NGS Single Cell RNA Analysis

NEBNEXT® SINGLE CELL/LOW INPUT LIBRARY PREP KIT

NEW ENGLAND BioLabs®

be INSPIRED
drive DISCOVERY
stay GENUINE

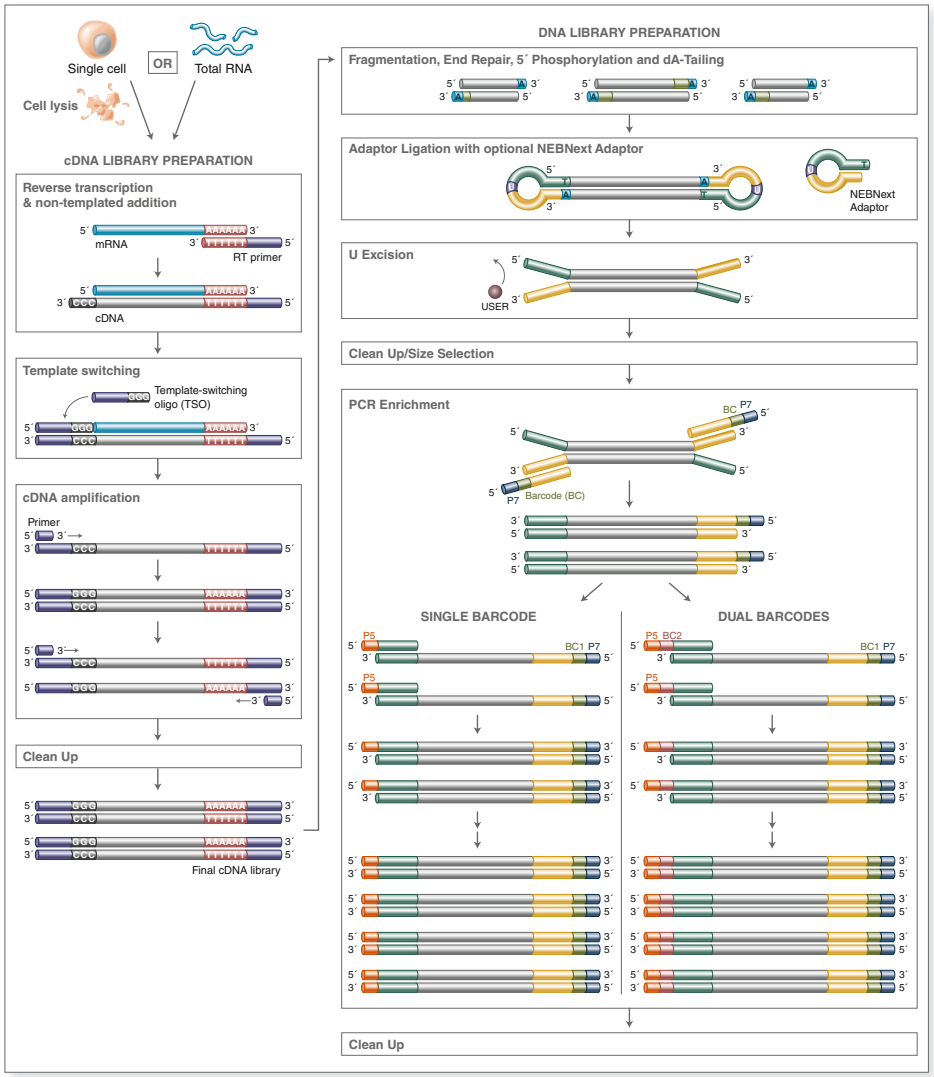
NEBNext® Single Cell/Low Input RNA Library Prep

High-quality transcript sequencing from a single cell or ultra-low input RNA

This unique workflow meets the demand for a highly sensitive, yet robust method that consistently generates high-quality, full-length transcript sequencing data from a single cell or ultra-low input RNA.

Optimized cDNA synthesis and amplification steps incorporate template switching, as well as utilize a unique protocol and suite of reagents. Even low-abundance transcripts are represented in the high yields of cDNA obtained. Subsequent library construction incorporates the Ultra™ II FS enzymatic DNA fragmentation/end repair/dA-tailing mix in a simple and efficient workflow.

NEBNext Single Cell/Low Input RNA Library Prep workflow



Advantages

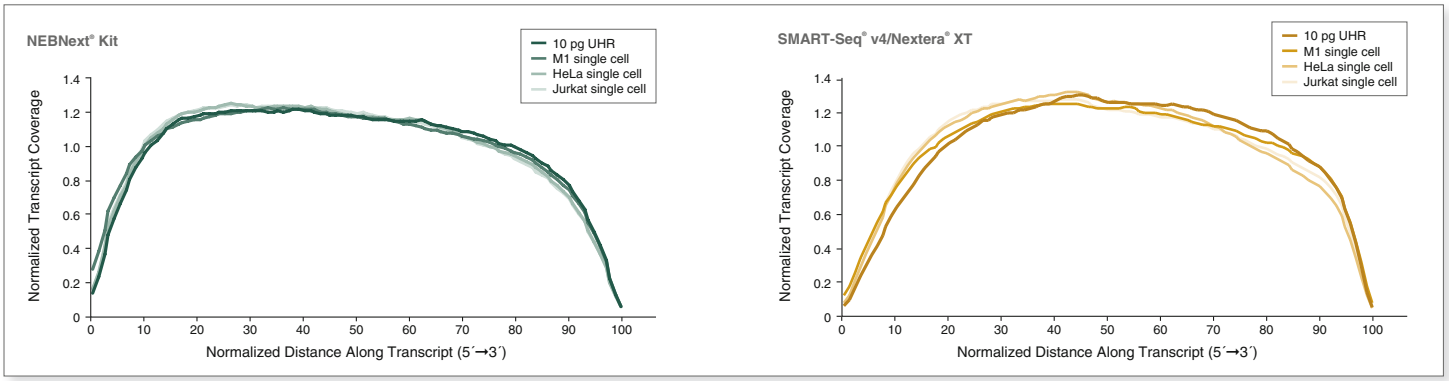
- Generate the highest yields of high-quality full-length transcript sequencing libraries from single cells, or as little as 2 pg – 200 ng total RNA
- Experience unmatched detection of low abundance transcripts
- Rely on consistent transcript detection for a wide range of input amounts and sample types
- Obtain full length, uniform transcript coverage, regardless of input amount or sample type
- Use with cultured or primary cells, or total RNA
- Save time with a fast, streamlined workflow, minimal handling steps and hands-on time
  - Single-tube protocol from cell lysis to cDNA
  - Enzymatic DNA fragmentation, end repair and dA-tailing reagents in a single enzyme mix, with a single protocol, regardless of GC content
- Available with or without library construction reagents

To learn more about the technology, please visit:

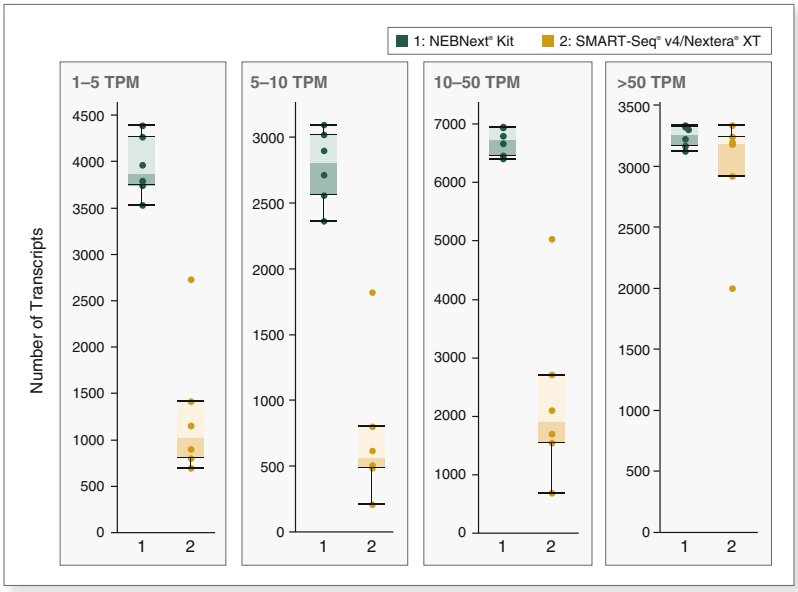
www.neb.com/nebnextsinglecell

and download the extensive technical note.

The NEBNext Single Cell/Low Input RNA Library Prep Kit provides uniform coverage across the length of transcripts

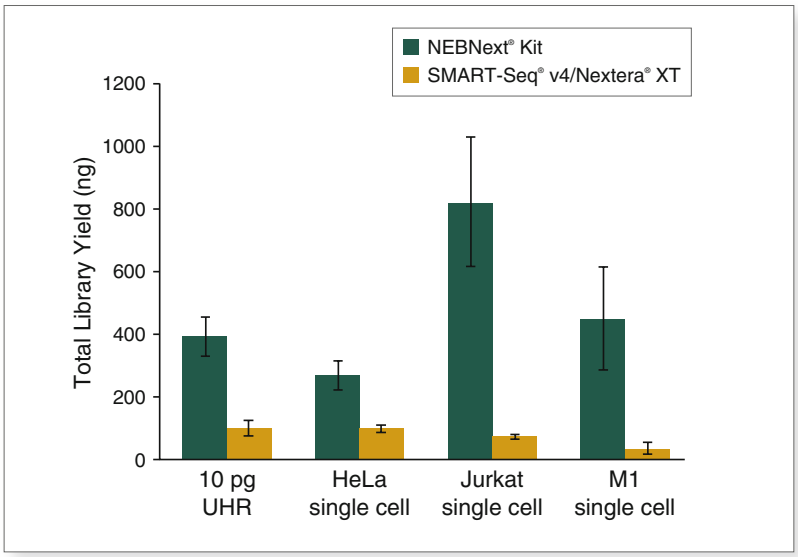


Sequencing libraries were generated from HeLa, Jurkat and M1 single cells, or 10 pg of Universal Human Reference (UHR) RNA (Agilent® #740000) with recommended amounts of ERCC RNA Spike-In Mix I (Thermo Fisher Scientific® #4456740). The NEBNext Single Cell/Low Input RNA Library Prep Kit, or the SMART-Seq v4 Ultra® Low Input RNA Kit for Sequencing (Clontech® # 634891) plus the Nextera XT DNA Library Prep Kit (Illumina® #FC-131-1096) were used. Libraries were sequenced on an Illumina NextSeq® 500 using paired-end mode (2x76 bp). Gene body coverage shown is an average of four replicates and was calculated using Picard tools. The global view of the 5´ to 3´ coverage of the RefSeq transcripts reveals both consistency across different sample types and uniformity across the transcript length in the NEBNext libraries.



The NEBNext Single Cell/Low Input RNA Library Prep Kit increases transcript detection

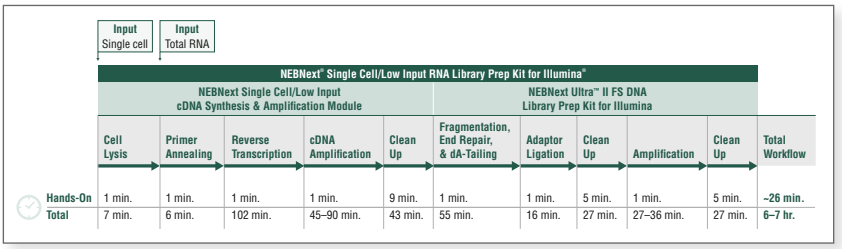
Sequencing libraries were generated from Jurkat single cells (6 replicates) using the NEBNext Single Cell/Low Input RNA Library Prep Kit, or the SMART-Seq v4 Ultra® Low Input RNA Kit for Sequencing (Clontech® # 634891) plus the Nextera XT DNA Library Prep Kit (Illumina® #FC-131-1096). Libraries were sequenced on an Illumina NextSeq® 500 using paired-end mode (2x76 bp). TPM = Transcripts per Kilobase Million. Each dot represents the number of transcripts identified at the given TPM range, and each box represents the median, first and third quartiles per replicate. Salmon 0.6 was used for read mapping and quantification of all GENCODE v25 transcripts. Panels show the number of transcripts detected within the following TPM ranges: 1-5, 5-10, 10-50 and >50 TPM. Increased identification of low abundance transcripts is observed with the NEBNext libraries.



Generate higher library yields with the NEBNext Single Cell/Low Input RNA Library Prep Kit

Sequencing libraries were generated from HeLa, Jurkat and M1 single cells or 10 pg of Universal Human Reference (UHR) RNA (Agilent® #740000) with recommended amounts of ERCC RNA Spike-In Mix I (Thermo Fisher Scientific® #4456740). The NEBNext Single Cell/Low Input RNA Library Prep Kit, or the SMART-Seq v4 Ultra® Low Input RNA Kit for Sequencing (Clontech® #634891) plus the Nextera XT DNA Library Prep Kit (Illumina® #FC-131-1096) were used. For the NEBNext workflow ~80% of the cDNA was used as input into sequencing library preparation, and libraries were amplified with 8 PCR cycles. For the SMART-Seq v4/Nextera XT workflow, as recommended, 125 pg of cDNA was used as input in sequencing library preparation and 12 PCR cycles were used for amplification. Error bars indicate standard deviation for 6-11 replicates.

Fast NEBNext Single Cell/Low Input RNA Library Prep workflow with only 26 min hands-on time!



What users are saying:

> *The implementation of this new single cell RNA-seq pipeline is an important addition to our core Scientific Operations and will enable our scientists to resolve at scale the transcriptional variation within a wide variety of single cells.*
>
> – Sarah Teichmann, PhD, head of cellular genetics at the Wellcome Sanger Institute

> *NEB libraries show greater consistency between cells across all metrics studied (number of reads, mapping rate, 5'-3' coverage, genome features, and transcript detection).*
>
> – Technical Note Fluidigm C1™ https://www.fluidigm.com/c1openapp/scripthub/script/2018-07/nebnext-mrna-sequencing-1530810776075-7

To request a free sample, please visit www.neb.com/nebnextsinglecell

ORDERING INFORMATION

PRODUCTS	NEB #	SIZE
NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina	E6420S/L	24/96 rxns
NEBNext Single Cell/ Low Input cDNA Synthesis & Amplification Module	E6421S/L	24/96 rxns

ALSO AVAILABLE	NEB #	SIZE
NEBNext Multiplex Oligos for Illumina (Index Primers Set 1, 2, 3, 4)	E7335, E7500, E7710, E7730S/L	24/96 rxns
NEBNext Multiplex Oligos for Illumina (96 Index Primers)	E6609S/L	96/384 rxns
NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1)	E7600S	96 rxns
NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs)	E6440S/L	96/384 rxns

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GERMANY & AUSTRIA
New England Biolabs GmbH
Brüningstr. 50, Geb B852
65926 Frankfurt/Main, Germany
Tel: +49/(0)69/305-23140
Fax: +49/(0)69/305-23149

Free Call: 0800/246 5227 (Germany)
Free Call: 00800/246 52277 (Austria)
info.de@neb.com

www.neb-online.de

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

HEADQUARTERS:
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

www.neb.com

AUSTRALIA
New England Biolabs, Inc.
Toll Free: +61 1800 934 218
info.au@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

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

SINGAPORE
New England Biolabs, PTE. Ltd.
Telephone: +65 63859623
sales.sg@neb.com

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

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