Next Generation Sequencing Sample Preparation Utilizing the Echo® Liquid Handler

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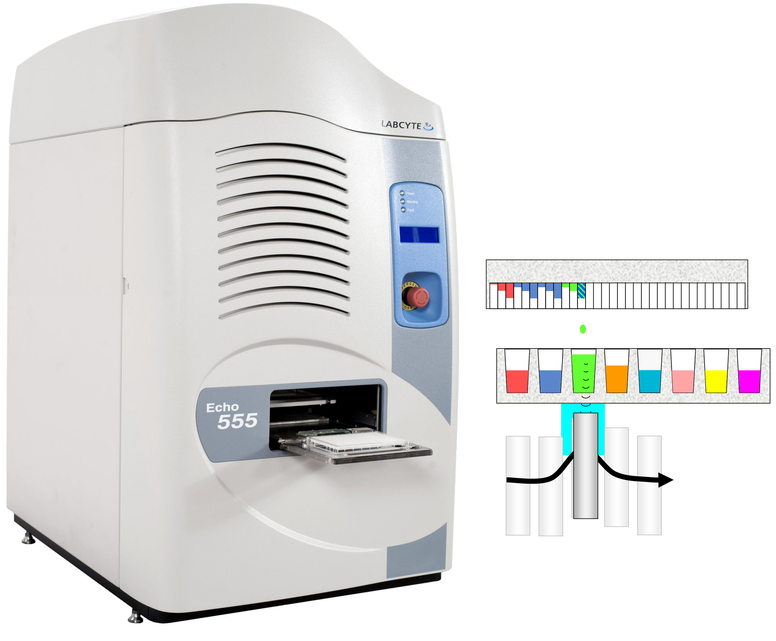
Abstract

The advent of Next-generation sequencing (NGS) has enabled researchers to overcome the limitations in resolution, scalability, and throughput experienced with capillary electrophoresis-based Sanger sequencing. While these technological advances have lowered the cost of sequencing, upstream library preparation remains a significant bottleneck and a prime target for automated liquid handling. The ability of Echo liquid handlers to acoustically transfer samples and reagents without tips or contact provides an efficient, contamination-free solution for genomic library preparation. The precision and accuracy of sub-microliter transfers from any microplate well to any microplate well accelerates and improves library pooling and normalization with less setup time in comparison to methods utilizing manual pipetting. In this work, the Echo 555 liquid handler was used to prepare libraries produced from *E.coli* for sequencing with the Illumina® MiSeq sequencer.

Echo Liquid Handler

The Labcyte Echo 500 series revolutionizes liquid transfer by using acoustic energy to transfer fluids. The Echo 500 series allows for access to any well of a microplate and acoustically eject liquids to a standard microplate, cell culture microplates, or slides from 2.5 nL to 50 µL volumes with high precision and accuracy. Transfer is non-contact and tipless, with increased cost savings from elimination of tip costs and washing fluids.

In collaboration with SeqMatic LLC, the Echo liquid handler was used to prepare libraries from *E.coli* for sequencing. Unlike traditional liquid handlers, Echo systems do not require operators to develop a set of calibrations or liquid handling techniques to transfer a variety of fluid types. The Echo system uses Dynamic Fluid Analysis™ a process by which the Echo system determines the power requirements to transfer each reagent, at runtime, on a well-by-well basis. The rapid acoustic transfer of DNA oligos (adaptors, primers, index barcodes) and enzymes (ligation mix, PCR master mix) to assay microplates at variable volumes greatly accelerates assay optimization and assembly. The Echo system's 2.5 nL resolution also eliminates the need to dilute amplified libraries for normalization and pooling.



The Echo 555 liquid handler uses acoustic energy to transfer fluids. A transducer uses acoustic energy to transfer reagents in a non-contact, tipless manner from a source microplate to an inverted destination microplate. Reagents can be transferred from any well to any well, at any volume. Empty and full wells can be addressed.

Acoustic Library Preparation

Methods

Genomic libraries produced from *E.coli* were prepared for sequencing with the Echo 555 liquid handler using the Illumina Nextera™ XT kit (FC-131-1096) in a procedure developed by SeqMatic LLC. The Echo 555 liquid handler was used to transfer 10 µL of TD (Tagment DNA Buffer), 5 µL of ATM (Amplicon Tagment Mix), and water to purified genomic samples (1 ng) in a 96-well PCR microplate. The final assay volume was 20 µL. The PCR microplate was sealed, centrifuged at 1000 rpm for 1 minute, and incubated at 55°C for 5 minutes. The Echo 555 liquid handler was used to transfer 5 µL of NT (Neutralize Tagment Buffer) to the PCR microplate. After transfer the microplate was sealed, centrifuged at 2000 rpm, and incubated at room temperature for 5 minutes.

The fragmented and labeled libraries were enriched by PCR amplification. Corresponding index 1 and index 2 primers were added at 5 µL using the Echo 555 liquid handler. 15 µL of Nextera PCR Master Mix was added manually. The PCR microplate was sealed, and centrifuged at 2000 rpm. Using the Veriti PCR system from Life Technologies, libraries were heated to 72°C for 3 minutes, then to 95°C for 30 seconds followed by thermocycling at 95°C for 10 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, for 12 cycles. After cycling, libraries were incubated at 72°C for 5 minutes.

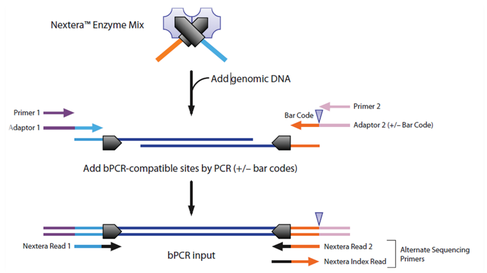


Figure 1. Schematic of Illumina Nextera library preparation reaction. Nextera enzyme mix was incubated with the genomic DNA, followed by the addition of adapters. The fragmented and labeled libraries were then ready for PCR enrichment with index primers.

A gel-based extraction was performed to purify and size-select libraries prepared with the Echo liquid handler. Libraries were manually added to 10 µL of 6% TBE PAGE gel and 2 µL 6X loading dye mix, then processed at 145 Volts for 60 minutes. The gel was then stained with SYBR Gold and visualized under UV light in comparison to a gel processed under identical conditions with manually prepared libraries. A band around 350-500 base pairs was extracted and transferred to a Gel Breaker tube inside a 2 mL microfuge tube, and centrifuged at 20,000xg for 2 minutes. The Gel Breaker tube containing the selected libraries was removed leaving behind the extruded gel inside the 2 mL microfuge tube. 100 µL of TE with 0.1% Tween-20 was added into the tube, which was capped and incubated at 55°C while shaking for 30 minutes.

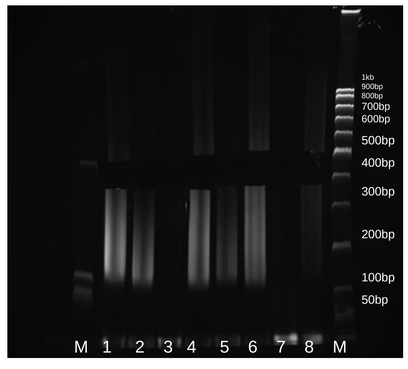


Figure 2. Gel image of manually prepared samples on lanes 1 to 8 with the 350 to 500 bp region of interest cut out.

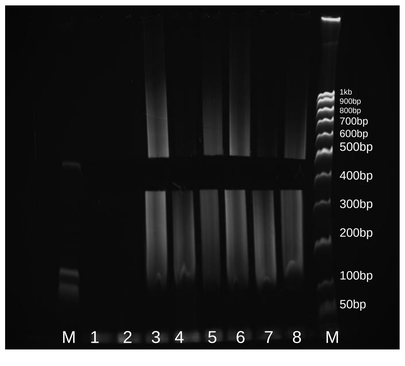


Figure 3. Gel image of Echo-prepared samples on lanes 3 to 8 with the 350 to 500 bp region of interest cut out.

Results

A comparison of gel-based extractions of libraries prepared manually and with the Echo liquid handler indicated more sample uniformity across libraries prepared by the Echo system.

Library Normalization and Pooling

Methods

Twelve enriched libraries were normalized and pooled with the Echo 555 liquid handler in a single transfer protocol without intermediate dilution steps. Libraries were quantified using the Agilent Bioanalyzer. From the estimated concentrations, the volume required to normalize each sample to a 2 nM concentration was calculated. An input file listing the sample wells and corresponding transfer volumes was used to build an Echo transfer protocol using the Echo® Cherry Pick application software. The Echo liquid handler then transferred the calculated volumes from each library to a single well of a destination plate. After transfer, 9 µL of water was manually added to the pooled library. The pooled was denatured, diluted to 10 pM and transferred to an Illumina flow cell cartridge for sequencing on an Illumina MiSeq sequencer.

Index	Raw Conc. [nM]	Vol. (nL)
501	7.7	260
502	11.1	180
503	1.7	1186
504	19.6	102
505	15.5	129
506	9.4	213
507	9.3	215
508	10.0	200
509	11.5	174
510	14.4	139
511	27.7	72
512	11.6	173

Table 1. Transfer volumes required for normalization of 12 libraries.

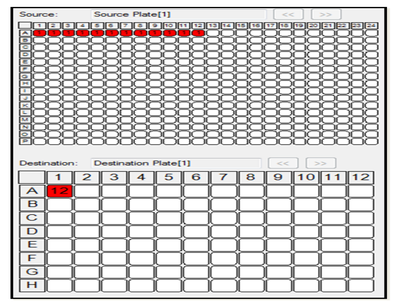


Figure 4. Screenshot from Echo Cherry Pick software. The volume transferred from each sample to a single well according to the volumes listed in Table 1.

Manual	Echo liquid handler
12 Samples	12 Samples
Library Normalization • Hands-on: >10 minutes Library Pooling • Hands-on: 5 minutes	Library Normalization and Pooling • Hands-on: < 2 minutes
Total Time > 15 minutes	Total Time < 5 minutes

Table 2. The normalization and pooling of libraries at SeqMatic were separate manual steps. The study with the Echo liquid handler combined the steps and enabled the work to be done in a shorter amount of time.

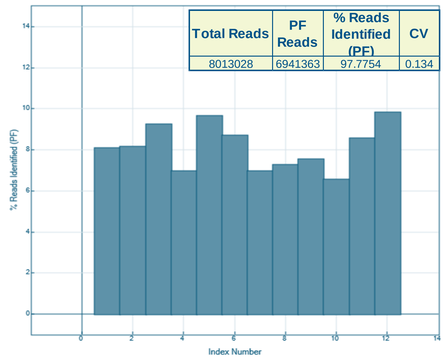


Figure 5. Graph displaying reads for each library prepared by the Echo system with each having a theoretical 8.3% (1/12) of total reads. Coefficient of variation for the group was at 13.4%. Percent reads identified as passing the Illumina filter (PF) was at 86.6%.

Results

The time required to normalize and pool libraries with the Echo liquid handler was significantly reduced in comparison to traditional manual preparation times recorded by SeqMatic. The prepared libraries were successfully sequenced with 97.8% of reads identified as passing the Illumina filter.

Summary

- The Echo liquid handler successfully transferred each component of the Illumina Nextera XT Library preparation kit to improve the reproducibility of gel-based extraction across libraries.
- The Echo liquid handler eliminates intermediate dilution steps to normalize and pool libraries to reduce the overall time for library preparation.