Recovery of Dislodged Cells via an Integrated Biopsy Workflow Improves Nucleic Acid Integrity and NGS Results Compared to FFPE

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Highlights

- Transform biopsy waste into insight. Convert discarded cellular material into actionable sequencing data—enhancing precision oncology without requiring extra tissue or re-biopsy.
- Boost NGS power from every biopsy. The Crow's Nest® Biopsy Catchment System and Zymo Research's integrated molecular workflow recover lost tumor cells and stabilize nucleic acids at the point of collection for maximum quality and yield.
- Deliver deeper, cleaner data. Pairing Crow's Nest™ with DNA/RNA Shield®, *Quick*-DNA/RNA™ HT kits, and Zymo Research's next generation sequencing services drives higher coverage, fewer repeats, and more confident variant calls.

Summary

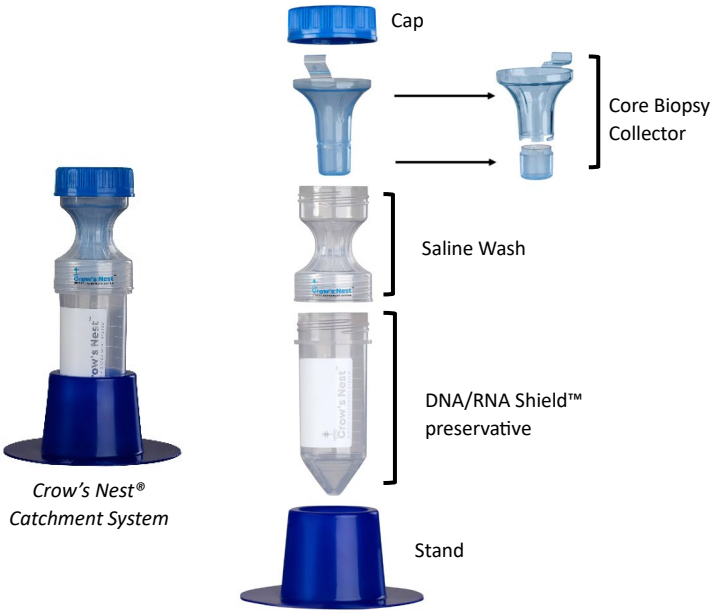
Next-generation sequencing (NGS) is foundational to precision oncology, yet insufficient nucleic-acid yield and suboptimal read coverage from small formalin-fixed, paraffin-embedded (FFPE) core needle biopsy (CNB) specimens frequently limit test success. The Crow's Nest® Biopsy Catchment System (Virchow Medical Inc.) is designed to rescue dislodged tumor cells (DTCs) from biopsy needles immediately post-CNB and preserve them in DNA/RNA Shield® (Zymo Research), a stabilization solution optimized for nucleic-acid integrity. By pairing Crow's Nest® (CN) with Zymo Research's complete workflow that includes DNA/RNA Shield™, *Quick*-DNA/RNA™ HT extraction kits, and Zymo Research Services for RNA-Seq and DNA-Seq analysis, laboratories can achieve higher nucleic-acid yields, superior read coverage, and more reliable variant detection. This whitepaper outlines the combined approach, its clinical rationale, workflow integration, and supporting evidence.

Clinical Problem

- Limited tissue volume. Many CNBs yield <10 mm of tissue across one to three cores. After histology and ancillary IHC, residual FFPE content may be marginal for multi-gene panels.
- FFPE-related degradation. Formalin crosslinking, fragmentation, and chemical adducts reduce library complexity and can introduce artifacts, compromising on-target rate and uniformity.
- Consequences. Failed libraries, low on-target bases, and uneven per-amplicon depth drive repeat testing, expanded panels failure, delays to therapy, and occasional re-biopsy.

Integrated Solution Concept: Front-End Capture with Molecular Stabilization

During CNB, tumor cells adhere to the needle cannula/stylet and are ordinarily discarded with the device. The Crow's Nest provides a sterile, multi-chamber catchment and controlled wash procedure to transfer those DTCs into DNA/RNA Shield™, which instantly lyses cells and inactivates nucleases and pathogens while preserving nucleic acids at ambient temperature. The resulting liquid specimen becomes: 1) An adjunct input combined with FFPE-extracted DNA/RNA to increase total mass and library diversity, or 2) A stand-alone input when FFPE is exhausted or unsuitable (e.g., decalcified bone, heavy necrosis).



Crow's Nest® Catchment System

What is the Crow's Nest?

The Crow's Nest® Biopsy Catchment System is a simple device that is used after solid tumor core needle biopsies of solid tumors. Its purpose is to collect the dislodged tumor cells that are otherwise wasted with the needle when the disposable needle is thrown away.

The device consists of six (6) interlocking plastic parts, and two (2) fluid-filled chambers. The lower chamber is pre-filled with DNA/RNA Shield™. DNA/RNA Shield™ is a key feature of the device, because it lyses (bursts) the cells that are collected from the needle, and preserves the expressed DNA at room temperature for at least two years.

As a result, any patient who undergoes a core needle biopsy procedure can have a second specimen without taking any additional tissue, which is ideal for molecular testing because it has not been damaged by formalin fixation.

Device and Workflow Overview

- Use case. Immediately post-biopsy, the interventionalist inserts the used needle into the Crow's Nest® upper chamber and following the steps detailed in the instruction for use (IFU) package insert.
- Specimen stabilization. Using the Crow's Nest® delivers the eluate directly into DNA/RNA Shield™, preserving nucleic acids at ambient temperature for transport and storage.
- Extraction. Laboratories utilize the *Quick*-DNA/RNA™ HT kits for high-throughput, dual extraction of DNA and RNA from both DTC and FFPE inputs.
- Sequencing services. Samples can be analyzed through Zymo Research Services, which provide validated RNA-Seq and DNA-Seq pipelines optimized for Shield™-preserved specimens, or other third-party reference labs that have validated this specimen type.
- Chain of custody. Each Crow's Nest® unit is marked with a 2D barcode that contains its serial number. This allows each 5 mL liquid specimen to be logged into the Virchow Vault online portal to maintain sample traceability from suite to lab, and from lab to the Virchow Vault Liquid Specimen Biorepository.

Devices serialized via 2D Barcodes

Molecular Rationale for Improved Read Coverage

- **Higher unique molecule counts.** Supplementing FFPE with DTC-derived DNA/RNA stabilized in DNA/RNA Shield™ increases total template diversity and usable input.
- **Reduced fixation damage.** Nucleic acids derived from liquid-captured cells bypass formalin, reducing crosslink-induced fragmentation and deamination, yielding longer insert sizes and even amplicon/probe performance.
- **Preservation advantage.** DNA/RNA Shield™ maintains nucleic acid integrity at ambient temperature without cold-chain dependency, ensuring consistent performance for downstream sequencing.

Recommended Laboratory Workflow

1. **Accessioning:** Register Crow's Nest + DNA/RNA Shield™ liquid specimen as a companion to the patient's FFPE block(s).
2. **Aliquoting & QC:** Spin at low g to pellet cells; quantify nucleic acids using fluorometric methods; assess DIN/RIN equivalents.
3. **Extraction:** Employ **Zymo's *Quick*-DNA/RNA™ HT kits** for simultaneous extraction of DNA and RNA from Shield-preserved material.
4. **Library Prep:**
    - Single-Stranded library method employed by **Zymo-Seq SPLAT DNA Library Kit** to generate libraries from both ssDNA and dsDNA to accommodate FFPE samples.
    - Whole transcriptome RNA-Seq using **Zymo-Seq RiboFree Total RNA Library Prep Kit**.
5. **Input Strategy:** Combine DTC and FFPE libraries during analysis, leveraging Zymo Research's bioinformatics pipelines for integrated variant calling.
6. **Sequencing & Analysis:** Perform the entire NGS workflow through **Zymo Research Services**, ensuring consistent QC metrics and variant reproducibility.
7. **Reporting:** Highlight improvements in coverage and variant detection enabled by the combined workflow.

Materials and Methods

Sample Collection

CNB specimens were obtained from de-identified breast cancer cases under approved institutional protocols. Immediately after each CNB, the biopsy needle, including the stylet and cannula, was inserted into the Crow's Nest® Biopsy Catchment System (Virchow Medical Inc.), and the integrated wash procedure was performed to recover DTCs adhering to the needle lumen. The resulting eluate was transferred to a sterile 5 mL screw-cap transport tube, labeled with a unique 2D barcode linked to the device serial number. The device's lower chamber, preloaded with 5 mL of DNA/RNA Shield™, lysed and stabilized the collected material at the point of capture, instantly inactivating nucleases and preserving nucleic acids at ambient temperature. Shield-preserved liquid specimens were transported at room temperature. Matching FFPE tissue scrolls from the same biopsies were collected in parallel to enable direct comparison of nucleic acid yield and integrity between Crow's Nest–derived and FFPE samples.

Sample Preparation and Nucleic Acid Extraction

A total of 5 breast cancer samples collected with the Crow's Nest Device were processed using the high-throughput *Quick*-DNA/RNA™ HT (R2150) (Figure 1). Duplicates of 300 µL from each sample were mixed with 600 µL of DNA/RNA Buffer HT (1:2 ratio) and 15 µL of HT MagBinding Beads. Each sample was mixed for 15 minutes at room temperature. The HT MagBinding Beads were washed following the kit's protocol, using 500 µL for each wash. DNA and RNA were eluted in 30 µL of DNase/RNase-free water. Half (15 µL) of the sample was used for downstream DNA Sequencing, while the other half went through DNase I treatment following the kit's protocol for downstream RNA Sequencing.

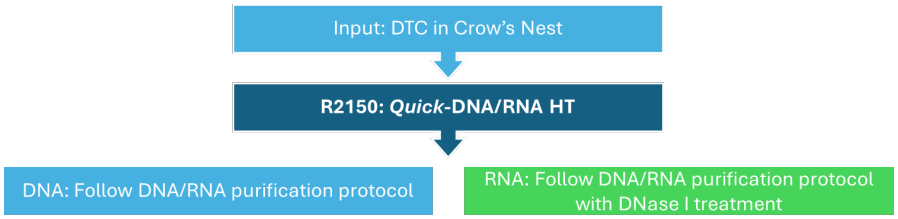


Figure 1: Schematic overview of Crow's Nest sample DNA/RNA extraction workflow.

Three matching FFPE (scrolls) samples were processed using the Zymo Research high-throughput FFPE tissue DNA/RNA extraction workflow (R2130) (Figure 2). Duplicates of each sample were processed in parallel. Scrolls for both DNA and RNA extraction were heated at 55°C for 10 minutes using Deparaffinization Solution to melt the paraffin. After spinning down to pellet the liberated tissue, Deparaffinization Solution was removed. 100 µL of a Proteinase K reaction mixture was added to help further break down the sample. Tissue for RNA analysis was digested for 1 hour at 55°C while tissue for DNA analysis was digested for 3 hours. Digested DNA lysate was de-crosslinked at 94°C for 20 minutes while RNA lysate was de-crosslinked at 65°C for 15 minutes. DNA/RNA Lysis Buffer was then added to all lysates at a 3:1 ratio (300 µL to 100 µL). For the following nucleic acid purification steps, samples were processed using the *Quick*-DNA/RNA™ MagBead protocol (R2130, Appendices, DNA/RNA Purification from FFPE Samples). DNA was purified from prepared lysates using the protocol for total nucleic acids; RNA was purified using the same protocol but incorporating DNase treatment to remove DNA contamination. DNA and RNA were eluted in 25 µL of DNA Elution Buffer or DNase/RNase-Free Water, respectively.

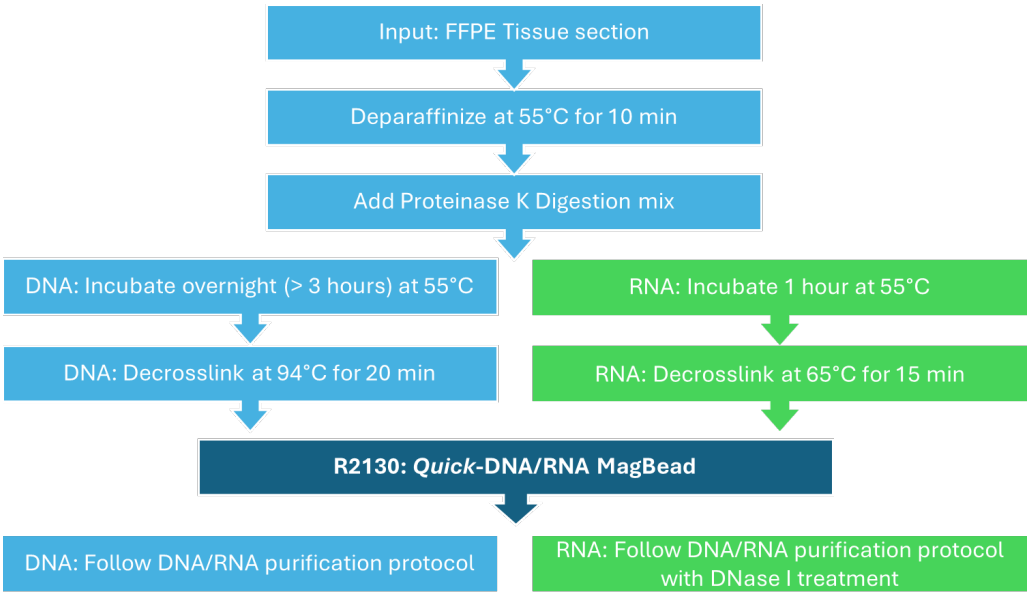


Figure 2: Schematic overview of FFPE tissue DNA/RNA extraction workflow.

Purified DNA and RNA were quantified using the HS dsDNA Qubit and HS RNA Qubit assays, respectively. Nucleic acid quality was imaged using the Genomic DNA and HS RNA TapeStation assays on the TapeStation 4200.

NGS Library Preparation and Sequencing

A novel single-stranded ligation method, which generates libraries from both ssDNA and dsDNA similar to reverse cross-linked and purified FFPE DNA, was selected for whole-genome sequencing library preparation using the Zymo-Seq SPLAT DNA Library Kit (D5464). Purified genomic DNA samples were normalized to 56 ng, followed by a single-strand adapter ligation and library amplification using Zymo-Seq™ UDI Index Primers (D3096). Library

quality control was performed on the Agilent 4200 TapeStation. Whole Genome libraries were sequenced on the NovaSeq X Plus (Illumina) using 300-cycle reagent kit

RNA was normalized to approximately 40 ng and prepped using the Zymo-Seq RiboFree Total RNA Library Prep Kit (R3000). For the rRNA depletion procedure, samples were incubated for 2 hours. The libraries were amplified for 13 PCR cycles. RNA-seq libraries were sequenced on AVITI (Element Biosciences) using 300-cycle reagent kit.

Bioinformatics Analysis

Sequence reads from whole-genome sequencing libraries were identified using standard Illumina base calling software. Raw paired-end FASTQ files were assessed for overall quality using FastQC, and adapter- and quality-trimmed using Fastp. Reads shorter than 15 bp were discarded. Library insert size distribution was calculated using Fastp. Trimmed reads were aligned to the reference genome assembly using BWA-MEM, coordinate-sorted with Samtools, and duplicate reads were marked with GATK MarkDuplicates. Alignment metrics were summarized using Mosdepth , and percentage of mapped reads were calculated. Variant calling was performed using DeepVariant. Detected single-nucleotide polymorphisms (SNPs) and insertions/deletions (indels) were filtered and summarized using VCFtools. Annotated variant call files (VCFs) were generated using the Ensembl Variant Effect Predictor (VEP), which predicts the impact of variants on genes and proteins based on Ensembl gene models. The filtered VCFs were compared using bcftools to identify shared variants based on matching chromosome, position, reference, and alternate alleles. Concordance between the two call sets was quantified using the Jaccard index. The Zymo Research RNA-seq data analysis pipeline was applied to reads generated from libraries prepared with the Zymo-Seq RiboFree Total RNA Library Kit (R3000). In brief, the Zymo Research RNA-Seq pipeline was originally adapted from nf-core/rnaseq pipeline v1.4.2 (https://github.com/nf-core/rnaseq). The pipeline was built using Nextflow (https://www.nextflow.io/). Quality control of raw reads was carried out using FastQC v0.11.9 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc). Adapter and low-quality sample sequences were identified and removed from the raw FASTQ reads files using Trim Galore! v0.6.6 (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore). Trimmed reads were aligned to the reference genome using STAR v2.6.1d (https://github.com/alexdobin/STAR). BAM file filtering and indexing was carried out using SAMtools v1.9 (https://github.com/samtools/samtools). RNA-seq library quality control was implemented using RSeQC v4.0.0 (http://rseqc.sourceforge.net/) and QualiMap v2.2.2-dev (http://qualimap.conesalab.org/). Duplicate reads were marked using Picard tools v2.23.9 (http://broadinstitute.github.io/picard/). Library complexity was estimated using Preseq v2.0.3 (https://github.com/smithlabcode/preseq). Duplication rate quality control was performed using dupRadar v1.18.0 (https://bioconductor.org/packages/dupRadar/).

Results

Quantification and Qualification Assessment of DNA and RNA

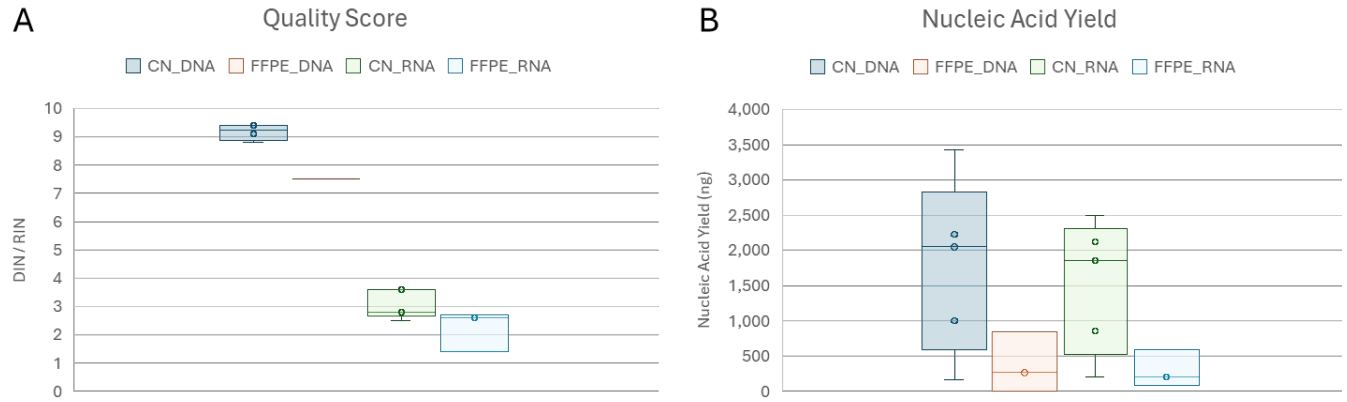


Figure 3. Boxplot of (A) quality score and (B) nucleic acid yield of DNA and RNA purified from CN and FFPE.

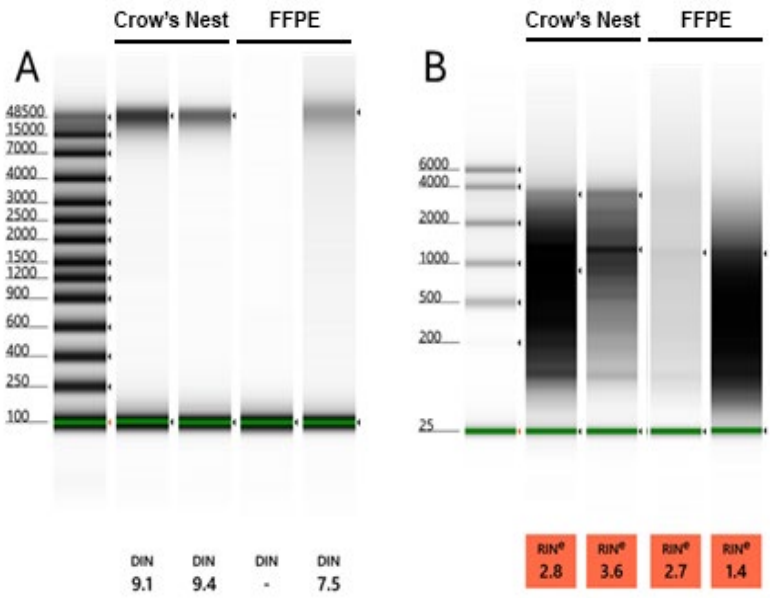


Figure 4. Representative Tapestation QC of A) gDNA and B) RNA extracted from CN and FFPE. Samples without DIN score are due to sample concentration outside of functional range for DIN calculation.

We first evaluated the quality and quantity of nucleic acids, and the results consistently demonstrated that CN samples yield substantially higher quality and quantity than FFPE samples. DNA recovery from CN ranged from 1,000 ng to 3,430 ng, far exceeding the 10.6 ng to 848 ng obtained from FFPE. Similarly, RNA recovery from CN ranged from 860 ng to 2,500 ng, compared to only 89.6 ng to 596 ng from FFPE. Due to insufficient material, nucleic acid extraction was not performed for FFPE in cases 3 and 4. Notably, Case 3–characterized histologically by extensive sclerosis–showed lower yield even from CN, consistent with its tissue condition. Importantly, CN samples exhibited greater DNA integrity, with an average DIN of 9.1 compared to 7.5 for FFPE (excluding NA). RNA integrity was also higher in CN, with an average RIN of 3.06 versus 2.23 in FFPE. Overall, these findings confirm that CN provides a markedly better source of nucleic acid in both quantity and quality than FFPE.

Whole Genome Sequencing (WGS) of DNA

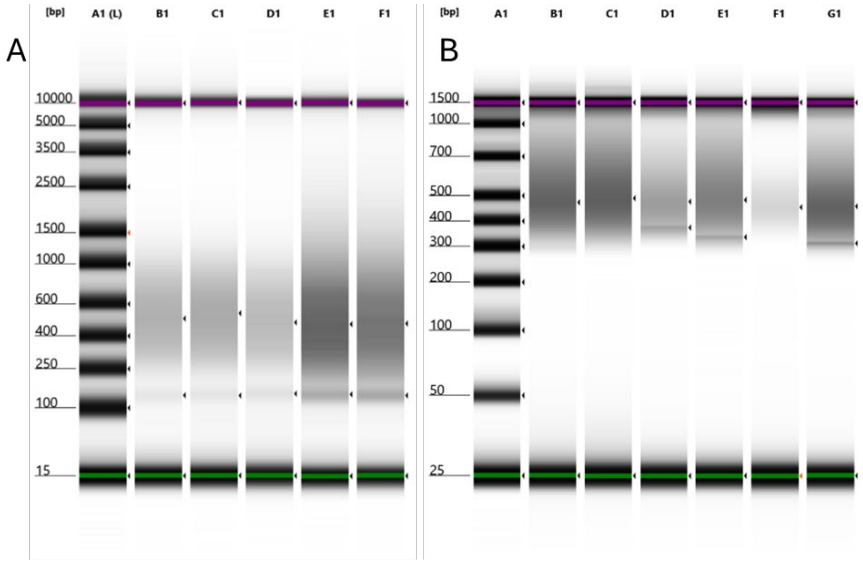


Figure 5. Tapestation QC of A) WGS libraries and B) RNA-seq libraries constructed from CN#1,2,5 and FFPE#1,2,5 loaded left to right across the image lanes.

5 WGS libraries were sequenced on an Illumina NovaSeq X, with approximately 3 billion 150 bp paired-end reads allocated per sample on average. For consistent evaluation, an equal read number (~2.6 billion 150 bp PE reads) was used across all samples. Only cases with matched CN and FFPE samples were included in the NGS analysis. As insufficient DNA was recovered from Case 2 FFPE, whole genome library preparation was omitted. Across all matched samples, CN consistently outperformed FFPE in sequencing quality metrics. CN achieved markedly higher average median read depth (83× vs. 43.5×), a greater percentage of mapped reads (91.5% vs. 70.9%), and substantially lower adapter content (32.4% vs. 70.8%) than FFPE (Table 1). Variant analysis was performed on 25 breast cancer biomarkers. A pathogenic deletion variant in ATM gene (rs1064793390) was observed at chr11, position 108293358 in both CN#1 and FFPE#1.

Despite, *Quick*-DNA/RNA purification chemistry used in our workflow efficiently recovers both ssDNA and dsDNA, and the WGS library preparation method employed here accommodates both forms—unlike many NGS library prep protocols that rely primarily on high-molecular weight dsDNA. CN-derived DNA is still more effectively utilized for high-quality NGS library construction and sequencing, leading to superior data output compared to FFPE. The combination of the CN Biopsy Catchment System and the Zymo Research NGS Service workflow likely acted synergistically to achieve the consistently high read depth (Figure 6) and sequencing quality observed in CN samples.

Table 1. General whole genome sequence data summary statistics.

Sample Name	≥ 30X	Median (X)	Reads mapped (M)	%Mapped	%Proper pairs	Total seqs (M)	GC content	% Adapter
CN#1	90.00%	83.0	2179.80	92.10%	90.50%	2365.7	42.00%	30.00%
CN#2	90.00%	82.0	2210.40	94.20%	92.60%	2345.6	41.20%	30.40%
CN#5	90.00%	77.0	2038.30	88.20%	86.50%	2310.2	43.20%	33.90%
FFPE#1	82.00%	45.0	1187.20	72.40%	70.50%	1639.1	42.50%	70.20%
FFPE#5	83.00%	42.0	1091.50	69.30%	67.30%	1575.2	42.80%	71.40%

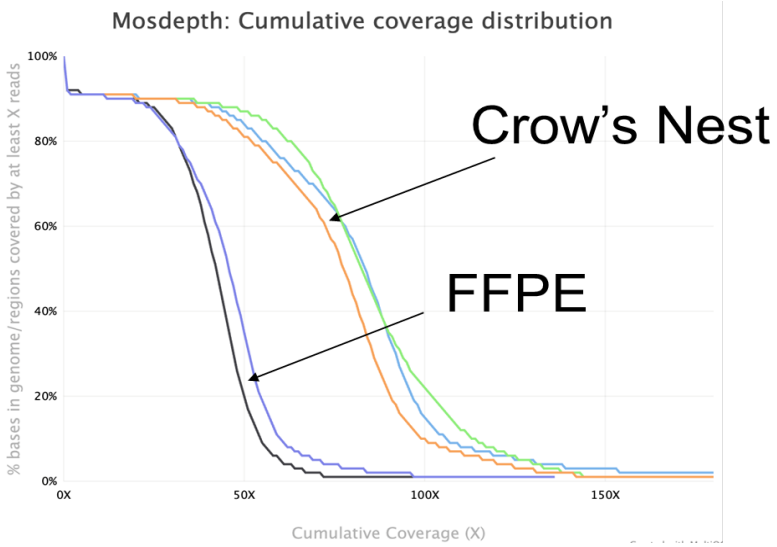


Figure 6. Cumulative coverage distribution.

Whole Transcriptome Sequencing (RNA-Seq)

We next evaluated RNA-seq library prep capability to support emerging studies that couple whole transcriptome profiling with genomic sequencing from the same DTCs collected in CN and FFPE tissue sample, supporting high-powered multi-omic research. Since RNA purified from FFPE tissue is highly degraded, we reasoned that a ribosomal (r)RNA depletion approach would yield better datasets compared to those designed to enrich the 3′ ends of mRNA molecules – for example, polyA enrichment approaches. Our rationale was that enriching around polyA tails would disproportionately incorporate only the very ends of the short, low molecular weight gene

transcripts, thus producing read data that makes bioinformatic analysis difficult. Others have drawn similar conclusions, yet most rRNA depletion protocols remain complicated, labor-intensive, or expensive (Liu et al., 2022; Pennock *et al.*, 2019; Jacobsen *et al.*, 2023), making them incompatible with high-throughput workflows. To overcome those challenges, we developed a customized workflow using the Zymo-Seq RiboFree® Total RNA Library Prep Kit (Zymo Research, R3000). The RiboFree kit simplifies RNA-seq library preparation by utilizing a novel enzymatic method to deplete abundant ribosomal RNA species. By avoiding probe-based depletion, the workflow is simplified, making it automation friendly while also reducing hands-on time and reagent costs.

We applied our custom RNA-seq workflow to RNA purified from the same DTCs collected in CN and FFPE tissues used for whole genome sequencing. As expected, FFPE RNA showed lower integrity compared to CN (Figure 3A). Nevertheless, both CN and FFPE samples produced high-quality RNA-seq libraries suitable for sequencing (Figure 4), yielding an average of approximately 20 million read pairs per library. Basic bioinformatics QC metrics demonstrated a robust and reproducible workflow across both sample types, with consistently high reads passing filter (>99%), strong alignment rates (>94%), broad gene detection (>25,000 genes; feature Counts), and minimal rRNA contamination (<1%) on average (Table 2). These results confirm that RNA isolated from CN performs equivalently well in downstream RNA-seq analysis as RNA from FFPE, despite differences in initial RNA quality. A heatmap of top gene expression profiles showed that matched CN and FFPE samples shared highly similar expression patterns, indicating strong concordance between the two preservation methods. Together, these findings support CN as a practical and effective alternative to FFPE for RNA-seq–based transcriptomic analysis.

Table 2. General RNA-sequencing data summary statistics.

Sample Name	M Seqs	% GC	% Reads PF	% Aligned	% Dups	% rRNA	% Assigned	M Assigned	rs of genes de
CN#1	16.3	42%	99.80%	96.70%	18.40%	0.10%	37.90%	6.1	25697
CN#2	29.3	43%	99.20%	96.00%	54.70%	0.15%	53.80%	15.4	25750
CN#5	16.1	43%	99.80%	96.50%	18.50%	0.04%	43.50%	6.9	27657
FFPE#1	21.3	44%	99.70%	94.70%	26.30%	0.84%	52.50%	10.8	26222
FFPE#2	18.4	43%	99.70%	96.50%	25.20%	0.29%	54.70%	9.9	25609
FFPE#5	16.3	45%	99.50%	95.50%	32.50%	0.36%	62.00%	9.8	25526

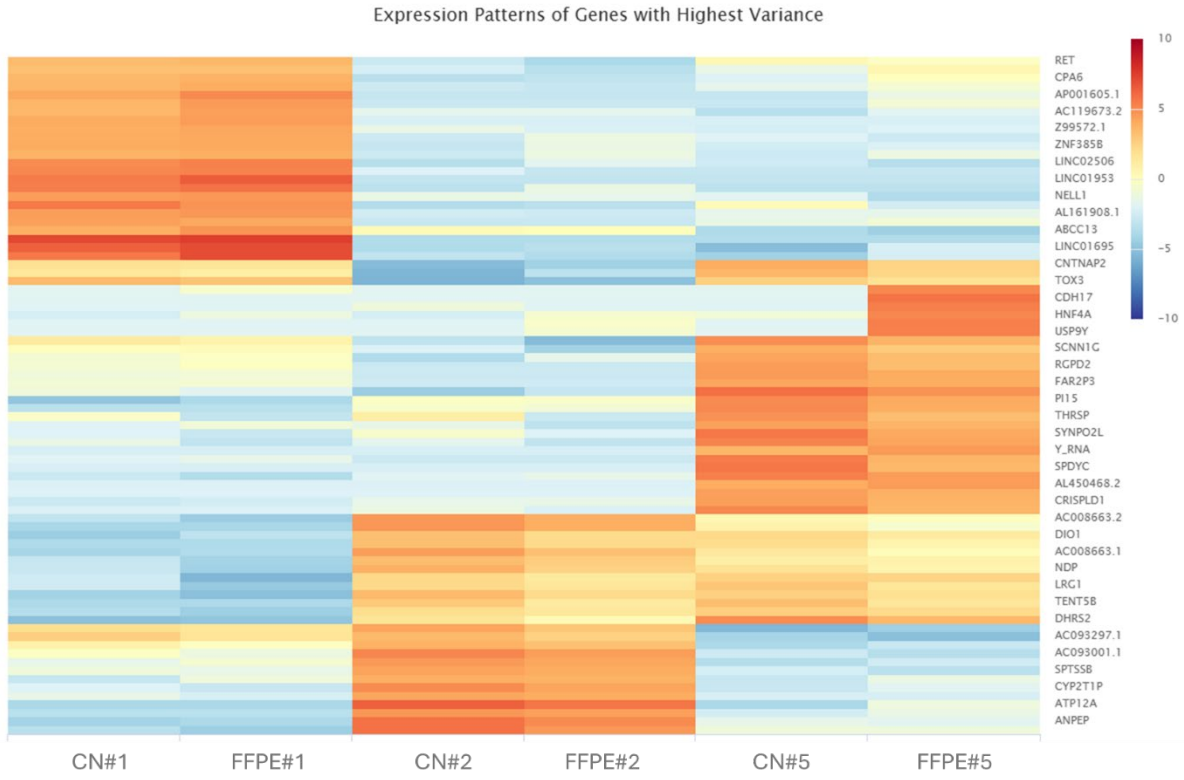


Figure 7. Top gene expression patterns heatmap from all 3 matching pairs of breast cancer tissue.

Discussion

The data from this study demonstrate how integrating the Crow's Nest® Biopsy Catchment System (that lyses and preserves DNA and RNA from DTCs) into core needle biopsy protocols then using Zymo Research's complete molecular workflow to extract the DNA from the liquid specimens, transforms what was normally considered biopsy waste into powerful, actionable insight. By recovering and stabilizing DTCs immediately after biopsy, this approach preserves the genetic and transcriptomic information critical for NGS without requiring additional tissue collection, depleting solid tissue blocks or compromising patient comfort.

Traditional FFPE samples remain a cornerstone of molecular testing, but they come with inherent drawbacks: formalin fixation damages nucleic acids, reduces library complexity, and introduces artifacts that can skew variant calling and lower sequencing success. In contrast, using the Crow's Nest® captures fresh DTCs directly and preserves their nucleic acids in DNA/RNA Shield™, maintaining nucleic acid integrity at room temperature – for at least two years for DNA and at least one month for RNA - and eliminating the degradation associated with FFPE processing. The result is a sample source that consistently delivers higher yield, greater integrity, deeper sequencing coverage, quicker turnaround times, and the opportunity for some patients who would otherwise miss out on the benefits of next generation sequencing due to tissue exhaustion.

When paired with Zymo's *Quick*-DNA/RNA™ HT kits and Zymo-Seq® library preparation technologies, CN-derived material generates clean, high-complexity libraries that perform exceptionally well in both DNA-Seq and RNA-Seq applications. Whole-genome sequencing data revealed superior read depth, higher mapping efficiency, and fewer artifacts compared with FFPE tissue samples, while transcriptome analysis confirmed that CN-derived RNA supports robust gene expression profiling. Together, these findings highlight how this integrated workflow enables confident variant detection and comprehensive molecular characterization, even from minimal biopsy input.

Beyond performance, this approach delivers significant practical and clinical advantages. It reduces the risk of failed NGS runs, reduces or eliminates the need for re-biopsy, and accelerates turnaround time—all while improving the reliability of results that guide precision oncology decisions. The standardized, automation-friendly workflow and secure chain of custody, convenient 5 mL liquid sample storage options in the Virchow Vault™ liquid specimen biorepository also make it easy for laboratories to implement at scale, ensuring consistent data quality from sample to report.

In short, the combination of Crow's Nest® and Zymo Research's molecular technologies redefines what's possible in biopsy-based molecular testing. By converting discarded cellular material into high-quality sequencing data, it empowers clinicians and researchers to extract maximum insight from every core needle biopsy. This fuels faster, more accurate, and more consistently available personalized cancer diagnostics and eligibility for targeted therapy.

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