples

Feature	Description		
No. of Samples per BeadChip	8		
DNA Input Requirement	8 µL restored DNA[b]		
Instruments Support	iScan and NextSeq 550 Systems		
No. of SNP Markers[a]	829,864		
Data Performance	**Product Specification**	**iScan System**	**NextSeq 550 System**
Call Rate	> 95%	99.6%	99.5%
Log R Dev	< 0.30	0.146	0.152

Due to the variability of FFPE tissue samples, specifications for these BeadChips are calculated at > 80% of the samples passing QC will achieve call rates of > 95%. Specifications are for normal samples only.

a. Final number of SNPs post Gentrain analysis for the FFPE tissue sample project.

b. Process modification is detailed in the "Analyzing FFPE Samples with Infinium Microarrays" technical note (www.illumina.com/content/dam/illumina-marketing/documents/products/technotes/technote-infinium-ffpe-sample-analysis.pdf).

Assay Protocol

Optimal assay performance with FFPE tissue samples requires a minor modification to the standard Infinium HD Super Protocol:[*] when preparing the MSA1 plate, use 8 µL of the repaired DNA instead of the standard 4 µL. This change will increase the effectiveness of the whole-genome amplification step. Following amplification, the remaining protocol steps are performed as recommended.

Data Analysis

Data analysis for Illumina BeadChips relies on GenCall scores made by the scanner that reflect the distance of a data point from the center of a "cluster" which represents signal intensities that correspond to genotype calls. Performance differences between normal and FFPE tissue samples can affect the cluster positions of the assayed SNPs, leading to indistinct genotyping and copy number calling. To account

for these clustering differences and increase the accuracy of copy number visualization using BlueFuse Multi Analysis Software, FFPE tissue sample–specific manifest and cluster files are available for the CytoSNP-850K v1.1 BeadChip. These files include reduced single nucleotide polymorphism (SNP) content in comparison to the standard manifest file, adjusting for the low performance of a small percentage of the SNPs due to poor restoration.

Use of the FFPE tissue sample–specific manifest and cluster files for the production of genotype call (GTC) files enables generation of reliable analytical data for copy number analysis from well-restored FFPE tissue samples (Figure 2). BlueFuse Multi Analysis Software offers validated algorithms and a suite of features that facilitate visualization and interpretation of identified copy number variants (CNV).

Accessing FFPE Tissue Sample–Specific Product Files

Download FFPE tissue sample–specific cluster and manifest files from the CytoSNP-850K BeadChip v1.1 support page (support.illumina.com/array/array_kits/cytosnp-850k_beadchip_kit/downloads.html). Available files include:

- FFPE Tissue Sample–Specific Manifest File (CytoSNP-850Kv1-1_FFPE.bpm)

- FFPE Tissue Sample–Specific Cluster File for the iScan System (CytoSNP-850Kv1-1_FFPE_iScan.egt)

- FFPE Tissue Sample–Specific Cluster File for the NextSeq 550 System (CytoSNP-850Kv1-1_FFPE_NSQ550.egt)

[*]For more information on the standard protocol, refer to the "Infinium HD Assay Super Protocol Guide" (support.illumina.com/content/dam/illumina-support/documents/myillumina/05340b1f-c179-495d-b790-fa91ecbb6ff2/inf_hd_super_assay_ug_11322427_revc.pdf).

Data Quality

The performance range of FFPE tissue samples in the Infinium Assay is highly dependent on the quality of the preserved tissue and restored DNA. Using the Infinium FFPE Restoration Solution and FFPE tissue sample–specific cluster file with these sample types provides clear profiling of chromosomal aberrations, such as duplications, deletions, and copy-neutral absence of heterozygosity (AOH) events, on the CytoSNP-850K v1.1 BeadChip. This high signal-to-noise ratio enables identification of low-level mosaic abnormalities and reproducible detection of CNVs.[6]

Summary

Archival FFPE tissue samples are potentially a rich source of genetic information that could inform many genomic studies, yet they often perform poorly in array-based assays due to highly degraded DNA. The Illumina FFPE Sample Restoration Solution for repairing damaged DNA enables effective and reproducible data analysis from these challenging samples. The provision of FFPE tissue sample–specific manifest and cluster files for the CytoSNP-850Kv1.1 BeadChip enables high-quality genotyping data for copy number analysis without the requirement to develop an internal data set for differential analysis. The streamlined workflow for processing FFPE tissue samples on the CytoSNP-850K v1.1 BeadChip coupled with BlueFuse Multi Analysis Software provides a reliable method for identifying and databasing CNV and AOH cytogenomic events.

Learn More

To learn more about the Infinium FFPE DNA Restoration Solution, visit www.illumina.com/products/infinium_ffpe_dna_restoration_solution.html

To learn more about the Infinium CytoSNP-850K BeadChip, visit www.illumina.com/products/infinium-cytosnp-850k.html

To learn more about BlueFuse Multi Analysis Software, visit www.illumina.com/clinical/clinical_informatics/bluefuse.html

Contact Technical Support at techsupport@illumina.com for addiitonal information.

References

1. Pokholok DK, Le JM, Steemers FJ, et al. Analysis of restored FFPE samples on high density SNP arrays. Presented at American Association of Cancer Research Meeting, April 2010.

2. Mukherjee S, Ma Z, Wheeler S, et al. Chromosomal microarray provides enhanced targetable gene aberration detection when paired with next generation sequencing panel in profiling lung and colorectal tumors. *Cancer Genet.* 2016; 209(4): 119–129. doi: 10.1016/j.cancergen.2015.12.011.

3. Sahoo T, Dzidic N, Strecker MN, et al. Comprehensive genetic analysis of pregnancy loss by chromosomal microarrays: outcomes, benefits, and challenges. *Genet Med.* 2016; Jun 23. doi: 10.1038/gim.2016.69. [Epub ahead of print].

4. Clinical Genome Resource. www.clinicalgenome.org. Accessed July 6, 2016.

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