HIGH MOLECULAR WEIGHT DNA FOR LONG-READ SEQUENCING
Laying the groundwork for third-generation sequencing

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Introduction

- DNA sequencing has become an important tool in diagnostic pathology. Starting with Sanger sequencing in 1977 followed by the “Next Generation Sequencing (NGS) technologies”.
- Short-read NGS features sequencing of short fragments of clonally amplified DNA molecules (100-300 bp).
- Recent technology allowing long-read sequencing is emerging, generating sequences of >10 kb of native DNA.
- The most common clinical samples sent for molecular genetics are formalin-fixed paraffin-embedded (FFPE) tissue. DNA quantity is adequate. However, the DNA is usually fragmented and of lower quality than non-FFPE samples.

Methods & Objectives

- A total of 38 samples were selected, 13 FFPE and 25 non-FFPE.
- Non-FFPE samples included variable preservation methods, such as smears from fresh tissue in 95% ethanol (8/25), previously stained cytology smears (8/25), OCT-embedded fresh tissue (5/25), and FNA samples collected in RNALater (4/25).
- DNA extractions were performed using Maxwell® nucleic acid extraction kits (DNA FFPE Plus) and Genexus™ multisample DNA purification kit.
- DNA quantity and quality were assessed using Qubit fluorometric quantification and the Agilent Tape Station System.
- We assessed the quantity and quality of DNA extracted from FFPE and non-FFPE samples.

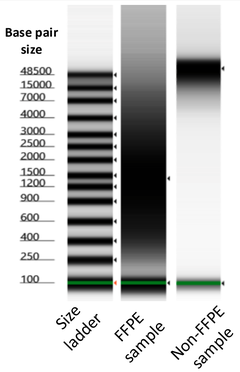


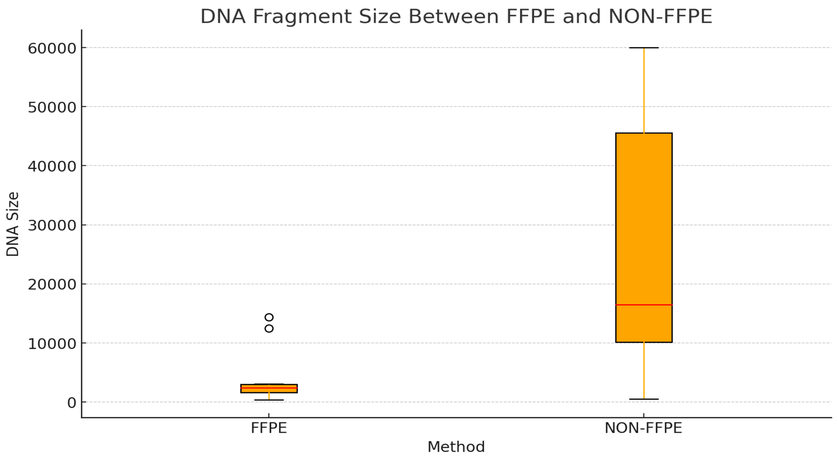
Figure 1. Agilent TapeStation system. Automated electrophoresis showing DNA quality based on base pair size. FFPE sample appears smeared given the fragmented condition of the DNA. The non-FFPE sample appears as a demarcation line beyond the upper size ladder limit.

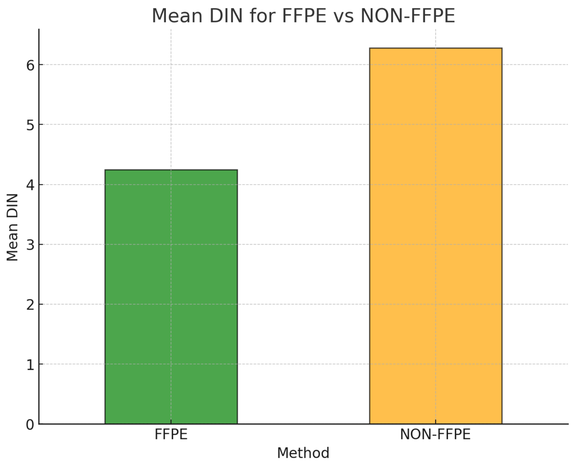
Results

- The FFPE DNA samples showed a mean quantity of 140.48 ng/µL. **The average DNA Integrity Number (DIN) for FFPE samples was 4.2.** The fragment size of FFPE samples ranged from 364 to 14,352 base pairs (bp), with an average of **3,645 bp**.

- In contrast, the non-FFPE DNA samples demonstrated a mean quantity of 58.7 ng/µL **(p 0.0165). The average DIN for non-FFPE samples was 6.2 (p 0.0034).** The fragment size for non-FFPE samples ranged from 504 to 60,000 bp, with an average of **26,079 bp (p<0.0001).**

- Of the 25 non-FFPE samples only one case showed fragment sizes of <1kb. 17 out of 25 non-FFPE samples, showed fragments of DNA of >10kb. 2 out of 13 FFPE samples, showed fragments of DNA of >10kb and <20kb.





Conclusions

- Long-read sequencing offers several advantages, including: detection of larger structural variants, methylation detection, haplotype phasing and transcriptome sequencing.
- To fully leverage these features, abundant DNA of high molecular weight is required. While FFPE specimens can be used for long-read sequencing, the lower quality and smaller DNA fragments can limit the detection of larger molecular alterations.
- Non-FFPE specimens, such as cytology smears, fresh OCT-frozen tissue sections or cells collected via FNA into preservative media, typically exhibit a higher quality and average DNA size compared to FFPE specimens, making these specimen types ideal for long-read sequencing.

References & Acknowledgements

We have no conflict of interest to disclose.

Warburton PE, Sebra RP. Long-Read DNA Sequencing: Recent Advances and Remaining Challenges. Annu Rev Genomics Hum Genet. 2023