



Featuring:  
NEBNext® Enzymatic Methyl-seq v2

# 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 ever-decreasing 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:



## 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](http://www.neb.com).

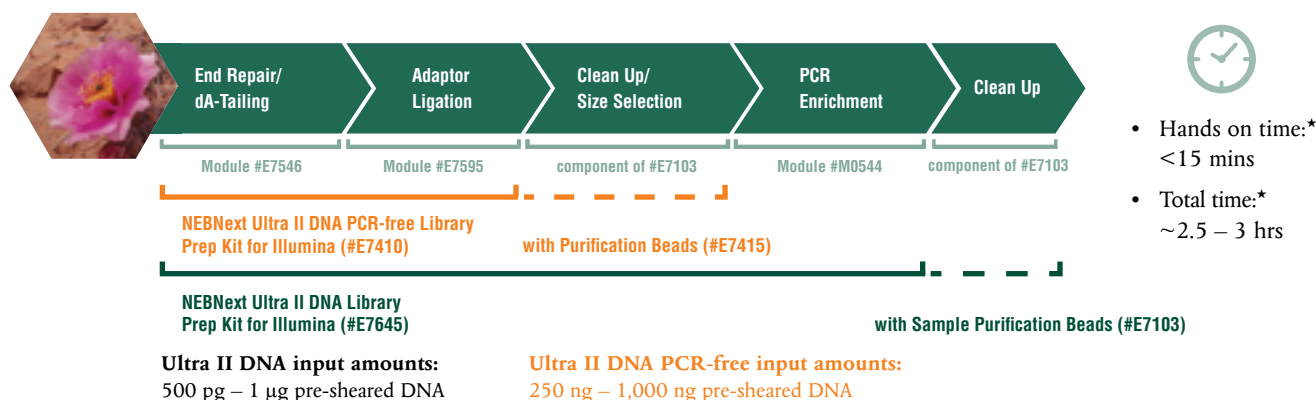
## Choose the convenient NEBNext Ultra II DNA Library Prep Kit for:

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

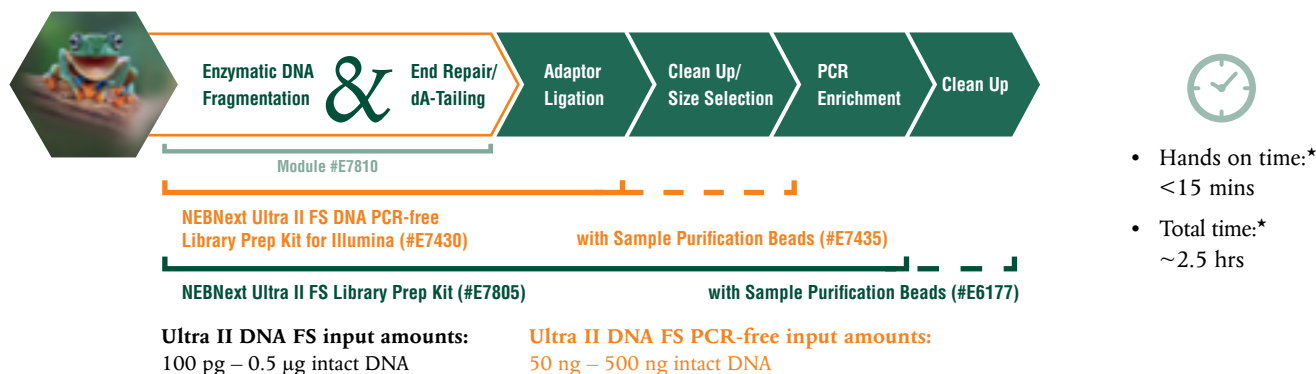
## Features

- 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:

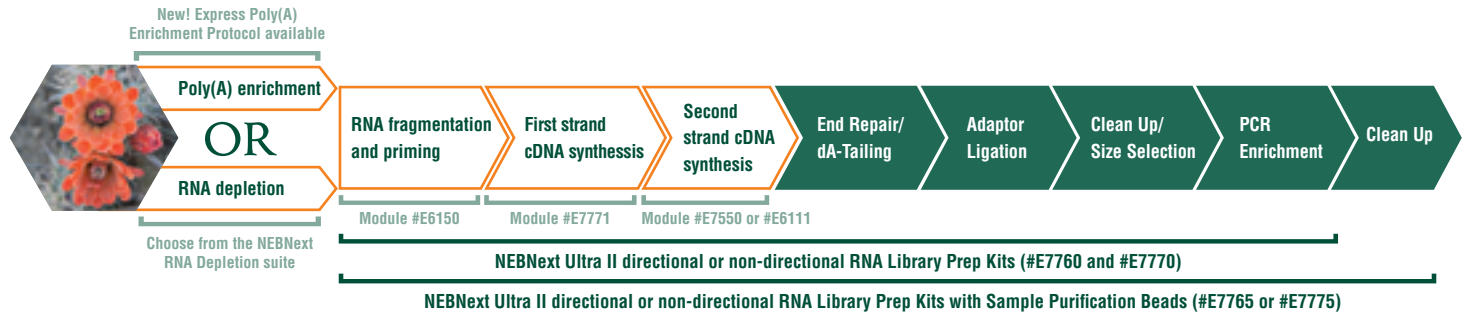


## NEBNext Ultra II FS DNA Library Prep Workflow:



\*Including PCR

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



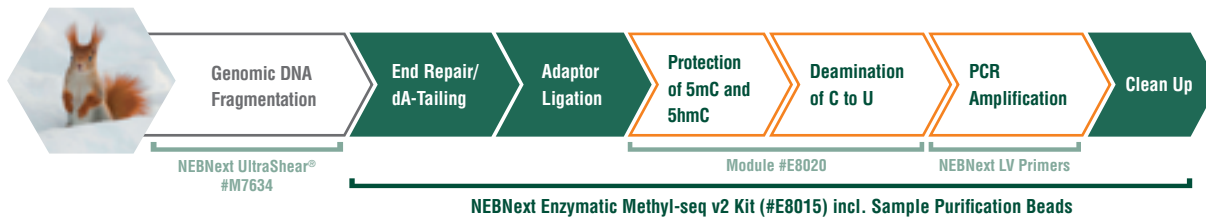
### Ultra II RNA input amounts:

10 ng – 1 µg total RNA (RNA depletion)  
10 ng – 1 µg total RNA (poly(A) mRNA)



- Hands on time: <30 mins
- Total time: ~ 5.5 – 6.5 hrs

## NEBNext Enzymatic Methyl-seq v2 Workflow:



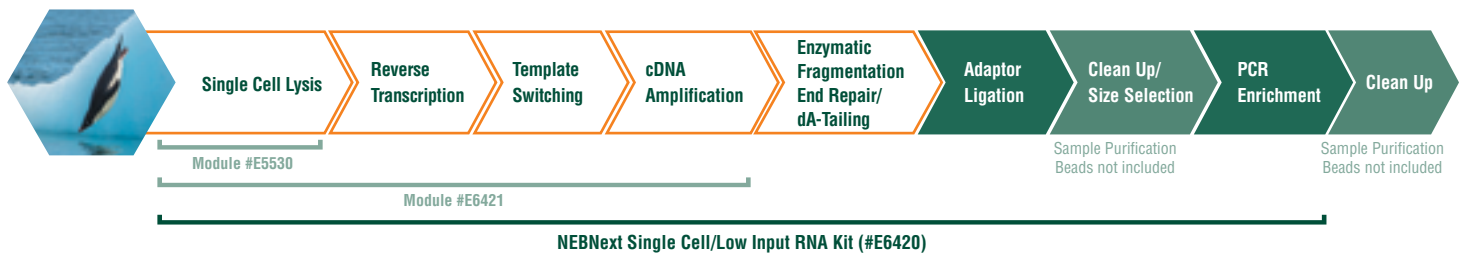
### EM-seq v2 input amounts:

100 pg – 200 ng



- Total time: 6 – 7 hrs

## NEBNext Single Cell/Low Input RNA Workflow:



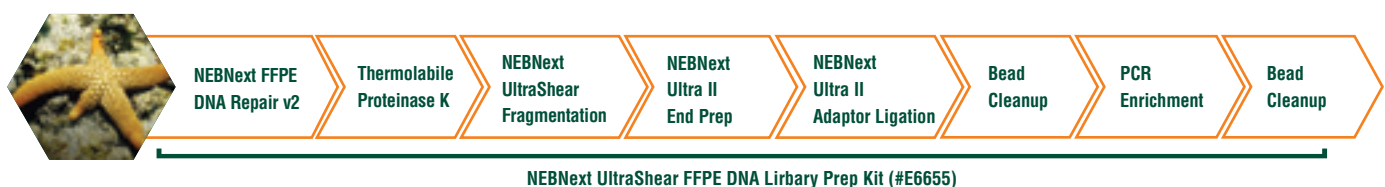
### Single Cell/Low Input RNA input amounts:

2 pg – 200 ng total RNA



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

## NEBNext UltraShear® FFPE DNA Library Prep Kit Workflow:



### DNA input amounts:

5 ng – 250 ng



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

## Ordering Information

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	E6177S/L	24/96 rxns
	NEBNext Ultra II FS DNA PCR-free Library Prep Kit for Illumina	E7430S/L	24/96 rxns
	NEBNext Ultra II FS DNA PCR-free Library Prep Kit with Sample Purification Beads	E7435L	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	E7410S/L	24/96 rxns
	NEBNext Ultra II DNA Library Prep with Sample Purification Beads	E7103S/L	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	E6655S/L	24/96 rxns
RNA KITS		NEB #	SIZE
Directional RNA	NEBNext Ultra II Directional RNA Library Prep Kit for Illumina	E7760S/L	24/96 rxns
	NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads	E7765S/L	24/96 rxns
Non-directional RNA	NEBNext Ultra II RNA Library Prep Kit for Illumina	E7770S/L	24/96 rxns
	NEBNext Ultra II RNA Library Prep with Sample Purification Beads	E7775S/L	24/96 rxns
Single Cell	NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina	E6420S/L	24/96 rxns
MODULES & ENZYMES		NEB #	SIZE
DNA	NEBNext Enzymatic Methyl-seq v2 Conversion Module	E8020S/L	24/96 rxns
	NEBNext Enzymatic 5hmC-seq Conversion Module	E3365S/L	24/96 rxns
	NEBNext FFPE DNA Repair v2 Module	E7360S/L	24/96 rxns
	NEBNext Ultra II End Repair/dA-Tailing Module	E7546S/L	24/96 rxns
	NEBNext Ultra II Ligation Module	E7595S/L	24/96 rxns
	NEBNext Ultra II FS DNA Module	E7810S/L	24/96 rxns
	NEBNext UltraShear	M7634S/L	24/96 rxns
FFPE DNA	NEBNext FFPE DNA Repair v2 Module	E7360S/L	24/96 rxns
RNA	NEBNext Single Cell Lysis Module	E5530S	96 rxns
	NEBNext Magnesium RNA Fragmentation Module	E6150S	200 rxns
	NEBNext Ultra II RNA First Strand Synthesis Module	E7771S/L	24/96 rxns
	NEBNext Ultra II Directional RNA Second Strand Synthesis Module	E7550S/L	24/96 rxns
	NEBNext Ultra II Non-directional RNA Second Strand Synthesis Module	E6111S/L	20/100 rxns
	NEBNext Single Cell/Low Input cDNA Synthesis and Amplification Module	E6421S/L	24/96 rxns
Poly(A) mRNA Enrichment	NEBNext High Input Poly(A) mRNA Isolation Module	E3370S	24 rxns
	NEBNext Poly(A) mRNA Magnetic Isolation Module	E7490S/L	24/96 rxns
Amplification	NEBNext Ultra II Q5 Master Mix	M0544S/L	50/250 rxns
	NEBNext Q5U Master Mix	M0597S/L	50/250 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)	E7416S/L	96/384 rxns
	NEBNext Multiplex Oligos for Enzymatic Methyl-seq (Unique Dual Index Primer Pairs)	E7140S/L	24/96 rxns
	NEBNext LV Unique Dual Index Primer Set (2A, 2B)	E3390S E3392S	24 rxns
	NEBNext LV Unique Dual Index Primer Set (1, 2, 3, 4, 5)	E3400L E3402L E3404L E3406L E3408L	96 rxns
	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
RNA Depletion	NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat)	E7750S/L/X	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](http://www.neb.com/oligos).



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## ATCG NEBNext® Selector<sup>v1.0</sup>

For help with choosing the best reagents for your next generation sequencing sample preparation, try our NEBNext selector at [NEBNextselector.neb.com](http://NEBNextselector.neb.com).



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