FFPE Tissue Samples and the Significance of Its Nucleic Acid Quality and Quantity

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FFPE tissue samples have been widely used for disease research, including diagnosis and drug treatment studies. However, it is often difficult to extract nucleic acids of sufficient quality for downstream applications from this highly valuable source of patient samples. A number of factors are known to affect both the quality and quantity of DNA/RNA extracted from FFPE tissues. This paper aims to discuss many of these factors and also to provide suggestions for maximizing the quality of the extracts.

Introduction

A plethora of biological tissue samples from patients have been used in medical research in the hopes of curing all kinds of diseases. In particular, Formalin-Fixed Paraffin-Embedded (FFPE) tissue samples have been studied most extensively because majority of clinical tissue samples are in this format for stability and long-term storage considerations. Due to their stability and long-term storage, Formalin-Fixed Paraffin-Embedded (FFPE) tissues were mainly used by pathologists to observe tissue morphology in the diagnosis of disease and in immunohistochemistry (IHC) to find protein biomarkers that may be applied to finding appropriate drug treatments. However, with the advent of qPCR and NGS technologies in life science research, scientists have begun obtaining more genetic information from FFPE samples and applied the information for patients' treatment and diagnosis purposes. To successfully collect the right genetic information, isolating good quality FFPE DNA and RNA with high yields is critical. The following conditions had been investigated aspects to this have been studied:

1. FFPE sample age. Since FFPE samples can be stored for well over ten years, there are tens of thousands of very old archived FFPE samples available from clinical resources. Most scientists, however, consider blocks of old age to be of inferior quality as compared to freshly collected samples. Therefore, many research projects require FFPE samples to be collected and prepared within the past 5 years or an even more recent time frame. Interestingly, this assumption might not be accurate. 100% correct since we were able to extract good quality DNA/RNA from thyroid tumors that were well over 20 years old, whereas we failed to isolate similarly good quality DNA/RNA from brain tumor samples that were less than 5 years old. This observation indicates that many other aspects are involved.

2. Fixative and fixation time. In general, most FFPE samples are fixed with 10% NBF (Neutral buffered formalin), but there are other fixatives available for various purposes. Because these fixatives can cause DNA/RNA to cross-link, they will indeed affect the quality of nucleic acids isolated from FFPE samples (Singh et. al., 2020). To maintain the best morphology, the tissue should be immediately placed into the fixative upon dissection (biopsy/resection). However, this time frame may also vary due to many different situations that may occur. In addition, the ideal fixation time is between 4 hours to 24 hours based on the size of the tissue. Variations in fixation time may also be another aspect affecting the DNA/RNA quality (Figure 1).

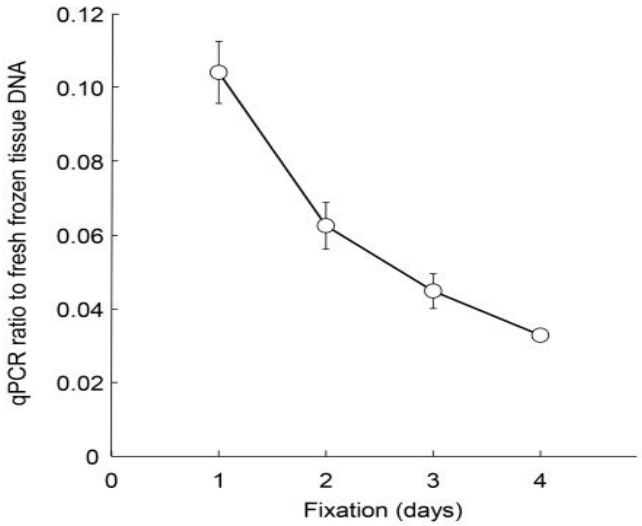

Figure 1. FFPE DNA quality is reduced with increasing formalin fixation times. In this study, matched sets of FFPE DNA samples from rat liver were compared to DNA obtained from fresh frozen tissues using qPCR. The average CT ratio of FFPE to frozen DNA is denoted by a circle and the standard deviation by an error bar. A lower ratio represents poorer quality FFPE DNA. (Einaga et. al., 2017)

3. Tissue type. We have observed that the DNA quality from FFPE samples is tissue-dependent (McDonough et. al., 2019). For example, the percentage of good quality lung tumor FFPE DNA samples for a successful NGS experiment from Illumina's TruSeq 48 gene panel is only about 30% while the rates for colon and breast cancer samples were much higher. However, we do not know the exact reasons for this difference. Also, some tissues such as adipose and normal breast which also contain large amounts of adipose, may result in very low yields of DNA due to the nature of these tissue types.

4. Extraction methods. Various commercial FFPE DNA/RNA extraction kits are available from different vendors. Qiagen's AllPrep and Thermofisher's RecoverAll™ are perhaps the two most popular kits that can simultaneously extract both DNA and RNA from the same sample. For automation platforms, Thermofisher's MagMAX™ and MagJET kits may be applied on their Kingfisher platform, and Promega's Maxwell has its own FFPE DNA/RNA extraction kits. BioChain's AnaPrep automation platform's FFPE DNA extraction kit also performs very well and is recognized by several core facilities for NGS services (Figure 2). Nevertheless, the FFPE DNA/RNA quality and yield vary between different extraction methods, and it is difficult to indicate which one performs better.

5. Tissue size and yield. The yield is also very important for downstream applications. In general, extractions need roughly 5 to 8 curls of tissue sections at 10µm thickness, but the yield varies due to differences in tissue size and type. Lower yields may still be sufficient for qPCR but may not be enough for NGS.

6. IRB Approved. BioChain has established an Institutional Review Board (IRB) compliant to 45 CFR part 46, 21 CFR part 56. The IRB is registered with the Office for Human Research Protections (OHRP) with the registration number of IRB00008283. Good Clinical Practice includes approval of the protocol by the IRB, the informed consent form for donors or participants, confidentiality/privacy of related information, and quality assurance in the process.

QC and Repair

Comparing with the DNA/RNA isolated from fresh frozen tissues or cell lines, FFPE DNA/RNA's quality should be compromised significantly due to the aforementioned variations (Figure 3). Furthermore, we can consider FFPE DNA/RNA as degraded to certain levels. To determine just how much degradation is present, two pair of primers can be designed for a house keeping gene. One pair is for short amplicons and the other pair is for long amplicons. The scale of DNA degradation may then be determined by using qPCR and comparing the ΔCT of the two pairs of primer. Higher ΔCT means DNA are degraded into more smaller DNA fragments than longer fragments. Various products are available on the market using this type of QC in preparation for NGS library preparation or qPCR kits to determine if the FFPE DNA/RNA can be used for their application. Certain biotech companies, such as New England Bio., have also developed FFPE DNA repair reagents that may salvage poor quality FFPE DNA/RNA. An example of this would be the NEBNext® FFPE DNA Repair Mix.

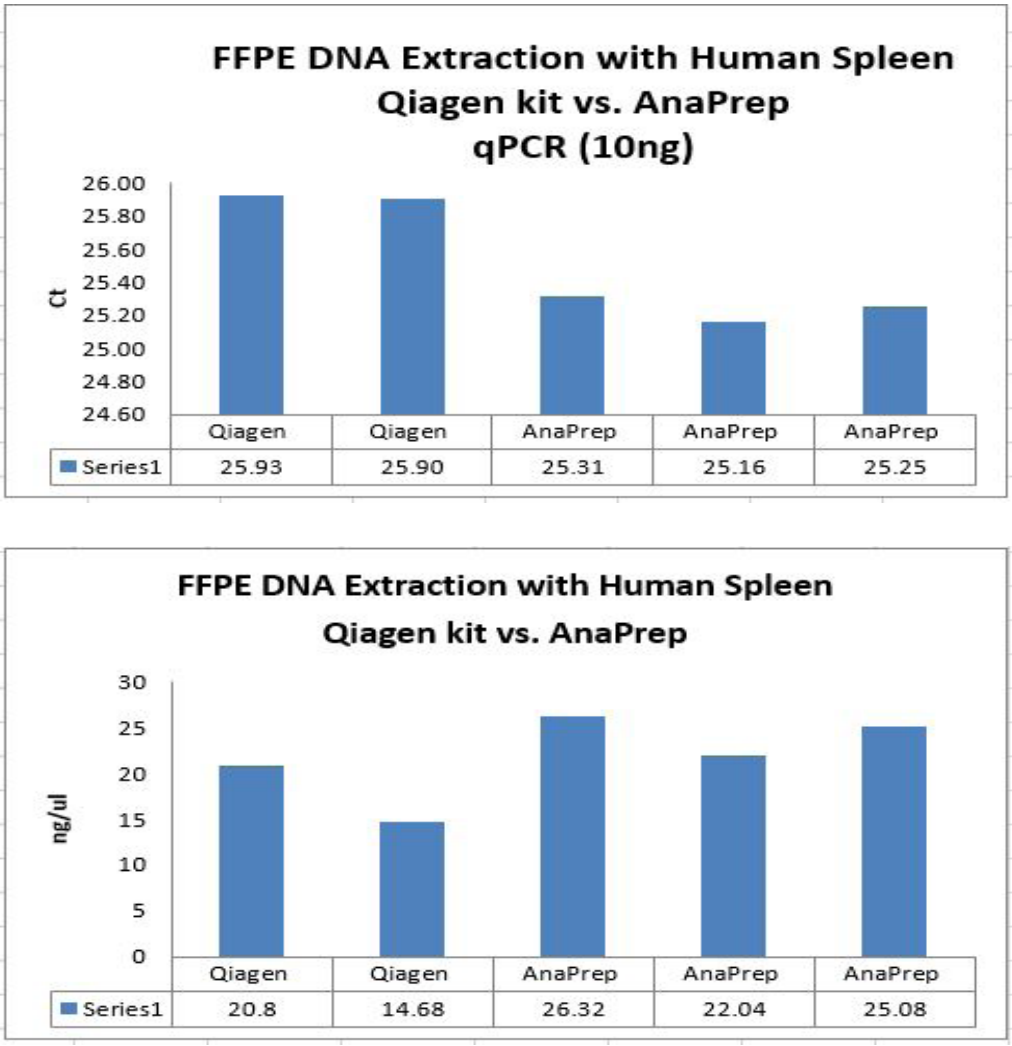

Figure 2. BioChain's AnaPrep FFPE DNA Extraction kit extracts DNA from FFPE samples with yields as good as or better than the competition. (A) Six human spleen sections were extracted using Qiagen's kit in duplicate and BioChain's kit in triplicate. The concentrations of the final elution were measured using Qubit. (B) 10 ng of each elution were used as template with β-actin as target in qPCR. A lower CT value reflects better quality FFPE DNA. Therefore, it is difficult to ascertain the reason why some FFPE samples do not yield good quality FFPE DNA/RNA.

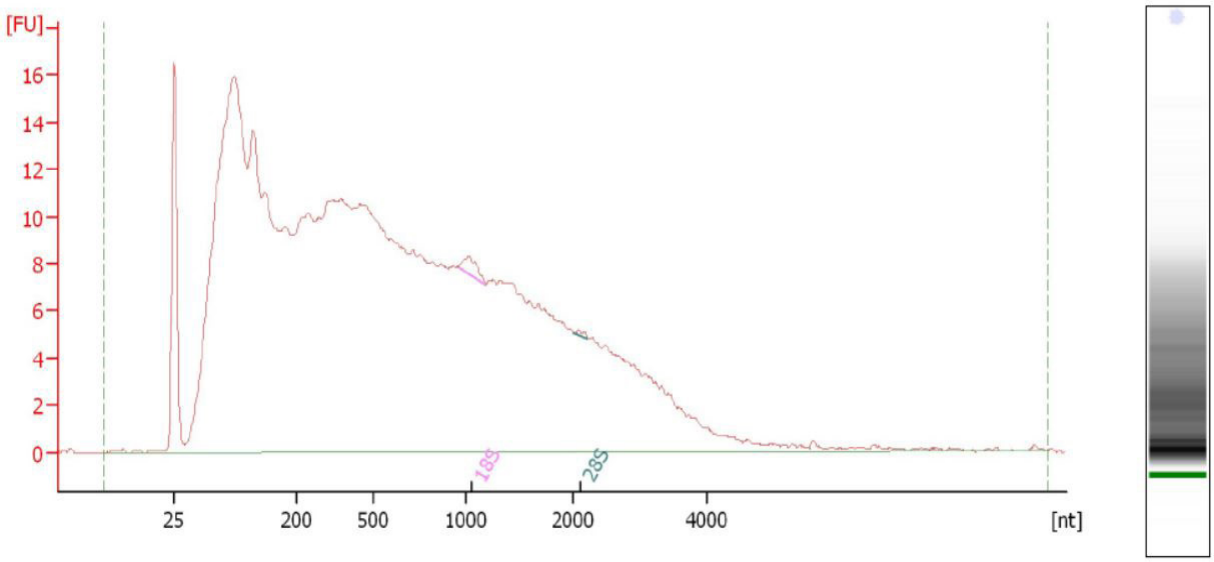

Figure 3. A BioAnalyzer image showing the profile of FFPE RNA from a human liver sample. Compared to total RNA extracted from frozen tissue, FFPE RNA exhibits a broad smear with a significant percentage of the product at <200 nt.

Summary

Various aspects may affect FFPE DNA/RNA quality and quantity. Therefore, it is difficult to ascertain the reason acceptable FFPE DNA/RNA could not be obtained from a certain FFPE sample. In this case, a thorough investigation should be initiated as follows:

1. Select several tissue types.
2. For each type, choose different times between dissection and fixation.
3. Purchase a few popular fixatives.
4. For each fixative, set up several fixing times.
5. Choose 2-3 different areas and sizes from the tissue.
6. Make 10 blocks at each condition by following the same procedure after fixation.
7. Isolate FFPE DNA/RNA from all blocks using different extraction kits every 2-3 years.
8. Determine the DNA/RNA quality and quantity from all samples mentioned above.

The investigation stipulated above is a long-term project, and to our knowledge, no one has yet undertaken such a project (or no publication has yet demonstrated and established the relationship between various FFPE quality to DNA/RNA quality). Thus, most scientists can only request FFPE samples from common sense assumptions or based on previous experiences. For example, if they want to have good quality FFPE DNA/RNA, they would like to have FFPE blocks collected within 5 years or less. However, for the scientists who are developing DNA/RNA repair reagents, they may want to purchase much older blocks instead and demonstrate that their reagents can repair poor quality DNA/RNA from very old samples.

BioChain has been providing a vast number of FFPE samples for more than 25 years, and we are also the main provider for extracted FFPE DNA/RNA samples. Many customers have used BioChain's FFPE DNA/RNA successfully in their project and have come back as repeat buyers. In addition, BioChain has incorporated their customers' feedback and dedicated itself to constant improvement of this product type. For a list of available FFPE samples for purchase and to further learn how these samples will help your research, please visit our website at https://www.biochain.com.

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