Active Deparaffinization Drives Superior Yields, Quality and Sequencing Coverage from FFPE Tissue Samples

Tissue samples are commonly preserved by Formalin Fixation and Paraffin Embedding (FFPE) to allow for extended storage. Starting with cores, sections, or slides, paraffin is removed from the FFPE tissue sample by emulsification driven by the very finely controlled and reproducible acoustic energy provided by Covaris Focused-ultrasonicators. The process is highly efficient with the microstreaming resulting from the high frequency acoustic waves generated by Adaptive Focused Acoustics (AFA) stripping paraffin away from the sample. This results not only in effective paraffin removal, but also promotes sample rehydration benefitting both tissue digestion by proteinase K as well as enhancing the dissociation of biomolecules for improved extraction of DNA.

Active paraffin removal for complete tissue rehydration

Tissue handling

FFPE Kidney and uterus tissue blocks were stored at 4°C upon delivery from CHTN, and frozen matched tissue sections were stored at -80°C. Prior to sectioning, excess paraffin from tissue sections was trimmed, and a microtome used to section tissue to 20 µm scrolls. 25 mg of the matched frozen tissue were cut using a scalpel for DNA extraction.

Effective, high yield, and high quality extraction of DNA from FFPE tissues for NGS-based applications is made possible by the use AFA. The focused short acoustic wavelength generated by Covaris AFA not only allows for an active non-organic solvent based paraffin removal, but it also allows for the efficient delivery of proteinase K into the tissue matrix for the effective digestion of proteins and efficient release of DNA. Although higher yield, and better qPCR results from FFPE extracted DNA are metrics used to assess the quality of extracted DNA from FFPE tissues, information gathered from sequencing results provide the best indication of quality. Analysis of the sequencing results from QIAGEN FFPE, Covaris FFPE, and matched fresh frozen samples indicated a clear distinction in quality between the DNA extraction methods.

Whole genome sequencing results from Covaris extracted DNA generated greater coverage depth across the genome, and coverage uniformity similar to that of DNA extracted from fresh frozen tissues. Gene rich regions of the genome seem to lack consistent coverage depth in QIAGEN extracted samples, indicating a bias in the library representation for regions of the chromatin with low abundance of genes.

As the utilization of FFPE extracted DNA in a clinical setting is becoming more prevalent, and the genetic analytic tools more sensitive, FFPE DNA extraction with Covaris is a unique method that matches the sensitivity and analytical requirement of the application.

CONCLUSION

FFPE Kidney and uterus tissue blocks were loaded on the IGV viewer, and the coverage analyzed for chromosome 19. Coverage of >10x is indicated in dark colors, coverage of <10X are indicated in light colors. Chromosomal view of coverage indicates that Covaris extracted DNA quality resembles that of the DNA extracted from fresh frozen tissue.

FFPE,

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There were consistent differences observed in the DNA yields across the different DNA extraction methods. Covaris samples had consistently lower than 10X coverage across the genome as compared to the Covaris samples.

In order to obtain 5 µg of total DNA using the QIAGEN QIAamp FFPE tissue kit, eight 20 µm sections of kidney FFPE, and ten sections of uterus tissue were processed. For Covaris truXTRACT™ FFPE DNA Extraction Kit, two kidney sections, and three uterus sections were processed to achieve a 5 µg yield.

DNA concentration and quality assessment

The concentration of each extracted DNA was determined using the Qubit Quant-It dsDNA BR assay kit using either 5 or 10 µl of the extracted DNA sample. qPCR quantification of the DNA, and quality assessment was carried out using KAPA Human Genomic DNA Quantification and QC kit according to the kit protocol.

96 samples processed in a 96 well microTUBE FFPE rack

- Individually 2D barcoded 8 microTUBE strip
- Rapid active paraffin removal of 96 FFPE samples (<1 hour with LE220 Focused-ultrasonicator)

Fully automatable & scalable vacuum purification

- From bench to handler load without further validation
- Scripts available for liquid handling robots

M-series

- Circular transducer
- Single sample processing
- Integrated temperature control

S-series

- Circular transducer
- Single sample processing

E-series

- Circular transducer
- 1 to 96 sample processing
- Robot integration for full automation

L-series

- Linear transducer – Parallel processing
- Highly uniform acoustic field along a row treats a full 96 plate 8x faster

Focused-ultrasonicators with AFA™

- NIST traceable calibration
- Scalable from single sample to high throughput
- Robust & proven protocols

Quality assessment by qPCR

<table>
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<th>Kidney</th>
<th>Uterus</th>
<th>QIAGEN</th>
<th>Covaris</th>
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<tr>
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<tr>
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<tr>
<td>Fresh</td>
<td>1.5</td>
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</tbody>
</table>

Quality assessment by qPCR

- Normalized yields of DNA extracted from FFPE kidney and uterus samples.
- DNA quantification was carried out by qPCR using KAPA Human Genomic DNA Quantification. Results displayed are for the 4-10K amplicon.

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