Scaled Preparation of FFPE Tumor Samples for Epigenetic Studies

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Characterizing FFPE Nuclear Material and Adapting Sequencing Workflows

- Cancer samples in formalin-fixed paraffin-embedded (FFPE) blocks contain a wealth of untapped epigenetic information. **There is a need to understand which samples are eligible to yield clinically informative results.**
- **EpiCypher, in collaboration with Charles River Laboratories,** is developing extraction and characterization protocols for FFPE nuclear material to assess compatibility through two sequencing assays.
  - One-pot UniNicE-seq (nicking enzyme-assisted sequencing)(Vishnu et al. (2021)) is an improved open chromatin mapping approach that minimizes DNA degradation through dsDNA nicking 5mC incorporation compatible with crosslinked material.
  - CUTAC (Cleavage Under Targeted Accessible Chromatin) is an antibody-targeted chromatin accessibility mapping protocol based on CUT&Tag (Henikoff *et al.* (2023)) also compatible with crosslinked material.
- **EpiCypher employs a comprehensive approach** to qualitative and quantitative sample assessment. Factors assessed include tissue type, FFPE block age, nucleic acid integrity, and library prep and/or sequencing metrics to determine FFPE sample eligibility for epigenetic assessment **with the ultimate goal to generate datasets that inform clinical research and improve patient outcomes.**

- **Grant support:** R44HG011006, R43HG013071
- **Related publication:** Kumary et al. (2024) Emerging Approaches to Profile Accessible Chromatin from Formalin-Fixed Paraffin-Embedded Sections. Epigenomes 8:20.

Extracting FFPE Nuclei for CUTANA™ CUT&Tag-inspired Workflows

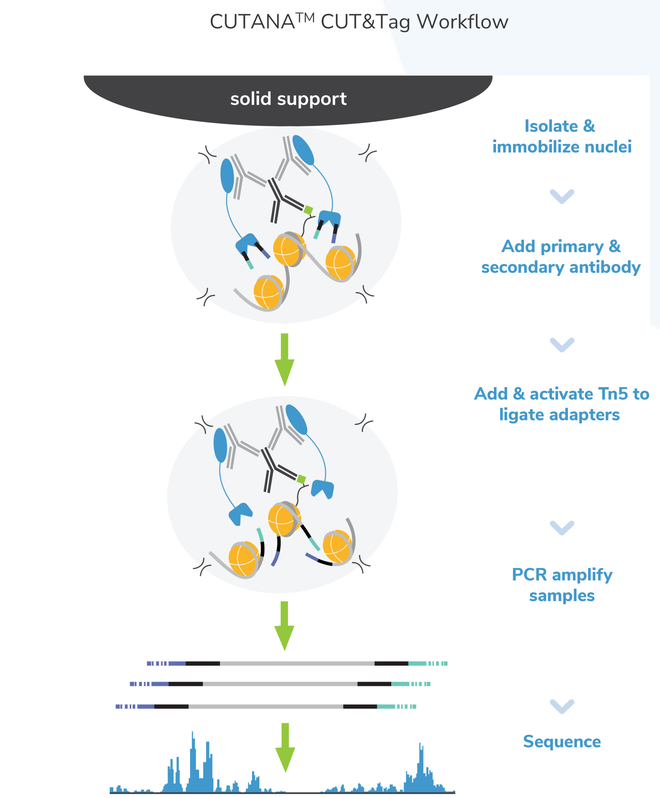

Figure 1. The CUTANA™ CUT&Tag streamlined workflow releases antibody-bound chromatin into solution, leaving superfluous genetic and biological material in bead-immobilized nuclei. **We intend to adapt our nuclear extraction, characterization, and cryopreservation protocols for nuclear material from FFPE scrolls and cores to enable parallel histological, spatial, and bulk or single-cell sequencing approaches for any compatible block.**

Workflow and Extracted Material from Healthy and Tumor Tissue Samples

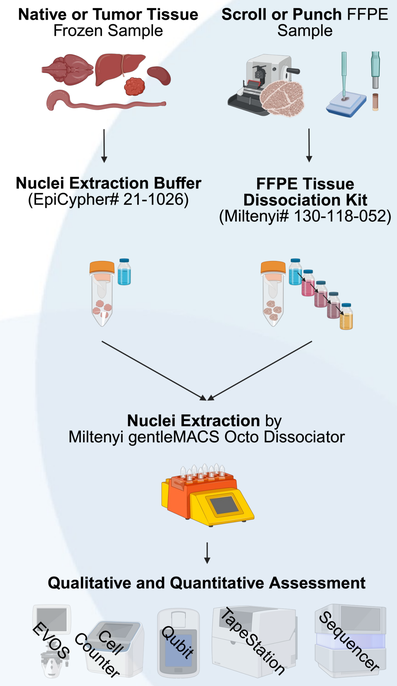

Figure 2. Streamlined workflows to extract and characterize nuclear material. **Objective: Compare extracted frozen and FFPE nucleic acid quality before and after sequencing to determine quality thresholds.** Created with BioRender.com.

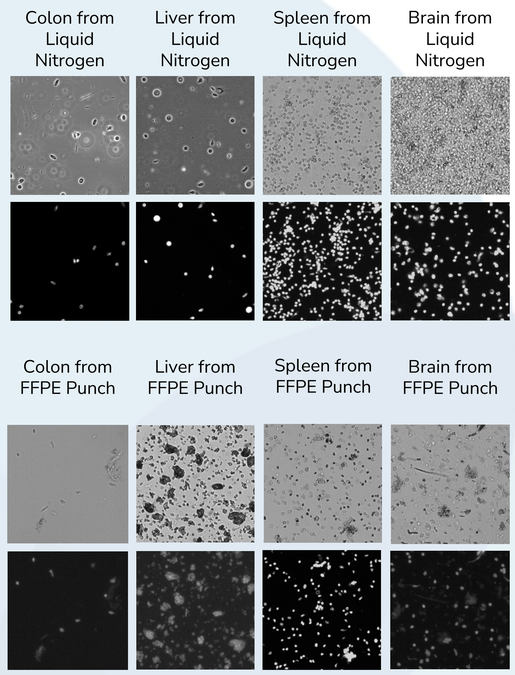

Figure 3. Representative images of nuclear material from frozen vs. FFPE samples. Species: Mouse. Top Representative Image: Brightfield Debris. Bottom Representative Image Nuclear Label: Propidium Iodide. Block age: 0.5-1-year-old.

FFPE block age negatively correlates with gDNA quality. Sample quality heterogeneity cannot be observed qualitatively.

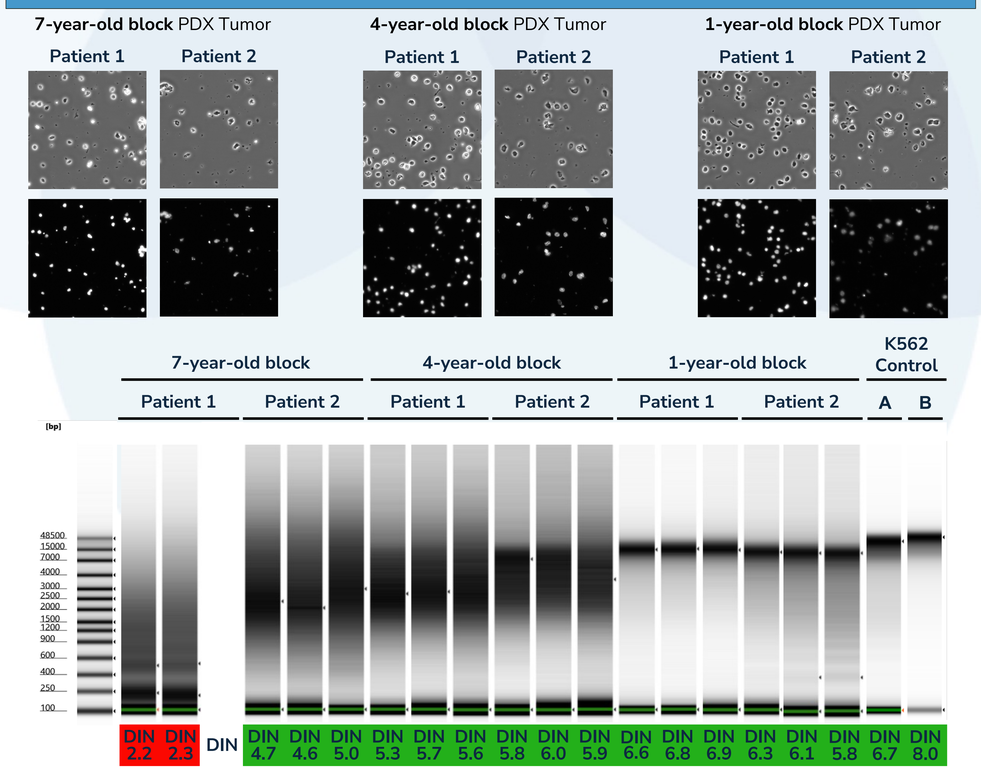

Figure 4. Charles River Laboratories shared FFPE scrolls of mouse model patient-derived xenografts (PDX). PDX tumors derived from 2 patient samples were embedded in paraffin blocks aged 1 to 7 years old. gDNA was extracted from nuclear material using a Qiagen AllPrep DNA/RNA FFPE Kit and assessed for DNA integrity score (DIN) by Agilent TapeStation. K562 Control A (unfixed) gDNA was extracted by the Qiagen AllPrep DNA/RNA FFPE Kit and K562 Control B (unfixed) gDNA was extracted by the Zymo Quick-DNA/RNA Purification Kit to provide examples of high-quality samples derived from 2 DNA extraction kits. Top Representative Image: Brightfield Debris. Bottom Representative Image Nuclear Label: Propidium Iodide.

Tissue type and preparation method may impact gDNA quality. RNA quality is consistently poor in FFPE samples.

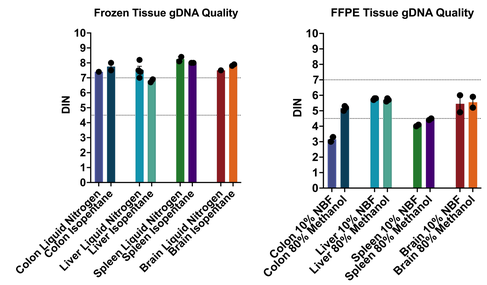

Figure 5. DIN values were calculated for 2 flash freezing and 2 fixation plus paraffin-embedding approaches. Typical DIN quality cut-off is 7, and frozen samples consistently yield high-quality gDNA (extracted by Zymo Quick-DNA/RNA Purification Kit). Others have successfully sequenced gDNA material from FFPE samples with DIN > 4.5. Methanol fixation prior to paraffin-embedding improves gDNA DIN scores compared to 10% neutral buffered formalin (NBF) fixation. Graphs of mean with SEM.

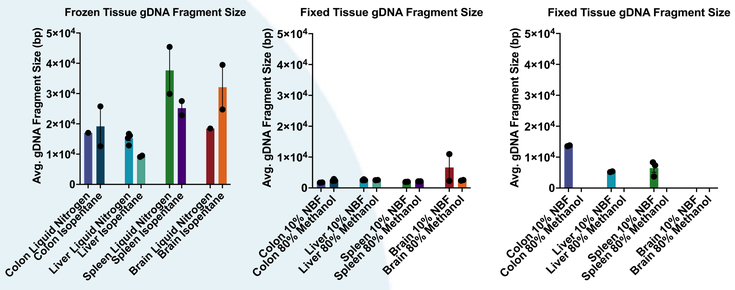

Figure 6. One-pot UniNicE-seq and CUTANA™ CUT&Tag approaches produce cleaved gDNA products for sequencing around 300 bp, so fragments going into the assay should be significantly larger to reduce sequencing bias. Frozen tissue gDNA fragment sizes are greatly increased over FFPE tissue fragment sizes. Ongoing assessment of matched fixed samples without paraffin-embedding suggest initial gDNA damage driven by 24-hour fixation that is further exacerbated by the high temperature paraffin-embedding process. Graphs of mean with SEM.

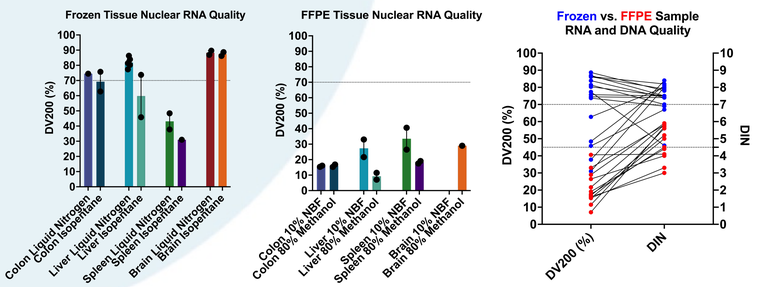

Figure 7. PCR amplification products and RNA quality are often used to assess nucleic acid quality from FFPE samples. Typical DV200 quality cut-off is 7, and preliminary readouts of DV200 (the percentage of RNA fragments >200 nucleotides) indicate that nuclear RNA quality is higher in frozen samples than in FFPE samples with either fixation approach. Graphs of mean with SEM. Matched RNA (DV200) and DNA (DIN) quality are illustrated.

Next Steps

- Correlate block age, tissue type, DIN metrics, and sequencing stats from One-pot UniNicE-seq, CUTANA™ CUT&Tag, and CUTAC assays to set thresholds for sample eligibility to contribute to high-quality datasets.
- Work with Charles River Laboratories to identify existing biomarkers in the epigenetic data that indicate successful characterization of the PDX tumor model.
- Utilize the optimized workflows to identify novel genetic markers previously inaccessible in Charles River Laboratories' FFPE PDX sample bank.

Other EpiCypher Posters at VAI

- Exploring antibodies and rationally designed readers for genomic mapping of histone monoubiquitin.
- Chromatin remodeling research and epigenetic high-throughput strategies for advancing cancer drug discovery.
- Automated CUT&RUN enables scalable multiomic mapping of chromatin proteins and DNA methylation.
- The CUTANA™ CUT&Tag platform for streamlined, ultra-sensitive epigenomics.