The Biomek Echo One High-Throughput Genomics Workstation and seqWell's ExpressPlex™ 2.0 Enable Miniaturized, Fully Automated NGS Library Preparation for Ultra High Throughput Sequencing

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Abstract

High-throughput plasmid sequencing is foundational to progress in antibody engineering, synthetic biology, protein expression, and vaccine development. The rapid expansion of AI-guided design workflows has driven an exponential increase in the number of plasmids requiring high-throughput plasmid purification and sequence verification, making plasmid purification and library preparation a critical bottleneck in modern wet lab pipelines. Truly scalable sequencing workflows require fast liquid handling, miniaturized reactions, and streamlined, end-to-end automation.

Here we describe a fully automated plasmid-to-sequence workflow implemented on the Biomek Echo One High-Throughput Genomics Workstation, which combines nanoliter-scale acoustic dispensing with macroscale liquid handling. At the core of this platform are the Biomek i7 liquid handler and Echo 525 acoustic dispenser, enabling high-throughput plasmid purifications at macro-scale, followed by highly miniaturized (3 µL) library preparation reactions in 384-well PCR plates, on-deck thermocycling, automated pooling, and SPRI purification. The full protocol automates the bead-based CosMCPrep Plasmid Purification kit and seqWell's one-step ExpressPlex™ 2.0 library preparation chemistry. Plasmid purification takes 1 hour 16 minutes for 96 samples, and the end-to-end library preparation is completed in approximately three hours from start to sequencer-ready libraries.

This fully automated plasmid purification and miniaturized sequencing workflow dramatically reduces hands-on time while delivering robust, scalable, and economical plasmid sequence verification. Future integration with higher-speed sequencing platforms is expected to further shorten sample-to-answer turnaround times, accelerating design-build-test-learn cycles across synthetic biology and biologics development.

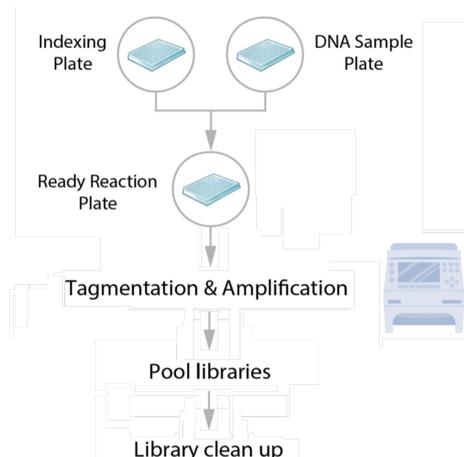## The ExpressPlex Workflow and the Biomek Echo One Genomics Workstation

Figure 1: The ExpressPlex workflow using seqWell's next-generation TnX™ transposase consists of reaction set up (combining indexing reagent, sample DNA, and polymerase master mix), one-step tagmentation & amplification, sample pooling of all wells into a single tube, and library clean up using magnetic beads. All necessary reagents are included within ExpressPlex kits.

ExpressPlex provides auto-normalization, fragments DNA into sizes suitable for Illumina sequencers, adds combinatorial dual-indexed adapters, and amplifies samples within a single step. Sample pooling and a SPRI cleanup complete the workflow. Sequencing results in uniform read-count statistics for hundreds to thousands of samples per run.

Figure 2: The Biomek Echo One solution incorporates the Echo 525 acoustic liquid handler with the Biomek i7 workstation, a tip-based liquid handler. In this study, 750 nL of transposase, 750 nL of sample DNA, and 1500 nL of polymerase master mix were combined into 3 µL reactions by the Echo 525 instrument and then incubated in an ODTC on the Biomek instrument deck. Cycled reactions were diluted in 10 mM Tris to raise their volumes, pooled, and SPRI-purified by the Biomek workstation's macroscale pipettors.

Results

For library preparation, two independent 384-well sample sets were assessed. The first consisted of the reference plasmid pUC19 at a single input concentration, designed to assess reaction uniformity and reproducibility across the full plate. Sequencing results were nearly completely uniform, demonstrating highly reproducible reaction setup at the 3 µL scale. The second set was comprised of reference plasmids isolated with the CosMCPrep Plasmid Purification kit, containing inserts spanning a broad GC content range (22–74%) to challenge sequencing performance under representative, real-world conditions. The automated plasmid prep on the Biomek i7 resulted in plasmids with 7.89 µg to 20.8 µg total yield, average 260/280 ratios of 1.9, and average 260/230 ratios of 2.2.
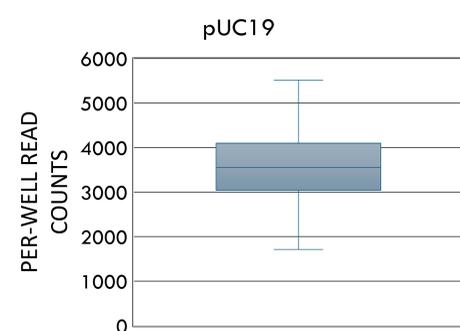

Figure 3: The CV of pUC19 read counts across all wells (n = 384) is ~ 22%. When downsampled to 2,000 read pairs per sample, 98% of pUC19 replicates were assembled *de novo*.

Clone ID	BaCD00068446	BaCD00064849	BaCD00067367	ScCD00008904*	ScCD00013271*	VcCD00060986*	YpCD00013559	YpCD00016721*	VcCD00061278*	PaCD00008367	PaCD00007490*	PaCD00006587*
Yield (µg)	15.32	17.54	16.16	20.89	10.77	12.25	16.92	16.2	12.45	8.2	7.89	14.29
Insert GC%	22.07	23.66	36.63	37.42	40.81	46.25	46.89	50.00	53.02	62.89	66.43	74.72
Insert length	213	93	789	4962	5508	3996	1719	2016	4464	291	414	1056
Full plasmid length	2763	2643	3339	7512	8058	6546	4269	4566	7014	2561	2684	3326
Vector	pDONR221	pDONR221	pDONR221	pDONR221	pDONR221	pDONR221	pDONR221	pDONR221	pDONR221	pDONR201	pDONR201	pDONR201
% Assembled	80	100	100	100	100	100	100	100	100	100	100	100

Table 1: For the set of 12 reference plasmids with GC content varying from 22 to 75%, sequencing reads for 11 out of the 12 plasmids were assembled *de novo* completely and correctly for 100% of the technical replicates (n=16 across two input concentrations). The outlier plasmid, containing an insert with an exceptionally low GC content (~22%) was assembled *de novo* for 80% of the technical replicates.

Summary

- We have developed a fully automated plasmid-to-sequence workflow on the Biomek Echo One solution that combines nanoliter-scale acoustic dispensing with conventional liquid handling. This workflow combines the bead-based CosMCPrep Plasmid Thurification kit and seqWell's ExpressPlex 2.0 library preparation chemistry.
- The automated plasmid purification method for 96 samples yields plasmids with high yield and quality in 1 hour and 16 minutes.
- Libraries were sequenced on the Illumina MiSeq platform using 2 × 151 bp paired-end reads, yielding an overall de novo assembly success rate of 99.2%.
- ExpressPlex 2.0 supports up to 6,144 unique indexes (16 × 384), enabling ultra-high-throughput workflows compatible with continuous, pipelined operation or seamless integration downstream of automated plasmid-preparation systems. Miniaturization reduces NGS library preparation costs to <$3 per sample.