



Now includes
NEBNext Enzymatic Methyl-seq (EM-seq™)

NEBNext® for DNA Sample Prep

FOR THE ILLUMINA® PLATFORM



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BioLabs® Inc.

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drive DISCOVERY
stay GENUINE

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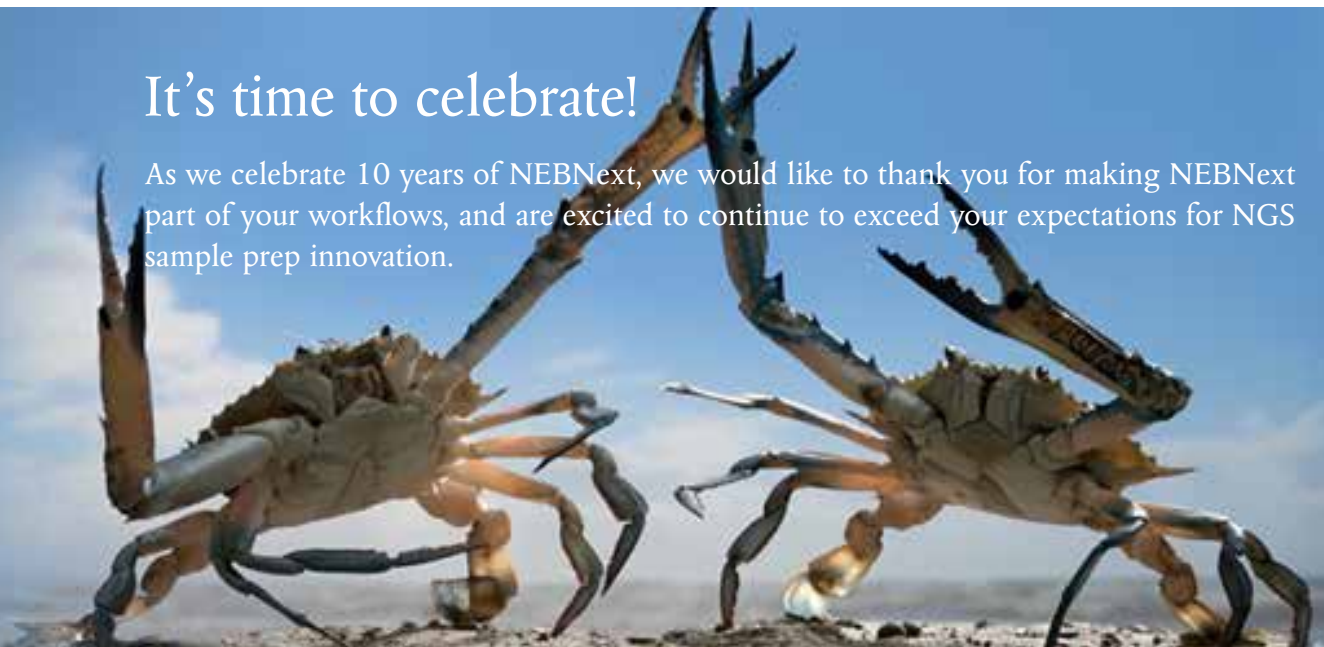
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It's time to celebrate!

As we celebrate 10 years of NEBNext, we would like to thank you for making NEBNext part of your workflows, and are excited to continue to exceed your expectations for NGS sample prep innovation.



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 these 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, as well as a broad range of input amounts (100 pg–1 µg). 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 6,000 peer-reviewed 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.

WHAT'S NEW IN NEBNext?

- **NEBNext Enzymatic Methyl-seq (EM-seq)** – a new alternative to bisulfite sequencing
- Rapid customization of targeted gene panels with **NEBNext Direct Custom Ready**
- Increased multiplexing options with **Unique Dual Index Primer Pairs**
- The **NEBNext Magnetic Separation Rack**: custom-designed for NGS magnetic bead separations



DNA Library Preparation Overview

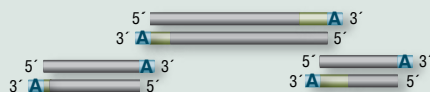
PRODUCT	INPUT AMOUNTS
NEBNext Ultra II FS DNA Library Prep Kit for Illumina	100 pg – 0.5 µg DNA
NEBNext Ultra II FS DNA Library Prep with Sample Purification Beads	
NEBNext Ultra II DNA Library Prep Kit for Illumina	500 pg – 1 µg DNA
NEBNext Ultra II DNA Library Prep with Sample Purification Beads	
NEBNext Oligos (including unique dual index, dual index and single index primers)	N/A

DNA	P5 Primer	NEBNext Adaptor
Uracil	P7 Primer	
Barcode (BC)	USER Enzyme	

Ultra II FS DNA Workflow

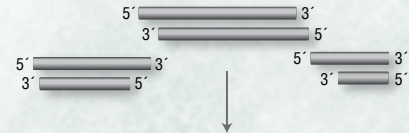
1 and 2 DNA Fragmentation, End Repair & dA-Tailing

- 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



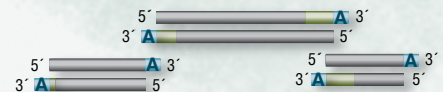
1 DNA Fragmentation

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



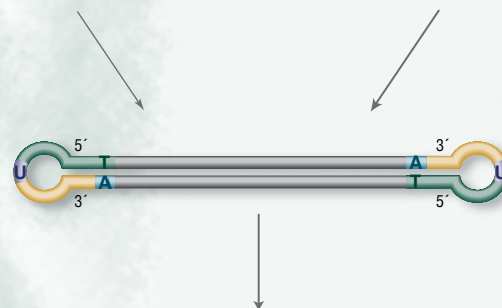
2 End Repair & dA-Tailing

- 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



3 Adaptor Ligation

- Ligation of short adaptors (contain sequences required downstream)
- The novel hairpin loop structure of the (optional) NEBNext Adaptor increases ligation efficiency & minimizes adaptor-dimer formation

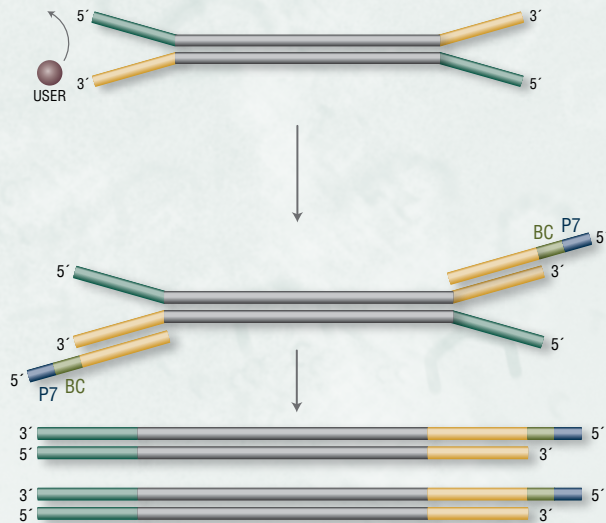


4 U Excision

- Removal of uracils in NEBNext Adaptor loop by USER® Enzyme (to make accessible for PCR)

5 PCR Enrichment

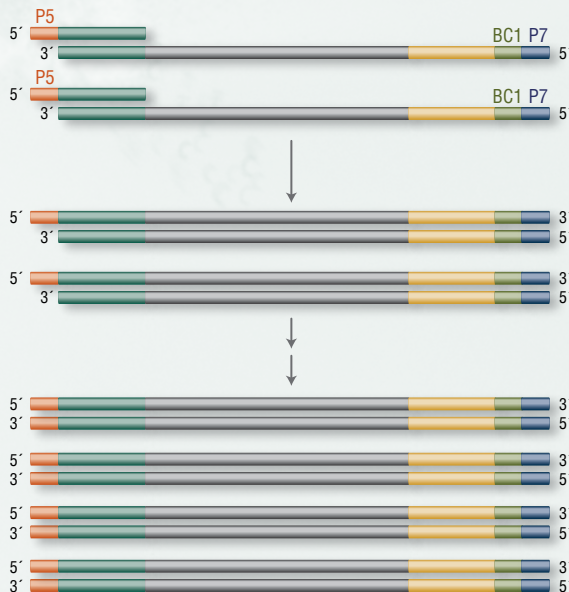
- Amplification using a high-fidelity polymerase:
 - Selects for molecules with an adaptor at each end
 - Increases library yield
 - Incorporates barcodes/indices to enable multiplexing, and P5 & P7 sequences required downstream



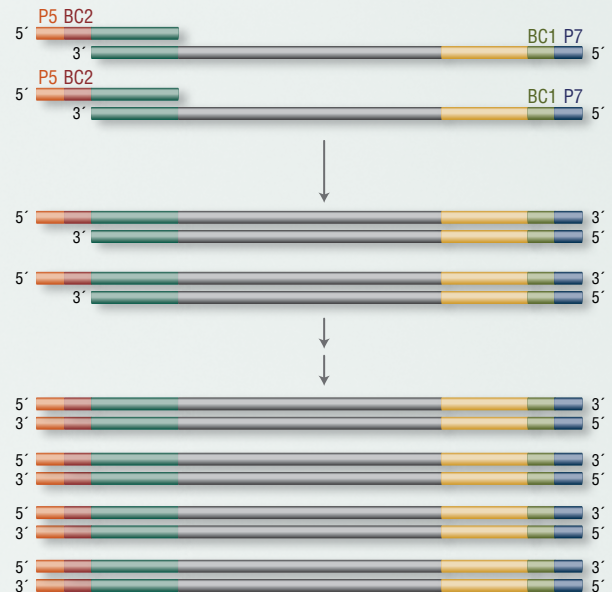
NEBNext Oligos

- Barcodes incorporated using NEBNext primers
- Single- or dual-barcode primer options available, including unique dual barcodes

SINGLE BARCODE



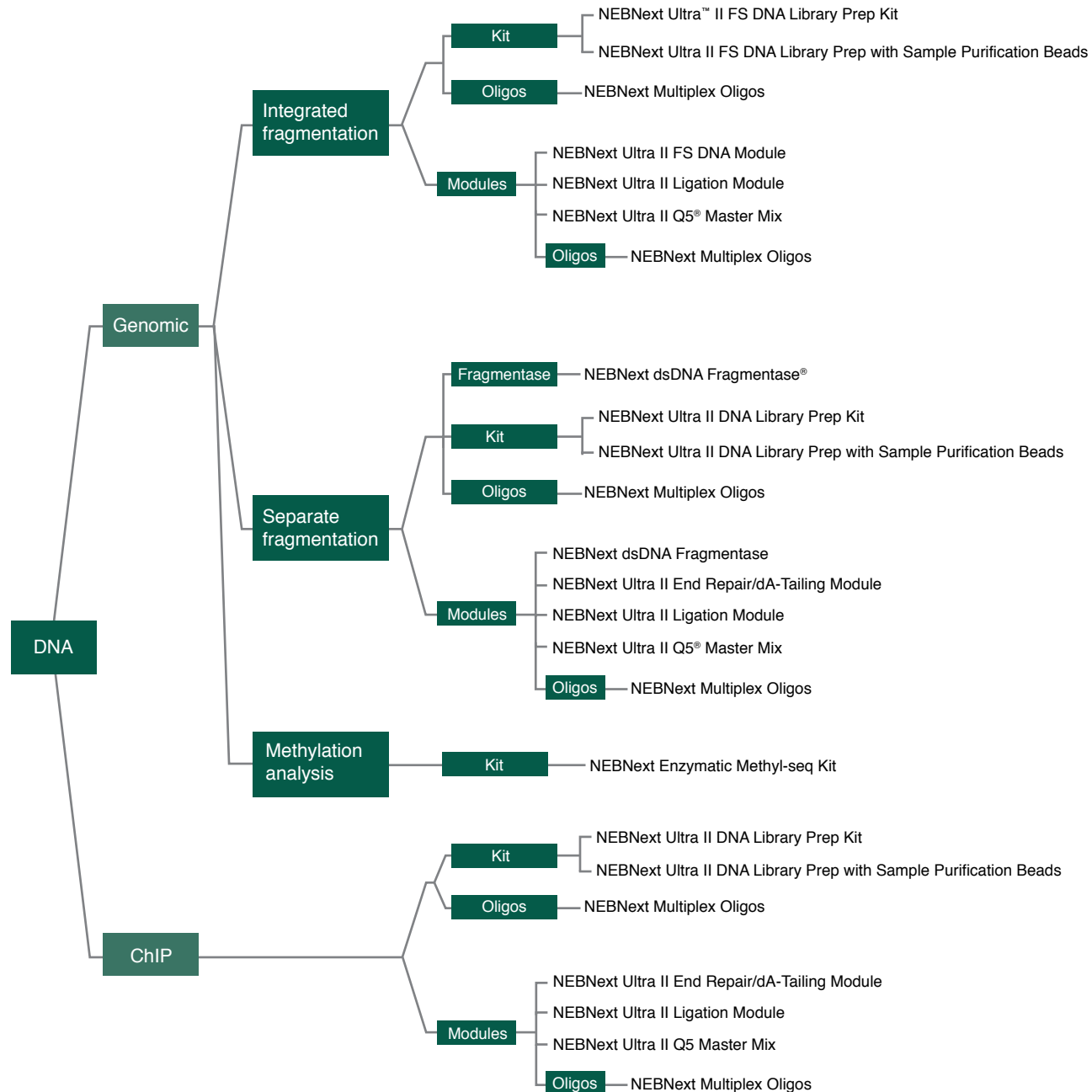
DUAL BARCODES



DNA Product Selection

For DNA, NEBNext kits are available 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 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, to choose the best products for your needs.



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

NEBNext for DNA Library Prep & Tips for working with DNA

DNA Sample Input Guidelines

Integrity of DNA

- Start with as high quality DNA as possible. The quality of the input material directly affects the quality of the library. DNA quality and purity can be assessed using absorbance measurements from UV spectrometry. 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 pH of between 7 and 8.5 for the input sample is preferable.

Quantitation of 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.

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.
- Open only one index primer 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 seals. To minimize the risk of contamination, do not pipette from a single well more than once.

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

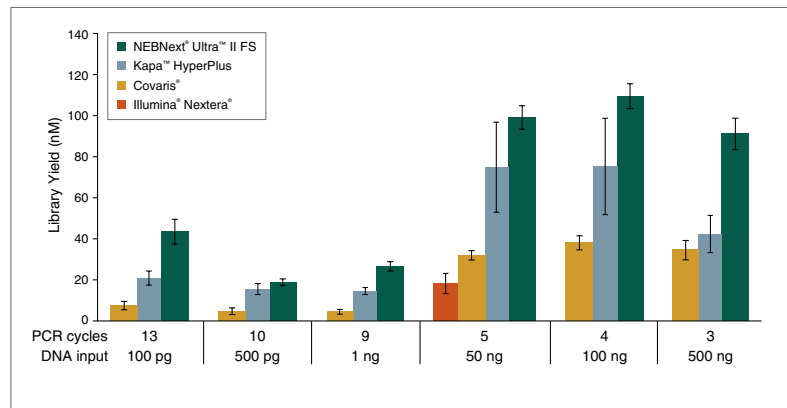


NEBNext MAGNETIC SEPARATION RACK

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.

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

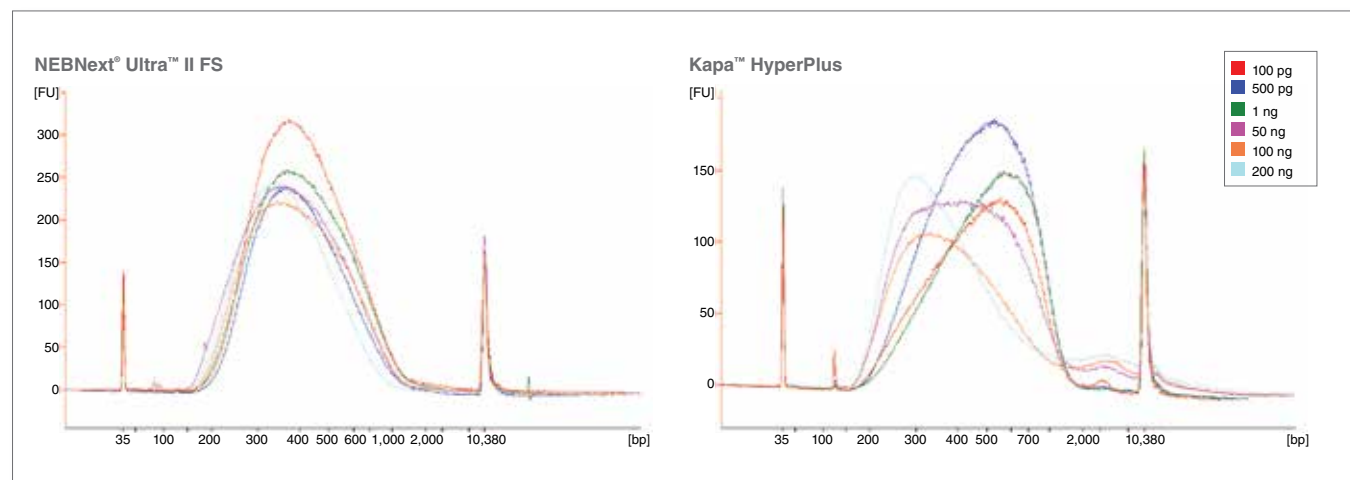


Libraries were prepared from Human NA19240 genomic DNA using the input amounts and numbers of PCR cycles shown. For NEBNext Ultra II FS, a 20-minute fragmentation time was used. For Kapa HyperPlus libraries, input DNA was cleaned up with 3X beads prior to library construction, as recommended, and a 20-minute 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. Error bars indicate standard deviation for an average of 3–6 replicates performed by 2 independent users.

You'll be thrilled to pieces.

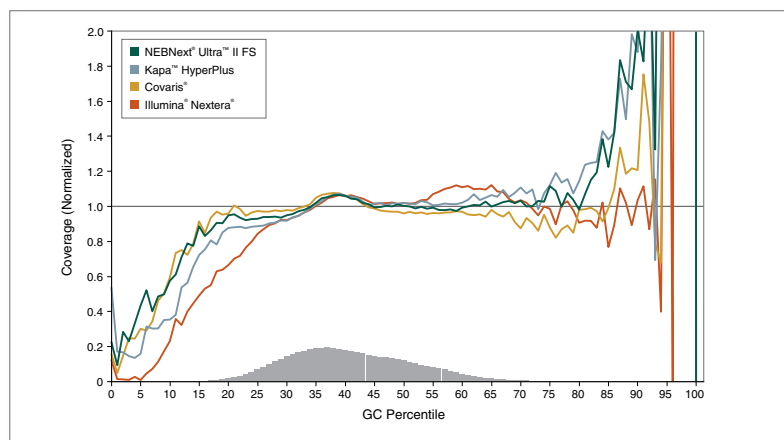
- Perform fragmentation, end repair and dA-tailing with a **single enzyme mix**
- Experience **reliable fragmentation with a single protocol**, regardless of DNA input amount or GC content
- Prepare high quality libraries from a **wide range of input amounts**: 100 pg–0.5 µg
- Generate high yields with 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

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



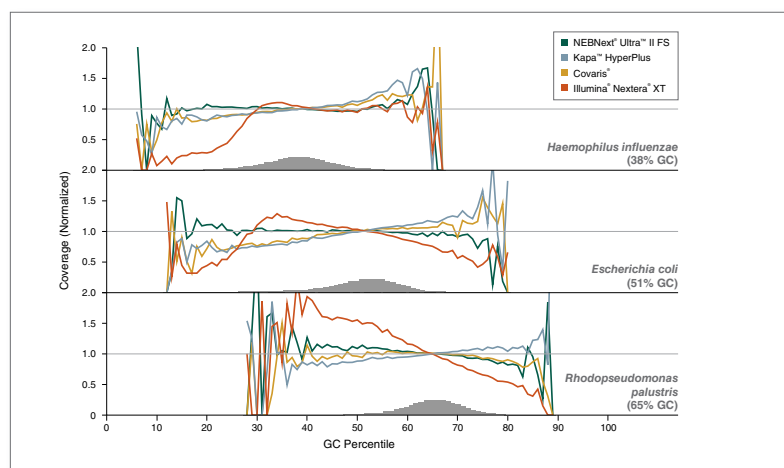
Libraries were prepared from Human NA19240 genomic DNA using the input amounts shown. NEBNext Ultra II FS libraries were prepared using a 20-minute fragmentation time. For Kapa HyperPlus, input DNA was cleaned up with 3X beads prior to library construction, as recommended, and a 20-minute fragmentation 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. 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.

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. 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.

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

"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."

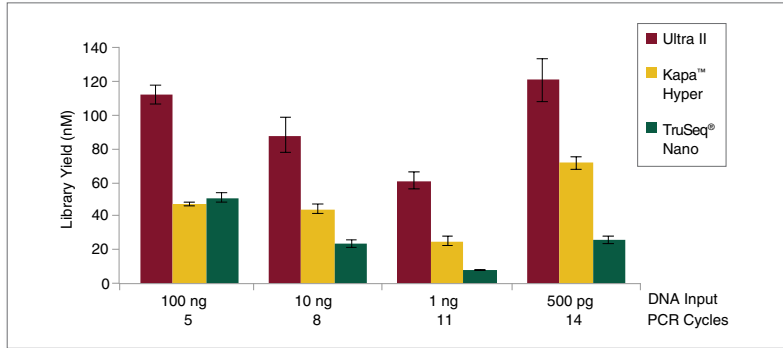
— João Anes, Ph.D.,
NGS specialist in Food Safety,
UCD-Centre for Food Safety,
Dublin, Ireland

"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

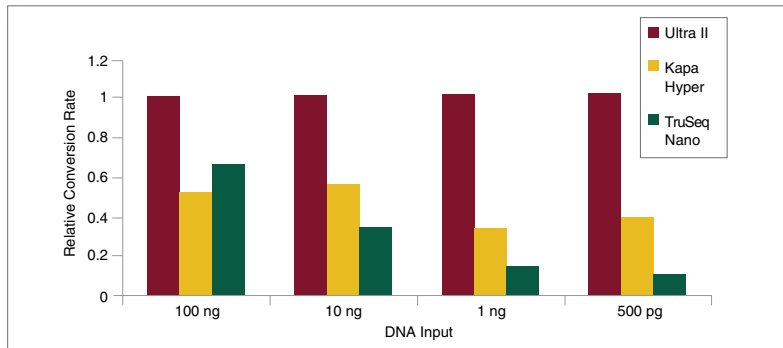
NEBNext Ultra II DNA Library Prep Kit – for pre-sheared DNA

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.

Even more
from less.

- 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
- 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.

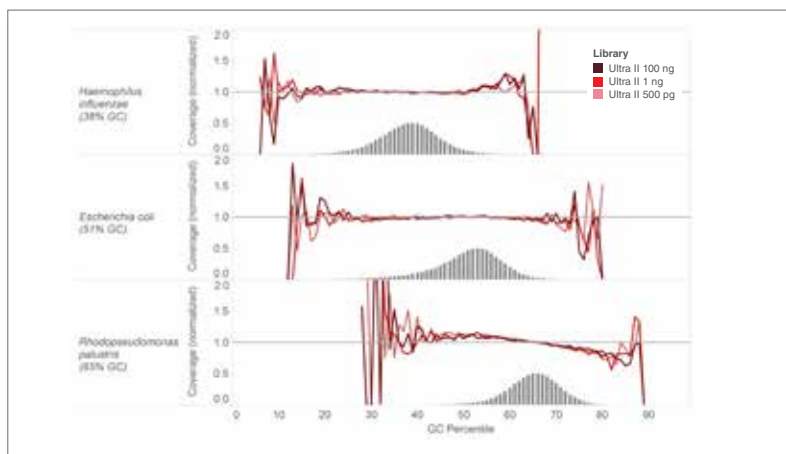


View the NEBNext Ultra II DNA protocol video for protocol steps, and tips for optimization



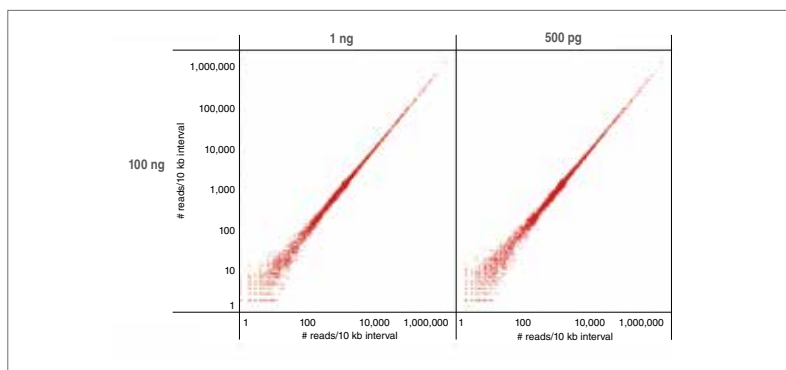
Find hundreds of peer reviewed publications citing use of NEBNext Ultra II DNA on the product pages

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



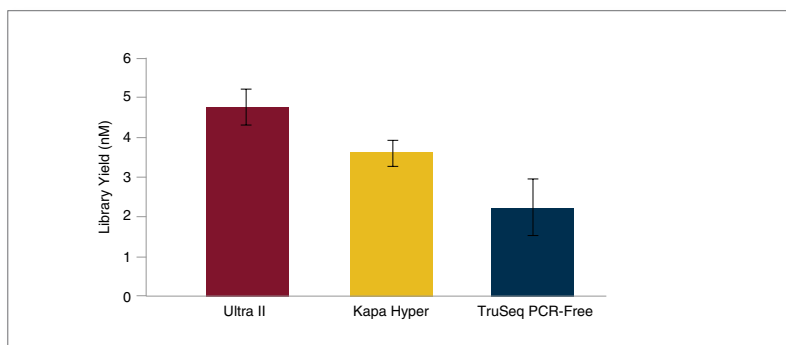
Libraries were made using 500 pg, 1 ng and 100 ng of the genomic DNAs shown and the Ultra II DNA Library Prep Kit and sequenced on an Illumina MiSeq. Reads were mapped using Bowtie 2.2.4 and GC coverage information was calculated using Picard's CollectGCBiasMetrics (v1.117). Expected normalized coverage 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 provides superior yields in PCR-free workflows

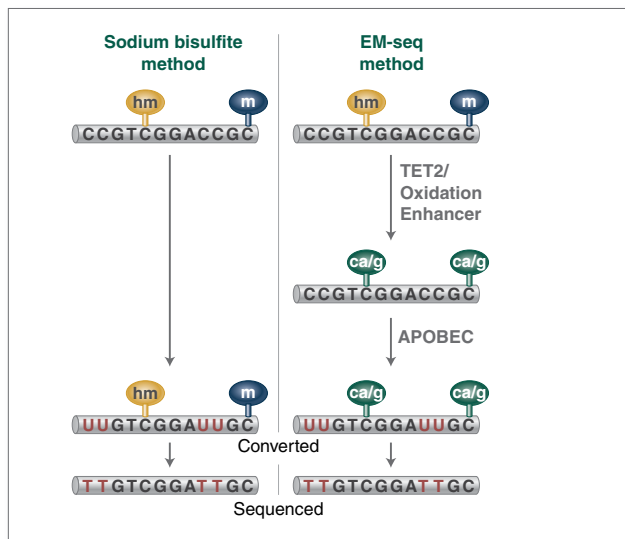


Libraries were generated from 100 ng of Human NA19240 genomic DNA using the library prep kits shown, following manufacturers' recommendations, and with no amplification step. Library yields were determined by qPCR using the NEBNext Library Quant Kit for Illumina. The NEBNext Ultra II Kit produces the highest yields.

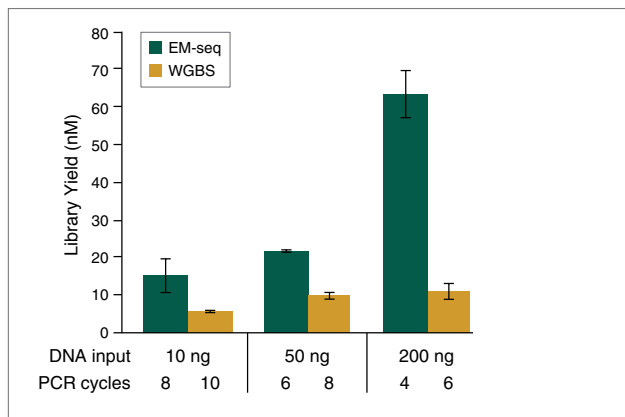
NEBNext Enzymatic Methyl-seq (EM-seq™) – a new method for identification of 5mC and 5hmC

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



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

What users are saying:

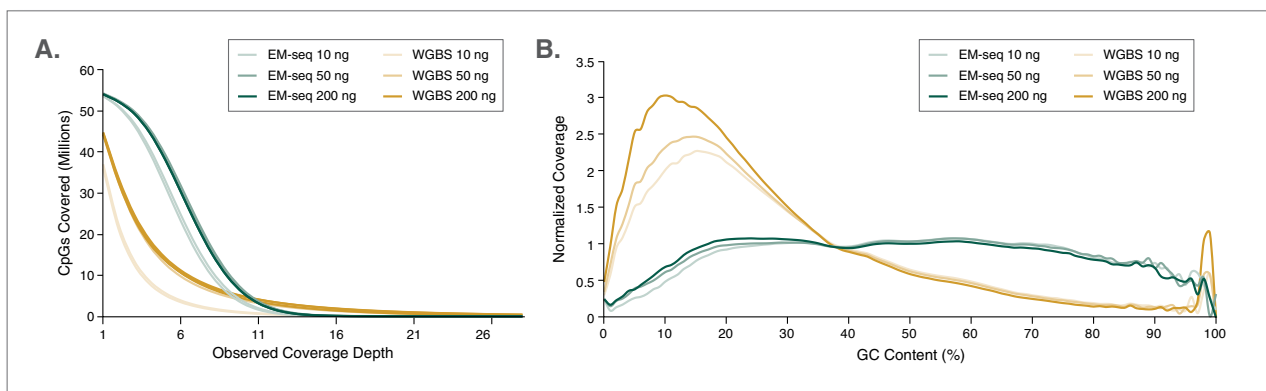
We've been testing EM-seq on a variety of inputs, platforms, and samples, and it shows more even coverage across CpG islands, the whole genome, and also greater detection of CpG sites across the genome vs. WGBS.

– Christopher Mason,
Weill Cornell Medical School
New York

Whole genome bisulfite sequencing is the workhorse technique in our laboratory and we have tested range of different kits. NEB's EM-seq Kit provides an excellent alternative that causes far less damage to the DNA and results in larger fragments which make the process of sequencing more cost effective. We found that the kit also produces libraries with very low biases in nucleotide coverage and methylation estimates.

– Duncan Sproul,
MRC Human Genetics Unit
Edinburgh

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

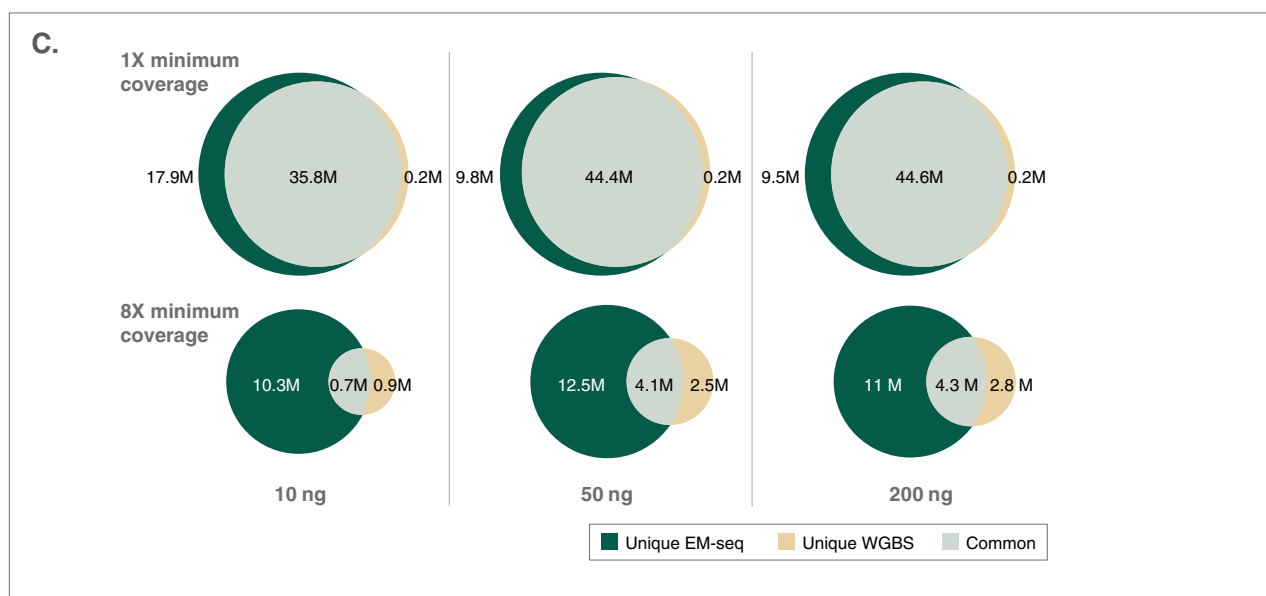


10, 50 and 200 ng Human NA12878 genomic DNA was sheared to 300 bp using the Covaris S2 instrument and used as input into EM-seq and WGBS protocols. For WGBS, NEBNext Ultra II DNA was used for library construction, followed by the Zymo Research EZ DNA Methylation-Gold Kit for bisulfite conversion. Libraries 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.”

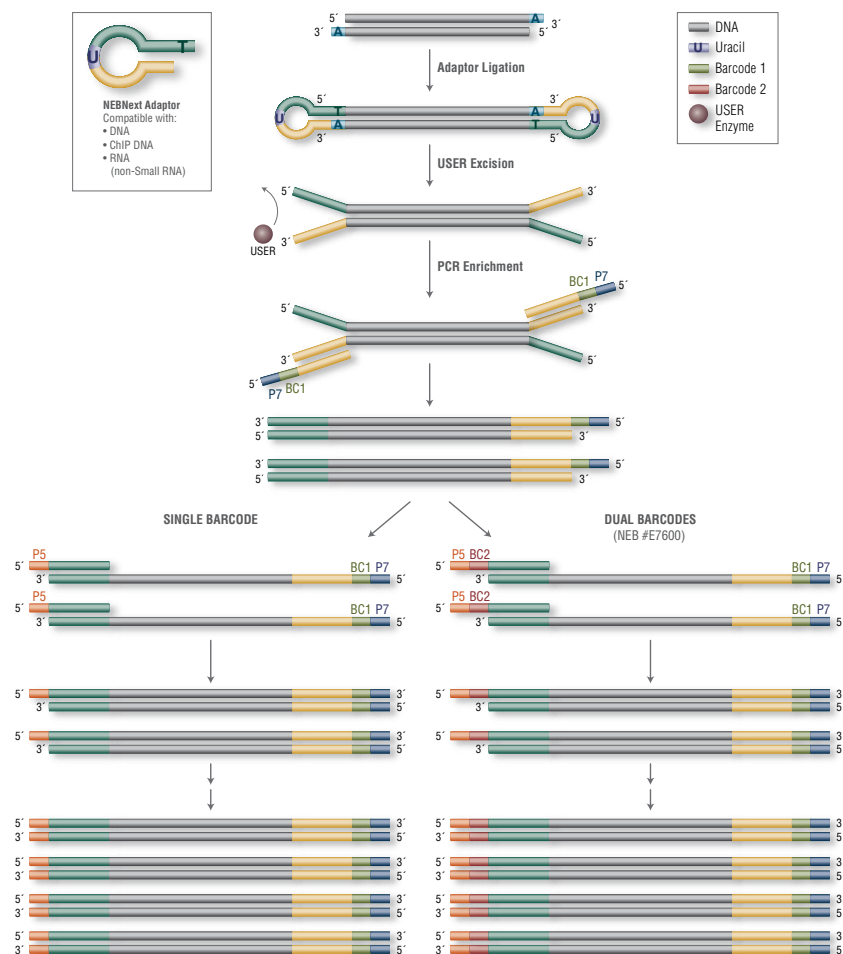
— Vladimir Benes,
Head Genomics Core Facility
at EMBL Heidelberg

NEBNext Adaptors and Primers

Designed for use in library prep for DNA, ChIP DNA and RNA (but not Small RNA), the NEBNext Adaptors enable high-efficiency adaptor ligation and high library yields, with minimized adaptor-dimer formation. Incorporating a novel hairpin loop structure, the NEBNext Adaptor ligates with increased efficiency to end-repaired, dA-tailed DNA. The loop contains a U, which is removed by treatment with USER® Enzyme (a mix of UDG and Endo VIII), to open up the loop and make it available as a substrate for PCR. During PCR, barcodes can be incorporated by use of the NEBNext index primers, thereby enabling multiplexing. NEBNext Oligos can be used with NEBNext products, and with other standard Illumina-compatible library preparation protocols. Single- or dual-barcode primer options are available. An optimized version of the NEBNext Adaptor is also available for use with EM-seq and bisulfite sequencing protocols.

Also the Unique Dual Index Primer Pairs address the “index hopping” seen with certain Illumina sequencing instruments. The unique combination of dual indices allows identification and complete filtering of index-swapped reads.

Workflow demonstrating the use of NEBNext adaptors and index primers



ADVANTAGES

- Increased ligation efficiency
- Minimized adaptor-dimer formation
- Increased library yields
- Multiple options
 - Unique dual indices (2 sets of 96 pairs)
 - Dual indices (2 sets)
 - Single indices (4 sets of 12 indices, one set of 96 indices)
- Convenient formats
 - Vials
 - Single-use 96-well plates with a pierceable foil seal
- Index pooling guidelines and sample sheets are provided

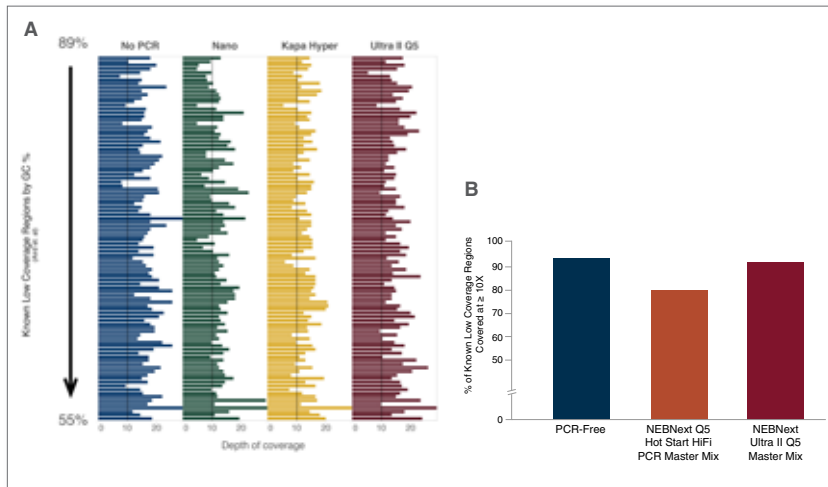
PRODUCT	# INDICES	SIZE
NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs or 96 Unique Dual Index Primer Pairs Set 2)	96 unique pairs	96/384 rxns
NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1 or 2)	8 x 12	96 rxns
NEBNext Multiplex Oligos for Illumina (96 Index Primers)	96	96/384 rxns
NEBNext Multiplex Oligos for Illumina (Index Primers Set 1, 2, 3 or 4)	12	24/96 rxns
NEBNext Multiplex Oligos for Enzymatic Methyl-seq (Unique Dual Index Primer Pairs)	96	24/96 rxns
NEBNext Multiplex Oligos for Illumina (Methylated Adaptor, Index Primers Set 1)	12	24/96 rxns
NEBNext Adaptor Dilution Buffer		1 x 9.6 ml

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.

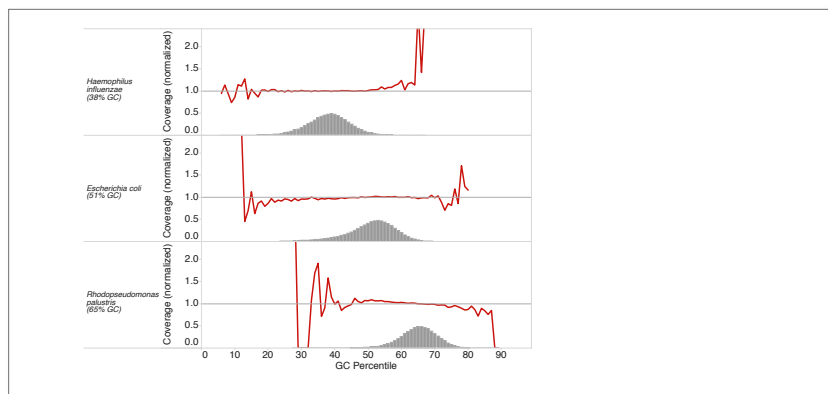
The NEBNext Ultra II Q5 Master Mix is the latest formulation of Q5 DNA polymerase that has been optimized for robust, high-fidelity amplification of next-generation sequencing (NGS) libraries. This formulation further improves the uniformity of amplification of libraries, including superior performance 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 or with NEBNext Ultra II Q5 Master Mix. 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	50/250 rxns

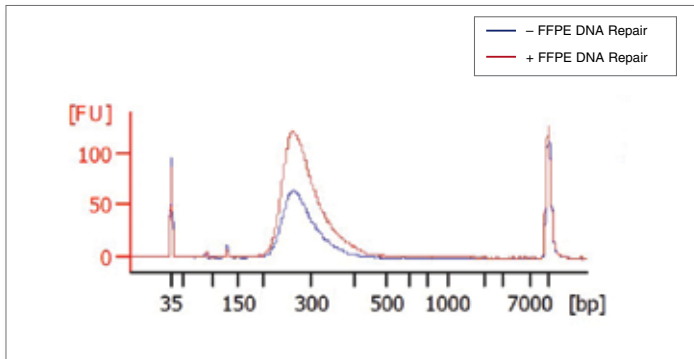
Reference:

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

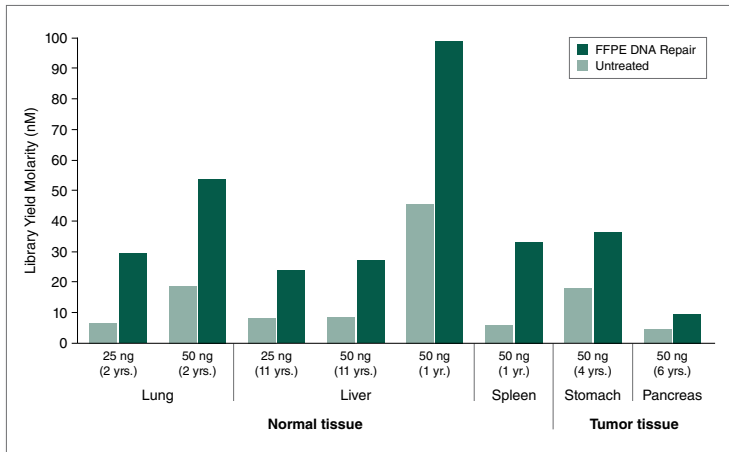


An example of Agilent Bioanalyzer traces of libraries prepared from stomach tumor FFPE DNA that was treated with the FFPE DNA Repair Mix, or was untreated, before library construction. Yield improvements of 101% to 458% have been observed.

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

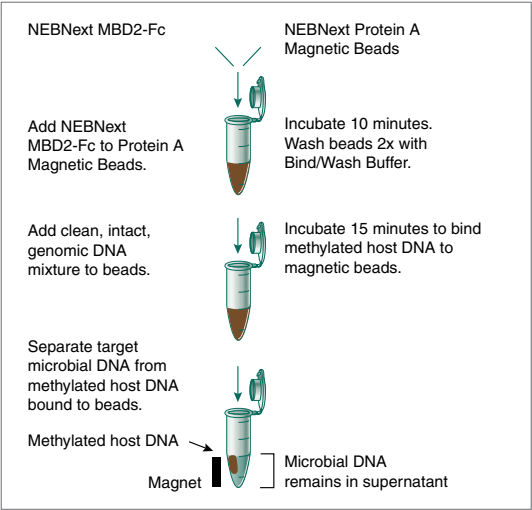
FFPE DAMAGE TYPE	REPAIRED BY THE FFPE DNA ENZYME REPAIR MIX?
Deamination of cytosine to uracil	Yes
Nicks and gaps	Yes
Oxidized bases	Yes
Blocked 3' ends	Yes
DNA fragmentation	No
DNA-protein crosslinks	No

PRODUCT	SIZE
NEBNext FFPE DNA Repair Mix	24/96 rxns

NEBNext Microbiome DNA Enrichment Kit

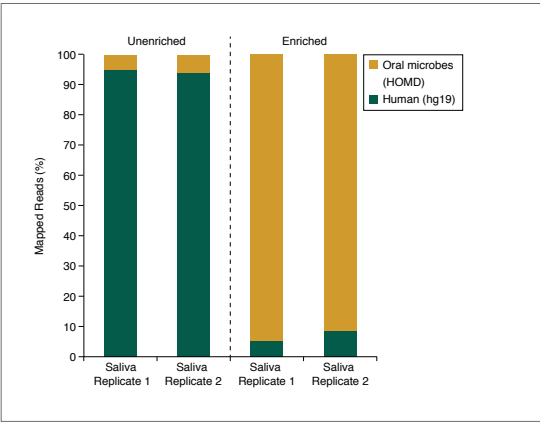
Microbiome DNA analysis can be challenging due to the high percentage of host DNA present in many samples. The NEBNext Microbiome DNA Enrichment Kit facilitates enrichment of microbial DNA from samples containing methylated host DNA (including human), by selective binding and removal of the CpG-methylated 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.

Salivary Microbiome DNA Enrichment



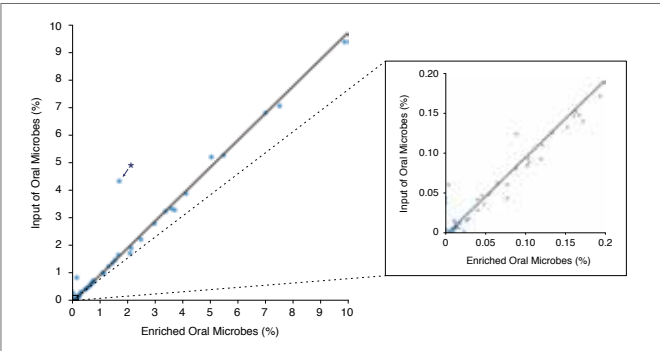
DNA was purified from pooled human saliva DNA (Innovative Research) and enriched using the NEBNext Microbiome DNA Enrichment Kit. Libraries were prepared from unenriched and enriched samples and sequenced on the SOLiD 4 platform. The graph shows percentages of 500 M-537 M SOLiD 4 50 bp reads that mapped 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.).

PRODUCT	SIZE
NEBNext Microbiome DNA Enrichment Kit	6/24 rxns

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)

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. elongata*) are represented, but do not exhibit this anomalous enrichment.

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

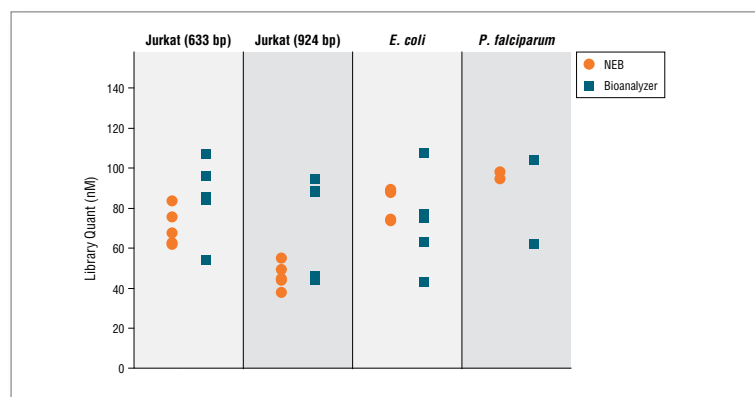
NEBNext Library Quant Kit for Illumina

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

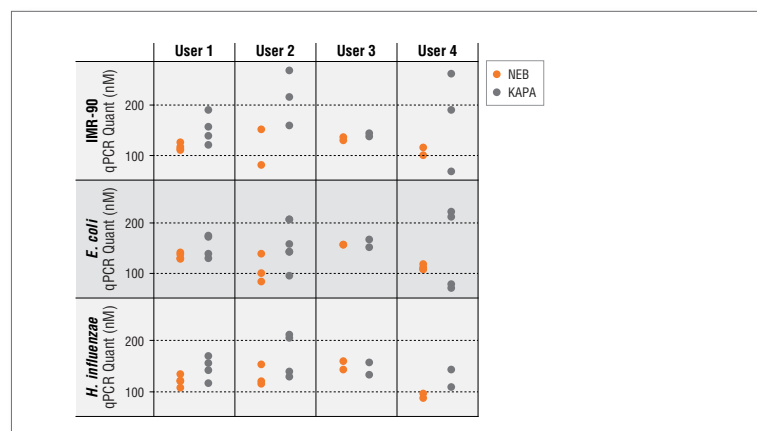
	Time					Workflow Time
	Reagent Preparation	Library Dilution	Set Up	qPCR	Data Analysis	
Hands-On	5 min.	10 min.	25 min.	1 min.	10 min.	51 min.
Total	5 min.	10 min.	25 min.	60 min.	10 min.	1 hr. 50 min.

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 Agilent 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).

Count on it.

- 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, 4 standards and ROX dye
- Simplify your reaction setup with **fewer pipetting steps** and a **single extension time** for all libraries
- Quantitate more libraries per kit, **as only 4 standards are required**
- **Use with all your libraries**, regardless of insert size, GC content and preparation method
- Save money with our value pricing

TOOLS & RESOURCES



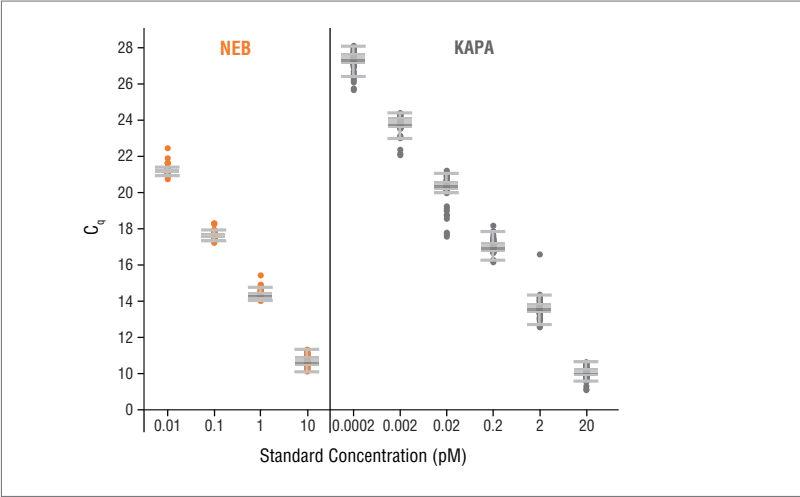
Use NEBioCalculator to calculate your qPCR-based library quant values



Download our application note, "Improved library quantitation for a broad range of library types using the NEBNext Quant Kit for Illumina"

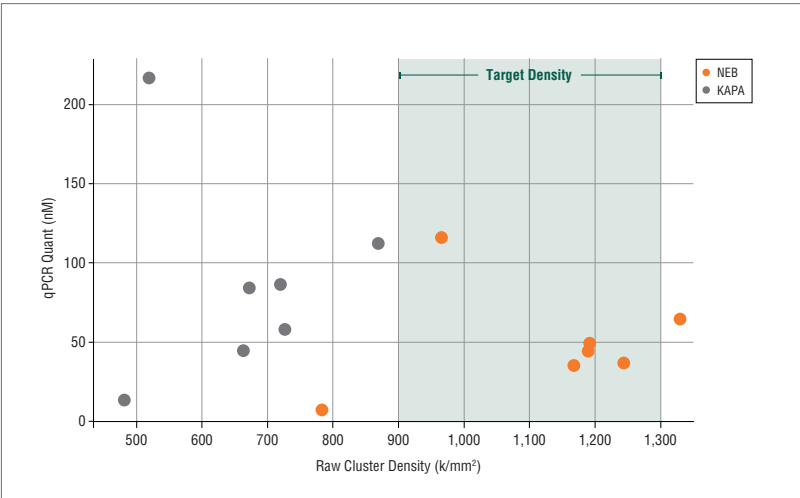
PRODUCT	SIZE
NEBNext Library Quant Kit for Illumina	100/500 rxns
NEBNext Library Dilution Buffer	7.5 ml

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



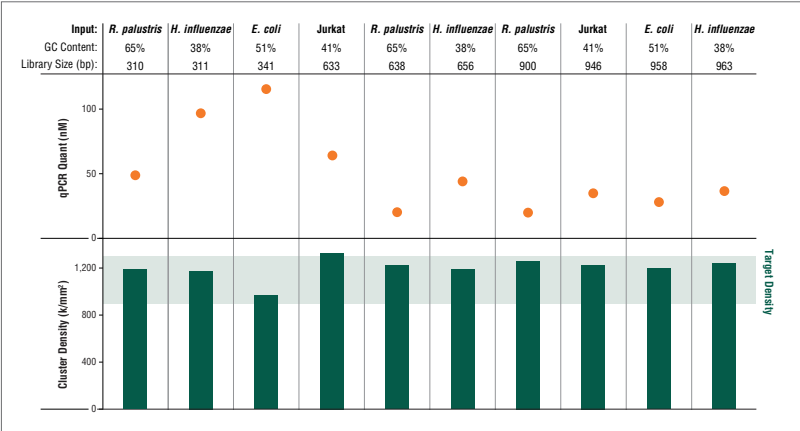
Accurate qPCR quantitation requires the use of high-quality DNA standards with known concentrations. The NEBNext Library Quant Kit contains 4 standards produced with a high level of both quantitation accuracy and consistency. This figure shows data from > 70 total runs from 4 lots of both NEBNext (orange) 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



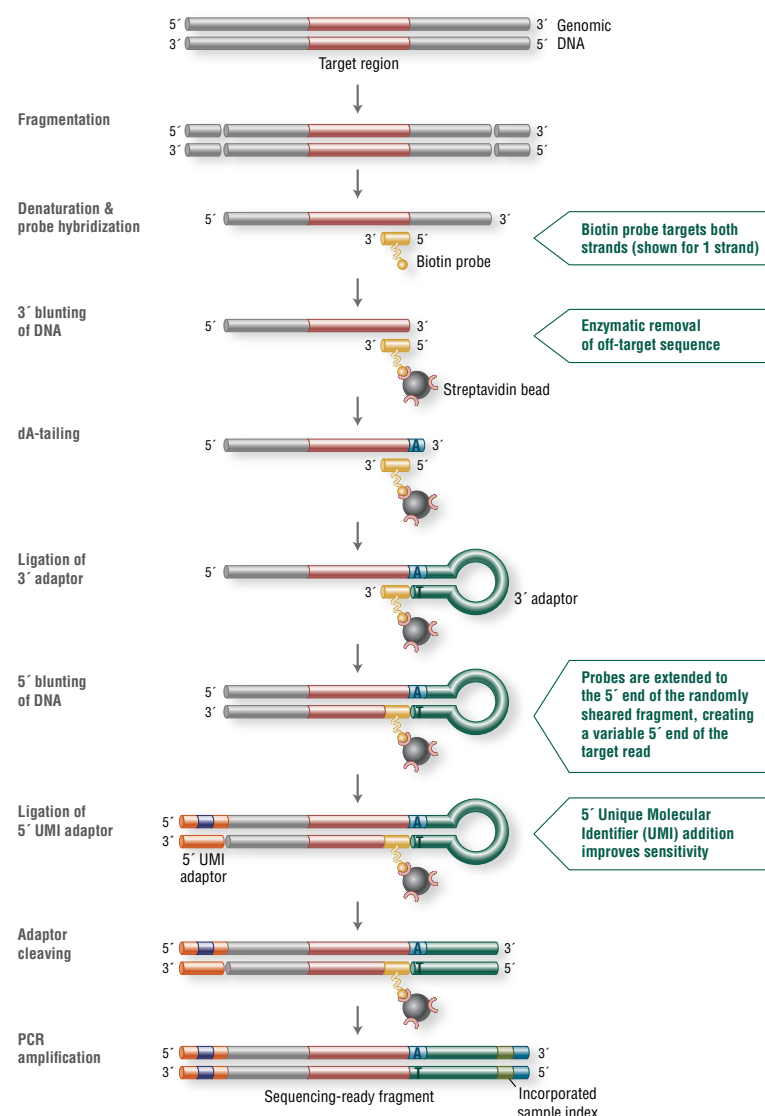
Libraries of 310–963 bp from the indicated sources were quantitated using the NEBNext Library Quant Kit, then diluted to 8 pM and loaded onto a MiSeq (v2 chemistry; MCS v2.4.1.3). Library concentrations ranged from 7–120 nM, and resulting raw cluster density for all libraries was 965–1300 k/mm² (ave.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

NEBNext Direct[®] for Target Enrichment

NEBNext Direct uses a unique technology to enable highly specific target enrichment of genomic regions of interest. This unique hybridization-based target enrichment technology enables high sensitivity variant calling with the speed and precision previously only feasible using multiplex PCR-based approaches. This flexibility allows a single workflow for assays ranging from single gene tests to comprehensive panels including several hundred genes. Regardless of sample type or assay content, NEBNext Direct enables enrichment of targets with precision.

For flexibility and fast turnaround, NEBNext Direct Custom Ready Panels allow rapid customization of targeted gene panels for Illumina sequencing. Select from a list of genes for which baits have been carefully designed and optimized to produce complete coverage of the full coding regions. High quality panels can be designed by you and rapidly delivered from any combination of genes.

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



Flexible precision.

NEBNext Direct:

- No upfront library prep: enriches targets and converts into a **sequence-ready library in 1 day**
- Generate a **higher percentage of your sequencing reads** aligning to your targets
- Eliminate the need to over-sequence, **reducing cost per sample**
- Obtain **uniform sequencing of all targets**, regardless of GC content
- Generate high quality libraries with **limited input amounts and degraded DNA samples**, including FFPE and ctDNA
- Improve sensitivity with Unique Molecular Identifiers (UMIs)

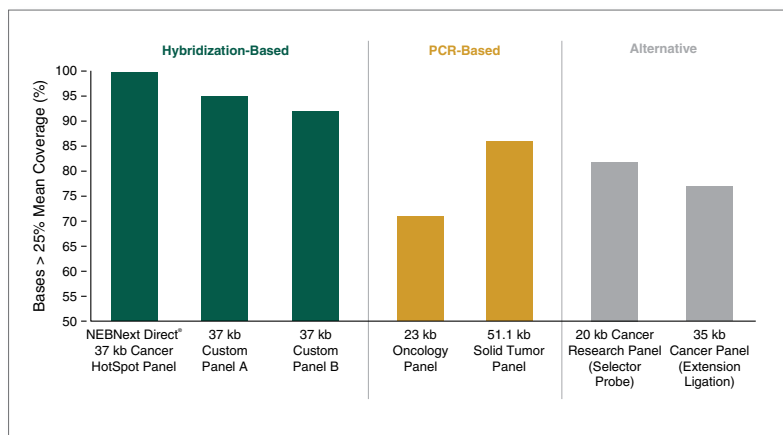
Custom Ready Panels:

- Choose from a single gene to hundreds of genes
- Eliminate synthesis and optimization steps for faster turnaround

PRODUCT	SIZE
NEBNext Direct Custom Ready Panels	8/24/96 rxns
NEBNext Direct Cancer HotSpot Panel	8/24/96 rxns
NEBNext Direct BRCA1/BRCA2 Panel	8/24/96 rxns

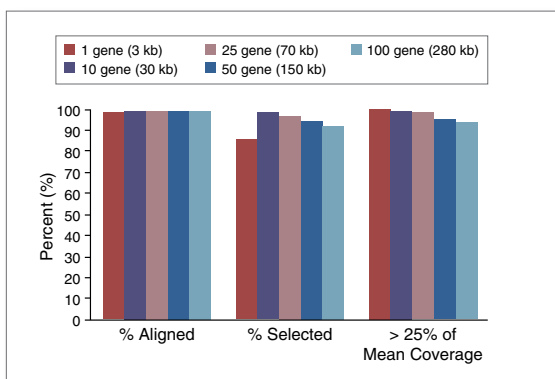
For research use only, not intended for diagnostic use.

NEBNext direct delivers higher coverage uniformity than alternative approaches.



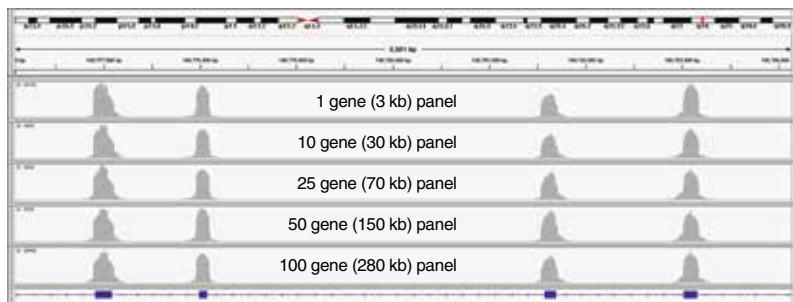
Plot shows the uniformity across targets for each panel, measured as the percentage of bases below 25% of the mean target coverage. Samples were processed in duplicate according to the manufacturer's suggested protocol using the recommended amount of DNA input. DNA used was a blend of 24 HapMap samples. Samples were sequenced on an Illumina MiSeq per the manufacturer recommendation. Representative data across 2 replicates are shown.

NEBNext Direct Custom Ready Panels demonstrate optimum performance across a wide range of panel sizes



Key target enrichment metrics demonstrate consistent performance across a range of panel sizes. 100 ng of DNA was tested against panels of 1, 10, 25, 50 and 100 genes, and sequenced using Illumina paired-end 150 bp sequencing. Larger panels included all genes present in smaller panels.

NEBNext Direct Custom Ready Panels demonstrate retention of target behavior across panel sizes



IGV image of coverage profile for 4 BRAF exons included in panels of 1, 10, 25, 50 and 100 genes, demonstrate consistent target behavior with the addition of gene targets. 100 ng of DNA was used as input for NEBNext Direct enrichment using the 5 panels, including the BRAF gene. Libraries were sequenced using Illumina 2 x 150 basepair sequencing.

What users are saying:

“NEBNext Direct Custom Ready Panels have allowed my research to focus on the specific genes we need to explore. In addition to the convenience of easily selecting genes for focused panels, NEBNext Direct enrichment has provided the necessary reliability and depth of coverage to enable robust somatic variant calling.”

– Guang Peng, M.D., Ph.D.,
MD Anderson Cancer Center

“NEBNext Direct enrichment technology is by far the fastest and most automation friendly protocol available today. I can have samples on the sequencer in 6 hours starting from genomic DNA. The technology produces very high on target percentages (> 90%) for even very small panels, and in combination with molecular barcoding produces low duplication rates. From an optimization perspective, NEBNext Direct enrichment allows me to assign individual captured fragments to a probe unambiguously, thus giving the opportunity for optimizing the coverage distribution of any target.”

– Eric C. Olivares,
Founder, SEQanswers.com

NEBNext Reagents for DNA Sample Preparation:

KITS FOR ILLUMINA DNA LIBRARY PREPARATION	VWR CAT. NO.	SIZE
NEBNext Ultra II DNA Library Prep Kit for Illumina	102969-110	24 reactions
	102969-108	96 reactions
NEBNext Ultra II DNA Library Prep with Sample Purification Beads	103258-560	24 reactions
	103258-558	96 reactions
NEBNext Ultra II FS DNA Library Prep Kit for Illumina	103529-140	24 reactions
	103529-168	96 reactions
NEBNext Ultra II FS DNA Library Prep with Sample Purification Beads	103529-166	24 reactions
	103529-164	96 reactions
NEBNext Enzymatic Methyl-seq Kit	76339-724	24 reactions
	76339-722	96 reactions
NEBNext Ultra II DNA Library Prep Kit for Illumina	102969-110	24 reactions
	102969-108	96 reactions
NEBNext Ultra II DNA Library Prep with Sample Purification Beads	103258-560	24 reactions
	103258-558	96 reactions
NEBNext Ultra DNA Library Prep Kit for Illumina	102715-916	24 reactions
	102715-914	96 reactions
NEBNext DNA Library Prep Master Mix Set for Illumina	101640-830	12 reactions
	101640-828	60 reactions
NEBNext ChIP-Seq Library Prep Master Mix Set for Illumina	101640-902	12 reactions
	101640-900	60 reactions
MODULES & ENZYMES	VWR CAT. NO.	SIZE
NEBNext FFPE DNA Repair Mix	102902-482	24 reactions
	102902-480	96 reactions
NEBNext Microbiome DNA Enrichment Kit	102855-164	6 reactions
	102969-098	24 reactions
NEBNext Enzymatic Methyl-seq Conversion Module	76339-724	24 reactions
	76339-722	96 reactions
NEBNext Ultra II FS DNA Module	103529-144	24 reactions
	103529-142	96 reactions
NEBNext Ultra II End Repair/dA-Tailing Module	102969-102	24 reactions
	102969-100	96 reactions
NEBNext Ultra II Ligation Module	102969-106	24 reactions
	102969-104	96 reactions
NEBNext Ultra II Q5 Master Mix	102969-090	50 reactions
	102969-088	250 reactions
NEBNext Ultra End Repair/dA-Tailing Module	102855-134	24 reactions
	102855-136	96 reactions
NEBNext Ultra Ligation Module	102855-128	24 reactions
	102855-130	96 reactions
NEBNext dsDNA Fragmentase	101640-954	50 reactions
	101640-952	250 reactions
NEBNext End Repair Module	101640-834	20 reactions
	101640-832	100 reactions
NEBNext dA-Tailing Module	101640-838	20 reactions
	101640-836	100 reactions
NEBNext Quick Ligation Module	101640-842	20 reactions
	101640-840	100 reactions
NEBNext Q5 Hot Start HiFi PCR Master Mix	102902-486	50 reactions
	102902-484	250 reactions
NEBNext High-Fidelity 2X PCR Master Mix	102500-096	50 reactions
	102500-094	50 reactions
NEBNext Q5U Master Mix	76339-748	250 reactions
	76339-746	100 reactions
NEBNext dsDNA Fragmentase Reaction Buffer v2	102877-608	6 ml
ADAPTORS & PRIMERS	VWR CAT. NO.	SIZE
NEBNext Multiplex Oligos for Illumina (Index Primers Set 1)	102500-100	24 reactions
	102500-098	96 reactions
NEBNext Multiplex Oligos for Illumina (Index Primers Set 2)	102715-934	24 reactions
	102715-932	96 reactions
NEBNext Multiplex Oligos for Illumina (Index Primers Set 3)	103258-544	24 reactions
	103258-564	96 reactions
NEBNext Multiplex Oligos for Illumina (Index Primers Set 4)	103258-548	24 reactions
	103258-546	96 reactions
NEBNext Multiplex Oligos for Illumina (96 Index Primers)	103218-910	96 reactions
	103218-908	384 reactions

NEBNext Reagents for DNA Sample Preparation (cont.):

NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs)	76284-670	96 reactions
	76284-192	384 reactions
NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 2)	76407-330	96 reactions
	76407-328	384 reactions
NEBNext Multiplex Oligos for Illumina (Methylated Adaptor, Index Primers Set 1)	102877-574	24 reactions
	102877-572	96 reactions
NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1)	102877-580	96 reactions
NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 2)	76284-672	96 reactions
NEBNext Multiplex Oligos for Enzymatic Methyl-seq (Unique Dual Index Primer Pairs)	76339-728	24 reactions
	76339-726	96 reactions
NEBNext Singleplex Oligos for Illumina	102500-110	12 reactions
	102500-108	60 reactions
NEBNext Adaptor Dilution Buffer	76326-062	1 x 9.6 ml
TARGET ENRICHMENT	VWR CAT. NO.	SIZE
NEBNext Direct Cancer HotSpot Panel	103218-926	8 reactions
	103218-924	24 reactions
	103218-928	96 reactions
NEBNext Direct BRCA1/BRCA2 Panel	76196-408	8 reactions
	76196-406	24 reactions
	76196-410	96 reactions
NEBNext Direct Custom Ready Panels	Contact Rep	8 reactions
	Contact Rep	24 reactions
	Contact Rep	96 reactions
LIBRARY QUANTITATION	VWR CAT. NO.	SIZE
NEBNext Library Quant Kit for Illumina	102942-110	100 reactions
	102942-108	500 reactions
NEBNext Library Dilution Buffer	102969-094	7.5 ml
DNA ENRICHMENT	VWR CAT. NO.	SIZE
NEBNext Microbiome DNA Enrichment Kit	102855-164	6 reactions
	102929-098	24 reactions
DNA REPAIR	VWR CAT. NO.	SIZE
NEBNext FFPE DNA Repair Mix	102902-482	24 reactions
	102902-480	96 reactions



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