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FFPE Tissue Sample DNA Extraction Kit

This article records the extraction of genomic DNA from FFPE using Allsheng® FFPE tissue sample DNA extraction kit (hereinafter referred to as Allsheng kit), and the extraction results are compared in detail with those of the same type of kits in the market through the precise quantitation extraction efficiency by Fluo-200 fluorometer as well as the analysis of fragment sizes and distributions by a bioanalyzer. The results show that the DNA extraction rate and the fragment distribution of the product are consistent with the performance of the same type of kits at home and abroad, and the extraction on some tissues is even better than that of similar products.

In addition, we use the Allsheng kit for manual extraction and fully automated extraction of continuous FFPE on Leap-Pure S24. The result shows that the yield of the above two methods is equivalent, and the nucleic acid quality for downstream applications (such as using the Agilent Bioanalyzer system for DNA and RNA analysis, as well as qPCR analysis) is also basically consistent. Paired with the Allsheng Leap-Pure series fully automated nucleic acid extractor, it reduces personnel operation time and greatly improves extraction efficiency.

Introduction

FFPE (Formalin fixation and paraffin embedding), i.e. formalin-fixed and paraffin-embedded tissue samples, are regarded as the "jewels" of the department of pathology, because they preserve a large amount of disease-related information, provide a valuable source of data for medical research, and are the main method of long-term preservation of pathological samples, which has been used to archive a large number of biological samples around the world over the past few decades. However, due to technological limitations, FFPE has not been fully utilized in the past decades. With the advancement of technology and the development of research, the application of FFPE has now become more and more widespread. From the initial use of immunohistochemistry for disease diagnosis and pathotyping, to the fact that it has now been applied to a variety of histological studies, the value of FFPE has been more fully explored.

Each FFPE is precious and unique. With a limited sample size, we should release the biological information contained in the sample while minimizing the loss. Extracting nucleic acids from FFPE is still challenging at this stage. For example, the degradation of nucleic acids during the production of paraffin samples makes it difficult to be compatible with downstream applications such as qPCR and sequencing, as well as the cumbersome and time-consuming extraction process, xylene in the reagents can be hazardous to the health of the experimenter, and secondly, xylene affects the quality of the tissue sections, and if it is not used appropriately, it is likely to cause the tissue to shrink, harden and become brittle, and other changes. Therefore, we need to extract as much high-quality nucleic acid as possible from limited samples, and at the same time realize the automation of the extraction operation. Here we show the method of extracting genomic DNA from rat paraffin-embedded tissue samples and human tissue samples by Allsheng kits, and compare it with the extraction effect of the same type of kits in the market, and the results show that the DNA extraction concentration and completeness of DNA extraction are consistent with the performance of the same type of products at home and abroad.

Material and Method

Comparison of Reagent Extraction Efficiency

Prepare 3 different batches of reagents, scrape 5-10 rolls with a surface area of 25-30 mm^2 according to the size of FFPE section / roll tissue area, and extract them manually, equal volume (50 μL) elution of all samples extraction process as shown in Figure 1. The purified DNA is accurately quantified for extraction concentration (dsDNA) by Fluo-200 fluorometer and dsDNA high-sensitivity quantitative analysis kit and DNA integrity is verified by agarose gel electrophoresis. Use GAPDH gene (the length of the amplification segment is 322 bp and 99 bp respectively) to conduct qPCR analysis on the DNA products, and the initial volume / quantity of all samples is consistent.

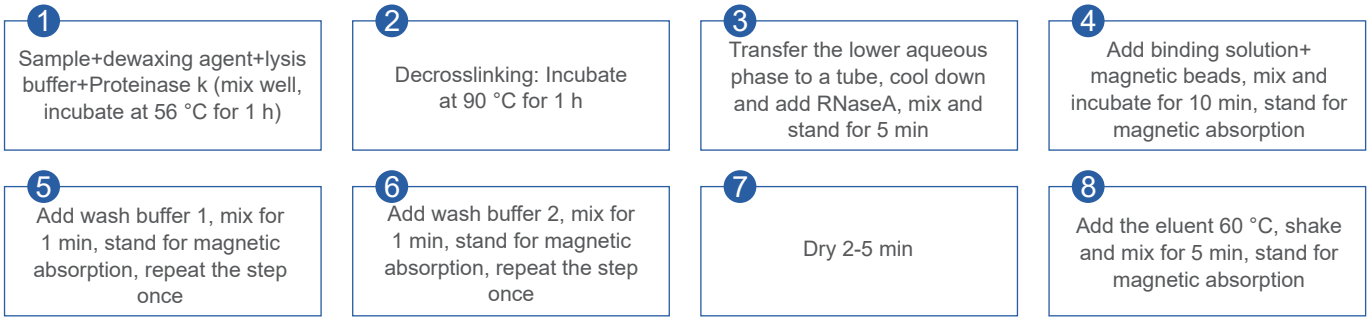


Figure 1 Overall Reagent Operation Flow

The above figure shows the operation flow of Allsheng kits. The manual extraction requires adding reagents in sequence according to the steps. With the automatic extraction method, you only need to add the sample directly into the designated wells, and the rest of the reagents are added to the other wells in advance, which is relatively more convenient.

Comparison of Extraction Effect of Various Brand Reagents

Allsheng kit and 3 other referenced kits at home and abroad are used to extract FFPE from different human tissues, with equal volumes (50 μL) eluting all samples. The purified DNA is accurately quantified for extraction concentration (dsDNA) by Fluo-200 fluorometer and dsDNA high-sensitivity quantitative analysis kit and DNA integrity is verified by agarose gel electrophoresis. With Allsheng® NGS Universal DNA Library Prep Kit for Illumina is used to compare the library preparation and peak pattern analysis of different brands of extracted products. Comparison with similar products for extracting human paraffin embedded tissue samples.

Validation of Automated Extraction Effects

To determine whether fully automated and manual extraction methods can achieve the same nucleic acid yield and quality, we use Allsheng kits for manual extraction and fully automated extraction of continuous FFPE on Leap-Pure S24. The sample size is the same for each sample and the elution volume is 50 μL for comparison testing.

Result and Analysis

Validation of Reagent Extraction Efficiency

The analysis of 3 batches of extracted product concentration determination found that the concentration of nucleic acid extracted from different FFPE tissue samples is almost close to or exceeded that of the comparable reagents (Figure 2-1); the fragment distribution and fragment integrity (amplification of the product with primers of different sizes, and agarose gel electrophoresis of amplified products, which all shows 4 complete fragments) are consistent with that of the comparable products (Figure 2-2, Figure 2-3); and the qPCR analysis of DNA products for GAPDH gene (amplified fragment length of 322 bp and 99 bp, respectively) found no significant difference in the concentration of amplified fragments of different lengths (Figure 2-4).

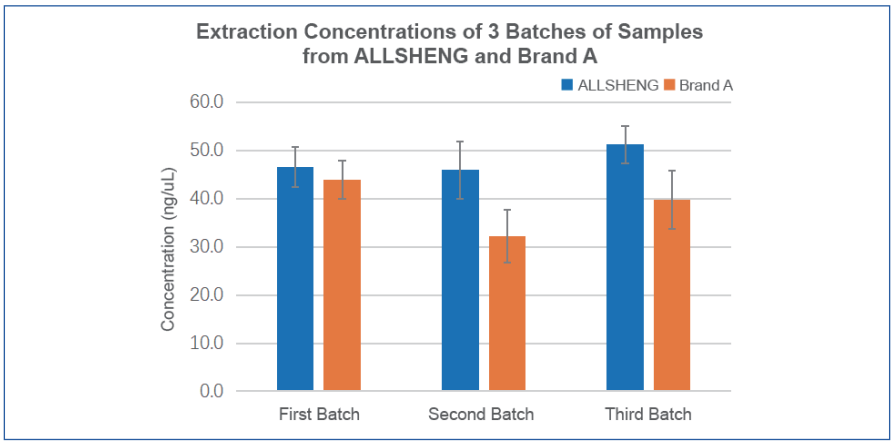


Figure 2-1 Extracted Product Concentration form Allsheng® FFPE Tissue Sample DNA Extraction Kits and Reference Kits

Figure 2-2 Results of Agarose Electrophoresis of Extracted Products from Allsheng® FFPE Tissue Sample DNA Extraction Kits and Reference Kits

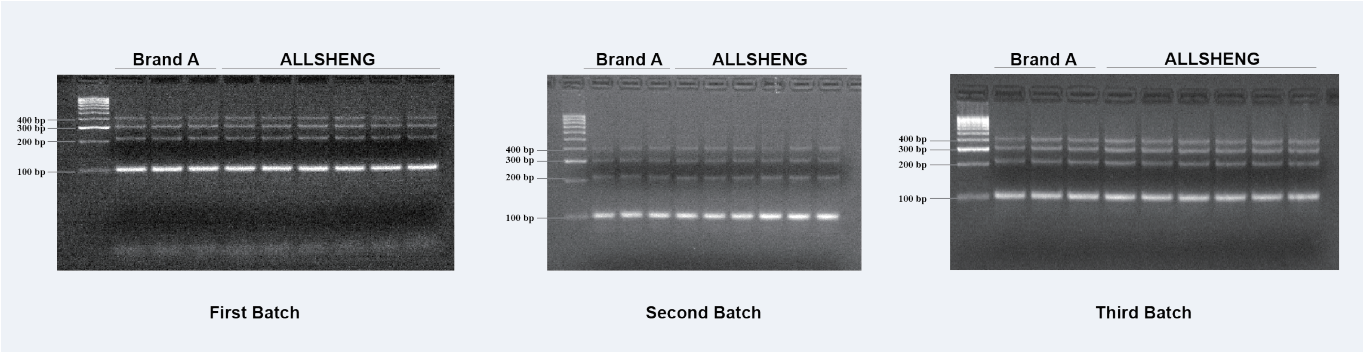


Figure 2-3 Fragment Integrity Analysis Results of Extracted Products from Allsheng® FFPE Tissue Sample DNA Extraction Kits and Reference Kits

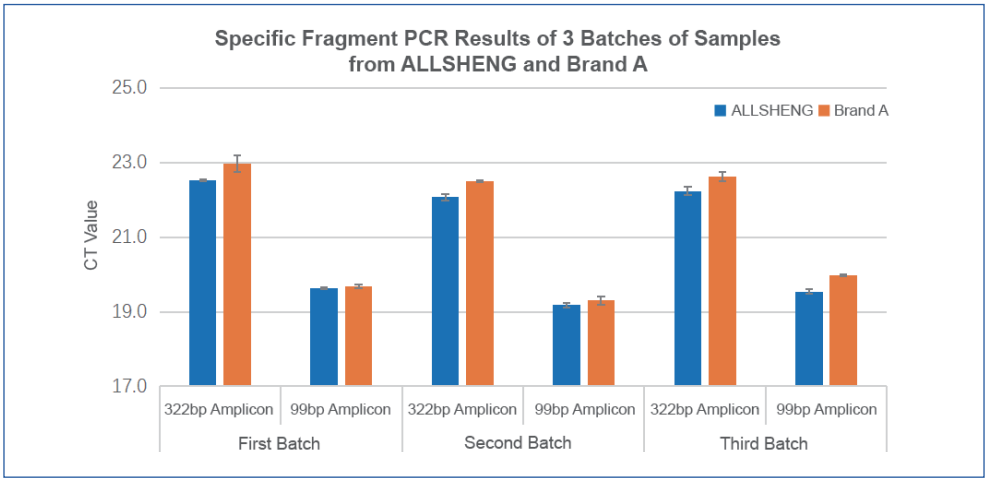


Figure 2-4 qPCR Results for Different Fragments of Extracted Products

Validation of Extraction Effects of Various Brand Reagents

Through FFPE extraction from different human organizations, it found that the extraction performance of the 4 kinds of kits is consistent for different samples. The extraction efficiency of the Allsheng kits and 3 referenced kits at home and abroad varies for different samples. In the extraction of sample C, the extraction efficiency of the Allsheng kits is significantly higher than that of the three referenced kits (Figure 3-1), and the fragment distribution and integrity of the 3 samples are consistent with the referenced products (Figure 3-2). The same amount of extracted products from the 4 kinds of kits is used to the library preparation, and there is no significant difference in the library production after Allsheng kits are built compared to the library production of the other 3 kinds of kits.

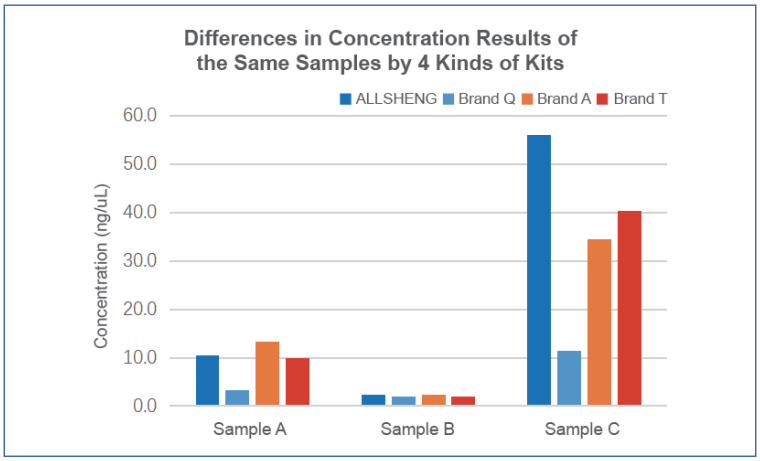


Figure 3-1 Concentration of Different Human Tissue FFPE Extracted by 4 Kinds of Kits

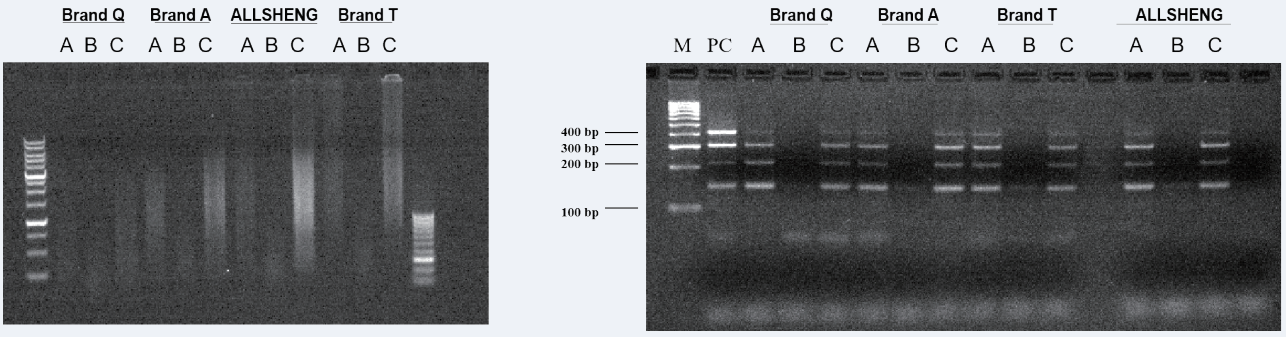


Figure 3-2 Agarose Gel Electrophoresis Diagram and Integrity Analysis Diagram of the Extracted Products of Different Human Tissue FFPE Extracted by 4 Kinds of Kits

Group	Qubit Concentration	Library Production
Brand T	17.8 ng/μl	445 ng
Brand Q	13 ng/μl	325 ng
Brand A	17.1 ng/μl	427.5 ng
ALLSHENG	17.5 ng/μl	437.5 ng

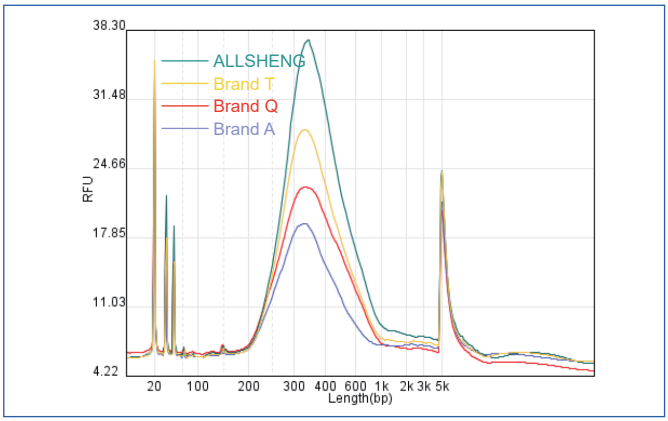


Figure 3-3 Results of Library Preparation Experiments of the Extracted Products by 4 Kinds of Kits

Validation of Automated Extraction Effects

Comparison between fully automated extraction on Leap-Pure S24 and manual extraction of FFPE. The results show that the concentration of Leap-Pure S24 extracted products can reach more than 90% of the manual method (Figure 4-2), the agarose gel electrophoresis effect shows that the two methods of extracted product fragments are distributed uniformly (Figure 4-3), and the nucleic acid quality for downstream applications (such as using Agilent Bioanalyzer system for DNA analysis, and qPCR and RT-PCR analysis) is basically consistent (Figure 4-4, 4-5). Using Leap-Pure S24 for nucleic acid extraction reduces manual intervention time by more than 90% compared to manual extraction throughout the process.

Well Position	Reagent Component	Volume (μL)
1	Dewaxing agent	700
	Lysis buffer	
	Proteinase k	
3	Binding buffer	300
4	Wash buffer 1	1000
5	Wash buffer 1	1000

Well Position	Reagent Component	Volume (μL)
6	Wash buffer 2	1000
6	Magnetic bead	10
7	Wash buffer 2	1000
9	RNase A (added by customer)	5
12	Eluent	50

Figure 4-1 Reagent Volume Required for Leap-Pure S24 Fully Automated Nucleic Acid Extraction and Reagent Well Position Distribution in Consumables

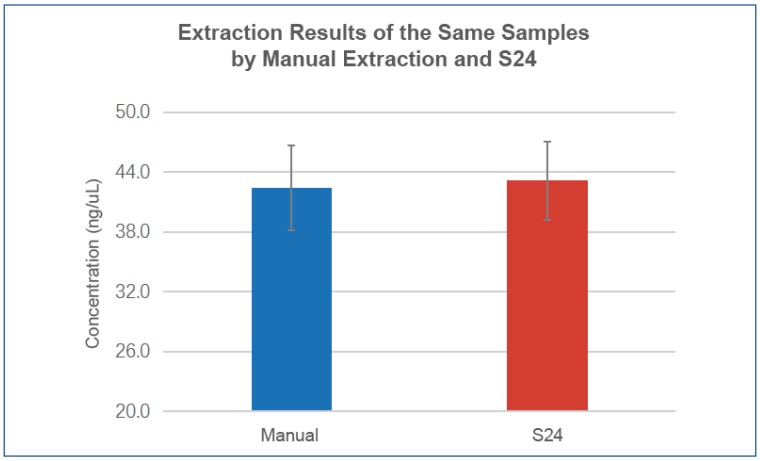


Figure 4-2 Comparison of Efficiency Between Fully Automated Nucleic Acid Extraction and Manual Extraction

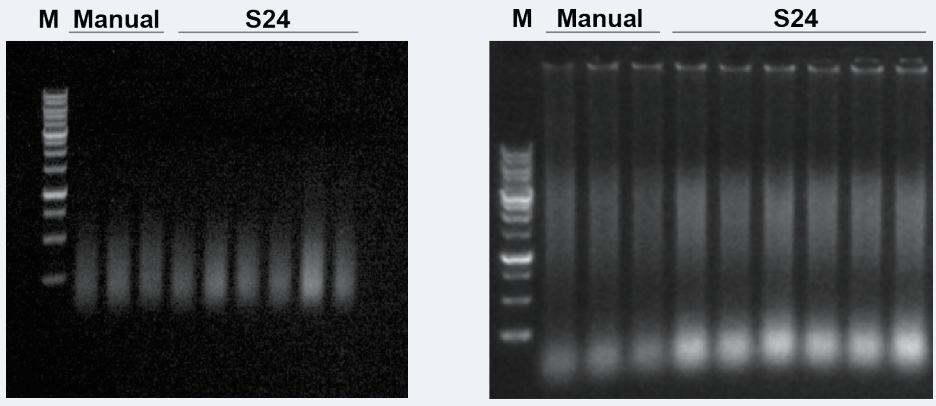
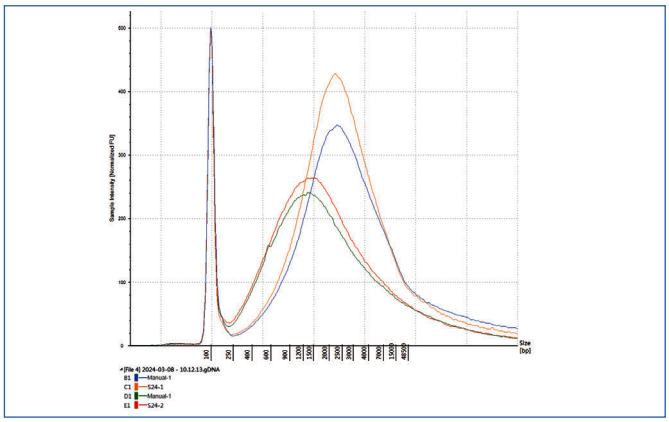


Figure 4-3 Agarose Gel Electrophoresis Results of Manual Method and S24 Extracted Products



Well	DIN	Conc.[ng/μl]	Sample Description
A1	/	89.1	Ladder
B1	4.5	80.2	Manual-1
C1	4.3	92.5	S24-1
D1	2.8	64.2	Manual-2
E1	2.9	69.5	S24-2

Figure 4-4 Fragment Analysis Results of Manual Method and S24 Extracted Products on Agilent 4150

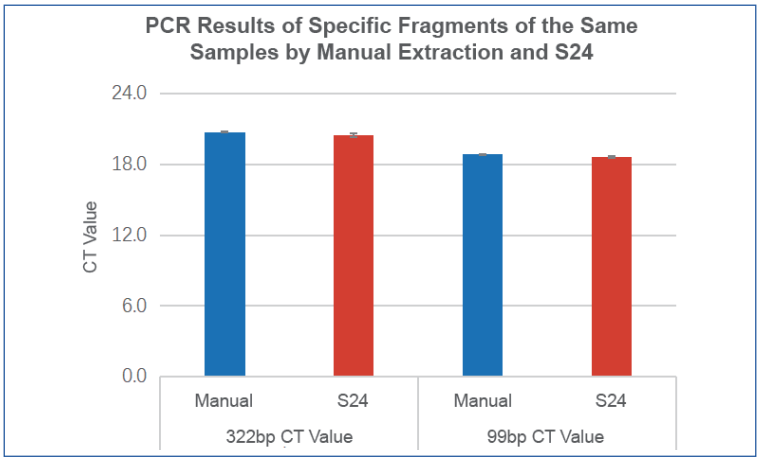


Figure 4-5 qPCR Results of S24 and Manual Method Extracted Products for Amplicons of Different Sizes

Summary

FFPE tissue samples are currently the most widely used form of tissue preservation. At present, molecular targeted therapy has gradually become the mainstream of tumor treatment. Detecting the gene mutation status of patients before clinical medication can correctly guide clinical individualized medication, reduce the economic burden of patients and improve the therapeutic effect of molecularly targeted drugs. Therefore, the extraction of high-quality DNA from FFPE for molecular targeting detection has also gradually attracted attention. The DNA extracted from FFPE tissue samples that can be effectively amplified is not only suitable for retrospective studies, but also important for the diagnosis and identification of diseases, assessment of disease prognosis and exploration of disease molecular mechanisms.

Allsheng® FFPE tissue sample DNA extraction kits use efficient tissue lysis buffer and non-toxic dewaxing agent for one-step dewaxing and lysis, and use silica hydroxy magnetic beads and nucleic acid separation and purification methods to quickly and efficiently extract DNA from fixed solution tissues such as paraffin embedded tissue slices, paraffin blocks, or formalin. The concentration, fragment distribution, and quality (downstream experiments) are consistent with the referenced reagents. The reagent does not contain harmful substances such as xylene, and is easy to operate without the need for multiple centrifugations. This kit is suitable for various tissue types of FFPE and can be manually extracted or fully automated with supporting instruments. Paired with ALLSHENG Leap-Pure S24 fully automated nucleic acid extractor, the extraction process can be fully automated, greatly reducing manual operation steps.

HANGZHOU ALLSHENG INSTRUMENTS CO., LTD.

Building 9 No.7 of Zhuantang Science and Technology Economic Zone,
Xihu District, Hangzhou City, 310024 Zhejiang, P.R. China

Tel: +86-571-88859758 Fax: +86-571-87205673

info@allsheng.com www.allsheng.com