Automating DNA Library Preparation of Small Genomes using Illumina ® DNA Preparation Kit on the Opentrons OT-2

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INTRODUCTION

The next-generation sequencing (NGS) workflow includes preparing DNA libraries using commercially available and cost-effective DNA preparation kits. Optimizing this workflow by using an automated OT-2 protocol helps to improve the efficiency for DNA library preparation. Here, we describe the efficiency of the Illumina® DNA preparation kit (Illumina, Cat. No. 20018705) automated on the OT-2, robotic liquid handling platform, to perform the tagmentation workflow. Automating this process with the high-performance capability of the OT-2 provides a fast and robust workflow for generating uniform DNA prep libraries for genome sequencing.

OVERVIEW OF THE ILLUMINA® DNA PREP KIT AND THE OPENTRONS OT-2 PLATFORM

Illumina® patented NGS Tagmentation workflow implements on-bead tagmentation which utilizes bead-linked transposomes. The chemistry of bead-linked transposomes is more efficient for DNA fragmentation and DNA end-repair compared to the previous in-solution tagmentation workflows. The fragmented DNA is tagged using Dual Index adapters, a unique barcode assigned to each DNA fragment. DNA Prep samples (n=8) were quantified, normalized to a 4nM library, pooled into a single multiplex(8-plex or 16-plex) sample, and then ran on a 2x75 flow cell on Illumina® MiSeq for genome sequencing. Our data showed a low variability for all DNA Prep samples measured before and after 5 cycles of PCR amplification; barcode balance, and a uniformed and high coverage for sequencing alignments to the reference genome, which demonstrates that automating tagmentation using the OT-2 protocol provides a quality DNA Prep library for genome sequencing.

SCHEMATIC OF THE OT-2 WORKFLOW PROTOCOL

DNA and IDT® for Illumina® DNA/RNA UD Indexes Set A (Illumina, Cat. No. 20027213) were incubated during a process referred to as tagmentation, on the OT-2, as shown in **(Figure 1)**. A tagmentation wash step was performed, to remove residual DNA and adapters. Next, DNA amplification by PCR was performed on an OT-2 on-deck thermocycler with a programmable lid and block temperatures.

FIGURE 1

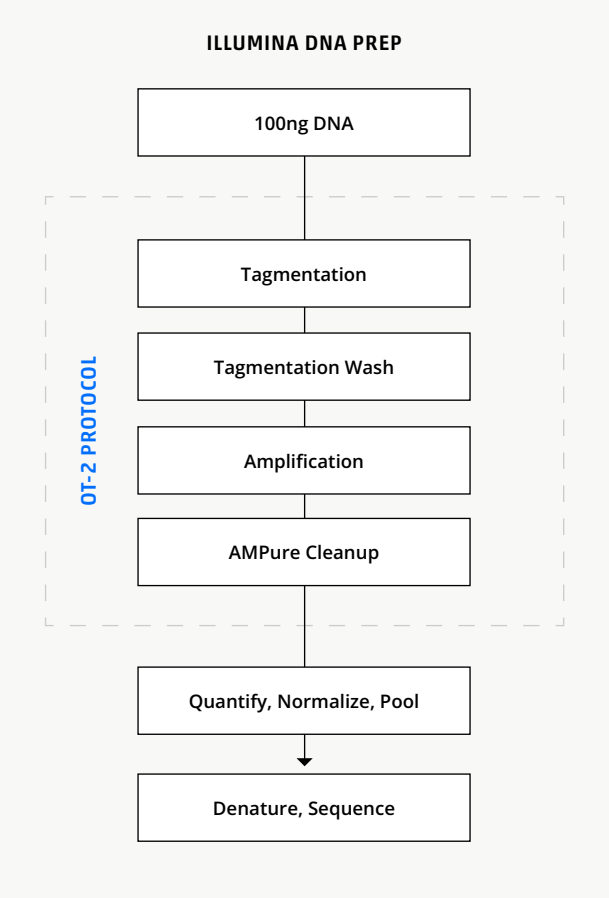

Figure 1. Illumina DNA Prep Workflow with OT-2.
The dotted line box indicate the steps of the Illumina DNA Prep workflow automated on the OT-2

Following the OT-2 protocol, a post-amplification cleanup step using AMPure (Beckman Coulter, Cat. No. a63881) was performed based on manufacturer recommendations. DNA Prep samples were quantified, normalized to 4nM (measured by qPCR using NEBNext® Library Quant Kit for Illumina® Library Quantification Kit, pooled as 8-plex or 16-plex into a single sample, and then ran on a 2x75 flow cell on the Illumina® MiSeq. Sequencing data was extracted and demultiplexed using Illumina® BaseSpace according to Illumina® DNA/RNA Unique Dual (UD) index adapter barcode sequence. The sequences aligned with the reference genome and coverage maps were produced through Geneious Prime 2021.2.2 **(https://www.geneious.com)**.

THE WORKFLOW LAYOUT

The layout of the Opentrons OT-2 platform includes the modules, labware, and Illumina® DNA Prep reagents, as shown in **(Figure 2)**. Although the workflow consists of automated pipetting on the OT-2, manual intervention is required to move the sample plate between the on-deck Thermocycler and the Magnetic Block and reset the pipette tip racks during the protocol.

PCR AMPLIFICATION OF DNA PREP SAMPLES

Illumina® DNA prep kit was used for constructing the Lambda DNA libraries (n=8, 100 ng) on the OT-2. The PCR concentration (ng/uL) was quantified before and after 5 cycles of PCR amplification, and DNA concentrations were measured using Quant-iT™ dsDNA High-Sensitivity Assay Kit (ThermoFisher Scientific, Cat.No.Q33120) on a Qubit® to determine the coefficient of variation, which is the standard deviation divided by the average across the samples. Pre-PCR average of 2.92 and a coefficient of variation (CV) of 10.4% across Lambda DNA samples and the Post PCR concentration after 5 cycles showed an average of 28.23 and a CV of 10.7%, as shown in **(Table 1)**. These results imply that the optimized OT-2 protocol can produce a quality and reliable DNA prep library for NGS.

SMALL GENOME SEQUENCING OF DNA PREP LIBRARIES

Illumina® DNA prep kit (M) Tagmentation for 96 samples (Illumina, Cat. No. 20018705) was used to prepare E. coli Non-Methylated Genomic DNA (Zymogen, Cat. No. D5016) DNA samples. Samples (n=16, 100ng) were prepared in

FIGURE 2

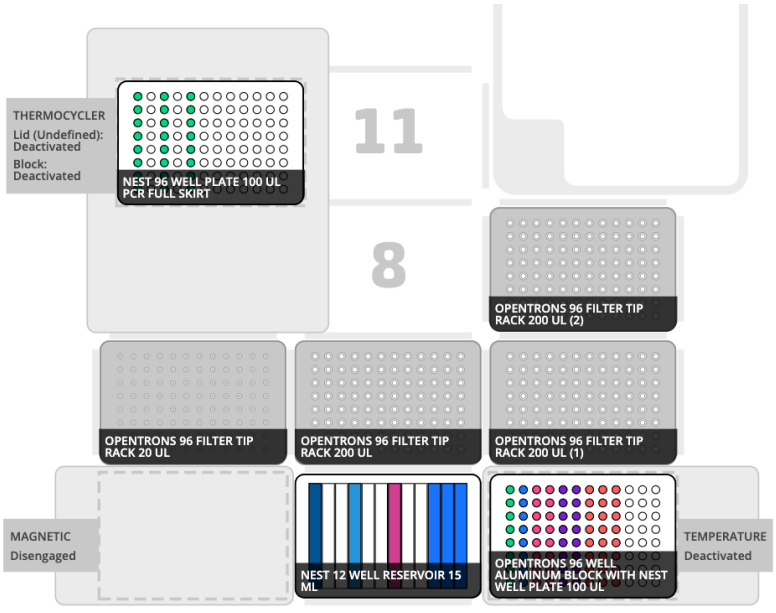

Figure 2. Opentrons OT-2 deck layout equipped with modules, labware, and Illumina DNA Prep reagents.

This workflow has all pipetting automated on the OT-2 and requires manual intervention to move the sample plate (1) between the on deck Thermocycler and Magnetic Block (2), as well as resetting tip racks during the run. The deck layout includes 1 x NEST 12 well reservoir, 1 x 96 well aluminum block, 1 x Eppendorf 200ul PCR Plate on the Thermocycler, and a 1 x NEST 0.1mL PCR plate on the Temperature module (3). Modules include a Magnetic Module, Temperature module, Thermocycler module, and P20 and P300 Multichannel pipettes.

TABLE 1

INPUT (NG)	INPUT (TYPE)	PRE PCR (NG/UL)	POST PCR (NG/UL)
100	Lambda	2.66	25.00
100	Lambda	2.89	31.40
100	Lambda	2.63	33.40
100	Lambda	3.22	24.60
100	Lambda	3.49	26.80
100	Lambda	2.66	27.10
100	Lambda	2.99	28.50
100	Lambda	2.85	29.00
	Average	**2.92**	**28.23**
	CV	**10.4%**	**10.7%**

Table 1. Low variability among libraries constructed on the Opentrons OT-2. *This table shows the yield of each sample after the Illumina Tagmentation washes, before and after 5 cycles of PCR amplification and AMPure cleanup.*

TABLE 2

SAMPLE TYPE	*E.COLI*	*E.COLI*	LAMBDA
1. Sequencing Batch	Plexity	8x	16x	8x
	Input (ng)	100	100	100
2. Sample Yields	Average	9.75	3.79	28.23
	CV	8.77%	6.2%	10.7%
3. Barcode Balance	Expected	12.5%	6.2%	12.5%
	Ave	11.2%	6%	12.31%
4. Coverage	Estimated	150x	45x	8000x
	Ave	106.75x	42.8x	7865x

Table 2. Sequencing batch specifications for small genomes shows low variation. *Comparison of 3 different batches prepared with the Illumina DNA Prep workflow on a OT-2 (1). Yields from different samples types are uniform (2), individual sample barcodes also demonstrated uniform representation (3). Average sample genome coverage is approximate to the calculated expected coverage from the Illumina coverage calculator.*

FIGURE 3

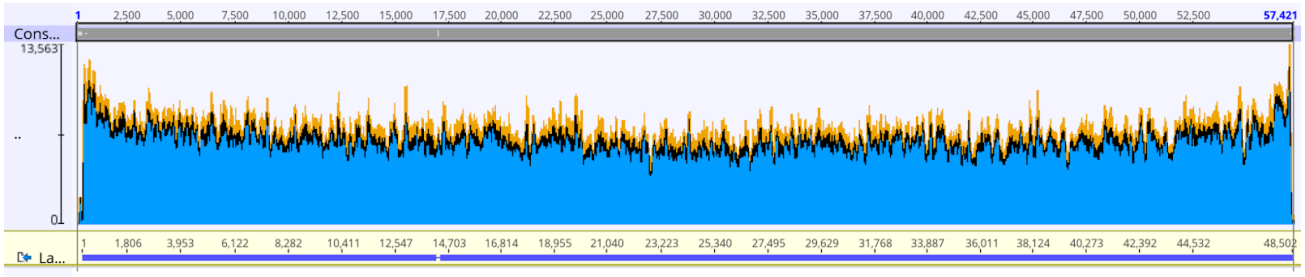

Figure 3. Highly consistent sequencing coverage across small genomes. *Coverage map from Geneious showing ~7800x coverage across the 48kb Lambda genome*

two batches. The second batch of E.Coli DNA samples (n=8, 100 ng) were processed on 2 separate columns on the OT-2—to account for variability that may arise within the column.

E.Coli DNA (n=16, 100 ng) and Lambda DNA (n=8, 100 ng) were amplified, normalized, and pooled into a single multiplexed (8-plex or 16-plex) sample for sequencing. The sequencing of the three DNA prep batches was compared to determine the sample yield, barcode balance, and coverage, as shown in **(Table 2)**. A uniform yield was observed for the DNA prep libraries. Calculated sample yields for E.Coli were 9.75 for 8-plex and 3.79 for 16-plex; with a CV value of 8.77% and 6.2% for 8-plex and 16-plex. Illumina® Adapter Barcodes' balance was accurate for the 8-plex and 16-plex samples, showing an average of 11.2%-12.31% for 8-plex, compared to the expected 12.5%. For the 16-plex, the expected value of 6.2% was observed based on the expected value of 6% based on Illumina® reference calculation. The average genome coverage for the DNA prep E.Coli sample was 150x for 8-plex and 45x for 16-plex compared to the 106.75x and 42.8x for 8-plex and 16-plex. Moreover, the coverage map of the Lambda 48kb genome showed a ~7800x coverage, which indicates a high level of aligned sequence reads **(Figure 3)**. Overall, this demonstrates that the DNA Prep libraries constructed on the OT-2 for sequencing showed uniformity compared to reference calculations **(Fig 4)**.

FIGURE 4

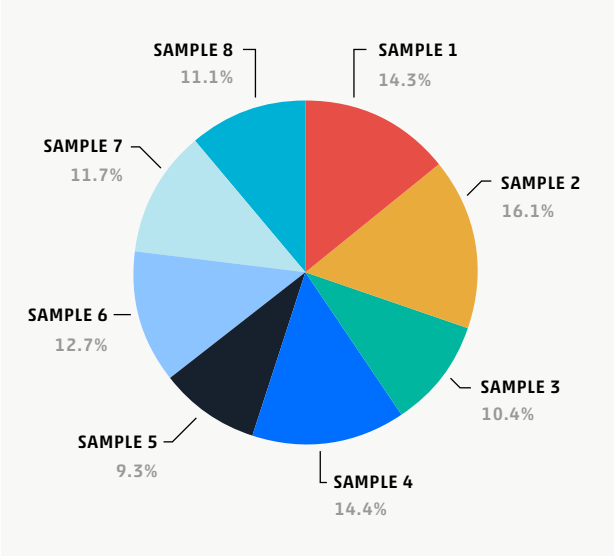

Figure 4. Uniform sample barcode representation. *This pie chart demonstrates the even sample barcode balance within the sequencing run by overall read percentage.*

CONCLUSION

We demonstrated the high-performance capability of automating the tagmentation process using the Illumina® DNA-Prep Kit on the Opentrons OT-2. We showed that DNA Prep samples displayed low variability, barcode balance, and high coverage of sequence alignments. Automating this comprehensive NGS workflow on the OT-2 minimizes the risks during DNA library preparation while ensuring a quality DNA library prep for genome sequencing.

REFERENCE DATA

Final Illumina DNA Prep Figures (Sept 2021) - Google Slides

REFERENCES

Geneious Prime 2021.2.2