The DNA Integrity Number (DIN) Provided by the Agilent 2200 TapeStation System is an Ideal Tool to Optimize FFPE Extraction

Application Note

Nucleic Acid Analysis

Authors

Christoph Kirsch
MACHEREY-NAGEL GmbH & Co. KG,
Düren, Germany

Eva Schmidt
Agilent Technologies, Inc.
Waldbronn, Germany

Introduction

One of the most widely used methods for clinical sample preservation and archiving is the preparation of formalin-fixed paraffin-embedded (FFPE) tissue. Thin sections of FFPE blocks are commonly subjected to histopathology with the remaining sample being archived. These FFPE tissue archives represent a valuable source for retrospective studies of gene expression and mutation analysis. However, DNA extraction from FFPE samples has proved to be challenging[1]. The most common issues are cross-linking and fragmentation of DNA during tissue processing and storage. Formalin, used for tissue fixation, causes cross-linking of nucleic acids and proteins, increasing the susceptibility of DNA to mechanical stress and decreasing the accessibility for enzymes. In addition, formalin may be oxidized to formic acid, which causes DNA depurination and DNA strand breaks. Therefore, the fixation conditions can significantly impact the quality of the extracted DNA[2]. In addition, the DNA extraction method is crucial for DNA integrity and the performance of downstream applications such as PCR[3]. Standard DNA isolation procedures often result in low amounts of suitable DNA. Therefore, specially tailored procedures were developed to extract DNA from FFPE tissue blocks. These procedures include steps to release the DNA from the FFPE tissue, but also to reverse the formalin cross-linking and, therefore, improve yield as well as DNA performance in downstream assays.

This Application Note highlights the utility of the DNA Integrity Number (DIN) for the comparative quality determination of gDNA extracted from FFPE tissue. Three different, commercially available FFPE DNA extraction kits using standard Proteinase K lysis conditions and an extended overnight lysis (Table 1) were used for gDNA extraction. The concentration and the quality of the obtained DNA samples was determined with the Agilent 2200 TapeStation system and the Genomic DNA ScreenTape assay. As a numerical measure of DNA integrity, the DIN was automatically determined for these extracted samples using the TapeStation Analysis Software (revision A01.05), where it is displayed directly under the gel image as well as in the sample table. The numerical assessment ranges from 1–10. A high DIN indicates highly intact gDNA, and a low DIN a degraded gDNA[4].

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Table 1. Overview of DNA extraction conditions

FFPE DNA extraction kit	Standard protocol	Extended lysis
A	3 hours at RT	Overnight at RT
B	1 hour at 56 °C	Overnight at 56 °C
C	1 hour at 56 °C	Overnight at 56 °C

Experimental

Materials

FFPE samples, mouse liver (~10-µm sections/0.4 × 0.9 cm), mouse spleen (~20-µm sections/ 0.4 × 0.1 cm), and mouse heart (~10-µm sections/0.5 × 0.7 cm) were kindly provided by MACHEREY-NAGEL GmbH & Co. KG (Düren, Germany). Three commercially available FFPE DNA extraction kits from different manufacturers were used (labelled here as A, B, and C). The 2200 TapeStation system (G2965AA) with the TapeStation Analysis Software (revision A01.05), Genomic DNA ScreenTape consumables (5067-5365) and Genomic DNA Reagents (5067-5366) were obtained from Agilent Technologies (Waldbronn, Germany).

DNA extraction from FFPE samples

Three DNA extraction kits were used according to the manufacturer's instructions following the standard protocol to extract DNA from the FFPE samples. Four sections of the FFPE blocks were used per kit. In addition to the standard protocol, sections from the same FFPE blocks were processed using an extended lysis protocol (Table 1).

Genomic DNA analysis with the Agilent 2200 TapeStation system

DNA analysis was performed according to the instructions[5]. In brief, 1 µL of gDNA was mixed with 10 µL of Genomic DNA Sample buffer. The Genomic DNA ScreenTape consumable, filtered loading tips, and the prepared samples were placed in the 2200 TapeStation instrument. The 2200 TapeStation system loaded, electrophoresed, imaged, and presented digitally analyzed results in less than 2 minutes per sample.

Results and Discussion

To compare the effect of DNA extraction method and the influence of tissue type on the quantity and quality of the extracted DNA, three different mouse tissues (heart, liver, and spleen) extracted by three commercially available DNA extraction kits were investigated. FFPE blocks were prepared under identical conditions to exclude any effects due to formalin fixation and paraffin embedding. All three extraction kits stated an optional longer incubation time with Proteinase K as a method to increase DNA yield. Together with the outlined standard conditions, additional sections from the same FFPE blocks were processed using an extended lysis protocol with overnight Proteinase K incubation (Table 1).

Figure 1 summarizes the results obtained for the DNA extracted from mouse spleen FFPE tissue using three different DNA extraction kits. The electropherograms obtained when analyzing gDNA extracted with the DNA extraction kit A showed the biggest difference between standard and overnight lysis protocols. There was almost no difference between the standard and overnight lysis protocol for DNA extraction kit B.

Comparing the electropherograms from all three DNA extraction kits highlights obvious differences in the overall profiles (Figure 1). The analysis of the DNA samples extracted with kit A resulted in electropherograms with a well-defined, prominent gDNA peak with some fragmented DNA quality, as reflected by the higher DIN. In contrast, DNA extracted with kit C migrated as a broadly distributed smear with lower gDNA integrity. The characteristic of broadly distributed smears is associated with insufficient DNA cross-linking removal during the extraction procedure, and is a known issue when preparing DNA from FFPE tissue[2].

Figure 2 compares the DNA concentrations obtained after DNA extraction with the three different kits for heart, liver, and spleen FFPE tissues. It is expected that the DNA yield is relatively low for FFPE tissue compared to fresh tissue or cells. DNA extracted from heart tissue in particular resulted in very low DNA amounts. DNA extraction from fibrous tissue, such as heart, is more challenging compared to other tissue. Nucleic acid and nuclease-rich tissues, such as spleen, are also known to be difficult for nucleic acid extraction, but, in this case, gave good results.

As shown in Figure 2, the DNA yield depended on the tissue, the DNA extraction method, and the lysis conditions. For mouse heart tissue, the measured DNA concentrations were below the quantitation range of the Genomic DNA ScreenTape assay for all three DNA extraction kits. For mouse liver tissue, the highest yields were achieved with DNA extraction kits A and B. For mouse spleen tissue, DNA extraction kits A and B with extended overnight lysis provided the highest yields. This clearly indicates that to achieve high DNA yields, it is required to optimize the DNA extraction for each respective tissue type for incubation time and amount of required starting material.

A highly efficient extraction of DNA based solely on quantity may be misleading if the DNA is highly degraded. Therefore, the samples were also measured for DNA integrity with the automatically determined DIN (Figure 3).

The highest DNA integrity, independent of the tissue, was obtained when using DNA extraction kit A with extended overnight lysis. DNA extraction kit B also resulted in good DNA integrity, and showed no difference for liver and spleen tissue when comparing the standard and the extended lysis protocol. For the DNA extracted from heart FFPE tissue, the DNA concentration was below the quantitation range of the Genomic DNA ScreenTape assay (10 ng/µL) and the majority of samples were below the functional quantitative range for DIN (5 ng/µL). For DNA concentrations below 3 ng/µL, no DIN is determined by the software[4].

The extended Proteinase K lysis did slightly improve the DNA integrity for DNA extraction kit A, however, it seemed to have no effect when using DNA extraction kit B, and it decreased the DNA integrity for DNA extraction kit C for liver and spleen tissue.

In summary, to optimize DNA extraction it is important not only to consider the DNA yield, but also the DNA integrity to best assess overall DNA quality.

Conclusion

The presented data show that tissue type can have a major influence on the extraction efficiency from FFPE tissues. In addition, DNA extraction protocol and lysis conditions affect the DNA yield and sample integrity and, thus, overall DNA quality. Therefore, depending on the requirements of the downstream application, it may be required to optimize the DNA extraction procedure for highest yield or highest DNA integrity. This Application Note demonstrates that the Agilent 2200 TapeStation system with the Genomic DNA ScreenTape assay and DIN provides a valuable and reliable tool for DNA extraction quality control, with quantification determination and automated sample integrity assessment. The DIN is presented automatically, and, thus, does not require a subjective integrity estimation or approximation based on user experience. Furthermore, DIN determination can help simplify inter-lab DNA integrity comparison.

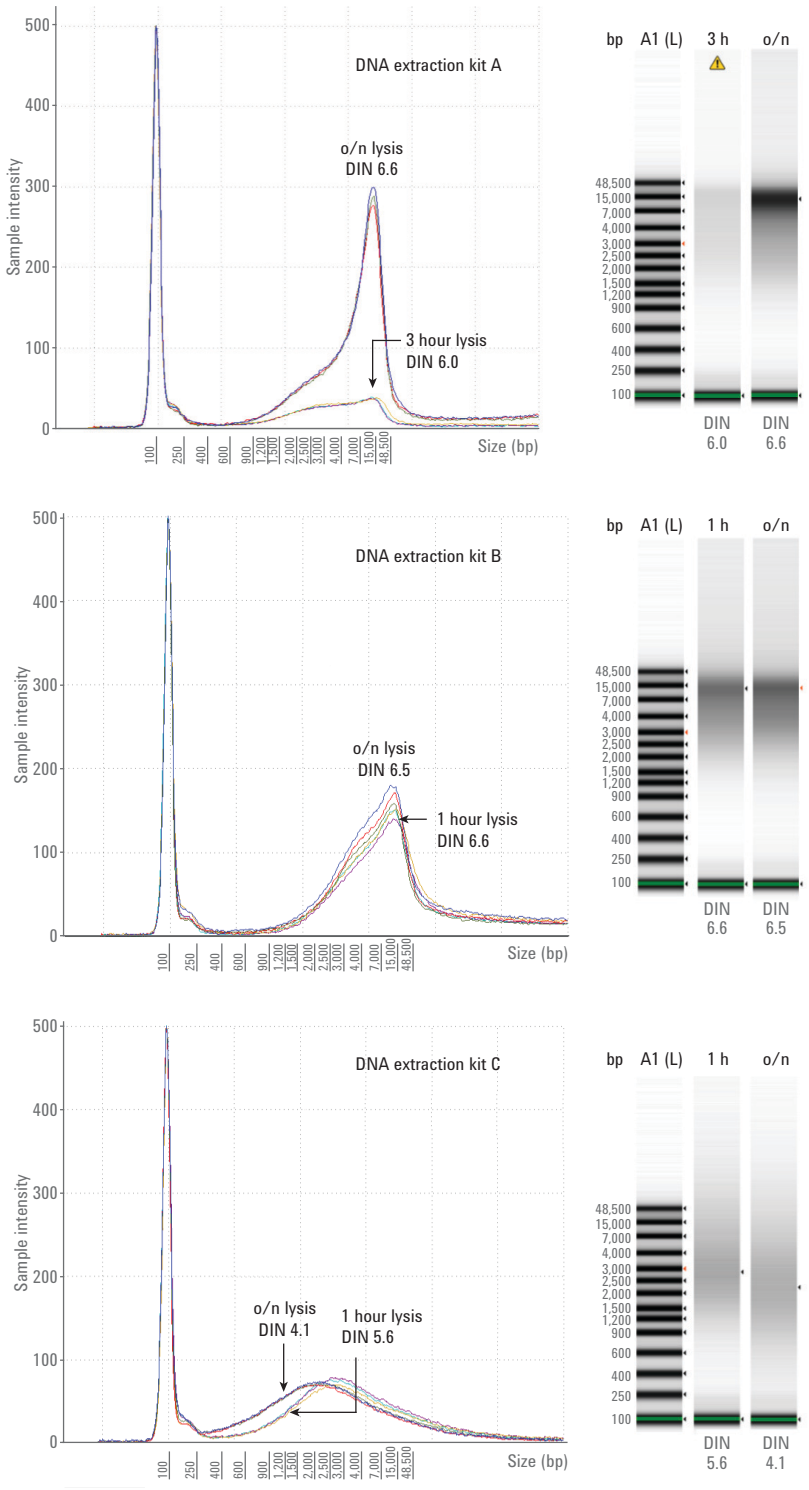

Figure 1. DNA extracted from mouse spleen FFPE tissue using three different DNA extraction kits. The six electropherograms obtained after analysis with the Agilent 2200 TapeStation system and Genomic DNA ScreenTape assay are overlaid and the average DIN (n = 3) is shown. The gel image shows a representative sample for standard lysis and extended lysis. For samples outside the quantitative specification range of the Genomic DNA ScreenTape assay (10–100 ng/µL) a yellow alert icon is presented in the gel image.

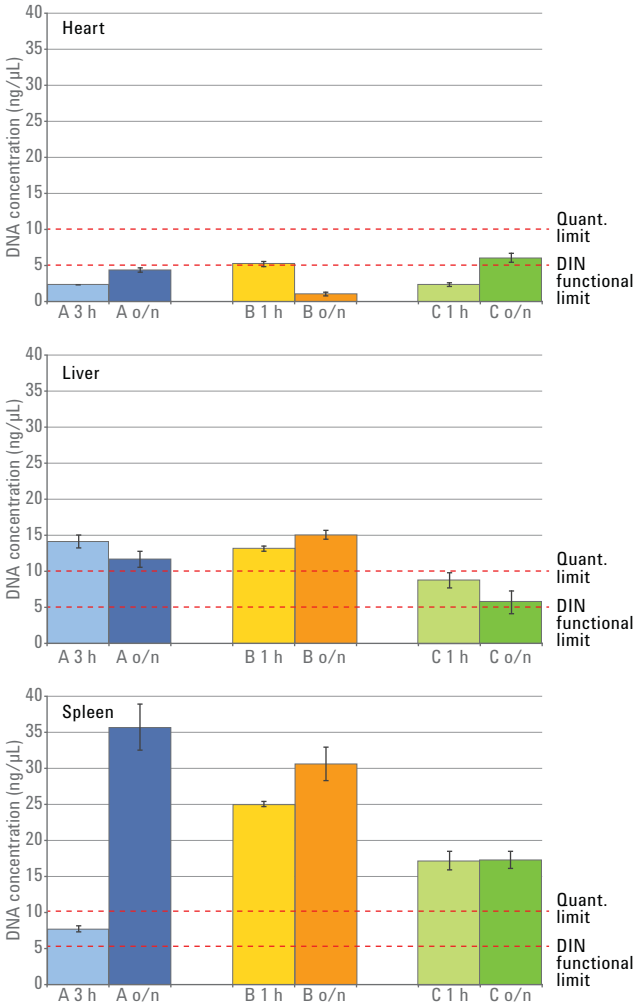

Figure 2. The DNA concentration was determined with the Agilent 2200 TapeStation system and the Genomic DNA ScreenTape assay for the DNA samples extracted from FFPE tissue from mouse heart, liver, and spleen using the three DNA extraction kits A, B, and C with the standard protocol and the extended lysis protocol (n = 3 for each data point). The lower quantitation limit of the Genomic DNA assay (10 ng/µL) and DIN functional quantitative limit (5 ng/µL) are indicated by red lines.

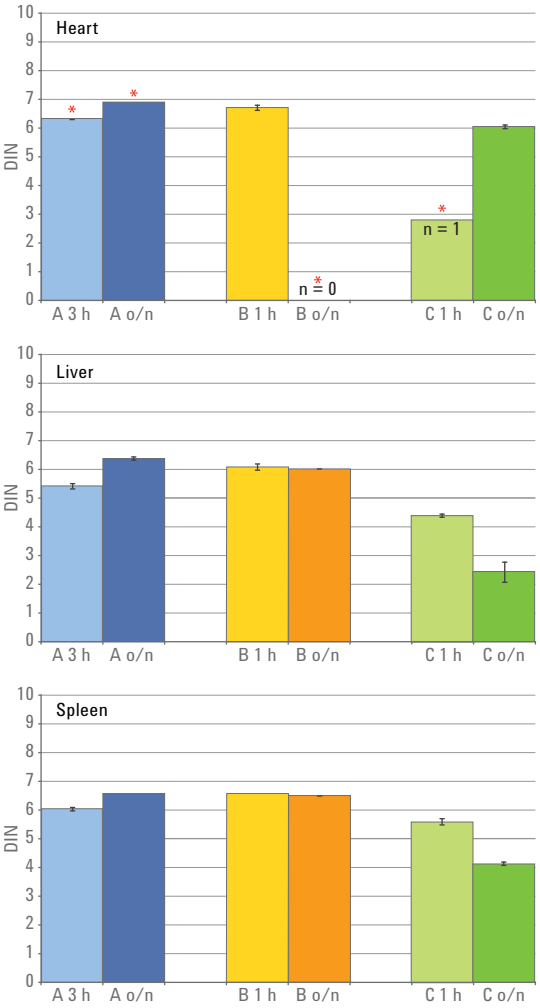

Figure 3. The DIN was determined with the Agilent 2200 TapeStation system for the DNA samples extracted from mouse liver, heart, and spleen FFPE tissue using the three DNA extraction kits A, B, and C with the standard protocol and the extended lysis protocol (n = 3 for each data point, unless stated otherwise). The red asterisk indicates that the DNA concentration for those samples was below the functional quantitative range required to determine a DIN.

References

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