NEXTERA® XT DNA LIBRARY PREPARATION ON PIPETMAX®

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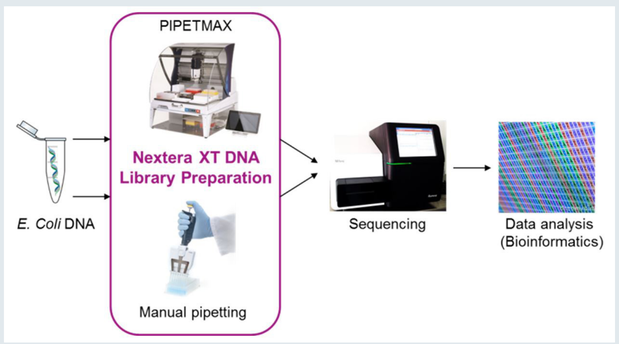
1. ABSTRACT

Reliable and reproducible library preparation is critical for successful next generation sequencing (NGS). In this project we automated the liquid handling steps of the Illumina Nextera XT DNA Library Preparation Kit workflow and then constructed replicate libraries using either a benchtop liquid handler or manual pipetting performed by an experienced technician. Five automated scripts were developed to correspond to the segments of the workflow: Tagmentation, Amplification, Library Cleanup, Library Normalization, and Library Pooling. PIPETMAX was equipped with two motorized, multichannel air displacement pipette heads that enable precise single channel or multichannel pipetting from 1 µL to 200 µL. The compact benchtop instrument permits up to nine bed elements to be employed during an automated script, including tips, microplates, tube racks, and accessories such as an on-bed orbital shaker. A custom rack was constructed to hold SLAS-footprint labware in the portrait orientation (as opposed to the landscape orientation on a standard PIPETMAX tray) and was used in the Amplification plate setup script to enable multichannel pipetting of Illumina TruSeq primers into the proper matrix. As part of the Library Cleanup and Library Normalization procedures, the Agencourt® AMPure® XP PCR purification system was automated using an on-bed magnetic bead separator rack. PIPETMAX can carry out all steps of the AMPure XP PCR cleanup procedure without manual intervention, including raising and lowering the magnets, resuspending paramagnetic beads, and transferring all liquids such as sample, wash solution, 80% ethanol, and resuspension buffer. The automated AMPure XP script also allows the end user to modify several variables such as number of samples to process, sample volume, bead volume, number of wash steps, and incubation times. The wizard-style TRILUTION® micro software interface provides real-time updates on the run status and produces run reports that help reduce errors associated with manual data entry. To assess the library quality for NGS libraries constructed using the automated procedure versus manual liquid handling, twelve Nextera XT DNA libraries were constructed using PIPETMAX and an additional twelve were prepared using manual pipetting. Each library was prepared from 1 ng of *E. coli* genomic DNA. Library size was assessed using an Agilent BioAnalyzer. All 24 libraries were pooled and sequenced in one lane of an Illumina MiSeq instrument. Each library was downsampled to 312,500 reads, which yielded >8x coverage for all 24 libraries. Both library preparation methods generated high quality data with >95% mapped reads and optimal quality scores. The percentage of mapped reads was almost identical for libraries prepared with automated liquid handling or manual liquid handling. The libraries prepared with PIPETMAX exhibited smaller standard deviation and variance, consistent with the reproducibility of liquid handling on this system.

2. EXPERIMENTAL DESIGN

Twelve libraries were prepared with PIPETMAX, and twelve with manual pipetting. All 24 normalized libraries were pooled and sequenced in one MiSeq lane.



3. MATERIALS AND METHODS

E. coli K12 genomic DNA, obtained from a commercial source, was diluted to the recommended concentration (0.2 ng/ µL) before use. Each library was prepared from 1 ng of *E. coli* genomic DNA. Automated liquid handling was performed with a Gilson PIPETMAX® 268. Five automated scripts, corresponding to the Tagmentation, Amplification, Library Cleanup, Library Normalization, and Library Pooling portions of the workflow, were written using TRILUTION® micro Protocol Builder software. Gilson TRILUTION® micro software running on a tablet PC was used to control PIPETMAX® and the on-bed accessories including the magnetic bead separator and orbital shaker.

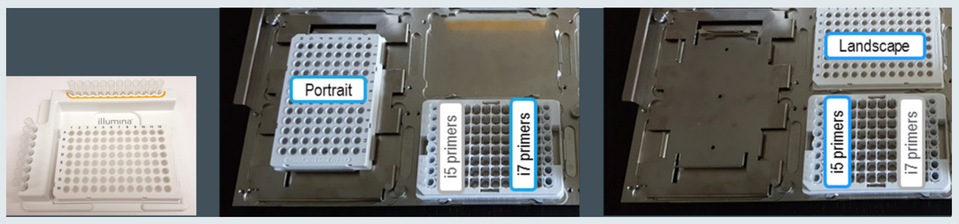
Libraries were prepared using Illumina reagents (Illumina p/n FC-131-1096 and FC-131-1001) according to manufacturer's guidelines. Subsequent to library cleanup with Agencourt® AMPure® XP beads (Beckman Coulter p/n A63880), library size was assessed using an Agilent BioAnalyzer. For sequencing, all 24 libraries were pooled and run on an Illumina MiSeq® system by an Illumina Certified Service Provider (Lucigen Corp., Madison, WI). Sequencing reads were downsampled to 312,400 reads per library and mapped to the *E. coli* K12 genome.

4. AUTOMATION OVERVIEW

Each blue rounded rectangle represents one script for PIPETMAX. Each script corresponds to a portion of the Nextera XT System workflow. The TRILUTION® micro wizard-style software interface provides real-time updates during the run, provides prompts for user intervention when needed to reposition labware, and produces run reports that can be incorporated into laboratory notebooks or electronic laboratory notebooks.

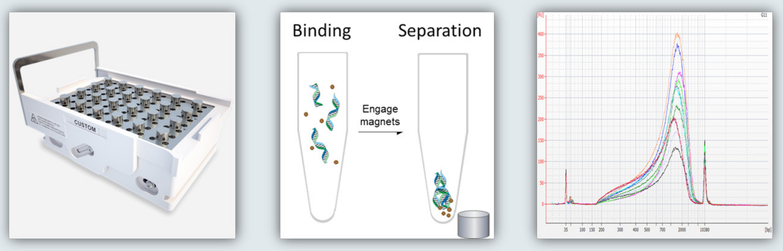
5. AMPLIFICATION PLATE SETUP

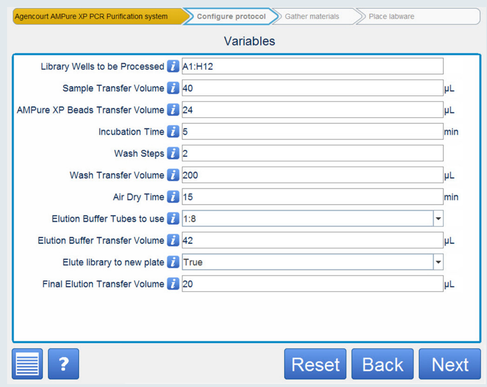
The manual procedure involves positioning primers in an Illumina Index Plate Fixture (left panel) and rotating the pipette to transfer cartain index primers into rows and others into columns. Since the motorized multichannel pipette heads on PIPETMAX are in a fixed orientation, this presented a challenge for automating this procedure on PIPETMAX. To address the need for multichannel addition of index primers across rows and columns we developed a custom rack that rotates the labware 90 degrees. This custom rack occupies two deck positions and holds an item of SLAS-footprint labware in the portrait orientation (as opposed to the landscape orientation on a standard PIPETMAX tray). This enables multichannel pipetting of Illumina TruSeq primers into the proper matrix in the Amplification plate setup script. The i7 primers are first added to the plate held in the portrait orientation. The user then repositions the plate to the landscape position before the i5 primers are added.



6. AMPURE XP BEAD CLEANUP

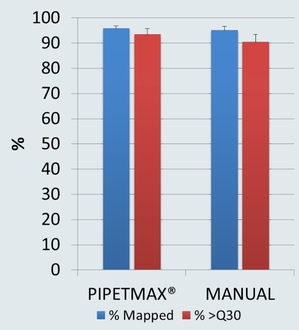
An on-bed magnetic bead separator rack was used to automate the Agencourt® AMPure® XP PCR purification system for the Library Cleanup script. The instrument can toggle the magnets (middle panel) between a lower (disengaged) position to permit binding of samples to paramagnetic beads and an upper (engaged) position to pellet the paramagnetic particles. PIPETMAX carries out all steps of the cleanup procedure without manual intervention, including engaging and disengaging the magnets, resuspending paramagnetic beads, and liquid handling steps including transferring the eluted material to a new plate. After cleanup, library size was assessed using an Agilent BioAnalyzer. The automated AMPure XP script includes several variables that can be changed at run time, including the number of samples to process, sample volume, bead volume, number of wash steps, and incubation times (refer to figure below).





7. 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 twelve libraries prepared with manual pipetting and the twelve libraries prepared with PIPETMAX were pooled and all 24 libraries were sequenced in one lane of an Illumina MiSeq instrument. Data from each library was downsampled to 312,500 reads. We observed >95% mapped reads and >8x coverage of the genome regardless of whether libraries were constructed with automated or manual liquid handling. The variance observed for libraries constructed with the automated workflow were slightly smaller than those prepared manually (30.8% vs. 34.1%), indicating the technical replicates prepared with automated liquid handling were more uniform than the technical replicates prepared with manual pipetting.

8. SUMMARY

We compared sequencing results for *E. coli* whole genome sequencing libraries prepared with the Illumina Nextera XT kit, with liquid handling carried out either manually or automated with PIPETMAX. The figure above shows the percentage of mapped reads and the quality scores (% >Q30) averaged across twelve replicate libraries prepared either with automated or manual pipetting. The automated methods provided reproducible liquid handling, resulting in smaller standard deviations and lower variance between replicates.

9. ACKNOWLEDGEMENTS

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