

Automated, Multiplexed Preparation of up to 384 Plasmid Libraries Supports a 24-Hour End-to-End Sequencing Workflow



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INTRODUCTION

- High-throughput genomic analysis is an indispensable tool in life sciences research.
- The seqWell ExpressPlex™ Library Kit offers a robust one-pot solution for high-throughput multiplexed library preparation of plasmids and amplicons.
- Carried out manually, library prep of 96 samples is completed in 90 mins, with 30 mins hands-on time.
- In this work, we aimed to compare automated preparation of libraries using the ExpressPlex Library Kit on the Opentrons Flex™ robotic system with manual preparation.
- The Opentrons Flex™ liquid handler has demonstrated success in automating a myriad of tedious NGS tasks.
- Preparing seqWell ExpressPlex™ libraries using the Flex liquid handler will significantly reduce turnaround time and increase throughput.

MATERIALS AND METHODS

Libraries were prepared on the Flex liquid handler according to the ExpressPlex protocol. 16 ng of pUC19 was used as input. After all components were added into the Ready Reaction plate, the mixture was shaken at 3000 rpm for 90 seconds followed by subsequent library preparation and pooling steps. The final library pool was then sequenced on a NextSeq 2000 using the 2X150 bp sequencing protocol.

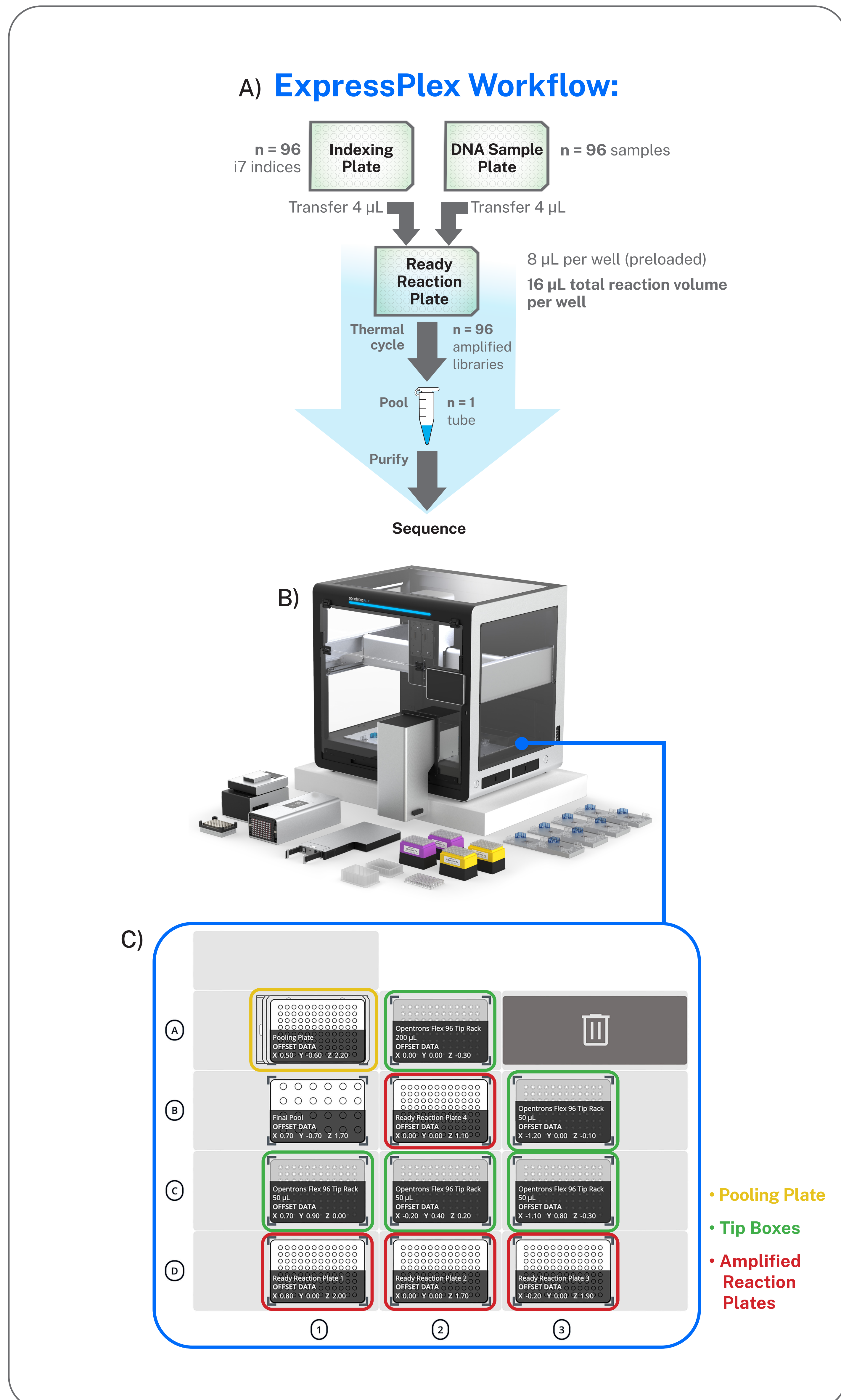
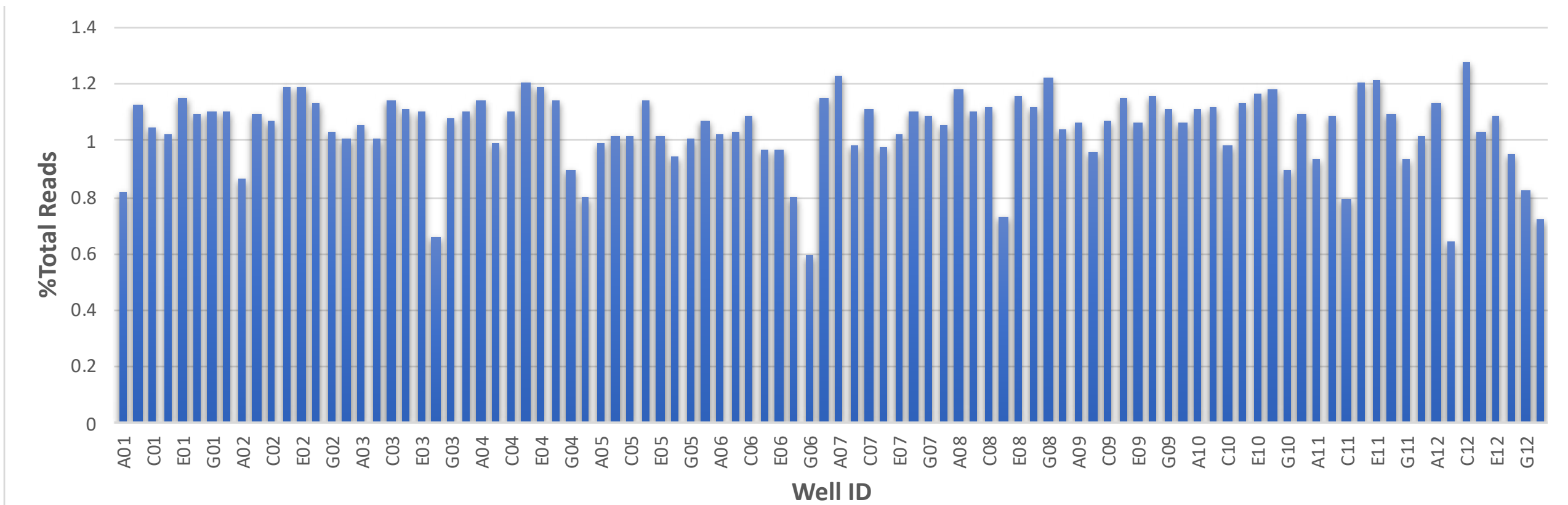


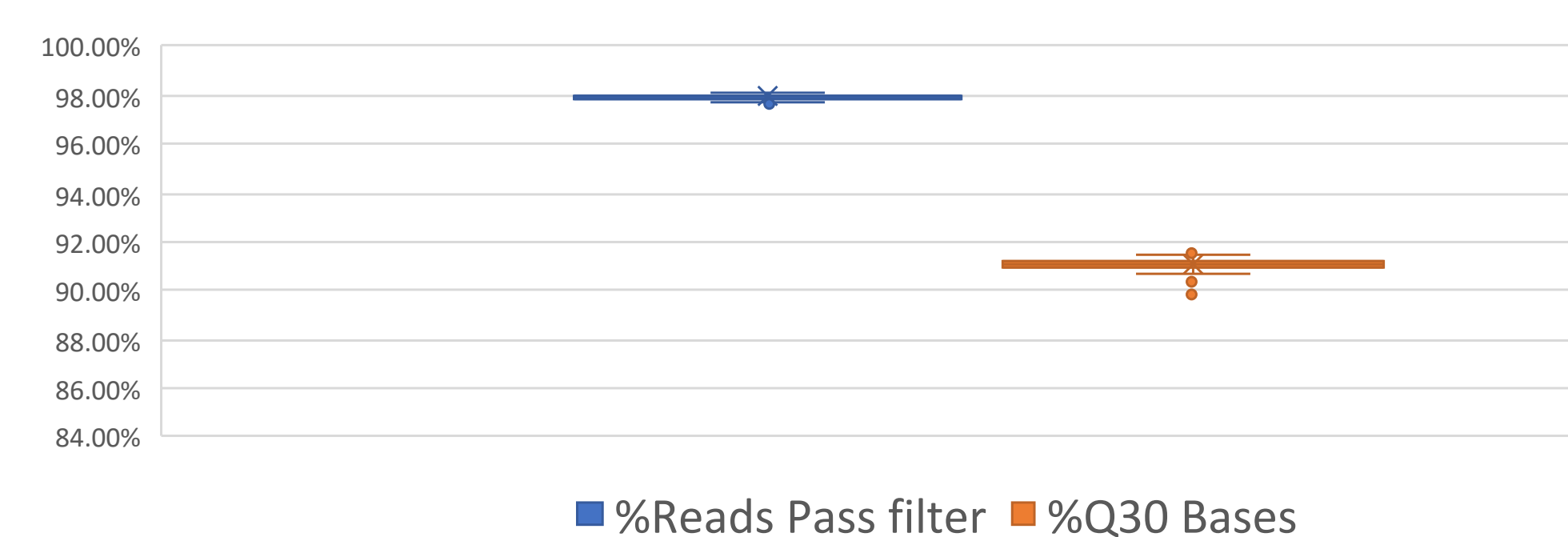
Figure 1. - A) The ExpressPlex workflow consists of adding indices and DNA into a Ready Reaction plate (containing a mixture of enzymes for library preparation), followed by amplification, pooling and MAGwise bead cleanup. B) Flex liquid handler including tip boxes, modules, gripper and other accessories. C) Flex liquid handler deck setup for pooling 4 reaction plates after amplification.

RESULTS

A) Read Count Balance Across 96 libraries with 16 ng input



B) Sequencing QC Metrics for 96 ExpressPlex libraries with Opentrons Flex



Library Type	Concentration (ng/ul)	Molarity (nmol)
Opentrons Flex	114	223
Manual Control	95.8	198

Figure 2. A) Read balance across the 96 well plate yielded a CV of 12.7%. B) 95% of reads passed filter and most Q30 scores exceeded 90%. C) The final library pool prepared by Flex revealed a higher concentration than manually prepared libraries.

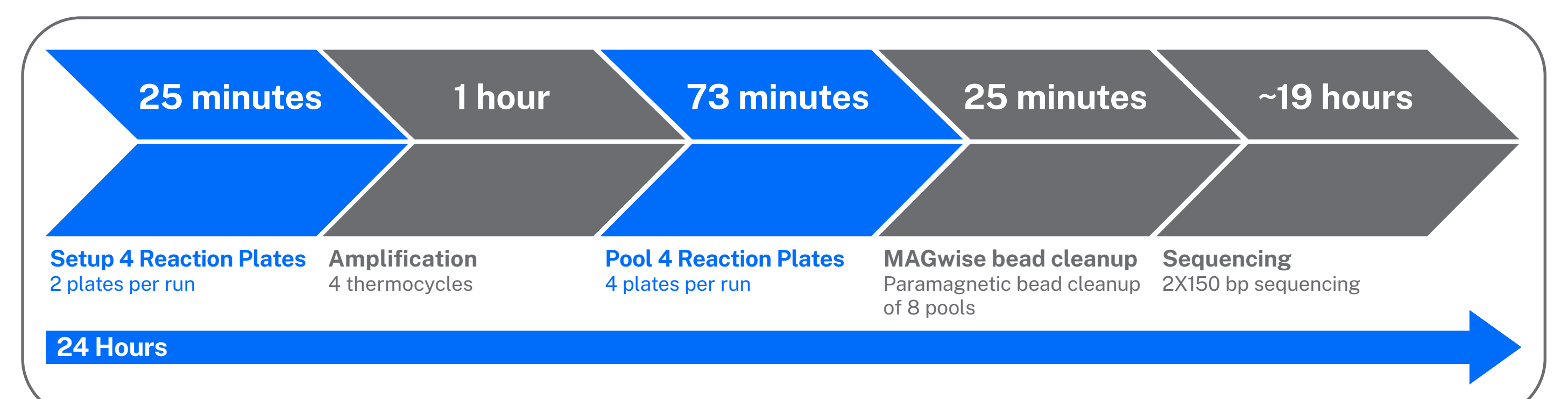


Figure 3. 8 individual 96-plex pools can be prepared in under 3 hours on Opentrons Flex by utilizing staggered start times. Sequencing data for 768 libraries can be available in 24 hours after setting up the first reaction plate on Flex.

CONCLUSIONS

- The Flex constructs highly uniform and balanced libraries across 96 pUC19 plasmid samples.
- Automated multiplexed library preparation yields robust sequencing performance with higher molarity and over 95% of reads above the pass filter threshold when compared to manual preparation.
- The Flex can support processing of up to 384 samples in a single run. In concert with a staggered workflow, it is possible to generate sequencing data for 768 libraries in 24 hours.
- Automating the ExpressPlex library preparation workflow on the Flex supports an end-to-end workflow from extracted plasmid/ amplicon DNA to data generation within 24 h.

REFERENCES

1. ExpressPlex™ Library Prep Kit Single-Step Workflow Demo for High-Throughput Multiplexed Sample Prep. (2023). YouTube. <https://www.youtube.com/watch?v=gB4IsVH0AIA>
2. Leonard, J. T., Feeley, R., Huang, S., Wang, L., & Smith, J. (n.d.). A transformational one-step library preparation method for multiplexed plasmid and amplicon sequencing. Library Prep Simplified through Scalable Workflows | seqWell. https://seqwell.com/wp-content/uploads/2022/06/20220519_ExpressPlex-Poster.pdf
3. Leonard, J. T., Huang, S., Feeley, R., Couture, J., Palys, J., Smith, J., & Liu, Y. (n.d.). An Optimal Strategy for Internalizing Ultra-high Throughput Plasmid Sequencing. Library Prep Simplified through Scalable Workflows | seqWell. https://seqwell.com/wp-content/uploads/2023/05/SW2023_Optimal-Strategy-HTP-Sequencing-11x8.5-05192023.pdf
4. Palys, J., Pearson, A., Whitmore, I., & Rees, H. (2023). High-Throughput NGS Library Prep for Plasmids and Amplicons with the ExpressPlex™ Library Preparation Kit on the SPT Labtech firefly®.
5. SeqWell Inc. (2023). ExpressPlex™ Library Prep Kit. Library Prep Simplified through Scalable Workflows | seqWell. https://seqwell.com/wp-content/uploads/2023/04/SW2023_ExpressPlex-Product-Flyer-04202023.pdf