BECKMAN COULTER
Life Sciences

A Complete Workflow for High-Throughput Isolation of DNA and RNA from FFPE Samples for Robust, Scalable Cancer Research and Biomarker Discovery

Summary

Formalin-fixed paraffin-embedded tissues are an invaluable resource for histological and genetic testing for cancer. Current next-generation sequencing (NGS) technologies and extraction methods make it possible to study and identify cancer-causing alterations at genomic and transcriptomic levels. However, challenges exist in most FFPE workflows, especially those requiring high-throughput extractions. First, FFPE samples generally provide low-quality nucleic acids, especially the RNA, as RNA undergoes more severe degradation and chemical and covalent modifications due to the effects of formalin-fixation compared to DNA. Second, consistent results for most high-throughput and automated extraction methods for FFPE are difficult to achieve due to inherent differences in the variety of tissue and disease types. A solution that offers the ability to apply high-throughput and reliable genomic applications to FFPE samples can help accelerate cancer research and biomarker discovery.

This application note demonstrates the use of the FormaPure XL Total kit, a total nucleic acid extraction kit for FFPE samples, in conjunction with the KingFisher™ Duo Prime Sample Purification system, as a potential solution that mitigates some of the challenges with FFPE workflows. the FormaPure XL Total kit has been demonstrated to obtain high-quality FFPE-derived nucleic acids . By automating FormaPure XL Total chemistry on the KingFisher™ Duo Prime system, the difficulty of working with FFPE tissue is reduced. Automating the chemistry can also reduce the risk of human error and hands-on time, thereby giving the user the ability to run more samples in a day.

Method

Manual processing

In this study, three FFPE tissue samples were used to assess the differences between manual and semi-automated RNA and DNA extraction. 10 µM curls were cut and three samples consisting of 3 curls were processed. For manual processing of each tissue type, 450 µL of mineral oil was added to each sample. The samples were then incubated at 80°C for 5 minutes. Then 200 µL of LBD was added and the samples were centrifuged according to the protocol. The samples were incubated again at 80°C for 5 minutes. Following the incubation, the tubes were allowed to cool for 5 minutes before the addition of 30 µL of proteinase K. The samples were then incubated at 60°C for 2 hours, after which 100 µL of the lysate was removed and added to a KingFisher™ Duo Prime 96-well plate for RNA processing.

The remaining lysate was incubated at 60°C for another hour followed by an 80°C incubation for 1 hour. After the incubation, 100 µL was removed and added to a KingFisher™ Duo Prime 96-well plate. RNase A was added to the 100 µL lysate and mixed 10 times. The mixture was incubated at room temperature for 5 minutes. The plate was then moved to the KingFisher™ Duo Prime System for DNA processing.

Automated processing

The parameters for mix times and speeds, and the collection times were optimized for the magnetic beads on the KingFisher™ Duo Prime system. The optimized parameters and reagent volumes for RNA processing are shown in Table 1, and DNA processing are shown in Table 2. The automation for RNA extraction is a two-step process. First, the protocol is automated up to the DNase I treatment. After the incubation on the DNase treatment, the user must add 150 µL of RBA; then automation can move on from there.

RNA Purification Step	Plate Row	Reagent	Volume (µL)	Automation parameters		
				Mixing time/Mixing Speed/ Pause Time	Collect Count/ time [s]	
DNase Treat	A	DNase I solution	100 µL	20 sec / medium / 20 min	5 / 30 sec	Heating 37°C
Bind	B	BBA	150 µL	20 sec / medium / 5 min	5 / 30 sec	
		Lysate	100 µL			
Wash	C	80% Ethanol	375 µL	20 sec / medium / 5 min	5 / 30 sec	
Wash	D	80% Ethanol	375 µL	20 sec / medium / 5 min	5 / 30 sec	
NA	E	NA	NA	NA		
NA	F	NA				
NA	G	KingFisher™ Duo 12-Tip Comb				
Elute	H	Nuclease Free Water	40 µL	20 sec / medium / 5 min	5 / 30 sec	

Table 1. RNA processing

DNA Purification Step	Plate Row	Reagent	Volume (µL)	Automation parameters	
				Mixing time/Mixing Speed/ Pause Time	Collect Count/ time [s]
Elute	A	Nuclease Free Water	40 µL	20 sec / medium / 20 min	5 / 30 sec
Bind	B	BBA	150 µL	5 min / medium / NA	5 / 30 sec
		Lysate	100 µL		
		RNase	2.5 µL		
Wash	C	WBA	200 µL	20 sec / medium / NA	5 / 30 sec
Wash	D	80% Ethanol	375 µL	20 sec / medium / NA	5 / 30 sec
NA	E	KingFisher™ Duo 12-Tip Comb	NA	NA	
NA	F	Empty			
NA	G	Empty			
NA	H	Empty			

Table 2. DNA Processing

Results

DNA and RNA were extracted from three 10 µM curls of breast, intestine, and lung FFPE tissue. The average yields are shown in Figure 1. The yields for RNA and DNA are similar between manual and automation. The differences in yields with RNA from lung tissue and DNA from breast tissue can most likely be attributed to differences in tissue distribution within an FFPE block.

The percent DV200 value is an important quality score to keep in mind for NGS. The percent DV200 is the percent of nucleic acid fragments that are larger than 200 nucleotides. In making FFPE tissue, the formalin can cross-link DNA or RNA to proteins. This causes the nucleic acids to fragment and to be very delicate after and during extraction. Various external forces can cause DNA and RNA to fragment more, such as the pressure it takes to move through a column matrix, or vigorous pipetting. The percent DV200 values for RNA and DNA extracted from the three tissues using the FormaPure XL Total kit manually and on the KingFisher™ Duo Prime system are not significantly different from each other (t-test RNA p=0.8 and DNA p=0.3). The average percent DV200 values for RNA for each tissue type is reported in Table 3 and representative traces are in Figure 2; all percent DV200s are greater than 65%. The average percent DV200 values for DNA are presented in Table 3 and representative traces are in Figure 3.

Lastly, we compared the hands-on time between the manually and semi-automated methods of the FormaPure XL Total kit. When manually processing 12 samples, the hands-on time is about 3.5 hours, while processing on the KingFisher Duo Prime system is approximately 1.5 hours (Table 4). Total time is also decreased by about 1.5 hours with processing on the KingFsher™ Duo Prime system.

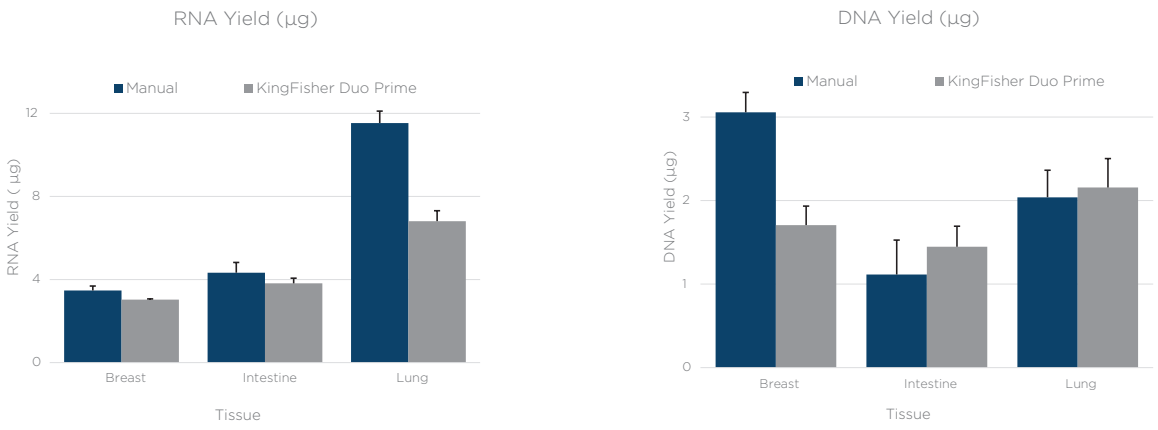
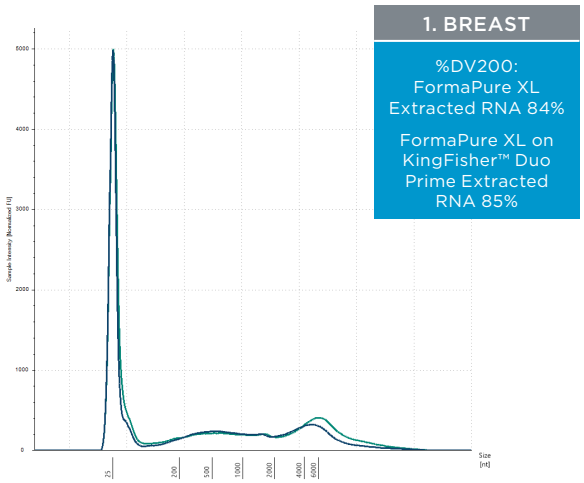
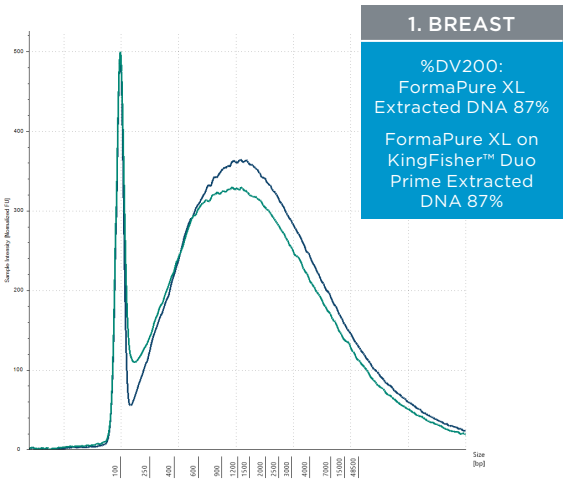


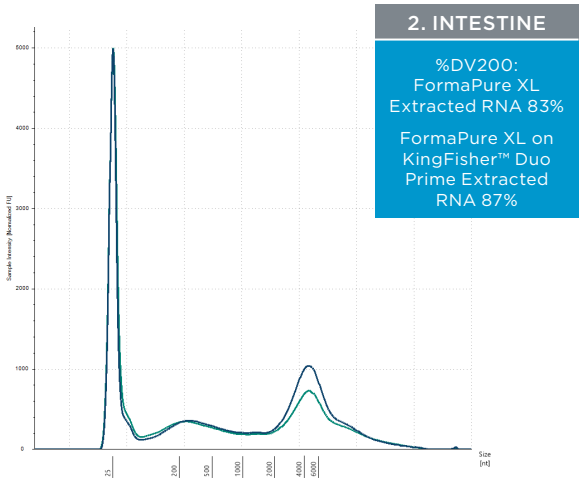
Figure 1. The average yield from three technical replicates of RNA and DNA extraction done manually or on a KingFisher Duo Prime system. The bars are an average yield of three technical replicates as calculated by Quant-iT assay (Thermo Fisher Scientific). Error bars are the standard deviation of three technical replicates.

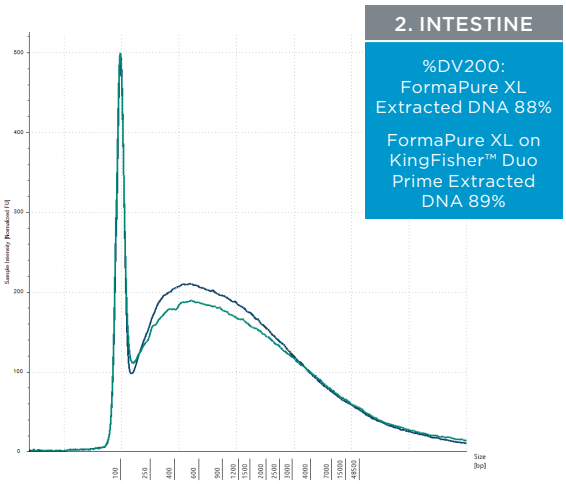
	RNA		DNA	
	Manual	KingFisher Duo Prime	Manual	KingFisher Duo Prime
Breast	84.7	88.5	88.8	86.3
Intestine	76.3	84.8	86.8	88.7
Lung	72.4	69.5	83.0	82.2

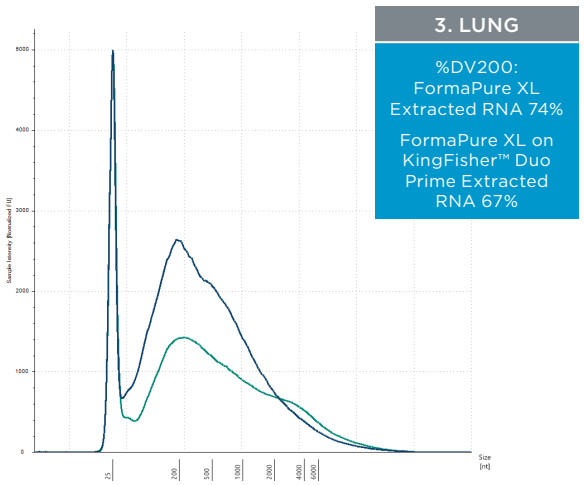
Table 3. The average percent DV200 values for RNA and DNA extracted from three tissue types. RNA and DNA was extracted from three 10 µM curls manually and using the KingFisher Duo Prime system with the FormaPure XL Total kit. The percent DV200 values were not significantly different between the KingFisher Duo Prime system and manually.

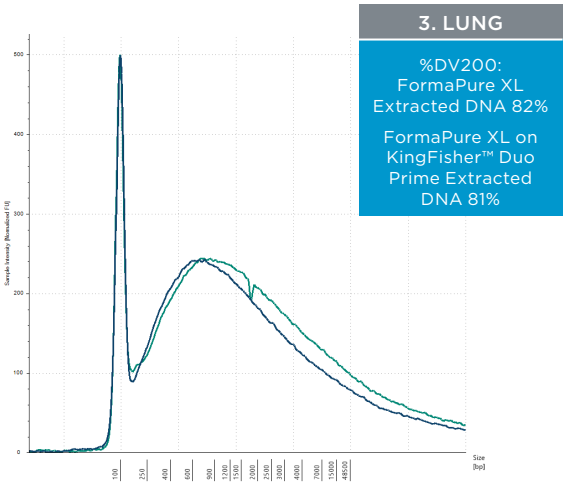












Manual KingFisher™ Duo Prime

Figure 2. Representative electropherograms of RNA isolated using the FormaPure XL Total kit manually (green traces) and using the KingFisher™ Duo Prime system (blue traces) from breast, intestine and lung FFPE samples are shown.

Figure 3. Representative electropherograms of DNA isolated using the FormaPure XL Total kit manually (green traces) and using the KingFisher™ Duo Prime system (blue traces) from breast, intestine and lung FFPE samples are shown.

Conclusion

This study demonstrates a solution that enables a semi-automated workflow for reliable isolation of DNA and RNA from single FFPE samples. The data presented suggests that the yield and integrity of DNA and RNA extracted from FFPE samples are comparable between manual and semi-automated extractions using the FormaPure XL Total kit. There are several advantages of using a KingFisher™ Duo Prime system to semi-automate the FormaPure XL Total chemistry. First, the semi-automated workflow does not significantly alter the yield and quality of DNA and RNA compared to a manual workflow as shown with the three tissue types. Up-front manual processing of sample deparaffinization, tissue digestion, decrosslinking and lysate splitting enables researchers to identify and resolve many of the challenges that typically lead to sample loss or dropouts.

Second, once these upstream steps are meticulously performed, the latter half of the extractions, which involves the bind, wash and elute steps, can be automated in a fraction of the time that it takes to process manually. Semi-automated processing of FFPE samples using the FormaPure XL Total kit and KingFisher™ Duo Prime system provides a viable solution to accelerate biomarker discovery. Table 4 outlines the three main differences between the manual vs semi-automated workflows.

	Throughput per run	Hands-on Time (12 samples)	Processing time (12 samples)
Manual	24	~3.5 hours	6.5 hours
Semi-automated KingFisher™ Duo Prime	12	~1.5 hours	5 hours

Table 4. The time and throughput abilities for both manual and semi-automated extraction.

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