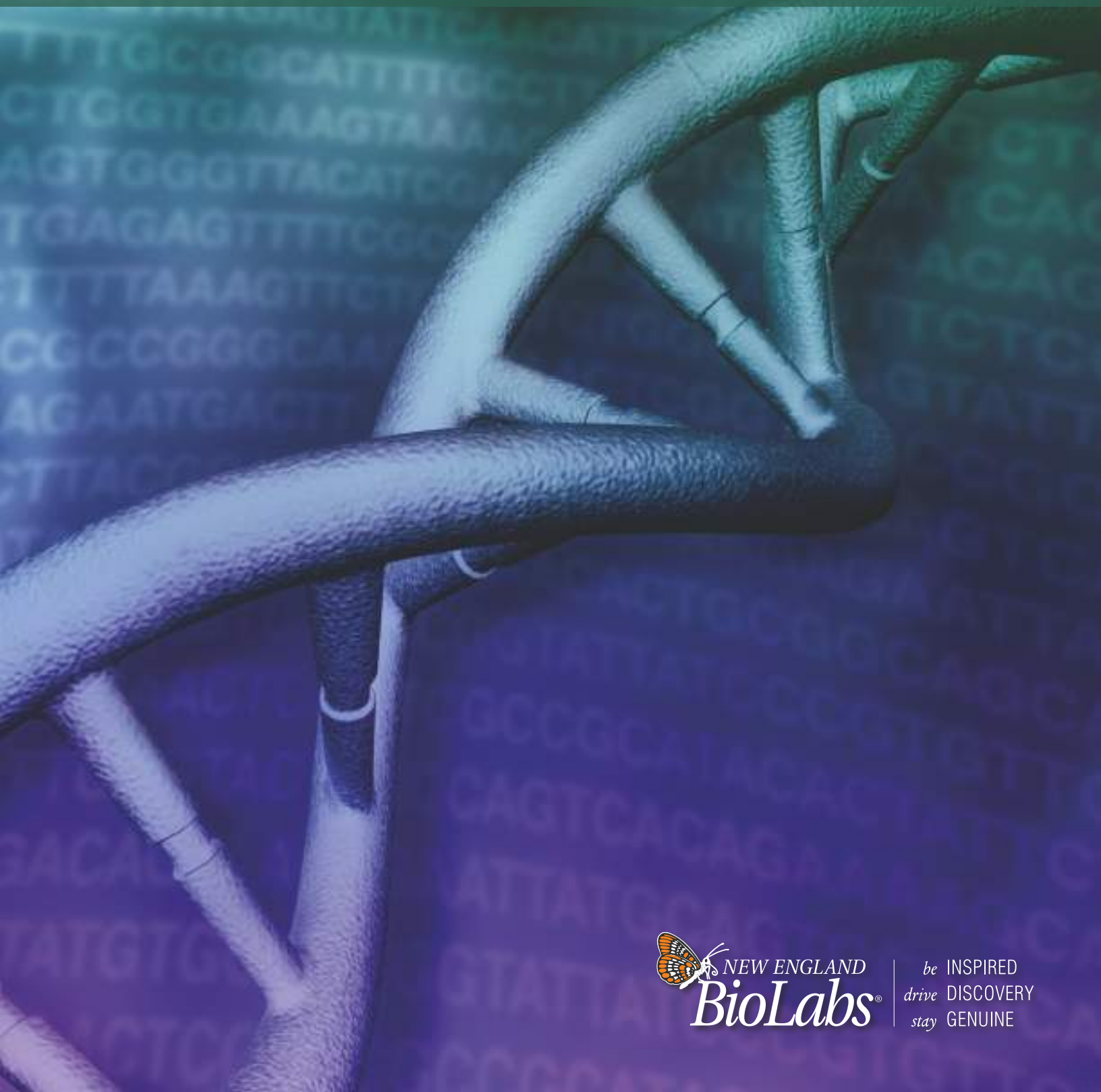
Now includes
NEBNext Ultra™ II DNA & FS DNA PCR-free Library Prep

# NEBNext® for DNA Sample Prep

## FOR THE ILLUMINA® PLATFORM



NEW ENGLAND BioLabs®

*be* INSPIRED
*drive* DISCOVERY
*stay* GENUINE

# Table of Contents



**TOOLS & RESOURCES**

**Visit NEBNext.com to find:**

- The full list of products available
- Video protocols
- Online tutorials to help with product selection, general handling tips and more
- Access to **NEBNext Selector Tool**, our online tool for help with selecting the right NEBNext product
- NEBNext citations
- Product Manuals & FAQs

# Why Choose NEBNext for DNA?

Now that NGS is being used in all types of laboratories, there is no shortage of options available for nearly any step of any NGS workflow. We know you have choices, so below are just a few of the reasons to choose NEBNext.

## High Performance and User Friendly

The NEBNext suite of products supports DNA sequencing on the Illumina platform with sample preparation tools that streamline workflows and minimize inputs, while improving library yields and quality.

NEBNext DNA library prep kits are driven by our Ultra II technology and are compatible with high- and low-quality samples, PCR-free and standard workflows, as well as a broad range of input amounts [100 pg–1 µg (standard), 250 ng–1,000 ng (Ultra II DNA PCR-free), 50 ng–500 ng (Ultra II FS DNA PCR-free)]. Our growing selection of indices (barcodes) provides a wide selection of options for library multiplexing. Beyond library construction, NEBNext also enables 5mC and 5hmC analysis, target enrichment, repair of FFPE DNA, enrichment of microbiome DNA, and qPCR-based library quantitation.

## Reliable and Time Tested

Since our first product release in 2009, the NEBNext brand has stood for quality you can count on. In addition to the extensive QCs performed on individual kit components, all NEBNext kits for Illumina are functionally validated by preparation of a library, followed by Illumina sequencing. Additionally, NEBNext products have been cited in over 15,000 publications.

## Flexible Formats

NEBNext library prep reagents are available in multiple kit and workflow formats, for maximum convenience and flexibility.

### Kits and modules

Kits are the most convenient option, as they include reagents for the entire library prep workflow. Many kits are available with SPRISelect® beads for clean-up and size-selection steps.

With flexibility as a priority, NEBNext modules contain reagents for the individual steps in library preparation. These modules can be combined to cover the entire library prep workflow, or a subset of NEBNext modules can be combined with other reagents to enable a customized workflow for your specific needs.

Adaptors and primers are supplied separately from the NEBNext kits (as NEBNext Oligos modules)*, allowing for increased flexibility in multiplexing options.

**except in the case of the EM-seq Kit, which includes adaptors and primers.*

### Bulk & custom formats:

When your reagent needs exceed standard volumes, or you require a specialized formulation or kit, consider NEBNext's Customized Solutions options. As reagent manufacturers, we are able to provide customized components, kits and modules to meet your specific needs. For more information, please contact Custom@neb.com.

**WHATS NEW IN NEBNEXT?**

- **Ultra II DNA and Ultra II FS DNA PCR-free** workflows for minimal amplification bias
- **384 Unique Dual Index Primer Pairs** for increased multiplexing
- Minimize library errors with **Unique Dual Index UMI Adaptors DNA Set 1**
- The **NEBNext Direct Genotyping Solution** delivers cost-effective, high-throughput, sequence-based target genotyping

Visit **NEBNextSelector.neb.com** to access the **NEBNext Selector Tool**, our online tool for help with selecting the right NEBNext product


**DOWNLOAD THE NEB AR APP***

Find an overview of NGS library preparation.

**see back cover for details*

# NEBNext Ultra II DNA/FS DNA Library Prep Workflow

## with the NEBNext Adaptor and NEBNext Index Primer Pairs

### Ultra II FS DNA Workflow

#### DNA Fragmentation, End Repair & dA-Tailing

- Enzymatic fragmentation
- Generation of blunt-ended fragments (filling in/ chewing back 3´ & 5´ overhangs)
- 5´ phosphorylation
- Creation of single A 3´ overhang enables ligation to adaptors with single T overhangs

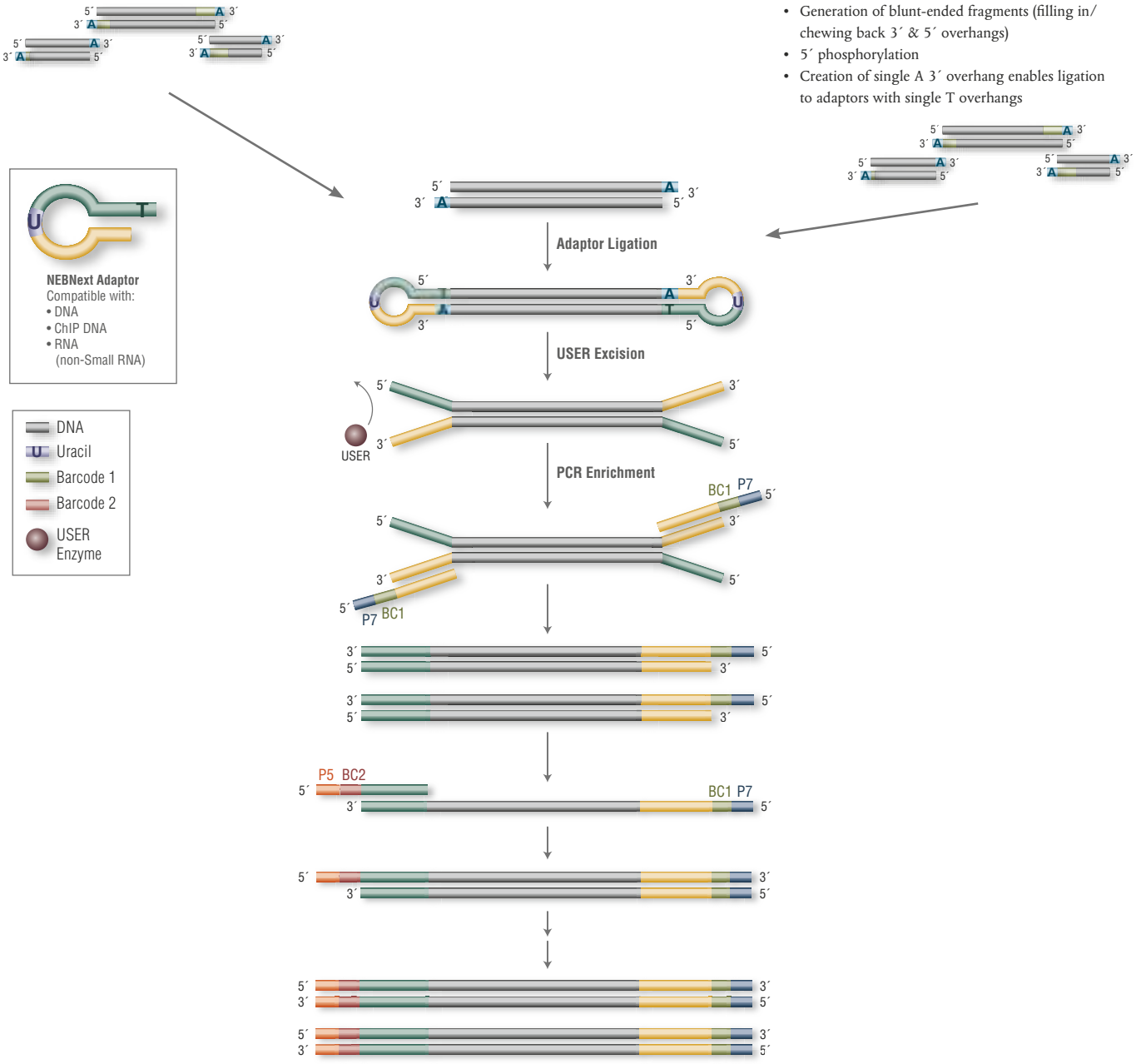
### Ultra II DNA Workflow

#### DNA Fragmentation

- Fragmentation by acoustic shearing, nebulization or enzyme-based methods

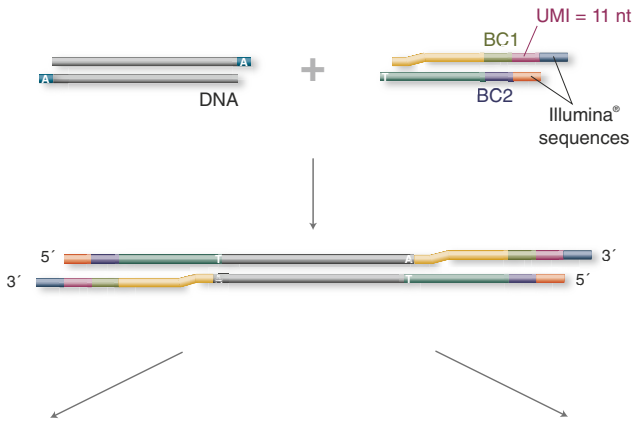
#### End Repair & dA-Tailing

- Generation of blunt-ended fragments (filling in/ chewing back 3´ & 5´ overhangs)
- 5´ phosphorylation
- Creation of single A 3´ overhang enables ligation to adaptors with single T overhangs

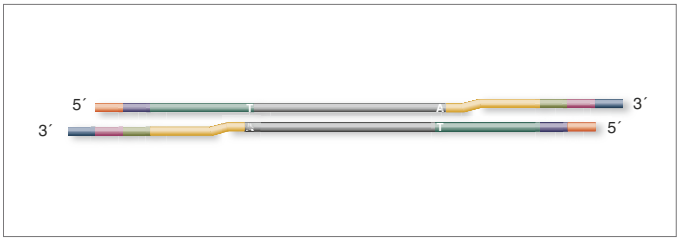


# NEBNext Ultra II DNA/FS DNA Library Prep Workflow

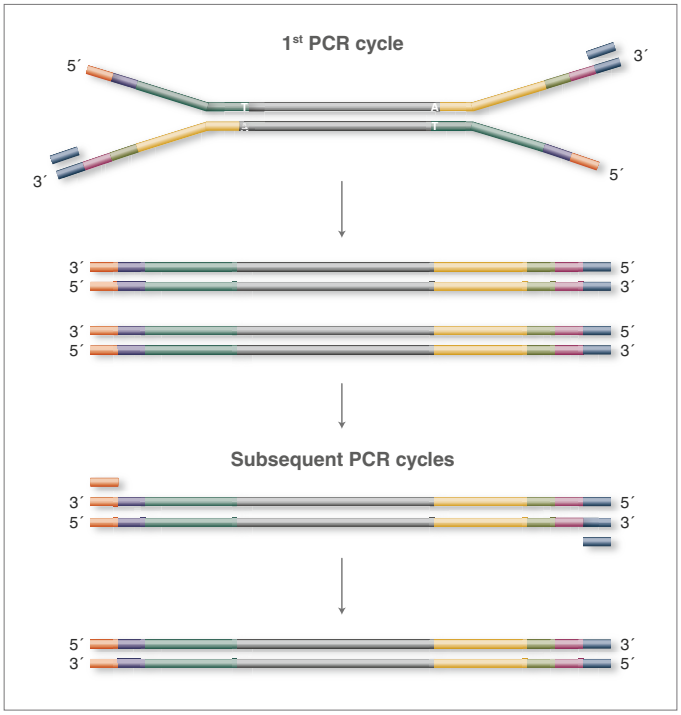
## with the NEBNext Unique Dual Index UMI Adaptor (with Optional Universal Adaptors for Amplification)




**PCR-FREE (READY FOR SEQUENCING)**




**PCR WITH UNIVERSAL PRIMER PAIRS**

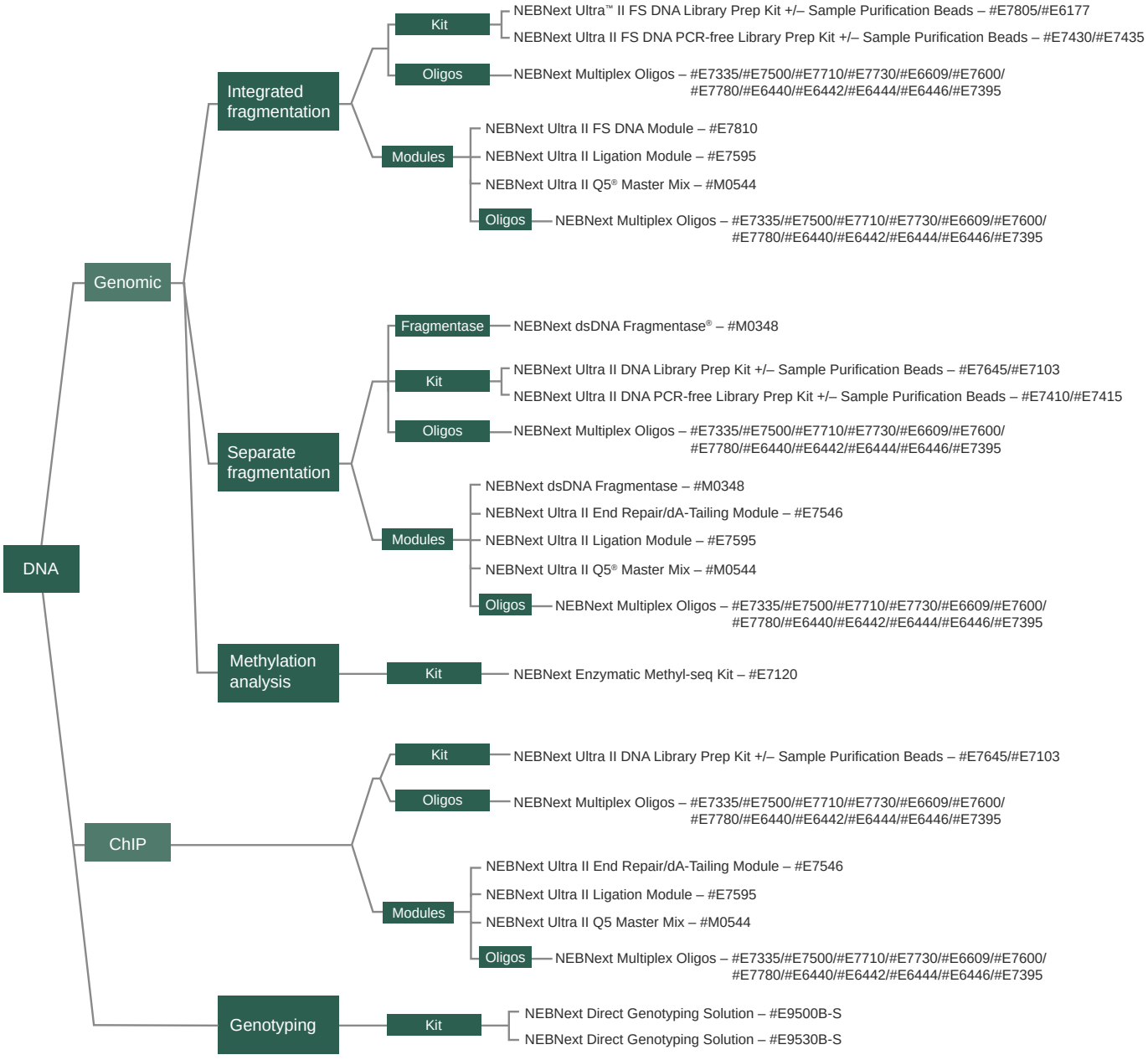



| PRODUCT | INPUT AMOUNTS |
|---|---|
| NEBNext Ultra II FS DNA Library Prep Kit for Illumina (NEB #E7805) | 100 pg – 0.5 µg DNA |
| NEBNext Ultra II FS DNA Library Prep with Sample Purification Beads (NEB #E6177) | |
| NEBNext Ultra II DNA PCR-free Library Prep w/wo Sample Purification Beads (NEB #E7410, #E7415) | 250 ng – 1,000 ng DNA |
| NEBNext Ultra II DNA Library Prep Kit for Illumina (NEB #E7645) | 500 pg – 1 µg DNA |
| NEBNext Ultra II DNA Library Prep with Sample Purification Beads (NEB #E7103) | |
| NEBNext Ultra II FS DNA PCR-free Library Prep w/wo Sample Purification Beads (NEB #E7430, #E7435) | 50 ng – 500 ng DNA |
| 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 |

# DNA Product Selection

For DNA, NEBNext kits are available with and without an amplification step, with and without integrated enzymatic DNA fragmentation, and for methylome analysis. Kits are based on our Ultra II technology and are compatible with samples including genomic DNA, ChIP DNA and FFPE DNA. They utilize fast, streamlined and automatable workflows with novel master mixes that have been designed for performance with a broad range of input amounts, from pg to µg of DNA. NEBNext DNA workflows are also available in module format, which provide the ability to easily customize sample preparation. Adaptors and primers (NEBNext Multiplex Oligos) and FFPE DNA repair reagents are supplied separately.

This chart will help you to determine the best NEBNext product for your Illumina DNA library preparation. You can also use our online tool, **NEBNext Selector at nebnextselector.neb.com**, to choose the best products for your needs.




*Reagents for the original Ultra workflow are also available. See ordering information.*

# NEBNext for DNA Library Prep & Tips for working with DNA

## DNA Sample Input Guidelines

### Integrity of DNA

- The quality of the input material directly affects the quality of the library. Absorbance measurements can be used as an indication of DNA purity. Ideally, the ratio of the absorbance at 260 nm to 280 nm should be between 1.8 – 2.0. However, measurements can be affected by the presence of RNA or small nucleic acid fragments. A DNA Integrity Number can be determined using the Agilent TapeStation® or similar instrumentation, and qPCR-based methods can also provide a measurement of DNA integrity.

### Quantitation of Input DNA

- It is important to quantify accurately the DNA sample prior to library construction. Fluorescence-based detection which utilizes dsDNA-specific dyes, such as the Qubit® from Thermo Fisher Scientific, is more accurate than UV spectrometer based measurements, as the presence of RNA or other contaminants can result in overestimation of the amount of the DNA sample by the latter. This can result in use of non-optimal adaptor dilutions and numbers of PCR cycles, compromising library prep efficiency.

### Indices/Barcodes

- When using a subset of the indices supplied in a kit, or using indices from more than one kit, it is important to optimize the combination of indices used, in order to ensure balanced sequencing reads. We provide recommendations for NEBNext index combinations in the manuals for NEBNext Oligos products.
- For index primers provided in vials, open only one vial at a time, to minimize the risk of contamination
- Be sure to change pipette tips for each index primer
- For 96-well plate formats, NEBNext index primers are provided in single-use plates with pierceable foil lids. To avoid risk of contamination, do not pipette from a well more than one time.

# NEBNext Magnetic Separation Rack

Next generation sequencing library preparation workflows include magnetic bead-based purification and size-selection steps and it is important for library yield and quality that bead separation be highly efficient and fast.

The NEBNext Magnetic Separation Rack was designed for this application and contains rare earth Neodymium Iron Boron (NdFeB) magnets, the most powerful commercially available magnets, in an anodized aluminium rack. The rack holds 24 0.2 ml tubes, and is compatible with single tubes or strip tubes.

**ADVANTAGES**

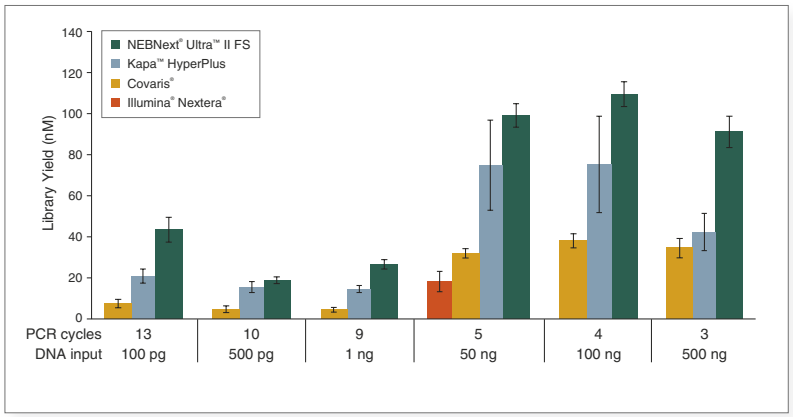
- Fast separations in purification and size-selection steps in next generation sequencing workflows
- 24 tube capacity

NEBNEXT MAGNETIC SEPARATION RACK

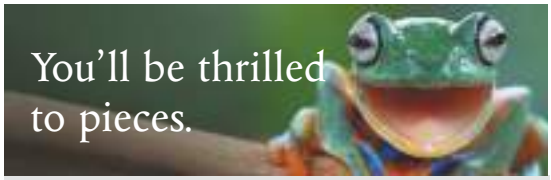
# NEBNext Ultra II FS enables fast, scalable and reliable library prep–*all with a user-friendly protocol*

The Ultra II FS kit includes a new DNA fragmentation reagent, which is also combined with end repair and dA-tailing reagents, enabling these steps to be performed in the same tube, with no clean-ups or sample loss. The same fragmentation protocol is used for any input amount (100 pg–0.5 µg), or GC content. Also available with a PCR-free workflow (50 ng–500 ng).

## NEBNext Ultra II FS DNA produces the highest yields, from a range of input amounts.




*Libraries were prepared from Human NA19240 genomic DNA using the input amounts and numbers of PCR cycles shown. For NEBNext Ultra II FS, a 20-minute fragmentation time was used. For Kapa™ HyperPlus libraries, input DNA was cleaned up with 3X beads prior to library construction, as recommended, and a 20-minute fragmentation time was used. Illumina recommends 50 ng input for Nextera™, and not an input range; therefore, only 50 ng was used in this experiment. "Covaris®" libraries were prepared by shearing each input amount in 1X TE Buffer to an insert size of ~200 bp using a Covaris instrument, followed by library construction using the NEBNext Ultra II DNA Library Prep Kit (NEB #E7645). Error bars indicate standard deviation for an average of 3–6 replicates performed by 2 independent users.*

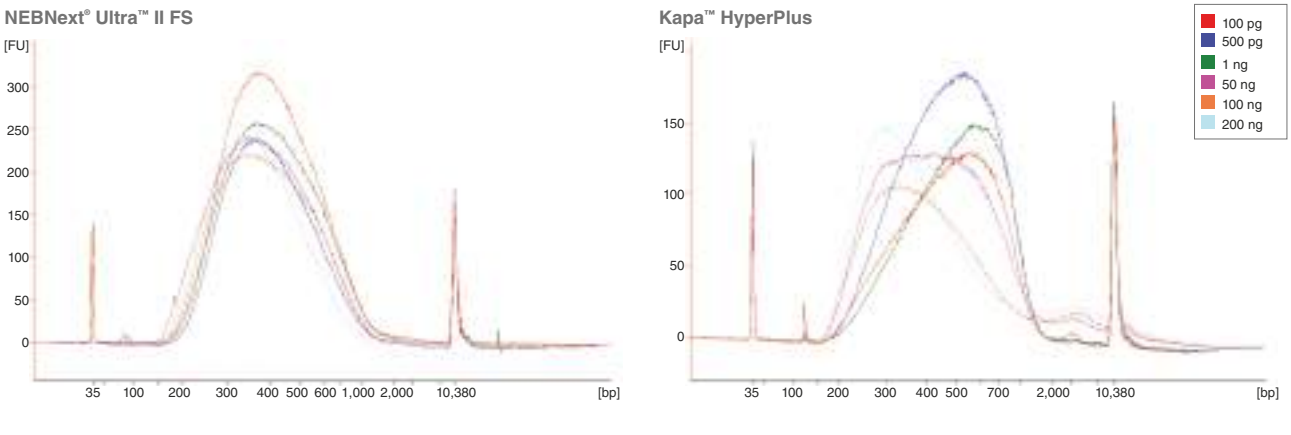



- Perform fragmentation, end repair and dA-tailing with a **single enzyme mix**
- Experience **reliable fragmentation with a single protocol**, regardless of DNA input amount or GC content
- Prepare high quality libraries from a **wide range of input amounts:** 100 pg–0.5 µg
- Prepare libraries **without an amplification step** with inputs from 50 ng–500 ng
- Generate high yields with increased reaction efficiencies and minimized sample loss
- Use with input DNA in standard buffers (TE, Tris-HCl) and water
- Save time with a **streamlined workflow:** ~ 2.5 hours, with < 15 minutes hands-on time
- Vary incubation time to generate a **wide range of insert sizes**
- Available with optional SPRIselect® beads for **gold standard size selection** and clean-up

### TOOLS & RESOURCES

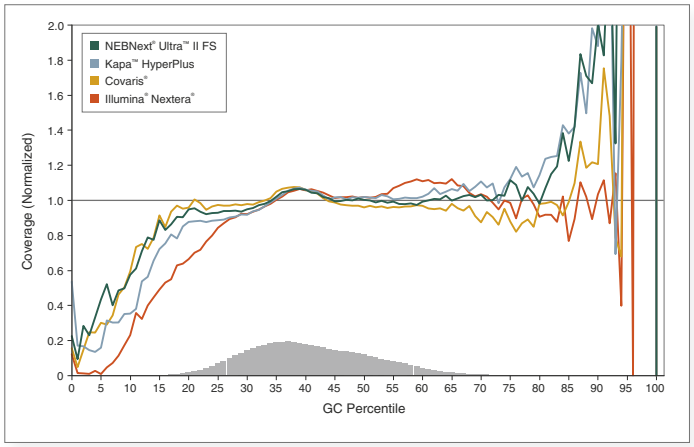
View and download performance data generated by NEBNext Ultra II FS DNA users at **NEBNextUltraII.com**

## Consistent and reliable library preparation with NEBNext Ultra II FS DNA.




*Libraries were prepared from Human NA19240 genomic DNA using the input amounts shown. NEBNext Ultra II FS libraries were prepared using a 20-minute fragmentation time. For Kapa HyperPlus, input DNA was cleaned up with 3X beads prior to library construction, as recommended, and a 20-minute fragmentation time. Library size was assessed using the Agilent® Bioanalyzer®. Low input (1 ng and below) libraries were loaded on the Bioanalyzer without a dilution. High input libraries were loaded with a 1:5 dilution in 0.1X TE.*

## NEBNext Ultra II FS DNA provides superior GC coverage



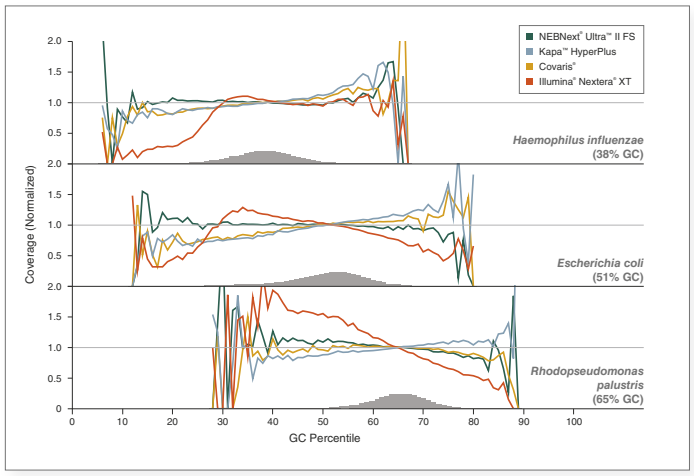

*Libraries were prepared from 50 ng Human NA19240 genomic DNA using the library prep kits shown and 5 PCR cycles. For NEBNext Ultra II FS, a 20-minute fragmentation time was used. For Kapa HyperPlus libraries, input DNA was cleaned up with 3X beads prior to library construction, as recommended, and a 20-minute fragmentation time was used. "Covaris" libraries were prepared by shearing input DNA in 1X TE Buffer to an insert size of ~200 bp using a Covaris instrument, followed by library construction using the NEBNext Ultra II DNA Library Prep Kit (NEB #E7645). Libraries were sequenced on an Illumina MiSeq® (2 x 76 bp). Reads were mapped to the hg19 reference genome using Bowtie 2.2.4 and GC coverage information was calculated using Picard's CollectGCBiasMetrics (v1.117). Expected normalized coverage of 1.0 is indicated by the horizontal grey line, the number of 100 bp regions at each GC% is indicated by the vertical grey bars, and the colored lines represent the normalized coverage for each library.*

## What users are saying:

> *"The Wellcome Sanger Institute currently processes thousands of DNA samples each month via its core DNA sequencing library construction pipelines. However, a recent requirement to generate high quality whole genome and targeted sequencing data from biopsy material led us to develop and implement a novel workflow enabled by NEBNext Ultra II FS. Our new automated workflow, coupled with the high efficiency of the NEBNext Ultra II FS reagent, is allowing us to routinely generate deep sequence data from as few as 100-1,000 human cells."*
>
> – Dr. Peter Ellis,
> Senior Staff Scientist,
> R&D Sequencing,
> Wellcome Sanger Institute

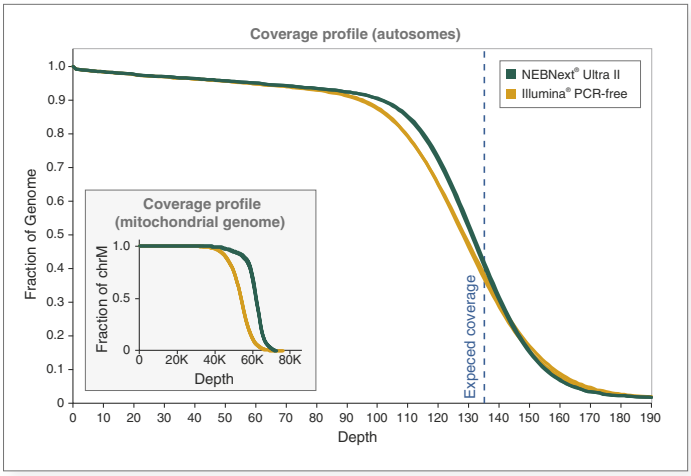
> *"I don't know of any other NGS library prep kit that works so well with small genomes/DNA fragments e.g., bacterial, phage genomes, plasmids, PCR products."*
>
> – Anton Bryksin, Ph.D., Director,
> Molecular Evolution Core, Parker H. Petit Institute for Bioengineering and Bioscience

## NEBNext Ultra II FS DNA provides uniform GC coverage for microbial genomic DNA over a broad range of GC composition.




*Libraries were prepared using 1 ng of a mix of genomic DNA samples from Haemophilus influenzae, Escherichia coli (K-12 MG1655), Rhodopseudomonas palustris and the library prep kits shown, with 9 PCR cycles for consistency across samples, and sequenced on an Illumina MiSeq. NEBNext Ultra II FS libraries were prepared using a 20-minute fragmentation time. For Kapa HyperPlus libraries, input DNA was cleaned up with 3X beads prior to library construction, as recommended, followed by a 25-minute fragmentation time. "Covaris" libraries were prepared by shearing 1 ng of DNA in 1X TE Buffer to an insert size of ~200 bp using a Covaris instrument, followed by library construction using the NEBNext Ultra II DNA Library Prep Kit (NEB #E7645). Reads were mapped using Bowtie 2.2.4 and GC coverage information was calculated using Picard's CollectGCBiasMetrics (v1.117). Expected normalized coverage of 1.0 is indicated by the horizontal grey line, the number of 100 bp regions at each GC% is indicated by the vertical grey bars, and the colored lines represent the normalized coverage for each library.*

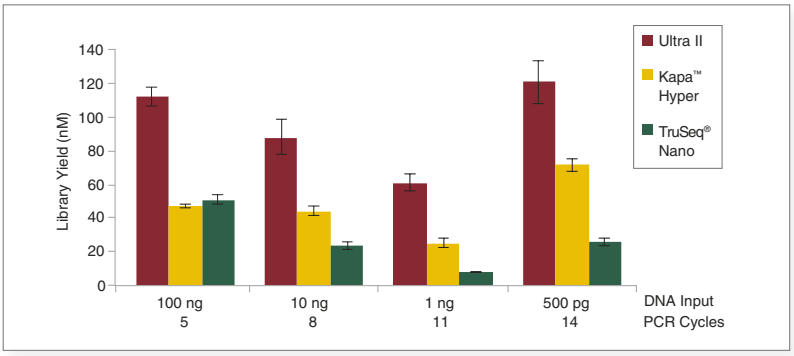
## NEBNext Ultra II FS DNA PCR-free Library Prep provides uniform genome coverage with human DNA.




*NEBNext Ultra II FS DNA PCR-free libraries have more even genome coverage than Illumina DNA PCR-free. 2.8B reads were randomly sampled from each bam (sambamba view -s 0.6.8). Coverage of primary, pass-filter alignments was assessed using mosdepth (v0.3.1, -F 772) and plotted for autosomes or the mitochondrial genome (inset). Expected coverage (num_reads*read length/ genome size) is indicated.*

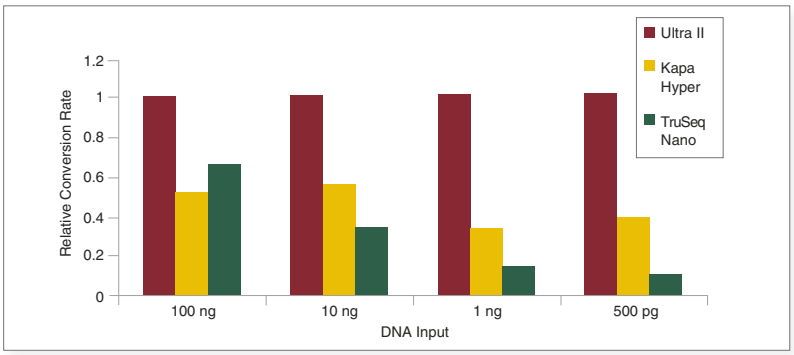
# NEBNext Ultra II DNA Library Prep Kit– *for pre-sheared DNA*

## The NEBNext Ultra II DNA Library Prep Kit produces high yield libraries from a broad range of input amounts, using pre-sheared input DNA.




*Libraries were prepared from Human NA19240 genomic DNA using the input amounts and numbers of PCR cycles shown. Manufacturers' recommended protocols were followed, with the exception that size selection was omitted.*

## NEBNext Ultra II DNA produces high rates of conversion to adaptor-ligated molecules from a broad range of input amounts.



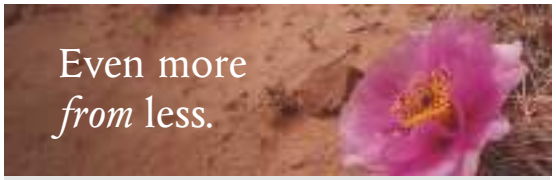

*Libraries were prepared from Human NA19240 genomic DNA using the input amounts and library prep kits shown without an amplification step, and following manufacturers' recommendations. qPCR was used to quantitate adaptor-ligated molecules, and quantitation values were then normalized to the conversion rate for Ultra II. The Ultra II kit produces the highest rate of conversion to adaptor-ligated molecules, for a broad range of input amounts.*

## Ultra II DNA libraries provide high quality sequencing data.

| DNA INPUT | LIBRARY KIT | TOTAL READS | % MAPPED | % DUPLICATION | % CHIMERAS |
|---|---|---|---|---|---|
| 100 ng | Ultra II | 419,093,838 | 96 | 1.87 | 0.48 |
| | Kapa Hyper | 419,097,926 | 96 | 2.00 | 0.60 |
| | TruSeq Nano | 419,086,546 | 97 | 1.91 | 0.53 |
| 1 ng | Ultra II | 226,860,968 | 96 | 3.96 | 0.44 |
| | Kapa Hyper | 226,857,578 | 96 | 11.40 | 0.53 |
| | TruSeq Nano | 226,857,754 | 97 | 34.80 | 0.41 |

*Libraries were prepared from Human NA19240 genomic DNA using the input amounts and library prep kits shown, following manufacturers' recommendations. Libraries were sequenced on the Illumina NextSeq® 500. Reads were mapped to the GRCh37 reference using Bowtie 2.2.4. This data illustrates that the NEBNext Ultra II DNA Library Prep Kit enables high quality sequence data, even with very low input amounts.*

***% Mapped:*** *The percentage of reads mapped to Human GRCh37 reference.*
***% Duplication:*** *The percentage of mapped sequence that is marked as duplicate.*
***% Chimeras:*** *The percentage of reads that map outside of a maximum insert size or that have the two ends mapping to different chromosomes.*




- Get more of what you need, with **high library yields**
- Use to generate high quality libraries with **inputs as low as 500 pg and as high as 1 µg**
- Improve library complexity with **fewer PCR cycles**
- Prepare libraries from ALL of your samples, including **GC-rich** and **FFPE** samples
- Prepare libraries **without an amplification step** with inputs from 250 ng–1,000 ng
- Improve library complexity with **fewer PCR cycles**
- Save time with **streamlined workflows**, reduced hands-on time, and automation compatibility
- Enjoy the flexibility and reliability of the gold standard **SPRIselect size selection and clean-up beads**, supplied in just the amounts you need

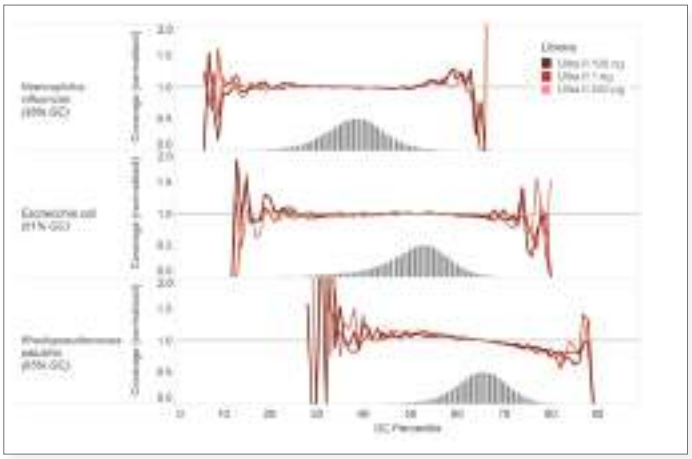
### TOOLS & RESOURCES

View additional performance data in our Technical Notes, which can be downloaded at **NEBNextUltraII.com**

View the NEBNext Ultra II DNA protocol video for protocol steps, and tips for optimization

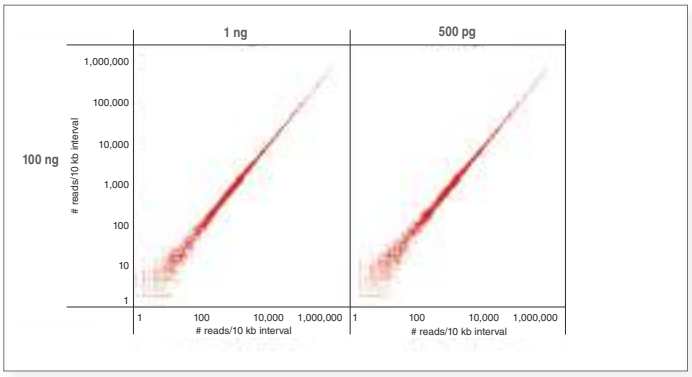
Over 1,000 peer-reviewed publications have been published citing use of **NEBNext Ultra II DNA**

## NEBNext Ultra II provides uniform GC coverage for microbial genomic DNA over a broad range of GC composition and input amounts.



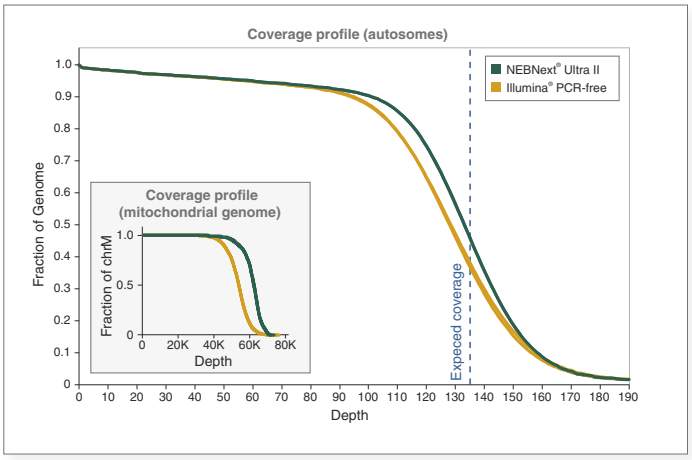
*Libraries were made using 500 pg, 1 ng and 100 ng of the genomic DNAs shown and the Ultra II DNA Library Prep Kit and sequenced on an Illumina MiSeq. Reads were mapped using Bowtie 2.2.4 and GC coverage information was calculated using Picard's CollectGCBiasMetrics (v1.117). Expected normalized coverage of 1.0 is indicated by the horizontal grey line, the number of 100 bp regions at each GC% is indicated by the vertical grey bars, and the colored lines represent the normalized coverage for each library.*

## Read depth correlation shows consistently high coverage for 500 pg–100 ng input amounts.




*Libraries were prepared with 100 ng, 1 ng and 500 pg of human NA19240 genomic DNA and sequenced on the Illumina NextSeq 500. Each library was downsampled (sambamba view -s) to include 423 M reads and mapped to GRCh37 using Bowtie 2.2.4. Coverage of each 10 kb region of GRCh37 (as determined by bedtools coverage) was compared between low (500 pg and 1 µg) and 100 ng input. Most regions are covered by ~1,000 reads, as expected. Low and high coverage regions are well correlated.*

## NEBNext Ultra II DNA PCR-free Library Prep provides uniform genome coverage with human DNA

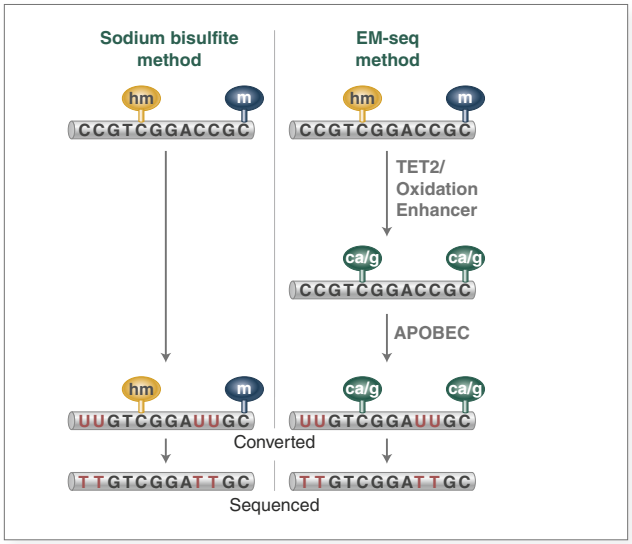



*NEBNext Ultra II DNA PCR-free libraries have more even genome coverage than do Illumina DNA PCR-free libraries. 2.8B reads were randomly sampled from each bam (sambamba view -s 0.6.8). Coverage of primary, pass-filter alignments was assessed using mosdepth (v0.3.1, -F 772) and plotted for autosomes or the mitochondrial genome (inset). Expected coverage (num_reads*read length/ genome size) is indicated.*

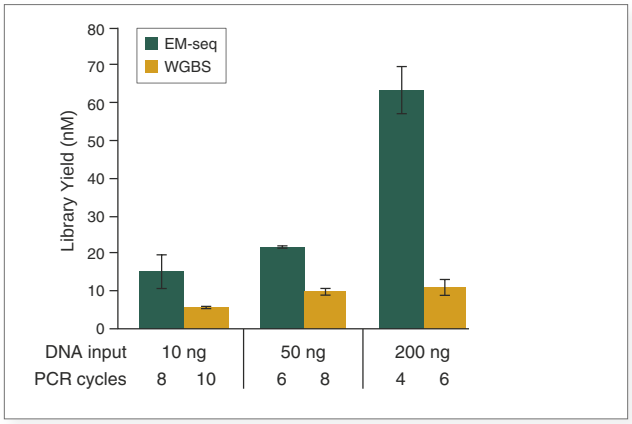
# NEBNext Enzymatic Methyl-seq (EM-seq™) – *a new method for identification of 5mC and 5hmC*

While whole genome bisulfite sequencing (WGBS) has been the gold standard for methylome analysis, it also damages DNA, resulting in fragmentation, loss and bias. In contrast, EM-seq enzymatic conversion minimizes damage to DNA and, in combination with the supplied NEBNext Ultra II Illumina library preparation reagents, produces high quality libraries that enable superior detection of 5mC and 5hmC from fewer sequencing reads.

## EM-seq and sodium bisulfite conversion methods




## EM-seq produces higher yields than WGBS using fewer PCR cycles




*10, 50 and 200 ng Human NA12878 genomic DNA was sheared to 300 bp using the Covaris S2 instrument and used as input into EM-seq and WGBS protocols. For WGBS, NEBNext Ultra II DNA was used for library construction, followed by the Zymo Research EZ DNA Methylation-Gold™ Kit for bisulfite conversion. For all input amounts, EM-seq library yields were higher, and fewer PCR cycles were required, suggesting greater DNA loss in the WGBS protocol. Error bars indicate standard deviation.*

## Heads up!

There's a new alternative to bisulfite sequencing

- Superior sensitivity of detection of 5mC and 5hmC
- Greater mapping efficiency
- More uniform GC coverage
- Detect more CpGs with fewer sequence reads
- Uniform dinucleotide distribution
- Larger library insert sizes
- High-efficiency library preparation
- Conversion module also available separately

### TOOLS & RESOURCES

View additional performance data in our Technical Notes, which can be downloaded at **NEBNext.com**

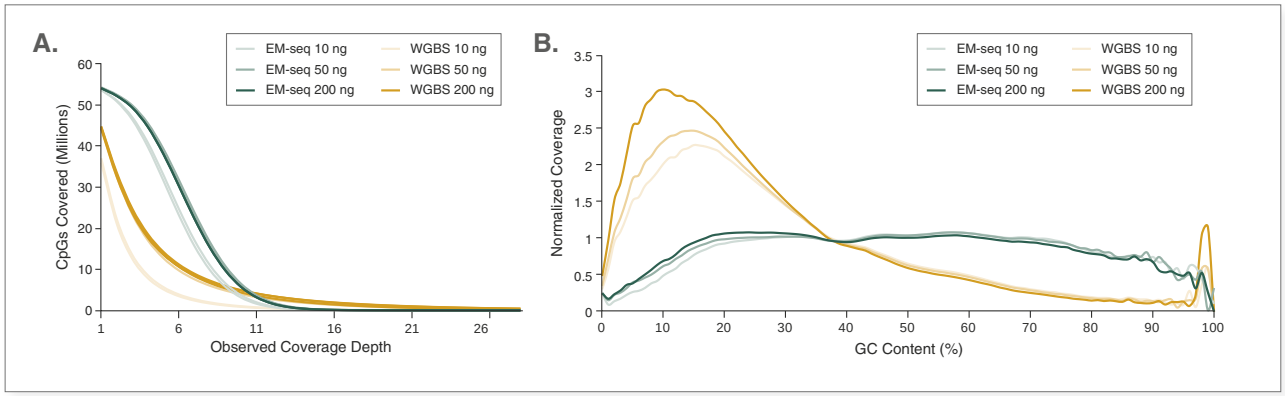
View the NEBNext EM-seq workflow animation and NEB TV episode that discusses challenges with methylome analysis.

## 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

> *"Enzymatic conversion of EM-Seq is THE alternative and our comparisons clearly showed, that the quality of data obtained is better than with conventional bisulfite conversion. We observed excellent conversion efficiency and extraordinary mapping rates."*
>
> – Alexander Vogt,
> Sequencing Specialist,
> Vienna BioCenter Core Facilities

## EM-seq identifies more CpGs than WGBS, at lower sequencing coverage depth with superior uniformity of GC coverage.

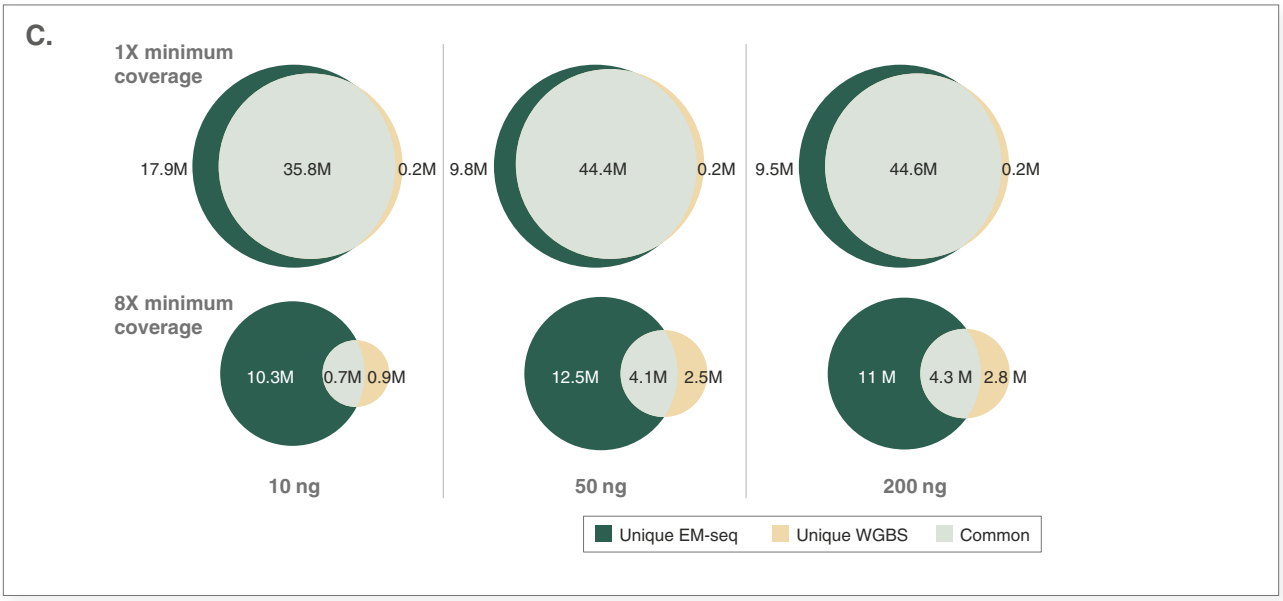



*10, 50 and 200 ng Human NA12878 genomic DNA was sheared to 300 bp using the Covaris S2 instrument and used as input into EM-seq and WGBS protocols. For WGBS, NEBNext Ultra II DNA was used for library construction, followed by the Zymo Research EZ DNA Methylation-Gold Kit for bisulfite conversion. Libraries were sequenced on an Illumina NovaSeq™ 6000 (2 x 100 bases). Reads were aligned to hg38 using bwa-meth 0.2.2.*

*A: Coverage of CpGs with EM-seq and WGBS libraries was analyzed using 324 million paired end reads, and each top and bottom strand CpGs were counted independently, yielding a maximum of 56 million possible CpG sites. EM-seq identifies more CpGs at lower depth of sequencing.*

*B: GC coverage was analyzed using Picard 2.17.2 and the distribution of normalized coverage across different GC contents of the genome (0-100%) was plotted. EM-seq libraries have significantly more uniform GC coverage, and lack the AT over-representation and GC under-representation typical of WGBS libraries.*

## EM-seq identifies more CpGs than WGBs, at lower sequencing coverage depth




*C. Coverage of CpGs with EM-seq and WGBS libraries was analyzed using 324 million paired end reads. The number of unique and common CpGs identified by EM-seq and WGBS at 1X and 8X minimum coverage for each input amount are shown. EM-seq covers at least 20% more CpGs than WGBS at 1X minimum coverage threshold. The difference in CpG coverage increases to two-fold at 8X minimum coverage threshold.*

> *"We were very excited by an opportunity to use the new EM-seq system launched now by NEB. In addition to its attractive features, such as user-friendliness and cleanliness of the process, for example, we have realized that it enables us to determine in precise and DNA sparing way the cytosine methylation status even at low integrity DNA. If bisulfite conversion were the only approach to apply, we would definitely fail to generate relevant results. The cool, biochemical approach to analyse cytosine methylation the system is utilizing, it also opens new avenues to explorations of methylation at intact long DNA fragments."*
>
> – Vladimir Benes,
> Head Genomics Core Facility
> at EMBL Heidelberg

# NEBNext Adaptors and Primers

Adaptors and Primers are an essential component of your NGS sample prep workflow, and NEBNext Multiplex Oligos offer flexibility in multiplexing; indexing options include unique dual indices (UDIs) with unique molecular identifiers (UMIs), unique dual indices (UDIs), combinatorial dual (CD) indices, and single indices in a range of formats and indexing strategies. For an overview of our Multiplex Oligos products, refer to the NEBNext Multiplex Oligos Selection Chart below.

## NEBNext Multiplex Oligos Selection Chart

| |  SINGLE INDEX |  DUAL INDEX |  UNIQUE DUAL INDEX |  UNIQUE DUAL INDEX UMIs |
|---|---|---|---|---|
| NEB PRODUCTS | NEB #E7335<br>NEB #E7500<br>NEB #E7710<br>NEB #E7730<br>NEB #E6609 | NEB #E7600<br>NEB #E7780 | NEB #E6440<br>NEB #E6442<br>NEB #E6444<br>NEB #E6446<br>NEB #E6448<br>NEB #E7140 | NEB #E7395 |
| Contains UMI | No | No | No | Yes |
| Addresses Index Hopping | No | No | Yes | Yes |
| Indexing Strategy | Index Primer | Index Primer | Index Primer | Index Adaptor |
| Applications | DNA-seq, RNA-seq (except small RNA) | DNA-seq, RNA-seq (except small RNA) | DNA-seq, RNA-seq (except small RNA) | PCR-free DNA-seq, RNA-seq (except small RNA) |
| Number of Indices for Multiplexing | up to 144 | up to 384 | up to 384 | up to 96 |
| Compatible with ARTIC sequencing for Illumina® | Yes | Yes | Yes | No |
| Compatible with EM-seq™ | Yes* | Yes* | Yes* | No |
| Compatible with EpiMark® Bisulfite Sequencing | Yes** | Yes** | Yes** | No |
| Number of Sets Available; Formats and Indices Available | Five;<br>Sets 1-4 (12 indices/set): Individual vials<br>96 Index: premixed plate | Two;<br>Individual vials containing 8 i5 primers and 12 i7 primers for combinitorial mixing | Four;<br>96 indices in premixed, foil-sealed 96-well plates, including a version for EM-seq (up to 120 indices, either 96-well plate or 24 vial format) | One;<br>96 indices in premixed, foil-sealed 96-well plate (DNA-seq OR RNA-seq) and primers |

** Requires the use of the EM-seq Adaptor; Single, dual and unique dual index are all compatible; NEB recommends using the Unique Dual Index Primers found in the NEBNext Enzymatic Methyl-seq Kit (NEB #E7120) or the NEBNext Multiplex Oligos for EM-seq (NEB #E7140), both supplied with the NEBNext EM-seq Adaptor; For higher levels of multiplexing, Unique Dual Index Primers Sets 3 and 4 (NEB #E6444 and #E6446) are also validated for EM-seq.*

*** Requires use of NEBNext EM-seq adaptor from NEBNext Multiplex Oligos for Enzymatic Methyl-seq (Unique Dual Index Primer Pairs, #E7140S/L or NEBNext methylated adaptor from NEBNext Multiplex Oligos for Illumina® (Methylated Adaptor, Index Primers Set 1, #E7535S/L).*

| PRODUCT | # INDICES | SIZE |
|---|---|---|
| NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors DNA Set 1) (NEB #E7395S/L) | 96 unique pairs | 96/384 rxns |
| NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs or 96 Unique Dual Index Primer Pairs Set 1, 2, 3, 4, 5) (NEB #E6440S/L, #E6442S/L, #E6444S/L, #E6446S/L, #E6448S/L) | 96 unique pairs | 96/384 rxns |
| NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1 or 2) (NEB #E7600S, #E7780S) | 8 x 12 | 96 rxns |
| NEBNext Multiplex Oligos for Illumina (96 Index Primers) (NEB #E6609S/L) | 96 | 96/384 rxns |
| NEBNext Multiplex Oligos for Illumina (Index Primers Set 1, 2, 3 or 4) (NEB #E7335S/L, #E7500S/L, #E7710S/L, #E7730S/L) | 12 | 24/96 rxns |
| NEBNext Multiplex Oligos for Enzymatic Methyl-seq (Unique Dual Index Primer Pairs) (#E7140S/L) | 96 | 24/96 rxns |
| NEBNext Multiplex Oligos for Illumina (Methylated Adaptor, Index Primers Set 1) (NEB #E7535S/L) | 12 | 24/96 rxns |
| NEBNext Adaptor Dilution Buffer (NEB #B1430) | | 1 x 9.6 ml |

### ADVANTAGES

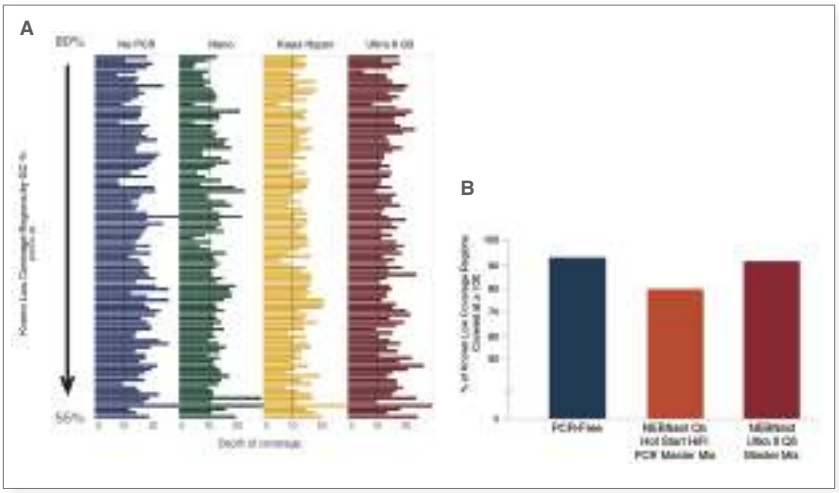
- Indexing strategies optimized by application
- Index Primers–For NGS Library Prep workflows that include an amplification step
- Index Adaptors–Enablement of PCR-free workflows and incorporation of UMIs for error correction/deduplication
- Extensively QC'd for purity and increased library yields
- Flexibility for use with NEBNext library preparation kits and other standard, Illumina-compatible library preparation methods
- Convenient formats (e.g., vials and single-use 96-well plates with pierceable foil seal)
- Provided with index-pooling guidelines and sample sheets

# High Yields and Minimized GC Bias with the NEBNext Ultra II Formulation of Q5® High-Fidelity DNA Polymerase

To ensure that sequence data reflects exactly the sequence of the original sample, it is essential that amplification of libraries be performed uniformly and with high fidelity. Historically, high-fidelity polymerases have been more susceptible to difficulties in PCR amplification of GC- rich and other challenging regions. If such bias occurs in library amplification, this can lead to uneven sequence coverage, challenges in sequence assembly and even "missing" sequence.

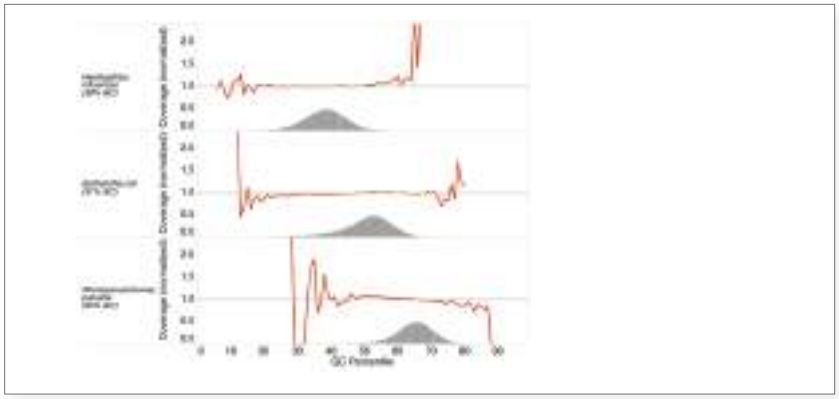
The NEBNext Ultra II Q5 Master Mix (NEB #M0544) is the latest formulation of Q5 DNA polymerase that has been optimized for robust, high-fidelity amplification of next-generation sequencing (NGS) libraries. This formulation further improves the uniformity of amplification of libraries, including superior performance with GC-rich regions.

## NEBNext Ultra II Q5 Master Mix provides improved coverage of known low coverage regions of the human genome




*Libraries were prepared from Human NA19240 genomic DNA. One library was not amplified. The other two libraries were amplified using 5 cycles of PCR with NEBNext Q5 Hot Start HiFi PCR Master Mix (NEB #M0543) or with NEBNext Ultra II Q5 Master Mix (NEB #M0544). Libraries were sequenced on an Illumina NextSeq 500. 420 million 75 bp reads were randomly extracted from each dataset, representing an average coverage of 10X. Reads were mapped to the hg19 reference genome using Bowtie 2.2.4. Reads on each region were counted using bedtools v2.19.1.* ***A:*** *The number of reads overlapping distinct low coverage regions of the human genome (1) are shown for each library.* ***B:*** *From the 420 million 75 bp reads randomly extracted from each dataset, 10X coverage was expected. The % of difficult regions covered at ≥ 10X are shown for each library. The NEBNext Ultra II Q5 Master Mix provides improved coverage of these known low coverage regions, without drop-outs, and shows similar coverage to the unamplified sample.*

## NEBNext Ultra II Q5 Master Mix provides uniform GC coverage with a broad range of GC composition



*Libraries were made using 100 ng of the genomic DNAs shown and the NEBNext Ultra II DNA Library Prep Kit. Libraries were amplified using the NEBNext Ultra II Q5 Master Mix, and sequenced on an Illumina MiSeq. GC coverage information was calculated using Picard's CollectGCBiasMetrics (v1.117). Expected normalized coverage of 1.0 is indicated by the horizontal grey line, the number of 100 bp regions at each GC% is indicated by the vertical grey bars, and the colored lines represent the normalized coverage for each library. NEBNext Ultra II Q5 Master Mix provides uniform GC coverage regardless of the GC content of the DNA.*

### ADVANTAGES

- Optimized for high yields in NGS library amplification
- Minimizes GC bias, with superior performance across the GC spectrum
- Ultra-high-fidelity amplification with Q5, the highest-fidelity polymerase (2)
- Aptamer-based hot start without a separate activation step, for room-temperature reaction set-up

| PRODUCT | SIZE |
|---|---|
| NEBNext Ultra II Q5 Master Mix (NEB #M0544S/L/X) | 50/250/500 rxns |

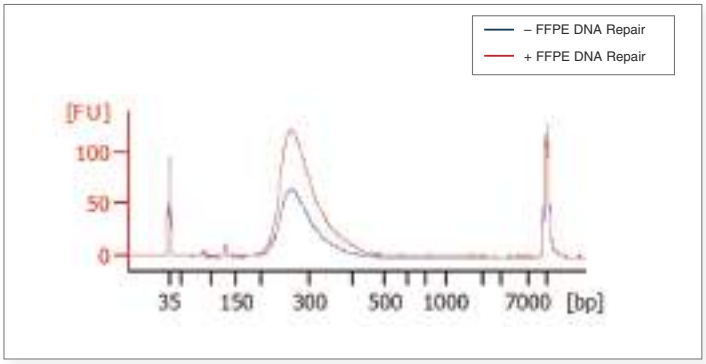
*Reference:*

1. *Aird, D. et al. (2011). Analyzing and minimizing PCR amplification bias in Illumina sequencing libraries. Genome Biology 12(2), R18.*
2. *Popatov, V. and Ong, J.L. (2017). Examining Sources of Error in PCR by Single-Molecule Sequencing. PLoS ONE. 12(1):e0169774.*

# NEBNext FFPE DNA Repair Mix

Archiving of clinical materials as Formalin-Fixed, Paraffin-Embedded (FFPE) samples is a common practice. However, the methods used for fixation and storage significantly damage and compromise the quality of nucleic acids from these samples. As a result, it can be challenging to obtain useful information, including high-quality sequence data, especially when sample amounts are limited. The NEBNext FFPE DNA Repair Mix is a cocktail of enzymes formulated to repair DNA, and specifically optimized and validated for repair of FFPE DNA samples. Incorporation of the FFPE DNA Repair Mix into Next Generation Sequencing (NGS) workflows can increase yields and overall library success rates, while also improving sequence quality (1).

## Effect of FFPE DNA Repair Mix on library yields

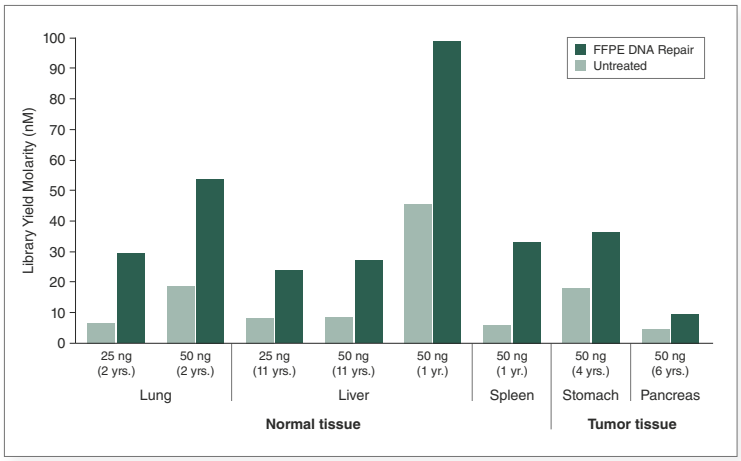



*An example of Agilent Bioanalyzer traces of libraries prepared from stomach tumor FFPE DNA that was treated with the FFPE DNA Repair Mix, or was untreated, before library construction. Yield improvements of 101% to 458% have been observed.*

### ADVANTAGES

- Increase library yield
- Increase library quality
- Use before library prep for any NGS platform
- No alteration of DNA sequence
- Rely on NEB's NGS validation process for FFPE DNA library prep

## FFPE DNA repair increases NGS library yields




*FFPE DNA samples (25 ng or 50 ng) from a variety of tissue types were treated with the NEBNext FFPE DNA Repair Mix, or were untreated, before library construction. Library yields were measured using the Agilent Bioanalyzer, and ratios of library yields with and without treatment with the NEBNext FFPE DNA Repair Mix were calculated. Yield improvements with these samples ranged from 101% to 458%.*

## Types of FFPE DNA damage and their ability to be repaired by the NEBNext FFPE DNA Repair Mix

| FFPE DAMAGE TYPE | REPAIRED BY THE FFPE DNA ENZYME REPAIR MIX? |
|---|---|
| Deamination of cytosine to uracil | Yes |
| Nicks and gaps | Yes |
| Oxidized bases | Yes |
| Blocked 3´ ends | Yes |
| DNA fragmentation | No |
| DNA-protein crosslinks | No |

| PRODUCT | SIZE |
|---|---|
| NEBNext FFPE DNA Repair Mix (NEB #M6630S/L) | 24/96 rxns |

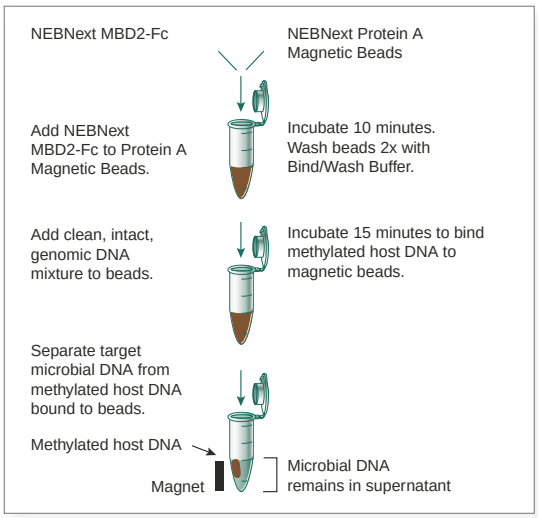
## NEBNext FFPE DNA Repair Mix v2 *Coming Soon.*

*Reference*
*1. Chen. L. et al. (2017) Science 355, 752-756.*

# NEBNext Microbiome DNA Enrichment Kit

Microbiome DNA analysis can be challenging due to the high percentage of host DNA present in many samples. The NEBNext Microbiome DNA Enrichment Kit facilitates enrichment of microbial DNA from samples containing methylated host DNA (including human), by selective binding and removal of the CpG-methylated host DNA. Importantly, microbial diversity remains intact after enrichment (1). If desired, the host DNA captured on the magnetic bead pellet can be eluted, and a protocol is provided for this.

## Microbiome DNA Enrichment Kit workflow

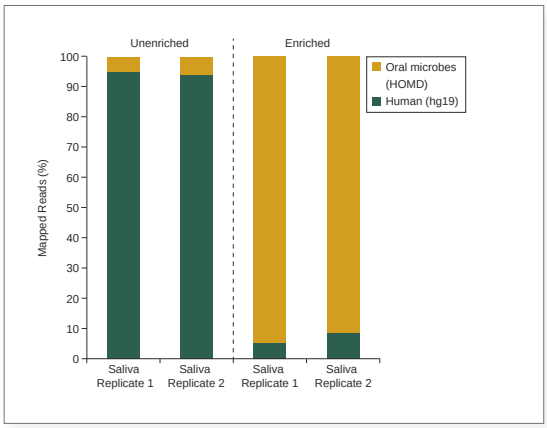



*The MBD2-Fc protein binds specifically to CpG methylated DNA. In the NEBNext Microbiome DNA Enrichment workflow, MBD2-Fc is attached to Protein A magnetic beads, enabling capture of methylated DNA, while the microbial DNA remains in the supernatant.*

## ADVANTAGES

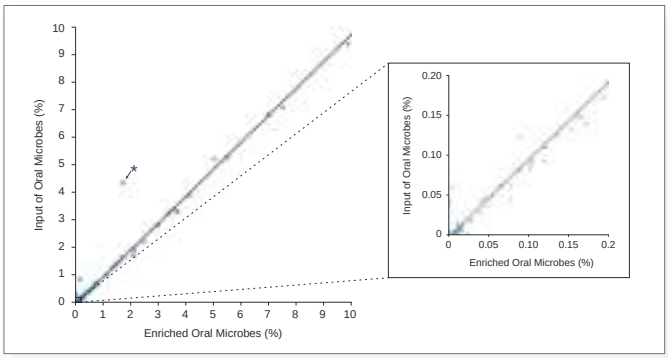
- Effective separation of microbial DNA from host DNA
- Fast, simple protocol
- Enables microbiome whole genome sequencing, even for samples with high levels of host DNA
- Compatible with downstream applications including next generation sequencing on all platforms, qPCR and end-point PCR
- Suitable for a wide range of sample types
- No requirement for live cells
- Optional protocol to retain separated host DNA
- Also effective for separation of organelle DNA (e.g. mitochondria, chloroplast) from eukaryote nuclear DNA (2)

## Salivary Microbiome DNA Enrichment




*DNA was purified from pooled human saliva DNA (Innovative Research) and enriched using the NEBNext Microbiome DNA Enrichment Kit. Libraries were prepared from unenriched and enriched samples and sequenced on the SOLiD 4 platform. The graph shows percentages of 500 M-537 M SOLiD 4 50 bp reads that mapped to either the Human reference sequence (hg19) or to a microbe listed in Human Oral Microbiome Database (HOMD)[3]. (Because the HOMD collection is not comprehensive, ~80% of reads in the enriched samples do not map to either database.) Reads were mapped using Bowtie 0.12.7[4] with typical settings (2 mismatches in a 28 bp seed region, etc.).*

## Microbiome diversity is retained after enrichment with the NEBNext Microbiome DNA Enrichment Kit




*DNA was purified from pooled human saliva DNA (Innovative Research) and enriched using the NEBNext Microbiome DNA Enrichment Kit. Libraries were prepared from unenriched and enriched samples, followed by sequencing on the SOLiD 4 platform. The graph shows a comparison between relative abundance of each bacterial species listed in HOMD[3] before and after enrichment with the NEBNext Microbiome DNA Enrichment Kit. High concordance continues even to very low abundance species (inset). We compared 501 M 50 bp SOLiD 4 reads in the enriched dataset to 537 M 50 bp SOLiD 4 reads in the unenriched dataset. Reads were mapped using Bowtie 0.12.7[4] with typical settings (2 mismatches in a 28 bp seed region, etc).*

** Neisseria flavescens – This organism may have unusual methylation density, allowing it to bind the enriching beads at a low level. Other Neisseria species (N. mucosa, N. sicca and N. elognata) are represented, but do not exhibit this anomalous enrichment.*

| PRODUCT | SIZE |
|---|---|
| NEBNext Microbiome DNA Enrichment Kit (NEB #E2612S/L) | 6/24 rxns |

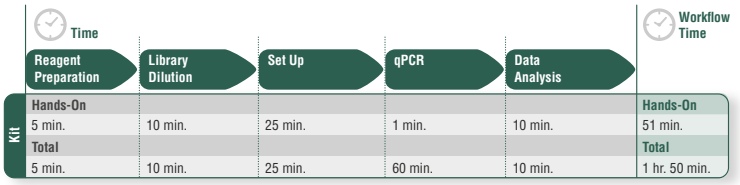
*References*
*1. Feehery, G. R. et al. (2013). PLoS One 8, e76096.*
*2. Yigit, E. et al. (2014). Applications in Plant Sciences 2014 2 (11): 1400064*
*3. Chen, T., et al. (2010) Database, Vol. 2010, Article ID baq013, doi: 10.1093/database/baq013*
*4. Langmead B., et al. (2009) Genome Biol. 10:R25 doi:10.1186/gb-2009-10-3-r25*

Updated

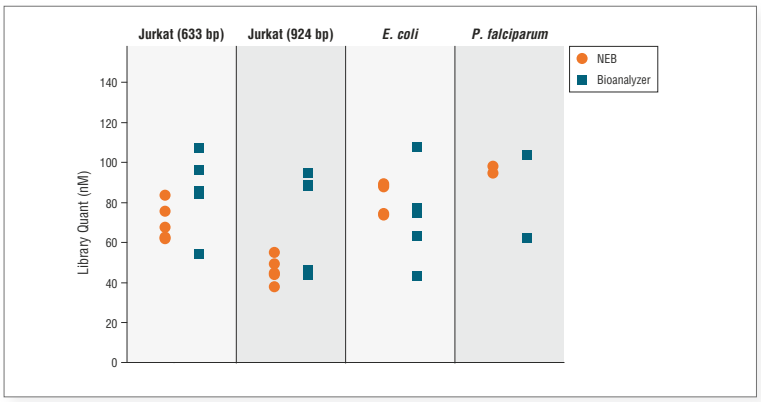
# NEBNext Library Quant Kit for Illumina

Accurate quantitation of next-generation sequencing libraries is essential for maximizing data output and quality from each sequencing run. For Illumina sequencing specifically, accurate quantitation of libraries is critical to achieve optimal cluster densities, a requirement for optimal sequence output. qPCR is considered to be the most accurate and effective method of library quantitation, providing considerably higher consistency and reproducibility of quantitation. qPCR-based methods quantitate only those molecules that contain both adaptor sequences, thereby providing a more accurate estimate of the concentration of the library molecules that can be sequenced. The NEBNext Library Quant Kit delivers significant improvements to qPCR-based library quantitation for next-generation sequencing.

## NEBNext Library Quant Kit for Illumina workflow

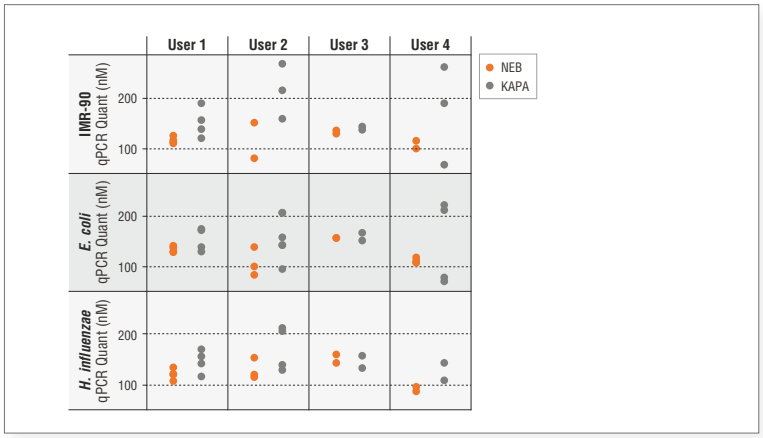



## Comparison of quantitation by qPCR and electrophoretic methods

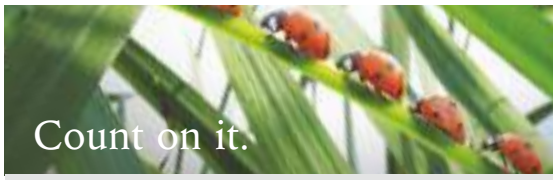



*Concentrations of 4 libraries were determined by the NEBNext Library Quant Kit (orange) and compared to values measured using the Aglient Bioanalyzer (blue). Compared to NEBNext's qPCR-based method, the Bioanalyzer concentrations displayed a greater level of variation.*

## Greater reproducibility of library quantitation with the NEBNext Library Quant Kit




*Three 340–400 bp libraries were quantitated by 4 different users 2–4 times using either the NEBNext or Kapa Library Quantification Kit (Universal). A notable improvement in quantitation consistency was observed for concentrations determined by the NEBNext Kit (orange) versus those from the Kapa kit (gray).*




- Be confident in your quant values, as our kit provides **more accurate and reproducible results** than other methods and kits
- Get up and running quickly with our **easy-to-use kit**, containing Library Dilution Buffer, optimized master mix, 6 standards and ROX dye
- Simplify your reaction setup with **fewer pipetting steps** and a **single extension time** for all libraries
- Enjoy the flexibility to use **4 or 6 standards**
- **Use with all your libraries**, regardless of insert size, GC content and preparation method
- Save money with our value pricing

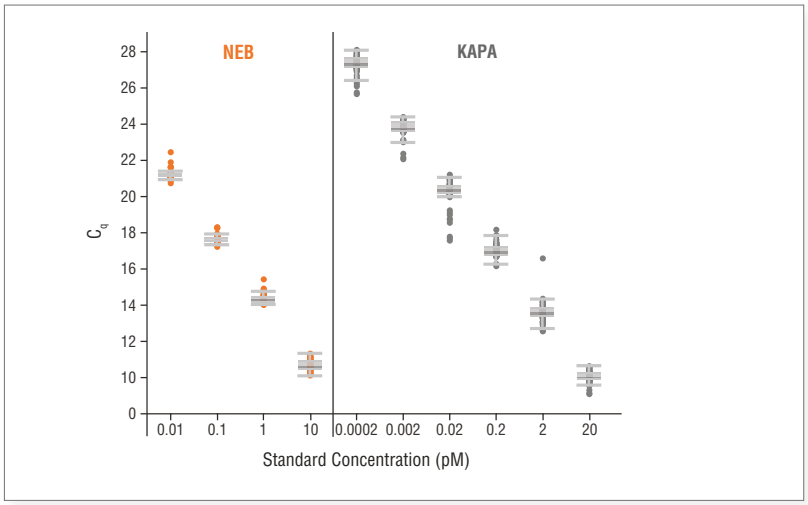
### TOOLS & RESOURCES

Use NEBioCalculator at **NEBioCalculator.neb.com** to calculate your qPCR-based library quant values

Download our application note, "Improved library quantitation for a broad range of library types using the NEBNext Quant Kit for Illumina" at **www.neb.com/E7630**

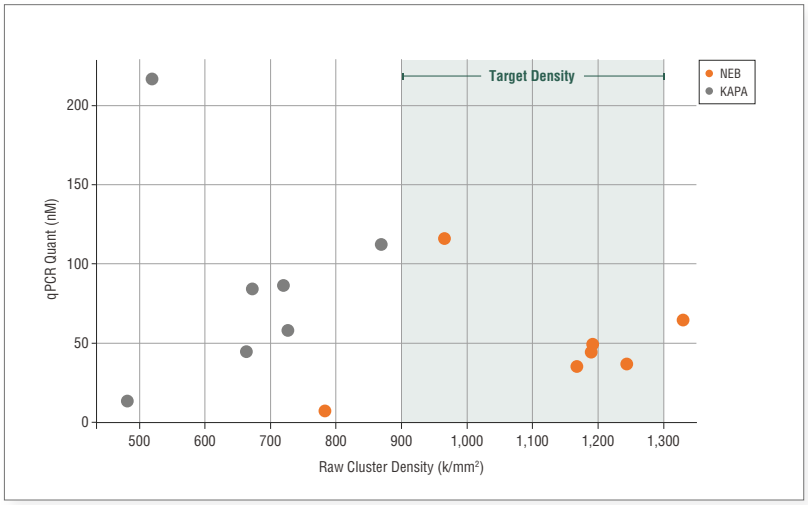
| PRODUCT | SIZE |
|---|---|
| NEBNext Library Quant Kit for Illumina (NEB #E7630S/L) | 100/500 rxns |
| NEBNext Library Dilution Buffer (NEB #B6118S) | 7.5 ml |

## Greater lot-to-lot consistency of standards with the NEBNext Library Quant Kit



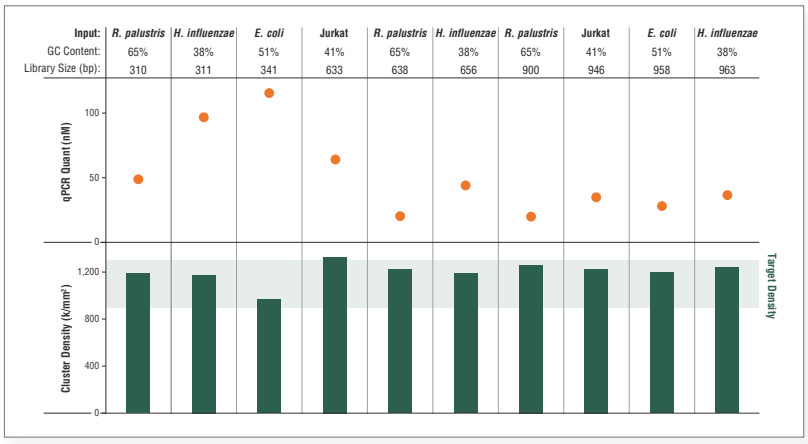

*Accurate qPCR quantitation requires the use of high-quality DNA standards with known concentrations. The NEBNext Library Quant Kit contains 6 standards produced with a high level of both quantitation accuracy and consistency. This figure shows data from > 70 total runs from 4 lots of both NEBNext (orange) and Kapa (gray) standards, with all Cq values plotted. Box and whiskers indicate mean and quartiles. The NEBNext Library Standards displayed much lower variation in Cq, resulting in more consistent quantitation performance.*

## The NEBNext Library Quant Kit values enable optimal cluster densities




*Seven different libraries were quantitated using either the NEBNext Library Quant Kit (orange) or the Kapa Library Quantification Kit (Universal) (gray). Undiluted library concentrations ranged from 2–200 nM. Libraries were diluted to 8 pM and loaded onto a MiSeq instrument (v2 chemistry; MCS v2.4.1.3). Libraries quantitated with the NEBNext kit resulted in a raw cluster density average of 1160 k/mm², directly in the optimal range of 900–1300 k/mm². In contrast, libraries loaded based on the Kapa quantitation averaged only 660 k/mm².*

## With NEBNext, optimal cluster density is achieved from quantitated libraries with a broad range of library size and GC content



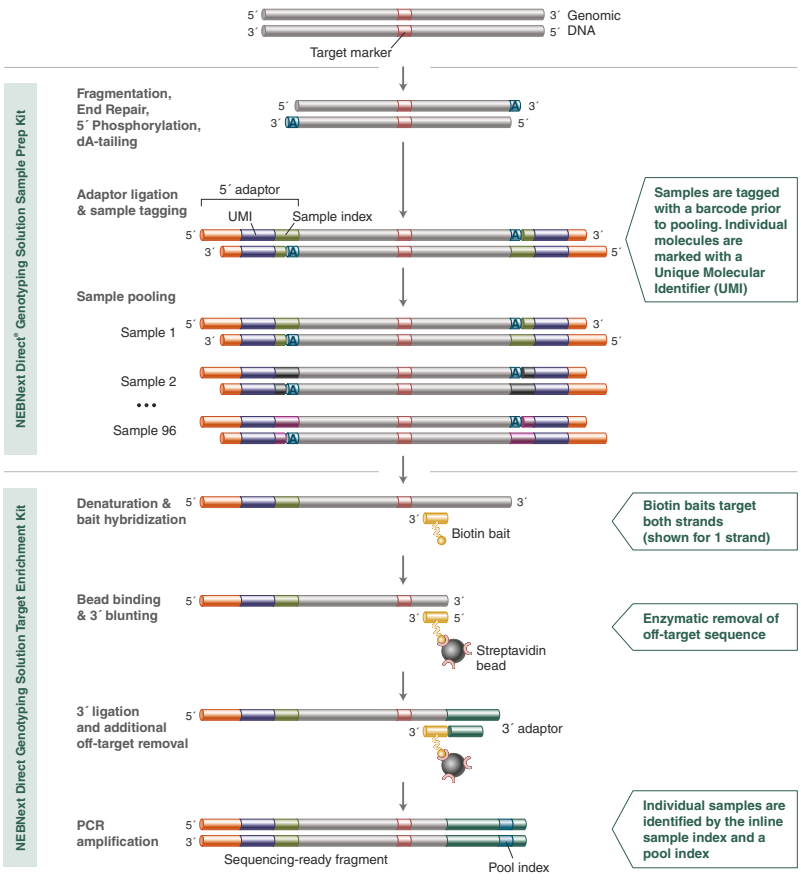

*Libraries of 310–963 bp from the indicated sources were quantitated using the NEBNext Library Quant Kit, then diluted to 8 pM and loaded onto a MiSeq (v2 chemistry; MCS v2.4.1.3). Library concentrations ranged from 7–120 nM, and resulting raw cluster density for all libraries was 965–1300 k/mm² (ave. =1199). Optimal cluster density was achieved using concentrations determined by the NEBNext Library Quant Kit for all library sizes.*

# NEBNext Direct Genotyping Solution

Do you need high-throughput targeted genotyping for Illumina Sequencing? The NEBNext Direct Genotyping Solution combines highly multiplexed, capture-based enrichment with maximum efficiency next generation sequencing to deliver cost-effective, high-throughput genotyping for a wide variety of applications. Applicable for ranges spanning 100-5,000 markers, pre-capture multiplexing of up to 96 samples combined with dual indexed sequencing allows over 3.8 million genotypes in a single Illumina sequencing run.

The NEBNext Direct Genotyping Solution begins with 25-100 ng of purified genomic DNA. The DNA molecules are enzymatically fragmented and 5´ tagged with an Illumina-compatible P5 adaptors, incorporating both an inline sample index to tag each sample prior to pooling and an inline Unique Molecular Identifier (UMI) to mark each unique DNA fragment within the samples. Up to 96 samples are subsequently pooled together prior to hybridization-based enrichment using biotinylated baits and captured on streptavidin beads. For the remainder of the protocol, up to 96 samples are processed as a single pool through ligation of a 3´ adaptor, removal of off-target sequence and final PCR, which amplifies the material and adds a second pool index to produce the final sequencing-ready fragment.

## NEBNext Direct employs a fast hybridization–based workflow that combines capture with library preparation





**Features:**

- Single-day workflow
- 96-plex pre-capture sample multiplexing of hundreds to thousands of markers
- Bait design and sample multiplexing to maximize sequencer efficiency
- High specificity and coverage uniformity

| PLANT | ANIMAL | HUMAN |
|---|---|---|
| Marker assisted selection / breeding | Mouse Genotyping | Biobanking |
| Quantitative Trait Locus (QTL) Screening | Livestock Breeding | NGS Sample Tracking |

| PRODUCT | SIZE |
|---|---|
| NEBNext Direct Genotyping Solution (NEB #E9500B-S) | 96 rxns |
| NEBNext Direct Genotyping Solution (NEB #E9530B-S) | 8 rxns |

For research use only, not intended for diagnostic use.


**DOWNLOAD THE NEB AR APP***

View the NEBNext Direct Workflow.

*see back cover for details

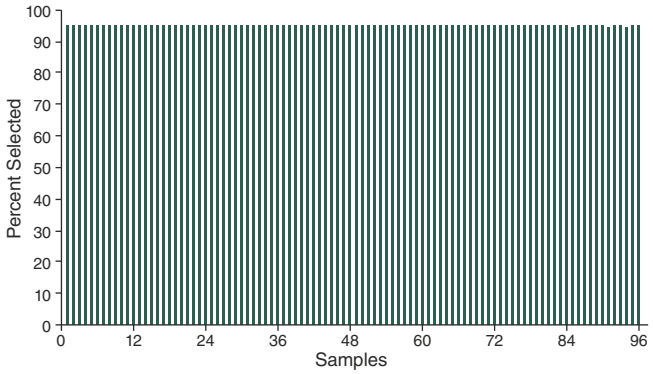
# Performance

The NEBNext Direct Genotyping Solution produces reliable and accurate genotyping calls using fewer sequencing reads. This is achieved through the combination of highly specific and highly consistent enrichment across targeted loci, reducing excess sequencing data due to off-target sequence, while ensuring more markers can be included in analyses.

## Highly specific enrichment across 96 pooled samples

Pre-capture pooling of 96 samples provides additional advantages, both in the significant reduction in pipetting steps, reducing plastic waste and consumables cost, as well as in the benefits from leveraging higher-throughput, lower cost sequencing platforms and configurations.

**Specificity of enrichment of NEBNext Direct Genotyping Solution across 96 pooled samples**

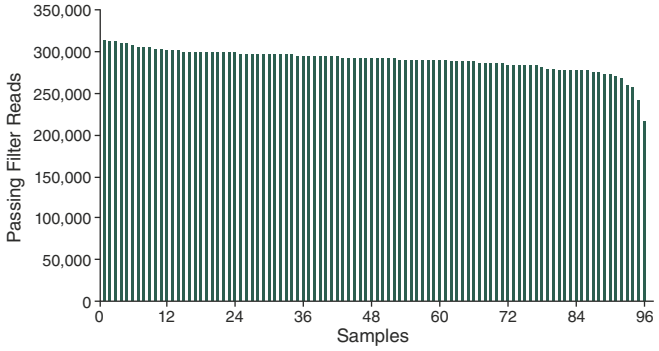



*The percent of passing filter reads mapping to targeted regions demonstrates high specificity across 96 multiplexed samples using the NEBNext Direct Genotyping Solution. 25 ng of purified tomato DNA was used as input for each sample. Samples were index-tagged and pooled prior to hybridization and Libraries were sequenced on an Illumina MiSeq® with 20 cycles of Read 1 to sequence the 12 base UMI and 8 base sample index, and 75 cycles of Read 2 to sequence the targets.*

## Consistent coverage across 96 samples

Capture-based enrichment has distinct advantages over amplification-based methods in producing highly even coverage across targeted loci. Even coverage across the targeted loci reduces the total amount of sequencing data required, and maximizes the number of markers included in analyses.

**Passing filter reads across 96 pooled samples**

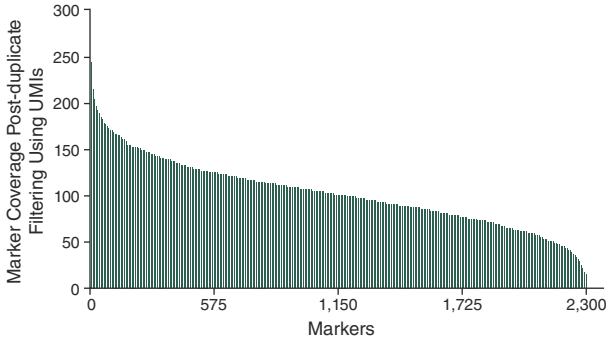



*Passing filter reads across 96 tomato DNA samples that were enriched using a genotyping panel consisting of 2,309 publicly available SolCAP markers and the NEBNext Direct Genotyping Solution. 25 ng of purified tomato DNA was used for each sample. Samples were index-tagged and pooled prior to hybridization and libraries were sequenced on an Illumina MiSeq with 20 cycles of Read 1 to sequence the 12 base UMI and 8 base sample index, and 75 cycles of Read 2 to sequence the targets.*

## Superior coverage uniformity

NEBNext Direct produces highly consistent, highly specific enrichment across samples, with >90% of sequencing reads mapping to the targeted regions. High specificity is achieved through the unique combination of capture-based enrichment conditions optimized for genotyping applications with proprietary enzymatic removal of off-target sequence.

**Mean Coverage across 2309 markers within a single sample**



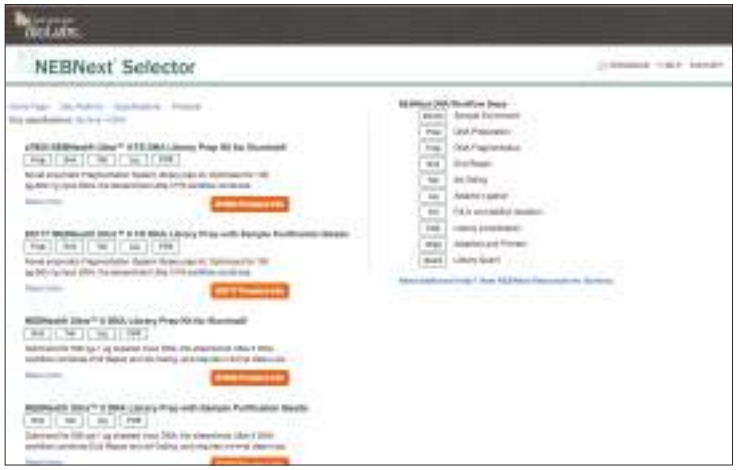

*Histogram of coverage across each of the 2,309 SolCAP markers demonstrates evenness of enrichment across targets and coverage levels sufficient for genotyping calls. These data represent enrichment of a single tomato sample pooled with 95 others prior to hybridization. 25 ng of purified tomato DNA was used for each sample. Samples were index-tagged and pooled prior to hybridization and libraries were sequenced on an Illumina MiSeq with 20 cycles of Read 1 to sequence the 12 base UMI and 8 base sample index, and 75 cycles of Read 2 to sequence the targets.*

**Illumina Platform:**

| KITS FOR ILLUMINA DNA LIBRARY PREPARATION | | NEB # | SIZE |
|---|---|---|---|
| DNA & ChIP | NEBNext Ultra II DNA Library Prep Kit for Illumina | E7645S/L | 24/96 rxns |
| | NEBNext Ultra II DNA Library Prep with Sample Purification Beads | E7103S/L | 24/96 rxns |
| | 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 DNA PCR-free Library Prep Kit for Illumina | E7410S/L | 24/96 rxns |
| | NEBNext Ultra II DNA PCR-free Library Prep with Sample Purification Beads | E7415S/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 with Sample Purification Beads | E7435S/L | 24/96 rxns |
| | NEBNext Enzymatic Methyl-seq Kit | E7120S/L | 24/96 rxns |
| | NEBNext Ultra DNA Library Prep Kit for Illumina | E7370S/L | 24/96 rxns |
| **MODULES & ENZYMES** | | **NEB #** | **SIZE** |
| DNA & ChIP | NEBNext Enzymatic Methyl-seq Conversion Module | E7125S/L | 24/96 rxns |
| | NEBNext FFPE DNA Repair Mix | M6630S/L | 24/96 rxns |
| | NEBNext Microbiome DNA Enrichment Kit | E2612S/L | 6/24 rxns |
| | NEBNext Ultra II FS DNA Module | E7810S/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 Q5 Master Mix | M0544S/L/X | 50/250/500 rxns |
| | NEBNext Ultra End Repair/dA-Tailing Module | E7442S/L | 24/96 rxns |
| | NEBNext Ultra Ligation Module | E7445S/L | 24/96 rxns |
| | NEBNext dsDNA Fragmentase | M0348S/L | 50/250 rxns |
| | NEBNext End Repair Module | E6050S/L | 20/100 rxns |
| | NEBNext dA-Tailing Module | E6053S/L | 20/100 rxns |
| | NEBNext Quick Ligation Module | E6056S/L | 20/100 rxns |
| | NEBNext Q5 Hot Start HiFi PCR Master Mix | M0543S/L | 50/250 rxns |
| | NEBNext High-Fidelity 2X PCR Master Mix | M0541S/L | 50/250 rxns |
| | NEBNext Q5U Master Mix | M0597S/L | 50/250 rxns |
| | NEBNext dsDNA Fragmentase Reaction Buffer v2 | B0349S | 6 ml |
| **ADAPTORS & PRIMERS** | | **NEB #** | **SIZE** |
| | NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors DNA Set 1) | E7395S/L | 96/384 rxns |
| | NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs) | E6440S/L | 96/384 rxns |
| | NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 2) | E6442S/L | 96/384 rxns |
| | NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 3) | E6444S/L | 96/384 rxns |
| | NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 4) | E6446S/L | 96/384 rxns |
| | NEBNext Multiplex Oligos for Enzymatic Methyl-seq (Unique Dual Index Primer Pairs) | E7140S/L | 24/96 rxns |
| | NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1) | E7600S | 96 rxns |
| | NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 2) | E7780S | 96 rxns |
| | NEBNext Multiplex Oligos for Illumina (Index Primers Set 1) | E7335S/L | 24/96 rxns |
| | NEBNext Multiplex Oligos for Illumina (Index Primers Set 2) | E7500S/L | 24/96 rxns |
| | NEBNext Multiplex Oligos for Illumina (Index Primers Set 3) | E7710S/L | 24/96 rxns |
| | NEBNext Multiplex Oligos for Illumina (Index Primers Set 4) | E7730S/L | 24/96 rxns |
| | NEBNext Multiplex Oligos for Illumina (Index Primers Set 5) *available soon* | E6448S/L | 24/96 rxns |
| | NEBNext Multiplex Oligos for Illumina (96 Index Primers) | E6609S/L | 96/384 rxns |
| | NEBNext Multiplex Oligos for Illumina (Methylated Adaptor, Index Primers Set 1) | E7535S/L | 24/96 rxns |
| | NEBNext Adaptor Dilution Buffer | B1430S | 1 x 9.6 ml |
| **TARGET ENRICHMENT** | | **NEB #** | **SIZE** |
| | NEBNext Direct Genotyping Solution | E9500B-S | 96 rxns |
| | NEBNext Direct Genotyping Solution | E9530B-S | 8 rxns |
| **LIBRARY QUANTITATION** | | **NEB #** | **SIZE** |
| | NEBNext Library Quant Kit for Illumina | E7630S/L | 100/500 rxns |
| | NEBNext Library Dilution Buffer | B6118S | 7.5 ml |
| **MAGNETIC SEPARATION** | | **NEB #** | **SIZE** |
| | NEBNext Magnetic Separation Rack | S1515S | 24 tubes |

# NEBNext Selector

Use this tool to guide you through selection of NEBNext reagents for next generation sequencing sample preparation. Try it out at **NEBNextSelector.neb.com**

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## NEBNext® Selector

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

x/f/yNEB109
NEBNEXT_DNA_ILL – Version 7.0 – 11/21

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