P01.114.F Sensitive Gene Fusion Detection and Comprehensive Transcriptome Profiling from Degraded FFPE Samples

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Introduction

Solving FFPE Challenges in RNA-Seq

- Whole Transcriptome Sequencing (WTS) is increasingly used to capture actionable transcriptomic data in precision oncology.
- However, FFPE processing causes RNA fragmentation, chemical modifications, and cross-linking, which degrade sample quality and challenge standard poly-A enrichment methods. Ribosomal RNA depletion and optimized library preparation can mitigate these issues.
- This study compares three library preparation workflows, one using mechanical acoustic shearing and two using chemical fragmentation, across metrics including workflow efficiency, rRNA depletion, genomic coverage, and fusion detection sensitivity.

Materials and Methods

- Total RNA was isolated from FFPE tissues and reference standards, with RNA input amounts of 10–500 ng and DV_{200} scores between ~ 37–81%.
- Three workflows were evaluated: Method A (mechanical acoustic shearing, ~350 nt fragments), Method B (chemical fragmentation), and Method C (chemical fragmentation, alternate protocol).
- Sensitivity and precision were assessed using the Seraseq FFPE Tumor Fusion RNA v4 Reference Material (16 known fusions, 2 alternative splicing events).
- Sequencing was performed on a NextSeq 2000 at ~20–25 M reads per sample.

Results

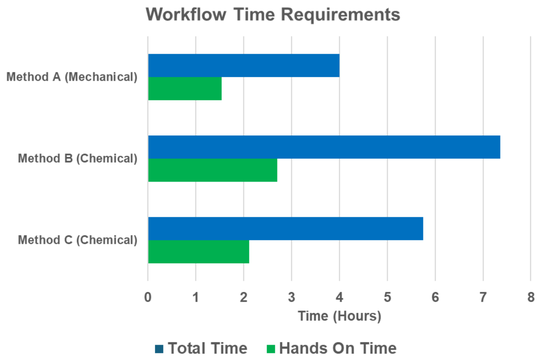


Figure 1. Method A required approximately four hours total, with reduced hands-on time relative to Methods B and C.

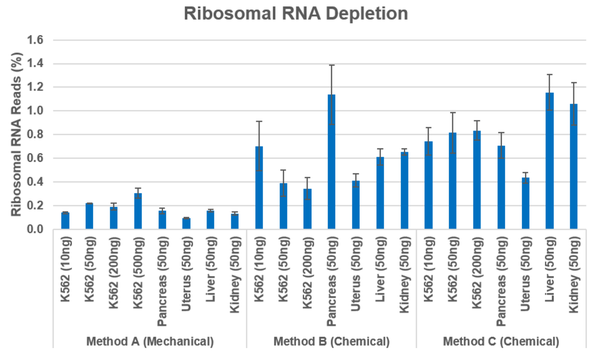


Figure 2. All methods achieved rRNA depletion below 1%. Method A showed the lowest residual rRNA across tissue types.

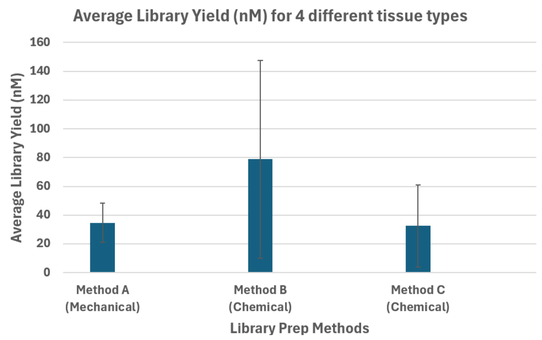


Figure 3. Mechanical shearing produced more consistent library yields across tissue types (Pancreas, Uterus, Liver, and Kidney), using 50 ng input with three technical replicates per condition.

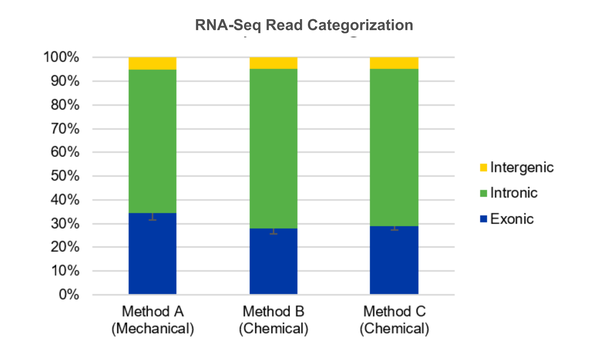
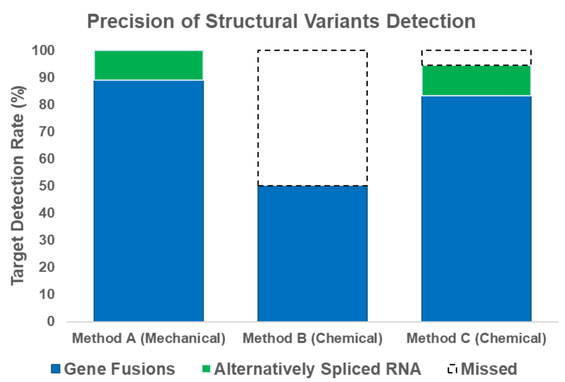


Figure 4. Method A yielded a consistently higher proportion of exonic reads (30–40%) across FFPE tissue types compared to Methods B and C.



Target	Method A	Method B	Method C
CCDC6-RET	Yes	Yes	Yes
CD74-ROS1	Yes	Yes	Yes
EGFR-SEPTIN14	Yes	No	No
EML4-ALK	Yes	Yes	Yes
ETV6-NTRK3	Yes	No	Yes
FGFR3-BAIAP2L1	Yes	Yes	Yes
FGFR3-TACC3	Yes	No	Yes
KIF5B-RET	Yes	Yes	Yes
LMNA-NTRK1	Yes	Yes	Yes
NCOA4-RET	Yes	No	Yes
PAX8-PPARG1	Yes	No	Yes
SLC34A-ROS1	Yes	Yes	Yes
SLC45A3-BRAF	Yes	No	Yes
TFG-NTRK1	Yes	Yes	Yes
TMPRSS2-ERG	Yes	No	Yes
TPM3-NTRK1	Yes	No	Yes
EGFR Variant III	Yes	No	Yes
MET ex 14	Yes	No	Yes

Figure 5. At ~25 M reads, Method A detected 100% of known structural variants and alternative splicing events from the reference standard; Methods B and C missed several targets at this depth.

Conclusions

- Method A reduced total preparation time to approximately four hours.
- All three workflows achieved effective rRNA depletion (<1%), though Method A showed the lowest residual rRNA levels.
- Mechanically sheared libraries had a higher proportion of exonic reads across tissue types.
- At 25M read depth, Method A detected all reference fusion and splicing targets, while chemical methods did not at equivalent depth.
- These results suggest mechanically sheared libraries offer advantages for degraded FFPE samples, though performance under different laboratory conditions and with broader sample types warrants further investigation.

References

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