# PIPETMAX®: Automation of the Illumina® Nextera® XT DNA Library Preparation Kit

**APPLICATION NOTE AN1011**

**APPLICATION BENEFITS**

Library preparation, including magnetic bead-based cleanup, is an important procedure in the Next generation sequencing (NGS) workflow.

**SOLUTIONS**

Automation of the Nextera XT System workflow with PIPETMAX reduces hands-on time and offers reproducible liquid handling as well as sample traceability. Custom accessories enable optimal multichannel pipetting of index primers, and automated handling of magnetic bead cleanup.

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## ABSTRACT

The Illumina® Nextera® XT DNA Library Preparation Kit workflow was automated on PIPETMAX®. For comparison, 12 technical replicate libraries were prepared side by side, using manual liquid handling or automated on PIPETMAX. The 24 libraries were pooled and sequenced on an Illumina MiSeq® sequencing system. Both library preparation methods generated high quality data with >95% mapped reads and optimal quality scores. Variance was lower for libraries generated with PIPETMAX, consistent with the reproducible performance of this automated pipetting system. Automation of the Nextera XT System workflow with PIPETMAX allows libraries to be prepared in parallel and provides walk-away time, freeing up the researcher to carry out other tasks. More importantly, this application demonstrates the reliability and process control that is important for complex workflows, such as next generation sequencing.


**Figure 1**
PIPETMAX® is a benchtop, automated liquid handler equipped with motorized, multichannel, air displacement pipettes.

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## INTRODUCTION

The Illumina Nextera® XT DNA Library Preparation Kit[1] is designed to rapidly prepare sequencing-ready libraries of plasmids or small genomes for next-generation sequencing (NGS), also referred to as massively parallel sequencing or deep sequencing.[2] To automate this workflow, five automated scripts were developed for PIPETMAX®[3,4] (Figure 1). These scripts, accessed via TRILUTION® micro software, automate the liquid handling steps of the tagmentation, amplification plate setup, library cleanup, library normalization, and library pooling procedures (Figure 2).

In this application note we compare sequencing-ready libraries that were generated using either manual liquid handling or the automated PIPETMAX liquid handling instrument.

For each method (manual and PIPETMAX) 12 replicate libraries were prepared from *E. coli* genomic DNA. The 24 libraries were pooled and then sequenced in one lane of an Illumina MiSeq® system.


**Figure 2**

Schematic representation of the five PIPETMAX® scripts for automation of the Illumina® Nextera® XT DNA Library Preparation Kit workflow. Each blue rounded rectangle represents one PIPETMAX® script, each of which corresponds to a portion of the Nextera™ XT System workflow. Some scripts include repositioning the plate or off-bed steps accomplished through user intervention, such as centrifugation of a microplate between liquid handling steps.

## MATERIALS AND METHODS

### Nucleic Acid Samples

*E. coli* K12 genomic DNA was obtained from a commercial source and diluted to the recommended concentration (0.2 ng/µL) before use. The same stock of genomic DNA was used for both manual and automated library preparation.

## AUTOMATED LIQUID HANDLING

Automated liquid handling was performed with a PIPETMAX® equipped with multichannel pipette heads (MAX8x20 and MAX8x200). The removable tray on PIPETMAX can hold up to nine ANSI/SLAS-footprint items, including Gilson PIPETMAN® DIAMOND tips, microplates, microcentrifuge tubes or PCR tubes in racks, reservoirs, tip waste, and accessories such as the magnetic bead separator rack or orbital shaker. PIPETMAN DIAMOND tips in tip adapter blocks were used for all automated liquid handling.

Gilson TRILUTION® micro software running on a tablet PC was used to control PIPETMAX and on-bed accessories including the magnetic bead separator and orbital shaker. To obtain the PIPETMAX scripts for the automated Illumina® Nextera® XT DNA Library Preparation Kit discussed in this application note, please contact techsupport@gilson.com.

### Library Preparation, Sequencing, and Bioinformatics

Library preparation and sequencing was performed by an Illumina Certified Service Provider (Lucigen Corp.; Madison, WI). Twelve libraries were constructed using manual liquid handling, with *E. coli* gDNA (1 ng) as input material and i5 and i7 index primers, following manufacturer's guidelines (Nextera XT System reagents FC-131-1096 and FC-131-1001). Twelve additional libraries were created on PIPETMAX, using the same source and concentration of *E. coli* gDNA and a different combination of i5 and i7 index primers. Following library cleanup with Agencourt® AMPure® XP beads (Beckman Coulter A63880), libraries were checked for size distribution and sample purity via an Agilent Bioanalyzer. All 24 libraries were then pooled and run on an Illumina MiSeq® sequencing system. Sequencing reads were downsampled to 312,400 reads per library and mapped to the *E. coli* K12 genome.

### Equipment and Labware

A list of Gilson-supplied items can be found in the Setup Guide for this application note (Gilson technical document LT309166). Additionally, the following labware was used in the automated procedures:

**Table 1**

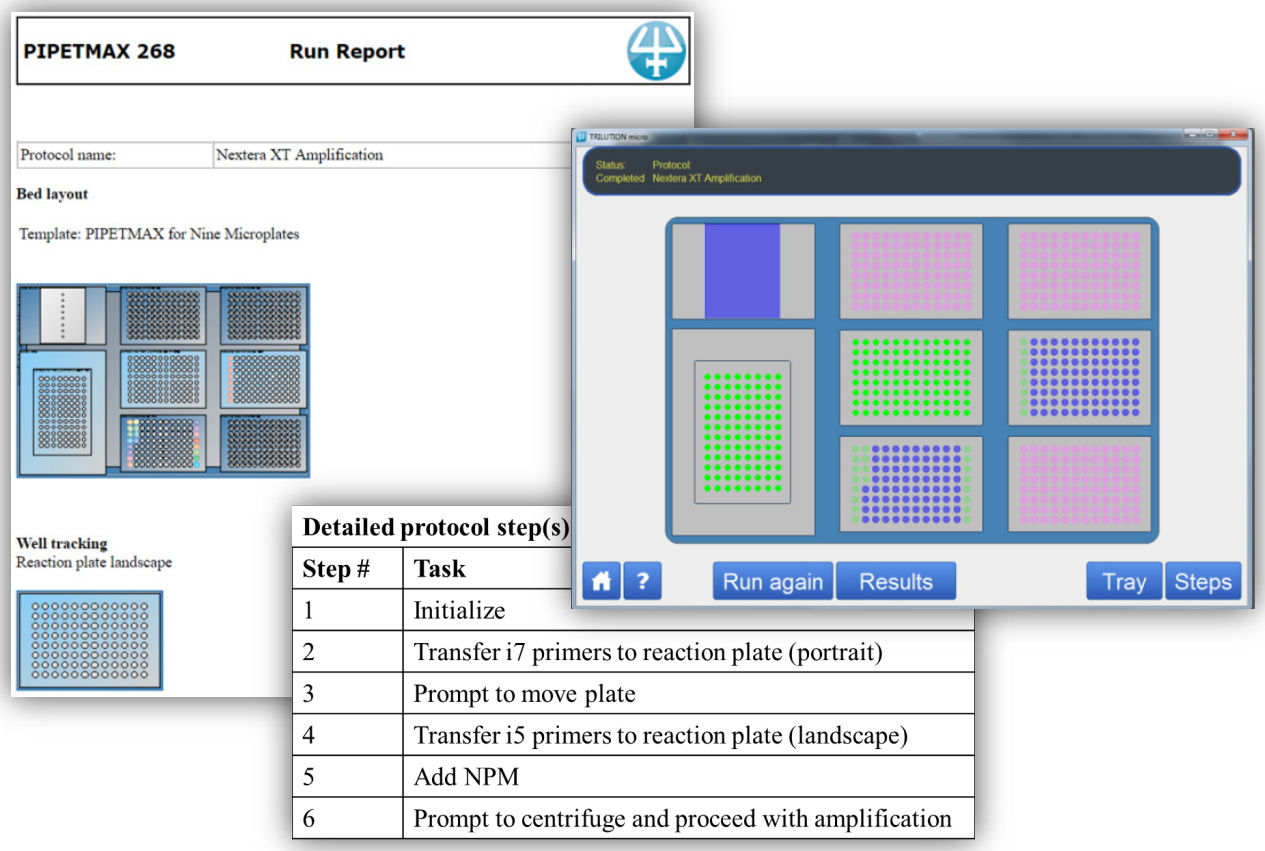
Labware used in the automated procedures.

| DESCRIPTION | PART NUMBER | NOTES |
|---|---|---|
| Labware for Tagmentation & Amplification | Eppendorf twin. tec 0030133307 | 96-well, unskirted microplate |
| Reaction Plate | Bio-Rad HSP-9601 | 96-well, hard shell, skirted microplate |
| Sample Plate | Greiner 659101 | 96-well, clear, V-bottom polystyrene, low binding microplate |
| Reservoir | Agilent Seahorse 201256-100 | 12-column reservoir |
| Strip Tubes | Thermo AB-0451 | 0.2 mL strip tubes |
| Midi Plate | Thermo AB-0859 | 96-well, deep well plate used for library normalization script |
| RoboRack | Micronic MPW51001BC3 | Rack to hold Illumina TruSeq Index primers for amplification plate setup script |
| 2 mL Tube | Greiner 623201 | 2 mL flip cap microcentrifuge tube |

## RESULTS AND DISCUSSION

Sequencing-ready normalized libraries were generated with the Illumina® Nextera® XT DNA Library Preparation Kit from an input of 1 ng per library using manual liquid handling or a Gilson PIPETMAX liquid handler. A PIPETMAX equipped with two motorized, multichannel air displacement pipette heads was used to carry out automated liquid handling for the tagmentation of *E. coli* genomic DNA, as well as PCR amplification reaction setup, bead cleanup, library normalization, and library pooling. The system enables precise single or multichannel pipetting from 1 µL to 200 µL and was outfitted with on-bed accessories for orbital shaking, magnetic bead cleanup, and dispensing of index primers into the proper positions. The efficient layout of the compact benchtop instrument permits up to nine bed elements to be employed during an automated script.

The intuitive TRILUTION® micro software provides a “wizard”-style interface to guide the user through instrument and script setup. The ease of use of the software decreases user-to-user variability and built-in help files reduces the need for training. The TRILUTION micro graphical interface walks users through run setup. Scripts include prompts when off-bed steps are required, and detailed run reports (Figure 3) help with sample tracking.




**Detailed protocol step(s)**

| Step # | Task |
|---|---|
| 1 | Initialize |
| 2 | Transfer i7 primers to reaction plate (portrait) |
| 3 | Prompt to move plate |
| 4 | Transfer i5 primers to reaction plate (landscape) |
| 5 | Add NPM |
| 6 | Prompt to centrifuge and proceed with amplification |

**Figure 3**
Example images from PIPETMAX® run report, TRILUTION® micro graphical interface, and list of script steps. Example shown: Nextera® XT amplification plate setup.

## PIPETMAX® Accessories

Some of the PIPETMAX® accessories that were employed in the amplification, cleanup, and normalization scripts are of note because they helped to increase efficiency in the workflow. These are shown in Figure 4.

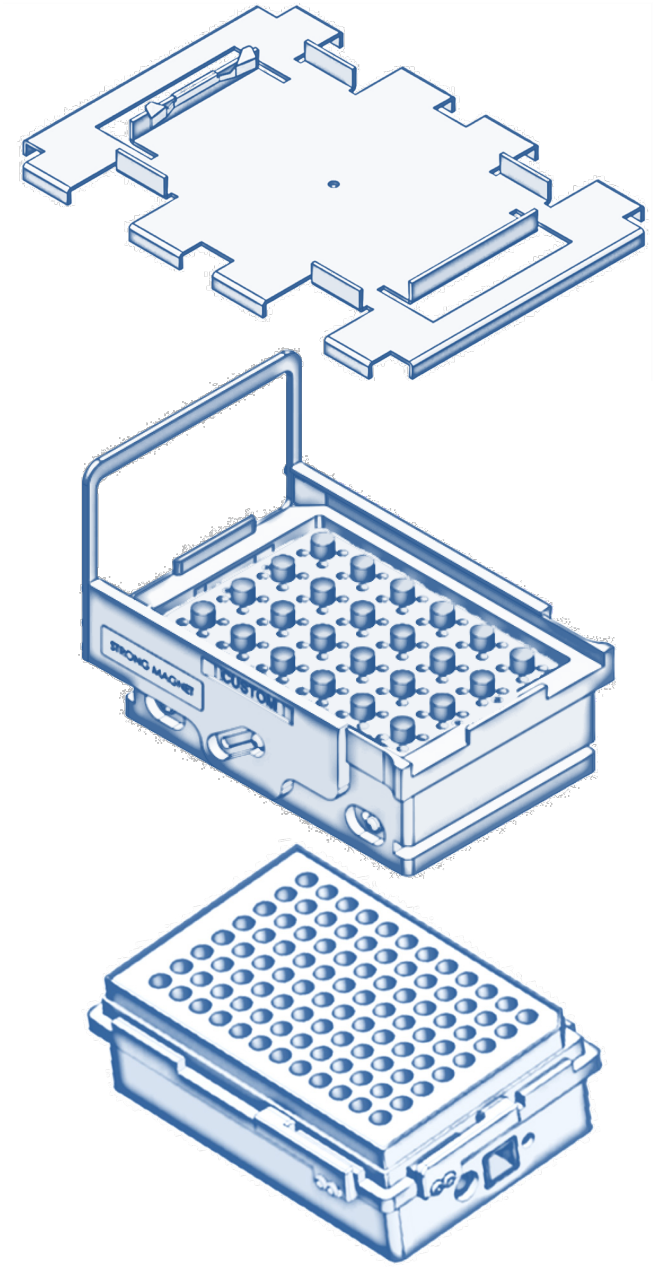



**Figure 4**
Some of the PIPETMAX® accessories used in this workflow. From top to bottom: portrait orientation rack, magnetic bead separator rack, and orbital shaker.

1. The portrait orientation rack (SPL-2141C-HDW) is used in the amplification script. This rack allows the user to manually rotate the reaction plate 90°, which enables multichannel addition of the index primers.
2. A Micronic Roborack-96 (not shown) was employed as an on-bed rack for index primer tubes in the amplification script. The primers are held in this automation-friendly rack that fits the TruSeq® Index Primer tubes.PIPETMAX can access the primer tubes and transfers primers to the correct position in the reaction plate.
3. The Gilson magnetic bead separator rack is used in both the library cleanup and library normalization scripts. The magnets in this on-bed device can be automatically toggled between disengaged and engaged positions, facilitating washing and elution steps.
4. The orbital shaker for PIPETMAX is used in the library normalization script. The user manually places the labware on the shaker, and PIPETMAX regulates the speed and time of shaking according to the needs of the application as designated in the script.

## Sequencing Results

For each method (manual and PIPETMAX) twelve replicate libraries were prepared from *E. coli* genomic DNA. The 24 libraries were then pooled and sequenced on an Illumina MiSeq® system. Sequencing results demonstrated the reliability of the automated Nextera XT System script.

The percentage of mapped reads was almost identical for libraries prepared with automated liquid handling or manual liquid handling. In general, the libraries prepared with PIPETMAX exhibited smaller standard deviation and variance, consistent with the reproducibility of liquid handling on this system. Each library was downsampled to 312,500 reads, which yielded >8x coverage for all 24 libraries.

Libraries constructed with either PIPETMAX or manual liquid handling performed well, achieving >95% mapped reads and >8x coverage of the genome (refer to Table 2).

**Table 2**
Summary of Sequencing Results

| | | AVG | STDEV | VARIANCE |
|---|---|---|---|---|
| PIPETMAX® | Total reads | 736,016 | 226,252 | 30.8% |
| | % Mapped | 95.7% | 1.2% | |
| | Fold coverage | 8.311x | 0.135 | 1.6% |
| | % reads >Q30 | 93.5% | 2.2% | 4.3% |
| MANUAL | Total reads | 853,963 | 291,140 | 34.1% |
| | % Mapped | 95.1% | 1.5% | |
| | Fold coverage | 9.395x | 0.174 | 1.9% |
| | % reads >Q30 | 90.3% | 3.2% | 9.3% |

The variance observed for libraries constructed with the PIPETMAX automated workflow were slightly smaller than those prepared manually (30.8% vs. 34.1%), indicating the technical replicates prepared with PIPETMAX were more uniform than the technical replicates prepared with manual pipetting.

Figure 5 compares the percentage of mapped reads and the quality scores (% >Q30) averaged across twelve replicate libraries prepared either with PIPETMAX or manual pipetting. Automation of Nextera XT System DNA preparation with PIPETMAX provided reproducible liquid handling, resulting in smaller standard deviations and lower variance between replicates.

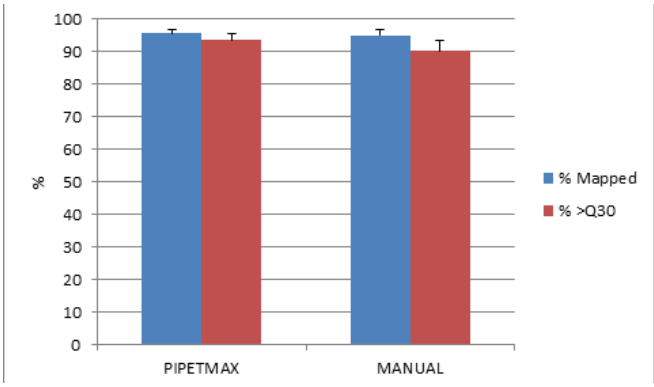



**Figure 5**
Comparison of % mapped reads (blue columns) and % >Q30 (red columns) values.

## REFERENCES


1. Illumina. (2016) Nextera® DNA Library Prep Reference Guide. Document # 15027987 v01. Accessed April 2016. Available from: http://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/samplepreps_nextera/nexteradna/nextera-dna-library-prep-reference-guide-15027987-01.pdf.
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## CONCLUSIONS

- The Illumina® Nextera® XT DNA Library Preparation Kit workflow was automated as five PIPETMAX® scripts, each of which corresponds to a portion of the Nextera XT System workflow.
- Libraries generated using the PIPETMAX automated Nextera XT System DNA Library Kit scripts are ready for downstream Illumina sequencing.
- Automation of the Nextera XT System workflow with PIPETMAX allows libraries to be generated in parallel, reduces the need for hands-on time, and offers reproducible liquid handling, thereby enabling verifiable science.
- Custom accessories were implemented to enable fast, optimal multichannel pipetting as well as manipulation of magnetic beads:
    - Portrait adapter rack
    - Orbital shaker for PIPETMAX®
    - Magnetic bead separator rack