Enhanced DNA Extraction and Whole Genome Sequencing Library Preparation from FFPE Samples

Authors: Bella Pfeiffer, Gabriel Lipof, Alaina Villareal, Kristopher Amirault, Sameer Vasantgadkar, Madan Ambavaram, Vanessa Process, Sushant Khanal, Martina Werner, Greg Endress, Ulrich Thomann, Eugenio Daviso | **Affiliation**: Covaris, LLC, Woburn, MA, USA

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Introduction

NGS Library Prep: Challenges & Solution

- As WGS gets more widely adopted, optimizing workflows to address fragmentation inconsistencies, poor library conversion, input variability, and sequencing bias is critical for accurate and scalable variant calling. [1]
- For low input or low-quality material such as FFPE, conventional amplification techniques often introduce biases, leading to non-uniform coverage, preferential amplification of regions with extreme GC content, and a subsequent compromise in data quality and variant calling precision. [2]
- Covaris truCOVER® Library Prep offers precise, mechanical DNA fragmentation along with optimized reagents and workflow to address all above challenges.

Methods

- Detailed protocols, including Covaris Adaptive Focused Acoustics® (AFA®) settings, library quantification procedures, and workflow specifics are described in the truCOVER WGS Library Prep Kit product manual [3] and in the associated Application Note. [1]

Figure 1. Workflow for truCOVER Library Preparation with Amplification module.

- **DNA Sources:** Horizon Discovery FFPE GIAB Samples of HG002, HG003, HG004, and HG005 WGS libraries prepared using truCOVER Library Prep and Amplification Kit. Comparison data was prepared with LP Supplier A Library Preparation Kit with Amplification.
- **FFPE Processing:** HG002, HG003, HG004, and HG005 extracted with both the Covaris truXTRAC® FFPE Total NA Auto 96 Kit [4] automated on Sonication STAR & Supplier α FFPE DNA/RNA extraction kit.

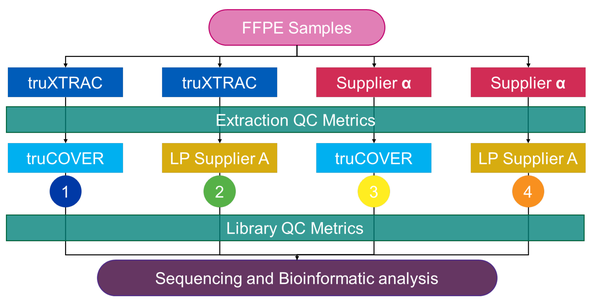

Figure 2. Experimental design for truCOVER and truXTRAC FFPE through WGS solution.

- FFPE libraries were pooled equimolar by mass (Qubit) and sequenced on a NovaSeq® X Plus to 30x coverage.
- Sequencing data was analyzed using the CLC Genomics® Workbench (Qiagen).

Results

Extraction Yield

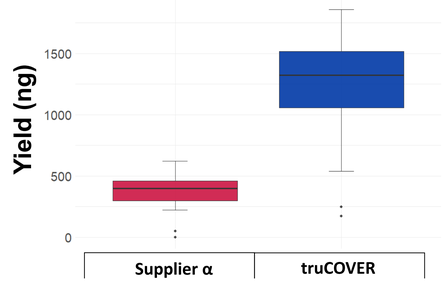

Figure 3. FFPE Extraction yield from Horizon Discovery FFPE samples extracted with truXTRAC and Supplier α.

Result Highlights

- **Improved Extraction Yield**: DNA extracted with truXTRAC is ~3X higher yield compared to Supplier α (***Figure 3***).
- **Maximize Data from FFPE Samples:** Achieves >200 bp Median Insert Size from challenging FFPE samples, delivering more usable data (***Figure 4***).
- **Superior Data Quality Post-Processing:** Retains more high-quality, usable reads after bioinformatic filtering (***Figure 5***).
- **Improved Variant Calling:** The truCOVER (library prep) and truXTRAC (extraction) workflow provides superior INDEL and SNP detection compared to Supplier α (***Figure 6***).

Median Insert Size

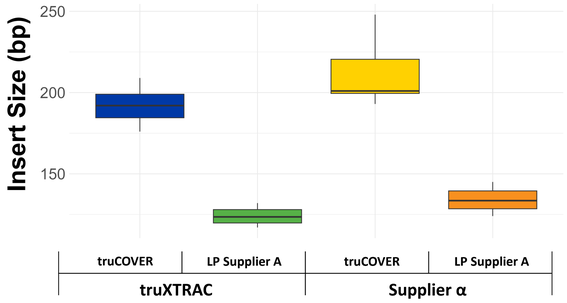

Figure 4. Median insert size of reads from FFPE samples extracted with truXTRAC or Supplier α. Libraries were prepared using either truCOVER or LP Supplier A from 100 ng input with 8 cycles of amplification.

Data Loss Through QC Pipeline

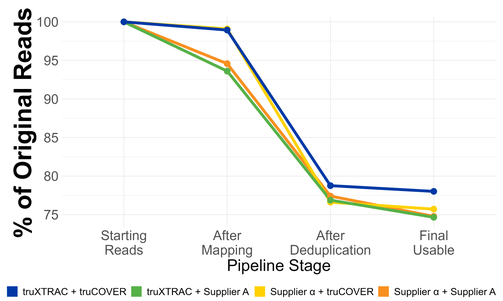

Figure 5. Data loss throughout the pipeline, showing final takeaway of usable data for further analysis and variant calling.

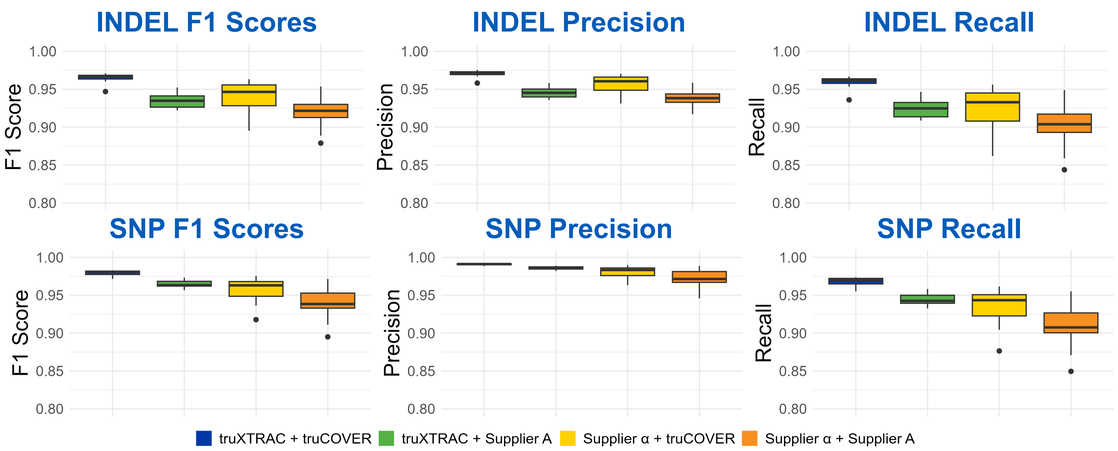

Figure 6. F1 score (harmonic mean of precision and recall), precision, and recall scores for INDELs and SNPs resulting from FFPE samples extracted with truXTRAC and Supplier α extraction with libraries prepared from either truCOVER or LP Supplier A. Libraries were prepared from 100 ng input with 8 cycles of amplification. F1 Analysis performed using hap.py tools from Illumina.

Conclusions

Nucleic Acids Yield

- **Simplified & Scalable Workflow:** truXTRAC and truCOVER offers a fast, modular workflow with minimal manual steps.
- **High Yield:** Nucleic Acids DNA extraction with truXTRAC minimize QNS by ensuring optimal and maximum yield.

Insert Size and Usable Data

- **Superior Data Quality for Deeper Insights:** The truCOVER and truXTRAC workflow provides high-integrity data post-bioinformatics, enabling more robust variant calling analysis.
- **Significantly Higher Read Retention:** Post-bioinformatic QC (merging, trimming, and duplicate marking) ensures more usable data is available for final analysis compared to LP Supplier A.

Variant Calling

- **truXTRAC Extraction Boosts Performance:** Shows higher precision and recall for SNPs and INDELs compared to LP Supplier A.
- **Additive Workflow Delivers Confident Calling:** The benefits of truXTRAC (extraction) and truCOVER (library prep) are additive, combining to achieve a >0.95 F1 Score for confident variant calling from FFPE samples.
- **The combined truXTRAC + truCOVER System:** consistently outperforms other tested workflows (LP Supplier A and extraction supplier α) across all key metrics (F1, precision, and recall) for both SNP and INDEL detection.

References

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US: +1 781.932.3959 customerservice@covaris.com
EU: +44 (0)845 872 0100 emeacustomerservice@covaris.com
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