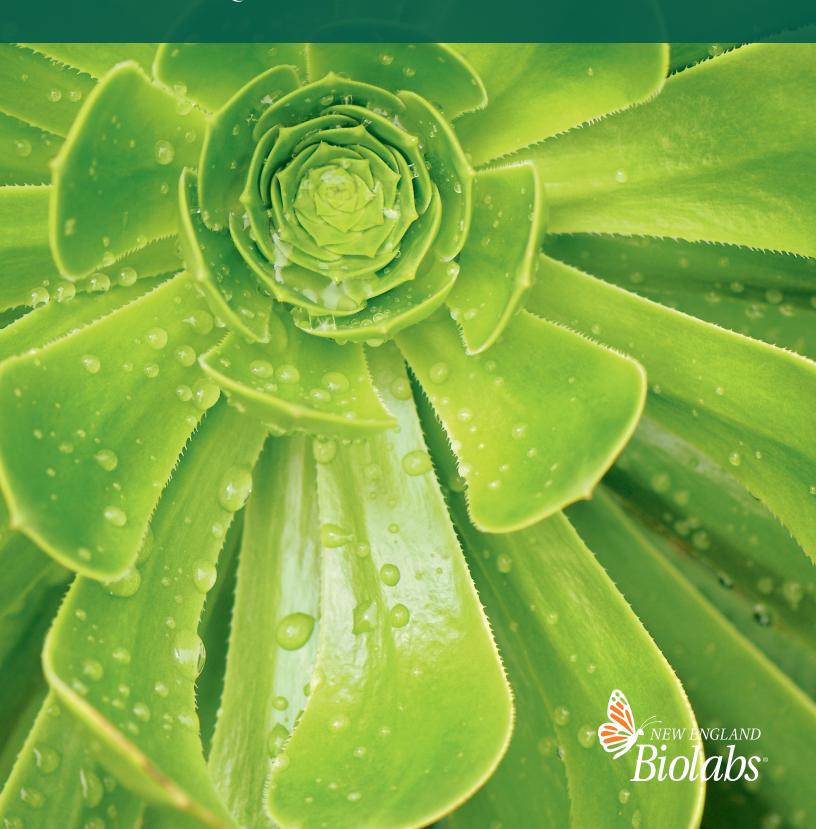
NEBNext® Ultra™ II Reagents & Kits

FOR THE HIGHEST QUALITY DNA AND RNA LIBRARIES FROM A BROAD RANGE OF SAMPLES



The heart of the matter

As sequencing technologies improve and applications expand, the need for compatibility with everdecreasing input amounts and sub-optimal sample quality grows. Scientists must balance reliability and performance with faster turnaround, higher throughput and automation compatibility.

The NEBNext Ultra II workflow lies at the heart of NEB's portfolio for next generation sequencing library preparation. NEBNext Ultra II kits and modules for Illumina® are the perfect combination of reagents, optimized formulations and simplified workflows, enabling creation of DNA or RNA libraries of highest quality and yield, even when starting from extremely low input amounts.

The Ultra II workflow is central to many of our NEBNext solutions, including:

- Ultra II DNA & FS DNA Library Prep, with PCR-free workflow options
- Enzymatic Methyl-seq

- UltraShear FFPE DNA Library Prep
- Single Cell/Low Input RNA Library Prep
- Module products for each step in the workflow

The NEBNext Ultra II Workflow:

End Repair/
dA-Tailing

Adaptor Clean Up/
Size Selection Enrichment

Clean Up

Advantages of the Ultra II Workflow:

- Learn one central workflow and apply it to a suite of different applications
- Save time with streamlined, modular workflows, reduced hands-on time, and automation compatibility
- Benefit from low input amount requirements, fewer PCR cycles, and uniform GC-coverage in all applications

What users are saying:

We've been testing EM-seq on a variety of inputs, platforms, and samples, and it shows more even coverage across CpG islands, the whole genome, and also greater detection of CpG sites across the genome vs. WGBS.

Christopher Mason,
 Weill Cornell Medical School
 New York

NEBNext Ultra II FS performance is exceptional. The possibility to start with higher DNA concentrations allows for less consumption of reagents for initial QC. Obtaining the desired fragment length and less hands-on time are also key factors when preparing genomic libraries and NEBNext Ultra II FS can provide that. The use of fewer PCR cycles also decreases the bias associated with the PCR step.

João Anes, Ph.D.,
 NGS specialist in Food Safety,
 UCD-Centre for Food Safety, Dublin, Ireland

The NEBNext Ultra II Workflow

The Ultra II workflow is available in a convenient kit format, or as separate modules – it is easily scalable and automated on a range of liquid handling instruments. The NEBNext Ultra II workflow has been cited in thousands of publications, as well as a growing number of preprints and protocols. Citation information and extensive performance data for each product is available on www.neb.com.

Choose the convenient NEBNext Ultra II DNA Library Prep Kit for:

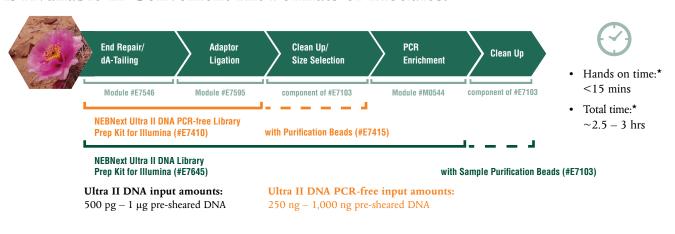
- Whole Genome Sequencing
- Standard & Low Input Sequencing
- ChIP-seq, NICE-seq, Cut&Run-seq
- Exome Capture

- Targeted Sequencing
- FFPE Material
- cfDNA

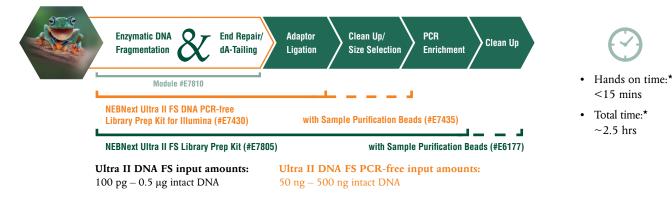
Features

- · Highest library yields and quality
- Fewer PCR cycles, including PCR-free options
- Broad input range (100 pg to 1 μg DNA)
- · Less than 15 minutes of hands-on time
- Workflow complete in ~2.5 − 3 hours, including PCR

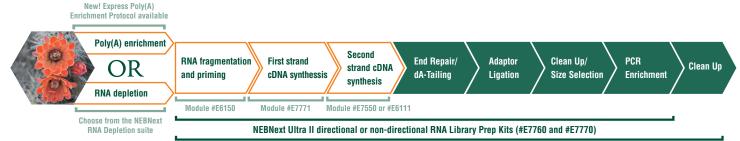
The Ultra II DNA Workflow is Available in Convenient Kit Formats or Modules:



NEBNext Ultra II FS DNA Library Prep Workflow:



NEBNext Ultra II Directional RNA or Non-directional RNA Workflow:



NEBNext Ultra II directional or non-directional RNA Library Prep Kits with Sample Purification Beads (#E7765 or #E7775)

Ultra II RNA input amounts:

 $10 \text{ ng} - 1 \text{ }\mu\text{g} \text{ total RNA (RNA depletion)}$ $10 \text{ ng} - 1 \text{ }\mu\text{g} \text{ total RNA (poly(A) mRNA)}$



- Hands on time: <30 mins
- Total time: $\sim 5.5 6.5$ hrs

NEBNext Enzymatic Methyl-seq v2 Workflow:



NEBNext Enzymatic Methyl-seq v2 Kit (#E8015) incl. Sample Purification Beads

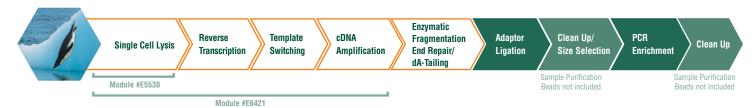
EM-seq v2 input amounts:

100 pg – 200 ng



• Total time: 6 − 7 hrs

NEBNext Single Cell/Low Input RNA Workflow:



NEBNext Single Cell/Low Input RNA Kit (#E6420)

Single Cell/Low Input RNA input amounts:

2 pg - 200 ng total RNA



- Hands on time: <30 mins
- Total time: 6 7 hrs

NEBNext UltraShear® FFPE DNA Library Prep Kit Workflow:



NEBNext UltraShear FFPE DNA Lirbary Prep Kit (#E6655)

DNA input amounts:

5 ng - 250 ng



- Hands on time: <1 hr
- Total time: 3 4 hrs

Ordering Information

Ordering in		Mark Street	
DNA KITS		NEB#	SIZE
DNA	NEBNext Ultra II FS DNA Library Prep Kit for Illumina	E7805S/L	24/96 rxns
	NEBNext Ultra II FS DNA Library Prep with Sample Purification Beads	<u>E6177S/L</u>	24/96 rxns
	NEBNext Ultra II FS DNA PCR-free Library Prep Kit for Illumina	<u>E7430S/L</u>	24/96 rxns
	NEBNext Ultra II FS DNA PCR-free Library Prep Kit with Sample Purification Beads	<u>E7435L</u>	96 rxns
	NEBNext Ultra II DNA Library Prep Kit for Illumina	E7645S/L	24/96 rxns
	NEBNext Ultra II DNA PCR-free Library Prep Kit for Illumina	<u>E7410S/L</u>	24/96 rxns
	NEBNext Ultra II DNA Library Prep with Sample Purification Beads	<u>E7103S/L</u>	24/96 rxns
	NEBNext Enzymatic Methyl-seq v2 Kit	E8015S/L	24/96 rxns
	NEBNext Enzymatic 5hmC-seq Kit	E3350S/L	24/96 rxns
FFPE DNA	NEBNext UltraShear FFPE DNA Library Prep Kit	<u>E6655S/L</u>	24/96 rxns
RNA KITS		NEB #	SIZE
Directional RNA	NEBNext Ultra II Directional RNA Library Prep Kit for Illumina	<u>E7760S/L</u>	24/96 rxns
	NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads	<u>E7765S/L</u>	24/96 rxns
Non-directional RNA	NEBNext Ultra II RNA Library Prep Kit for Illumina	<u>E7770S/L</u>	24/96 rxns
	NEBNext Ultra II RNA Library Prep with Sample Purification Beads	<u>E7775S/L</u>	24/96 rxns
Single Cell	NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina	<u>E6420S/L</u>	24/96 rxns
MODULES		NEB#	SIZE
& ENZYMES		NED #	O.L.L
& ENZYMES	NEBNext Enzymatic Methyl-seq v2 Conversion Module	E8020S/L	24/96 rxns
& ENZYMES			
& ENZYMES	Conversion Module NEBNext Enzymatic 5hmC-seq	<u>E8020S/L</u>	24/96 rxns
	Conversion Module NEBNext Enzymatic 5hmC-seq Conversion Module	E8020S/L E3365S/L	24/96 rxns 24/96 rxns
	Conversion Module NEBNext Enzymatic 5hmC-seq Conversion Module NEBNext FFPE DNA Repair v2 Module NEBNext Ultra II End Repair/dA-Tailing	E8020S/L E3365S/L E7360S/L	24/96 rxns 24/96 rxns 24/96 rxns
	Conversion Module NEBNext Enzymatic 5hmC-seq Conversion Module NEBNext FFPE DNA Repair v2 Module NEBNext Ultra II End Repair/dA-Tailing Module	E8020S/L E3365S/L E7360S/L E7546S/L	24/96 rxns 24/96 rxns 24/96 rxns 24/96 rxns
	Conversion Module NEBNext Enzymatic 5hmC-seq Conversion Module NEBNext FFPE DNA Repair v2 Module NEBNext Ultra II End Repair/dA-Tailing Module NEBNext Ultra II Ligation Module	E8020S/L E3365S/L E7360S/L E7546S/L E7595S/L	24/96 rxns 24/96 rxns 24/96 rxns 24/96 rxns 24/96 rxns
	Conversion Module NEBNext Enzymatic 5hmC-seq Conversion Module NEBNext FFPE DNA Repair v2 Module NEBNext Ultra II End Repair/dA-Tailing Module NEBNext Ultra II Ligation Module NEBNext Ultra II FS DNA Module	E8020S/L E3365S/L E7360S/L E7546S/L E7595S/L E7810S/L	24/96 rxns 24/96 rxns 24/96 rxns 24/96 rxns 24/96 rxns 24/96 rxns
DNA	Conversion Module NEBNext Enzymatic 5hmC-seq Conversion Module NEBNext FFPE DNA Repair v2 Module NEBNext Ultra II End Repair/dA-Tailing Module NEBNext Ultra II Ligation Module NEBNext Ultra II FS DNA Module NEBNext Ultra II FS DNA Module	E8020S/L E3365S/L E7360S/L E7546S/L E7595S/L E7810S/L M7634S/L	24/96 rxns 24/96 rxns 24/96 rxns 24/96 rxns 24/96 rxns 24/96 rxns 24/96 rxns
DNA	Conversion Module NEBNext Enzymatic 5hmC-seq Conversion Module NEBNext FFPE DNA Repair v2 Module NEBNext Ultra II End Repair/dA-Tailing Module NEBNext Ultra II Ligation Module NEBNext Ultra II FS DNA Module NEBNext Ultra II FS DNA Module NEBNext FFPE DNA Repair v2 Module	E8020S/L E3365S/L E7360S/L E7546S/L E7595S/L E7810S/L M7634S/L E7360S/L	24/96 rxns 24/96 rxns 24/96 rxns 24/96 rxns 24/96 rxns 24/96 rxns 24/96 rxns 24/96 rxns
DNA FFPE DNA	Conversion Module NEBNext Enzymatic 5hmC-seq Conversion Module NEBNext FFPE DNA Repair v2 Module NEBNext Ultra II End Repair/dA-Tailing Module NEBNext Ultra II Ligation Module NEBNext Ultra II FS DNA Module NEBNext UltraShear NEBNext FFPE DNA Repair v2 Module NEBNext Single Cell Lysis Module NEBNext Magnesium RNA Fragmentation Module NEBNext Ultra II RNA First Strand Synthesis Module	E8020S/L E3365S/L E7360S/L E7546S/L E7595S/L E7810S/L M7634S/L E7360S/L E5530S	24/96 rxns 24/96 rxns 24/96 rxns 24/96 rxns 24/96 rxns 24/96 rxns 24/96 rxns 24/96 rxns 96 rxns
DNA	Conversion Module NEBNext Enzymatic 5hmC-seq Conversion Module NEBNext FFPE DNA Repair v2 Module NEBNext Ultra II End Repair/dA-Tailing Module NEBNext Ultra II Ligation Module NEBNext Ultra II FS DNA Module NEBNext UltraShear NEBNext UltraShear NEBNext FFPE DNA Repair v2 Module NEBNext Single Cell Lysis Module NEBNext Magnesium RNA Fragmentation Module NEBNext Ultra II RNA First Strand Synthesis Module NEBNext Ultra II Directional RNA Second Strand Synthesis Module	E8020S/L E3365S/L E7360S/L E7546S/L E7595S/L E7810S/L M7634S/L E7360S/L E5530S E6150S	24/96 rxns
DNA FFPE DNA	Conversion Module NEBNext Enzymatic 5hmC-seq Conversion Module NEBNext FFPE DNA Repair v2 Module NEBNext Ultra II End Repair/dA-Tailing Module NEBNext Ultra II Ligation Module NEBNext Ultra II FS DNA Module NEBNext Ultra II FS DNA Module NEBNext UltraShear NEBNext FFPE DNA Repair v2 Module NEBNext Single Cell Lysis Module NEBNext Magnesium RNA Fragmentation Module NEBNext Ultra II RNA First Strand Synthesis Module NEBNext Ultra II Directional RNA Second	E8020S/L E3365S/L E7360S/L E7546S/L E7595S/L E7810S/L M7634S/L E5530S E6150S E7771S/L	24/96 rxns
DNA FFPE DNA	Conversion Module NEBNext Enzymatic 5hmC-seq Conversion Module NEBNext FFPE DNA Repair v2 Module NEBNext Ultra II End Repair/dA-Tailing Module NEBNext Ultra II Ligation Module NEBNext Ultra II FS DNA Module NEBNext Ultra II FS DNA Module NEBNext UltraShear NEBNext FFPE DNA Repair v2 Module NEBNext Single Cell Lysis Module NEBNext Magnesium RNA Fragmentation Module NEBNext Ultra II RNA First Strand Synthesis Module NEBNext Ultra II Directional RNA Second Strand Synthesis Module NEBNext Ultra II Non-directional RNA	E8020S/L E3365S/L E7360S/L E7546S/L E7595S/L E7810S/L M7634S/L E5530S E6150S E7771S/L E7550S/L	24/96 rxns 200 rxns 24/96 rxns 24/96 rxns
DNA FFPE DNA	Conversion Module NEBNext Enzymatic 5hmC-seq Conversion Module NEBNext FFPE DNA Repair v2 Module NEBNext Ultra II End Repair/dA-Tailing Module NEBNext Ultra II Ligation Module NEBNext Ultra II FS DNA Module NEBNext UltraShear NEBNext UltraShear NEBNext Single Cell Lysis Module NEBNext Magnesium RNA Fragmentation Module NEBNext Ultra II RNA First Strand Synthesis Module NEBNext Ultra II Directional RNA Second Strand Synthesis Module NEBNext Ultra II Non-directional RNA Second Strand Synthesis Module NEBNext Ultra II Non-directional RNA Second Strand Synthesis Module	E8020S/L E3365S/L E7360S/L E7546S/L E7595S/L E7810S/L M7634S/L E5530S E6150S E7771S/L E7550S/L E6111S/L	24/96 rxns 200 rxns 24/96 rxns 24/96 rxns 201 rxns
DNA FFPE DNA RNA	Conversion Module NEBNext Enzymatic 5hmC-seq Conversion Module NEBNext FFPE DNA Repair v2 Module NEBNext Ultra II End Repair/dA-Tailing Module NEBNext Ultra II Ligation Module NEBNext Ultra II FS DNA Module NEBNext Ultra II FS DNA Module NEBNext UltraShear NEBNext FFPE DNA Repair v2 Module NEBNext Single Cell Lysis Module NEBNext Magnesium RNA Fragmentation Module NEBNext Ultra II RNA First Strand Synthesis Module NEBNext Ultra II Proctional RNA Second Strand Synthesis Module NEBNext Ultra II Non-directional RNA Second Strand Synthesis Module NEBNext Single Cell/Low Input cDNA Synthesis and Amplification Module NEBNext High Input Poly(A) mRNA	E8020S/L E3365S/L E7360S/L E7546S/L E7595S/L E7810S/L M7634S/L E5530S E6150S E7771S/L E7550S/L E6111S/L E6421S/L	24/96 rxns 200 rxns 24/96 rxns 24/96 rxns 24/96 rxns 24/96 rxns 24/96 rxns
DNA FFPE DNA RNA Poly(A) mRNA	Conversion Module NEBNext Enzymatic 5hmC-seq Conversion Module NEBNext FFPE DNA Repair v2 Module NEBNext Ultra II End Repair/dA-Tailing Module NEBNext Ultra II Ligation Module NEBNext Ultra II FS DNA Module NEBNext Ultra II FS DNA Module NEBNext UltraShear NEBNext FFPE DNA Repair v2 Module NEBNext Single Cell Lysis Module NEBNext Magnesium RNA Fragmentation Module NEBNext Ultra II RNA First Strand Synthesis Module NEBNext Ultra II Directional RNA Second Strand Synthesis Module NEBNext Ultra II Non-directional RNA Second Strand Synthesis Module NEBNext Single Cell/Low Input cDNA Synthesis and Amplification Module NEBNext High Input Poly(A) mRNA Isolation Module NEBNext Poly(A) mRNA Magnetic	E8020S/L E3365S/L E7360S/L E7546S/L E7595S/L E7810S/L M7634S/L E5530S E6150S E77771S/L E7550S/L E6111S/L E6421S/L	24/96 rxns 200 rxns 24/96 rxns 24/96 rxns 24/96 rxns 24/96 rxns 24/96 rxns 24/96 rxns

COMPANION Products		NEB #	SIZE	
Oligos	NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs, Sets 1-5)	E6440S/L E6442S/L E6444S/L E6446S/L E6448S/L	96/384 rxns	
	NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors DNA Sets 1-4)	E7395S/L E7874S/L E7876S/L E7878S/L	96/384 rxns	
	NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors RNA Set 1)	<u>E7416S/L</u>	96/384 rxns	
	NEBNext Multiplex Oligos for Enzymatic Methyl-seq (Unique Dual Index Primer Pairs)	<u>E7140S/L</u>	24/96 rxns	
	NEBNext LV Unique Dual Index Primer Set (2A, 2B)	<u>E3390S</u> <u>E3392S</u>	24 rxns	
	NEBNext LV Unique Dual Index Primer Set (1, 2, 3, 4, 5)	E3400L E3402L E3404L E3406L E3408L	96 rxns	
RNA Depletion	NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat)	E7400S/L/X	6/24/96 rxns	
	NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) with RNA Sample Purification Beads	E7405S/L/X	6/24/96 rxns	
	NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat)	<u>E7750S/L/X</u>	6/24/96 rxns	
	NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads	E7755S/L/X	6/24/96 rxns	
	NEBNext rRNA Depletion Kit (Bacteria)	E7850S/L/X	6/24/96 rxns	
	NEBNext rRNA Depletion Kit (Bacteria) with RNA Sample Purification Beads	E7860S/L/X	6/24/96 rxns	
	NEBNext RNA Depletion Core Reagent Set	E7865S/L/X	6/24/96 rxns	

To view the selection chart for all oligos, please visit: www.neb.com/oligos.

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For help with choosing the best reagents for your next generation sequencing sample preparation, try our NEBNext selector at **NEBNextselector.neb.com**.



Did you know that many of these products can be purchased in large volumes and custom formats?

Learn more at www.neb.com/customizedsolutions

