Deep Clonal Profiling of Purified Tumor Cell Populations From FFPE Samples

SurePrint G3 Human CGH Microarrays and SureSelect facilitated high definition genomic profiling of purified tumor cell populations from FFPE samples

Customer Success Story

Translational Genomics Research Institute; University of Basel; NUGen; Mayo Clinic Arizona; Virginia Piper Cancer Center; Johns Hopkins Univ.

The Challenge:

- The variable quality of DNA extracted from FFPE tissues limits their utility in cancer research.
- Many FFPE samples, notably tumors arising in solid tissues, exhibit high degrees of tissue heterogeneity, with varied admixtures of non-tumor and polyclonal neoplastic cells.
- Current FFPE approaches for the analyses of cancer genomes are thus limited in their ability to advance translational genomics for improving patient management and clinical outcomes.

The Solution:

Flow cytometry-based methods were used to isolate pure populations of tumor cell nuclei from FFPE tissues, and a DNA extraction methodology compatible with oligonucleotide array CGH and whole exome sequencing analyses was developed.

The CGH array assays provided discrimination of single copy loss from homozygous loss and the mapping of amplicon and deletion boundaries in each tumor genome. Furthermore, the SureSelect exome enrichment kit provided at least 20x coverage for almost 80% of targeted loci across the FFPE-derived sample and its matching fresh tissue and derived cell line ensuring accurate variant detection1.

Formalin fixed paraffin embedded (FFPE) tissues are a vast resource of clinically annotated samples. High definition genomics of these informative materials could improve patient management and provide a molecular basis for the selection of personalized therapeutics. A collaboration between several research groups around the world, including the laboratory of Dr. Michael T. Barrett at Translational Genomics Research Institute (TGen), has developed a method based on flow cytometry cell-sorting to isolate individual tumor cell populations from FFPE tissues, as well as methods for their accurate genomic analysis.

Recent studies have described various methods to interrogate FFPE samples with array and sequencing technologies. These methods typically select for samples that exceed a threshold for tumor cell content using histological methods.

Solid tumors, however, exhibit high degrees of tissue heterogeneity. Current approaches for enriching tumor samples prior to analysis of cancer genomes in FFPE tissue, such as laser capture microdissection (LCM), are limited in their ability to sufficiently distinguish and isolate different cell types in a timely manner, making them less suitable for clinical research applications of highly sensitive single molecule-resolution approaches such as NGS.
Recent advances in cytometry-based cell sorting technology facilitate the detection of relatively rare events in dilute admixed samples, enabling DNA content-based flow cytometry assays for high definition analyses of human cancer biopsies. These assays provide intact nuclei for DNA extraction, eliminate the need and bias to preselect samples based on tumor content and non-quantitative morphology-based measures, and greatly increase the number of samples for analyses.

To evaluate the use of sorted solid tissue FFPE samples, fresh frozen (FF) pancreatic ductal adenocarcinoma (PDA) tissue samples were compared to matching FFPE samples (Figure 1). The width of the histograms for the diploid and aneuploid (3.2N) peaks was greater for the FFPE sample, likely reflecting the lower quality of the sample relative to the FF sample. Genomic intervals for ADM2 were used to measure the reproducibility of aCGH data in the matching FFPE and FF samples. The top 20 ranked amplicons in the FFPE sample were used for this analysis. In 19 of these, the overlap was >90% with the same ADM2-defined interval in the sorted fresh frozen sample. The global utility of the CGH assay was determined with different tissues, including triple negative breast cancer, bladder carcinoma, glioblastoma and small cell carcinoma of the ovary. The CGH assay was able to discriminate homozygous and partial deletions, map breakpoints, and amplicon boundaries to the single gene level in these sorted samples, regardless of tumor cell content.

**Figure 1.** Flow sorting and aCGH analysis of matching fresh frozen (FF) and formalin fixed paraffin embedded (FFPE) samples from a pancreatic ductal adenocarcinoma. Flow sorting histogram and detection of 3.2N tumor population in a) FF tissue 11164 and b) FFPE tissue B3733. c–d) Whole genome aCGH plots of 3.2N population sorted from each tissue. Red arrow denotes highly aberrant region on chromosome 2. Blue arrow denotes highly aberrant region on chromosome 9. Light blue shaded areas in lower left of a) and b) show sample and cell debris in flow data.1
Accurate Next Generation Sequencing Data on FFPE Clonal Populations

NGS analysis of even highly tumor cell-enriched bulk cancer samples, including those prepared by LCM, cannot accurately distinguish whether aberrations in the tumor DNA are present in a single cancer genome or if they are distributed in multiple clonal populations in each biopsy.

In contrast, NGS analysis of these highly defined clonal populations can provide accurate sequence information on specific tumor cell types. To demonstrate this, an analysis was performed on sorted FFPE samples prepared from a PDA cell line whose exome has been extensively studied.

The PDA cell line, primary FF tissue from which the cell line was derived, and the corresponding FFPE blocks were used to validate the sorting-based NGS analyses. A comparison of the paired end reads alignments against the reference genome in each of the 3 samples showed that almost 80% of the target areas had at least 20X coverage in all three samples. The overlap of unique reads and the detection of known mutations across the three independent sample preparations demonstrated that sorted FFPE samples can generate accurate NGS Data, using the SureSelect Kit (Figure 2).

**Figure 2.** Combined aCGH and whole exome sequencing of sorted fresh frozen (FF) and formalin fixed paraffin embedded (FFPE) pancreatic ductal adenocarcinoma (PDA) tissue.

a) Flow sorted 3.0N tumor population from PDA tissue.

b) Homozygous TP53 mutation in sorted FF and FFPE tissues, and matching cell line.

c) Chromosome 17 CGH plot of 3.0N population from sorted FF sample.

d) Heterozygous KRAS mutation in sorted FF and FFPE tissues, and matching cell line.

e) Chromosome 12 aCGH plot of 3.0N population from sorted FF sample.

Conclusions

These highly sensitive and quantitative sorting assays provide pure and objectively defined populations of neoplastic cells prior to analysis. The deep and unbiased clonal profiling of sorted FFPE samples by aCGH and NGS provides a valuable methodology with broad application for cancer research which can advance the development of personalized patient therapies.

Reference
