INTEGRATED MICROFLUIDIC HUB FOR AUTOMATED PREPRATION OF DNA LIBRARIES FOR PERSONALIZED SEQUENCING SYSTEMS

Mais J. Jebrail[1], Hanyoup Kim[1], Numrin Thaitrong[1], Michael S. Bartsch[2], Ronald F. Renzi[2], and Kamlesh D. Patel[2]

[1]Department of Biotechnology & Bioengineering, Sandia National Laboratories, USA
[2]Advanced Concepts & Microsystem Engineering, Sandia National Laboratories, USA

ABSTRACT

While DNA sequencing technology is advancing at an unprecedented rate, sample preparation technology still relies primarily on manual bench-top processes, which often can be slow, labor-intensive, inefficient, or inconsistent. To address these disadvantages, we developed an integrated microfluidic platform for automated preparation of DNA libraries for next generation sequencing. This sample-to-answer system has great potential for rapid characterization of novel and emerging pathogens from clinical samples.

KEYWORDS

Digital microfluidics, capillary interface, sample preparation, DNA sequencing

INTRODUCTION

DNA sequencing has emerged as an important technique for enabling scientists to generate and use genetic information for applications in forensics, medicine and pharmaceutical research [1]. With the advancement and personalization of whole genome sequencing, there is a critical need for automating protocols for DNA preparation prior to analysis. We recently developed a novel integrated sample preparation system for robustly interconverting liquid samples between continuous-flow and discretized droplet formats for sample-to-answer functionality [2,3]. The central feature of our technology is the digital microfluidic (DMF) platform which functions as a central hub for distributing and routing samples and reagents. In DMF, discrete droplets are controlled (i.e., moved, merged, mixed, and dispensed from reservoirs) by applying a series of electrical potentials to an array of electrodes coated with a hydrophobic insulator [4]. We report here for the first time the successful application of our integrated platform to automate the Nextera™ DNA library preparation protocol for downstream sequencing with Illumina's MiSEQ® personalized sequencer. Relative to conventional manual methods, the new system can execute this protocol in less than two hours, reduce sample and reagents by 10-fold, and integrate quantitative library validation methods.

EXPERIMENTAL

DMF devices were fabricated in the Sandia National Laboratories Applied Biosystems cleanroom facility as described in detail elsewhere [2].

For DNA size analysis, Agilent 2100 Bioanalyzer System (Santa Clara, CA) was used with a high-sensitivity DNA kit. For DNA quantification, quantitative PCR based Kapa library kit (KAPA Biosystems, Woburn, MA) was used.

RESULTS AND DISCUSSION

As shown in Figure 1a-c, the central feature of our system is the digital microfluidic (DMF) platform which functions as a central hub for distributing and routing samples and reagents.

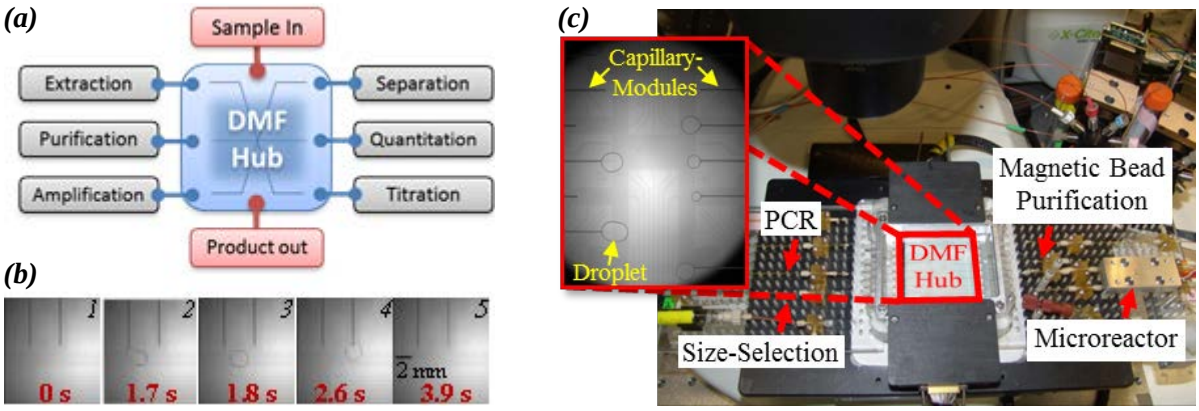

Figure 1: ***(a)*** *Schematic showing the concept of the central DMF Hub architecture with external processing modules connected together through the capillary-droplet interface.* ***(b)*** *A series of micrographs showing a droplet being dispensed from an in-plane capillary to the DMF platform, actuated to a second position, and aspirated from the device demonstrating the clean transfer of a small (~2 uL) fluid bolus from one module to another.* ***(c)*** *Picture of the setup used to perform the automated Nextera™ DNA library preparation with required reagents and processing modules.*

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In DMF, discrete microliter-size droplets are controlled (i.e., moved, merged, mixed, and dispensed from reservoirs) by applying a series of electrical potentials to an array of patterned electrodes coated with a hydrophobic insulator. Another crucial feature of the technology is the integration of multiple microchannel-based sample processing modules for enzyme reactions, bead-based purification, amplification and size-selection through an in-plane capillary-droplet interface. Using this platform, we have automated all the steps required for the Nextera™ transposase enzyme library preparation method, which simultaneously fragments the DNA and adds the appropriate priming adapters and barcodes to the fragment. Figure 2a shows the four protocol steps adapted to the platform to format the DNA for the sequencer, shows the resulting benchtop electrophoreograms for each step (Figure 2b), and illustrates the processing of the DNA fragments through each step (Figure 2c). The performance of our system was similar to manual benchtop approaches with 4-fold decrease in time and one-tenth of the amount of reagents and DNA required. Figure 2d shows an overview schematic and the series of micrograph that captures the execution of the Nextera protocol on our platform. Comparison of performance of sequencing results for the full genome coverage for E. coli showed no particular biasing and average of 50X coverage (data not shown) with an expected GC content and reads mapped to the reference genome.

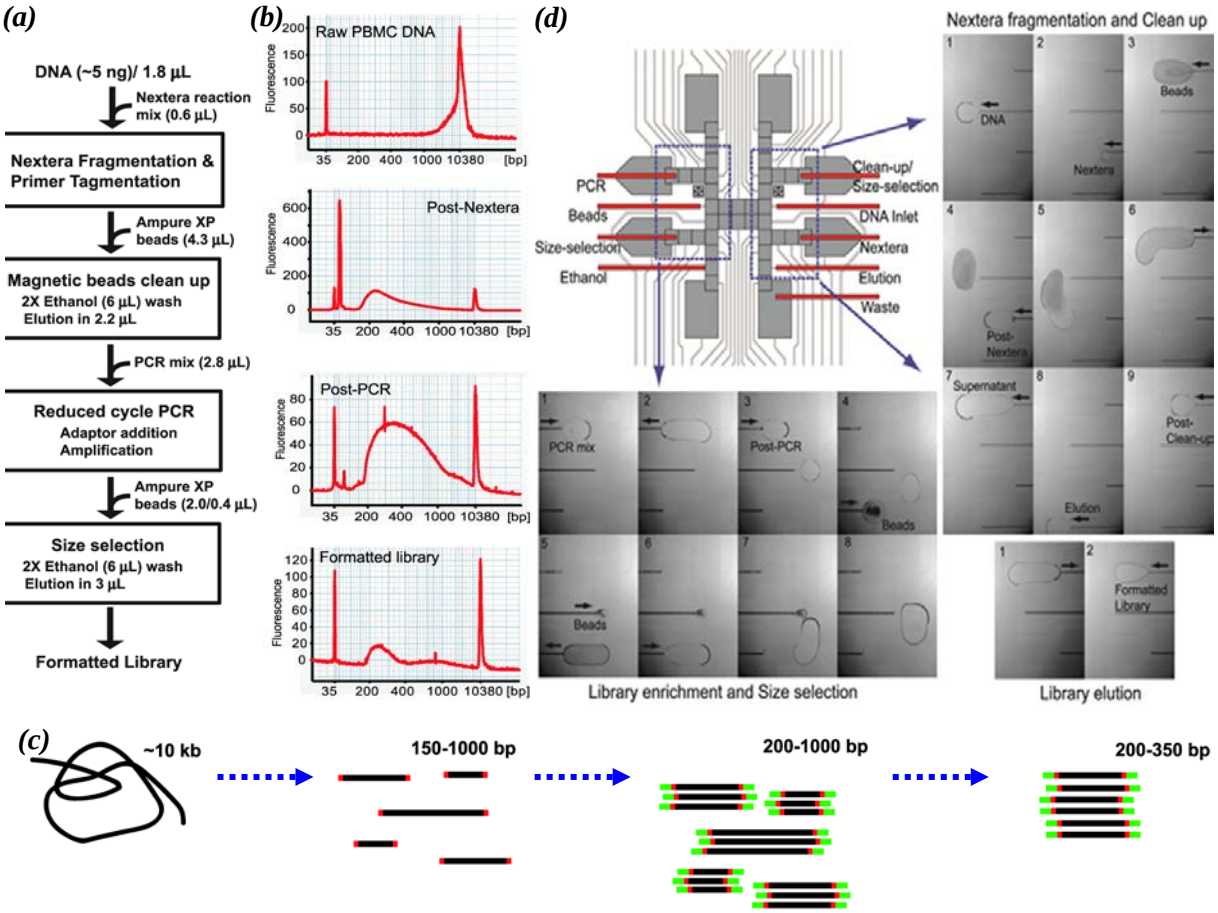

Figure 2: ***(a)*** *The four required steps for processing DNA for end-to-end library preparation. Starting amount of material on the DMF platform is only 5 ng of starting DNA amount (~1/10 of bench top amount).* ***(b and c)*** *corresponding benchtop bioanalyzer traces and schematic for the sample DNA at each step of the process.* ***(d)*** *Series of micrographs showing fragmentation, magnetic bead clean-up, library enrichment (PCR), and size-selection steps. The library was validated on a MiSEQ sequencer.*

CONCLUSIONS

In conclusion, we report a novel method for automating the Nextera™ DNA library preparation protocol for downstream sequencing and analysis. We propose that this technique can mature to a complete, easily configurable, sample-to-answer system that can aid in the rapid characterization of novel and emerging pathogens from clinical samples.

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CONTACT

K.D. Patel, tel: +1 925-294-3737; kdpatel@sandia.gov