Novel Solvent-Free Deparaffinization Method for FFPE Sample Prep Enabling a More Convenient Workflow

Angie Cheng and Xingwang Fang

ThermoFisher SCIENTIFIC

INTRODUCTION

Formalin-fixed paraffin embedded (FFPE) is the most common preservation method to archive solid tissue samples. Traditionally, FFPE samples have to be deparaffinized prior to proteinase digestion and nucleic acid extractions. The gold-standard for deparaffinizations involves a toxic and flammable organic solvent, xylene. In addition to the health hazards and burdensome of chemical waste disposal, it involves multiple rounds of washes with xylene and ethanol, which could lead to tissue loss. Alternative methods exist using less toxic solvents but still the protocol is tedious. Here, we present a novel solvent-free method to deparaffinize FFPE samples using the Autolys M spin tubes. These tubes eliminate the need for organic solvents, alcohols, and manual washing steps with only one pipetting step needed. Hands-on time is decreased resulting in more convenience and most importantly, tissue loss is minimized. These tubes can work upfront different extraction methods but it has been validated with the MagMAX FFPE DNA/RNA Ultra kit and the KingFisher Purification Systems.

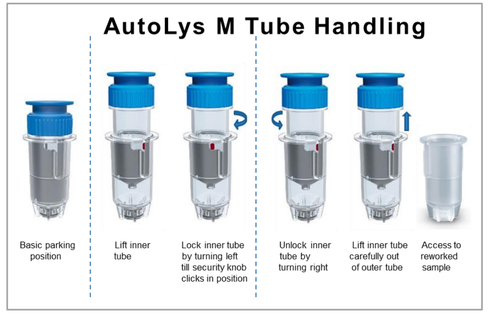
MATERIALS AND METHODS

Different deparaffinization methods (Autolys M tubes (A38738) versus xylene versus an organic separation method) were compared in lung, colon and breast FFPE cancer tissues. For the Autolys M tube method, the Autolys M TubeLifter (A37956) was used to process the tubes. After the different deparaffinization methods, the samples were treated together under the same extraction conditions and sequential DNA and RNA from the same sample were isolated using the MagMAX FFPE DNA/RNA Ultra kit (A31881) automated on the KingFisher Flex Purification instrument. DNA and RNA concentrations were measured with the Qubit and the nucleic acids were functionally tested by real-time PCR on the QuantStudio 12K Flex system and next-generation targeted sequencing on the Ion Torrent sequencing platform.

Figure 1. MagMAX FFPE DNA/RNA Ultra and the KingFisher Flex Purification System

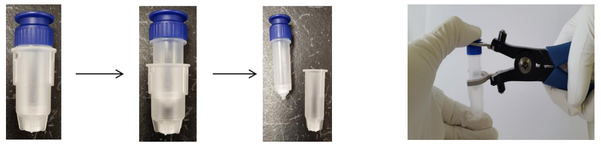
RESULTS

Figure 2. Overview of AutoLys M Tubes



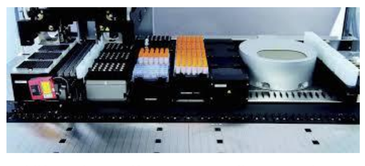
FFPE samples (curls, slides, cores) are placed in the tubes and protease digestion happens directly in them. After digestion is complete, the tubes are lifted from parking to the centrifuge position. Samples are spun allowing a physical separation of wax and tissue. Tubes are lifted to a final position so the lysate can be accessible for purification.

Figure 3. FFPE Workflow-3 options



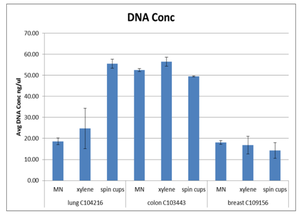
Manually lift and then centrifuge-**option 1**

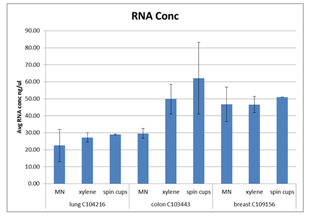
Semi-automated-lift 24 tubes simultaneously and then centrifuge-**option 2**



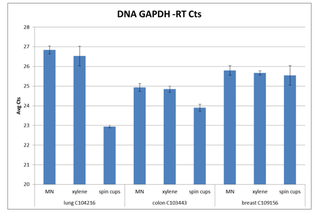
Fully-automated lift and centrifuge on Hamilton Autolys STAR deck- **option 3**

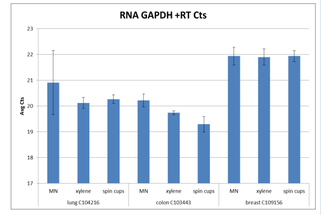
Figure 4. Comparison to Xylene and Paraffin Dissolver





Sequential DNA and RNA was isolated from 1x10um FFPE curls. Nucleic acid concentrations were measured with the Qubit 3.0 and respective high sensitivity reagents. Xylene is the gold standard solvent for deparaffinizations and Paraffin Dissolver is an alternative that phase separates the wax and tissue. Comparable concentrations were achieved.





Real-time PCR was performed to assess functionality of the nucleic acids. RT was performed with SuperScript VILO and samples were run on the QuantStudio 12K Flex Real-time PCR system.

Samples	Mapped Reads	Mean Read Lengths	% on target	% Uniformity
lung MN	1,265,341	113bp	95.30%	97.90%
lung xylene	1,518,161	112bp	96.16%	99.41%
lung spin cups	2,049,273	114bp	98.99%	98.63%
colon MN	1,294,640	111bp	94.74%	99.43%
colon xylene	1,453,346	112bp	98.81%	99.56%
colon spin cups	1,622,482	113bp	98.37%	99.60%
breast MN	1,007,587	112bp	97.19%	97.54%
breast xylene	1,165,427	111bp	93.22%	98.55%
breast spin cups	1,173,460	112bp	97.21%	98.36%

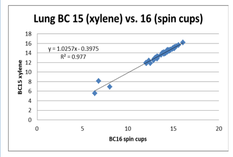
DNA libraries were made with the Ion AmpliSeq Cancer Hotspot Panel v2 and prepared with the Ion Chef and Ion S5 System. Here are results from the coverage analysis plug in.

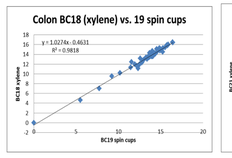
Sample Type	Variants	Allelic Frequency-MN	Allelic Frequency-xylene	Allelic Frequency-spin cups	Reference to Variant
Lung	IDH1	43.4%	46.4%	44.5%	G to A
	KRAS	11.8%	11.8%	16.7%	C to G
	SMARCB1	45.7%	52.4%	47.6%	G to A
Colon	PIK3CA	29.2%	28.5%	26.0%	G to A
	PDGFRA	49.2%	53.9%	51.2%	C to T
	FBXW7	31.1%	27.0%	29.4%	C to T
	HRAS	46.5%	51.3%	48.3%	A to G
	KRAS	50.0%	48.9%	42.5%	C to T
	SMAD4	30.3%	26.8%	29.4%	C to T
Breast	HRAS	15.4%	16.1%	16.6%	A to G
	SMARCB1	40.2%	40.0%	41.0%	G to A

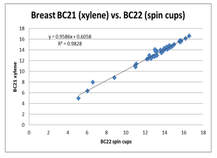
Variant calling analysis from the DNA libraries.

Samples	Mapped Reads	Mean Read Lengths	Valid Reads	Targets Detected
lung MN	651,627	141bp	99.66%	100%
lung xylene	649,535	150bp	99.51%	100%
lung spin cups	673,457	149bp	99.46%	100%
colon MN	625,569	135bp	99.73%	98%
colon xylene	694,485	135bp	99.80%	98%
colon spin cups	936,485	136bp	99.79%	98%
breast MN	875,669	132bp	99.62%	98%
breast xylene	648,966	139bp	99.61%	100%
breast spin cups	660,510	132bp	99.56%	100%

RNA libraries were made with the Ion AmpliSeq RNA Cancer Panel and the Ion Chef and Ion S5 System.







RNA gene expression correlation plots between the AutoLys spin cups and xylene.

CONCLUSIONS

The AutoLys M tubes provides a convenient FFPE deparaffinization workflow by decreasing hands on time and minimizing tissue loss while eliminating the use of hazardous chemicals. These tubes in combination with our MagMAX FFPE Ultra chemistry and our KingFisher instrumentation increases user's FFPE sample processing throughput.