

# NEBNext<sup>®</sup> Ultra<sup>™</sup> II Reagents & Kits

THE CORE OF YOUR NGS LIBRARY PREP



NEW ENGLAND  
*BioLabs*<sup>®</sup>

be INSPIRED  
drive DISCOVERY  
stay GENUINE



# 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 you to create 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
- Single Cell/Low Input RNA Library Prep
- Enzymatic Methyl-seq
- Module products for each step in the workflow
- Ultra II RNA & Directional RNA Library Prep

## 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

“NEB is currently the only company, which is offering really ‘automation friendly’ library preparation kits. The volumes of components are calculated to cover for unavoidable deadvolumes, reactant volumes are in range of most automated platforms. The stability of the NEBNext chemistry allows a broad range of automation strategies”

– Dr. Jürgen Zimmermann,  
Senior Engineer - Automation  
GeneCore EMBL Heidelberg, Germany

# 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 related to COVID-19. Citation information and extensive performance data for each product is available on [www.neb.com](http://www.neb.com).

## Choose the convenient

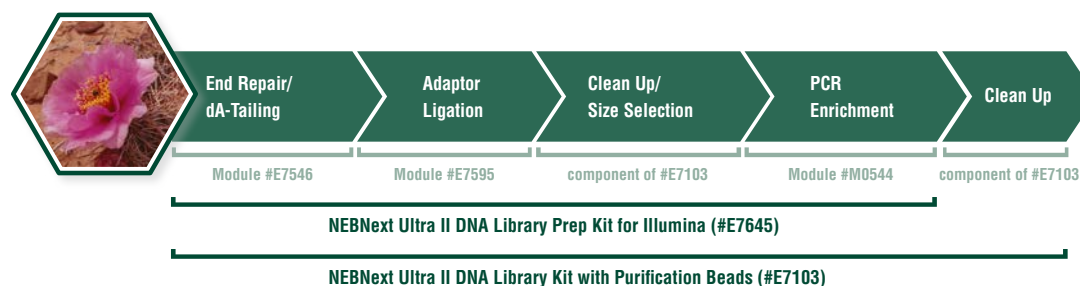
### NEBNext Ultra II DNA Library Prep Kit for:

- Whole Genome Sequencing
- Standard & Low Input Sequencing
- ChIP-seq, NICE-seq, Cut&Run-seq
- Exome Capture
- Targeted Sequencing
- FFPE Material
- cfDNA
- Infectious disease / surveillance sequencing ( e.g. SARS-CoV-2 )

## Features

- Highest library yields and quality
- Fewer PCR cycles
- Broad input range (500 pg to 1 µg sheared DNA)
- Less than 15 minutes of hands-on time
- Workflow complete in ~2:30 – 3:00 hours

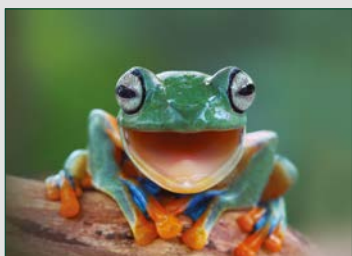
## The Ultra II DNA Workflow is Available in Convenient Kit Formats or Modules:



- Hands on time: <15 minutes
- Total time: ~2:30 – 3:00 hrs

## The Ultra II DNA Workflow is the Core of:

Enzymatic  
DNA Fragmentation  
Library Prep



You'll be thrilled  
to pieces.

Directional & Non-  
directional RNA-seq



Even more *from* less –  
now for RNA.

Enzymatic  
Methyl-seq  
(bisulfite-free)



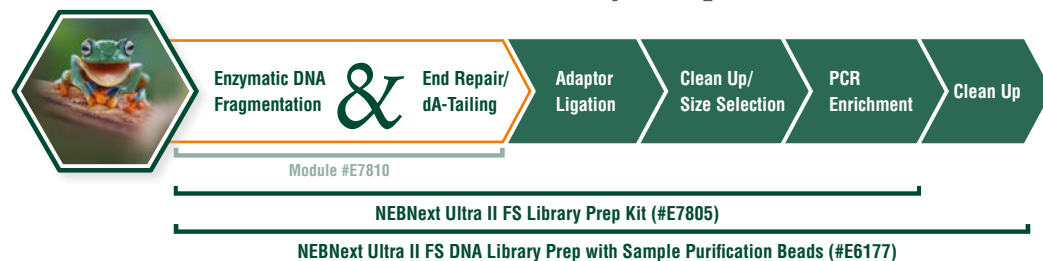
Heads up!

Single Cell/  
Low Input RNA  
Library Prep



How low can you go?

## NEBNext Ultra II FS DNA Library Prep Workflow:



**Ultra II DNA FS input amounts:**  
100 pg – 0.5 µg unshredded DNA



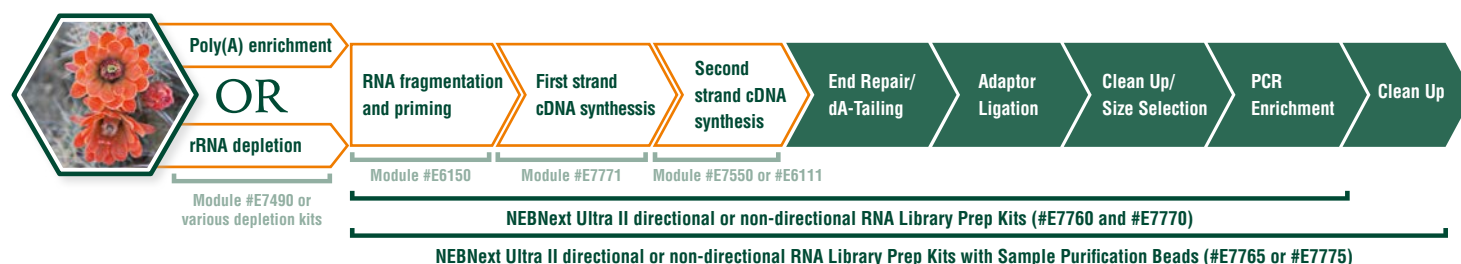
- Hands on time: <15 minutes
- Total time: ~ 2:30 hrs



Also being used in SARS-CoV-2 surveillance sequencing

For more information, visit [www.neb.com/ARTIC](http://www.neb.com/ARTIC)

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

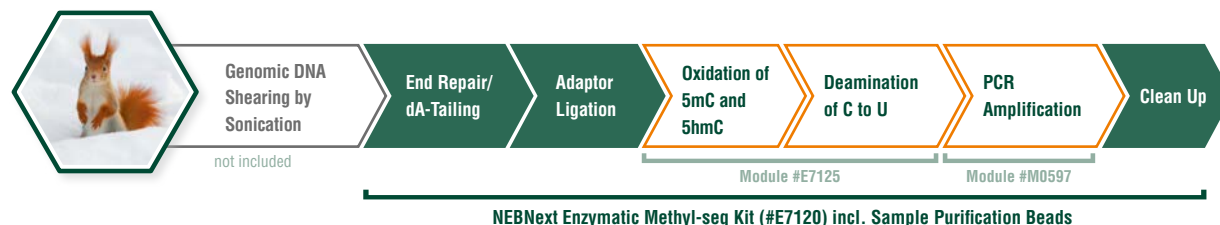


**Ultra II RNA input amounts:**  
5 ng – 1 µg total RNA (rRNA depletion)  
10 ng – 1 µg total RNA (poly(A) mRNA)



- Hands on time: <30 minutes
- Total time: ~ 5:30 – 6:30 hrs

## NEBNext Enzymatic Methyl-seq Workflow:

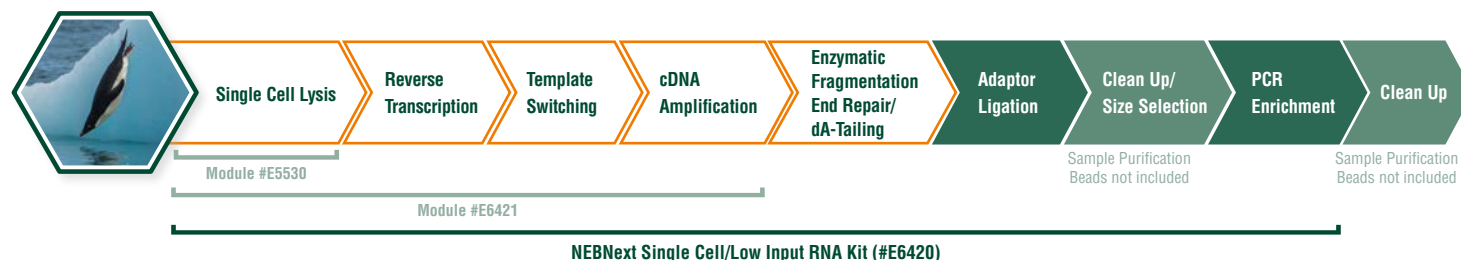


**EM-seq input amounts:**  
10 ng – 200 ng



- Total time: 6 hrs – 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 minutes
- Total time: 6 hrs – 7 hrs



## Ordering Information

DNA KITS		NEB #	SIZE
DNA	NEBNext Ultra II FS DNA Library Prep Kit for Illumina	E7805S/L	24/96 reactions
	NEBNext Ultra II FS DNA Library Prep with Sample Purification Beads	E6177S/L	24/96 reactions
	NEBNext Ultra II DNA Library Prep Kit for Illumina	E7645S/L	24/96 reactions
	NEBNext Ultra II DNA Library Prep with Sample Purification Beads	E7103S/L	24/96 reactions
	NEBNext Enzymatic Methyl-seq Kit	E7120S/L	24/96 reactions
RNA KITS		NEB #	SIZE
Directional RNA	NEBNext Ultra II Directional RNA Library Prep Kit for Illumina	E7760S/L	24/96 reactions
	NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads	E7765S/L	24/96 reactions
Non-directional RNA	NEBNext Ultra II RNA Library Prep Kit for Illumina	E7770S/L	24/96 reactions
	NEBNext Ultra II RNA Library Prep with Sample Purification Beads	E7775S/L	24/96 reactions
Single Cell	NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina	E6420S/L	24/96 reactions
MODULES & ENZYMES		NEB #	SIZE
DNA	NEBNext Enzymatic Methyl-seq Conversion Module	E7125S/L	24/96 reactions
	NEBNext Ultra II End Repair/dA-Tailing Module	E7546S/L	24/96 reactions
	NEBNext Ultra II Ligation Module	E7595S/L	24/96 reactions
	NEBNext Ultra II FS DNA Module	E7810S/L	24/96 reactions
RNA	NEBNext Single Cell Lysis Module	E5530S	96 reactions
	NEBNext Magnesium RNA Fragmentation Module	E6150S	200 reactions
	NEBNext Ultra II RNA First Strand Synthesis Module	E7771S/L	24/96 reactions
	NEBNext Ultra II Directional RNA Second Strand Synthesis Module	E7550S/L	24/96 reactions
	NEBNext Ultra II Non-directional RNA Second Strand Synthesis Module	E6111S/L	20/100 reactions
	NEBNext Single Cell/Low Input cDNA Synthesis and Amplification Module	E6421S/L	24/96 reactions
Amplification	NEBNext Ultra II Q5 Master Mix	M0544S/L	50/250 reactions
	NEBNext Q5U Master Mix	M0597S/L	50/250 reactions
COMPANION PRODUCTS		NEB #	SIZE
Oligos	NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs)	E6440S/L	96/384 reactions
	NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 2)	E6442S/L	96/384 reactions
	NEBNext Multiplex Oligos for Enzymatic Methyl-seq (Unique Dual Index Primer Pairs)	E7140S/L	24/96 reactions
	NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1)	E7600S	96 reactions
	NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 2)	E7780S	96 reactions
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 1)	E7335S/L	24/96 reactions
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 2)	E7500S/L	24/96 reactions
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 3)	E7710S/L	24/96 reactions
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 4)	E7730S/L	24/96 reactions
	NEBNext Multiplex Oligos for Illumina (96 Index Primers)	E6609S/L	96/384 reactions
RNA Depletion	NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat)	E7400S/L/X	6/24/96 reactions
	NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) with RNA Sample Purification Beads	E7405S/L/X	6/24/96 reactions
	NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat)	E7750S/L/X	6/24/96 reactions
	NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads	E7755S/L/X	6/24/96 reactions
	NEBNext rRNA Depletion Kit (Bacteria)	E7850S/L/X	6/24/96 reactions
	NEBNext rRNA Depletion Kit (Bacteria) with RNA Sample Purification Beads	E7860S/L/X	6/24/96 reactions

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## Featured Tools

**ATCG**

### NEBNext Selector

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).



### Read Coverage Calculator

For help with calculation of values associated with read coverage in NGS protocols, try our Read Coverage Calculator at [Readcoveragecalculator.neb.com](http://Readcoveragecalculator.neb.com).

## Distributors

For a complete list of all NEB subsidiaries and distributors, please go to:

[www.neb.com/international-support](http://www.neb.com/international-support)



# The heart of the matter.

## NEBNext<sup>®</sup> Ultra<sup>™</sup> II DNA Sample Prep Solutions – now featuring PCR-free workflows

As sequencing technologies continue to improve and applications expand, the need for compatibility with ever-increasing input amounts and sub-optimal sample quality grows. Reliability and high performance are critical, along with faster turnaround, higher throughput, and automation compatibility.

NEBNext's line of Ultra II DNA library prep reagents has expanded to include PCR-free workflows, powering high performance without the need for amplification. Be eliminating the risk of PCR bias, libraries are a clearer reflection of biology.

The NEBNext Ultra II workflow lies at the heart of NEB's portfolio for next generation sequencing library preparation, with kits and modules for optimal flexibility. You can be assured your DNA libraries will be of the highest quality and yield, even when starting from extremely low input amounts.

Use the selection chart below to determine which NEBNext Ultra II products best suit your needs.

FEATURES	ULTRA II DNA LIBRARY PREP	ULTRA II FS DNA LIBRARY PREP
	Unprecedented performance, enabling lower inputs and fewer PCR cycles	Perform fragmentation, end repair, and dA-tailing with a single enzyme mix
ULTRA II INPUT AMOUNTS	500 pg – 1 µg of sheared DNA	100 pg – 500 ng of intact DNA
Available with or without beads?	<ul style="list-style-type: none"><li>• With beads: NEB #E7103</li><li>• Without beads: NEB #E7645</li></ul>	<ul style="list-style-type: none"><li>• With beads: NEB #E7435</li><li>• Without beads: NEB #E7805</li></ul>
Modules available?	<ul style="list-style-type: none"><li>• Ultra II End Repair/dA-tailing Module (NEB #E7546)</li><li>• Ultra II Ligation Module (NEB #E7595)</li></ul>	<ul style="list-style-type: none"><li>• Ultra II FS DNA Module (NEB #E7810)</li></ul>
ULTRA II PCR-FREE INPUT AMOUNTS	250 ng – 1,000 ng of sheared DNA	50 ng – 500 ng of intact DNA
Available with or without beads?	<ul style="list-style-type: none"><li>• With beads: NEB #E7415</li><li>• Without beads: NEB #E7410</li></ul>	<ul style="list-style-type: none"><li>• With beads: NEB #E7435</li><li>• Without beads: NEB #E7430</li></ul>
Modules available?	<ul style="list-style-type: none"><li>• Ultra II End Repair/dA-tailing Module (NEB #E7546)</li><li>• Ultra II Ligation Module (NEB #E7595)</li></ul>	<ul style="list-style-type: none"><li>• Ultra II FS DNA Module (NEB #E7810)</li></ul>
COMPATIBLE WITH FFPE DNA?	Yes – for improved performance, consider the NEBNext FFPE DNA Repair Mix (NEB #M6630)	Yes – for improved performance, consider the NEBNext FFPE DNA Repair Mix (NEB #M6630)
COMPATIBLE WITH METHYLOME ANALYSIS?	Yes – Methylome analysis is supported; however, we recommend NEBNext EM-seq <sup>™</sup> (NEB #E7120)	No – not compatible due to potential for loss of methyl marks
COMPATIBLE WITH OXFORD NANOPORE TECHNOLOGIES?	Yes. We recommend the NEBNext Companion Module for Oxford Nanopore Technologies <sup>®</sup> Ligation Sequencing (NEB #E7180).	

Note: NEBNext Multiplex Oligos are available separately. Visit [www.neb.com/oligos](http://www.neb.com/oligos) for options.



# NEBNext Ultra II DNA Sample Prep Solutions – now featuring PCR-free workflows



## Ordering Information

PRODUCT	NEB #	SIZE	PRICE
NEBNext Ultra™ II DNA Library Prep Kit for Illumina®	E7645S/L	24/96 reactions	545 € / 2.085 €
NEBNext Ultra II DNA Library Prep with Sample Purification Beads	E7103S/L	24/96 reactions	626 € / 2.394 €
NEBNext Ultra II FS DNA Library Prep Kit for Illumina	E7805S/L	24/96 reactions	665 € / 2.526 €
NEBNext Ultra II FS DNA PCR-free Library Prep with Sample Purification Beads	E7435S/L	24/96 reactions	693 € / 2.620 €
NEBNext Ultra II End Repair/dA-Tailing Module	E7546S/L	24/96 reactions	262 € / 835 €
NEBNext Ultra II Ligation Module	E7595S/L	24/96 reactions	395 € / 1.270 €
NEBNext Ultra II DNA PCR-free Library Prep Kit for Illumina	E7410S/L	24/96 reactions	518 € / 1.980 €
NEBNext Ultra II DNA PCR-free Library Prep with Sample Purification Beads	E7415S/L	24/96 reactions	594 € / 2.253 €
NEBNext Ultra II FS DNA PCR-free Library Prep Kit for Illumina	E7430S/L	24/96 reactions	632 € / 2.399 €
NEBNext Ultra II FS DNA PCR-free Library Prep with Sample Purification Beads	E7435S/L	24/96 reactions	693 € / 2.620 €
NEBNext Ultra II FS DNA Module	E7810S/L	24/96 reactions	290 € / 1.036 €
NEBNext FFPE DNA Repair Mix	M6630S/L	24/96 reactions	173 € / 604 €
NEBNext Enzymatic Methyl-seq Kit	E7120S/L	24/96 reactions	895 € / 3.360 €
NEBNext Enzymatic Methyl-seq Conversion Module	E7125S/L	24/96 reactions	185 € / 675 €
NEBNext Companion Module for Oxford Nanopore Technologies® Ligation Sequencing	E7180S	24 reactions	920 €
NEBNext Oligos (including unique dual index, dual and single index primers, as well as unique dual index UMI adaptors) (NEB #E6440, #E6442, #E6444, #E6446, #E6609, #E7140, #E7335, #E7395, #E7500, #E7535, #E7600, #E7710, #E7730, #E7780)	N/A	N/A	N/A

For more information on our NEBNext portfolio for DNA and RNA library prep, and to request a sample, visit [NEBNext.com](https://www.neb.com)

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