Automated Library Preparation using NEBNext Ultra DNA Library Prep Kit for Illumina (E7370) on the Hamilton STARline

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ABSTRACT

Library preparation from low amounts of input DNA is a key requirement for many Next Generation Sequencing (NGS) applications such as sequencing precious clinical samples or ChIP-seq libraries. Reagents from New England Biolabs® provide an ideal solution for generation of indexed NGS libraries over a broad range of input amounts from 5 ng to 1µg of genomic DNA. In this application note, we describe the automation of the NEBNext® Ultra™ DNA Library Prep Kit for Illumina® on a Hamilton Microlab STARlet instrument. The design allows a capacity of 8-48 samples per run. Ready to sequence libraries with high yield and quality can be obtained from different sample sources.

System Benefits

- ***Optimized throughput from 8 to 48 libraries per run***
- ***Wide range of input from 5 ng to 1 µg genomic DNA***
- ***Automated master mix generation on deck***
- ***Independent size selection per sample in a single run***

Figure 1: Deck Layout of the HAMILTON Starlet platform required for automated NEBNext DNA library preparation

INTRODUCTION AND HARDWARE DESCRIPTION

Library preparation for NGS is a time-consuming process and a bottleneck for many laboratories. Automation can increase data output and consistency of results even for a small number of samples. A standardized NGS workstation, based on the Hamilton Microlab STARlet platform was developed, to cope with that demand. The workstation is equipped with eight 1ml channels, an InHeco® CPAC peltier module for heating and cooling of the reaction plate, a cooling module for safe storage of enzymes, buffers, and master mixes, as well as carriers for tips, reagents, and 96 well plates.
The closed housing of the instrument provides a built-in safety feature for the user and protects samples against environmental influences. Magnetic bead-based purification is performed with an Alpaqua® Super Magnet. The STARlet provides sufficient deck space for the preparation of up to 48 libraries in one run with minimal hands-on time. The method is programmed in the Hamilton control software, Venus.
All liquid classes are set for minimal chemical consumption; the pipetting parameters are optimized for maximum recovery and purity of the libraries and the scripts are written to use the minimum number of filter tips. A user-friendly graphical user interface is implemented to guide the operator through the setup process (Figure 1), displaying the status during the run and offering advanced tip management.
The tracing and reporting feature of the software allows strict process control and documentation. Sample-dependent size selection in the library process is controlled by a simple excel working list.
The application was validated using 5 ng – 1 µg *E. coli* and human DNA. Initial tests using ~ 1ng of human ChIP DNA were made. Additional methods are currently under development.

HAMILTON

METHOD DESCRIPTION

In general the automated process follows the manual method (see flowchart), with several modifications. Input materials can be provided in microtiterplates (MTPs) or microcentrifuge tubes. The system is preparing all master mixes automatically, depending on the number of samples in the work list on a cooled deck position. The stock chemicals can safely be removed at any time. End repair, dA-Tailing, Adapter Ligation, size selection and preparation for PCR were performed consecutively. The PCR is performed off deck. After reload the purification is performed prior to sequencing. The NEBNext® Ultra™ kits reduce the number of reaction steps by combination of end repair and dA tailing as well as the number of purifications steps, resulting in time gain and positive cost effects compared to other protocols.

RESULTS

1. In plate reproducibility

48 replicates of Covaris-sheared *E. coli* DNA with 5 ng input material were processed with the automated method using the NEBNext® Ultra™ DNA Library Prep Kit for Illumina® and the NEBNext® Multiplex Oligos for Illumina® (Primer Set 1) with a size selection for 250 bp fragments. An aliquot (1µL) of each purified library was analysed on an Agilent Bioanalyzer using a DNA 1000 chip according to the manufacturer's instructions. The data show a reproducible size selection of 250 bp as well as an average concentration of 29 ng/µl; total yield above 900 ng) over the replicates. (Figure 2, shows 8 randomly selected libraries).

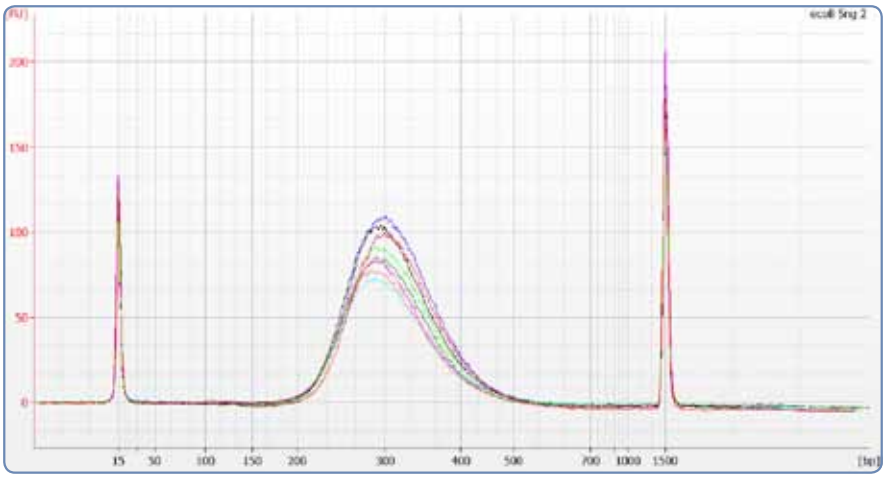

Figure 2: Bioanalyzer trace of 8 libraries generated from 5ng *E. coli* DNA demonstrating the reproducibility of the STARlet system.

2. In plate cross contamination

All liquid handling stations, like the Hamilton STARlet, are processing unsealed MTPs. To detect cross contaminations 24 Covaris-sheared *E. coli* DNA samples and 24 water samples were arranged in a checker-board pattern in a 96 well plate and processed with the automated method. Figure 3 shows representative results of the wells A1 to A6. The data reveal no cross contamination between the individual wells.

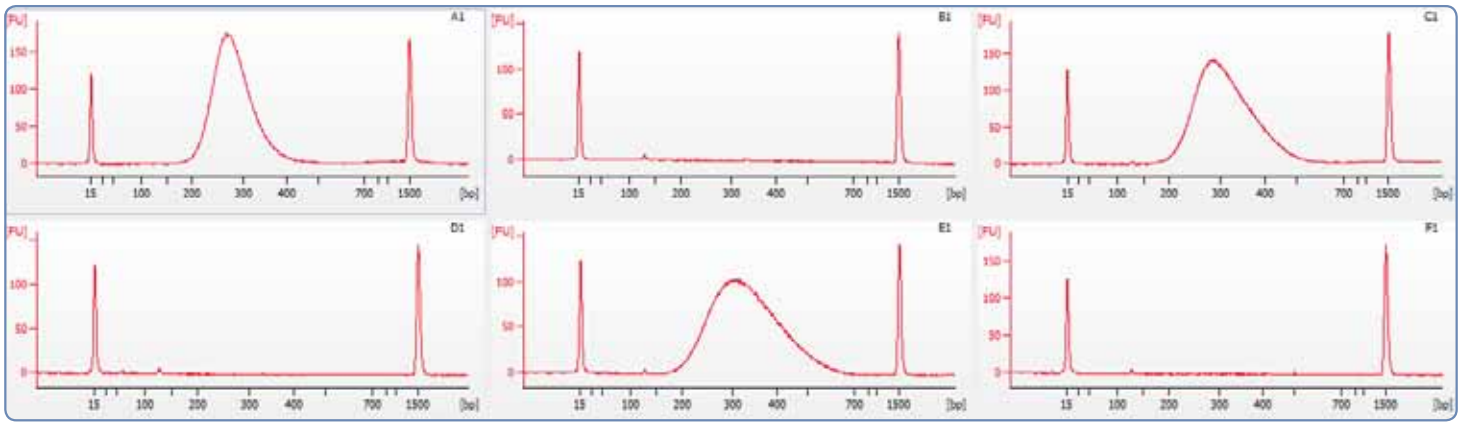

Figure 3: Bioanalyzer DNA 1000 traces from wells A1-A6 of the checker board test. The representative data of a 48 sample run reveal no cross contamination between individual wells.

3. Manual library preparation vs. automated library preparation

Two sets of 6 samples each containing 5 ng of sheared *E. coli* genomic DNA were provided for indexed library generation and subsequently sequenced on an Illumina® MiSeq in a 36 paired end run. The generated data were analysed for number of reads, mapped reads, mean library sizes as well as duplicate reads (Fig. 4). The number of total reads varies around 3 Million reads per library [manual (green), automated (blue)] according to the different indices. The data reveal a slightly higher percentage of mapped reads for the automatically generated libraries (> 96%) than for the manually generated ones (> 91%). The percentage of duplicate reads as well as the insert lengths is superior in case of the automated protocol in this test.

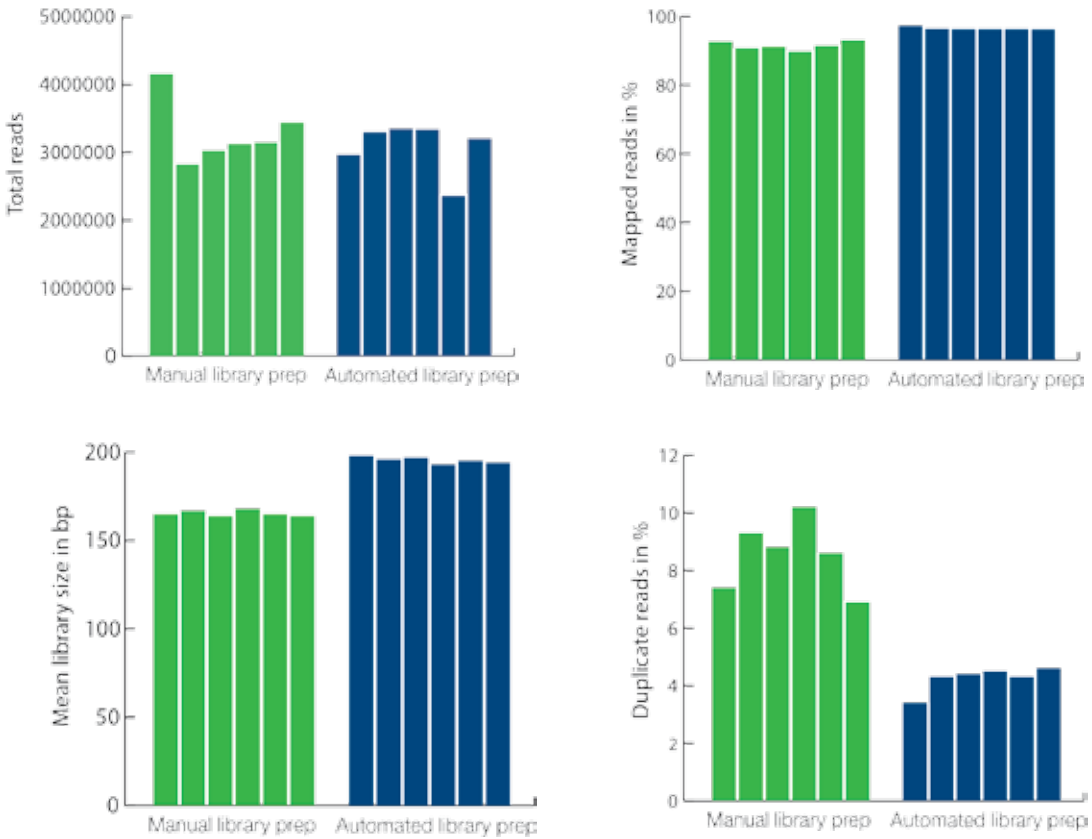

Figure 4: Manually (green) and automatically (blue) generated libraries were analyzed for total and mapped reads. Libraries generated on the STARlet show an equivalent number of reads (upper left) and a higher mapping rate than manually prepared libraries (upper right), as well as a higher mean library size (lower left) and lower duplicate rates (lower rights) in this test

DISCUSSION

We have demonstrated that the Hamilton Microlab STARlet workstation, using the NEBNext® Ultra™ DNA Library Prep Kit for Illumina® is capable of generating libraries for successful NGS from amounts as low as 5ng input DNA.
The application design consisting of hardware, software and chemistry constitutes an easy to use generalized platform for NGS library preparation.
The utilisation of working lists allows the flexible selection of process parameters during the run, such as size selection.

The Hamilton Microlab STARlet

System Configuration	
Microlab STARlet, manual load, modular arm	
8 x 1ml channels with CO-RE technology	
INHECO CPAC Ultraflat HT 2-TEC	
Mutiflex Cooling Module	
Carriers for tips, plates, microcentrifuge tubes, reagents	
System Dimensions	
Width: 1124mm, Height: 903mm, Depth: 795mm	
Reagents and accessories	**Part number / Provider**
NEBNext® Ultra™ DNA library prep kit for Illumina®	E7370 / NEB
NEBNext® Multiplex Oligos for Illumina (Primer Set 1)	E7335 / NEB
NEBNext® Multiplex Oligos for Illumina (Primer Set 2)	E7500 / NEB
96S Super Magnet Plate	A001322 / Alpaqua

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