High-Throughput NEBNext Library Preparation Solutions Enabled Through Laboratory Automation

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Introduction

Advances in next generation sequencing (NGS) technologies in the last decade have driven countless discoveries, impacting everything from basic research to advances in drug discovery, diagnostics and human health.

Automated NGS library prep, enabled by robotic liquid handlers, meets the needs of NGS as a tool, unlocking high-throughput library preparation while allowing scientists time to focus on data analysis, developing new protocols, and investing time in the science behind their work.

- New England Biolabs and NEBNext® NGS solutions are designed with automation in mind by addressing several factors:
  - Fewer components simplify automation setup.
  - Streamlined workflows minimize tip consumption and the risk of pipetting errors.
  - Less cleanup-bead consumption reduces cost-per-reaction.
  - Lower input amounts enable improved sequencing results with less sample.
  - Modularity of workflows allows maximum flexibility with minimal effort.

NEBNext® Library Prep Kit Automation Support

Looking to automate your NEBNext library prep kit? Knowing where to look for support with your library prep workflow can save both time and money.

- Learn which of our DNA and RNA library prep kits have been automated!

DNA	Revvity® (PerkinElmer)				Beckman Coulter®			Hamilton®		Agilent®		Tecan®		Eppendorf®	SPT Labtech®	Opentrons®
	Sciclone® NGS/ NGSx	Sciclone NGS iQ	Zephyr®	Bioqule®	Biomek® i7	FXp	Ngenius	NGS Star	NGS STARlet	Bravo® A	Bravo® B	Dream® Prep	EVO Freedom Plus	5075 5073	Firefly	NGS
NEBNEXT ULTRA™ II																
NEBNext Ultra II DNA	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
NEBNext Ultra II FS DNA	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
NEBNEXT ULTRAEXPRESS®																
NEBNext UltraExpress DNA	↗				✓			↗				↗		↗	✓	
NEBNext UltraExpress FS DNA	↗				✓			↗				↗		↗	✓	
ENZYMATIC METHYL-SEQ (EM-SEQ)™																
NEBNext Enzymatic Methyl-seq (EM-seq)	✓	✓			✓	↗	✓	↗								
EM-seq + NEBNext UltraShear®	✓	✓			↗											

RNA	Revvity® (PerkinElmer)			Beckman Coulter®			Hamilton®	Agilent®		Tecan®	Eppendorf®	SPT Labtech®	Opentrons®
	Sciclone® NGS/ NGSx	Sciclone NGS iQ	Zephyr®	Biomek® i7	FXp	Ngenius	NGS Star	Bravo® A	Bravo® B	Dream® Prep	5075 5073	Firefly	OT2
NEBNEXT ULTRA™ II													
NEBNext Ultra II Directional RNA	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓
NEBNext Ultra II RNA	✓	✓	✓	✓	✓	✓	✓	✓			✓	✓	✓
NEBNEXT ULTRAEXPRESS®													
NEBNext UltraExpress RNA	↗			↗			✓		✓	↗	✓		
RNA ENRICHMENT/DEPLETION													
NEBNext Poly(A) mRNA Enrichment Module	✓	✓	✓	✓	✓	✓	✓	✓			✓	✓	✓
NEBNext rRNA Depletion Module + Poly(A)	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓
NEBNEXT INFECTIOUS DISEASE SEQUENCING													
NEBNext ARTIC SARS-CoV-2 Kit	✓	✓	✓	✓	✓	✓	✓	✓		✓			

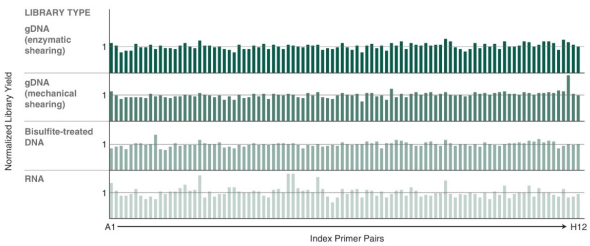
Support currently available ✓ Via NEB ✓ Via manufacturer

Future support, in development ↗ Via NEB ↗ Via manufacturer

Maximize your throughput with NEBNext Multiplexing Oligos

Indexing primers are an essential component of your NGS library prep workflow, and NEBNext Multiplex Oligos offer flexibility in multiplexing. Indexing options include unique dual indices (UDIs) with and without unique molecular identifiers (UMIs), combinatorial dual (CD) indices, and single indices, and are available in a range of formats.

- Rigorous QC ensures each well contains the expected indices, and quantity.
- Plated formats enable streamlined integration into automation workflows.
- Unique Dual Indices are compatible across all sets, allowing pooling of up to 480-libraries, while UMI containing indexes are compatible for pooling of up to 384-libraries.



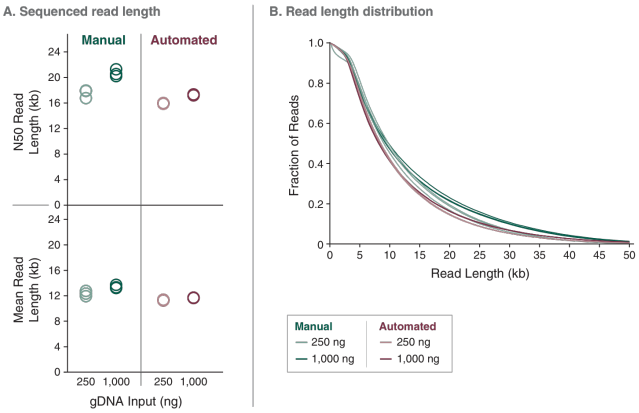
Human NA19240 genomic DNA (Coriell Institute) was used to prepare 96 libraries using either the NEBNext Ultra II FS DNA library prep kit, Covaris-sheared DNA with the NEBNext Ultra II DNA library prep kit, the Ultra II DNA library prep kit combined with bisulfite conversion, or the NEBNext Ultra II Directional RNA library prep kit. Libraries were PCR-amplified using the NEBNext 96 Unique Dual Index Primer Pairs to produce libraries containing unique i5 and i7 indices. Library yields were quantified (Agilent® TapeStation® 4200) and normalized by summing the total yield of all 96 libraries and calculating the contribution from each library (expected fraction per library = 1.04%). Library amplification efficiency was robust with each library prep method and efficiency was uniform across all 96 unique index primer combinations. Each bar represents the average of at least 2 technical replicates.

Novel Workflows that are Automation Compatible by Design

New England Biolabs recognizes the growing need for products that are not just permissive to automation, but instead, are designed with automation in mind. The upcoming NEBNext UltraShear® Long Read (UltraShear LR), demonstrates this, as automation compatibility, including adequate reagent volume and demonstrations of comparability, were integral to product development.

Current methods for DNA fragmentation include mechanical shearing and enzymatic fragmentation. Mechanical shearing (e.g., Covaris® g-TUBE and Megaruptor®) is the gold standard method for DNA fragmentation upstream of long read library preparation, however it does require expensive consumables and/or instruments, is not automation-friendly, and results in sample loss. In comparison, enzymatic fragmentation methods do not require expensive instruments and are automation friendly; unfortunately, the fragment sizes generated may not be compatible with long read sequencing.

To address these constraints, we developed a novel enzymatic fragmentation solution, UltraShear LR, that is quick, tunable, and automation friendly. Enzymatic fragmentation is time-dependent and can be used to generate a wide-range of DNA fragment sizes (2 to 30 kb) suitable for different sequencing platforms and applications. UltraShear LR is robust across a wide range of gDNA input amounts (250 to 5,000 ng) as well as different gDNA samples and species (e.g., animal, plant, and human). Please see our other in-suite poster for more extensive data using this workflow



250 ng and 1,000 ng of gDNA (DQN = 9.9), extracted from GM12878 human cells using the Monarch® HMW DNA Extraction Kit, was fragmented with UltraShear LR using either a manual workflow or an automated workflow on the SPT Labtech FireFly®. Fragmentation conditions were 5 minutes at 30°C followed by 15 minutes at 65°C. (A) Sequenced N50 and mean read lengths obtained using UltraShear LR fragmentation under manual or automated conditions. (B) S-shaped curve plotting the read length distributions. Manual fragmentation with UltraShear LR generated longer read lengths than the automated workflow, due to potential additional shearing by the instrument, which could be gDNA or platform dependent. However, automated fragmentation was more consistent across replicates. Three technical replicates are shown for each condition.

Customized Solutions for High-Throughput and Automation

Reaching the highest-throughput for a specific NGS workflow may require custom formats where reagent volume, labware, labeling, and quality is purpose built for a laboratory and automation based on applications and workflows.

- NEB's Customized Solutions Team will help with access to novel products, meet quality specifications, speed time to market, and streamline supply chain needs.

Aliquoting, Kitting, Packaging, & Private Label | Large Volume Packaging | Custom Formulations & Concentrations | Lyophilization Capabilities

Purpose Built for Laboratory Automation

New England Biolabs' expertise in NGS library preparation goes beyond streamlined and innovative workflows.

- Custom plated versions of any NEBNext Library Preparation solution can be created with support from Customized solutions and Application Development teams.
- Bespoke for a specific workflow, automation, and throughput needs.
  - Varied reagent arrangement, maximizing deck space (single, or multiple reagents on a single plate).
  - Mixed labware, plates, vials, and bottles, to meet workflow requirements.
  - Unique overages and even "no-overage" fills, down to 5 µl, allowing pipetting from, or addition directly to the next reagent required in the workflow.
  - Custom labeling with plain-text, barcodes, private label and more!