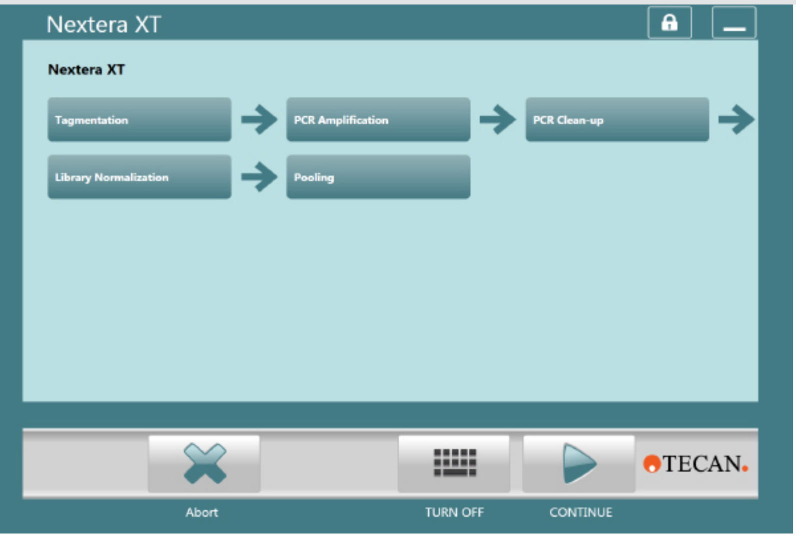
Application Note



Automated Illumina® Nextera® XT sample preparation

NGS library preparation on the Freedom EVO® NGS workstation

Contributed by Piotr Mieczkowski, Research Assistant Professor and Director of the High-Throughput Sequencing Facility (HTSF), and Ewa Malc, Postdoctoral Researcher, Department of Genetics, University of North Carolina, Chapel Hill, USA

Introduction

Illumina's Nextera XT DNA Library Preparation Kit enables the effective construction of high quality libraries from PCR amplicons, plasmids and small genomes, using a protocol optimized for DNA inputs from as little as 1 ng. Automation of the protocol on the Freedom EVO NGS workstation allows up to 96 libraries to be generated in parallel, addressing a variety of different thoughput needs, and reducing the extent of user intervention. The intuitive TouchTools™ interface guides the operator through instrument loading and set-up, decreasing training needs and operator-to-operator variability, enabling the preparation of highly reproducible, sequencing-ready libraries.

This application note describes the automated protocol for preparation of 96 normalized, sequencing-ready DNA libraries using the Nextera XT DNA Library Preparation Kit on the Freedom EVO NGS workstation (Figure 1).

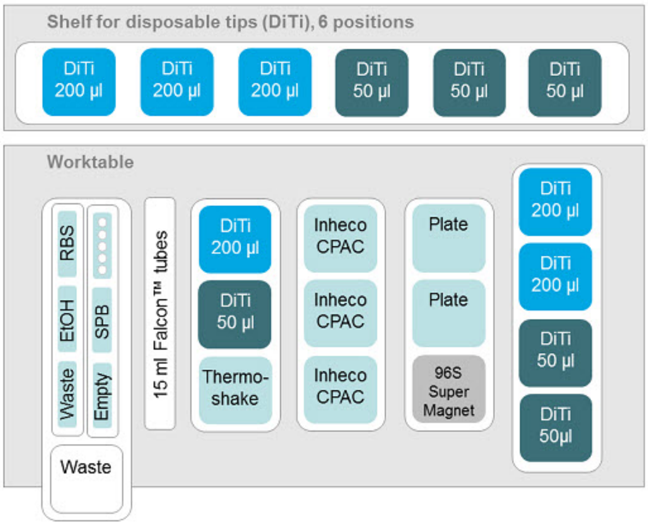


Figure 1: Deck layout of the Freedom EVO NGS workstation set-up for Illumina library preparation, including the Nextera XT DNA Library Preparation protocol.

Experimental design

Automated liquid handling for tagmentation of plasmid DNA, PCR amplification set-up, bead clean-up and normalization with magnetic beads was performed on a Freedom EVO NGS workstation equipped with advanced air displacement pipetting technology, enabling precise eight-channel pipetting from 1,000 µl down to just 0.5 µl. The system also included three INHECO CPAC thermal devices – allowing reagents to be kept cool and providing optimal conditions for the enzymatic steps – an INHECO Thermoshake heated shaker, a 96-position magnetic plate separator (Alpaqua® 96S Super Magnet) and a Robotic Manipulator Arm. In addition, the compact worktable offers storage space for up to 12 tip boxes, allowing longer unattended runs.
A total of 96 libraries were prepared from 24 different plasmids, with a starting input of 1 ng, using the Nextera XT DNA Library Preparation Kit. All 96 libraries prepared were then pooled and sequenced (paired-end clustering and 2x250 sequencing cycles) across one lane of an Illumina MiSeq™ system. In addition, libraries were prepared from 41 *E.Coli* genomes.

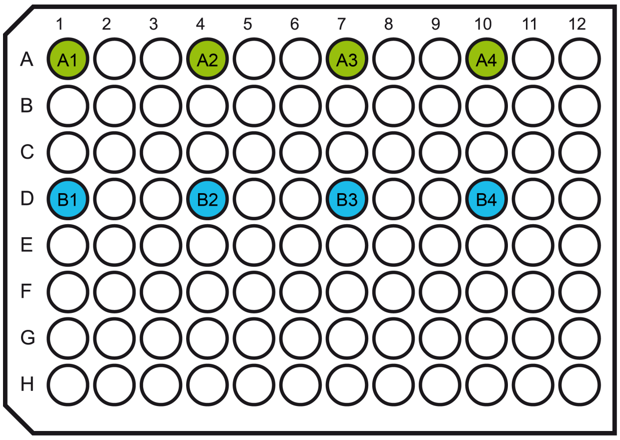


Figure 2: Positions in which different sequenced plasmids were processed.

Analysis and results

Sequencing data was analyzed with the CLC Genomics Workbench package (Qiagen), using adapter sequence trimming, de novo assembly and alignment software. Libraries were successfully generated from all four replicates of 24 plasmid samples and 41 *E.Coli* genomes. The size of the resulting libraries was consistent between samples and replicates (Figure 3).

Quality control sequencing results demonstrated the reliability of the automated Nextera XT Library Preparation protocol, with averages of up to 98.8 % alignment and 91 % of paired reads (as shown in Table 1).

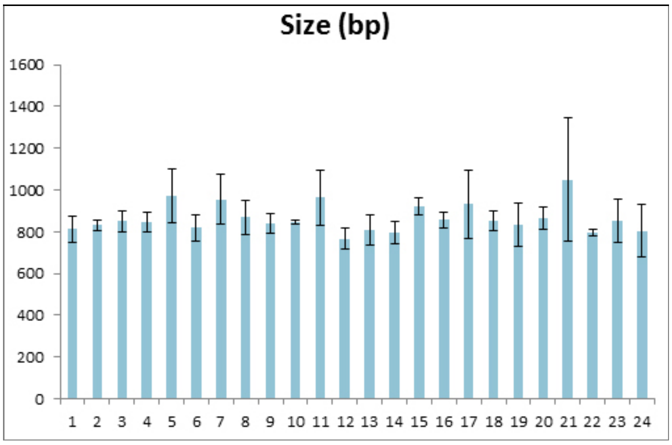


Figure 3: Average size of library fragments was homogenous between samples. Each value is an average of four replicates of the same sample distanced by 24 wells in the same plate. Error bars show standard variation.

Sample	Reference size (bp)	Position in plate	Average size (bp)	Total reads	Mapped reads (%)	Paired reads (%)
Plasmid A-1	5'929	A1	870	502'194	91.27	68.03
Plasmid A-2	5'929	A4	821	395'402	90.58	68.94
Plasmid A-3	5'929	A7	831	463'179	88.83	68.32
Plasmid A-4	5'929	A10	726	442'990	88.54	66.66
Plasmid B-1	10'515	D1	807	441'024	98.81	83.22
Plasmid B-2	10'515	D4	824	383'040	98.76	82.71
Plasmid B-3	10'515	D7	915	433'588	98.69	91.07
Plasmid B-4	10'515	D10	837	551'972	98.64	90.07

Table 1: Plasmids A and B were selected for quality analysis of the sequenced libraries. For both plasmids, very homogeneus results were observed troughout the plate, even with replicates up to 72 wells (9 columns) away from each other.

Summary

The results show that automation of the Nextera XT DNA Library Preparation workflow on the Freedom EVO NGS workstation is a rapid, efficient solution for library preparation. The user-friendly TouchTools interface simplifies the instrument set-up process, and minimizes operator-to-operator variability.

Learn more

To obtain the automated Nextera XT DNA Library Preparation protocol for the Freedom EVO NGS workstation discussed in this application note, contact your Tecan sales representative, visit www.tecan.com/ngs or contact NGSprep@tecan.com.

To learn more about Nextera XT DNA Library Preparation Kits, visit http://www.illumina.com/products/nextera_xt_dna_library_prep_kit.html.

Acknowledgments

The data contained in this application note was kindly contributed by Piotr Mieczkowski, Research Assistant Professor and Director of the High-Throughput Sequencing Facility (HTSF), and Ewa Malc, Postdoctoral Researcher, Department of Genetics, University of North Carolina, Chapel Hill, USA.

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