

Automating NEBNext® UltraExpress® NGS Library Prep with SPT Labtech's firefly® for rapid, high-throughput NGS library generation at Harvard Medical School's core facility

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Introduction

At the Biopolymer Facility genomics core at Harvard Medical School (BPF) we have successfully automated the NEBNext® UltraExpress® RNA Library Prep kits with options for both polyadenylated mRNA enrichment and rRNA depletion workflows using SPT Labtech's firefly® liquid handling platform.

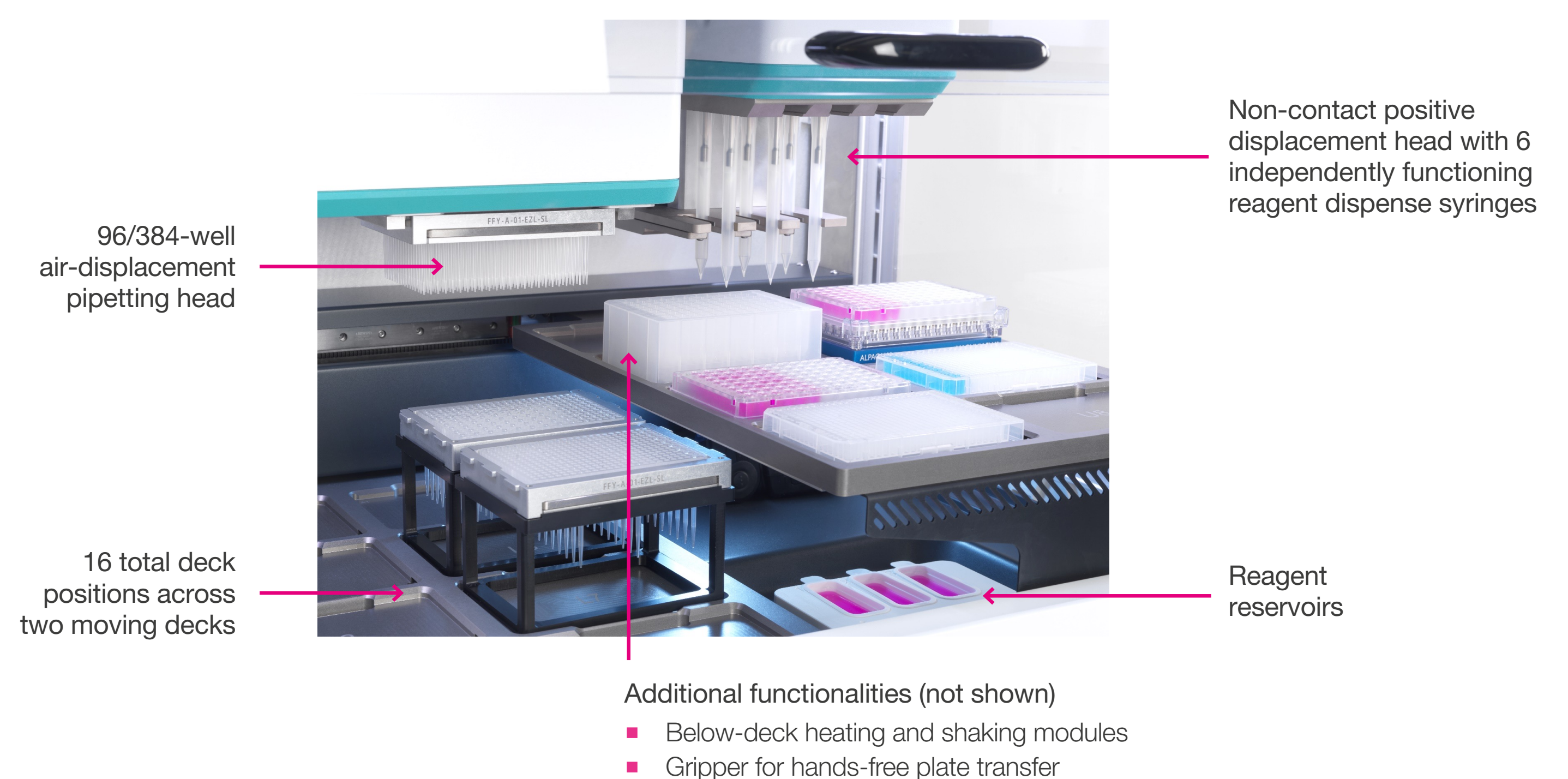
The new NEBNext UltraExpress RNA Library Prep kits feature a single, uniform adapter dilution and one PCR cycling condition across the entire input range, along with fewer bead clean-up steps for an addition-only library construction process and automation-friendly workflows. This results in a faster process and reduced plastic consumption.

We demonstrate the ease of automating NEB's UltraExpress® kits with the user-friendly firefly® system, achieving high-quality and reproducible libraries and sequencing data. Libraries were prepared from 25ng to 250ng of total RNA, consistently showing high data quality across this input range.

Rapid production of RNA libraries enhances the sequencing services provided by genomics core facilities like the BPF.

Automating genomics liquid handling with firefly

firefly has been designed specifically to streamline NGS library preparation by bringing together multiple liquid handling capabilities in a single benchtop instrument.



NEBNext UltraExpress RNA workflow

The NEBNext UltraExpress RNA Library Prep Kit is the latest generation of NEBNext RNA library preparation, and it has a fast, streamlined workflow.

The kit allows the use of a single protocol for all RNA inputs (25-250 ng total RNA) and incorporates master mixed reagents, reduced incubation times and fewer clean-up steps, reducing total time and consumables used.

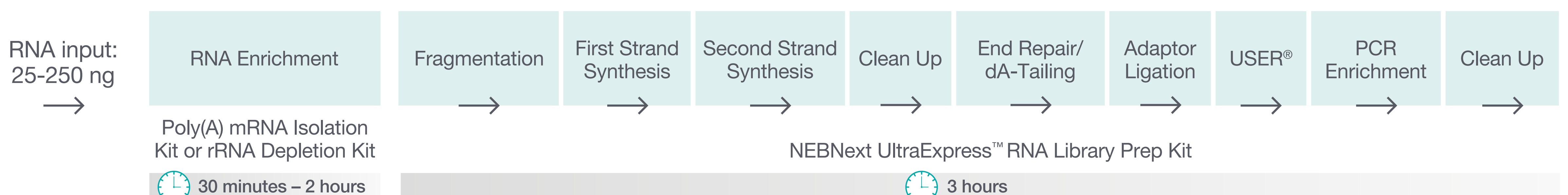


Figure 1. The NEBNext UltraExpress RNA workflow.

Bead-based RNA purification

firefly was used to perform all dispense, aspiration and mixing steps in a 96-well PCR plate (Biorad Hard Shell Plate). The non-contact dispense head was used to dispense RNA purification beads (SPRIselect, Beckman Coulter), 80% ethanol in water, all master mixes (including the USER® enzyme), and elution buffer (0.1X TE). The pipetting head was used to perform the initial RNA transfer, all mixing steps, and all transfers of supernatant to waste. A 96-well plate magnet (MagnumFLX, Alpaqua) was used for all bead separation steps.

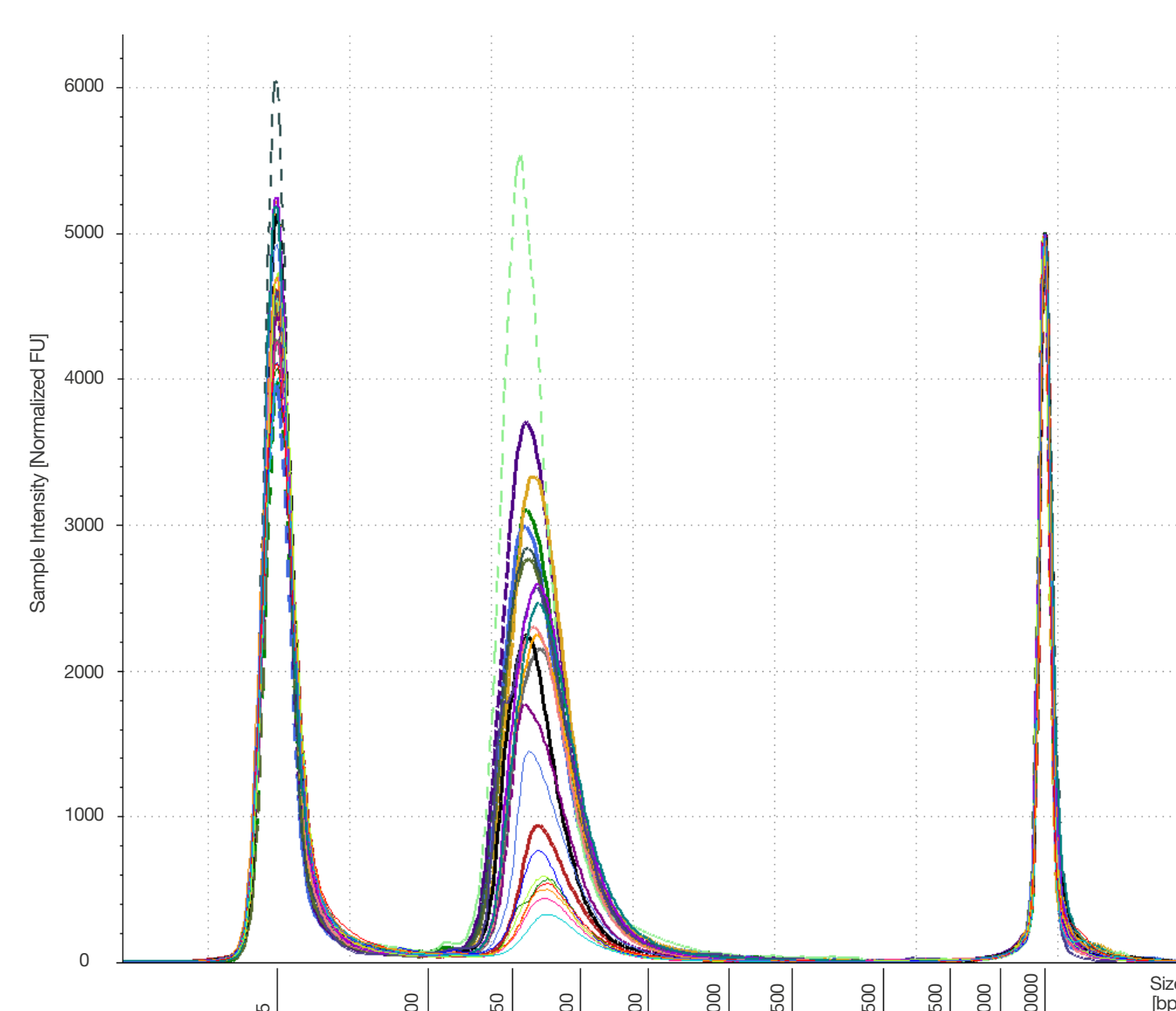


Figure 2. TapeStation results of 24 undiluted sample libraries

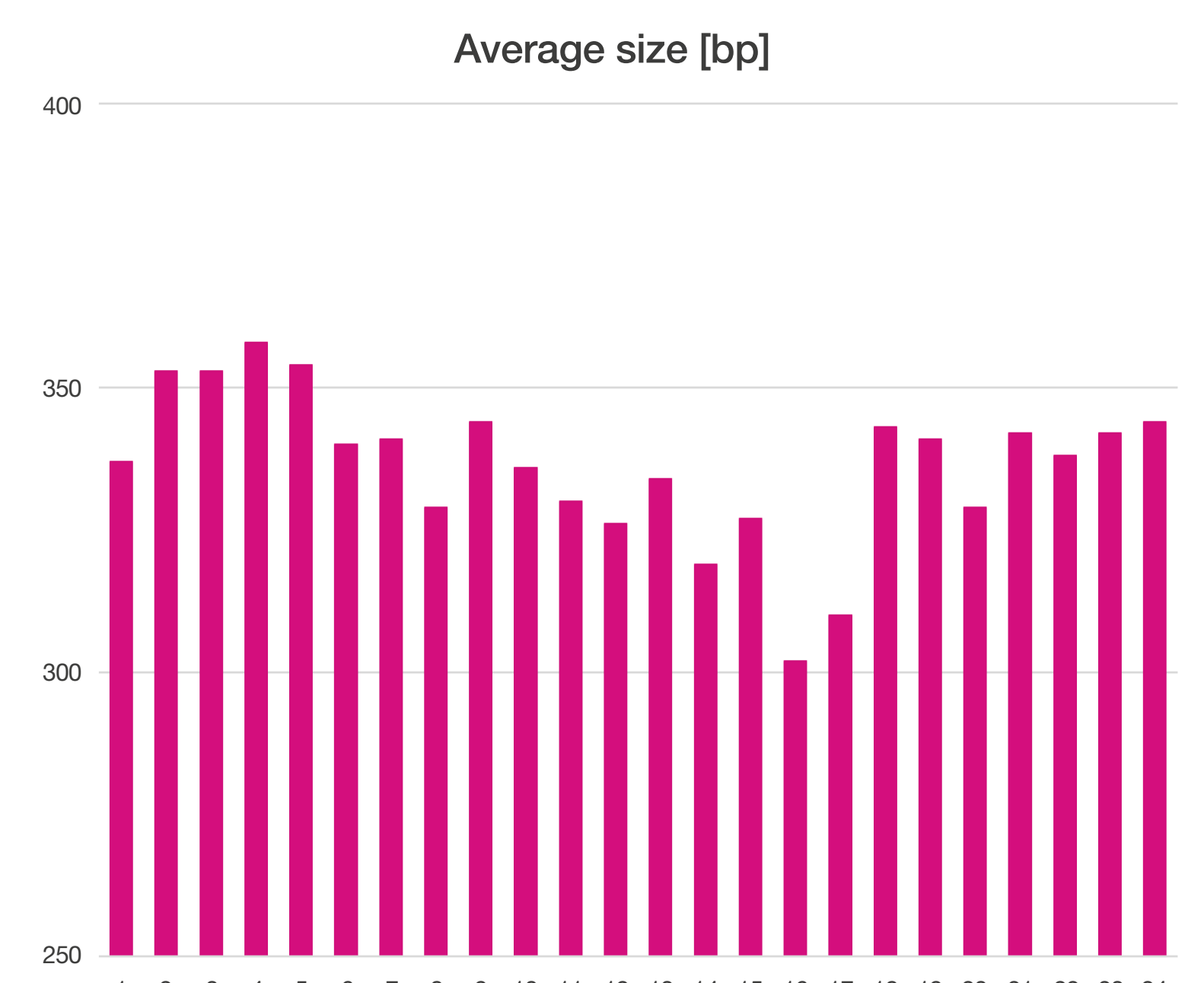


Figure 3. Average size of library per sample.

Conclusions

The combination the NEBNext® UltraExpress® NGS Library Prep kit and firefly has yielded a robust, efficient, accessible and streamlined method for generating high-quality and reproducible libraries for Illumina® sequencing ranging from 25 – 250 ng of total input RNA.

As a high-throughput genomics core facility, automation is essential for delivering high-quality NGS data with quick turnaround times for our diverse projects. The automation of the NEBNext UltraExpress kits on firefly has resulted in our fastest method for producing RNA libraries for Illumina sequencing, enhancing our sequencing services and making NGS faster and more accessible to our research community.

References

- <https://www.sptlabtech.com/products/firefly>
- <https://www.neb.com/en-us/products/e3330nebnext-express-rna-library-prep-kit>