



Biomek Automated Genomic Sample Prep Accelerates Research

Biomek i-Series Automated Illumina TruSeq® Nano DNA Library Prep Kit

Introduction

Illumina TruSeq Nano DNA Library Prep Kit enables preparation of uniquely indexed DNA libraries for single end and paired end sequencing (Figure 1). Especially designed for low sample input, this method is known to deliver high coverage across the genomes and reduce library bias (Illumina TruSeq® Nano DNA Library Prep Guide15041110-d). The method avoids the sample loss associated with gel-based selection by the use of a bead-based size selection strategy. Therefore, the Nano DNA Library Prep Kit is widely used across many NGS applications. In this technical note, we will demonstrate automated performance of the Kit on the Biomek i7 Dual Hybrid (Multichannel 96, Span-8) Genomics Workstation.

When compared to manual pipetting, the Illumina TruSeq Nano DNA Library Prep Kit automated on a Biomek platform provides:

- · Reduced hands-on-time and increased throughput
- · Reduction in pipetting errors
- · Reduction of cost by using low reagent volumes
- Standardized workflow for improved results
- · Quick implementation with demonstrated methods
- · Knowledgeable support for reagents, automation and methods all from a single vendor

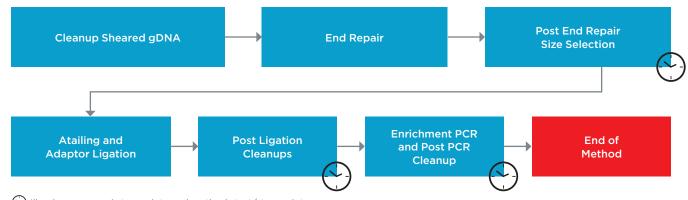
Spotlight: Biomek i7 Dual Hybrid (Multichannel 96, Span-8) **Genomics Workstation**

System features deliver reliability and efficiency to increase user confidence and walk-away time, compared to manual operation.

- 300uL or 1200uL Multichannel head with 1-300uL and 1-1200uL pipetting capability
- · Span-8 pod with fixed and disposable tips
- Enhanced Selective Tip pipetting to transfer custom array of samples
- Independent 360° rotating gripper with offset fingers optimize access to high density decks
- · High deck capacity with 45 positions
- Orbital Shakers, peltiers, span-8 and 96 channel Tip washing for controlling sample processing
- Spacious open platform design to integrate on-deck and off-deck elements (e.g. thermocyclers)







() Illumina approved stop points and method start/stop points

Figure 1. Illumina TruSeq® Nano DNA Library Prep Kit protocol.

Process	Time		
	24 Samples	96 Samples	
Prepare Reagents, Set up Instrument	15 min	30 min	
Method Run (walk away time)	4 hr, 52 min	5 hr, 51 min	
Total*	5 hr, 7 min	6 hr, 21 min	

^{*}Timing estimate includes incubations and thermocycling. Timing estimate does not include reagent thawing.

Table 1. Estimated run times for Illumina TruSeq® Nano DNA Library Prep Kit on the Biomek i7 Dual Hybrid Genomics Workstation.

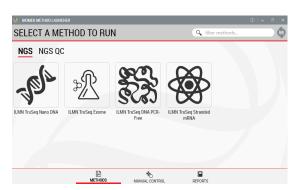


Figure 2. Biomek Method Launcher provides an easy interface to start the method

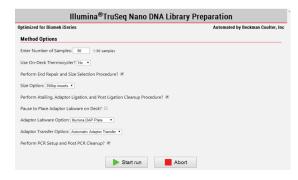


Figure 3. Biomek Method Options Selector indicate sample number and processing options

Automated Method

Automation provides increased efficiency, reducing the hands-on time (Table 1). The automated TruSeq Nano DNA Library Prep Kit protocol includes logical start and stop points assigned based on Illumina's recommendations, providing the users flexibility in scheduling their day (Figure 1).

1. Biomek Method Launcher (BML)

BML is a secure interface for method implementation without introducing errors during method setup (Figure 2). Within BML, the method steps are organized in a modular manner for workflow optimization (Figure 1).

2. Method Options Selector (MOS)

MOS enables selection of sample number and sample processing options (e.g. insert size selection, adaptor transfer options) to maximize flexibility, adaptability and the ease of method execution (Figure 3). The thermocycling steps can be done either off-deck or on-deck using an automated thermocycler (ATC Thermo Fisher) (Figure 4).

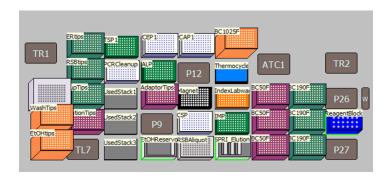


Figure 4. Deck Layout for Illumina TruSeq® Nano DNA Library Prep Kit protocol on Biomek i7 Dual Hybrid for 96 samples with on-deck thermocycling option

3. Guided Labware Setup (GLS)

GLS provides the user specific text and graphical setup instructions with reagent volume calculation and step by step instructions to prepare reagents (Figure 4). The automated method supports placing Illumina low throughput and high throughput adaptor labware on deck along with custom adaptor plates (Figure 5). The GLS steps are generated based on the options selected in MOS. For instance, selecting automatic adaptor transfer creates dataset driven adaptor ID logs. Users also have the ability to customize adaptor assignments by uploading a .csv file.

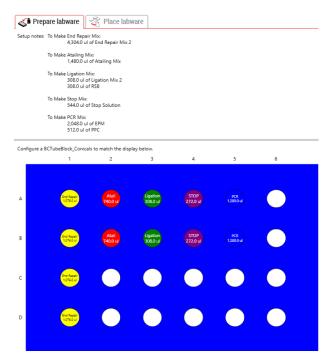


Figure 5. Guided Labware Setup indicates calculated reagent volumes required to make reagent mixes and guides the user for correct deck setup

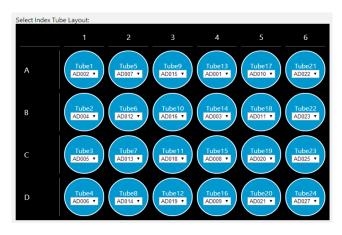


Figure 6. Guided Labware Setup enables selecting index tube layout

Experimental Design

Promega Human gDNA (200 ng/µl) was used for the automation of the Nano DNA Library Prep kit protocol on the Biomek i7 Dual Hybrid (Multichannel 96, Span-8) Genomics Workstation. After the preparation, the libraries were analyzed on Agilent TapeStation 2200 with High Sensitivity D5000 Kit and by qPCR (Applied Biosystems 7900HT Fast real time PCR system) using KAPA Library Quantification Kit.

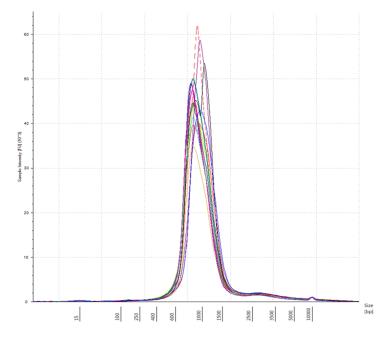
Results

Agilent TapeStation results indicated that the prepared libraries are of expected size (Approximately around 900 base pairs, Illumina TruSeq® Nano DNA Library Prep Guide15041110-d; Table 2; Figure 7). The yields according to qPCR show that the libraries are suitable for sequencing (Approximately 10nM of samples are used for the proceeding steps, Illumina TruSeq® Nano DNA Library Prep Guide15041110-d; Table 2).

Sample ID	Mass (ng)	Insert Size (bp)	Index	TapeStation Size (bp)	qPCR Yield (nM)
Promega Human gDNA rep1	200	550	AD001	845	452
Promega Human gDNA rep2	200	550	AD003	917	383.4
Promega Human gDNA rep3	200	550	AD008	960	383.4
Promega Human gDNA rep4	200	550	AD009	547	365.3
Promega Human gDNA rep5	200	550	AD010	958	549.8
Promega Human gDNA rep6	200	550	AD011	849	398.8
Promega Human gDNA rep7	200	550	AD020	879	389.8
Promega Human gDNA rep8	200	550	AD022	842	453.5
Promega Human gDNA rep9	200	550	AD023	838	476.6
Promega Human gDNA rep10	200	550	AD025	846	481.3
Promega Human gDNA rep11	200	550	AD027	837	454.5
Promega Human gDNA rep12	200	550	AD002	812	401.6
Promega Human gDNA rep13	200	550	AD004	852	404.9
Promega Human gDNA rep14	200	550	AD005	905	393.9
No Template Control	0	550	AD006	0	0

Table 2. Library quantification of automated Illumina TruSeq* Nano DNA Library Prep kit protocol using Agilent TapeStation 2200 and qPCR using the Kapa Illumina Library Quantification Kit.

Figure 7. Electropherogram (Sample intensity vs. size in base pairs) of Agilent TapeStation corresponding to UHR250 replicate3 showing the libraries around expected size of the marker



Summary

We demonstrated the automation of Illumina TruSeq® Nano DNA Library Prep kit on Biomek i7 Dual Hybrid (Multichannel 96, Span-8) Genomics Workstation. The quality and the quantity of prepared libraries indicate that they are appropriate for sequencing. Automation increases the library preparation efficiency by reducing the hands-on time. In addition, by the use of Biomek Method Launcher the user can easily customize (i.e. change sample number and select a module to run) and run the method.



Data obtained during development

Biomek i-Series Automated Workstations are not intended or validated for use in the diagnosis of disease or other conditions.

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