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Whole Transcriptome Sequencing from Degraded FFPE Samples: A Fast Workflow to Deliver Robust Gene Fusion Detection

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Covaris truCOVER® Total RNA Library Prep Kit Features & Benefits

Features

- **Powered by Adaptive Focused Acoustics® (AFA®) Technology:** Unique, tested and proven mechanical shearing that ensures consistent insert sizes and unbiased treatment of RNA regardless of its quality and size.
- **Superior Read Distribution:** Higher proportion of exonic reads, maximizing the high-value data available for Differential Gene Expression (DGE).
- **Broad Input Compatibility:** Validated workflows over a wide range of total RNA (10–500 ng), including RNA isolated from highly degraded FFPE samples.
- **Integrated Ultra-efficient rRNA Depletion:** Efficiently removes cytoplasmic and mitochondrial ribosomal RNA in a single, easy workflow step.
- **Single-step cDNA Synthesis:** Utilizes a single enzyme and incubation step, replacing complex multi-stage protocols.
- **Automation-ready Design:** Simplified plate-based workflow with fewer handling steps and bead cleanups compared to traditional methods.

Benefits and Critical Outcomes for Laboratories

- **Confident Biomarker Detection:** Detects 100% of known gene fusions and alternative splicing from FFPE samples, regardless of quality.
- **Rapid Turnaround Time Ensuring Efficiency:** Complete library prep in ~4 hours, reducing hands-on time by up to 40% when compared with traditional approaches.
- **Data Clarity:** Depletes residual rRNA to <1%, maximizing sequencing capacity for informative transcripts.
- **Reduced Cost/Sample:** Enables cost-efficient library preparation by minimizing workflow failures and reagent use, while optimizing read utilization to avoid unnecessary over-sequencing.
- **High Reproducibility:** Delivers consistent library yields and sequencing metrics across variable sample qualities ($DV_{200} \geq 30\%$).

Introduction

Precision oncology is rapidly evolving from a focus on static genomic mutations to a dynamic understanding of the actionable transcriptome. While DNA-based gene panels have been the standard for years, recent data indicates that integrating Whole Transcriptome Sequencing (WTS) with genomic profiling significantly enhances diagnostic yield. In a direct comparison of Whole Genome Sequencing (WGS) in combination with WTS versus the use of comprehensive gene panels in advanced cancers, approximately one-third of therapy recommendations derived from WGS/WTS were based on biomarkers not covered by the panel, particularly those relying on RNA expression levels and complex structural variants (Kerle *et al.*, 2025). Furthermore, the expanding repertoire of histology-agnostic therapies targeting cell-surface proteins, such as antibody-drug conjugates (ADCs) and bispecifics, requires precise and reliable RNA expression profiling to identify overexpression of oncogenes or loss of tumor suppressors that DNA sequencing alone cannot reveal (Johnson *et al.*, 2026).

The clinical utility of WTS relies heavily on the performance of the library preparation, particularly regarding strand specificity and fusion detection. Strand-specific protocols are essential for accurately resolving overlapping genes and identifying antisense transcripts, which play critical regulatory roles and are often confounded in standard non-stranded libraries (Levin *et al.*, 2010). Moreover, accurate detection of gene fusions, major drivers in precision oncology, is highly dependent on library insert size; protocols that generate longer, consistent inserts are superior for spanning fusion junctions and avoiding data loss due to read overlaps (Jaksik *et al.*, 2021). RNA-based sequencing is now preferred over DNA-based NGS for fusion detection in guidelines for non-small cell lung cancer (NSCLC) due to its ability to identify events involving intronic breakpoints that DNA panels frequently miss (Johnson *et al.*, 2026).

Beyond diagnostic precision, the economic argument for upfront comprehensive profiling is becoming indisputable. In patients with advanced NSCLC, the use of WES/WTS was shown to drastically reduce total care costs per patient compared to sequential single-gene testing, while simultaneously improving median overall survival by identifying more patients who are eligible for approved targeted therapies and clinical trials (Ortendahl *et al.*, 2025).

The Evolving Reimbursement Landscape

The reimbursement landscape for WTS is rapidly maturing as leading reference labs move from laboratory-developed tests (LDTs) toward formal FDA-approved or 510(k)-cleared IVD status. This shift provides the rigorous clinical evidence required to secure stable, national reimbursement coverage, particularly through the Centers for Medicare & Medicaid Services (CMS) (Centers for Medicare & Medicaid Services, 2011).

Key Leaders and Product Status

- **Caris Life Sciences:** Recently achieved a major milestone with FDA approval for MI Cancer Seek® (2024), the first simultaneous Whole Exome Sequencing (WES) and Whole Transcriptome Sequencing (WTS) assay with companion diagnostic (CDx) indications. This approval facilitates broad payer coverage by demonstrating non-inferiority to other FDA-approved tests for identifying patients eligible for targeted therapies.
- **TempusAI:** Received FDA 510(k) clearance in September 2025 for its Tempus xR® IVD, an RNA-based NGS diagnostic tool. This clearance specifically positions xR as a tool for drug development and clinical trials while the company continues to ramp up reimbursement for its xT genomic profiling tests.
- **Veracyte:** Operates an RNA WTS platform utilized across its thyroid (Afirma®), lung (Percepta®), and idiopathic pulmonary fibrosis (Envisia®) classifiers. These tests are heavily covered by Medicare and private payers, with some like the Envisia classifier receiving Advanced Diagnostic Laboratory Test (ADLT) status (2020).
- **Exact Sciences:** While primarily known for Cologuard®, the company is expanding its Medicare coverage for molecular residual disease (MRD) tests like OncoDetect™ (2025). Their acquisition of Ashion Analytics (2021) and internal R&D focused on WTS further integrates comprehensive transcriptomics into their oncology portfolio to support therapy selection.

The integration of WTS into price-sensitive, value-based care models is largely driven by its ability to identify gene fusions and pathways that DNA-only tests might miss, directly impacting therapeutic outcomes. By leveraging the CMS MolDX program, many of these companies secure technical assessment approvals that pave the way for private insurer adoption.

To realize these workflow and economic benefits in routine practice, however, laboratories require a library preparation workflow that is not only cost-effective but also capable of rescuing high-quality data from challenging, degraded FFPE samples. The truCOVER Total RNA Library Prep Kit addresses this need, providing a robust solution for deriving comprehensive transcriptomic insights from cancer samples.

Materials and Methods

RNA Input and Shearing

Total RNA was isolated from FFPE tissue blocks (including reference standards such as K562 cell pellets) using the Covaris truXTRAC® FFPE Total NA Auto 96 Kit. RNA quality was assessed using the DV_{200} metric, which is a representation of RNA fragments >200 nucleotides. For library preparation, input amounts ranging from 10–500 ng were utilized. Mechanical fragmentation was performed using Covaris, AFA technology to generate consistent fragment distributions (~350 nucleotides) without the bias associated with chemical fragmentation of degraded samples. For performance benchmarking, libraries were also prepared using two commercially available library prep kits (***denoted Supplier A and B***) following their respective manufacturers' instructions.

Library Quantification and Sequencing

Libraries were prepared using the truCOVER Total RNA Library Prep Kit following the manufacturer's protocol, which integrates rRNA depletion, cDNA synthesis, and unique dual indexing (UDI) to prevent index hopping. Final library concentrations were quantified using fluorometric methods (Qubit™) and fragment size distribution was verified via a tape-based automated electrophoresis system (Agilent TapeStation®). Sequencing was performed on an Illumina NextSeq® 2000 platform (P4 XLEAP-SBS Reagent Kit, 300 cycles) targeting approximately 20–25M reads per sample.

Data Analysis

Raw sequencing reads were aligned to the human reference genome using the STAR aligner. Post-alignment metrics were calculated to assess:

- **Alignment Rate:** Percentage of reads mapping to the genome.
- **rRNA Depletion:** Percentage of reads aligning to ribosomal RNA regions.
- **Strand Specificity:** Ratio of reads mapping to the sense strand vs antisense strand.
- **Gene Body Coverage:** Distribution of reads across exon, intron, and intergenic regions.
- **Fusion Detection & Alternative Splicing:** Sensitivity was evaluated using the Seraseq FFPE Tumor Fusion RNA. Reference Material v4 containing 16 known fusions and 2 alternative splicing that are prevalent across different types of solid tumors.

Results and Discussion

Streamlined Workflow Efficiency and Automation Compatibility

The truCOVER Total RNA Library Prep Kit addresses a critical bottleneck in transcriptomics: workflow complexity. By utilizing a single-enzyme, single-incubation step for cDNA synthesis and optimizing magnetic bead clean-ups, the protocol shows a total workflow time of approximately 4 hours for 8 samples (***Figure 1***). This workflow represents a reduction in hands-on time and touchpoints by up to 40% compared to traditional methods (both Supplier A and Supplier B), minimizing user error and facilitating integration into automated liquid handling systems.

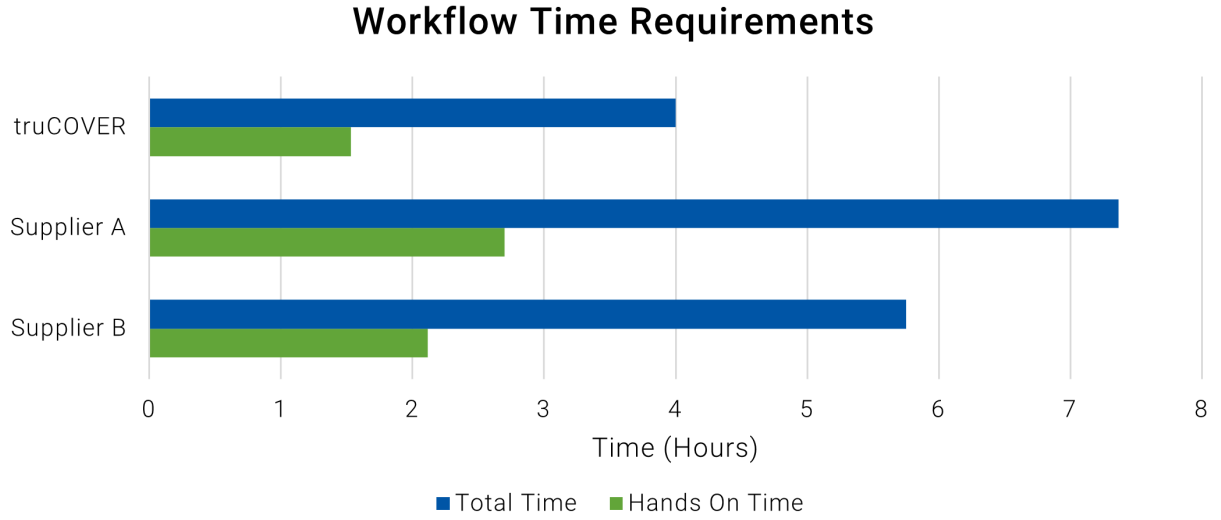

Figure 1. Workflow Time Requirements. The amount of time necessary to complete truCOVER and standard industry kit workflows is presented, with hands on time and total time distinguished by **green** and **blue** bars respectively.

Robust Performance Across Variable FFPE Quality

A major challenge in clinical research is the variability of RNA derived from FFPE tissues. Data demonstrates that truCOVER Total RNA Library Prep maintains high performance even with degraded inputs (DV_{200} scores >30%). Unlike chemical fragmentation, which requires optimization based on sample degradation levels, the AFA-based shearing provides an agnostic treatment of RNA. This results in highly reproducible insert sizes and library yields across a broad input range (10–500 ng), ensuring that valuable low-input samples are not lost to preparation failure (Covaris data on file).

Accurate Sequencing Metrics

The fidelity of the sequencing data is paramount for accurate differential gene expression (DGE) profiling, transcript annotation, and variant calling. The truCOVER Total RNA Library Prep kit demonstrates robust performance, yielding >87% alignment rates with strand specificity exceeding 84%. High strand specificity is critical for accurately quantifying gene expression, particularly in regions with overlapping sense and antisense transcripts, ensuring that regulatory antisense RNAs are not misidentified as protein-coding expression (Covaris data on file).

Superior rRNA Depletion and Transcriptome Clarity

Efficient removal of ribosomal RNA is critical for cost-effective WTS. The truCOVER Total RNA Library Prep kit demonstrates ultra-efficient rRNA depletion, consistently achieving <1% residual rRNA (combined cytoplasmic and mitochondrial). In comparative studies, truCOVER Total RNA Library Prep maintained depletion rates between 0.01–0.5%, ensuring most sequencing reads are dedicated to informative protein-coding and non-coding RNA rather than transcriptional noise (Covaris data on file).

High Precision in Gene Fusion Detection

Detecting structural variants in FFPE samples is often hindered by short fragment lengths and poor coverage. The truCOVER Total RNA Library Prep workflow preserves library complexity and insert size, which is critical for spanning fusion junctions. In validation studies using Seraseq FFPE Tumor Fusion RNA Reference Material, the truCOVER kit successfully detected 18 out of 18 (100%) target fusions and alternatively spliced variants (***Figure 2***). This performance, demonstrated by truCOVER Total RNA Library Prep enables precise identification of druggable targets and biomarkers, outperforming competing kits that failed to detect multiple targets at sequencing depths centered around 25M and capped at 50M reads, while matching the performance of other commercially available kits that require a significantly greater number of reads.

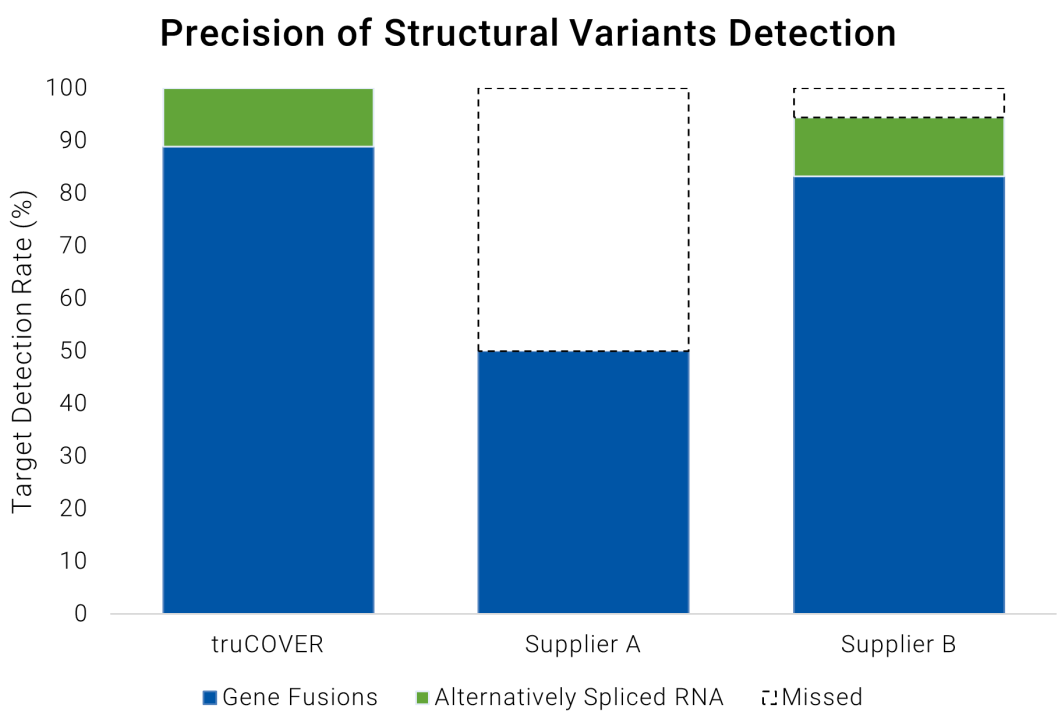

Figure 2. Precision of structural transcript variants calls. The total number of detected target variants from a known reference standard is presented for libraries generated using truCOVER and alternative commercial kits. Results are categorized into correctly identified gene fusions (**blue**), alternatively spliced RNA transcripts (**green**), and missed calls (white with dash line).

Superior Genomic Coverage and Read Distribution

Read distribution analysis across diverse sample types (K562 cell line and various FFPE tissues including pancreas, uterus, liver, and kidney) highlights the superior coverage of truCOVER Total RNA Library Prep. As shown in ***Figure 3***, truCOVER Total RNA Library Prep captures a consistently higher proportion of exonic reads (ranging from approximately 30–40%) across all tested sample types compared to standard industry kits, maximizing the high-value data available for DGE. Importantly, this exonic enrichment is achieved while keeping uninformative intergenic reads to an absolute minimum and retaining a robust amount of intronic data. This precise balance ensures comprehensive capture of the actionable transcriptome, enabling highly sensitive DGE analysis while preserving the intronic reads necessary for the detection of complex alternative splicing events and intronic fusions.

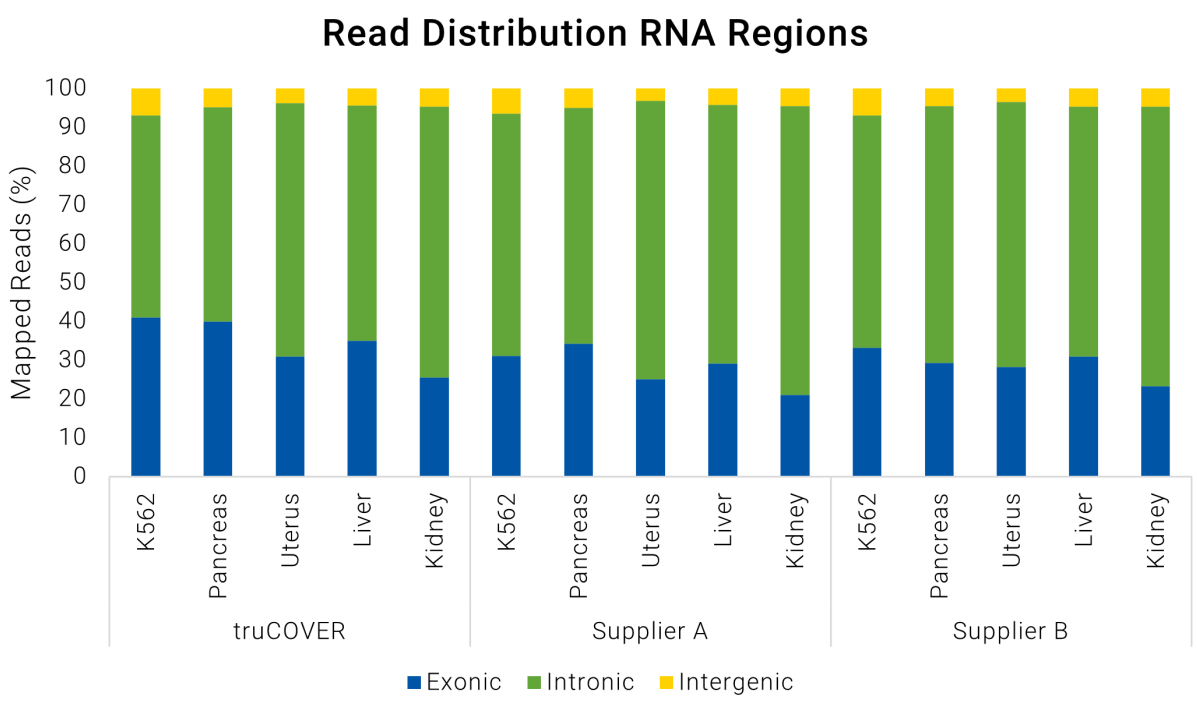

Figure 3. Read distribution across distinct genomic regions. Stacked bar charts display the percentage of mapped reads aligning to exonic (**blue**), intronic (**green**), and intergenic (**yellow**) regions for libraries prepared from K562 cells and various FFPE tissue types (Pancreas, Uterus, Liver, Kidney). When compared to Supplier A and Supplier B, the truCOVER kit consistently yields a higher proportion of valuable exonic reads and minimizes intergenic waste across all sample types, optimizing the sequencing data for comprehensive transcriptomic analysis.

Conclusion

The Covaris truCOVER Total RNA Library Prep Kit is a transformative solution for WTS, specifically optimized for the challenges typically experienced in working with FFPE samples. By combining the physical precision of AFA-based shearing with a streamlined, unbiased, and optimized workflow, the kit delivers high-quality libraries in under 4 hours. With validated performance in rRNA depletion (<1%), fusion detection (100% sensitivity on reference panels), and broad input flexibility (10–500 ng), truCOVER Total RNA Library Prep empowers researchers to extract maximum biological insight from complex and even degraded samples, bridging the gap between limited sample availability and reliable data generation. This workflow is compatible with downstream sequencing applications, including WES and custom enrichment panels. Furthermore, the superior read distribution achieved by truCOVER Total RNA Library Prep maximizes highly valuable exonic data while minimizing intergenic waste, ensuring that every sequencing run yields cost-effective transcriptomic insights.

For reference laboratories, contract research laboratories, and academic core laboratories, the benefits of truCOVER RNA Library Prep demonstrated in this report translate into reduced sample QC failures, simplified streamlined training, and optimized costs. Ultimately, this solution transforms the processing of FFPE samples from an experimental challenge into a reliable and scalable methodology, enabling researchers to confidently pursue high-impact genomic discoveries from archival samples with consistent quality and high efficiency.

References

1. Caris Life Sciences. (2024). Caris Life Sciences Receives FDA Approval for MI Cancer Seek™ as a Companion Diagnostic (CDx) Test. Retrieved from https://www.carislifesciences.com
2. Centers for Medicare & Medicaid Services. (2011). MolDX: Molecular Diagnostic Services Program. Retrieved from https://palmettogba.com/moldx
3. Exact Sciences. (2021). Exact Sciences to Acquire Ashion Analytics and Enter Research Collaboration with City of Hope's Genomics Institute, TGen. Retrieved from https://investor.exactsciences.com
4. Exact Sciences. (2025). Exact Sciences Announces Medicare Coverage for Oncodetect™ Molecular Residual Disease Test. Retrieved from https://www.exactsciences.com
5. Jaksik, R., *et al*. (2021). RNA-seq library preparation for comprehensive transcriptome analysis in cancer cells: The impact of insert size. Genomics, 113(6): 4149-4162.
6. Johnson, A., *et al*. (2026). The actionable transcriptome: a framework for incorporating RNA sequencing into precision oncology. Nature Reviews Clinical Oncology, 23: 1-17.
7. Kerle, I. A., *et al*. (2025). Translational and clinical comparison of whole genome and transcriptome to panel sequencing in precision oncology. npj Precision Oncology, 9:9.
8. Levin, J. Z., *et al*. (2010). Comprehensive comparative analysis of strand-specific RNA sequencing methods. Nature Methods, 7(9): 709-715.
9. Ortendahl, J. D., *et al*. (2025). Costs and Benefits of Whole-Exome, Whole-Transcriptome Sequencing in Patients With Advanced Non-Small Cell Lung Cancer. JCO Precision Oncology, 9:e2400640.
10. Tempus AI. (2025). Tempus Receives U.S. FDA 510(k) Clearance for Tempus xR IVD, its RNA NGS In Vitro Diagnostic Device. Retrieved from https://investors.tempus.com
11. Veracyte. (2020). Veracyte Receives ADLT Status for Envisia Genomic Classifier from Centers for Medicare and Medicaid Services. Retrieved from https://investor.veracyte.com

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