Improving High-Throughput Automated NGS Library Generation in a Core Sequencing Lab

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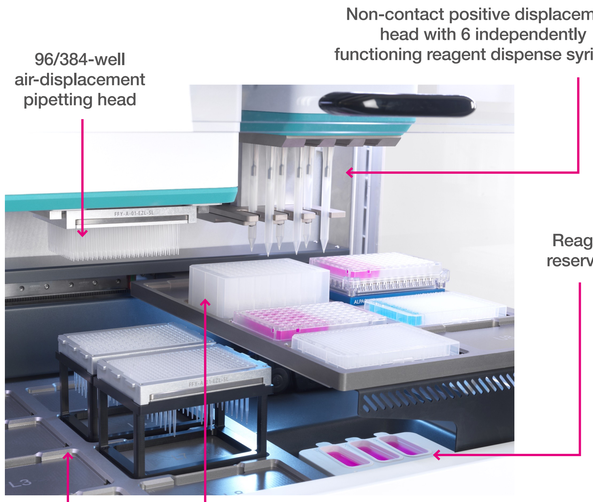
Introduction

To answer genomic research needs for a faster, streamlined DNA prep workflow that delivers high quality libraries from a variety of sample types, New England Biolabs developed the NEBNext UltraExpress™ DNA Library Prep Kits which have straightforward protocols for all DNA inputs, either pre-sheared or enzymatically sheared. The workflow incorporates master mixed reagents, reduced incubation times, and fewer cleanup steps in comparison to previous kits. Thanks to an improved chemistry and a wide tolerance of inputs, the kit allows for the generation of libraries in under 2 hours and has a uniform protocol that streamlines preparation of diverse DNA sample inputs. With an automated infrastructure, this new kit is able to provide some of the most accessible, high-throughput, quality NGS library prep of DNA samples available.

The Biopolymers Genomics Core Facility (BPF) researchers at Harvard Medical School have successfully automated the versatile NEBNext UltraExpress DNA Library Prep Kits using SPT Labtech's firefly® liquid handling platform to generate high-quality NGS libraries from a broad input range of DNA. The accessible, streamlined NEBNext UltraExpress workflow in tandem with user-friendly firefly creates one of the most accessible means for generating NGS libraries for Illumina sequencing of DNA.

Automating genomics liquid handling with firefly

firefly has been designed specifically to streamline NGS library preparation by bringing together multiple liquid handling capabilities.



Method

NEBNext UltraExpress Library Prep Kit

firefly was used to perform all dispense, aspiration and mixing steps to execute bead-based DNA purification in a 96-well PCR plate (Biorad Hard-Shell® Plate).

- Dispensing of DNA purification beads (Ampure XP, Beckman Coulter), ethanol in water and elution buffer (0.1X TE), and enzyme mixtures was performed using the non-contact dispense head.
- DNA sample and index transfers, all mixing steps and all transfers of supernatant to waste were performed using the pipetting head.
- A 96-well plate magnet (MagnumFLX, Alpaqua) was used for all bead separation steps.

NEBNext UltraExpress DNA Library Prep with FS:

- 11.8 ng of Illumina PhiX in all 12 columns. No shearing necessary as this kit includes enzymatic shearing.

NEBNext UltraExpress DNA Library Prep with pre-sheared DNA:

- 74.7 ng/µL (concentration from Qubit) of human gDNA (promega) sheared to 200bp using Covaris M220. Sheared libraries ran on TapeStation D5000 assay to confirm desired size was reached. 1:20 dilution and Qubit QC performed to get concentration of 6.96 ng/µL. Final input was 104.4 ng of sheared human gDNA in each well. Two wells of NTC (DNase/Rnase-free water) included.

Pre-sheared DNA method steps – part 1

End prep and adaptor ligation

Dispense head | Pipetting head | Process module | Off-deck

Location	Head / Process module	Reagent	Volume (µL)	Destination	Incubation times
On-deck	Dispense	End-prep Mastermix	5	Frag DNA	
	Pipetting: Tip Mix			Frag DNA	
Off-deck	Incubation		11	Frag DNA	30 mins
On-deck	Dispense	Universal adaptors		Frag DNA	
	Pipetting: Tip Mix			Frag DNA	
	Dispense	Ligation Mastermix	10	Frag DNA	
	Pipetting: Tip Mix			Frag DNA	
	Incubate			Frag DNA	20 mins
	Dispense	USER enzyme	2	Frag DNA	
	Pipetting: Tip Mix			Frag DNA	
Off-deck	Incubate			Frag DNA	5 mins

On-deck time: 30 mins

Pre-sheared DNA method steps – part 2

PCR enrichment

Dispense head | Pipetting head | Process module | Off-deck

Location	Head	Reagent	Volume (µL)	Destination
On-deck	Dispense	High Yield Mastermix	40	Ligated DNA plate
	Pipetting: Transfer	Indexes/Universal Primers	6	Ligated DNA plate
	Pipetting: Tip Mix			Ligated DNA plate
Off-deck	Thermocycler PCR			Ligated DNA plate

On-deck time: 3 minutes

Pre-sheared DNA method steps – part 3

Phased bead clean-up

Location	Head / Process module	Reagent	Volume (µL)	Destination	Magnet
On-deck	Incubation			Samples	Off-magnet
	Gripper plate move			Samples → magnet	On-magnet
	Incubation			Samples	On-magnet
	Pipetting	Supernatant → waste		Waste	On-magnet
	Dispense	Ethanol	20 + 60	Samples	On-magnet
	Pipetting	Supernatant → waste		Waste	On-magnet
	Dispense	Ethanol	20 + 60	Samples	On-magnet
	Pipetting	Supernatant → waste		Waste	On-magnet
	Gripper plate move			Samples → off-magnet	Off-magnet
	Dispense	0.1X TE buffer (resuspension)	33	Samples	Off-magnet
	Pipetting: Tip Mix			Samples	Off-magnet
	Gripper plate move			Samples → magnet	On-magnet
	Pipetting	Eluted DNA libraries		Final libraries plate	On-magnet

Addition and incubation of DNA and beads, followed by addition of wash and resuspension buffers

Location	Head / Process module	Reagent	Volume (µL)	Destination	Magnet
On-deck	Dispense	SPRI Beads	56 (0.7X)	Samples	Off-magnet
	Pipetting: Tip Mix			Samples	Off-magnet
	Incubation			Samples	Off-magnet
	Gripper plate move			Samples → magnet	On-magnet
	Incubation			Samples	On-magnet
	Pipetting	Supernatant → waste		Waste	On-magnet
	Gripper plate move			Samples → off-magnet	Off-magnet
	Dispense	0.1X TE buffer	50	Samples	Off-magnet
	Pipetting: Tip Mix			Samples	Off-magnet
	Dispense	Bead Resuspension buffer	40	Samples	Off-magnet
	Pipetting: Tip Mix			Samples	Off-magnet

Results

DNA library preparation with enzymatic fragmentation system

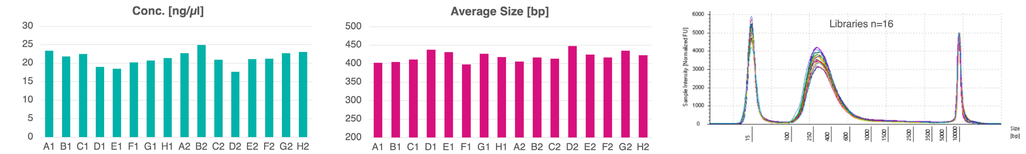


Figure 1. TapeStation results showed successful and consistent size generation of libraries between all wells down two columns.

DNA library preparation with pre-sheared input DNA

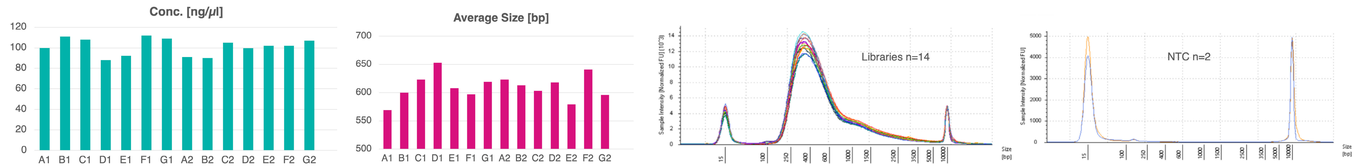


Figure 2. TapeStation results showed successful and consistent size genertaion of libraries between all wells down two columns. NTC controls show no contamination.

Conclusions

In this work, we show that we have successfully automated the NEBNext UltraExpress DNA Library Prep workflow both with and without enzymatic fragmentation systems on firefly, with a uniform fragment size distribution demonstrated for samples in a 96-well plate. Combining the accessibility of firefly and the simplicity of the NEBNext UltraExpress DNA Library Prep Kits makes the generation of sequencing libraries from DNA easier than ever before. The rapid NEBNext UltraExpress DNA Library Prep workflow allows for the quick and effective automation of DNA in only a few hours from initial sample to readable libraries, saving valuable time and resources compared to more labor-intensive kits, which take longer via manual or automated executions. firefly is among the most compact liquid handlers in its class, enabling the automation of straightforward NGS library preparations with minimal bench space requirement. This, combined with its ease-of-use, allows laboratories facing size and personnel constraints to fulfil their NGS library preparation demands.

References

1. NEBNext UltraExpress™ DNA Library Prep Kit https://www.neb.com/en-us/products/e3325nebnext-express-dna-library-prep-kit#Product%20Information
2. Biopolymers Facility, Harvard Medical School https://genome.med.harvard.edu/

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