

Simultaneously DNA and RNA extraction from formalin-fixed paraffin embedded (FFPE) tissue

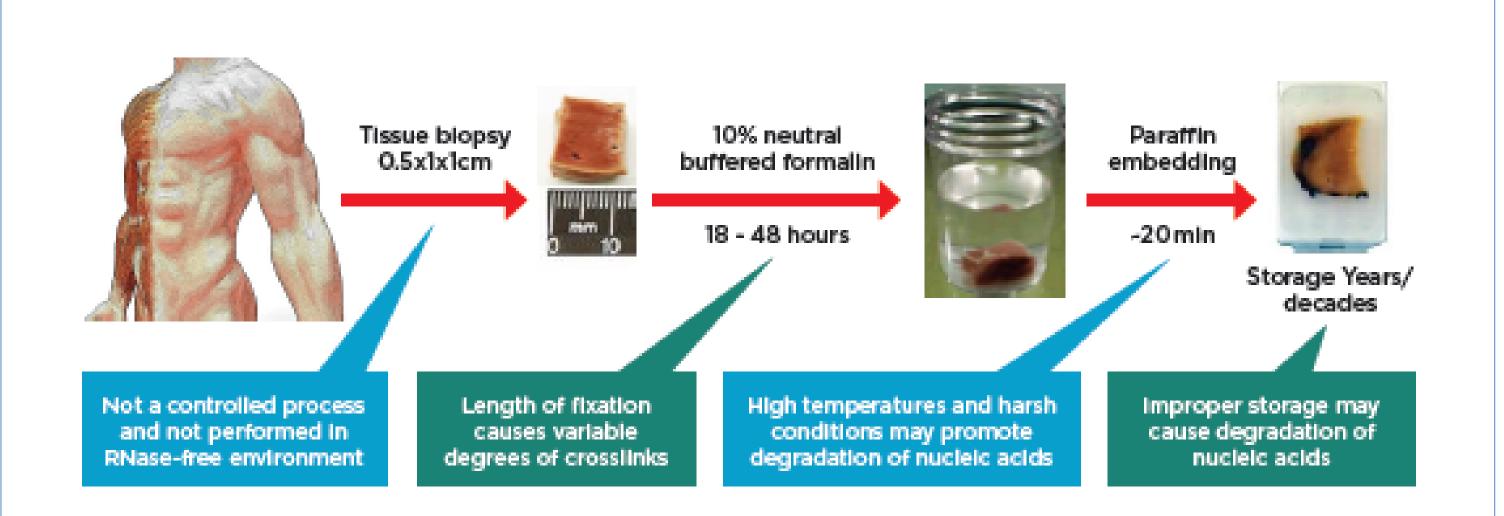
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Introduction

Formalin-fixed, paraffin-embedded (FFPE) tissue is an invaluable study resource for research, especially for biomarker detection. The advances in Next Generation Sequencing (NGS) allows researchers to study genomes, epigenomes and transcriptomes using limited sample material such as FFPE tissue. However, there are challenges for nucleic acids extraction from FFPE tissue due to how FFPE samples are prepared. The nucleic acid quality and integrity are often affected by duration of tissue fixation, age, and storage condition of tissue blocks, and the extraction method.

Here we present a streamlined NGS ready to use sample prep workflow for FFPE tissue using a product in current development, FormaPure XL Total* (Beckman Coulter Life Science). FormaPure XL Total uses SPRI (Solid Phase Reverse Immobilization) paramagnetic beads to isolate DNA and RNA simultaneously. Up to 7 x 10 µm/curls of FFPE tissue can be processed by FormaPure XL Total in a single reaction. For researchers who have high throughput needs, we implemented an automated FFPE extraction method on a Biomek i-Series using FormaPure XL Total. A total of 7 FFPE tissues that represent 4 different tumor types: liver, large intestine, breast and brain were demonstrated on a Biomek i5**. The work flow takes less than 6.5 hours with only 30 minutes hands on time. FormaPure XL Total provides consistent and efficient extraction performance from FFPE tissues, and it is easy to accommodate tissue input variations.

Challenges with FFPE Samples



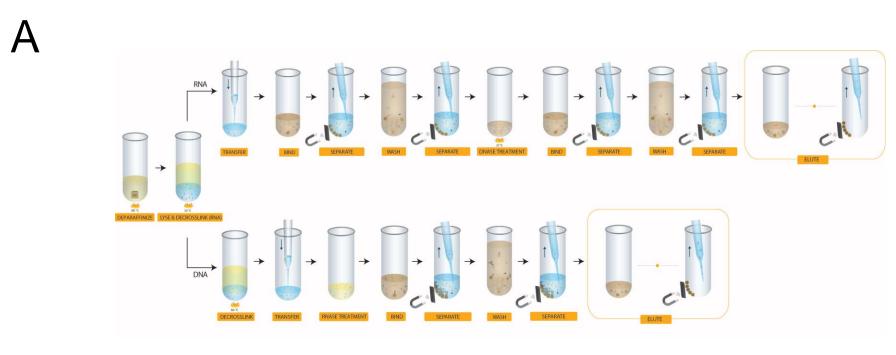
Why trust Beckman extraction

SPRI Technology



Figure 1. AMPure XP chemistry is based on SPRI (Solid Phase Reverse Immobilization) paramagnetic bead technology, where the Nucleic acid is immobilized on beads, contaminants washed away, then eluted. FormaPure XL Total uses SPRI paramagnetic bead technology to isolate DNA and RNA from FFPE samples.

FormaPure XL manual performance data



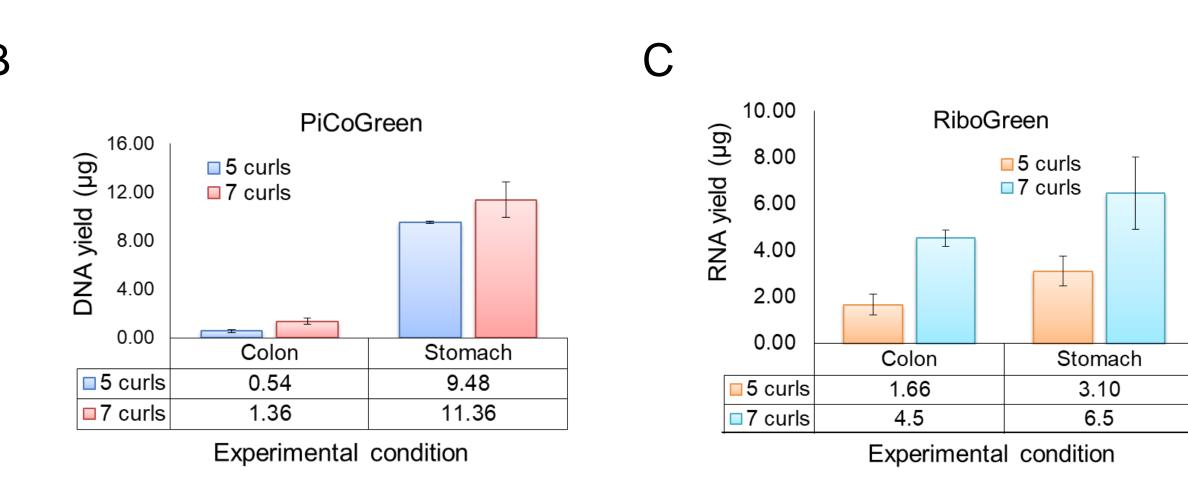


Figure 2. FormaPure XL workflow. FormaPure XL Total uses SPRI paramagnetic beads to isolate DNA and RNA simultaneously. DNA (B) and RNA (C) manual extraction using FormaPure XL. 5 and 7 curls of Colon and stomach cancer FFPE tissues were used in this demonstration. DNA and RNA were quantified by Quant-iT PicoGreen dsDNA Assay and Quant-iT RiboGreen RNA Assay, respectively. Increasing the number of curls can increase the total nucleic acid extracted.

Automated FormaPure XL performance data

96 sample process time

	Total time	Hands on time	Maximum sample/run
Manual	6 h	2h	96
Automation	7 h	0.5h	192

B DNA RNA 30.0 25.0 20.0 20.0 10.0 5.0 0.0 Liver Large Intestine Breast Liver Brain Brain DNA 11.5 12.3 11.9 1.5 6.0 1.2 RNA 5.6 11.7 24.9 5.9 19.4 3.3

C TapeStation			
Sample ID	DNA DV200	RNA DV200	
Liver	93.7	42.5	
Large intestine	87.8	42.9	
Breast	87.0	85.9	
Liver	95.7	24.5	
Brain	97.9	68.8	
Brain	87.1	78.6	

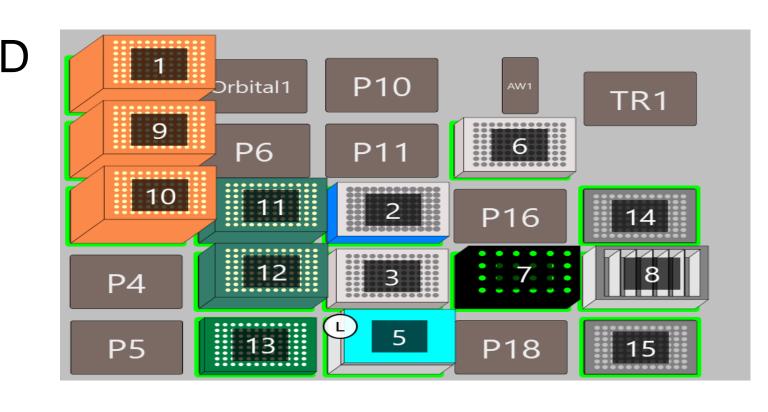
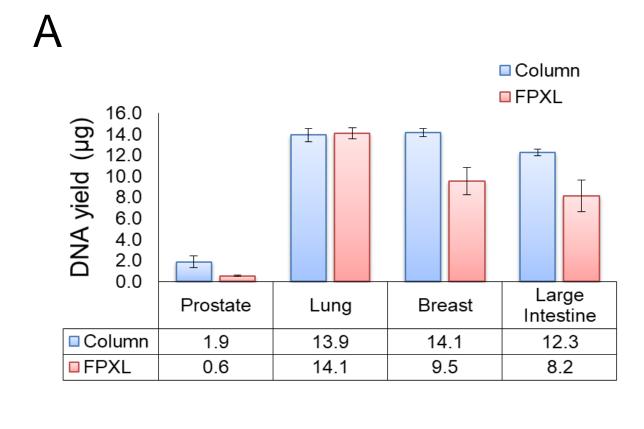
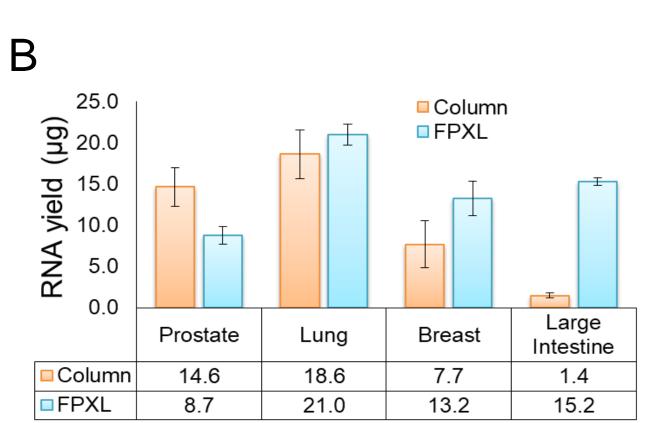
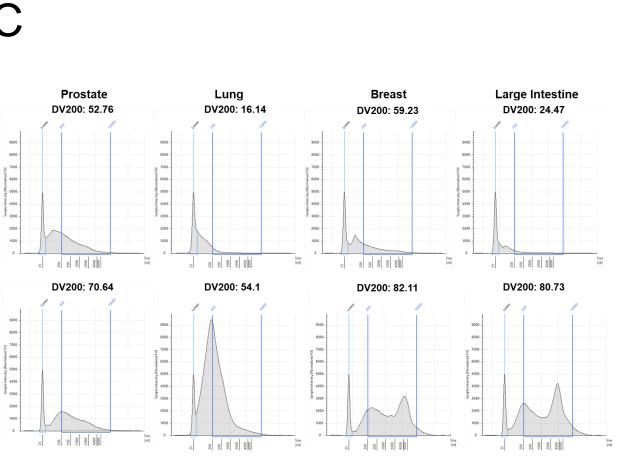


Figure 3. FormaPure XL Total is automated on Biomek i-Series. 7 FFPE tissues that represent 4 different tumor types: liver, large intestine, breast and brain were demonstrated on a i5 Span-8 platform. Sample process time comparison between manual and Biomek automation (A). DNA and RNA were quantified by Quant-iT PicoGreen dsDNA Assay and Quant-iT RiboGreen RNA Assay, respectively (B). The nucleic acid integrity was measured by DV200 (C). Biomek i5 deck layout for nucleic acid extraction from FFPE tissues (D).

FormaPure XL performance comparison







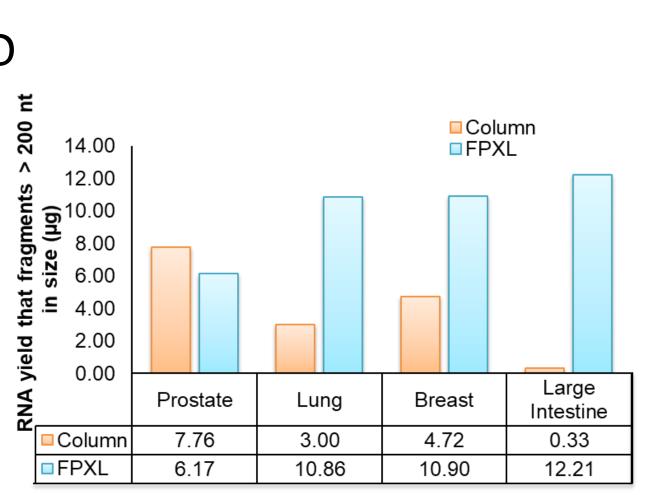


Figure 4. FFPE-derived nucleic acid yield and integrity comparison. Column based method and FormaPure XL total were used in this demonstration. DNA and RNA were quantified by Quant-iT PicoGreen dsDNA Assay and Quant-iT RiboGreen RNA Assay, respectively (A and B). DNA and RNA yields can be balanced by adjusting lysis times. The nucleic acid integrity was measured by DV200 (C). RNA yield that fragments > 200 nt in size (D) was calculated use Total RNA yield * DV200 (%).

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** Not intended or validated for use in the diagnosis of disease or other conditions.

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