APPLICATION NOTE 26 JUL 2016

RNA and DNA extraction using Thermo Fisher Scientific tissue sectioning and nucleic acid extraction instruments and consumables

Automated extraction of RNA and DNA from fresh frozen and formaldehyde fixed paraffin embedded lung and breast tissues for cancer research

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ABSTRACT

The Thermo Scientific™ HM 355S automatic microtome, Thermo Scientific™ CryoStar™ NX70 cryostat and the Thermo Scientific™ KingFisher™ Flex Purification System were tested for nucleic acid extraction performance. Fresh frozen (FF) and formalin fixed paraffin embedded (FFPE) tissues from breast cancer and lung cancer tissues were fixed and processed and/or sectioned followed by nucleic acid extraction and QC analyses. Nucleic acid quantity, purity, and quality are reported to guide cancer researchers using the applicable KingFisher and MagMAX™ kits.

Thermo Scientific™ Cryostar™ NX70 Cryostat

Thermo Scientific™ HM 355S Microtome

INTRODUCTION

Extraction of nucleic acids (NA) from fresh frozen (FF) and formaldehyde fixed paraffin embedded (FFPE) tissues is a critical and daily routine for biomedical researchers. Utilizing magnetic bead technology, the Thermo Scientific™ KingFisher Flex Purification System purifies RNA and/or DNA from cell lines and tissues enabling high throughput, robust, and repeatable NA extraction from FF and FFPE tissues. To provide a benchmark for KingFisher Flex (KF) purification of nucleic acids, the yield of DNA and RNA from cancerous lung and breast FF and FFPE tissue was determined empirically using the Thermo Scientific™ KingFisher Pure RNA Tissue, Thermo Scientific™ Cell and Tissue DNA, and MagMAX™ FFPE Total Nucleic Acid Isolation Kits.

MATERIALS and METHODS

Tissue Selection and FFPE Preparation

Extractions were conducted on cancerous human tissue purchased from Asterand Bioscience (Detroit, MI). All tissues used were conformed to the following criteria; deidentified patient information, 2+ grams of >95% tumor volume and RNA integrity number (RIN) >9.5. Two different tissue types with significant clinical relevance were selected for this study. Lung cancers were non-small cell lung carcinoma (LCa) of adenocarcinoma subtype from three different patients. Breast cancers (BCa) were represented by infiltrating ductal adenocarcinoma tissues from three different patients. All frozen tissues were kept at -80C except for grossing and sectioning and immediately returned to -80C when possible. One section was saved from each tissue to serve as a FF control, the rest were fixed and embedded. Tissues were fixed in Richard Allan Scientific 10% Neutral Buffered Formalin (NBF) for 24 hours, grossed into 300 mm3 pieces, then processed for 8 hours upon a Thermo Scientific Excelsior ES Tissue Processor and embedded in Histoplast LP paraffin at a Thermo Scientific™ HistoStar™ Embedding Workstation. Fixation, processing, and embedding took place under RNase-minimized conditions by sterilizing tools and surfaces with 75% Ethanol in nuclease-free water followed by RNaseAway.

Tissue Sectioning and NA Extraction Instrumentation and Kits

FF tissues were sectioned at -20°C on a Thermo Scientific™ CryoStar™ NX70 cryostat cutting 7 µm curls. Each curl

was placed into 50 µl of respective lysis buffer with cold forceps. Tubes were kept inverted on ice until all sectioning was complete. After sectioning, all tubes were centrifuged at 12,000 rpm at 4°C for 1 minute to collect tissue and buffer at the bottom of the microcentrifuge tube in a Thermo Scientific™ Sorvall™ Legend™ Micro 21 microcentrifuge. Then, the remaining lysis buffer was added. FFPE tissues were sectioned on a Thermo Scientific™ HM 355S Automatic Microtome set at 7 µm cutting thickness. Microtome blades used were Thermo Scientific™ MX35 Premier Plus Low Profile. Each curl was placed directly into an empty sterile 1.5 or 2.0 mL microcentrifuge tube.

The Thermo Scientific™ KingFisher™ Flex Purification System was used with the following compatible kits for lysate preparation and NA extraction: Thermo Scientific™ KingFisher™ Pure RNA Tissue kit for RNA from FF tissue, Thermo Scientific™ KingFisher™ Cell and Tissue DNA kit for DNA from FF tissue, and MagMAX™ FFPE Total Nucleic Acid Isolation kit for both DNA and RNA from FFPE tissue. Methods for the above kits were performed according to the protocols listed in their manuals with Thermo Scientific™ KingFisher™ Flex™ sterile microtiter deepwell 96 plates. The MagMAX FFPE Total Nucleic Acid Isolation kit uses two separate workflows to isolate RNA and DNA but contains the reagents needed for both workflows.

Three independent lysis and digestion reactions were performed in separate experimental runs to account for day-to-day variability for each workflow. Different experimental runs from the same workflow were purified in the same KingFisher Flex operation. Each experimental run for each kit was conducted with three curls from each of three blocks of tissue from different donors. Experimental run number one for each kit however was composed of four curls from each tissue sample.

Nucleic Acid Assays:

Extracted RNA and DNA were quantified by Qubit™ 3.0 Fluorometer and the Thermo Scientific™ NanoDrop™ 2000c Spectrophotometer. Nucleic acid purity was measured by the NanoDrop focusing on 260/230 and 260/280 absorbance ratios. RNA quality measured using an Agilent 2100 Bioanalyzer (Santa Clara, CA) with RNA Pico chips, RNA integrity number (RIN) represents the condition of assayed RNA relative to intact total RNA on a scale of 1-10 with 10 containing completely intact 18s and 28s rRNA.

RESULTS

Two independent methods, fluorometry and spectrophotometry, were used to quantitate nucleic acids isolated from FF and FFPE LCa and BCa tissues. Fluorometric quantification for RNA isolated from FF and FFPE LCa, indicated that both had an average concentration of 13 ng/µl, whereas RNA isolated from FF and FFPE BCa had an average concentration of 10 ng/µl and 15 ng/µl, respectively (Fig. 1A). Fluorometric quantification of DNA isolated from FF and FFPE LCa had an average concentration of 14 ng/µl and 1.5 ng/ µl, respectively. Similarly, DNA isolated from FF and FFPE BCa had an average concentration of 19 ng/µl and 1 ng/µl, respectively (Fig. 1B).

Spectrophotometric quantification of RNA isolated from FF and FFPE LCa had an average concentration of 17 ng/µl and 19 ng/µl, whereas RNA isolated from FF and FFPE BCa had an average concentration of 14 ng/µl and 27 ng/µl, respectively (Fig. 1A). Spectrophotometric quantification of DNA isolated from FF and FFPE LCa had an average concentration of 29 ng/µl and 17 ng/µl, and DNA isolated from FF and FFPE BCa had an average concentration of 47 ng/µl and 15 ng/µl, respectively (Fig. 1B). Nucleic acid yields are summarized in Table 1.

NanoDrop absorbance ratios were used to measure the relative purity of RNA and DNA absorbing at 260 nm compared to protein at 280 nm and salt impurities at 230 nm (Fig. 2A and 2B, respectively). RNA extracted from FF LCa and BCa tissues had an average 260/280 absorbance of 2.0 which decreased to 1.8 with FFPE LCa and BCa tissues. 260/230 ratios were an average of 1.4 for FF LCa and BCa which further decreased to .8-1.0 for FFPE tissues. Average DNA 260/280 values for FF LCa and BCa are 1.8 whereas FFPE tissues are 1.6. For FF and FFPE, LCa and BCa tissues average 260/230 values range between 0.5-0.8. Average RIN for FF LCa was 6.3 and for BCa was 6.9. Both tissue types displayed a decreased RIN down to slightly above 2 from FFPE (Fig. 3).

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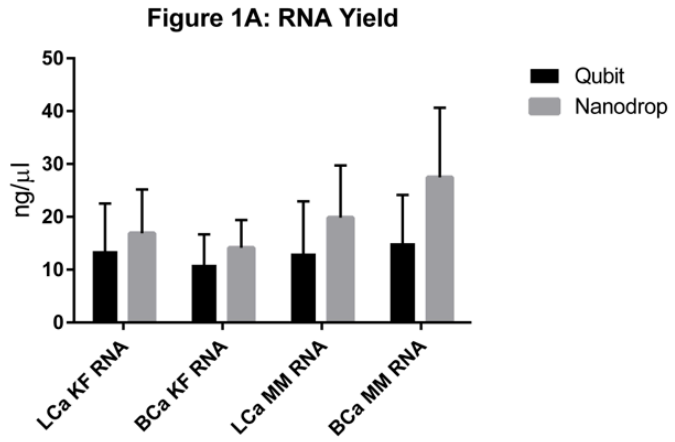

Figure 1A. Average yield of RNA quantified from LCa and BCa using the KingFisher Pure RNA Tissue kit (KF RNA) and the MagMAX FFPE Total Nucleic Acid kit (MM RNA). Averages and standard deviations are calculated from independent experiments and measurements, N=3.

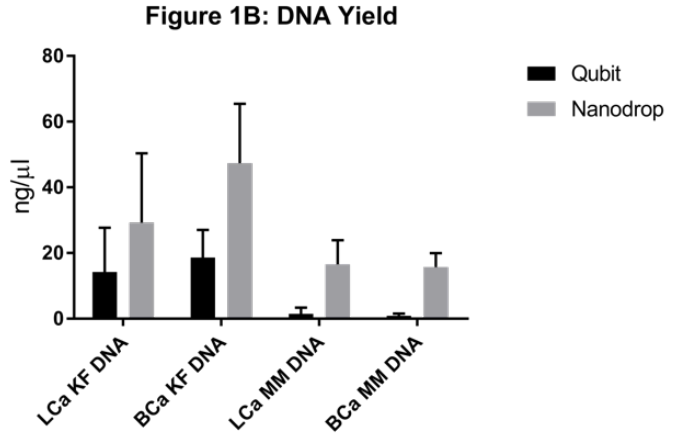

Figure 1B. Average yield of DNA quantified from LCa and BCa using the KingFisher Cell and Tissue DNA kit (KF DNA) and the MagMAX FFPE Total Nucleic Acid kit (MM DNA). Averages and standard deviations are calculated from independent experiments and measurements, N=3.

Table 1 NA Yield (ng/µl)		Qubit		NanoDrop	
		FF	FFPE	FF	FFPE
RNA	LCa	13.53	13.08	16.89	19.86
	BCa	10.92	15.02	14.13	27.46
DNA	LCa	14.20	1.53	29.22	16.59
	BCa	18.61	0.90	47.35	15.69

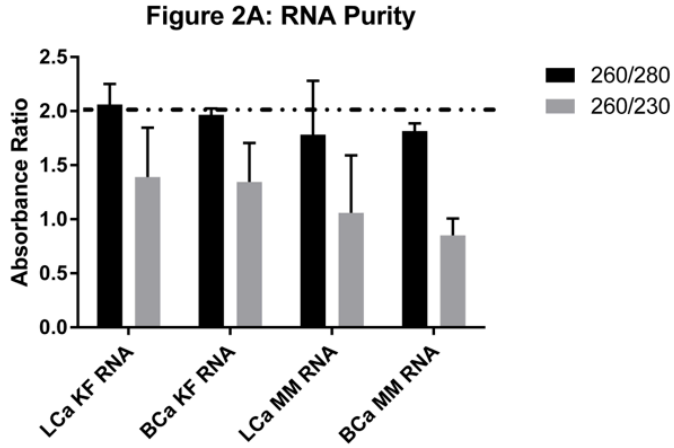

Figure 2A. Average RNA purity ratios from LCa and BCa using the KingFisher Pure RNA kit (KF RNA) and the MagMAX FFPE Total Nucleic Acid kit (MM RNA). The dashed and dotted line indicates an absorbance ratio of 2.0 which is optimal for RNA 260/280 and 260/230 ratios. Averages and standard deviations are calculated from independent experiments and measurements, N=3.

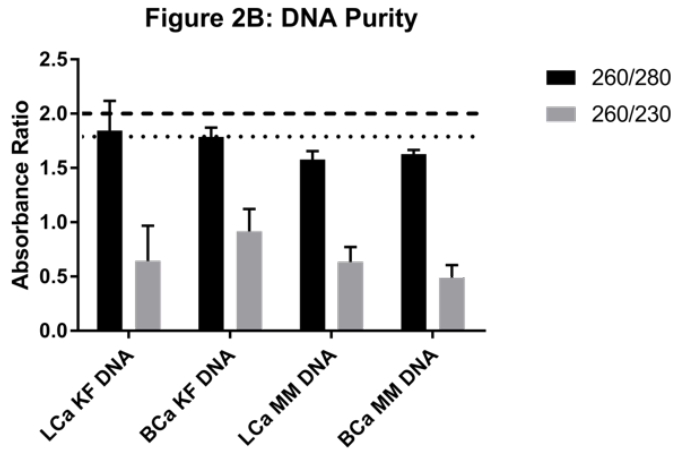

Figure 2B. Average DNA purity ratios from LCa and BCa using the KingFisher Cell and Tissue DNA kit (KF DNA) and the MagMAX FFPE Total Nucleic Acid kit (MM DNA). The dotted line indicates an absorbance ratio of 1.8 which is optimal for DNA 260/280 and the dashed line at 2.0 is optimal for 260/230 ratios. Averages and standard deviations are calculated from independent experiments and measurements, N=3.

Table 1. Average yield of nucleic acids (NA) quantified from LCa and BCa calculated from independent experiments and measurements, N=3.

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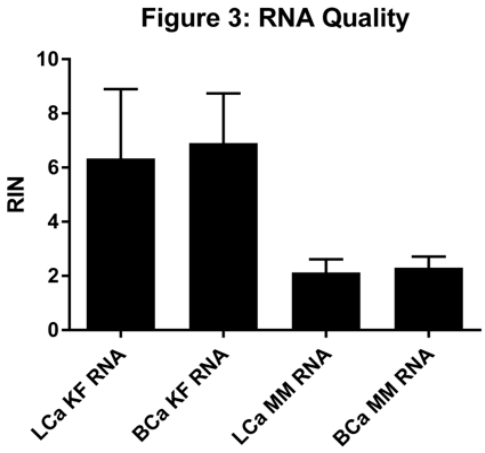


Figure 3. Average RIN values from LCa and BCa using the KingFisher Pure RNA kit (KF RNA) and the MagMAX FFPE Total Nucleic Acid kit (MM RNA). On a scale of 1-10 optimal RIN is at 10. Averages and standard deviations are calculated from independent experiments and measurements, N=3.

DISCUSSION

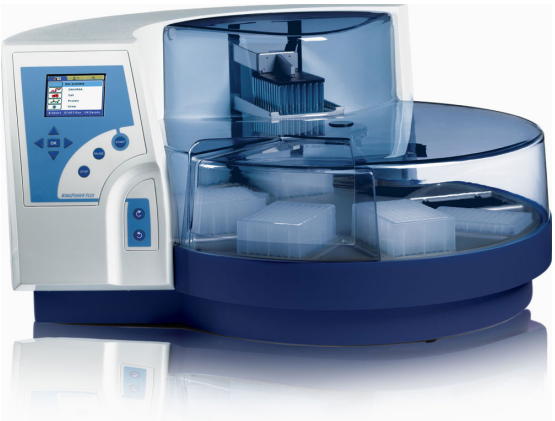
Qubit and NanoDrop measurements of nucleic acids were done to represent the available instrumentation for lab personnel. Due to specific dye binding chemistry, Qubit measurements are sensitive to distinguish between RNA and DNA species whereas, the NanoDrop measures all absorbance at 260 nm. RNA yield remained similar in both tissue types independent of whether the tissue had been fixed for 24 hours with NBF. This may be due to quantitation of degraded RNA that is not completely destroyed by tissue fixation and processing. Possibly, the RNA is fragmented but still present in similar quantities. Additionally, fixation may inactivate RNases while frozen tissue still contains RNases which become active as they thaw. Fixed DNA yield was expectedly lower than from fresh frozen for both tissue types, likely due to formaldehyde crosslinking resulting in degradation through the formation of methylene bridges.

Nucleic acid purity tended to decrease slightly when fixed but was optimal when extracting from fresh frozen tissue. Nucleic acid 260/230 purity measurements tended to be lower than expected, which may be attributable to the saltiness of buffers used in extraction. In terms of nucleic acid purity measures, 260/280 absorbance ratios are the prime consideration with 260/230 values being less significant. RNA quality is clearly lower in fixed tissue as expected as RNA degrades during the fixation and embedding process due to the action of RNase and formaldehyde methylene bridge formation.

In this study, we report the feasibility of a workflow to extract RNA and DNA from fixed and unfixed tissues. This workflow shows that sectioning lung and breast tissues using a cryostat or a microtome directly into microcentrifuge tubes is a viable solution for preparing NA for downstream genomic and transcriptomic analyses. The data reported here are intended to guide cancer researchers about the expected nucleic acid yield, purity and RIN from FF and FFPE LCa and BCa tissues using clinical pathology and molecular biology instruments and consumables.

CONCLUSION

Using the workflow outlined with Thermo Fisher Scientific instruments and consumables, biomedical researchers can extract RNA and DNA from fresh frozen and FFPE lung and breast cancer tissues with sufficient yield, purity and quality for downstream genomic and transcriptomic analyses.



Thermo Scientific™ KingFisher™ Flex Purification System

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Ordering information

Instruments	
Thermo Scientific Excelsior ES Tissue Processor	A78400006
Thermo Scientific™ HistoStar™ Embedding Workstation	A81000001
Thermo Scientific™ CryoStar™ NX70 Cryostat	957030H
Thermo Scientific™ HM 355S Automatic Microtome	905200
Thermo Scientific™ KingFisher™ Flex Purification System	5400630
Qubit™ 3.0 Fluorometer	Q33216
Thermo Scientific™ NanoDrop™ 2000c Spectrophotometer	ND2000C
Thermo Scientific™ Sorvall™ Legend™ Micro 21 Microcentrifuge	75002446

Consumables	
Richard Allan Scientific 10% Neutral Buffered Formalin	22-050-104
Histoplast LP Paraffin	8332
RNaseAway	10-328-011
Thermo Scientific™ MX35 Premier Plus Low Profile Blades	3052835
1.5 or 2.0 mL Microcentrifuge Tubes	02-681-271 or 05-408-129
Thermo Scientific™ KingFisher™ (KF) Pure RNA Tissue kit	98040496
Thermo Scientific™ KingFisher™ Cell and Tissue DNA kit	97030196
MagMAX™ FFPE Total Nucleic Acid Isolation kit	4463365
Thermo Scientific™ KingFisher™ Flex™ sterile microtiter deepwell 96 plates	95040460

Acknowledgement

The data presented in the graphs were created in GraphPad Prism 6. (GraphPad Software Inc., La Jolla, CA)

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