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BOLDLY FORWARD

Automation enablement with liquid handler and magnetic-bead based purification methods for ultra-high throughput processing of solid tumor samples in oncology research

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INTRODUCTION

Sample preparation can be easily streamlined with semi-automated bench top instruments like KingFisher™ sample purification systems such as KingFisher™ Duo Prime, KingFisher™ Flex, or KingFisher™ Apex across various research applications. Even with magnetic bead-based solutions, there is a need to support full automation with liquid handlers to remove tedious pre-processing and preparation steps before nucleic acid isolation. For workflows like sequential isolation of DNA and RNA with FFPE, MagMAX™ FFPE DNA/RNA Ultra workflows on KingFisher™ instruments with upfront deparaffinization using AutoLys M tubes, have significantly cut down time of manual processing while enabling consistency and reproducibility of sequential isolation from one single sample curl or slide. However, there is a need to prepare processing plates for two independent purification scripts on the KingFisher™ instruments. Here, we leverage the Hamilton Nimbus liquid handler with the KingFisher™ Presto system to enable full automation of sample preparation with ultra-high throughput workflows (multiple runs of 96 samples) per day using just one integrated KingFisher™ Presto system to isolate DNA and RNA sequentially using downstream oncology research workflows of NGS and dPCR.

MATERIALS AND METHODS

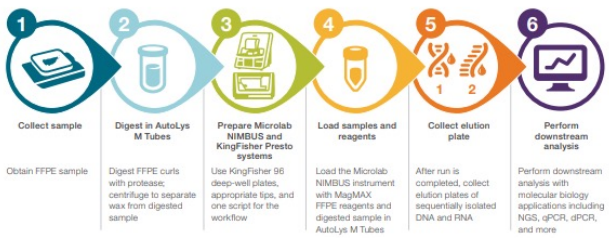
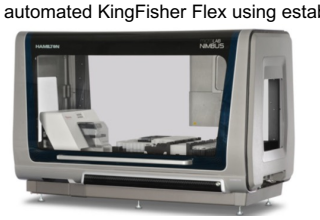


Figure: High throughput automated workflow for Sequential isolation of DNA and RNA from FFPE tissue.

FFPE tissue curls (up to 10µm) from low yielding (Breast cancer tissue) and high yielding (Uterus, Endometrial Cancer tissue) research samples were digested using Autolys M tubes. Sequential extraction of DNA and RNA using the MagMAX FFPE DNA/RNA Ultra kit was performed on the automated KingFisher Presto system integrated on the Hamilton Nimbus liquid handler using qualified Hamilton Protocol. For comparison, the same pooled samples were sequentially extracted using the semi-automated KingFisher Flex using established KingFisher Flex protocols.



Hamilton Nimbus integrated with Kingfisher Presto

Kingfisher Flex

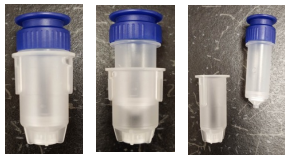
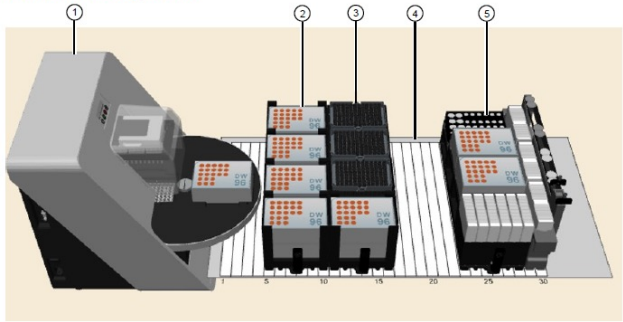


Figure: One step digestion of FFPE Tissues using Autolys M tube. Add Protease & Digestion buffer and incubate at 60°C for Protease digestion and 90°C for de-crosslinking. Digested sample in Autolys M tube can be used as input for Sequential DNA/RNA extraction.

Automation configuration

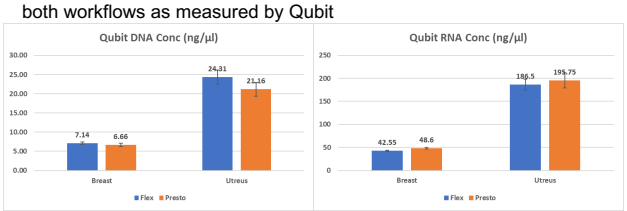


① KingFisher Presto instrument.
② Empty DW Plates.
③ Tips and DW Plates.
④ Space for 1.5 flip cap tube runers for AutoLys tube racks. Input tube type based on user selection.
⑤ Small reagent tubes, DW plates, small reagent troughs, and large reagent troughs.

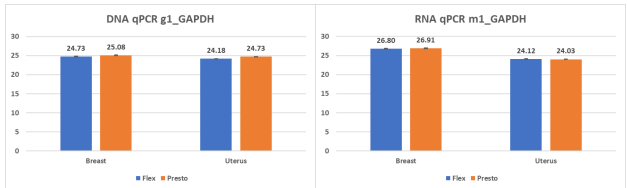
Figure: Hamilton Nimbus deck layout from the qualified Hamilton protocol for automated FFPE extraction.

RESULTS and DISCUSSION

Sequential isolation of DNA and RNA were performed using automated Hamilton Nimbus - Kingfisher Presto and Flex instruments and comparable concentrations of DNA and RNA were observed in both workflows as measured by Qubit



qPCR analysis was conducted on the DNA and RNA samples using TaqMan assay for housekeeping gene GAPDH. The results revealed comparable Ct values for both the Nimbus/Presto and Flex systems.



The negative control utilized in the Nimbus-Presto extraction exhibited no amplification in qPCR, providing evidence that there is no cross-contamination occurring during the extraction process when using the Hamilton Nimbus integrated with the Kingfisher Presto system.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample	Sample	40	40	40	Sample	Sample	39	40	40	40	Sample
B	40	40	40	40	40	40	40	40	40	40	40	Sample
C	40	40	40	40	40	40	40	40	40	40	40	40
D	40	40	40	Sample	40	40	40	40	Sample	Sample	40	40
E	40	40	40	Sample	40	40	40	40	40	40	40	40
F	40	40	40	40	40	40	40	40	40	40	40	40
G	40	40	40	40	40	40	39	40	40	40	40	Sample
H	Sample	Sample	40	40	40	Sample	Sample	40	40	40	40	Sample

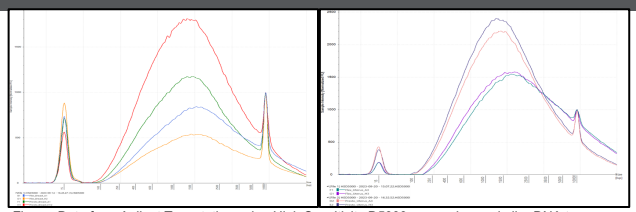


Figure: Data from Agilent Tapestation using High Sensitivity D5000 assay shows similar DNA trace across both platforms.

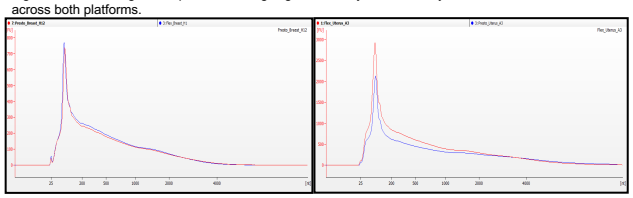
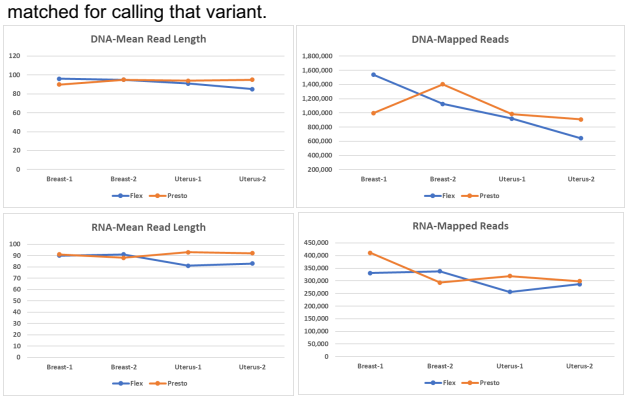


Figure: Data from Agilent Bioanalyzer using High sensitivity 6000 Pico RNA assay shows similar RNA trace across both platforms.

The impact of extraction method in the downstream oncology research was determined by performing a deep sequencing of the DNA and RNA using the Genexus Dx integrated sequencer. 30ng of either DNA or RNA were used for sequencing using the AmpliSeq™ HD target amplification assay in 2 separated runs using the same instrument. One variant has been detected in Breast tissue, both Flex and Presto matched for calling that variant.



Comparable sequencing metrics, such as mean read length and mapped reads, were observed for both DNA and RNA. DNA percent coverage uniformity was ~90% for both Presto and Flex instruments. We observed a mutation call in Breast tissue with a similar allele frequency across Presto and Flex.

Sample	Gene	Allele Frequency (replicate1)	Allele Frequency (replicate2)	Variant ID	AA Change
Breast cancer (Flex)	PIK3CA	37.8	38.2	COSM763	p.E545K
Breast cancer (Presto)	PIK3CA	40.4	41.5	COSM763	p.E545K

CONCLUSION

Current study shows the successful creation of automation workflow for sequential isolation of DNA and RNA from FFPE samples using the Hamilton Nimbus integrated with King Fisher Presto system.. This automation process allows for high-throughput extraction of DNA and RNA from up to 96 samples with in 3.5 hours. The extracted DNA and RNA were of high quality, suitable for qPCR and sequencing applications.

ACKNOWLEDGMENTS

Hamilton developed the script for Nimbus Automation.