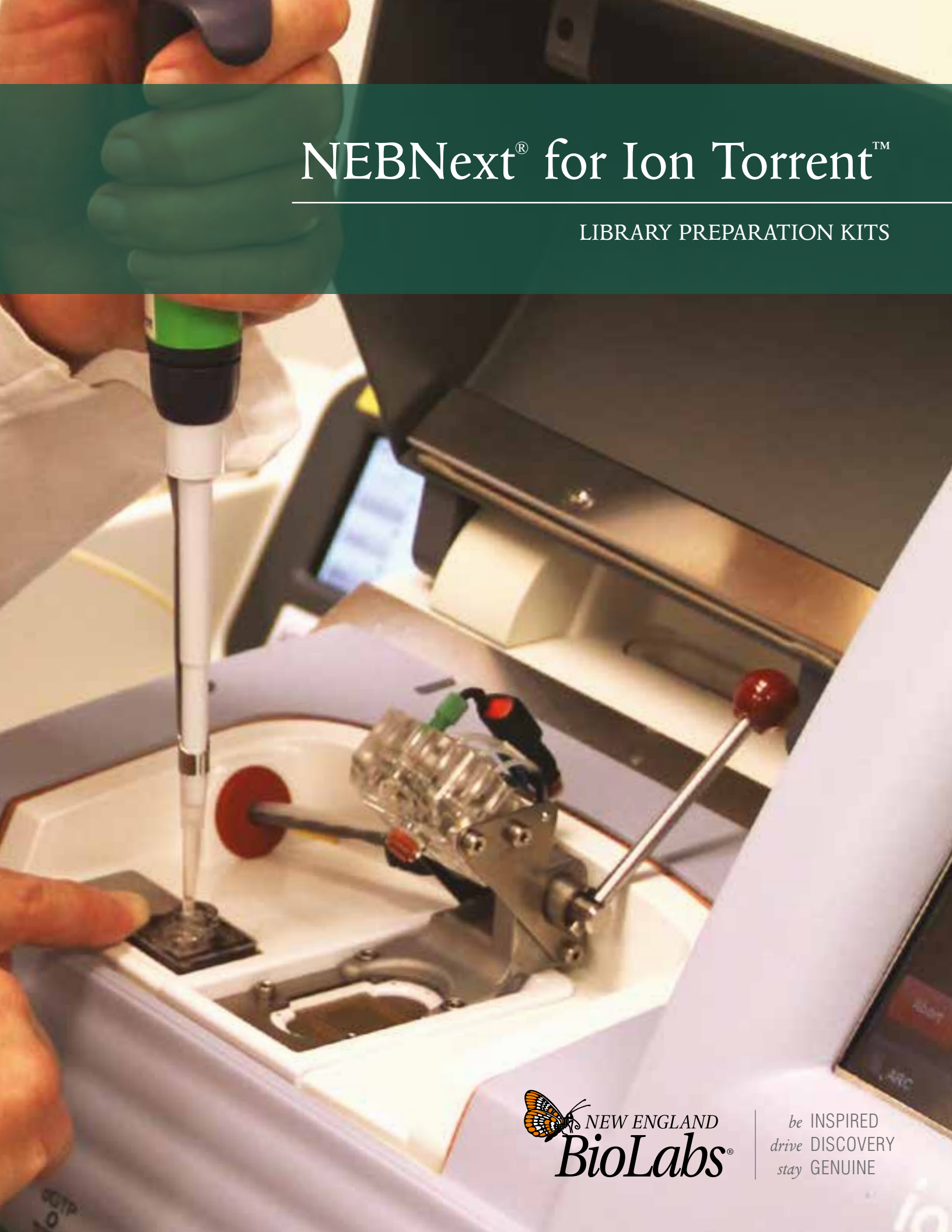


NEBNext® for Ion Torrent™

LIBRARY PREPARATION KITS

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
- Protocols & FAQs
- Online tutorials to help with product selection, general handling tips and more
- NEBNext citations

Access to the NEBNext Selector Tool, our online tool for help with selecting the right NEBNext product

The NEBNext suite of products supports Ion Torrent sequencing with library preparation tools that streamline workflows, minimize inputs, and improve library yields and diversity. DNA library preparation is complete in approximately 2 hours, with minimal hands-on time. In addition to the extensive QCs on individual kit components, all NEBNext kits for Ion Torrent are functionally validated by preparation of a library, followed by Ion Torrent sequencing.

NEBNext for Ion Torrent:

Kits include reagents for the entire library preparation workflow, and include master mix reagents that minimize the number of components and pipetting steps.

Bulk & Custom formats:

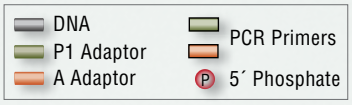
Choose NEBNext's bulk and custom formats when your reagent needs exceed standard volumes, or you require a specialized formulation. As enzyme manufacturers, we are able to easily provide customized reagents, kits and modules to meet your specific NGS library prep workflow needs. For more information, please contact **NEBsolutions@neb.com**.

THE NEBNEXT ADVANTAGE

- Broad range of input amounts, from low ng to µg
- Fast workflows with minimal hands-on time
- Gel-free workflows
- High yields
- High library diversity
- Minimized GC bias
- Convenient formats include kits and modules
- All reagents undergo stringent quality controls, plus sequencing validation
- Value pricing

Workflow for Ion Torrent DNA Library Preparation

RECOMMENDED INPUT AMOUNTS	TOTAL DNA
NEBNext Fast DNA Library Prep Set for Ion Torrent	10 ng – 1 µg
NEBNext Fast DNA Fragmentation & Library Prep Set for Ion Torrent	10 ng – 1 µg



1 DNA Fragmentation

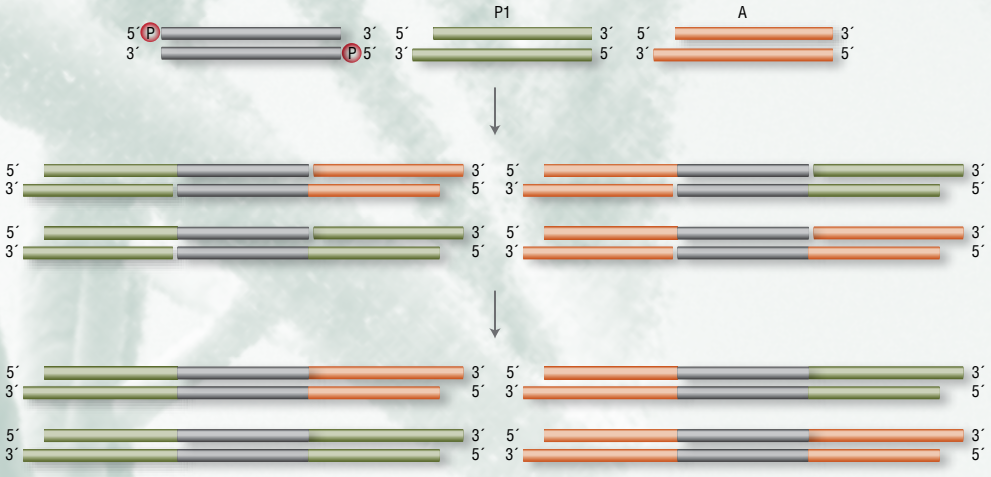
The first step in DNA library preparation is generally fragmentation of purified DNA. Fragmentation can be accomplished by a number of methods including acoustic shearing, nebulization and enzyme-based methods, such as dsDNA Fragmentase®. All of these methods leave a mix of 3´ and 5´ ends (recessed, overhang, blunt) which may or may not be phosphorylated. NEBNext kits for Ion Torrent are available with or without enzyme-based fragmentation reagents, allowing a choice of fragmentation method.

2 End Repair and 5´ Phosphorylation

Blunt-ended fragments are created by filling in or chewing back 3´ and 5´ overhangs. Phosphorylation of the 5´ ends ensures the fragments are suitable for ligation.

3 Adaptor Ligation and Nick Translation

Short adaptor sequences are added to the ends of the DNA fragments by blunt-end ligation. The adaptors are not 5´ phosphorylated, in order to minimize adaptor-dimer formation. Since ligases require a 5´ phosphate for ligation, this absence of 5´ phosphate results in a nick on one strand at each ligation site. This nick is repaired by nick translation, using *Bst* 2.0 WarmStart® DNA Polymerase. The four possible reaction products are shown below.

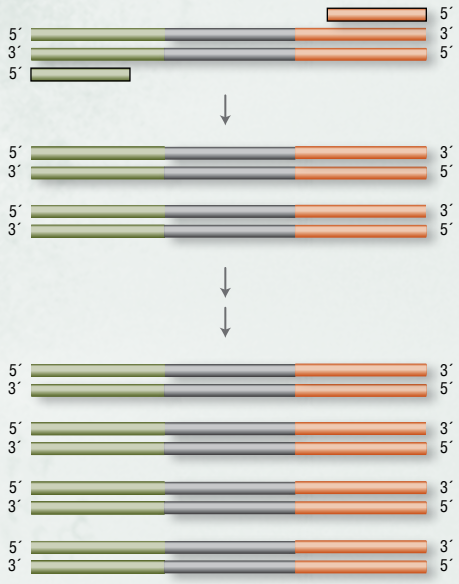


4 PCR Enrichment

Finally, amplification using a high-fidelity polymerase is performed, resulting in a robust library suitable for sequencing.

This step has multiple functions:

- Selection for molecules with the proper adaptor at each end
- Increase the amount of library
- Incorporation of sequences required downstream in the sequencing workflow, and, when desired, barcodes/indices to enable multiplexing. NEBNext kits for Ion Torrent are not supplied with barcode adaptors, but are compatible with adaptors from other sources, including Ion Xpress™ Barcode Adaptors from Life Technologies.

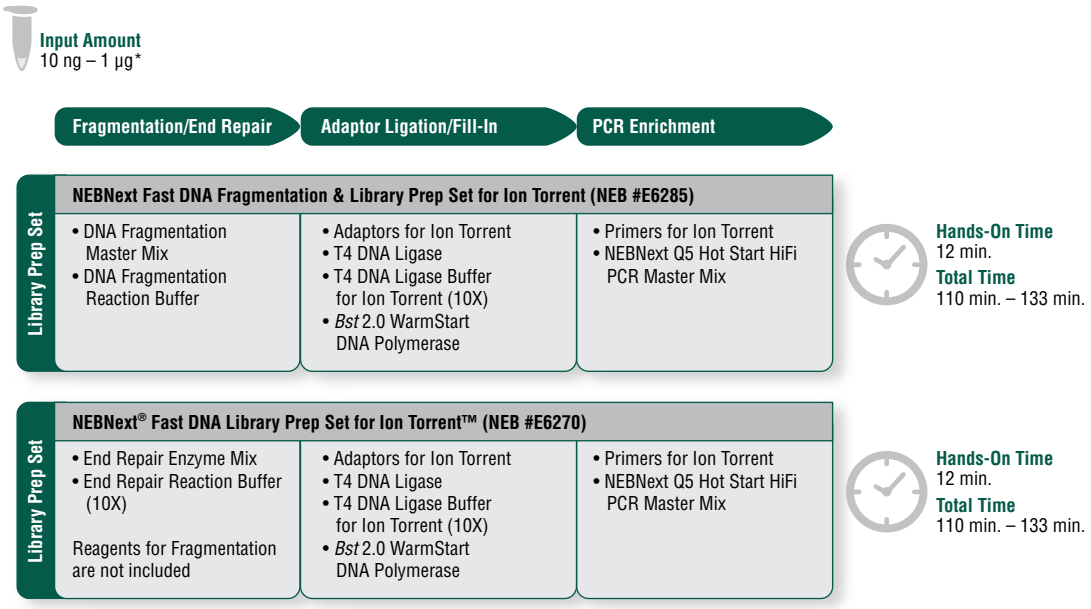


Product Selection

Designed with the user in mind, NEBNext kits maximize efficiency and convenience. For use with Ion Torrent, NEBNext kits are available with or without fragmentation reagents, allowing the user to choose the method of fragmentation. For maximum convenience and ease of scale-up, the NEBNext Fast DNA Fragmentation & Library Prep Set for Ion Torrent (NEB #E6285) includes a master mix for enzyme-based fragmentation. This set contains dsDNA Fragmentase, a mix of two enzymes for random DNA shearing, described on page 9, and this is combined with End Repair reagents for a streamlined workflow. When mechanical shearing methods, such as Covaris® acoustic shearing or nebulization are preferred, the NEBNext Fast DNA Library Prep Set for Ion Torrent (NEB #E6270) is the recommended choice. The components and protocol for this kit have been optimized for the volumes and concentrations of DNA sheared by mechanical methods. Both kits have the same fast workflow and low minimum input DNA requirements.

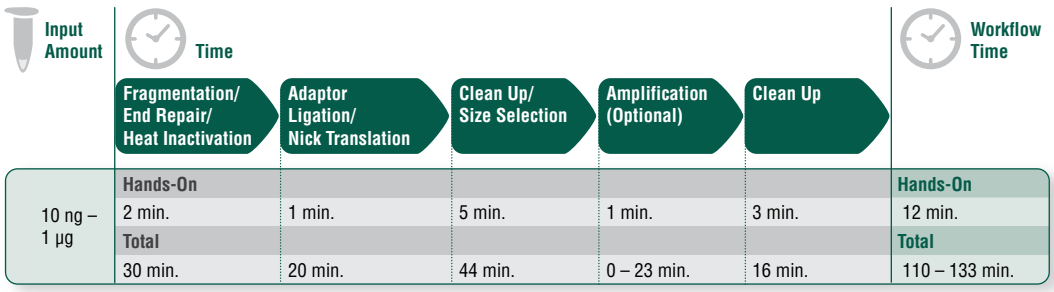
Product Details

In addition to stringent QCs on individual components, the NEBNext DNA kits are functionally validated by library preparation of a genomic DNA library, followed by Ion Torrent sequencing. Reagent lots are reserved specifically for inclusion in NEBNext kits. Most of these reagents are provided in master mix format, reducing the number of vials provided in the kits, and reducing pipetting steps. Adaptors and primers for singleplex libraries are supplied in the kits. For multiplexed libraries, the Ion XPress Barcode Adaptors from Life Technologies can be used.

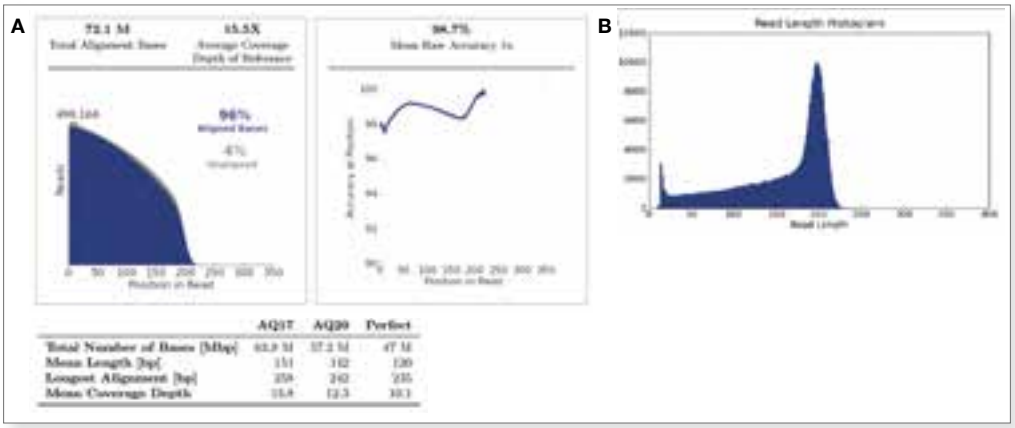


** Note that a minimum of 100 ng is recommended when used in conjunction with Ion Xpress Barcode Adaptors.*

Library Preparation workflow for Ion Torrent is complete in approximately 2 hours

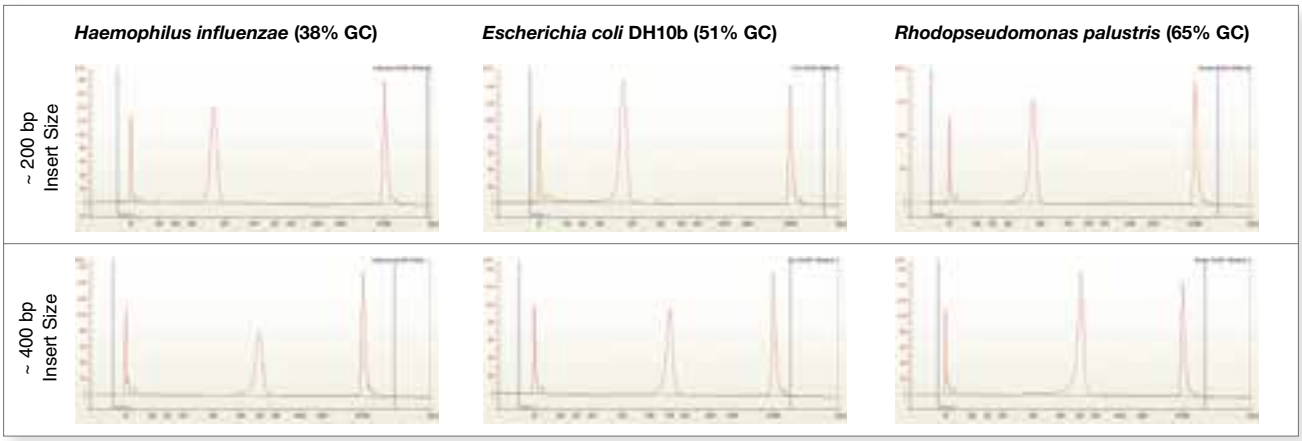


Sequencing Data



A typical Ion Torrent run report for libraries made from E. coli (K12 MG1655 strain) genomic DNA using NEBNext Fast DNA Fragmentation & Library Prep Set for Ion Torrent. A. Alignment Summary. 72 Mb of data was generated, with an average genome coverage of 15.5X, from approximately 0.5 Million 200 bp reads. B. Read length histogram from 200 bp run.

Varying GC Content Libraries

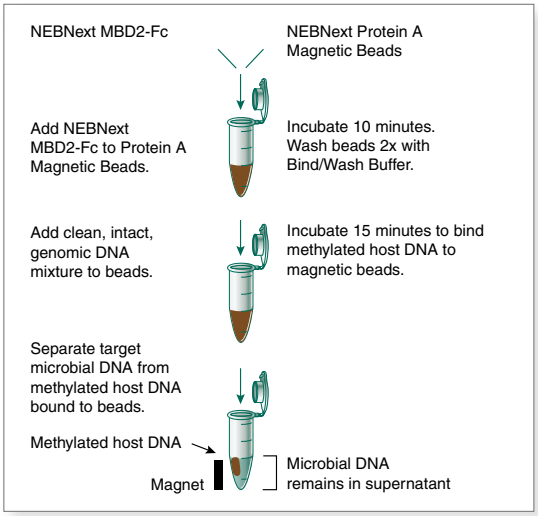


0.5 µg of DNA from 3 different genomes with varying GC content were used to construct 200 bp and 400 bp libraries using the NEBNext Fast DNA Fragmentation and Library Prep Set for Ion Torrent, analyzed by the Agilent® Bioanalyzer®.

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.

Microbiome DNA Enrichment Kit Workflow

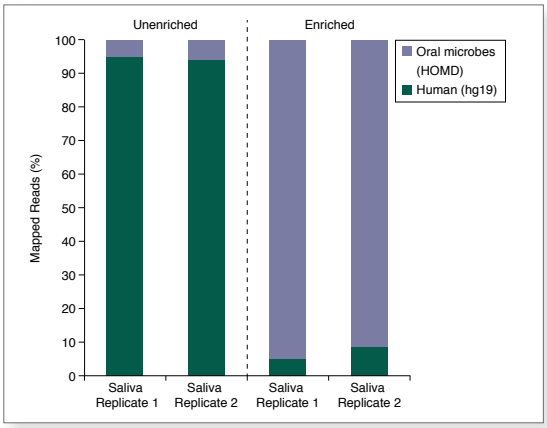


The MBD2-Fc protein binds specifically to CpG methylated DNA. In the NEBNext Microbiome DNA Enrichment workflow, MBD2-Fc is attached to Protein A magnetic beads, enabling capture of methylated DNA, while the microbial DNA remains in the supernatant.

ADVANTAGES

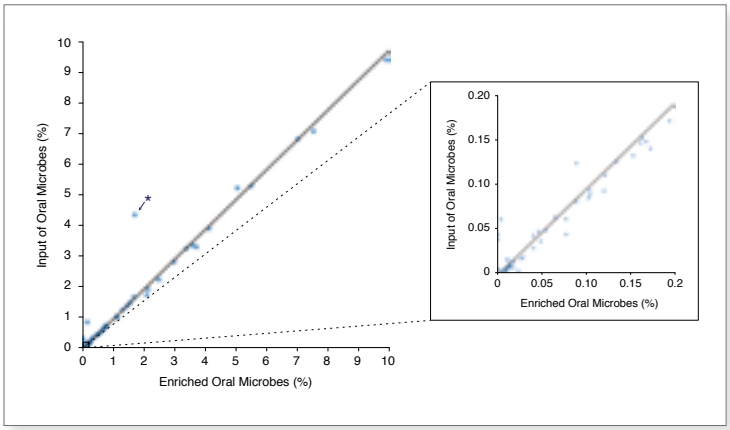
- Effective enrichment of microbial DNA from samples containing contaminating host DNA
- Fast, simple protocol
- Enables microbiome whole genome sequencing, even for samples with high levels of host DNA
- Compatible with downstream applications including next generation sequencing on all platforms, qPCR and end-point PCR
- Suitable for a wide range of sample types
- No requirement for live cells

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)[1]. (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[2] 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[10] 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[13] with typical settings (2 mismatches in a 28 bp seed region, etc).

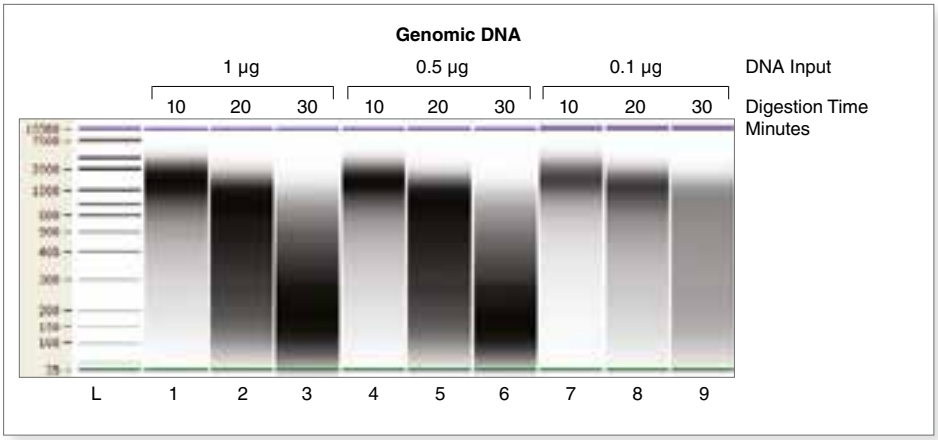
** Neisseria flavescens – This organism may have unusual methylation density, allowing it to bind the enriching beads at a low level. Other Neisseria species (N. mucosa, N. sicca and N. elognata) are represented, but do not exhibit this anomalous enrichment.*

DNA Fragmentation with NEBNext dsDNA Fragmentase

Methods for DNA fragmentation include mechanical shearing (such as acoustic shearing or nebulization) and enzyme-based fragmentation.

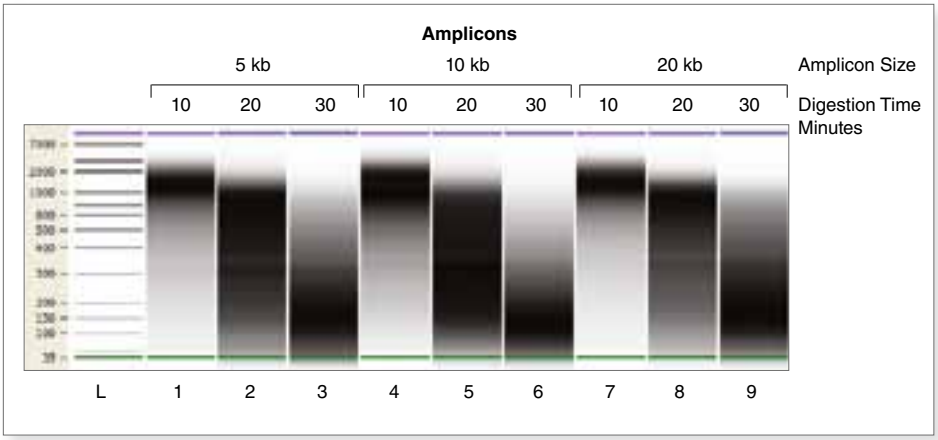
For this step in the DNA library preparation workflow, NEB provides NEBNext dsDNA Fragmentase, an enzyme-based reagent that generates fragments of the desired sizes for NGS libraries in a time-dependent manner. dsDNA Fragmentase contains two enzymes; one randomly generates nicks on dsDNA, and the other recognizes the nicked sites and cuts the opposite DNA strand across from the nick, producing dsDNA breaks. The resulting DNA fragments contain short overhangs, 5´-phosphates and 3´-hydroxyl groups. Randomness of shearing for this application is critical, and dsDNA Fragmentase provides random fragmentation, similar to mechanical methods (1,2). dsDNA Fragmentase is now supplied with an updated buffer (NEBNext dsDNA Fragmentase Reaction Buffer v2) and protocol for increased ease of use and robustness.

Fragmentation of *E. coli* gDNA using dsDNA Fragmentase



E. coli gDNA was fragmented with NEBNext dsDNA Fragmentase for the indicated times and purified on MinElute® columns.

Fragmentation of 5,10 and 20 kb amplicons with NEBNext dsDNA Fragmentase.



Fragmentation of 5,10 and 20 kb amplicons with NEBNext dsDNA Fragmentase.

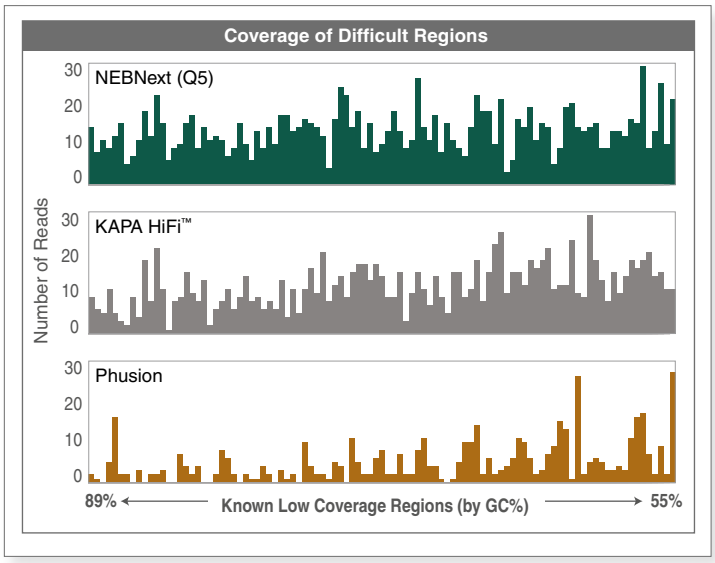
References
1. Knierim, E. et al. (2011) PLOS One 6, e28240.
2. Adey, A. et al. (2010) Genome Biology 11: R119.

Minimized GC bias with the New NEBNext Formulation of Q5® High-Fidelity DNA Polymerase

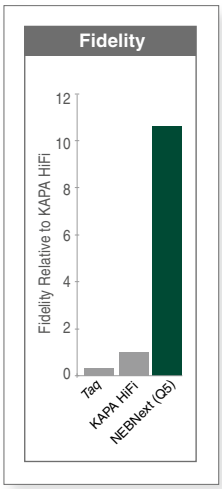
To ensure that sequence data reflects exactly the sequence of the original sample, it is essential that amplification of libraries be performed evenly and with high fidelity. Historically, high-fidelity polymerases have been more susceptible to difficulties in PCR amplification of GC-rich and other challenging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

To minimize GC bias, NEB offers the NEBNext High-Fidelity 2X PCR Master Mix (NEB #M0541). This master mix is specifically optimized for robust, high-fidelity amplification of next-generation sequencing libraries, regardless of GC content. This unique formulation combines the ultra low error rates of Q5 High-Fidelity DNA Polymerase with specific buffers and reaction enhancers to support robust amplification of a broad range of templates, even those with high GC content. The buffer component of the master mix has been optimized for robust, even amplification of NGS libraries, including GC-rich amplicons. This combination makes NEBNext High-Fidelity 2X PCR Master Mix an ideal choice for your NGS library construction.

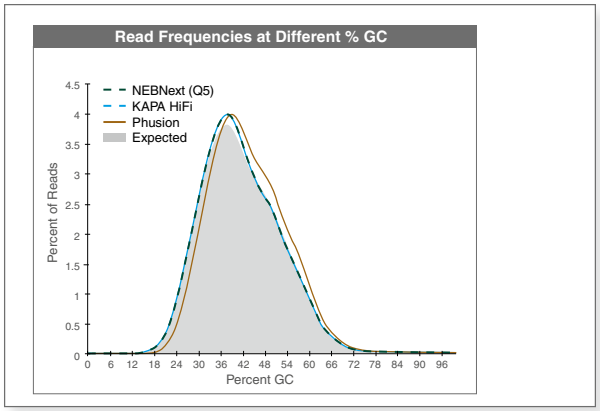
NEBNext High-Fidelity 2X PCR Master Mix minimizes GC bias and error rate



Indexed human IMR90 DNA libraries were split and amplified using 8 cycles of PCR with Phusion High-Fidelity PCR Master Mix with HF® Buffer, KAPA HiFi® HotStart ReadyMix or NEBNext High-Fidelity 2X PCR Master Mix, followed by sequencing on an Illumina HiSeq™ 2000. 180 million reads were randomly extracted from each data set and the number of reads overlapping distinct low coverage regions of the human genome (Aird et.al. Genome Biology, 2011) are shown for each library.



Fidelity measurements of Taq DNA Polymerase (in Standard Taq Buffer), KAPA HiFi HotStart ReadyMix and NEBNext High-Fidelity 2X PCR Master Mix were measured, side-by-side, in a PCR-based mutation screening assay using a lacZ method. Values (n ≥ 2) are expressed relative to KAPA HiFi HotStart ReadyMix.



Amplified libraries of human genomic DNA were generated using index primers and NEBNext High-Fidelity 2X PCR Master Mix, Phusion® High-Fidelity PCR Master Mix with HF Buffer or KAPA HiFi HotStart ReadyMix, and sequenced on an Illumina HiSeq 2000. An equal number of reads from each dataset were randomly selected and the percentage of reads was plotted against GC content. NEBNext High-Fidelity 2X PCR Master Mix and KAPA HiFi HotStart PCR ReadyMix aligned closely to the expected read frequencies (shaded grey), while Phusion did not.

General Handling Tips

- Spin down the vials before opening. This ensures that everything is at the bottom of the vial and not in the lid or on the sides.
- Use filter tips
- Use RNase- and DNase-free plastics, water, and solutions
- Thaw and keep all master mixes on ice
- Keep all sample and reagent vials capped when not in use
- Mix reaction components by pipetting gently up and down, and do not vortex (except when using Fragmentase, which should be vortexed as described in the protocol)
- Return enzymes to the freezer after use
- Always use freshly prepared 80% ethanol
- Keep all sample and reagent vials capped when not in use
- Always use aseptic technique
- Store final libraries in TE, in low-bind DNA tubes

TOOLS & RESOURCES

Visit **NEBNext.com** to:

- View the latest videos from NEB scientists discussing technical tips for library preparation

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 can be assessed using gel electrophoresis. Impurities such as detergents or probes, or damaged DNA can usually be seen as a smear on the gel. RNA impurities are often seen at the bottom of the gel. Absorbance measurements can also 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.

Quantitation of DNA

- It is important to accurately quantify the DNA sample prior to library construction. Fluorescence based detection which utilizes dsDNA specific dyes, such as the Qubit® from Life Technologies, are more accurate than UV spectrometer based measurements, as the presence of RNA or other contaminants can result in overestimation of the DNA sample.

Bead-based clean-ups and size selection

- Be careful when transferring material not to disturb the bead pellet
- Be sure to vortex the beads just before use – they should be a uniform suspension
- Do not over-dry the beads. This can make resuspension difficult and reduce yield.

Ordering Information

KITS FOR ION TORRENT DNA LIBRARY PREPARATION		NEB #	SIZE
DNA	NEBNext Fast DNA Library Prep Set for Ion Torrent	E6270S/L	10/50 rxns
	NEBNext Fast DNA Fragmentation & Library Prep Set for Ion Torrent	E6285S/L	10/50 rxns
MODULES & ENZYMES		**NEB #**	**SIZE**
DNA	NEBNext High-Fidelity 2X PCR Master Mix	M0541S/L	50/250 rxns
	NEBNext dsDNA Fragmentase	M0348S/L	50/250 rxns

France
New England Biolabs France SAS
Genopole Campus 1, Bâtiment 6
5 rue Henri Desbruères
91030 Evry cedex, France
Tel.: 0800 100 632 (Customer Service)
Tel.: 0800 100 633 (Technical Service)
FAX.: 0800 100 610
info.fr@neb.com
www.neb-online.fr

USA
New England Biolabs, Inc.
Telephone (978) 927-5054
Toll Free (USA Orders) 1-800-632-5227
Toll Free (USA Tech) 1-800-632-7799
Fax (978) 921-1350
info@neb.com
www.neb.com

Canada
New England Biolabs, Ltd.
Toll Free: 1-800-387-1095
info.ca@neb.com

China, People's Republic
New England Biolabs (Beijing), Ltd.
Telephone: 010-82378265/82378266
info@neb-china.com

Germany & Austria
New England Biolabs GmbH
Free Call: 0800/246 5227 (Germany)
Free Call: 00800/246 52277 (Austria)
info.de@neb.com

Japan
New England Biolabs Japan, Inc.
Telephone: +81 (0)3 5669 6191
info@neb-japan.com

Singapore
New England Biolabs, PTE. Ltd.
Telephone +65 6776 0903
sales.sg@neb.com

United Kingdom
New England Biolabs (UK), Ltd.
Call Free: 0800 318486
info.uk@neb.com

www.neb-online.fr

nqa ISO 9001 Registered Quality Management
nqa ISO 14001 Registered Environmental Management
nqa ISO 13485 Registered Medical Devices

NEBNEXT_ILL – Version 3.0 – 12/15