NEBNext® Sample Preparation Technologies
FOR NEXT GENERATION SEQUENCING

NEW ENGLAND BioLabs® Inc.

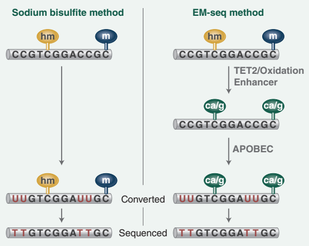
be INSPIRED
drive DISCOVERY
stay GENUINE

ILLUMINA®-COMPATIBLE SAMPLE PREP SOLUTIONS

NEW NEBNEXT ENZYMATIC METHYL-SEQ (EM-seq™)

- Protection of 5mC & 5hmC methylation marks with TET2/Oxidation Enhancer
- Conversion of non-methylated cytosines with APOBEC
- Methylome sequencing with Ultra II DNA (below)

PRODUCT	NEB #
NEBNext Enzymatic Methyl-seq Kit	E7120



GENOMIC DNA LIBRARY PREPARATION

PRODUCT	NEB #	RECOMMENDED INPUT AMOUNTS
NEBNext Ultra™ II FS DNA Library Kit for Illumina/ NEBNext Ultra II FS DNA Library Prep with Sample Purification Beads	E7805, E6177	100 pg – 0.5 µg DNA
NEBNext Ultra II DNA Library Prep Kit for Illumina/ NEBNext Ultra II DNA Library Prep with Sample Purification Beads	E7645, E7103	500 pg – 1 µg DNA
NEBNext Oligos (12-, 96-plex and dual index primers, including unique pairs)	E7335, E7500, E7710, E7730, E6609, E7600, E7780, E6440	

ULTRA II FS DNA WORKFLOW

DNA Fragmentation, End Repair & dA-Tailing

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

DNA Fragmentation (Not Required for ChIP)

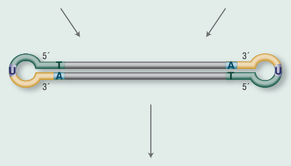
- Fragmentation by acoustic shearing, nebulization or enzyme-based methods

End Repair & dA-Tailing

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

Adaptor Ligation

- Ligation of short adaptors (contain sequences required downstream)
- A novel hairpin loop structure increases ligation efficiency & minimizes adaptor-dimer formation

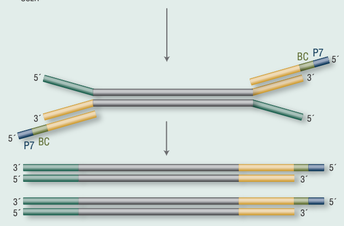


U Excision

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

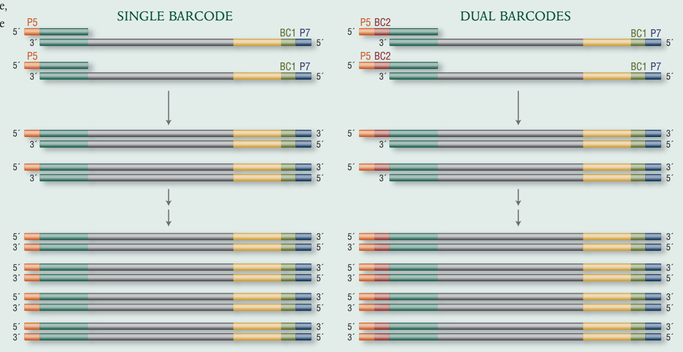
PCR Enrichment

- 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

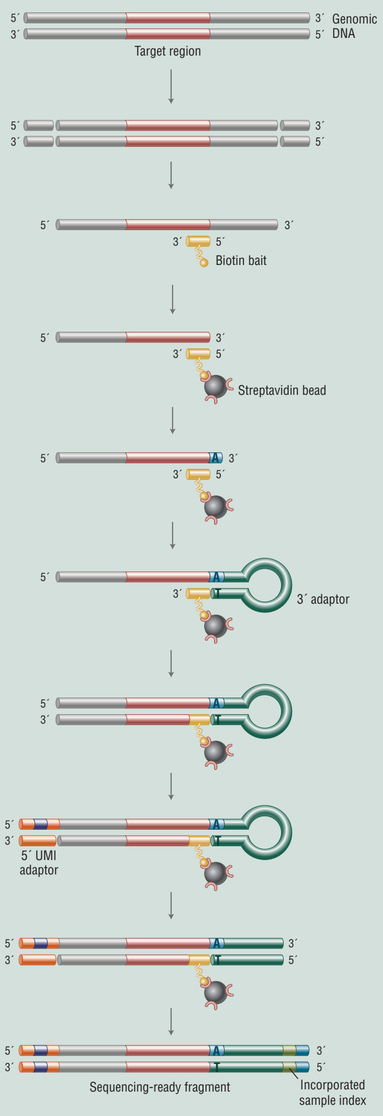
- Barcodes incorporated using NEBNext primers
- Single- or dual-barcode primer options available, including unique dual barcodes



NEBNEXT DIRECT® TARGET ENRICHMENT

This unique approach enriches a wide range of genomic targets directly from DNA, eliminating the need to generate a library prior to enrichment. Enriched regions are converted to a sequencer-ready library through simple enzymatic manipulations that remove off-target sequence and ligate universal adaptors. The short single-bait design enables flexible targeting and compatibility with degraded clinical samples, while incorporation of UMIs enables superior determination of allelic frequencies.

PRODUCT	NEB #
NEBNext Direct Custom Ready Panels	E6631
NEBNext Direct Cancer HotSpot Panel	E7000
NEBNext Direct BRCA1/BRCA2 Panel	E6627



Fragmentation

- Genomic DNA is fragmented, either mechanically or using included nicking reagents

Denaturation & bait hybridization

- Biotin bait targets both strands (shown for one strand)

3´ blunting of DNA

- Enzymatic removal of off-target sequence

dA-tailing

- Creation of single 3´ A overhang enables ligation to adaptor with a single T overhang

Ligation of 3´ adaptor

- 3´ hairpin loop adaptor is ligated

5´ blunting of DNA

- Bait is extended to the 5´ end of the randomly sheared fragment, creating a variable 5´ end of the target read

Ligation of 5´ UMI adaptor

- 5´ Unique Molecule Index (UMI) addition enables removal of PCR duplicate reads

Adaptor cleaving

- 3´ adaptor is cleaved

PCR amplification

- Sample index is added during PCR

NEBNEXT RNA DEPLETION

Efficient removal of ribosomal RNA (rRNA) from total RNA for human, mouse and rat samples. This method works well for both low-quality/degraded RNA (including FFPE RNA) and high-quality, intact RNA.

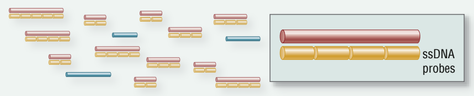
PRODUCT	NEB #
NEBNext rRNA Depletion Kit (Human/Mouse/Rat)/ NEBNext rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads	E6310, E6350
NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat)/ NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads	E7750, E7755

Total RNA

- Total RNA contains greater than 80% rRNA (red)

Binding of ssDNA probes

- Single-stranded DNA probes hybridize specifically to rRNA molecules



rRNA degradation by Ribonuclease H (RNase H)

- RNase H degrades the hybridized RNA (rRNA)

Probe degradation by DNase I & clean up

- DNase I degrades the DNA probes

rRNA-depleted RNA

- Non-rRNA species (blue) are enriched

RNA LIBRARY PREPARATION

PRODUCT	NEB #	RECOMMENDED INPUT AMOUNTS
NEBNext Ultra II Directional RNA Library Prep Kit for Illumina/ NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads	E7760, E7765	5 ng – 1 µg Total RNA (rRNA Depletion Workflow)
NEBNext Ultra II RNA Library Prep Kit for Illumina/ NEBNext Ultra II RNA Library Prep with Sample Purification Beads	E7770, E7775	10 ng – 1 µg Total RNA (poly(A) mRNA workflow)
NEBNext Oligos (12-, 96-plex and dual index primers, including unique pairs)	E7335, E7500, E7710, E7730, E6609, E7600, E7780, E6440	
NEBNext Poly(A) mRNA Magnetic Isolation Module	E7490	

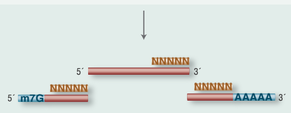
RNA Enrichment (RNA Depletion or Poly(A) mRNA Isolation)

- Removal of abundant RNAs (e.g., > 80% of total RNAs are rRNAs) or enrichment of mRNAs
- NEBNext Library Prep kits are compatible with either method

ULTRA II RNA WORKFLOW

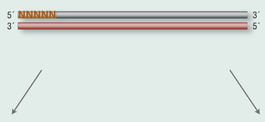
RNA Fragmentation & Random Priming

- Fragmentation by incubation with divalent cations (e.g., Mg^{++}) or enzymes (e.g., RNase III)
- Hybridization of random primers



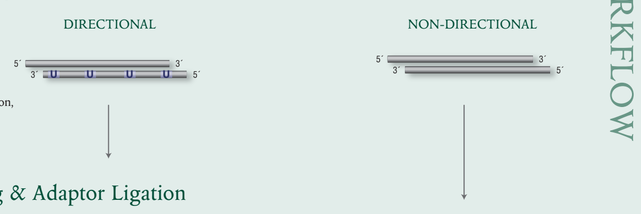
First Strand cDNA Synthesis

- Reverse transcriptase lacking RNase H activity is optimal (does not degrade RNA in RNA:DNA complex)
- For directional RNA library preparation, Actinomycin D is added:
  - To inhibit DNA-dependent DNA Polymerase activity of RT & inhibit second strand synthesis/increase strand specificity



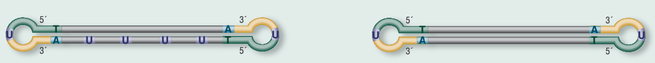
Second Strand cDNA Synthesis

- Generation of nicks & gaps in RNA by RNase H, enabling second strand synthesis by nick translation
- Sealing of breaks in second strand by *E. coli* DNA ligase
- For Directional RNA library preparation, second strand labeled with uracils by dUTP incorporation



End Repair, dA-Tailing & Adaptor Ligation

- Generation of blunt, phosphorylated ends
- Addition of single A 3´ overhang (enables ligation to adaptors with single T overhangs)
- Ligation of short adaptors (contain sequences required downstream)
- NEBNext adaptors increase ligation efficiency & minimize adaptor-dimer formation



U Excision

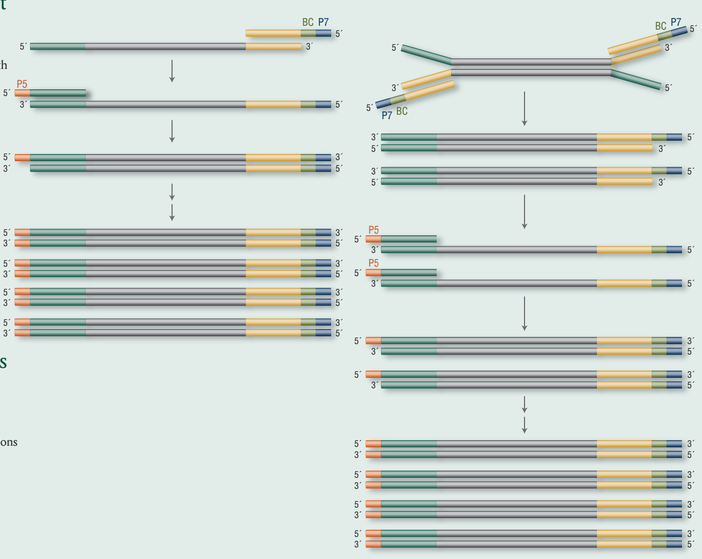
- Removal of uracils in NEBNext Adaptor loop by USER Enzyme (to make accessible for PCR)

Directional Only

- Selective removal of second strand through excision of uracils by USER Enzyme
- Result is single-stranded molecule with different adaptor-derived sequences on each end

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
- Unique dual-, dual-, and single-barcode primer options available

NEBNEXT FFPE DNA REPAIR MIX

Archiving of clinical materials as Formalin-Fixed, Paraffin-Embedded (FFPE) samples significantly damages the nucleic acids within these samples. It can be challenging to obtain high-quality sequence data, especially when sample amounts are limited. The NEBNext FFPE DNA Repair Mix is a cocktail of enzymes optimized and validated for repair of FFPE DNA samples.

PRODUCT	NEB #
NEBNext FFPE DNA Repair Mix	M6630

FFPE DNA Sample Preparation Workflow

Extract & Purify DNA → Shear DNA → FFPE DNA Repair → Library Construction

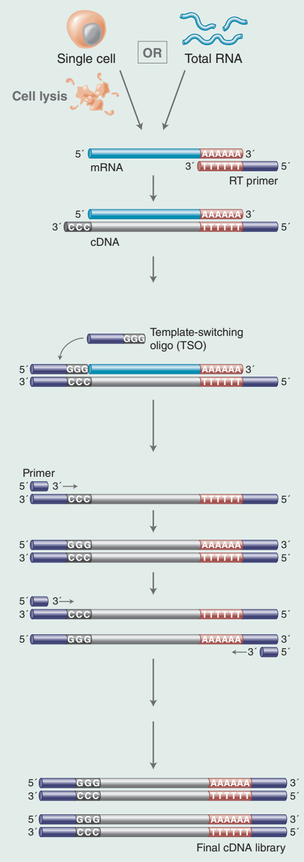
Ability of FFPE DNA damage to be repaired by the NEBNext FFPE DNA Repair Mix

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

NEW SINGLE CELL/LOW INPUT RNA

PRODUCT	NEB #	RECOMMENDED INPUT AMOUNTS
NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina/ NEBNext Single Cell/Low Input cDNA Synthesis & Amplification Module	E6420, E6421	Single cells or 2 pg – 200 ng RNA

SINGLE CELL/LOW INPUT RNA WORKFLOW



Reverse transcription & non-templated addition

- Generation of cDNA with a non-templated CCC tail

Template switching

- Template-switching enables production of full-length cDNAs with a common sequence at the 3´ end

cDNA amplification

- Amplification of the full-length cDNA provides better coverage of 5´ ends

Final cDNA library

- Following a clean-up step, the final cDNA library is ready to move into the Ultra II FS DNA workflow

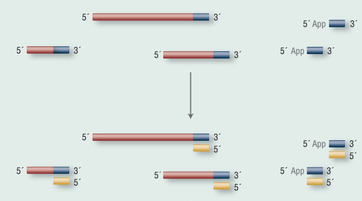
SMALL RNA

PRODUCT	NEB #	RECOMMENDED INPUT AMOUNTS
NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 1)/ NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 2)/ NEBNext Multiplex Small RNA Library Prep Kit for Illumina (Index Primers 1-48)	E7300, E7580, E7560	100 ng – 1 µg Total RNA

SMALL RNA WORKFLOW

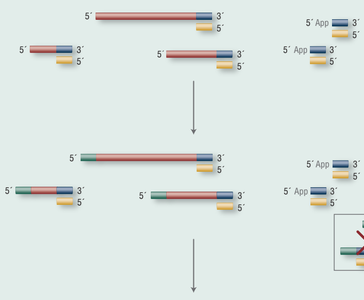
3´ Adaptor Ligation

- Input is purified total RNA
- Ligation of 5´-adenylated, 3´-blocked, single-stranded DNA adaptor to 3´ end of RNA



Primer Hybridization

- Hybridization of RT primer to 3´ adaptor-ligated molecules & any remaining 3´ adaptors

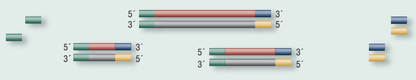


5´ Adaptor Ligation

- Preferential ligation of 5´ adaptor to single-stranded molecules (and therefore not to double-stranded 3´ adaptor:RT primer hybrid molecule)
- Result is minimized formation of adaptor-dimers

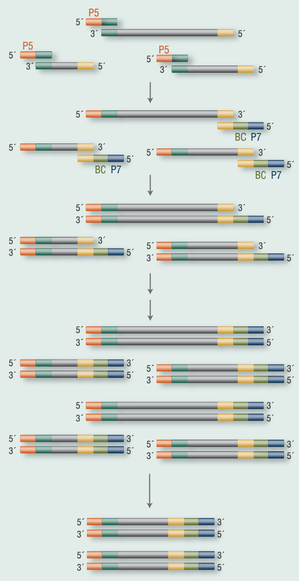
First Strand cDNA Synthesis

- Extension from RT primer synthesizes first strand cDNA
- Reverse transcriptase lacking RNase H activity is optimal (does not degrade RNA in RNA:DNA complex)



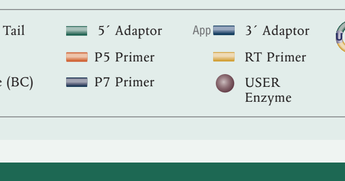
PCR Enrichment

- Amplification with 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



Size Selection

- Ensures that only Small RNAs of interest are included in final library



DNA · RNA · Random Primer · Poly(A) Tail · Uracil · Barcode (BC) · 5´ Adaptor · P5 Primer · P7 Primer · 3´ Adaptor · RT Primer · USER Enzyme · NEBNext Adaptor

NEBNEXT LIBRARY QUANT KIT

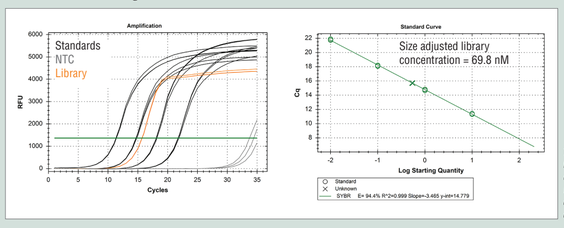
Accurate quantitation of NGS libraries is essential for maximizing sequencing data output and quality. qPCR is considered to be the most accurate and effective method of library quantitation, providing considerably higher consistency and reproducibility than electrophoresis or spectrophotometry, which measure total nucleic acid concentration. Amplification-based methods quantitate only those molecules that contain both adaptor sequences, thereby providing a more accurate estimate of the concentration of library molecules that can be sequenced.

PRODUCT	NEB #
NEBNext Library Quant Kit For Illumina	E7630

Library Quantitation Workflow

Reagent Preparation → Library Dilution → Set Up → qPCR → Data Analysis

4 standards are used to generate the standard curve



Typical results from the NEBNext Library Quant Kit with 4 standards on a Bio-Rad® CFX96 Touch™, with default settings. Amplification curve (left) and resulting standard curve (right).

For more information, visit NEBNext.com

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