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Automated FFPE DNA Extraction Using the FormaPure DNA Extraction Kit using the Biomek series of automated liquid handlers from Beckman Coulter, Inc.

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Introduction and Method Description

Extraction of high quality DNA from formalin fixed paraffin embedded (FFPE) tissue is essential for many cancer research applications. The FormaPure DNA extraction kit from Beckman Coulter, Inc. provides a convenient extraction process utilizing Beckman Coulter proprietary solid phase reversible immobilization (SPRI) chemistry to extract DNA from FFPE curls. Briefly, FFPE curls are deparaffinized, after which a lysis buffer is added in conjunction with Proteinase K to lyse the tissue. Following tissue digestion, the DNA is decrosslinked and the lysate is transferred to a new plate. RNase A is then used to digest unwanted RNA from the sample, after which the remaining DNA is bound to magnetic beads using a proprietary binding buffer. Following binding, the beads containing the DNA are washed to remove unwanted nucleotides and other contaminants, after which the DNA is eluted from the beads (Figure 1).

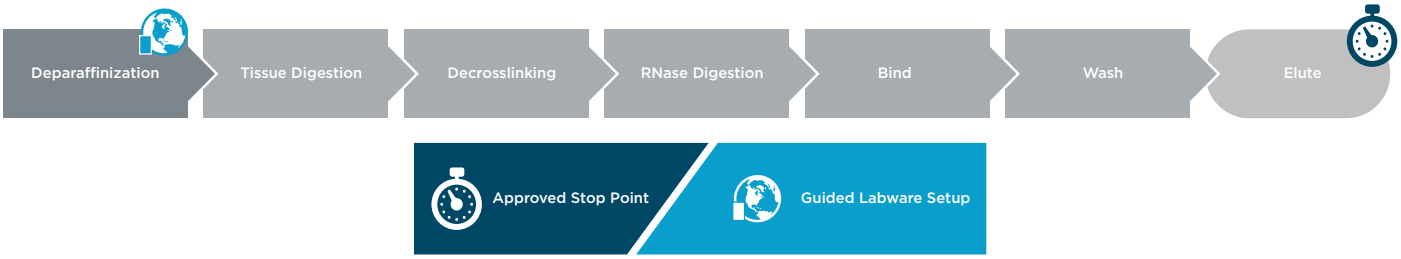

Figure 1. FormaPure DNA automation workflow

In this technical note, we describe the automation of the FormaPure DNA extraction kit on the Biomek FX^P Dual Arm Multi-channel 96 and Span-8 automated liquid handler (Biomek FX^P) as shown in Figure 2. The automation method utilizes an HTML-driven user interface (UI) in conjunction with Biomek Method Launcher software to provide a simplified and efficient user experience. The user may specify the number of samples to be processed (any number from one to 96). Additionally, the user can configure a number of method options at run-time, including deparaffinization time, deparaffinization temperature, decrosslinking time, decrosslinking temperature, ethanol drying time, and elution volume to optimize the method for a variety of different tissue types. A screen-shot of the UI is shown in Figure 3. For ease of use, the method also utilizes Biomek Method Launcher's Guided Labware Step to guide the user through setting up the instrument with clear instructions for the placement of labware and reagents on the deck. Additionally, the method can be launched through the Biomek Method Launcher interface for a simplified run-time experience[1].

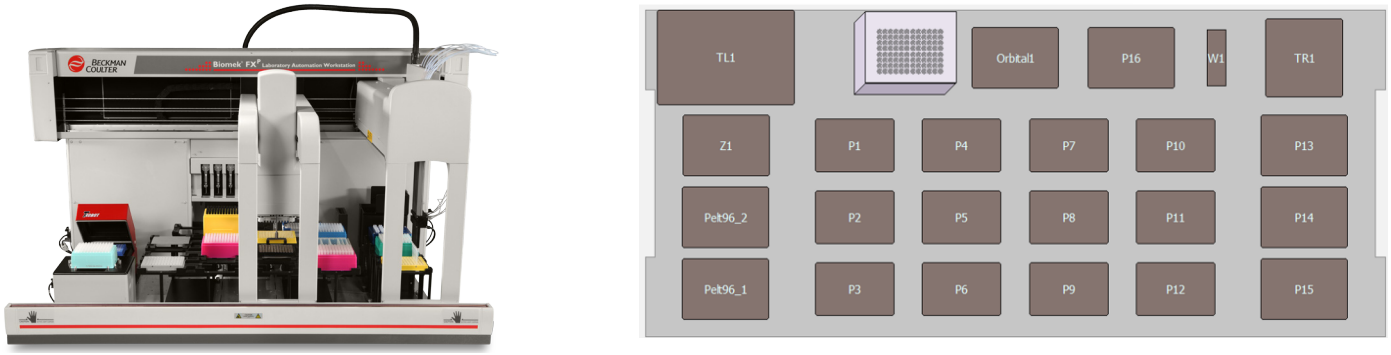

Figure 1. Beckman Coulter Biomek FX^P with deck layout.

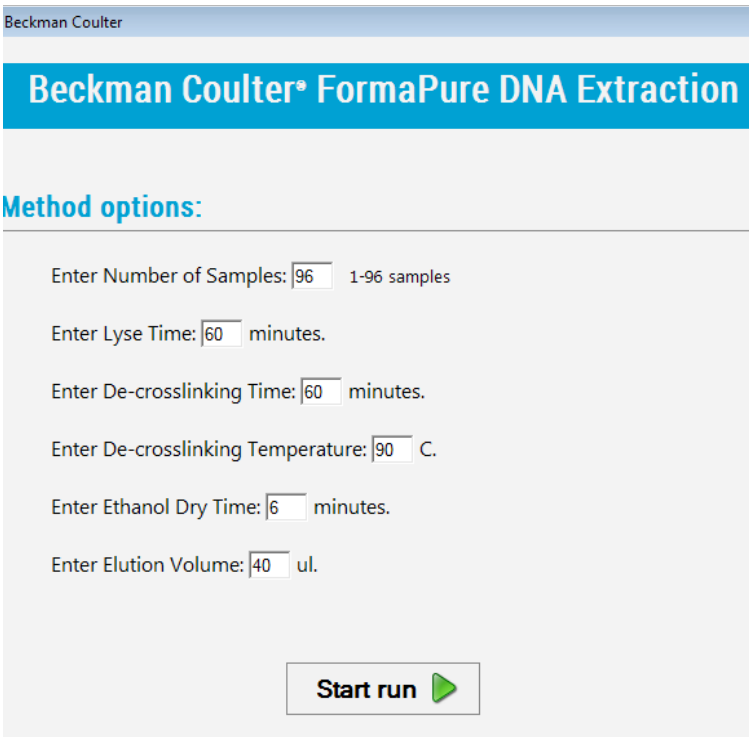

Figure 3. FormaPure DNA automation method UI

In addition to the flexibility provided by the UI, the automation method can be configured to accommodate the user's particular instrument. The method can be configured to utilize one or two static peltiers. Additionally, for the deparaffinization, tissue digestion, and decrosslinking the method can be configured to utilize either a 96 well plate or to use a custom tube rack with Matrix™ 2D Barcoded Clear Polypropylene Open-Top Storage 1.4 ml storage tubes OR Thermo Scientific MATRIX 1.4 2D storage tubes. These tubes come with etched 2D barcodes on the bottom of the tube, allowing for efficient sample tracking when combined with the Thermo Scientific VisionMate or Ziath Datapaq high speed barcode readers. The automation method can extract DNA from up to 96 FFPE curls in approximately 6 hours with no user interventions required (Figure 4). This protocol has also been automated on the Biomek 4000 automated liquid handler.

Major Process Description	Automated/ Hands on Time		
	24 Samples	48 Samples	96 Samples
FormaPure DNA			
Prepare Reagents/Set up Inst	15 min	20 min	30 min
Method Run	4hrs 45min	5hrs 1 min	5 hrs 26 min
Total	**5 hrs**	**5 hrs 21 min**	**5 hrs 56 min**

Figure 4. Time estimates for the FormaPure DNA automation method. Time estimates assume a one hour tissue digestion and decrosslinking incubations

Experimental Design and Results

To test the automation method, 48 Horizon Quantitative Multiplex FFPE Reference Standards (Horizon) were arrayed in an AB-0765 titer plate (ThermoFisher) in a checkerboard pattern as shown below in Figure 5. The automation method was set to process all 96 wells with default parameters except for an 85°C decrosslinking temperature instead of the default 90°C decrosslinking temperature.

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

Figure 5. FormaPure DNA sample plate layout

Extracted DNA was eluted in 40 ul of nuclease-free water and quantified using a Qubit 3.0 fluorometer (ThermoFisher) using the Qubit dsDNA HS Assay Kit. Qubit yields are shown below in Figure 6. Negative controls selected from the empty well were below Qubit detection limits. No significant variation was found in yields between columns (n = 48, p = 0.969) or rows (n = 48, p = 0.856). Expected yield from each Horizon Quantitative Multiplex FFPE Reference Standard is approximately 400ng.

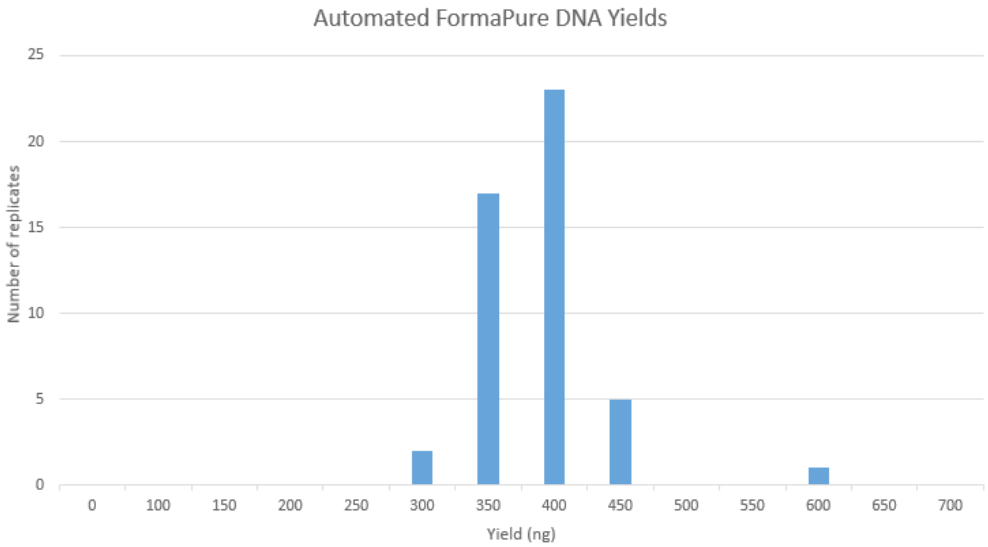

Figure 6. FormaPure DNA automated method Qubit yields from Horizon samples

In addition to Qubit quantification, eight randomly selected automated FormaPure DNA samples were subjected to the Kapa hgDNA Quantification and QC Kit (Kapa Biosystems) to determine sample quality. This kit allows the generation of "Q-ratios", which are calculated based on the amplification efficiency of three primer pairs that amplify targets of 41bp, 129bp, and 305bp respectively. Generally, high quality DNA will have a $Q_{129\ bp}/Q_{41\ bp}$ ratio around 1 and a $Q_{305\ bp}/Q_{41\ bp}$ ratio also around 1, while lower quality DNA will have $Q_{129\ bp}/Q_{41\ bp}$ ratio less than 1 and a $Q_{305\ bp}/Q_{41\ bp}$ ratio much less than 1. Q-ratios for the eight selected samples are presented in Figure 7.

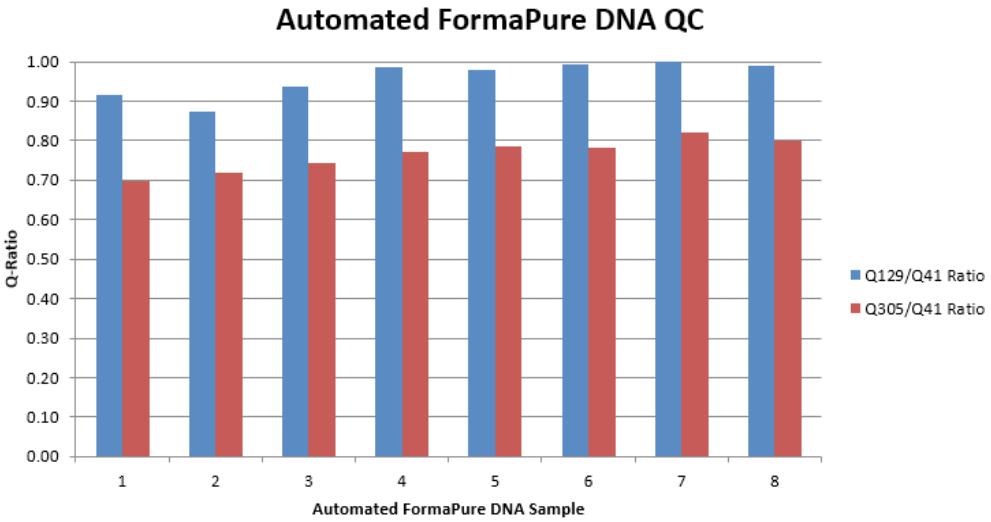

Figure 7. Q-ratios for selected automated FormaPure DNA samples

Conclusion

We have demonstrated that the Biomek FX^{P} automation method for extracting DNA from FFPE curls utilizing the FormaPure DNA Extraction Kit results in high quality DNA with expected yields from reference FFPE slices.

References

1. Biomek Method Launcher not compatible with Biomek Accounts and Permissions authentication.

For Beckman Coulter's worldwide office locations and phone numbers, please visit "Contact Us" at **beckman.com**

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