Application Note

High-Throughput NGS Library Prep for Plasmids and Amplicons with the ExpressPlex™ Library Preparation Kit on the SPT Labtech firefly®

Introduction

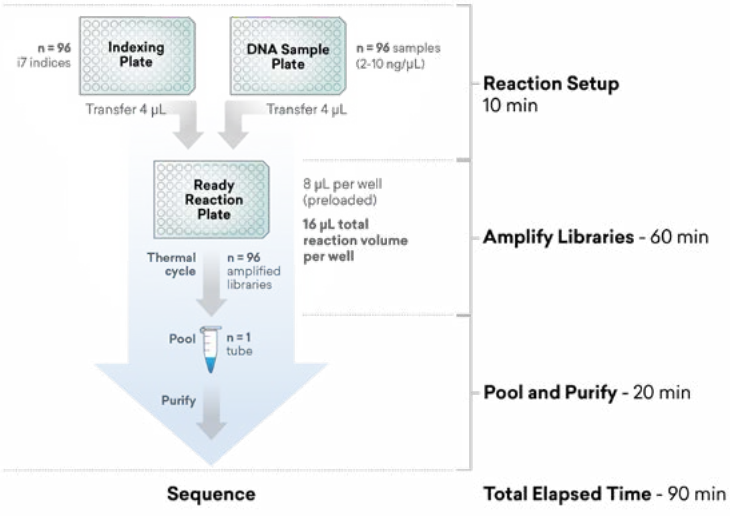
In the synthetic biology domain, the ability to rapidly manufacture large numbers of new constructs and confirm their sequences is of paramount importance. To leverage the ever-increasing capacity of sequencing instruments while minimizing the sequencing cost-per-sample, users need to be able to multiplex larger numbers of samples during every run. These dual requirements of reduced turnaround time and higher multiplexing demand highly efficient NGS library preparation methods and reliable, easy-to-implement, automated liquid handling systems. Herein we describe the automation of seqWell's ExpressPlex™ Library Preparation Kit on SPT Labtech's firefly® liquid handler to create compact and cost-efficient methods for fast screening of synthetic constructs.

ExpressPlex Library Preparation Kit

The ExpressPlex Library Preparation Kit* is designed to be the fastest and easiest library prep kit available for creating Illumina-compatible libraries from plasmid or amplicon DNA. Key features of ExpressPlex include:

- 90-minute total time to final library, with only 30 minutes hands-on (for 96 samples)
- Multiplexing up to 1,536 samples in a single run (6,144 barcodes expected early 2024)
- Only 2 reagent additions, followed by a single cleanup step
- Auto-normalization of read counts and insert sizes
- Up to 80% reduction in pipette tips required compared to other methods

ExpressPlex Workflow:



The ExpressPlex protocol requires only 2 pipetting steps per sample prior to placing the full plate onto the thermocycler. This enables the user to immediately proceed to setting up the next plate. Post-cycling, the user pools all samples from the plate into a single tube and performs one magnetic bead cleanup prior to quantifying the library and loading onto the sequencer. Technicians can easily prepare multiple sets of libraries and begin sequencing in less than half a day, and a high-throughput laboratory can easily go from plasmids or amplicons to sequenced data in 24 hours. However, to take full advantage of the multiplexing available to ExpressPlex users, most will want to incorporate a robotic liquid handling platform to maximize efficiency.

** Patents Pending*

firefly Liquid Handler

The firefly platform is SPT Labtech's newest, extremely compact automated liquid handling platform. It measures only 66cm (W) x 56cm (D) x 78cm (H), making it easy to place in almost any laboratory that wants to improve efficiency by automating manual workflows. Key features of firefly include:

- 2 moving decks with a total of 16 deck positions
- 2 liquid handling heads: an air-displacement pipetting head and a non-contact positive-displacement dispense head
- An air-displacement pipetting head comprising 384 pipetting channels that can aspirate and dispense from both 96 and 384 well plates, depending on the format of the tip array presented to the head
- A positive displacement head that can dispense up to 6 different reagents to 96 and 384 well plates on the deck
- A gripper to move plates
- A loading position for the dispense head reagents
- Optional application accessories for process modules such as heaters or shakers

Visit sptlabtech.com/products/firefly for more information.

ExpressPlex + firefly

To assess the compatibility of seqWell's library prep solution and SPT's robotic platform, we created a firefly method that performs the library setup, which is followed by manual sealing of the plate and off-deck thermo-cycling. Post cycling, the plate is then moved back to the firefly instrument for pooling, after which a simple paramagnetic bead cleanup was performed. Final libraries were sequenced on an Illumina MiSeq instrument and data were evaluated for equivalence of read count across all samples. For comparison purposes, the same samples were prepared and sequenced manually.

Materials and Methods for Automated and Manual Preparation of ExpressPlex Libraries

DNA Template
pUC19 plasmid DNA (New England Biolabs, Ipswich, MA) was diluted to 4 ng/uL and distributed across all wells of a 96-well plate.

Library Construction
Following the ExpressPlex Library Preparation Kit User Guide (seqWell, Beverly, MA), 4 uL of indexing reagent was transferred from each well of an Indexing Reagent Plate to a Ready Reaction Mix Plate, 4 uL of DNA was transferred to all wells of the Ready Reaction Mix Plate, the Ready Reaction Mix Plate was placed on a thermocycler for fragmentation/indexing and amplification, and 10 uL from each well was ultimately pooled into a 2 mL Eppendorf tube. **Note:** The firefly pools into a single column of a fresh plate, and the resulting libraries are combined manually prior to cleanup.

Post-Pooling Cleanup
After pooling, libraries were purified in a conventional SPRI cleanup protocol using a 0.75 X ratio of seqWell's MAGwise paramagnetic beads to pool volume, typically 720 uL MAGwise to 960 uL pooled libraries.

Quantification for Concentration
Final purified libraries were quantified using the Qubit 4 Fluorometer and the Qubit 1X dsDNA HS Assay kit (ThermoFisher Scientific, Waltham, MA).

Electrophoresis
Fragment size distributions were characterized using the Agilent TapeStation 4200 and the High Sensitivity D5000 ScreenTape System (Agilent Technologies, Santa Clara, CA). Key metrics provided by the TapeStation Analysis Software include the average fragment size within the range of clusterable (sequenceable) fragment sizes (400 – 1200 bp) and the fraction of the total which lies within the sequenceable range.

Quantification for Molarity
Libraries were diluted to 4 nM in Tris-HCl pH 8.

Final Preparation for Sequencing
Aliquots of each 4 nM library were pooled equi-volume and prepared for sequencing following the MiSeq System Denature and Dilute Libraries Guide (Illumina, San Diego, CA). Denatured libraries were diluted to a final loading concentration of 15 pM and sequenced on a MiSeq at 2 X 150 bp using the MiSeq Reagent Kit v2 Micro 300 cycle kit (Illumina).

ExpressPlex Library Preparation on the firefly

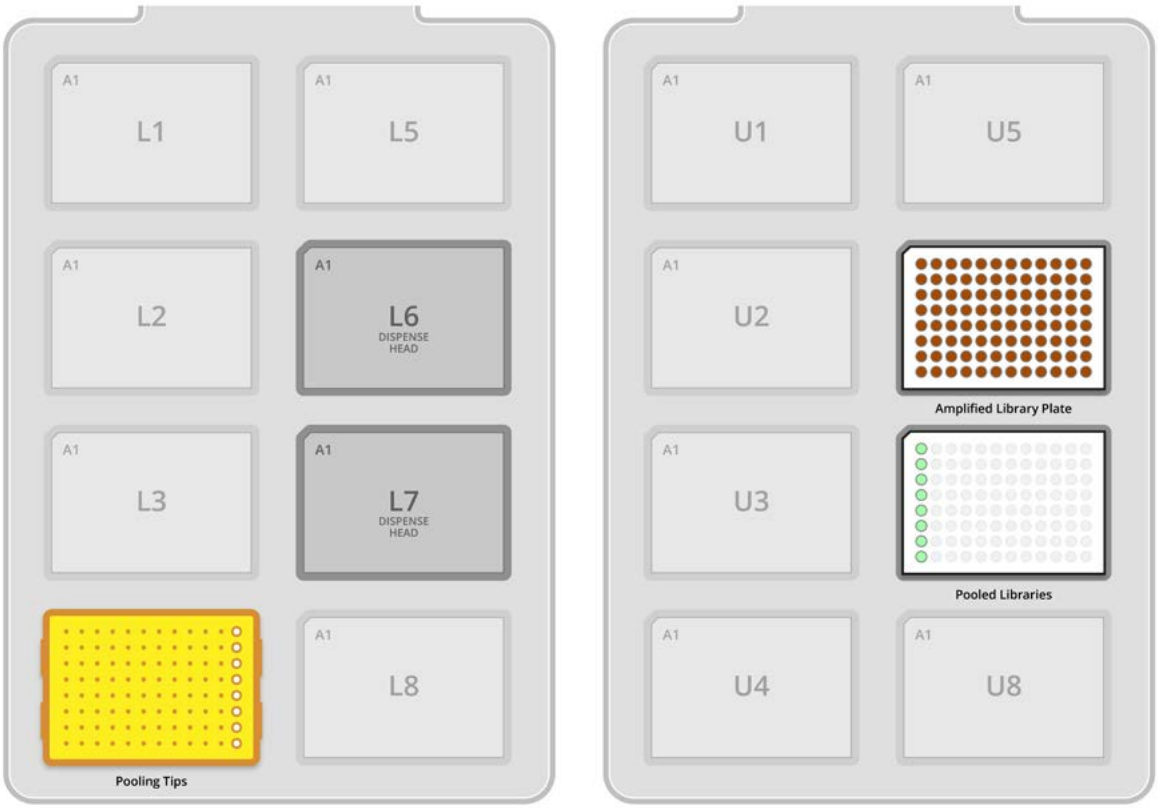
The deck layout for a single 96-well plate of ExpressPlex library preparation is shown below. For each DNA sample plate to be sequenced, one Indexing plate, one plate of Ready Reaction Mix, and two tipboxes are required, consuming a total of five deck positions. After library setup the plate is transferred to an off-deck thermocycler for fragmentation, indexing, and amplification. Space remains for an additional set of 96 to be prepared within the same run.

Lower Deck

Upper Deck

ExpressPlex Library Preparation on the firefly

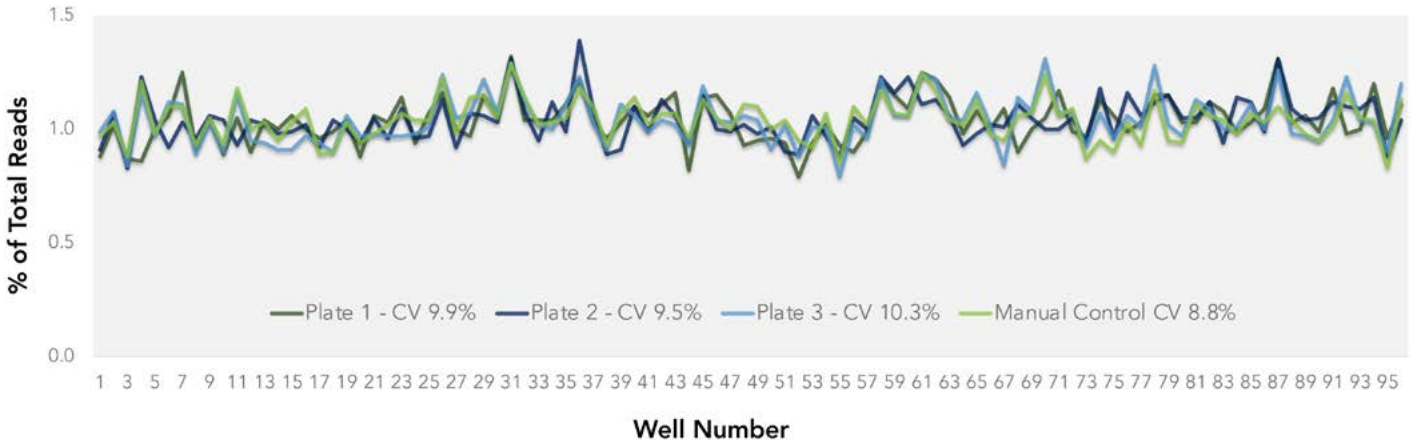
The deck layout for the sample pooling of a single 96-well plate is shown below. After thermocycling, the Amplified Library Plate is placed on the firefly for pooling. For each plate of samples, a single column of tips and a column on an empty DNA plate is required. Up to four library plates can be pooled in a single run.



Liquid handling on the firefly is rapid - the total time to prepare 2 x 96-well plates of libraries (prior to thermal cycling) is less than 6 minutes, and the total time to pool 2 x 96-well plates of libraries is less than 10 minutes.

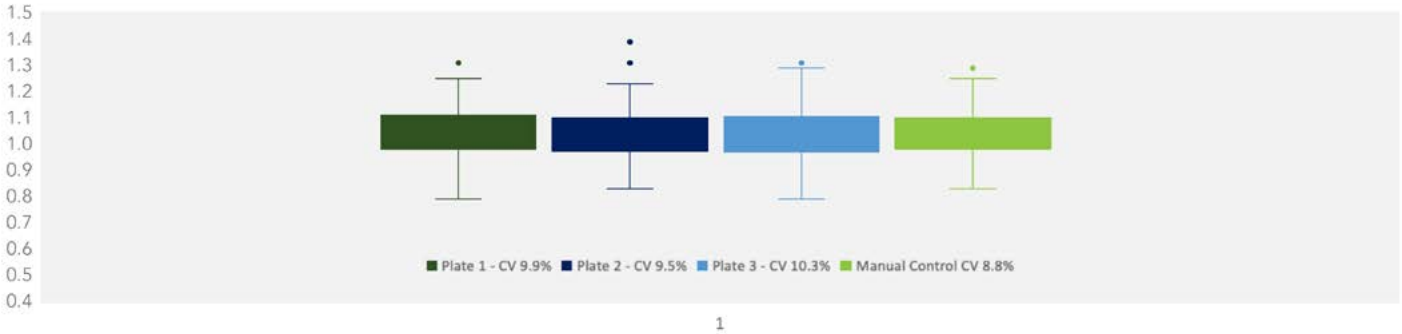
Results

A total of 288 individual libraries were prepared across three separate 96-well plates on the SPT Labtech firefly platform, as described above, and 96 libraries were prepared manually. The number of sequencing reads per well, as a percent of the total reads recovered, reflects the consistency of the library preparation across all libraries. As seen in Data Figure 1, the balance of sample reads is highly consistent, both inter- and intra-plate, for the automated and manual preparations, with coefficients of variation (CVs) of 9.9%, 9.5%, 10.3%, and 8.8%.



Data Figure 1. Read Balances for three plates of pUC19 samples prepared and pooled on the firefly, with manual control data. An ideal read balance would be 100%/96 wells, or 1.04% of total reads per well for all wells. The firefly produces read balances which are close to the best attainable in actual practice, and highly comparable to read balances produced manually.

Visualized differently, the medians and distributions about the medians of the read-balance data for libraries prepared using the firefly and libraries prepared manually are virtually indistinguishable (Data Figure 2).

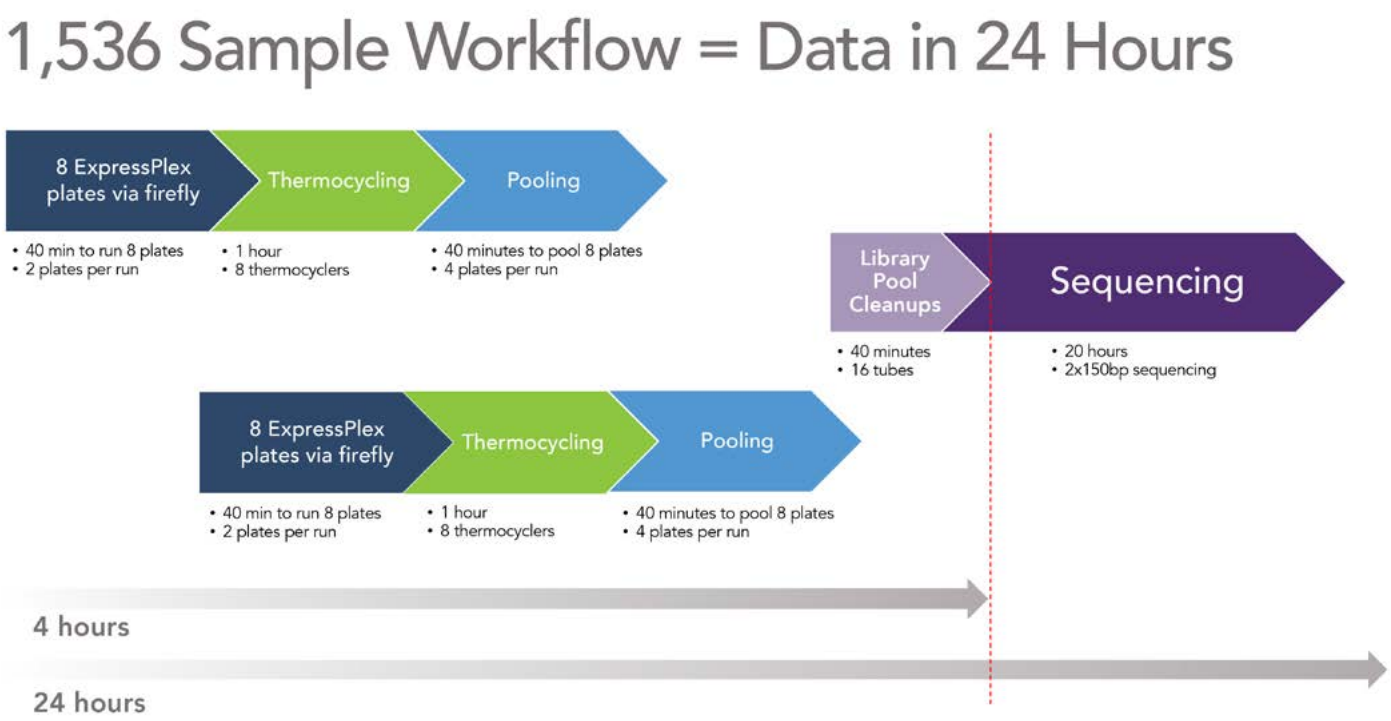


Data Figure 2. Read balances for libraries prepared using the firefly and libraries prepared manually are practically indistinguishable

Highly similar read counts for pooled sample libraries are a result of the auto-normalization engineered into the ExpressPlex Library Preparation Kit, and validates the ability to proceed directly from cleanup into final sequencing preparation without the need to normalize sample libraries on a well-by-well basis.

Discussion

Using the ExpressPlex Library Preparation Kit, the SPT Labtech firefly, and sufficient thermocyclers, a single user can easily prepare 1,536 total libraries and take them through sequencing in approximately 24 hours. One possible scenario divides the 16 plates comprising 1,536 samples into two cohorts of 8 plates apiece, and staggers the library preparation/thermocycling/pooling steps to maximize instrument and thermocycler usage. To expedite the library preparation process, up to two 96-well plates can be set up for sequencing in a single firefly run, and up to four plates may be pooled in a firefly pooling run (additional programming may be required).



Quick, reproducible sequencing of synthetic construct libraries can enable synthetic biology researchers to rapidly determine if their sequences of interest are present. With the strategy described in this application note, high-throughput laboratories can efficiently screen large numbers of constructs and focus on the important discovery aspects of their work.

Conclusions

- ExpressPlex libraries created on the SPT Labtech firefly are highly consistent, maintaining highly balanced read counts without normalization.
- Users can prepare and sequence 1,536 or more samples in 24 hours, which enables speedy discovery via rapid data turnaround.
- Through high-throughput screening of synthetic constructs, researchers can increase their rates of discovery, thus allowing them to focus on more important aspects of their work.

The following scientists are responsible for the work shown in this application note:
John Palys (seqWell), Anita Pearson (SPT), Ian Whitmore (SPT), and Huw Rees (SPT)

Would you like more information about ExpressPlex, firefly, or associated methods? Contact seqWell or SPT Labtech, respectively, at:

Visit: seqwell.com/contact

Visit: sptlabtech.com/contact