Automation of NEBNext® Ultra™ II RNA Library Prep Kit for Illumina® and the NEBNext® Poly(A) mRNA Magnetic Isolation Module® on SPT LabTech firefly® Workstation at Weil Cornell Medical School Genomics Core.

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SUMMARY

This work demonstrates the verified automation of the NEBNext® Ultra II RNA library preparation kit using the mRNA workflow probing performance with low-input total RNA sample mass on the SPT Labtech firefly® platform, expanding the reach of usable sequencing automation options for core facilities and enables consistent, high-throughput mRNA library production that is scalable for larger projects.

INTRODUCTION

The NEBNext Ultra II mRNA workflow for library prep was automated on the SPT Labtech firefly® system. Samples were evaluated on the system by preparing libraries using the workflow automated on the firefly down to 10ng of input. Results were quantitated by Qbit. Size distributions were measured on a Tape Station and samples were sequencing to determine consistency of gene expression levels observed across the input range. QC Metrics demonstrate that the firefly® is capable of readily generating usable libraries down to the lowest specified total RNA input concentration. Here we present initial QC data along with descriptions of the kit and the instrument used.

These results confirm that NEBNext® Ultra™ II RNA Library Prep Kit are successfully processed across a wide input range on the SPT Labtech firefly instrument in a core facility environment.

Ultra™ II RNA Library Prep Kit for Illumina® and the NEBNext® Poly(A) mRNA Magnetic Isolation Module

The NEBNext Ultra II RNA Library Prep Kit for Illumina contains the enzymes and buffers required to convert a broad range of input amounts of RNA into high quality non-directional libraries for next-generation sequencing on the Illumina platform. The fast, user-friendly workflow has minimal hands-on time and is compatible with poly(A) mRNA enrichment and rRNA depletion methods. The NEBNext Poly(A) mRNA Magnetic Isolation Module is designed to isolate intact poly(A)+ RNA from previously isolated total RNA. The technology is based on the coupling of Oligo d(T)25 to 1 µm paramagnetic beads which is then used as the solid support for the direct binding of poly(A)+ RNA. Thus, the procedure permits the manual processing of multiple samples and can be adapted for automated high-throughput applications.

SPT Labtech firefly® liquid handling platform

firefly is a compact automated liquid handling platform. The firefly platform consists of: ■ two moving decks with a total of 16 deck positions ■ two liquid handling heads: an air-displacement pipetting head and a non-contact positive-displacement dispense head ■ a gripper to move plates ■ positions for process modules such as heaters or shakers The volumetric performance underlies its ability to produce consistent results. Dispense performance of the firefly pipetting head and dispense head spans a wide range of different volumes. Viscous fluids, like those used in NGS library preparations, are dispensed with precision, regardless of viscosity, using positive displacement technology. Low volume precision (as low as 200nl) also provides a path to assay miniaturization, while the disposable tip head can also manage a full plate bead cleanup at normal NGS volumes.

- Protocol Library Access: Users can download pre-verified protocols from leading kit providers
- Software enables users to develop, edit, and run protocols through an intuitive, visual user interface that requires no coding knowledge.
- Ready path to assay miniaturization
- Fast low volume dispenser together with full plate, full volume bead cleanup capability

METHODS

- We tested the performance of firefly using Ultra™ II RNA Library Prep Kit protocols on low input samples
- We diluted one sample of a project (ID redacted) and used 100ng, 50ng, 20ng and 10ng as input amount respectively
- Libraries were diluted, quantitated and sized by capillary electrophoresis using an Agilent Tape Station running the high sensitivity protocol.
- We pooled these four libraries for a spike in sequencing test (data not shown)
- Sequencing data analysis results of these four samples were comparable (da

RESULTS

The Automated Ultra™ II RNA Library Prep Kit for Illumina® and the NEBNext® Poly(A) mRNA Magnetic Isolation on Firefly® produces expected insert sizes and expected library yields

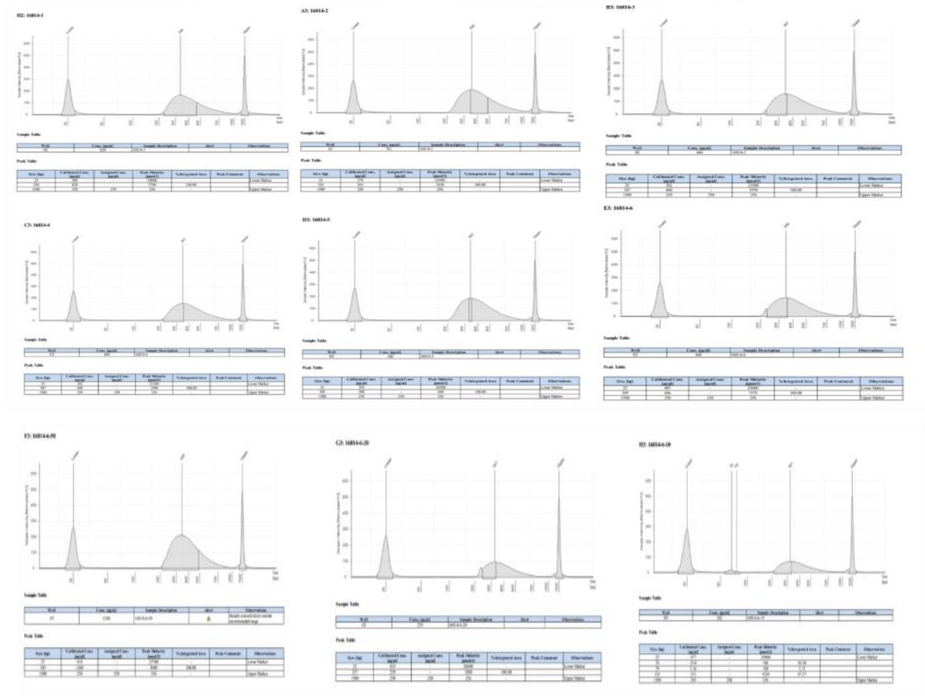
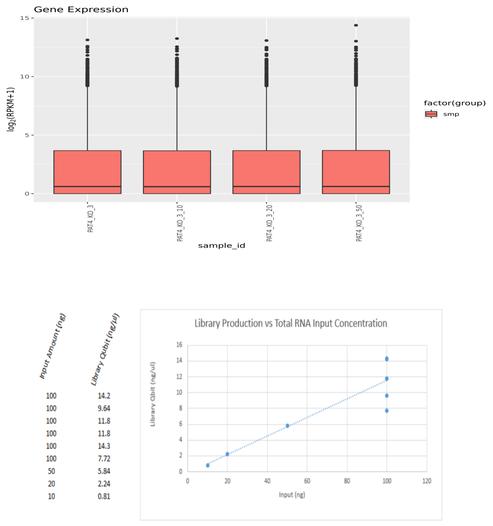


Figure 1. Tape station analysis for libraries



Input Amount (ng)	Library Qubit (ng/ul)
100	14.2
100	9.64
100	11.8
100	11.8
100	14.3
100	7.72
50	5.84
20	2.24
10	0.81

Ultra™ II RNA Library Prep Kit for Illumina® and the NEBNext® Poly(A) mRNA Magnetic Isolation on firefly® yeilds good quality library down to 10ng of total RNA input

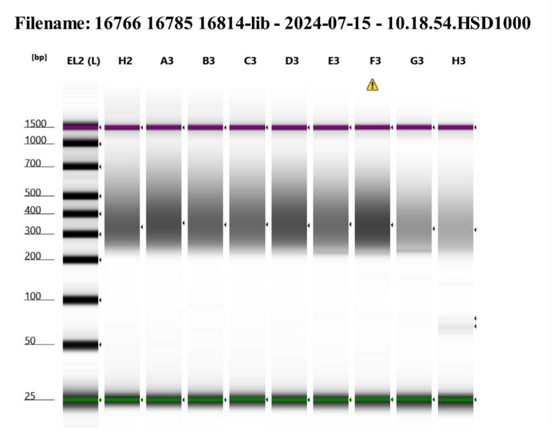


Figure 2: Yields and Expression Analysis across input range

CONCLUSIONS

- Automated Ultra™ II RNA Library Prep Kit for Illumina® and the NEBNext® Poly(A) mRNA Magnetic Isolation on firefly® produces expected insert sizes and expected library yields across a wide input range of total RNA
- Gene expression measurements are consistent across input dilutions, and the automated workflow can support heterogeneous samples using a uniform process within in a single plate along this range.