Automated library preparation of small genomes using DNA preparation kits

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ABSTRACT

Three library prep kits – Illumina® DNA Prep Kit, KAPA® HyperPrep kit, and NEBNext® Ultra™ II Kit – were automated with the Opentrons OT-2 for fast and robust preparation of high quality libraries for next generation sequencing.

- Libraries prepared with 100 ng input were prepared from lambda and human genomic DNA.
- Comparable performance was observed for libraries prepared with the three kits on the OT-2 in terms of sample variability and yield. Similar sequencing metrics such as barcode balance and sequence alignment coverage were also observed.

INTRODUCTION

DNA library preparation is a critical step for next-generation sequencing (NGS). Automating DNA library preparation provides a streamlined approach for constructing high-yield libraries for sequencing while minimizing hands-on time. Here, we describe the efficiency of automating the Illumina DNA Prep kit (Illumina, Cat. No. 20018705), KAPA HyperPrep kit (Roche®, Cat. No. 7962363001), and NEBNext Ultra II kit (New England Biolabs®, Cat. No. E7645L) on the OT-2 robotic liquid handling platform.

MATERIALS AND METHODS

Overview of the Illumina DNA Prep kit, KAPA HyperPrep Kit, and NEBNext Ultra II and the Opentrons OT-2 Platform

Illumina has a patented NGS tagmentation workflow that utilizes bead-linked transposomes, which differs from the KAPA HyperPrep and NEBNext Ultra II workflows-in solution process for DNA end-repair and A-tailing.

For the Illumina DNA Prep kit, the DNA was tagmented and tagged using Dual Index adapters on the OT-2. DNA Prep for lambda DNA samples (n=8, 100 ng) was quantified, normalized to a 4 nM library, pooled into a single multiplexed sample, and then ran on a 2x75 flow cell on Illumina MiSeq for genome sequencing. The workflow included generating DNA prep libraries for Lambda (n=24) and Human Genomic DNA samples (n=8).

For both HyperPrep and NEBNext Ultra II DNA prep kits, DNA libraries were constructed for fragmented lambda DNA (n=8, 100 ng) on an OT-2. Barcodes used were from KAPA Unique Dual Index Adapters for HyperPrep, and NEB Multiplex Dual Index Primers Set 1 for NEBNext Ultra II. Samples were quantified, normalized, and pooled into a 4 nM library and sequenced on a MiSeq with a 2x75 flow cell. The workflow included generating libraries for lambda fragmented DNA (n=24) and human genomic DNA (n=8).

The sequencing data were demultiplexed by Illumina's BaseSpace® according to the adapter barcode sequence and aligned to the reference genome. The coverage map of the genome was analyzed using Geneious® Prime 2021.2.2[1]. Our data showed low sample variability and reliable uniformity for libraries prepared using DNA prep kits on the OT-2.

SCHEMATIC OF THE OT-2 WORKFLOW PROTOCOL

For the Illumina DNA Prep kit, the DNA and IDT® for Illumina DNA/RNA UD Indexes Set A (Illumina, Cat. No. 20027213) were tagmented on the OT-2 **(Figure 1)**. A wash step is necessary for removing residual DNA and adapters. Next, PCR amplification was conducted using an OT-2 on-deck thermocycler with a programmable lid and block temperatures.

For both HyperPrep and NEBNext Ultra II DNA prep kits, fragmentation was performed with NEB Fragmentase for 16 minutes for lambda and 20 minutes for human genomic DNA, followed by a cleanup step with AMPure® XP Beads (Beckman Coulter, Cat. No. A63881). DNA end-repair/A tailing, followed by the AMPure XP beads cleanup step, barcode, or adapter ligation, was performed on the OT-2 **(Figure 1)**. Barcodes used were from KAPA Unique Dual Index Adapters (Roche, Cat. No. 08861919702) for HyperPrep and NEB Multiplex Dual Index Primers Set 1 (NEB, Cat. No. E7335L) for NEBNext Ultra II.

DNA amplification was performed using an on-deck thermocycler on the OT-2.

THE WORKFLOW LAYOUT

The layout of the Opentrons OT-2 platform includes the modules, labware, and DNA Prep reagents **(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.

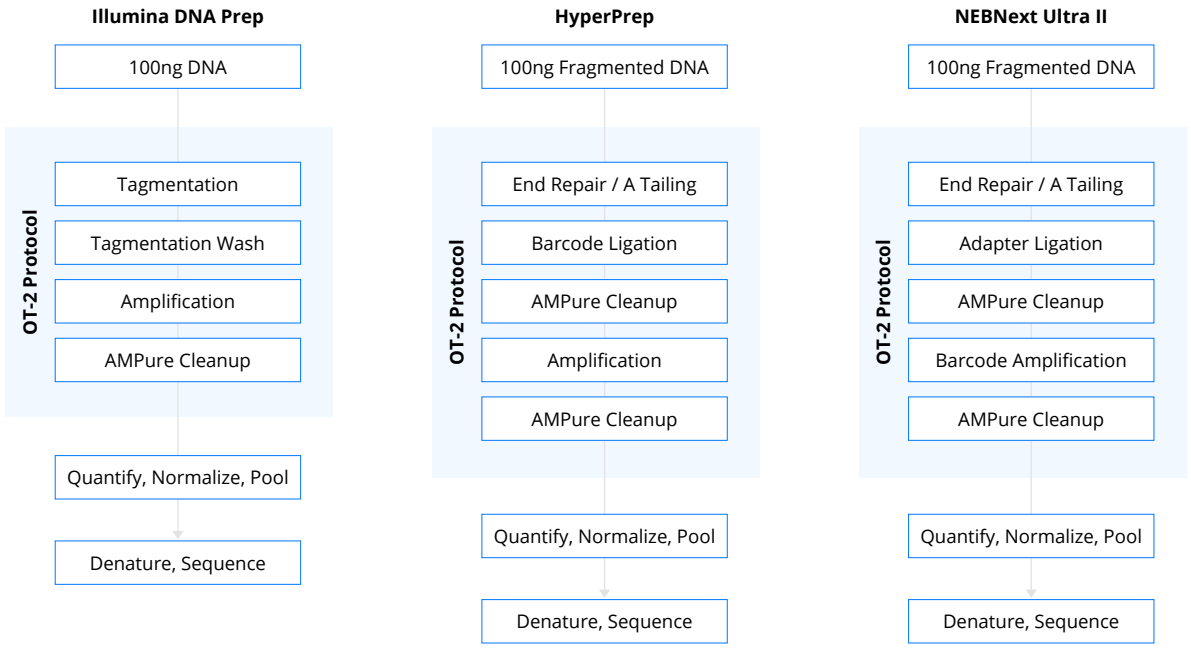

Figure 1: NGS Workflows with OT-2. The blue-shaded boxes indicate the steps of the DNA Prep workflow automated on the OT-2

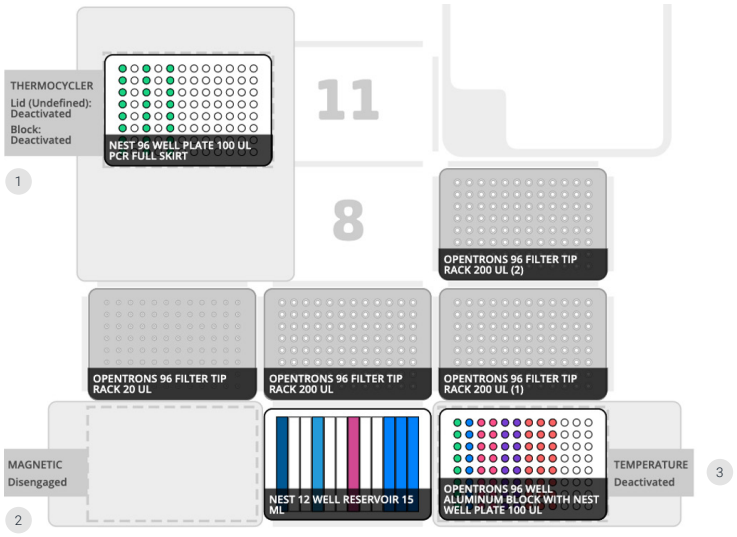

Figure 2: Opentrons OT-2 deck layout equipped with modules, labware, and DNA Prep reagents. This standardized deck layout is shared with HyperPrep and NEBNext Ultra II. This workflow has all pipetting automated on the OT-2 and requires manual intervention to move the sample plate (1) between the on-deck 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 200 µl 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 8-Channel pipettes.

RESULTS

DNA Library Amplification

DNA libraries were measured using PicoGreen® on a Qubit® 4 Fluorometer to determine concentration. The CV (coefficient of variation is the standard deviation divided by average). The final elution volume for all the libraries was 30 µL. For libraries prepared using the Illumina DNA Prep showed a CV of 10.7% for (n=8, 100 ng) and a CV of 16.6% for (n=24, 100 ng) **(Table 1A)**. For libraries prepared using the HyperPrep, showed a CV of 13.4% for (n=8, 100 ng) and a CV of 17.6 for (n=24, 100 ng) **(Table 1B)**. Libraries prepared using the NEBNext Ultra II showed a CV of 12.1% for (n=8) and a CV of 14.5% for (n=24) **(Table 1C)**. Comparisons for the yield (ng/µl), CV (%), and fragment size (bp) for lambda and human DNA libraries prepared on the OT-2 are shown in **Table 2**.

Small Genome Sequencing of DNA Prep Libraries

The multiplexed lambda DNA prep libraries (n=8, 100 ng) were sequenced using a 2x75 flow cell on a Illumina MiSeq instrument. The second batch of DNA prep libraries (n=24, 100 ng) were processed in 3 separate columns on the OT-2 to account for any inter-column variability within a batch. The Agilent® 5300 Fragment Analyzer calculated fragment sizes of the DNA prep libraries to determine the reliable uniformity for libraries prepared on the OT-2 **(Table 3A–3C)**. Similarly, consistent sequencing coverage of the 48 kb lambda genome showed uniformity across 8-plexed DNA prep libraries constructed on the OT-2 **(Table 4)**.

Barcode balance was accurate for the DNA Prep samples showing an average of 11.2%–12.31% for Illumina DNA Prep, 10.6%–14.1% for the KAPA HyperPrep, and 10.5%–14.6% for the NEBNext Ultra II **(Figure 3)**. The comparisons of the DNA prep kits allocation of tips and the duration of OT-2 protocol **(Table 5)**.

CONCLUSION

We validated Illumina DNA Prep, KAPA HyperPrep, and NEBNext Ultra II on the Opentrons OT-2 and demonstrated its use for library preparation for next generation sequencing. We showed that the DNA Prep samples displayed low variability across replicates and chemistries, even sample barcode balance, and high coverage of the sequence alignments, demonstrating that the OT-2 can be used to reliably automate preparation of high-quality DNA libraries.

ILLUMINA DNA PREP ON 100 NG LAMBDA						
	8x Replicates		**24x Replicates**			
	Column 1		Column 1	Column 2	Column 3	
	25.0		41.4	39.8	31.3	
	31.4		27.9	34.2	35.0	
	33.4		31.1	31.1	27.7	
	24.6		40.9	32.6	27.0	
	26.8		33.5	48.8	31.1	
	27.1		34.0	34.2	41.9	
	28.5		31.0	46.8	33.3	
	29.0		42.0	39.8	37.0	**Combined**
Average	28.2	**Average**	35.2	38.4	33.0	35.6
CV	10.7%	**CV**	15.5%	17.2%	14.9%	16.6%

Table 1A: Low variability among libraries constructed on the Opentrons OT-2.
Final eluted volume is 30 µl, values are ng/µl. This table shows the final yield of each sample after Illumina DNA Prep.

HYPERPREP (NG/µL)						
	8x Replicates		**24x Replicates**			
	Column 1		Column 1	Column 2	Column 3	
	19.7		7.1	7.8	11.4	
	17.5		10.1	7.8	9.6	
	17.3		9.4	10.1	12.0	
	15.5		8.7	10.8	11.7	
	13.2		7.1	7.1	8.2	
	15.8		8.1	7.2	10.4	
	14.5		7.8	9.5	11.9	
	14.0		8.0	8.5	9.4	**Combined**
Average	15.9	**Average**	8.3	8.6	10.6	9.1
CV	13.4%	**CV**	12.9%	15.9%	13.3%	17.6%

Table 1B: Low variability among libraries constructed on the Opentrons OT-2.
Final eluted volume is 30 µl, values are ng/µl. This table shows the final yield of each sample after HyperPrep.

NEBNEXT ULTRA II (NG/µL)						
	8x Replicates		**24x Replicates**			
	Column 1		Column 1	Column 2	Column 3	
	15.5		27.1	25.9	33.1	
	21.4		23.8	26.4	28.4	
	17.0		33.2	27.5	26.7	
	19.9		32.2	28.5	31.0	
	21.9		26.5	17.9	29.2	
	18.2		21.2	29.2	23.3	
	20.5		26.0	25.9	24.4	
	17.3		30.2	20.9	32.0	**Combined**
Average	19.0	**Average**	27.5	25.3	28.5	27.1
CV	12.1%	**CV**	15.0%	15.4%	12.4%	14.5%

Table 1C: Low variability among libraries constructed on the Opentrons OT-2.
Final eluted volume is 30 µl, values are ng/µl. This table shows the final yield of each sample after NEBNext Ultra II.

SUMMARY	INPUT	SAMPLE TYPE	SAMPLES	YIELD (NG/µL)	CV	FRAGMENT SIZE (BP)
Illumina DNA Prep	100 ng	Lambda	8	28.23	10.7%	310±15
Illumina DNA Prep	100 ng	Lambda	24	35.56	15.9%	316±15
Illumina DNA Prep	100 ng	Human Genomic	24	10.69	17.3%	247±15
HyperPrep	100 ng Fragged	Lambda	8	15.94	13.4%	280±10
HyperPrep	100 ng Fragged	Lambda	24	9.18	14.6%	280±10
HyperPrep	100 ng Fragged	Human Genomic	8	16.61	11.4%	283±10
NEB Ultra II	100 ng Fragged	Lambda	8	18.61	12.1%	259±12
NEB Ultra II	100 ng Fragged	Lambda	24	27.10	14.0%	255±12
NEB Ultra II	100 ng Fragged	Human Genomic	8	19.43	14.8%	243±12

Table 2: Comparison of NGS Sample Library Preps on the OT-2.

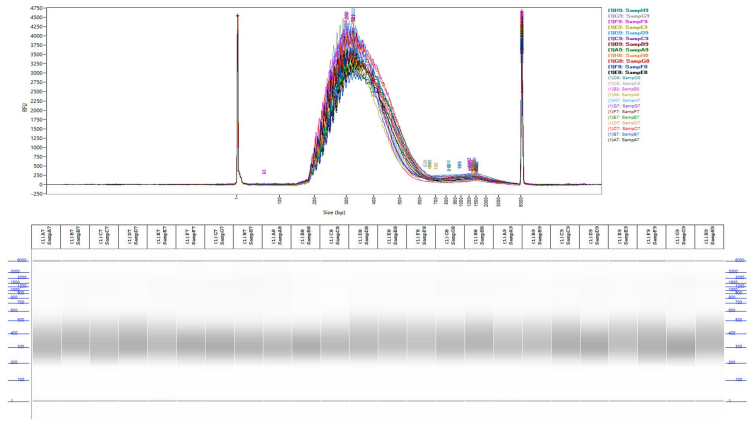

Table 3A: Illumina DNA Prep Sample Fragment Sizes

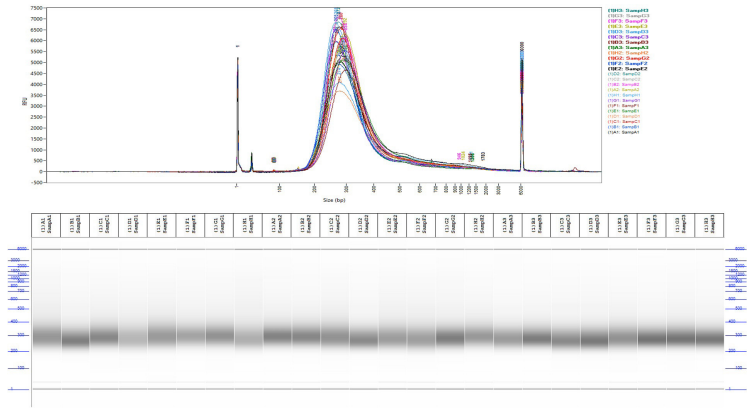

Table 3B: HyperPrep Sample Fragment Sizes

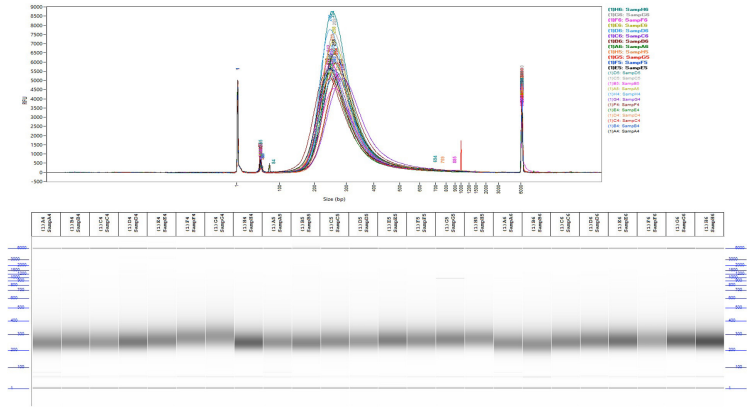

Table 3C: NEBNext Ultra II Sample Fragment Sizes

	ILLUMINA DNA PREP	HYPERPREP	NEBNEXT ULTRA II
Average	7865x	6883x	7589x

Table 4: NGS Sample Prep sequencing coverage. A comparison of Lambda Genome coverage of 2x75 Miseq runs of 8x Multiplexed sample libraries.

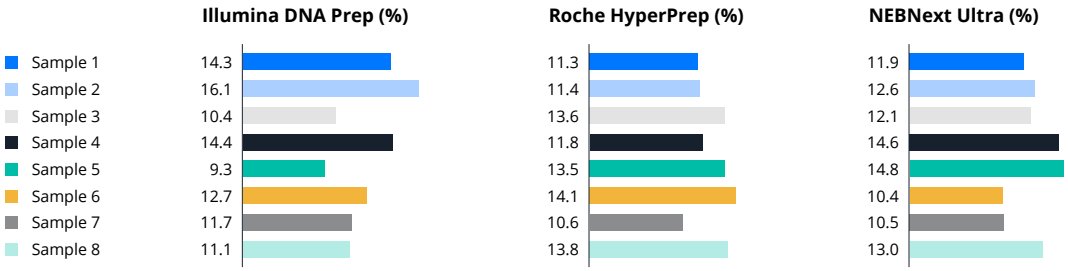

Figure 3: Uniform sample barcode representation. This chart demonstrates the even sample barcode balance within the sequencing run by overall read percentage.

	TIP BOXES REQUIRED (WITHOUT REUSING TIPS)		TIP BOXES REQUIRED (WITH REUSING TIPS)		
	p20	**p300**	**p20**	**p300**	**Time**
Illumina DNA Prep					
24x	1	5	1	3	2 Hrs 44 Min
16x	1	3	1	2	2 Hrs 14 Min
8x	1	2	1	1	1 Hrs 48 Min
HyperPrep					
24x	1	5	1	3	3 Hrs 9 Min
16x	1	4	1	2	2 Hrs 42 Min
8x	1	2	1	1	2 Hrs 20 Min
NEBNext Ultra II					
24x	2	5	1	3	3 Hrs 9 Min
16x	1	4	1	2	2 Hrs 42 Min
8x	1	2	1	1	2 Hrs 20 Min

Table 5: Comparison of NGS library prep configuration experiment duration and tip usage.

REFERENCES

1. Geneious Prime 2021.2.2