Active paraffin removal for complete tissue rehydration

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 benefiting both tissue digestion by protease K as well as enhancing the dissociation of biomolecules for improved extraction of DNA and RNA.

Automation ready – seamless integration with NGS workflow

- 96 samples processed in a 384 format microTUBE FFPE rack
- Individually 20 bar coded microTUBE strips
- Rigid active paraffin removal for FFPE samples
- (<1 hour with LE220 Focused-ultrasonicator)

High DNA and RNA extraction yield and quality

DNA/RNA quantity normalization

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 FFPE extraction, ten sections, and three uterus sections were processed to achieve a 5 µg yield. RNA from six kidney sections were extracted using QIAGEN RNAeasy kit, and 4 sections using Covaris truXtRAC™ FFPE RNA kit to achieve 5 µg total yield. A similar number of uterus sections were processed to obtain 5 µg of RNA for RNA-Seq DNA/RNA concentration and quality assessment

The concentration of each extracted DNA was determined using the Quanti-Fluor™ dNDA BR assay kit using either 5 or 10 µl of the extracted DNA sample. RNA quantitation was carried out using Qubit™ RNA BR assay. qPCR quantitation of the DNA, and quality assessment was carried out using KAPA Human Genomic DNA Quantification and QC kit according to the kit protocol. qPCR analysis of RNA was performed using a SYBR Green assay and house designed primers for amplification of 46bp and 191bp regions. human beta actin. DNA/RNA quantities and quality of total RNA were assessed using the Qubit™ assay.

The results clearly indicate that for the two tissues tested up to several fold more amplifiable RNA is recovered using the Covaris method for both the long and the short amplicon regions.

CONCLUSION

High yield, and high quality extraction of nucleic acids from FFPE tissues for NGS based applications is made possible by the use of 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 NGS-quality nucleic acids.

Although higher yield, and better qPCR results from FFPE extracted nucleic acids 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/RNA extraction methods.

Whole genome sequencing results from Covaris extracted FFPE 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. Covaris extracted RNA from two different FFPE tissue types indicated consistent coverage of the transcriptome comparable to RNA from matched fresh frozen tissue. Coverage greater than 2x was not observed for all tissue specific transcripts for both tissue types tested. These results are consistent with the findings that longer total RNA fragments are recovered and more amplifiable or “usable” total RNA is extracted using the Covaris FFPE method. As the utilization of FFPE extracted nuclear acids in a clinical setting is becoming more prevalent, and the genetic analytic tools more sensitive, DNA and RNA extraction with Covaris is a unique method that matches the sensitivity and analytical requirement of the application.

REFERENCES

1. FFPE Tissue Blocks and matched fresh frozen tissues were obtained from Theresa Kakumanu, PhD and Diane McGannery, Cooperative Human Tissue Network (CHTN), Eastern Division, University of Pennsylvania

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