

Automated Library Preparation using NEBNext[®] Ultra[™] DNA Library Prep Kit for Illumina[®] (E7370) on the HAMILTON[®] STAR[™] Line

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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 1 µg 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.



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

Introduction & 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

DNA CLONING

DNA AMPLIFICATION & PCR

EPIGENETICS

RNA ANALYSIS

SAMPLE PREP FOR NEXT GEN SEQUENCING

PROTEIN EXPRESSION & ANALYSIS

CELLULAR ANALYSIS

Applications

- Generation of genomic DNA NGS Libraries for Illumina Sequencing
- Input: as low as 5ng genomic DNA
- Throughput: 8 - 48 libraries/ run

Materials

- NEBNext ULTRA DNA Library Prep Kit for Illumina (NEB #E7370)
- NEBNext Multiplex Oligos for Illumina (Primer Set 1) (NEB #E7335)
- NEBNext Multiplex Oligos for Illumina (Primer Set 2) (NEB #E7500)

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.

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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 prepares all mastermixes 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, Adaptor Ligation, size selection and preparation for PCR were performed consecutively. The PCR is performed off deck. After the PCR step is finished, the samples are returned to the deck for the final clean up step.

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 (1 µL) 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).

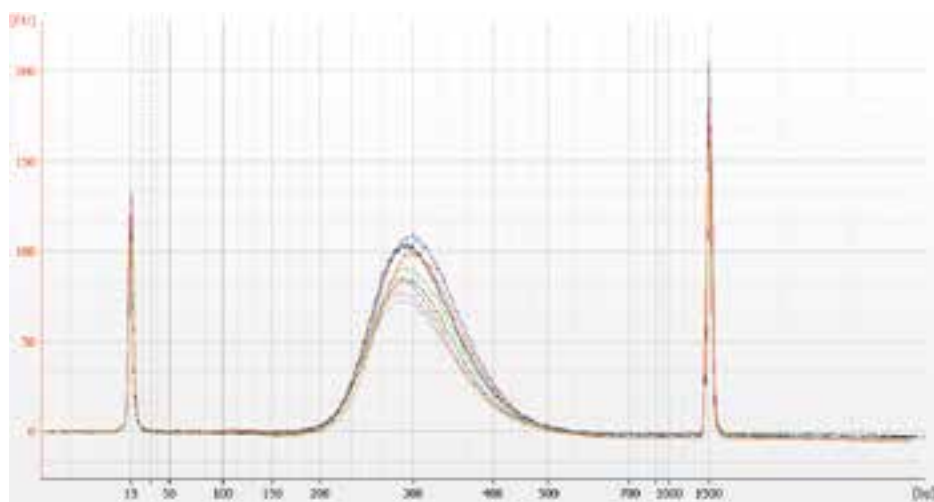


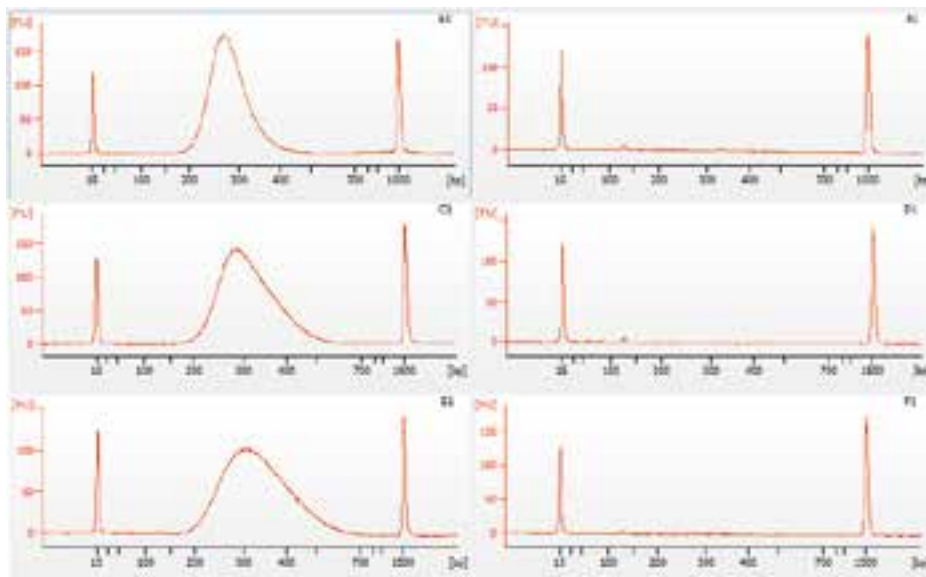
Figure 2: Bioanalyzer trace of 8 libraries generated from 5ng *E. coli* DNA demonstrating the reproducibility of the STARlet system.

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

2. In plate cross contamination

All liquid handling stations, like the Hamilton STARlet, are processing unsealed MTPs. To detect cross contamination 24 Covaris-sheared *E. coli* DNA samples and 24 water samples were arranged in a checkerboard pattern in a 96 well plate and processed with the automated method. Figure 3 shows representative results of the wells A1 to F1. The data reveal no cross contamination between the individual wells.



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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%) then 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.

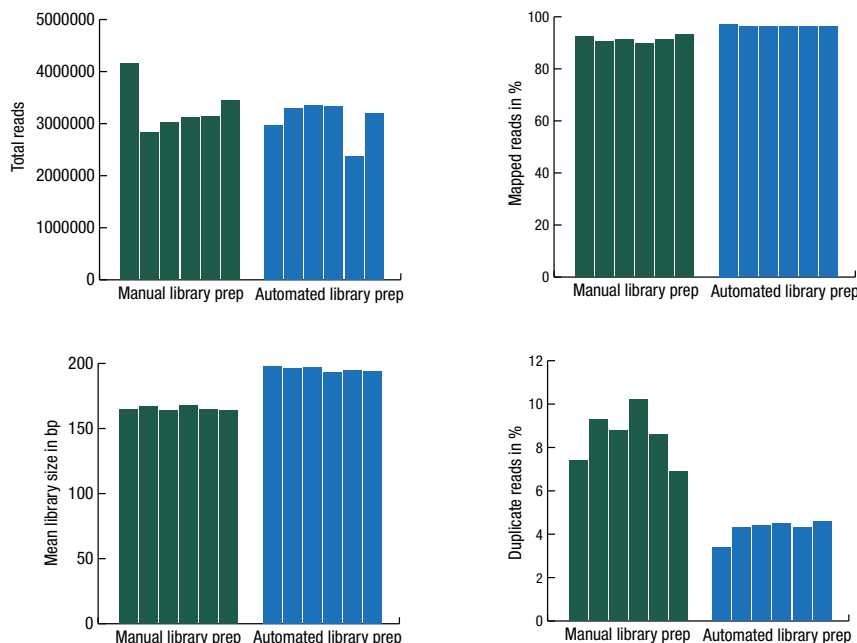


Figure 4: Manually (green) and automatically (blue) generated libraries were analyzed for total and mapped reads. Libraries generated on the STARlet show an equivalent number of reads (upper left) and a higher mapping rate than manually prepared libraries (upper right), as well as a higher mean library size (lower left) and lower duplicate rates (lower right) 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 (for heating and cooling of reaction plate) | | |
| Mutiflex Cooling Module (for safe storage of enzymes and buffers) | | |
| Carriers for tips, plates, microcentrifuge tubes, reagents | | |
| System Dimensions | | |
| Width: 1124mm, Height: 903mm, Depth: 795mm | | |
| Reagents and chemicals | Part N. | Provider |
| NEBNext Ultra DNA library prep kit for Illumina | E7370S/L | NEB |
| NEBNext Multiplex Oligos for Illumina (Primer Set 1) | E7335S/L | NEB |
| NEBNext Multiplex Oligos for Illumina (Primer Set 2) | E7500S/L | NEB |
| 96S Super Magnet Plate | A001322 | Alpaqua |

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