



Automated Illumina TruSeq DNA PCR-Free Sample Preparation on the Biomek 4000 Laboratory Automated Workstation

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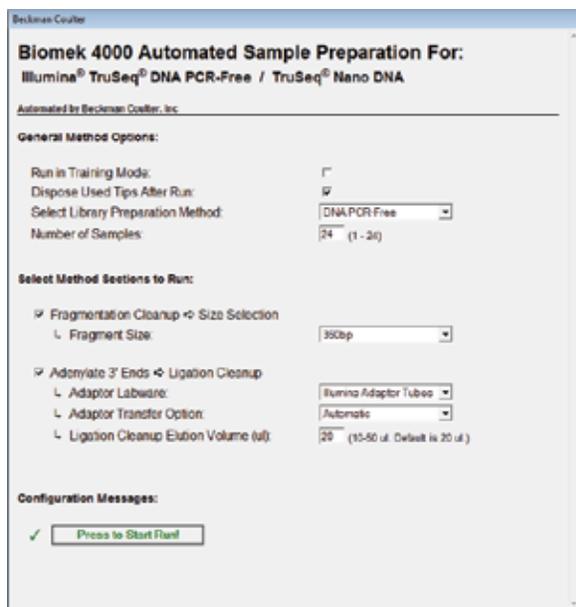
Introduction

The Illumina TruSeq DNA PCR-Free Sample Preparation workflow is designed to generate whole genome libraries without the need for PCR amplification, thereby reducing library bias and gaps and allowing for detection of the greatest number of variants in a wide variety of organisms. Solid Phase Reversible Immobilization (SPRI) bead chemistry is utilized throughout the protocol for size selection and sample cleanup steps. SPRI bead chemistry provides an accelerated workflow and greater sample recovery as compared to gel-based size selection.

The automated Biomek 4000 method enhances the benefits of the TruSeq DNA PCR-Free kit by adding walk-away capability for all pipetting and liquid handling operations, variable workflow options and temperature controlled reagent storage to deliver consistently high quality whole genome libraries. The automated method is compatible with either the LT or HT versions of the Illumina TruSeq DNA PCR-Free Sample Preparation kits (Illumina P/N FC-121-3001, FC-121-3002 or FC-121-3003).

Automated Method Features and Key Benefits

The automated Illumina TruSeq DNA PCR-Free Sample Preparation workflow features an intuitive User Interface (Figure 1) that allows for selection of customizable workflow options. The user may process any number of samples between 1 and 24 and may run the full method or start/stop the method at the Illumina recommended safe-stopping points to allow for maximum flexibility in planning their experiments. Options selected via the User Interface will dynamically update a Reagent Calculator which provides the user with instructions for preparation of Master Mixes and reagent labware setup (Figure 2).



Biomek 4000 Automated Sample Preparation For:
Illumina® TruSeq® DNA PCR-Free / TruSeq® Nano DNA

Automated by Beckman Coulter, Inc.

General Method Options:

Run in Training Mode:

Dispose Used Tips After Run:

Select Library Preparation Method: DNA PCR Free

Number of Samples: 24 (1 - 24)

Select Method Sections to Run:

Fragmentation on Cleanup ↔ Size Selection

Fragment Size: 350bp

Adenylyate 3' Ends ↔ Ligation Cleanup

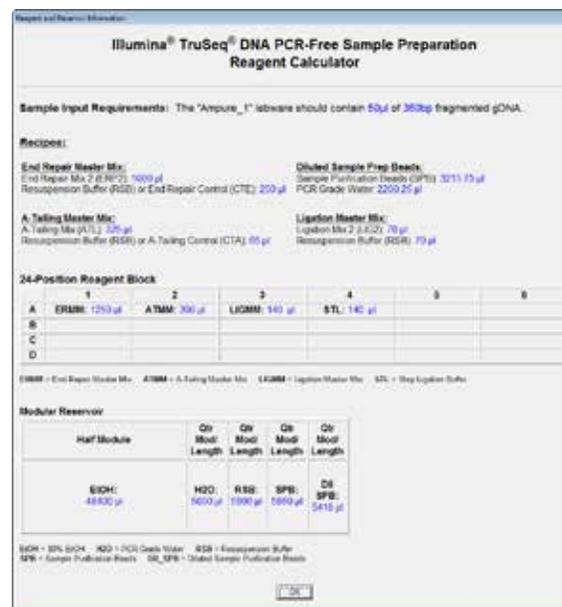
Adaptor Labware: Illumina Adaptor Tubes

Adaptor Transfer Option: Automatic

Ligation Cleanup Elution Volume (ul): 20 (10-50 ul. Default is 20 ul.)

Configuration Messages:

Figure 1. Automated Illumina TruSeq DNA PCR-Free Sample Preparation method User Interface



Illumina® TruSeq® DNA PCR-Free Sample Preparation Reagent Calculator

Sample Input Requirements: The "Ampure_1" labware should contain 50ul of 350bp fragmented gDNA.

Recipes:

End Repair Master Mix: End Repair Mix 2 (ER2) 5000 ul, Resuspension Buffer (RSB) or End Repair Control (ERC) 200 ul

Diluted Sample Prep Beads: Sample Purification Beads (SPB) 3215.75 ul, PCR Grade Water 2200.25 ul

A-Tailing Master Mix: A-Tailing Mix (ATL) 200 ul, Resuspension Buffer (RSB) or A-Tailing Control (ATC) 60 ul

Ligation Master Mix: Ligation Mix 2 (LIG2) 70 ul, Resuspension Buffer (RSB) 79 ul

24-Position Reagent Block:

	1	2	3	4	5	6
A	ER2B: 1250 ul	ATMM: 300 ul	LIGMM: 140 ul	STL: 140 ul		
B						
C						
D						

ER2B = End Repair Master Mix, ATMM = A-Tailing Master Mix, LIGMM = Ligation Master Mix, STL = Sticky End Ligation Buffer

Modular Reservoir

Half Module	Qty	Mod	Qty	Mod	Qty	Mod	
	Mod	Length	Mod	Length	Mod	Length	
ER2B	4800	ul	H2O	5000	ul	RSB	1900
			SPB	5900	ul	DS	5410

ER2B = 30% ER2, H2O = PCR Grade Water, RSB = Resuspension Buffer, SPB = Sample Purification Beads, DS_SPB = Diluted Sample Purification Beads

Figure 2. Automated Illumina TruSeq PCR-Free Reagent Calculator



A full 24-sample run can be completed in ~5 ½ hours including reagent and instrument setup (Figure 3). Options for either 350bp or 550bp library insert size are available as well as various adapter labware options, including use of the Illumina 96-well DNA adapter plate or custom adapter labware. Adapter transfers can be accomplished either through pre-set well-mapping patterns or via a user-defined transfer file.

The method employs both 8 channel and single channel pipetting to deliver the right balance of efficiency and reagent conservation during the method run. Customized pipette tip and labware utilization steps reduce consumable usage. A Peltier unit ensures that enzyme master mixes are kept cool during the course of the method.

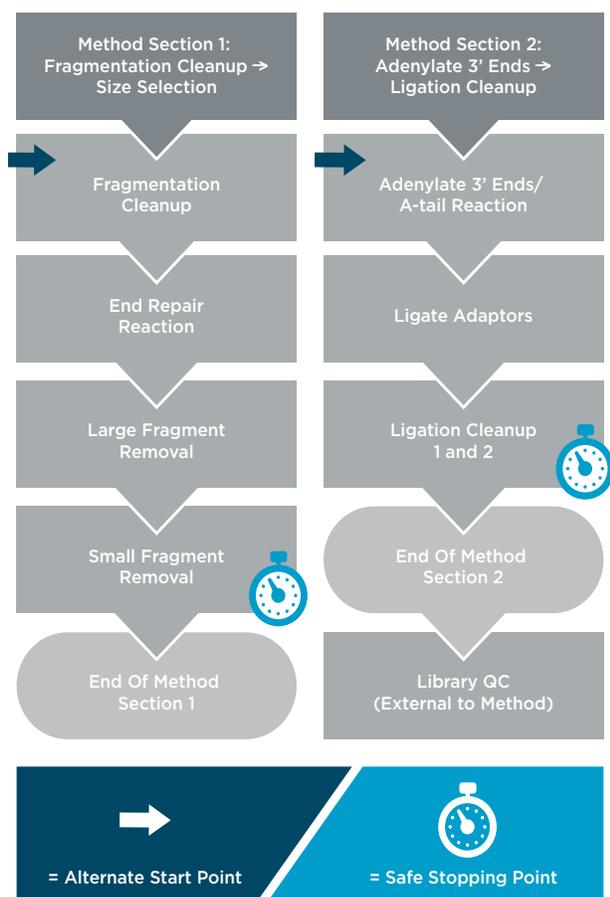


Figure 3A. Automated Illumina TruSeq DNA PCR-Free workflow.

Method Section	Sample Number* / Processing Time (h:m)	
Fragmentation Cleanup → Size Selection	8	24
Method Setup	0:15	0:15
Method Run	2:12	2:39
Total	2:27	2:54
Adenylylate 3' Ends → Ligation Cleanup	8	24
Method Setup	0:15	0:15
Method Run	2:23	2:58
Total	2:38	3:13
Full Method	8	24
Method Setup	0:15	0:15
Method Run	4:18	5:16
Total	4:33	5:31

Figure 3B. The entire method may be performed with a single instrument setup or for added flexibility; each Method Section may be executed separately. *Any number of samples may be run between 1 and 24.

Experimental Design and Results

Genomic DNA (Promega P/N: G3041) was sheared using a Covaris S220 focused Ultrasonicator utilizing the settings defined in the Illumina TruSeq DNA PCR-Free Sample Preparation Kit protocol for a 350bp insert size. 1µg of the sheared DNA was loaded in a volume of 50ul for each of 6 technical replicates. In addition, two non-template control samples (NTC) were processed alongside the positive control samples. Samples were processed using the full Illumina TruSeq DNA PCR-Free Sample Preparation protocol automated on the Biomek 4000 Laboratory Automated Workstation. Library quality was assessed using an Agilent DNA High Sensitivity chip assayed on the Bioanalyzer 2100 instrument and analyzed with the Agilent 2100 Expert Software (Figure 4). Samples were quantified using the KAPA SYBR Fast Universal 2X qPCR Master Mix library quantification kits (PN# KK4824, KAPA Biosystems) and assayed on a 7900 HT (Applied Biosystems) real-time PCR Instrument (Figure 4).

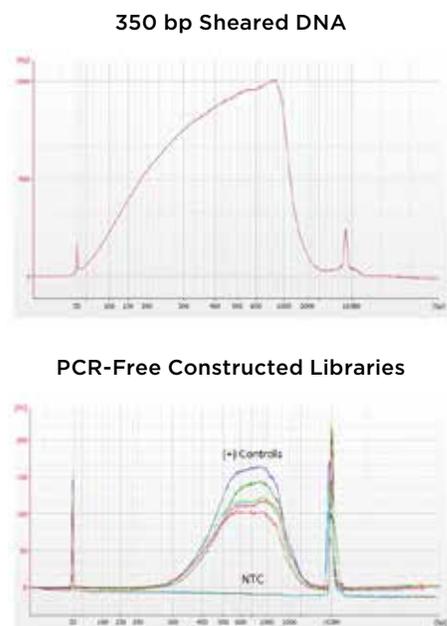


Figure 4A. 350bp sheared DNA and final prepared library tracings assayed on the Agilent 2100 Bioanalyzer on a DNA High Sensitivity chip.

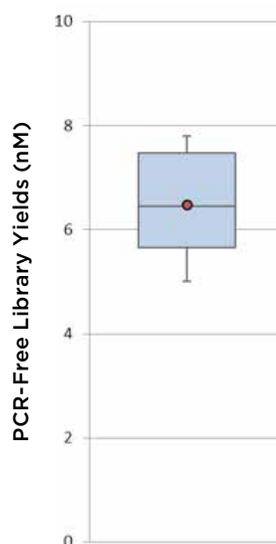


Figure 4B. PCR-Free library yields assayed with KAPA Biosystems Library Quantitation kit.

Summary

The Illumina TruSeq DNA PCR-Free Sample Preparation kit automated on the Biomek 4000 Laboratory Automated Workstation was demonstrated to provide a robust, flexible and efficient walk-away whole genome library preparation workflow. 24 high quality sequence-ready libraries can be prepared in as little as 5 ½ hours which are suitable for sequencing on all Illumina platforms. An intuitive User Interface guides the user through run configuration options while a dynamic Reagent Calculator provides reagent setup information based on user inputs.

To request more information about this technical note or access additional educational resources, please contact your local sales representative or visit beckmanngs.com



Biomek Automated NGS Library Preparation Methods

DEMONSTRATED/VALIDATED METHOD		BIOMEK FX [®] HYBRID	BIOMEK FX [®] MULTI-CHANNEL	BIOMEK FX [®] SPAN 8	BIOMEK NX [®] SPAN 8	BIOMEK NX [®] MULTI-CHANNEL	BIOMEK 3000	BIOMEK 4000
DNA SEQUENCING	SPRIworks HT - Illumina	■			■			
	SPRIworks HT with Bead	■						
	Illumina TruSeq [®] DNA PCR-Free	■						■
	Illumina TruSeq [®] Nano DNA	■			■			■
	Illumina Nextera [®] XT	●						
	NEBNext [®] Ultra DNA for Illumina NGS (ChIP-seq and HLA)	■						
	Illumina TruSight [®] HLA Sequencing Panel	●			■			
	Rubicon Genomics ThruPLEX [®] Plasma-seq Kit for Illumina NGS	■						
	KAPA Hyper Prep Library Prep Kit for Illumina NGS	▲						
	KAPA HyperPlus Library Prep Kit for Illumina NGS	▲						
	Rubicon Genomics ThruPLEX [®] DNA-seq Kit for Illumina NGS	■						
	Swift Biosciences Accel - NGS [®] 2S Plus DNA Library Kit for Illumina NGS	■						
	NEBNext [®] Ultra DNA II Kit for Illumina NGS	■						
	RNA SEQUENCING	Illumina TruSeq [®] RNA v2	■					
Illumina TruSeq [®] RNA Access		■						
Illumina TruSeq [®] Stranded mRNA		●						
Illumina TruSeq [®] Stranded Total RNA		●						
NEBNext [™] Ultra Directional RNA Library Kit for Illumina NGS		■						
NEBNext [™] II RNA Library Prep Set for Illumina NGS								■
NEBNext Small RNA Kit for Illumina NGS								■
TARGET/EXOME CAPTURE	Epicentre ScriptSeq [®] Complete Gold Low Input	●						
	Nimblegen SeqCap EZ [®] for Illumina NGS	■						
	Agilent HaloPlex [™] Target Enrichment - Ion Torrent	■			■			■
	Illumina Nextera [®] Rapid Capture	●						
	Agilent SureSelect XT [®]	■						
DNA AND RNA PURIFICATION/ SIZE SELECTION/ QUANTITATION/ NORMALIZATION/ POOLING	KAPA Biosystems Library Quantification Kit - Illumina	■			■			
	SPRIselect for DNA Size Selection	■			■			■
	qPCR setup 384	■			■			■
	qPCR setup 96	■			■			■
	Quantitation/Normalization	■			■			■
	Sample Pooling for Multiplexing	■						
	AMPure XP - PCR Purification	■	■		■	■	■	■
	RNAClean XP - Post cDNA Purification and Post-IVT cRNA Purification	■	■			■		■
NUCLEIC ACID EXTRACTION AND CLEANUP	CleanSEQ - Dye Terminator Removal	■	■			■	■	■
	CosMCPrep, High/Low Copy Plasmid Extraction	■	■			■		
	DNAdvance - DNA Isolation from Mammalian Tissue	■	■			■		■
	Genfind v2, gDNA from Blood	■		■	■			
	FormaPure - Nucleic Acid Isolation from FFPE Tissue	■		■	■			
	RNAdvance Blood - RNA from Paxgene Preserved Blood	■		■	■			■
	RNAdvance Tissue	■		■	■			
	RNAdvance Cell v2 - RNA Isolation from Tissues	■	■	■	■	■		



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