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APPLICATION NOTE

Workflow for FFPE-derived DNA shearing prior to Next Generation Sequencing

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Formalin fixation and paraffin embedding (FFPE) is a standard method for long-term preservation of most archived pathological specimens. Archival FFPE samples provide an invaluable repository of information for genetic analysis. These samples can be analyzed by next-generation sequencing for applications including biomarker discovery, drug development, and cancer research. However, DNA from archival tissues is challenging to use in NGS studies due to insufficient quality, including degradation, chemical modifications and the presence of single-stranded molecules.

DNA should be precisely fragmented prior to NGS library preparation. The size of the DNA fragments is a key parameter for successful library preparation and reliable sequencing data. The Diagenode Bioruptor® Pico ensures that the DNA is efficiently sheared to an appropriate and consistent fragment size. The successful FFPE-derived DNA shearing relies on the initial quality and size assessment of extracted DNA. It thus becomes critical to assess the size and the amount of DNA extracted from FFPE samples before proceeding to the shearing and the library preparation.

This application note describes the workflow (Figure 1) for FFPE DNA preparation and qualification prior to Next Generation Sequencing using the Diagenode FFPE deparaffinization and extraction method, the Bioruptor Pico for precise DNA shearing, and the Fragment Analyzer™ Automated CE System from AATI for DNA sizing.

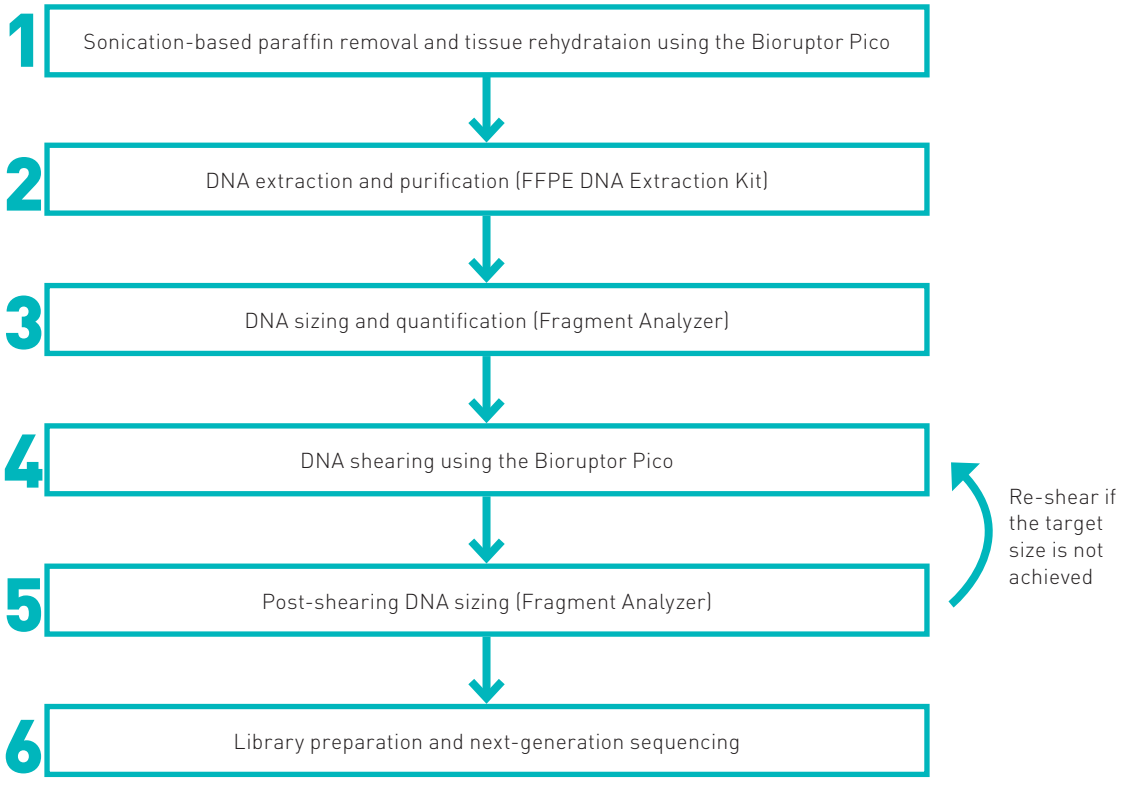

Figure 1. Workflow for FFPE DNA preparation and qualification prior to NGS

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STEP 1 and 2. Sonication-based paraffin removal and DNA purification.

FFPE samples were deparaffinized using a solvent-free sonication-based approach https://www.diagenode.com/files/protocols/bioruptor-deparaffinization.pdf. DNA was extracted and purified using the Diagenode FFPE DNA Extraction Kit (Cat. No. C20000030) which contains optimized reagents that are added directly to the FFPE samples to remove paraffin, digest tissues, and purify DNA with high yields and low sample degradation. Sonication-based deparaffinization eliminates laborious protocols using organic solvents. It allows removing paraffin and rehydrating tissue in just one step (Figure 2). The protocol preserves DNA integrity by using a mild cross-link reversal process with no high temperatures.

Figure 2. Efficient deparaffinization of FFPE sections by sonication using the Bioruptor Pico.
10 µm sections were sonicated for 3 cycles (30 sec ON/OFF at RT) with the Bioruptor Pico. The paraffin has been emulsified and completely dissociated from the tissue section.

STEP 3. DNA sizing and quantification (Fragment Analyzer)

The size and the amount of DNA extracted from FFPE have to be assessed at this step in order to ensure successful shearing for library preparation. Each sample should be assessed individually because the quality of DNA from FFPE samples varies (Figure 3, DNA size assessment prior sonication). The Fragment Analyzer™ Automated CE System together with the High Sensitivity Large Fragment Analysis Kit from AATI can be used for the automated sizing and quantification of large DNA fragments and smears of FFPE-derived DNA.

STEP 4. DNA shearing using the Bioruptor Pico

Extracted DNA is fragmented to the desired size using the Bioruptor Pico (B01060001), 0.2 ml Bioruptor microtubes (Cat. No. C30010020) and the corresponding holder (Cat. No. B0120004). Based on the size and the concentration of the extracted DNA (step 3), choose the appropriate sonication settings.

If extracted DNA is of high molecular weight above 10 kb, the standard DNA shearing protocol should be applied https://www.diagenode.com/files/protocols/protocol-dna-shearing-tubes-02ml.pdf. Smaller DNA fragments are more resistant to sonication and the total sonication time should be extended by additional cycles to reach the target size. Time course monitoring after 5, 10, 15 additional cycles is recommended. Re-shear sample in 5 cycle increments until desired size is reached. If target length is still not achieved, shear an additional 5 cycles. Do not shear more than an additional 15 cycles.

Example: The standard protocol recommends sonicating for 15 cycles 30" ON/30"OFF for a target size of 200 bp. When starting with degraded DNA (fragment sizes below 10 kb), the sample should be re-sheared in 5 cycle increments until desired size is reached. For example, if target length is 200 bp, shear by adding 5 cycles to the recommended number of cycles from https://pybrevet.typeform.com/to/o8cQfM and assess the size. If target length is still not achieved, shear an additional 5 cycles 30" ON/30"OFF. It is recommended to monitor the shearing efficiency after each round of 5 cycles to avoid over-sonication.

Step 5. Post-shearing DNA sizing (Fragment Analyzer)

Perform post-sonication DNA sizing to confirm that fragment length is in optimal range for library preparation (Figure 3). The final target size depends on the platform and protocol used, e.g. exome sequencing protocols require DNA insert lengths of 180-200 bp.

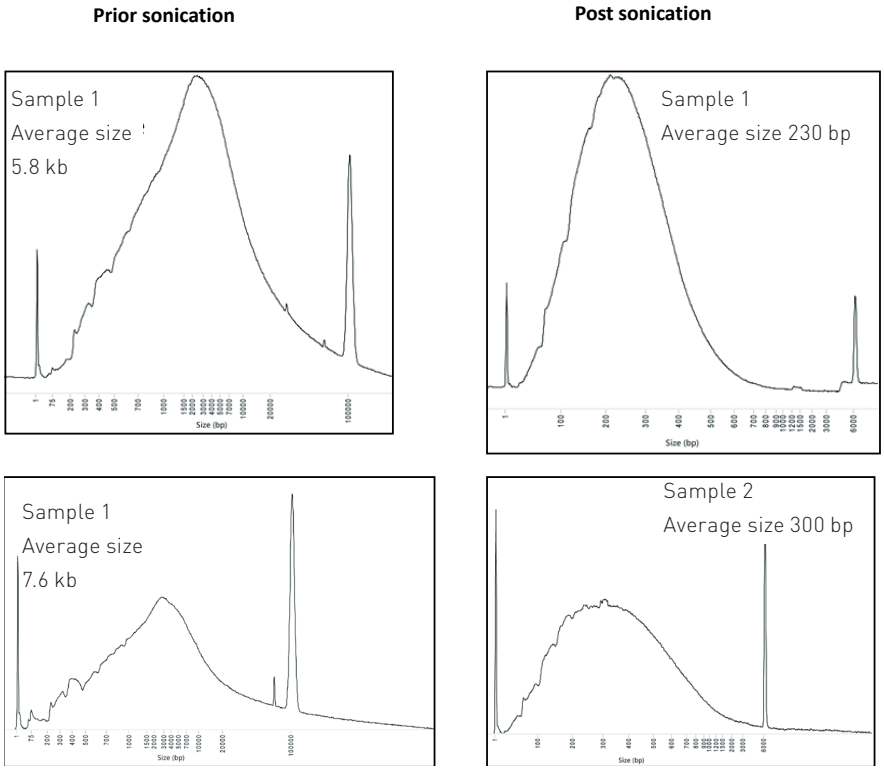

Figure 3. DNA from FFPE samples (human breast cancer, 4 year storage) was extracted using the Diagenode FFPE DNA Extraction Kit (Cat. No. C20000030). Isolated DNA was analyzed with the Fragment Analyzer and the DNA size was recorded. Samples were sheared using the Bioruptor Pico, 0.2 ml Bioruptor microtubes, and the corresponding holder for 30 cycles according to the initial size (less than 10 kb). Post-shearing fragmentation was assessed using the Fragment Analyzer.

Conclusion

The Bioruptor Pico was successfully used for FFPE-derived DNA shearing prior to NGS library preparation.