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LIBRARY PREPARATION FOR THE OXFORD MINION SEQUENCER WITH 'ASPIRE': AUTOMATED SAMPLE PREP BY INDEXED ROTARY EXCHANGE

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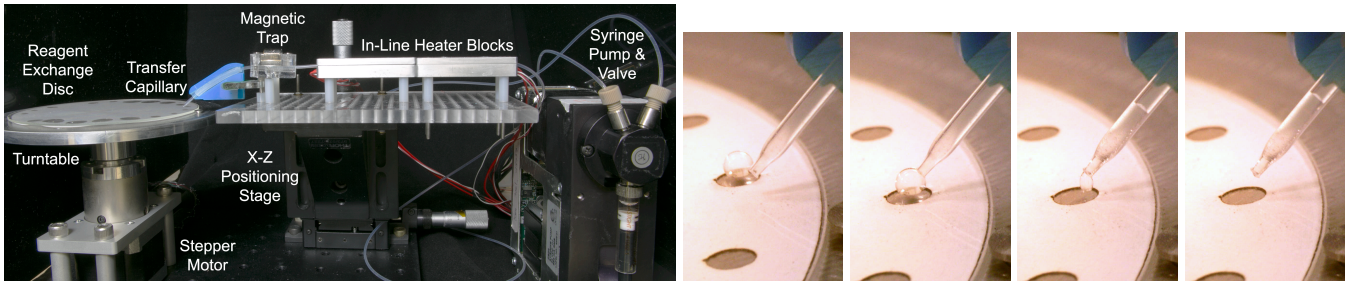
ABSTRACT

We report a prototype system to automate the DNA library preparation of bacterial genomes for analysis with the Oxford MinION nanopore sequencer as a first step towards a universal bacterial pathogen identification and biosurveillance tool. The ASPIRE (Automated Sample Preparation by Indexed Rotary Exchange) platform incorporates a rotary hydrophobic substrate that provides sequential delivery of sample and reagent droplets to heater and magnetic bead trapping modules via a single capillary coupled to a syringe pump. We have applied ASPIRE-based library preparation to lambda-phage and *E. coli* genomic DNA (gDNA) and verified its ability to produce libraries with DNA yield and ultimate sequenced read size distribution, quality, and reference-mapping percentages comparable to those obtained for benchtop prep methods.

KEYWORDS: library preparation automation, nanopore sequencer, long DNA, surface microfluidics

INTRODUCTION

The Oxford MinION is a long-read, portable DNA sequencer that detects changes in ionic current as single-stranded DNA translocates through a nanopore [1]. The small size and real-time analysis capabilities of the MinION make it particularly well suited for point-of-care and field-based applications like outbreak investigations and environmental metagenomics. Currently, DNA extraction and library preparation are still primarily performed using conventional benchtop methods, hampering the use of MinION sequencing in the field [2]. To address this challenge, we have developed ASPIRE, a simple, droplet-based, low-shear microfluidic platform that automates and streamlines the preparation of MinION-ready long fragment DNA libraries for sequencing.



(a) (b)

Figure 1: (a) Photo of the ASPIRE prototype. (b) Droplet aspiration from reagent exchange disc to transfer capillary.

EXPERIMENTAL

The first-generation ASPIRE prototype is shown in Figure 1a. The user initially pipettes sample and reagent droplets onto the Teflon AF coated glass surface of the 10 cm reagent exchange disc at locations defined by a template underneath. Under automated operation, a stepper motor rotates the turntable supporting the disc, bringing reagent droplets one at a time into contact with the thermo-mechanically pulled tip of the transfer capillary (Figure 1b), a 1/16 in perfluoroalkoxy (PFA) tube that is routed past a rare earth magnet bead trap and through temperature controlled heater blocks to a syringe pump. In addition to magnetic bead purifications and heated sample incubation, sample/reagent mixing can be accomplished by repeatedly dispensing/aspirating to/from the hydrophobic surface of the reagent exchange disc. These basic unit operations enable the semi-automated execution of the MinION 2D library preparation protocol (i.e., template and complement strands joined by a hairpin loop and read sequentially) including DNA clean-up, end-repair, dA-tailing, and hairpin/adapter ligation reactions. Moreover, the droplet-on-disc format and 1 mm ID capillary enable all operations to be done at standard benchtop protocol volumes.

To evaluate ASPIRE performance, Covaris g-TUBE sheared Lambda phage and unsheared *E. coli* genomic DNA were prepared with ASPIRE and with standard benchtop methods. Library quality was assessed by comparing DNA yield after the dA-tailing and adapter ligation steps and by evaluating 6-hour sequencing run results in detail.

RESULTS & DISCUSSION

Table 1 summarizes key figures of merit for Lambda phage and *E. coli* library preparation and sequencing. DNA yields following end-repair and dA-tailing were found to be comparable, while ASPIRE yielded slightly more DNA with less variation following adapter ligation, perhaps a benefit of automation. While variability between MinION flowcells may account for the observed disparity in read counts between ASPIRE and bench, both methods produced a usable number of reads and showed essentially equivalent size distributions, comparable mean read sizes, and maximum read lengths in excess of 75 kbp. In these experiments, ASPIRE also produced a significantly higher proportion of genome-mapped reads (both 2D template/complement and 1D template-only) than the bench prep, particularly for short reads. While more data are needed to establish whether the ASPIRE platform can consistently produce results that are better than benchtop methods, these experiments provide an encouraging proof of concept for this automation approach and its utility in providing high-quality sequencing libraries suitable for bacterial genomic analysis.

CONCLUSION

ASPIRE is a compact, inexpensive, semi-automated sample prep architecture that has been successfully applied to execute the Oxford MinION library preparation protocol, yielding sequence results comparable to those obtained by conventional methods. The simplicity and flexibility of the approach shows promise for automating a variety of sample prep tasks, particularly in low resource, field-forward, and point-of-care applications.

Table 1. Comparison of ASPIRE and bench library prep results

Metrics	λ DNA		*E. coli* gDNA	
	ASPIRE	Bench	ASPIRE	Bench
Post-End Prep Yield (ng DNA)	680 ± 187	642 ± 204	866	672†
Post-Ligation Yield (ng DNA)	118 ± 12.7	67.3 ± 12.6	100	60†
Total Reads	66,583	-	70,051	43,873
Mean Aligned Read (bp)	7,886	-	5,244	4,936
Longest Aligned Read (bp)	47,581	-	75,642	76,946
% Aligned Reads	88.0%	-	85.7%	68.0%

† Quantitation and sequence results are from two different preps

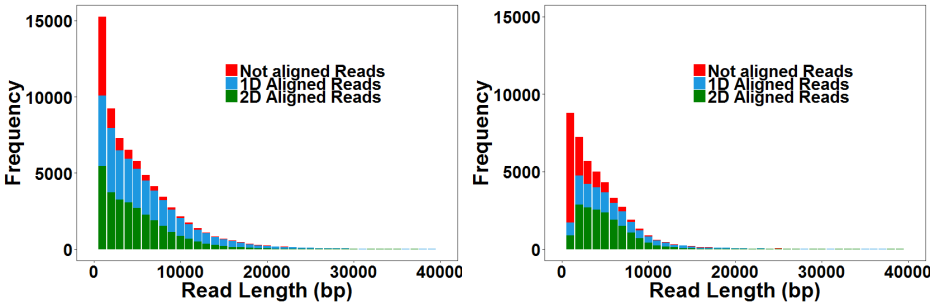


Figure 2: MinION-sequenced read length histograms for E. coli gDNA prepared on ASPIRE (left) and bench (right) using the 2D library prep protocol.

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REFERENCES

[1] H. Bayley, "Nanopore sequencing: from imagination to reality," *Clinical chemistry*, 61, 1, 2015.

[2] J. Quick et al., "Real-time, portable genome sequencing for Ebola surveillance," *Nature*, 530, 7589, 2016.

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