Echo™ Acoustic Liquid Handling and Next Generation TnX™ Transposase-based Technologies Enable Miniaturized, Automated NGS Library Preparation for Ultra High-Throughput Sequencing

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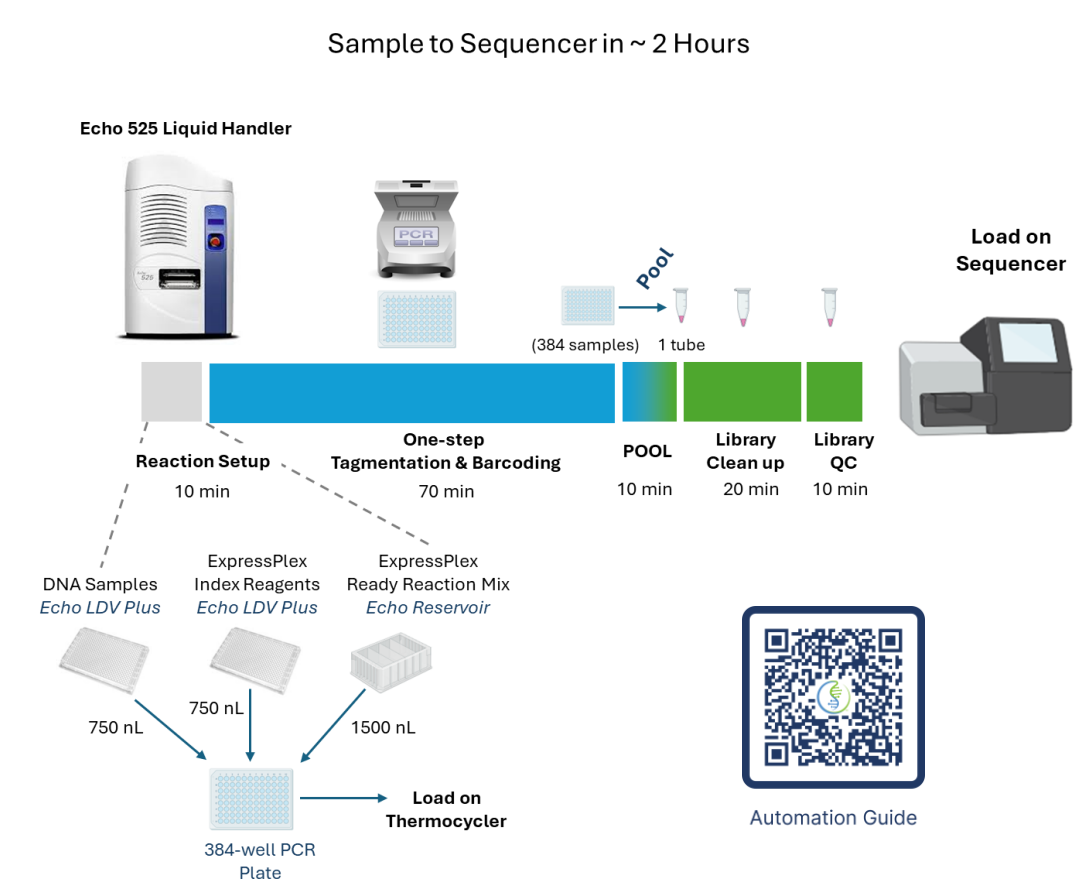
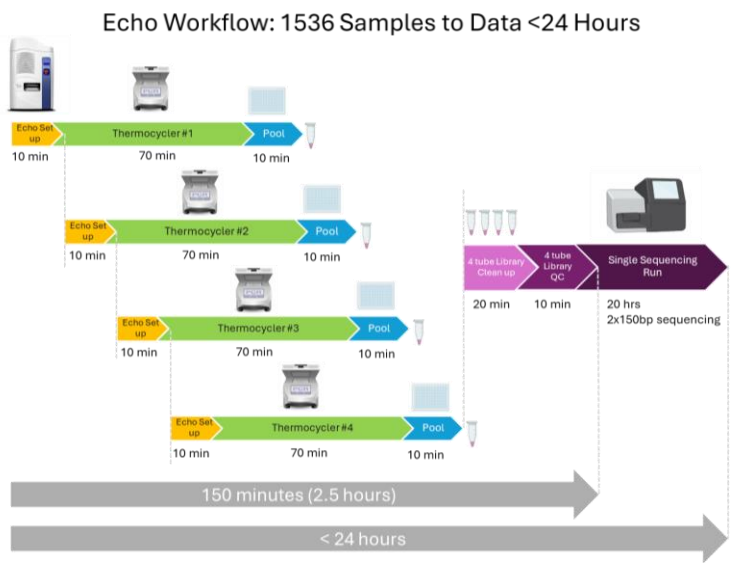
Introduction

ExpressPlex™ 2.0 is the fastest library preparation chemistry available. Using seqWell's high performance TnX transposase - which was specially engineered for NGS library preparation - it performs auto-normalization of sample input, fragments input DNA into sizes suitable for Illumina sequencers, and tags the DNA with combinatorial-dual-indexed adapters in a single step. Sequencing results in uniform read-count statistics for hundreds to thousands of samples. ExpressPlex 2.0 complements the Beckman Coulter Echo 525 by offering robust multiplexing capabilities for sequencing projects. It is designed to maximize throughput and data yield, as well as allows researchers to process thousands of samples simultaneously. This scalability is vital for large-scale efforts in synthetic biology, where the ability to sequence multiple samples in parallel can significantly accelerate discovery.

Here we demonstrate a proof-of-principle miniaturized (three-microliter) reaction setup on the Echo 525 for a single 384-well plate of pUC19 plasmid, resulting in highly consistent read counts (CV ~ 14% for 384/384 replicates). Using Echo's rapid contact-free dispensing, the fast ExpressPlex 2.0 chemistry, and miniaturized reactions, it is possible to achieve economies of scale and ultra-high-throughput workflows in plasmid sequencing.

The Echo 525 Enables Rapid Setup of Miniaturized ExpressPlex Reactions





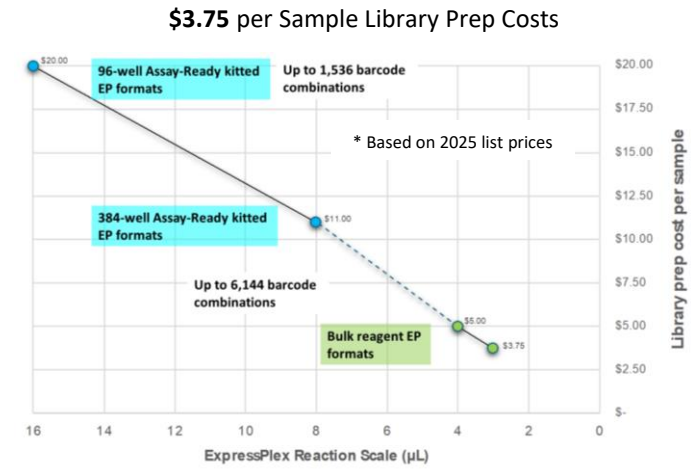


Figure 1: The Echo instrument performs acoustically-mediated contactless droplet dispensing. In the ExpressPlex 2.0 workflow, it rapidly and sequentially transfers DNA sample, Indexing Reagent, and Master Mix into a 384-well PCR plate. The plate is thermocycled, and then individual wells are pooled into a single tube, SPRI-purified, quantitated, and sequenced.

Data and Analysis

Read Counts are Uniform across all Samples

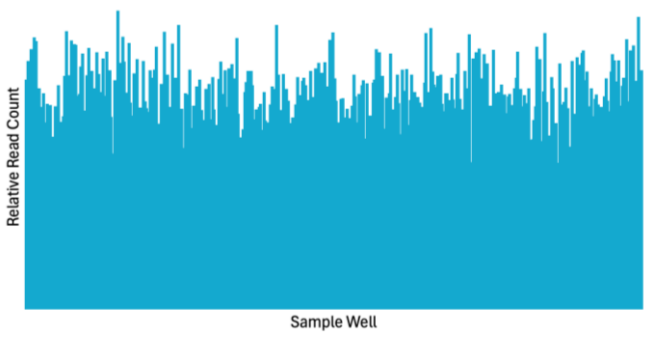


Figure 2: The CV of read counts across all wells is < 14% and there are no failed wells.

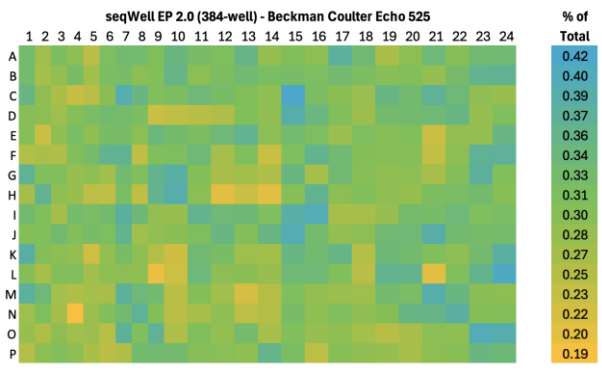
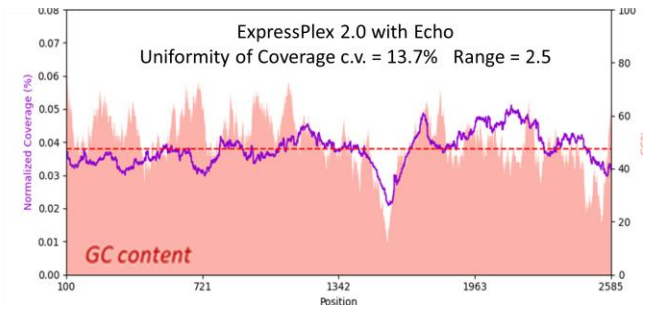


Figure 3: A color gradient image of read counts is even and devoid of systematic anomalies.

- pUC19 plasmid DNA was sourced from New England Biolabs (Ipswich, MA) and diluted to 1.25 ng/uL.
- Reactions were set up as described above, thermocycled, diluted with 10 mM Tris, pooled, purified by SPRI, and electrophoresed on an Agilent TapeStation.
- The resulting library pool was sequenced on an Illumina MiSeq.
- Bioinformatics analysis was carried out on seqWell's SNAP pipeline.
- Competitor uniformity-of-coverage data were acquired by seqWell under competitor's standard reaction conditions.



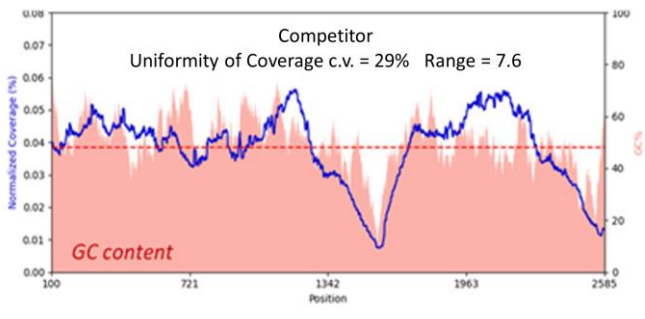


Figure 5: The benefits of the ExpressPlex 2.0 engineered transposase are seen in reduced insertion bias and greater uniformity of coverage. The figure on the left depicts a typical outcome for the Echo-derived 3 uL sequencing libraries. GC content of the pUC19 genome is shown in red, with normalized coverage overplotted. The figure on the right depicts the same parameters for libraries derived using a competitor kit under standard conditions. (**c.v.** = coefficient of variation; **Range** = average of the three highest read counts divided by the average of the lowest three read counts.)

100% of Plasmids were Assembled and Circularized

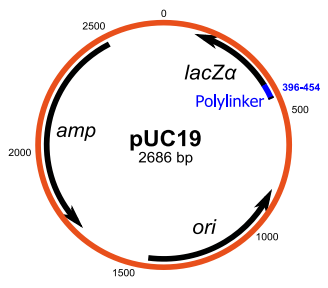


Figure 4: When downsampled to 2,000 read pairs per sample, all plasmids were successfully assembled *de novo*.

Summary

- Using Echo's rapid contact-free dispensing, the fast ExpressPlex chemistry, and miniaturized reactions, it is possible to achieve economies of scale and ultra-high-throughput workflows in plasmid sequencing.
- The scale of miniaturization demonstrated is approximately one fifth of a standard ExpressPlex reaction, resulting in significant savings in reagent costs.
- This approach should also be valid for amplicon sequencing and other construct-validation applications in protein engineering and synthetic biology.