COMPARISON OF MANUAL VS. AUTOMATED LIQUID HANDLING FOR NGS LIBRARY PREP

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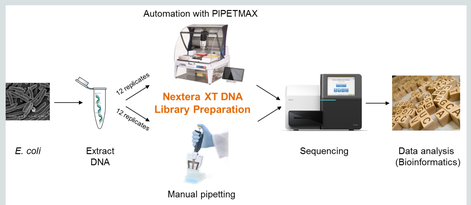
ABSTRACT

In this work we compare data from sequencing-ready normalized libraries that were generated either using manual liquid handling or an automated liquid handler equipped with multichannel motorized air displacement pipettes mounted on an XYZ robotic platform. Five scripts were developed to automate the liquid handling steps of the tagmentation, amplification plate setup, library cleanup, library normalization, and library pooling procedures in the Nextera XT DNA Library Preparation workflow. A custom magnetic bead separator was utilized to automate the Agencourt® AMPure® XP PCR purification system that is required as part of the library cleanup and library normalization procedures. The magnetic bead separator was used to carry out automated bead cleanup of up to 96 samples in a single run and the software interface allows the end user to adjust variables, such as number of samples to process, sample volume, bead volume, number of wash steps, and incubation times, depending on the needs of the application. 12 replicate libraries were prepared using manual liquid handling and an additional 12 replicate libraries were prepared using automated liquid handling. Each library was prepared from an input of 1 ng *E. coli* genomic DNA. The 24 libraries were then pooled and subjected to massively parallel sequencing in one lane of an Illumina MiSeq system. Each library was downsampled to a consistent number of reads, yielding >8x coverage for all 24 libraries. Both library preparation methods generated high quality data with >95% mapped reads. The variance observed for libraries constructed with the automated liquid handling were slightly smaller than those prepared manually.

EXPERIMENTAL DESIGN



Twelve libraries were prepared using manual pipetting and twelve libraries were prepared using PIPETMAX.

MATERIALS AND METHODS

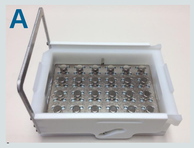
E. coli K12 genomic DNA was obtained from a commercial source and diluted to the recommended concentration (0.2 ng/ µL) before use. Automated liquid handling was performed with a Gilson PIPETMAX® 268. Gilson TRILUTION® micro software running on a tablet PC was used to control PIPETMAX® and on-bed accessories including the magnetic bead separator and orbital shaker.

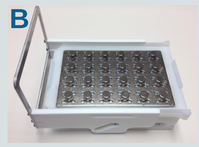
Libraries were prepared according to manufacturer's guidelines (Illumina p/n FC-131-1096 and FC-131-1001). Following library cleanup with Agencourt® AMPure® XP beads (Beckman Coulter p/n A63880), libraries were checked for size distribution and sample purity via an Agilent Bioanalyzer. All 24 libraries were pooled and run on an Illumina MiSeq® sequencing 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.

AUTOMATION OVERVIEW

Each blue rounded rectangle represents one PIPETMAX script, each of which corresponds to a portion of the Nextera XT System workflow. Some user intervention may be required to reposition labware or centrifuge a microplate between liquid handling steps.

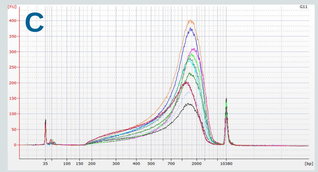
MAGNETIC BEAD CLEANUP





Custom magnetic bead separator used in the automated procedure. The handle of the device can be actuated by the liquid handler to move the magnets from the disengaged lower position (A) to the engaged upper position (B). When engaged, each magnet is in proximity to 4 wells of a 96 well plate.

The automated library cleanup procedure was carried out using Agencourt AMPure XP beads. Following PCR cleanup, DNA fragment size was assessed using an Agilent 2100 Bioanalyzer (C).

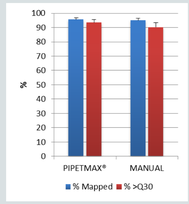


RESULTS

		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	95.10%	1.50%	
	Fold coverage	9.395X	0.174	1.9%
	% reads >Q30	90.3 %	3.2 %	9.3 %

The 24 libraries (12 prepared with manual pipetting and 12 prepared with PIPETMAX) were pooled and sequenced in one lane of an Illumina MiSeq instrument. Sequence data from each library was downsampled to 312,500 reads. Libraries constructed with either automated or manual liquid handling performed well, achieving >95% mapped reads and >8x coverage of the genome. 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.

SUMMARY



The percentage of mapped reads and the quality scores (% >Q30) were 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.

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