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Automated Illumina TruSeq Nano DNA Sample Preparation on the Biomek 4000 Laboratory Automated Workstation

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Introduction

The Illumina TruSeq Nano DNA Sample Preparation workflow is designed to generate whole genome libraries with sample input requirements as low as 100ng, enabling researchers to study samples with limited available DNA. Enhanced PCR chemistry reduces the number and average size of typical PCR-induced gaps in coverage, reduces library bias and improves coverage uniformity across the genome. 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 Nano DNA 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 Nano DNA Sample Preparation kits (Illumina P/N FC-121-4001, FC-121-4002 or FC-121-4003).

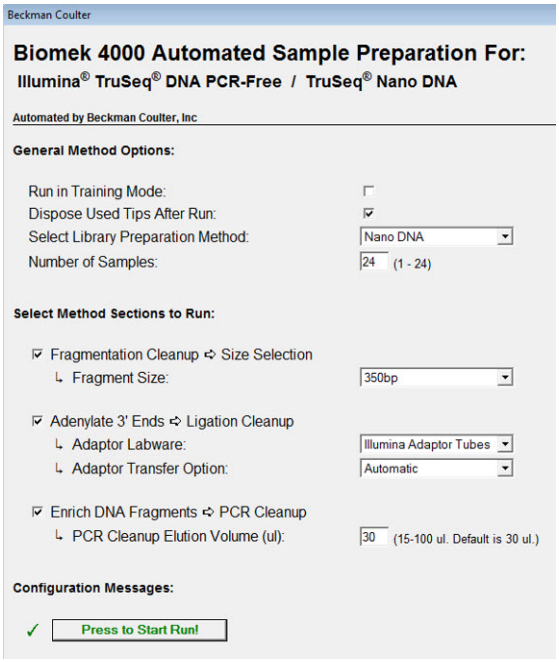

Figure 1. Automated Illumina TruSeq Nano DNA Sample Preparation method User Interface.

Automated Method Features and Key Benefits

The automated Illumina TruSeq Nano DNA 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. A full 24-sample run can be completed in ~7 hours including reagent and instrument setup (Figure 2). Options for either 350bp or 550bp library insert size selection 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. 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 3).

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.

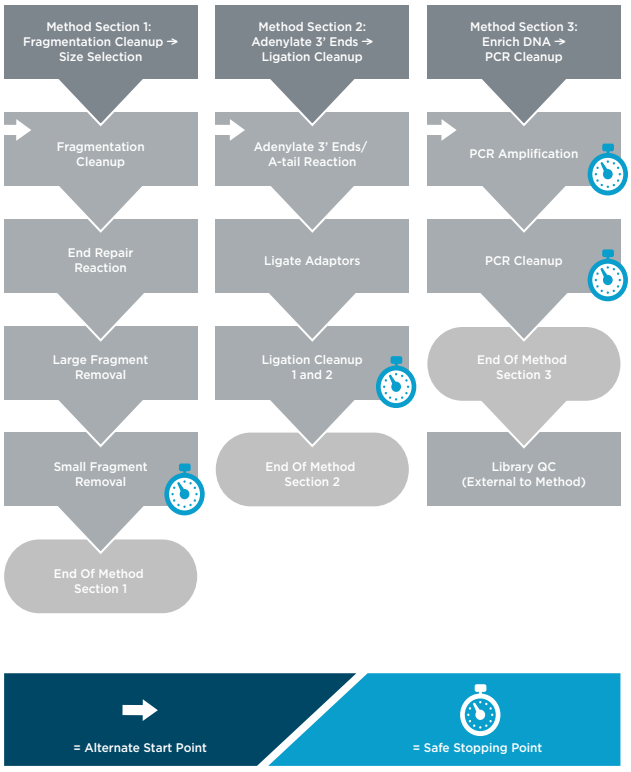

Figure 2A. Automated Illumina TruSeq Nano DNA workflow.

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**
Adenylate 3' Ends → Ligation Cleanup	**8**	**24**
Method Setup	0:15	0:15
Method Run	2:23	2:58
Total	**2:38**	**3:13**
Enrich DNA Fragments → PCR Cleanup	**8**	**24**
Method Setup	0:15	0:15
Method Run	1:17	1:35
Total	**1:32**	**1:50**
Full Method	**8**	**24**
Method Setup	0:15	0:15
Method Run	5:49	7:04
Total	**6:04**	**7:19**

Figure 2B. 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.

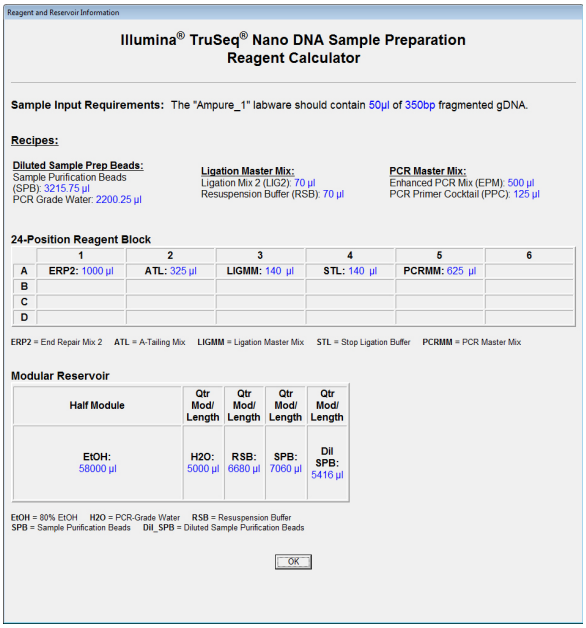

Figure 3. Automated Illumina TruSeq Nano DNA Sample Preparation Reagent Calculator.

Experimental Design and Results

Genomic DNA (Promega P/N: G3041) was sheared using a Covaris S220 focused Ultrasonicator using the settings defined in the Illumina TruSeq Nano DNA Sample Preparation protocol for a 350bp insert size. 100 ng of the sheared DNA was prepared in a volume of 50µl for each of 6 positive control technical replicates. In addition, two non-template control samples (NTC) were prepared. All control samples were processed using the full Illumina TruSeq Nano DNA 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).

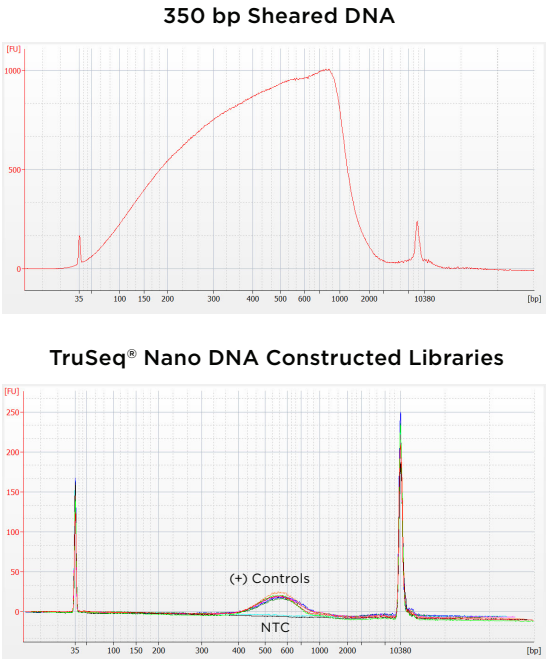

Figure 4A. 350bp sheared DNA and final prepared TruSeq Nano DNA libraries assayed on the Agilent 2100 Bioanalyzer on a DNA High Sensitivity chip.

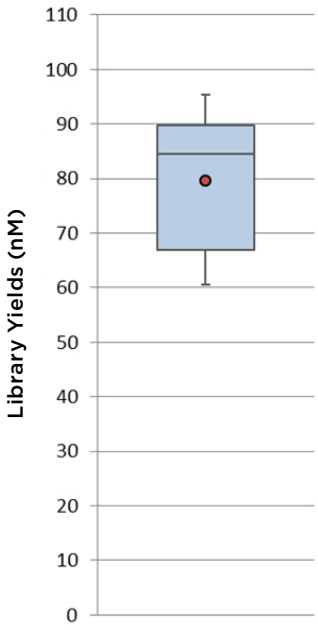

Figure 4B. TruSeq Nano DNA library yields assayed with KAPA Biosystems Library Quantitation kit.

Summary

The Illumina TruSeq Nano DNA Sample Preparation chemistry 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 from low-input samples can be prepared in as little as 7 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.

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