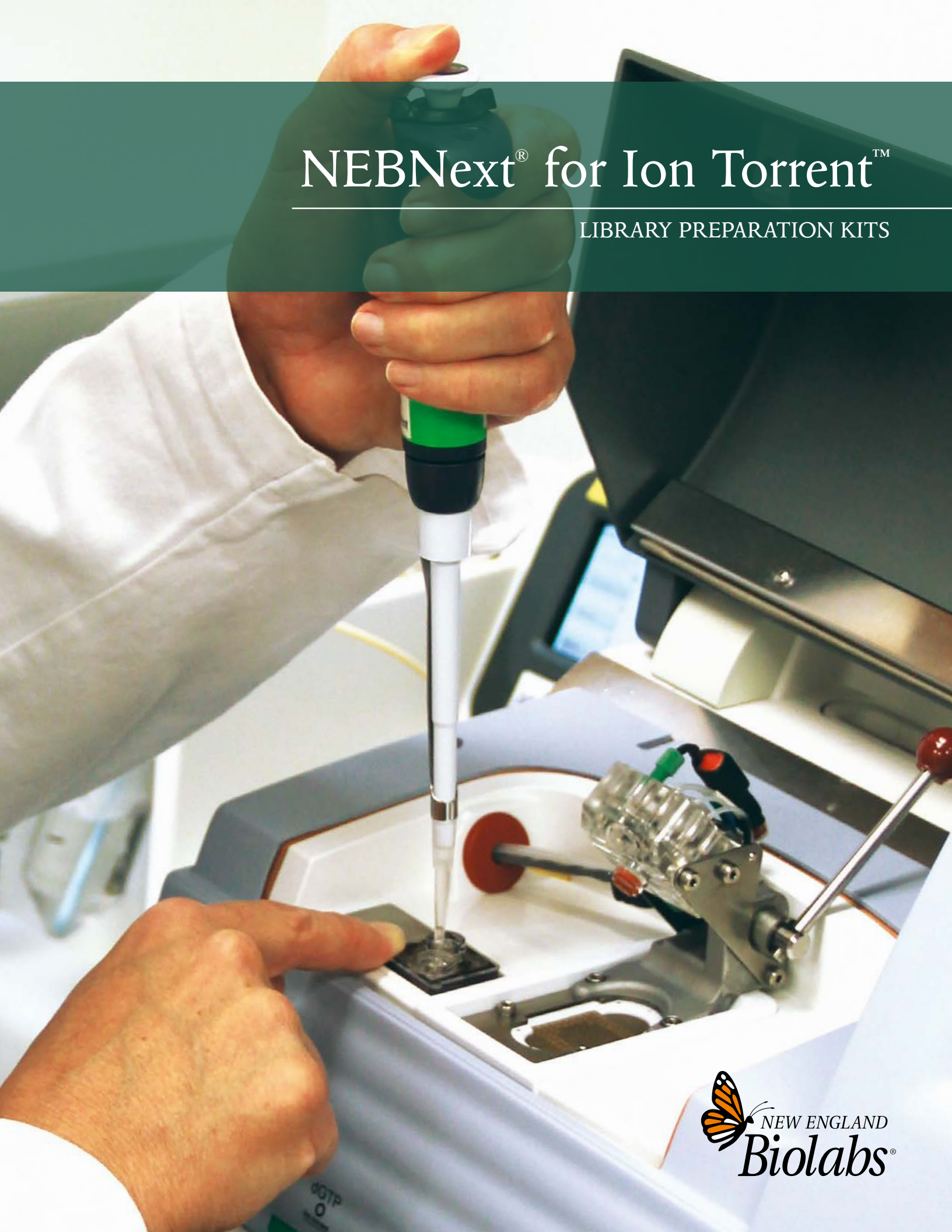
NEBNext® for Ion Torrent™

LIBRARY PREPARATION KITS



NEW ENGLAND Biolabs®

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Why choose NEBNext for Ion Torrent?

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 kits for Ion Torrent 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

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**.

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

Learn more about NEBNext
www.NEBNext.com

Tools & Resources

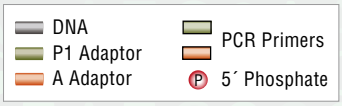
Visit **NEBNext.com** to find:

- 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.

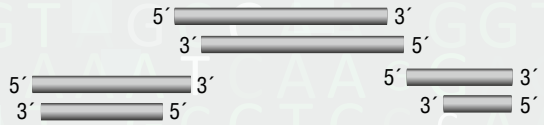
Workflow for Ion Torrent DNA Library Preparation

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



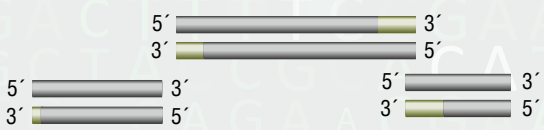
1 DNA Fragmentation

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.



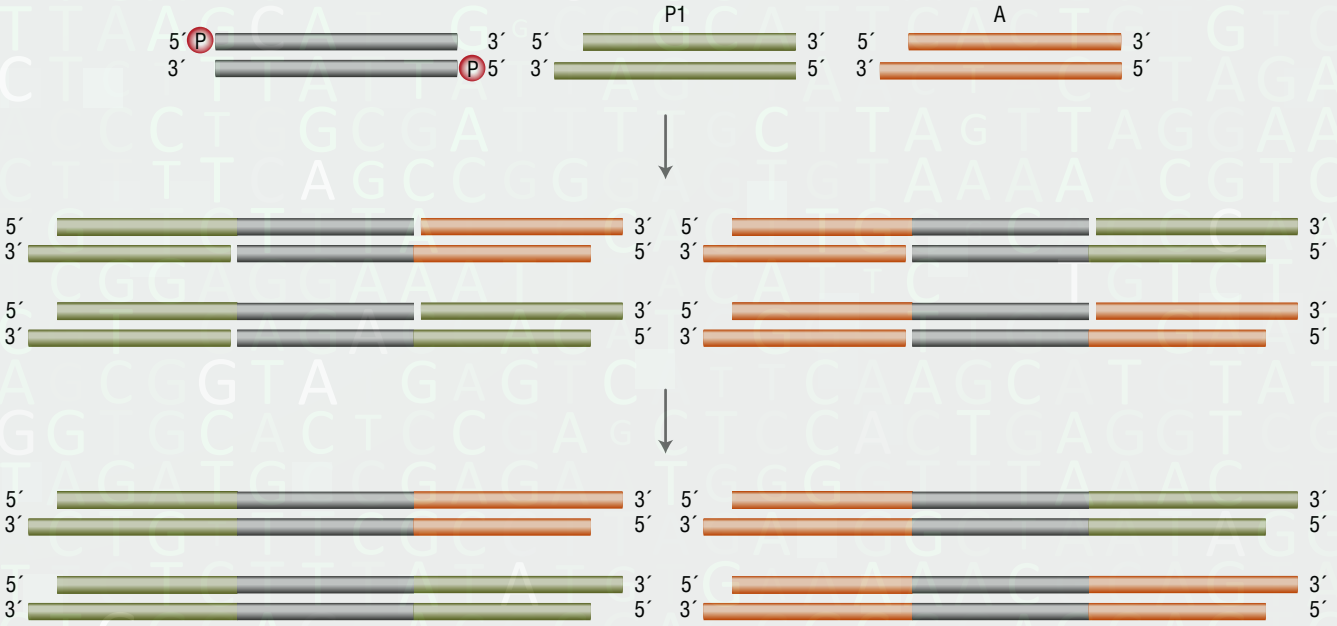
2 End Repair and 5´ Phosphorylation

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.

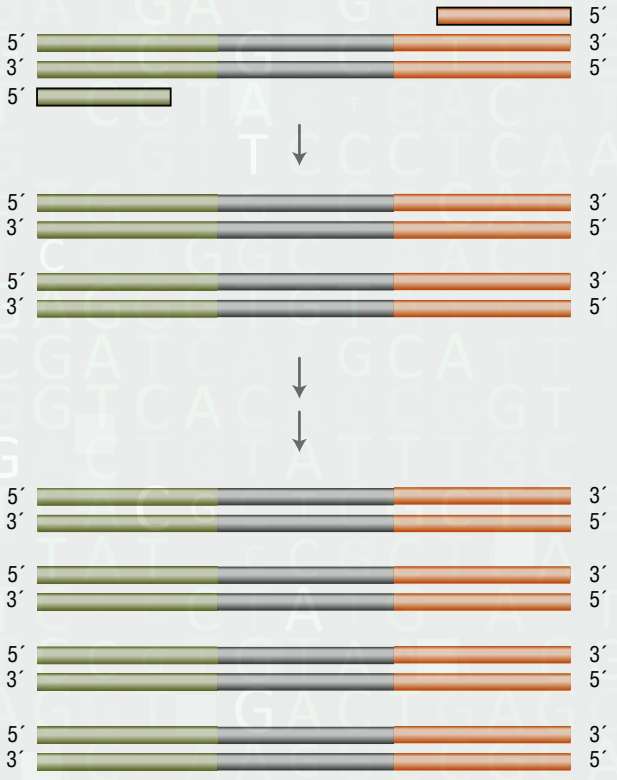


4 PCR Enrichment

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.

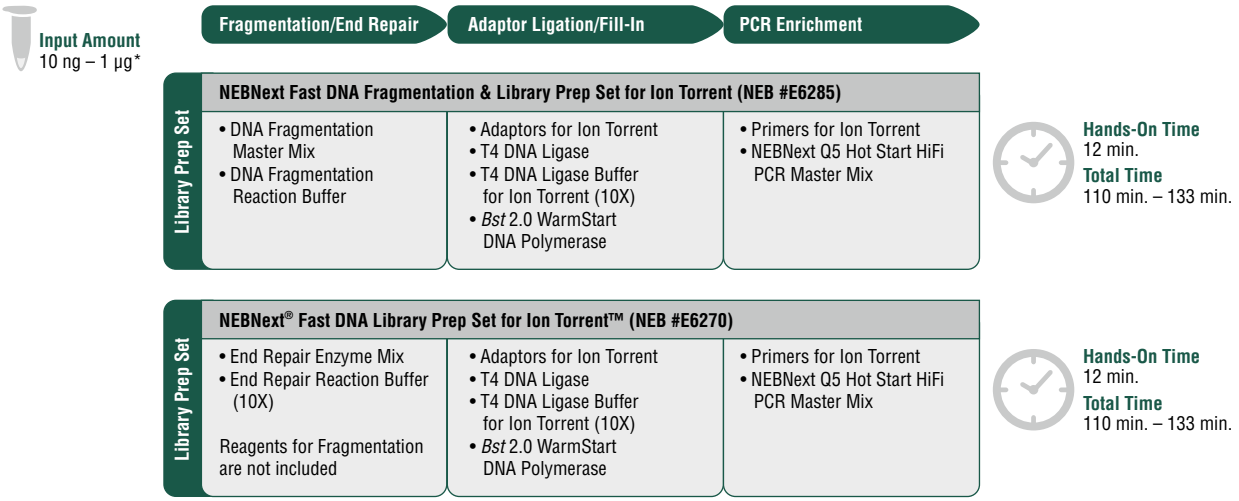


Product Selection

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, 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.



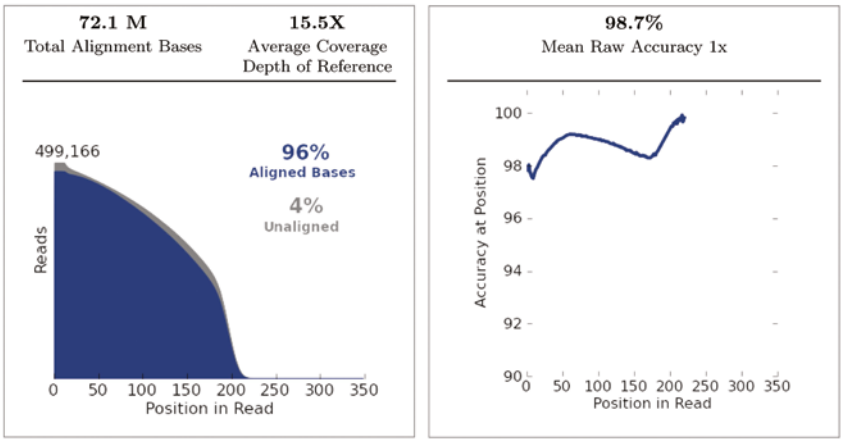
** Note that a minimum of 100 ng is recommended when used in conjunction with Ion Xpress Barcode Adaptors.*

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

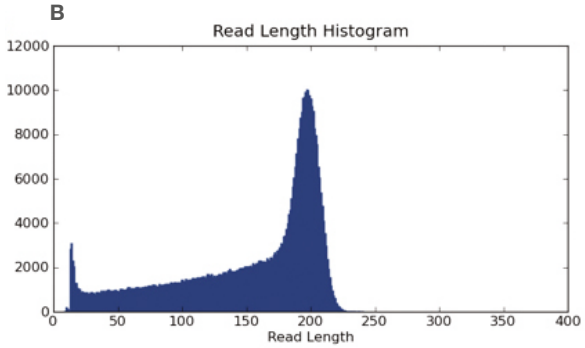
Input Amount	Time					Workflow Time
	Fragmentation/ End Repair/ Heat Inactivation	Adaptor Ligation/ Nick Translation	Clean Up/ Size Selection	Amplification (Optional)	Clean Up	
10 ng – 1 µg	Hands-On					Hands-On
	2 min.	1 min.	5 min.	1 min.	3 min.	12 min.
	Total					Total
	30 min.	20 min.	44 min.	0 – 23 min.	16 min.	110 – 133 min.

Sequencing Data

A



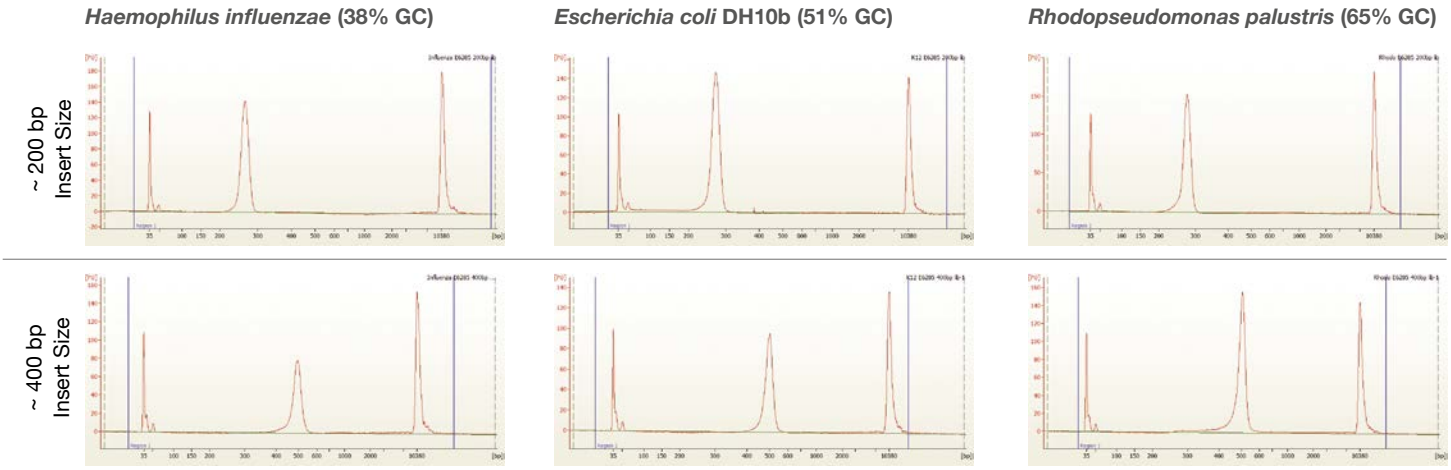
B



	AQ17	AQ20	Perfect
Total Number of Bases [Mbp]	63.9 M	57.2 M	47 M
Mean Length [bp]	151	142	120
Longest Alignment [bp]	258	242	235
Mean Coverage Depth	13.8	12.3	10.1

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®.

Ordering Information

KITS FOR ION TORRENT DNA LIBRARY PREPARATION	NEB #	SIZE
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

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