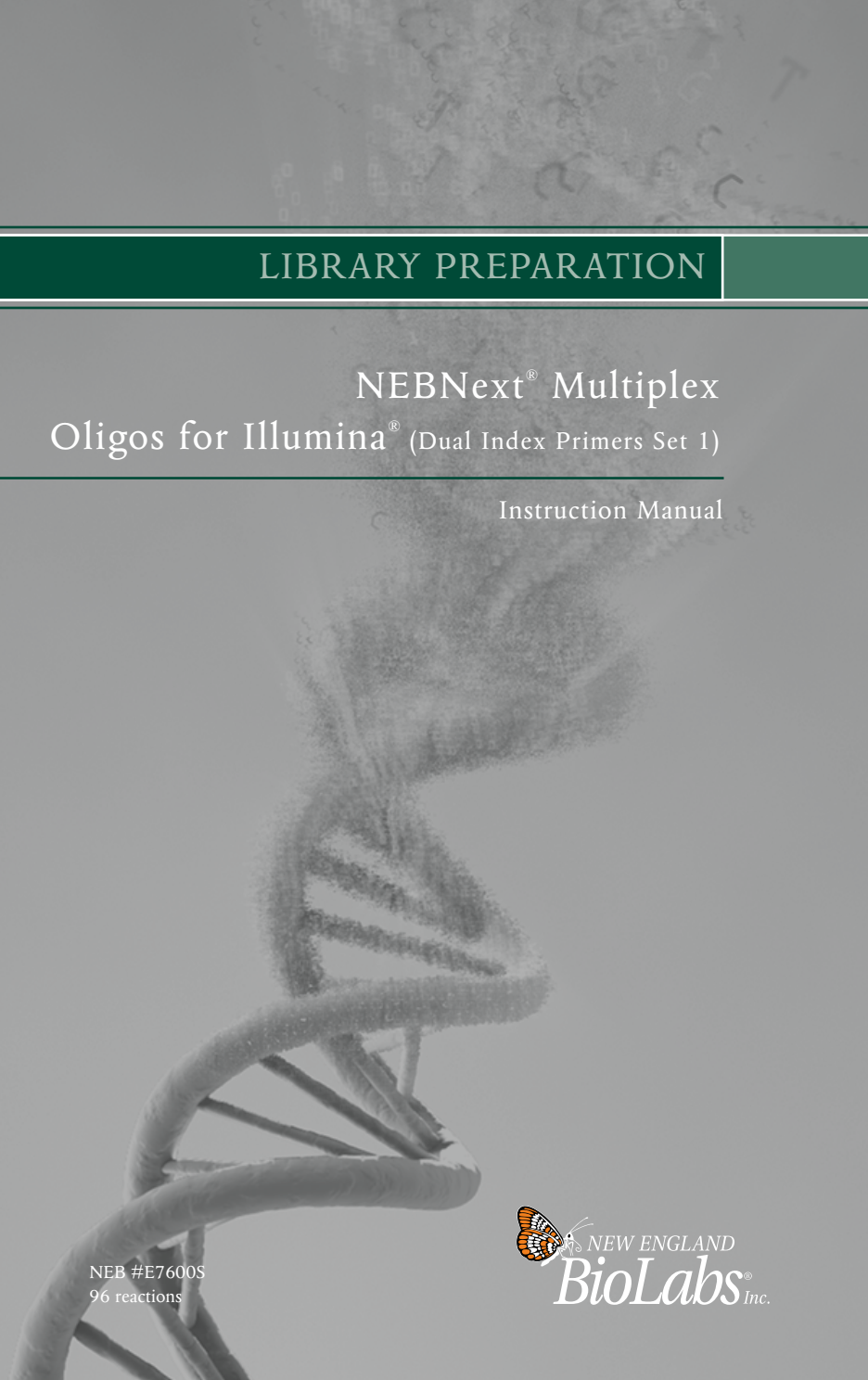
LIBRARY PREPARATION

NEBNext® Multiplex Oligos for Illumina® (Dual Index Primers Set 1)

Instruction Manual



NEB #E7600S
96 reactions

NEW ENGLAND BioLabs® Inc.

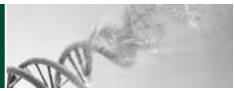


Table of Contents:

The Library Prep Kit Includes:

The volumes provided are sufficient for preparation of up to 96 reactions (NEB #E7600S). (All reagents should be stored at –20°C).

- (red) NEBNext Adaptor for Illumina
- (red) USER Enzyme
- (white) NEBNext i501 Primer
- (white) NEBNext i502 Primer
- (white) NEBNext i503 Primer
- (white) NEBNext i504 Primer
- (white) NEBNext i505 Primer
- (white) NEBNext i506 Primer
- (white) NEBNext i507 Primer
- (white) NEBNext i508 Primer
- (orange) NEBNext i701 Primer
- (orange) NEBNext i702 Primer
- (orange) NEBNext i703 Primer
- (orange) NEBNext i704 Primer
- (orange) NEBNext i705 Primer
- (orange) NEBNext i706 Primer
- (orange) NEBNext i707 Primer
- (orange) NEBNext i708 Primer
- (orange) NEBNext i709 Primer
- (orange) NEBNext i710 Primer
- (orange) NEBNext i711 Primer
- (orange) NEBNext i712 Primer

Required Materials Not Included for DNA or ChIP Libraries:

Enzymes and buffers appropriate for DNA or ChIP Illumina library preparation. (DNA: #E7645, #E7370, #E6040, #E6000 ChIP: #E7645, #E7370, #E6240, #E6200)

Agencourt® AMPure® XP Beads (Beckman Coulter, Inc. #A63881)

Magnetic Stand

DNA LoBind® Tubes (Eppendorf®)/PCR Tubes/96-well PCR Plates

Freshly prepared 80% Ethanol

Nuclease-free Water

0.1X TE (or 10 mM Tris-HCl, pH 7.5–8.0)

10 mM Tris-HCl, pH 8.0

10 mM NaCl

Bioanalyzer® (Agilent Technologies, Inc.)

Bio-Rad® Microseal® (Bio-Rad Laboratories, Inc.) "A" Film or equivalent

Recommended products not supplied:

TruSeq® Index Plate Fixture (Illumina #FC-130-1005) or similar setup (see page 6)

Required Materials Not Included for RNA Libraries:

Enzymes and buffers appropriate for RNA Illumina library preparation. (#E7420, #E7530, #E6110, #E6100)

NEBNext Poly(A) mRNA Magnetic Isolation Module (#E7490), or NEBNext rRNA Depletion Kit (Human, Mouse, Rat) (#E6310) or similar

Magnetic Rack (Alpaqua, cat #A001322 or equivalent)

80% Ethanol (freshly prepared)

0.1X TE (or 10 mM Tris-HCl, pH 7.5–8.0)

10 mM NaCl

Agencourt AMPure XP Beads (Beckman Coulter, Inc. #A63881)

Actinomycin D (Sigma# A1410, dissolved in dimethylsulfoxide [DMSO] to 5 µg/µl).

DNA LoBind Tubes/PCR Tubes/96-well PCR Plates

Bioanalyzer (Agilent Technologies, Inc.)

Bio-Rad Microseal (Bio-Rad Laboratories, Inc.) "A" Film or equivalent

Recommended products not supplied:

TruSeq Index Plate Fixture (Illumina #FC-130-1005) or similar setup (see page 6).

Additional materials required for use with #E6110 and #E6100:

3 M Sodium Acetate, pH 5.5

100% Ethanol

PCR Column Purification Kit (Qiagen or other)

DNA Gel Extraction Column Purification Kit

RNeasy® MinElute® Clean Up Kit (Qiagen® #74204)

Applications:

The NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1) contains the adaptor and index primers that are ideally suited for multiplex sample preparation for next-generation sequencing on the Illumina platform. Each of these components must pass rigorous quality control standards and are lot controlled, both individually and as a set of reagents.

Lot Control: The lots provided in the NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1) are managed separately and are qualified by additional functional validation. Individual reagents undergo standard enzyme activity and quality control assays, and also meet stringent criteria in the additional quality controls listed on each individual component page.

Functionally Validated: Each set of reagents is functionally validated together through construction and sequencing of genomic DNA libraries on the Illumina platform.

For larger volume requirements, customized and bulk packaging is available by purchasing through the OEM/Bulks department at NEB. Please contact OEM@neb.com for further information.

Please Refer to the Kit Specific Protocol for using the NEBNext Multiplex Oligos for Illumina:

The following kits are designed for use with the NEBNext Multiplex Oligos for Illumina:

- #E7645, NEBNext Ultra II DNA Library Prep Kit for Illumina
- #E7595, NEBNext Ultra II Ligation Module
- #E7420, NEBNext Ultra Directional RNA Library Prep Kit for Illumina
- #E7530, NEBNext Ultra RNA Library Prep Kit for Illumina
- #E7370, NEBNext Ultra DNA Library Prep Kit for Illumina
- #E7445, NEBNext Ultra Ligation Module
- #E6040, NEBNext DNA Library Prep Master Mix Set for Illumina
- #E6110, NEBNext mRNA Library Prep Master Mix Set for Illumina
- #E6240, NEBNext ChIP-Seq Library Prep Master Mix Set for Illumina
- #E6056, NEBNext Quick Ligation Module
- #E6000, NEBNext DNA Library Prep Reagent Set for Illumina
- #E6200, NEBNext ChIP-Seq Library Prep Reagent Set for Illumina
- #E6100, NEBNext mRNA Library Prep Reagent Set for Illumina

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Setting up the PCR Reaction

NEBNext Multiplex Oligos for Illumina
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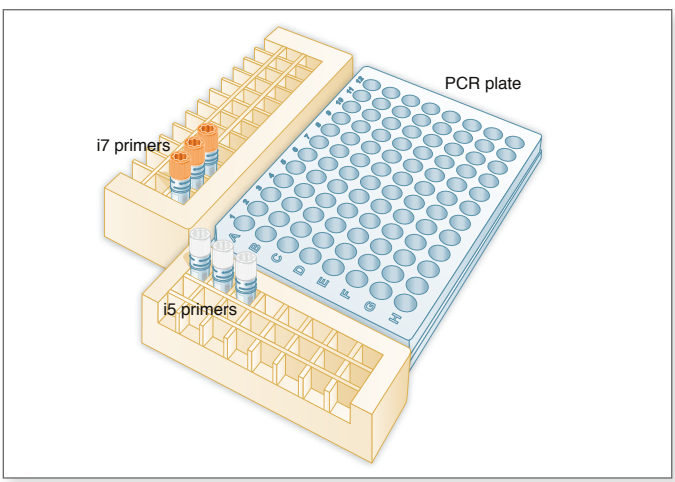
1.1 PCR Amplification

For < 96 samples, follow the protocol in Section 1.1A. For 96 samples, follow the protocol in Section 1.1B.

1.1A Setting up the PCR reactions (< 96 samples)

Note: We recommend using a PCR work-up rack such as the TruSeq Index Plate Fixture (Illumina #FC-130-1005) to assist in properly combining the index primers during the PCR amplification step. Alternatively, 96-well deep well plates can be used and aligned against a PCR plate as in the diagram below.



1. Ensure that a valid combination of i7 and i5 primers is used. See Appendix A to verify that correct primer combinations have been selected.

2. Arrange the index primers in the Index Plate Fixture as follows:

   a. Arrange the ● (orange) i7 primers in increasing order horizontally, so that the lowest number i7 index primer is in column 1, second lowest number i7 index primer is in column 2, etc).

   b. Arrange the ○ (white) i5 primers in increasing order vertically, so that the lowest number i5 index primer is in row A, second lowest number i5 index primer is in row B, etc).

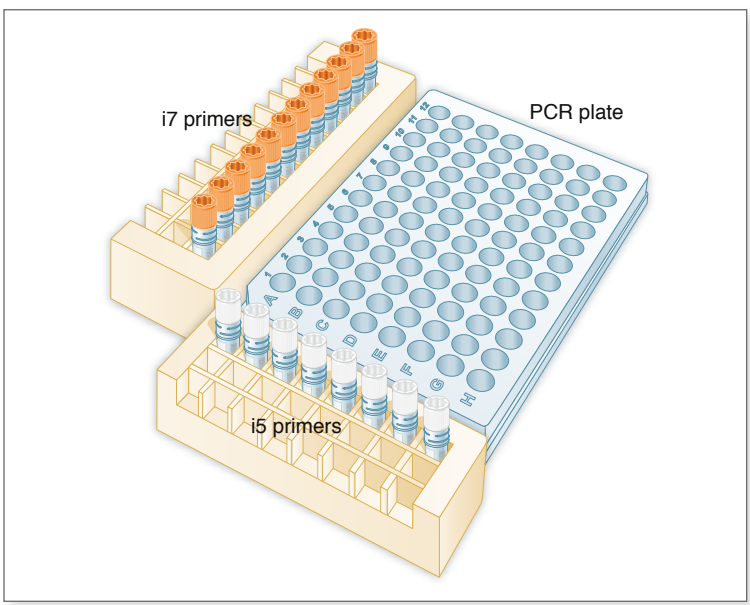
   c. Record their positions on the PCR setup template (see Appendix B).

3. Using a multichannel pipette, add desired volume of ○ (white) i5 primers to every column (as needed) of the PCR plate. **It is critical to change tips between columns to avoid cross-contamination.**

4. Discard the original i5 white caps and apply new caps to avoid index cross-contamination.
5. Using a multichannel pipette, add desired volume of ● (orange) i7 primers to every row (as needed) of the PCR plate. **It is critical to change tips between rows to avoid cross-contamination.**
6. Discard the original i7 orange caps and apply new caps to avoid index cross-contamination.
7. Add 25 µl ● (blue) NEBNext PCR Master Mix to each well that contains primers.
8. Add desired volume of adaptor ligated DNA for a final volume of 50 µl to the corresponding well. Gently pipette up and down 5–10 times to mix. **It is critical to change tips between samples to avoid cross-contamination.** Record each sample position on the PCR setup template (see Appendix B).
9. Cover the plate with Bio-Rad Microseal "A" Film, and seal with a rubber roller. Quickly centrifuge.
10. Perform PCR according to recommended cycling conditions.

1.1B Setting up the PCR reactions (96 samples)

Note: We recommend using a PCR work-up rack such as the TruSeq Index Plate Fixture (Illumina #FC-130-1005) to assist in properly combining the index primers during the PCR amplification step. Alternatively, 96-well deep well plates can be used and aligned against a PCR plate as in the diagram below.



1. Ensure that a valid combination of i7 and i5 primers is used. See Appendix A to verify that correct primer combinations have been selected.

2. Arrange the index primers in the Index Plate Fixture as follows:

   a. Arrange ● (orange) i7 primers in increasing order horizontally, so that i701 is in column 1, i702 is in column 2, i703 is in column 3, etc.

   b. Arrange the ○ (white) i5 primers in increasing order vertically, so that i501 is in row A, i502 is in row B, i503 is in row C, etc.

   c. Record their positions on the PCR setup template (see Appendix B).

3. Using a multichannel pipette, add desired volume of ○ (white) i5 primers to every column of the PCR plate. **It is critical to change tips between columns to avoid cross-contamination.**

4. Discard the original i5 white caps and apply new caps to avoid index cross-contamination.
5. Using a multichannel pipette, add desired volume of ● (orange) i7 primers to every row of the PCR plate. **It is critical to change tips between rows to avoid cross-contamination.**
6. Discard the original i7 orange caps and apply new caps to avoid index cross-contamination.
7. Add 25 µl ● (blue) NEBNext PCR Master Mix to each well.
8. Add desired volume of adaptor ligated DNA for a final volume of 50 µl to the corresponding well. Gently pipette up and down 5–10 times to mix. **It is critical to change tips between samples to avoid cross-contamination.** Record each sample position on the PCR setup template (see Appendix B).
9. Cover the plate with Bio-Rad Microseal "A" Film, and seal with a rubber roller. Quickly centrifuge.
10. Perform PCR according to recommended cycling conditions.

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Appendix A: Principle for Use and Pooling Guide

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The Principle of Dual Index Primers

The dual index primer strategy utilizes two 8 base indices within each primer; i7 primers contain indices that are adjacent to the P7 sequence; i5 primers contain indices that are adjacent to the P5 sequence. Dual indexing is enabled by adding a unique index to both ends of a sample to be sequenced. Up to 96 different samples can be uniquely indexed by combining each of the 12 i7 primers with each of the 8 i5 primers. Similarly, < 96 samples can be uniquely indexed by combining i7 primers with i5 primers as follows:

N = Number of samples = X(i7) * Y(i5) + other primers as needed

Examples:

1. **For N = 12 samples**

   Option 1: 4 (i7) * 3 (i5)

   From the i7 primers, choose a valid set of 4. From the i5 primers choose a valid set of 3. Use each i7 primer with each i5 primer to form 12 primer pairs for PCR amplification of 12 libraries. When setting up the sequencing run, select “Dual Index” and choose the indices used for each sample.

   Option 2: 3 (i7) * 4 (i5)

   From the i7 primers, choose a valid set of 3. From the i5 primers choose a valid set of 4. Use each i7 primer with each i5 primer to form 12 primer pairs for PCR amplification of 12 libraries. When setting up the sequencing run, select “Dual Index” and choose the indices used for each sample.

   Option 3: 6 (i7) * 2 (i5)

   From the i7 primers, choose a valid set of 4 and add any other two i7 primers, for a total of 6 primers. From the i5 primers choose a valid set of 2. Use each i7 primer with each i5 primer to form 12 primer pairs for PCR amplification of 12 libraries. When setting up the sequencing run, select “Dual Index” and choose the indices used for each sample.

   Option 4: 12 (i7) * 1 (i5)

   Use all 12 i7 primers. Use any i5 primer. Use each i7 primer with the i5 primer to form 12 primer pairs for PCR amplification of 12 libraries. When setting up the sequencing run, select “Single Index”, and choose the i7 index used for each sample.

1. For N = 26 samples

Option 1: 6 (i7) * 4 (i5) + 2 (i5)

From the i7 primers, choose a valid set of 4 and add any other two i7 primers, for a total of 6 primers. From the i5 primers choose a valid set of 4 and add any other two i5 primers, for a total of 6 primers. Use each i7 primer with four of the i5 primers to form 24 primer pairs. Use any of the six i7 primers with the remaining two i5 primers to form 2 primer pairs. This will give you a total of 26 primer pairs for PCR amplification of 26 libraries. When setting up the sequencing run, select “Dual Index” and choose the indices used for each sample.

Option 2: 6 (i7) * 5 (i5)

From the i7 primers, choose a valid set of 4 and add any other two i7 primers, for a total of 6 primers. From the i5 primers choose a valid set of 4 and add any other one i5 primer. Use each i7 primer with each i5 primer to form 30 primer pairs for PCR amplification. Use 26 of the 30 primer pairs to amplify 26 libraries. When setting up the sequencing run, select “Dual Index” and choose the indices used for each sample.

Low Plexity Pooling Guidelines

Illumina uses a red laser/LED to sequence A/C and a green laser/LED to sequence G/T. For each cycle, both the red and the green channel need to be read to ensure proper image registration (i.e. A or C must be in each cycle, and G or T must be in each cycle). If this color balance is not maintained, sequencing the index read could fail. The following table lists some valid combinations that can be sequenced together. Note: for 1-plex (no pooling), use any i7 primer with any i5 primer. In this case it is important to select “0” index reads in the Illumina Experiment Manager.

CAUTION: Sufficient primers are provided to generate 96 different samples if each i5 primer is used only once with each i7 primer.

If using subsets of i5 and i7 primers multiple times, you may have to readjust primer pairs to be able to generate 96 samples.

Table 2.1 Pooling: 2–12 libraries; Sequencing Workflow: Single Index (Select "1" Index Reads in the Illumina Experiment Manager).

PLEX	i7 PRIMERS	i5 PRIMERS
2	i701 and i702 i703 and i704 i705 and i706 i707 and i708 i709 and i710 i711 and i712	Any i5 Primer
3	i701, i702 and i703 i703, i704 and i705 i705, i706 and i707 i707, i708 and i709 i709, i710 and i711	Any i5 Primer
4	i701, i702, i703 and i704 i703, i704, i705 and i706 i705, i706, i707 and i708 i707, i708, i709 and i710 i709, i710, i711 and i712	Any i5 Primer
5–12	Any valid i7 4-plex with any other i7 Primers	Any i5 Primer

Table 2.2 Pooling: 7+ libraries; Sequencing Workflow: Dual Index (Select "2" Index Reads in the Illumina Experiment Manager).

PLEX	i7 PRIMERS	i5 PRIMERS
7–12	Any 3 plex combination from Table 2.1 with and any other i7 primer (as needed)	i501 and i502 i503 and i504 i505 and i506 i507 and i508
Greater than 12	Any 4 plex combination from the Table 2.1 with any other i7 primer (as needed)	i501, i502 and any other i5 primer (as needed) i503, i504 and any other i5 primer (as needed) i505, i506 and any other i5 primer (as needed) i507, i508 and any other i5 primer (as needed)

Tables 2.1 and 2.2 do not include an extensive list of all valid index combinations. Please check the sequences of each index to be used to ensure that you will have signal in both the red and green channels for every cycle. See example below:

GOOD			
i7 PRIMERS		i5 PRIMERS	
i7-PCR Index 1	ATTACTCG	i5-PCR Index 3	CCTATCCT
i7-PCR Index 2	TCCGGAGA	i5-PCR Index 4	GGCTCTGA
i7-PCR Index 3	CGCTCATT	i5-PCR Index 5	AGGCGAAG
i7-PCR Index 4	GAGATTCC	i5-PCR Index 6	TAATCTTA
	✓✓✓✓✓✓✓✓		✓✓✓✓✓✓✓✓

BAD			
i7 PRIMERS		i5 PRIMERS	
i7-PCR Index 1	ATTACTCG	i5-PCR Index 2	ATAGAGGC
i7-PCR Index 2	TCCGGAGA	i5-PCR Index 4	GGCTCTGA
i7-PCR Index 3	CGCTCATT	i5-PCR Index 6	TAATCTGA
i7-PCR Index 4	GAGATTCC	i5-PCR Index 8	GTACTGAC
	✓✓✓✓✓✓✓✓		✓✓✗✓✓✗✓✗

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Appendix B: PCR Setup Template

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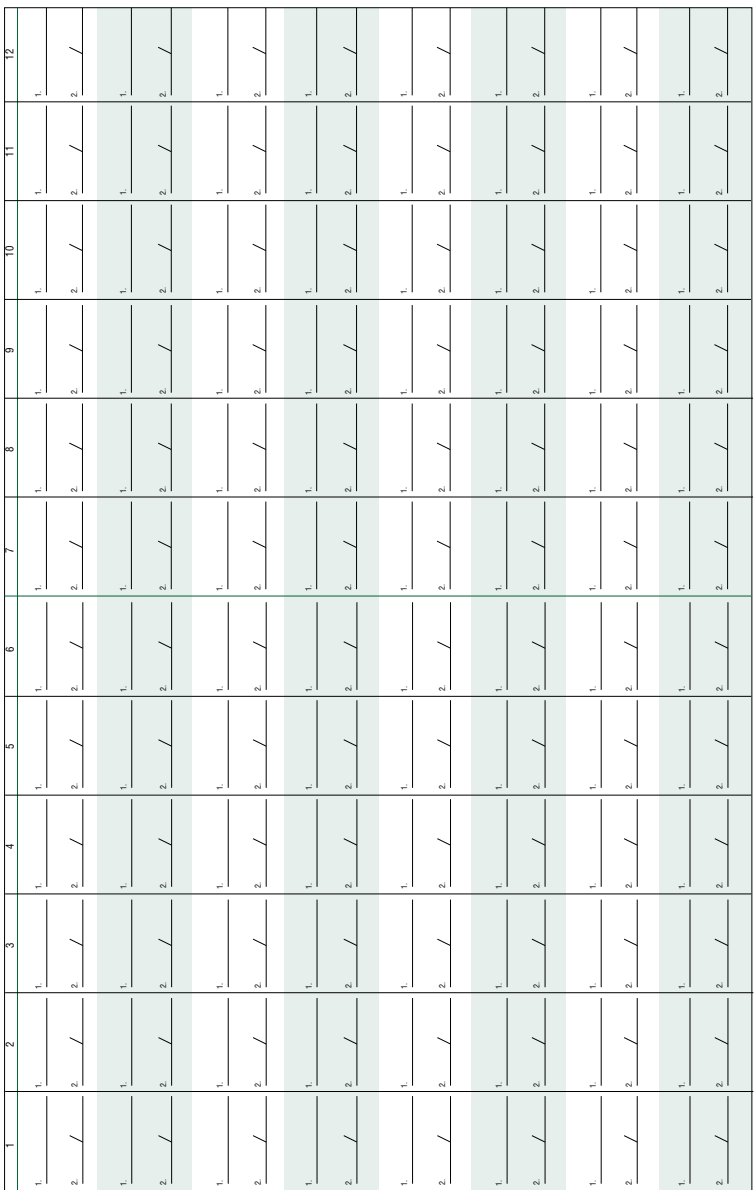
For each well, record: 1. DNA Sample Name _____

2. Index Primer Pairs _____



NEBNext Adaptor for Illumina

#E7601A: 0.96 ml Concentration: 15 µM

5´-/5Phos/GAT CGG AAG AGC ACA CGT CTG AAC TCC AGT C/ideoxyU/A CAC TCT TTC CCT ACA CGA CGC TCT TCC GAT C*T-3´

Store at –20°C

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing this adaptor at 1X concentration and 1 µg of HindIII digested Lambda DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis. 50 µl reactions containing this reaction buffer at 1X concentration and 1 µg T3 DNA incubated for 16 hours at 37°C also results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis.

Endonuclease Activity: Incubation of a 50 µl reaction of this adaptor at a 1X concentration with 1 µg of ϕX174 RF 1 DNA in assay buffer for 4 hours at 37°C in 50 µl reactions results in < 10% conversion to RF II as determined by agarose gel electrophoresis.

Phosphatase Activity: Incubation of a minimum of 10 µl of this adaptor in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) containing 2.5 mM *p*-nitrophenyl phosphate at 37°C for 4 hours yields no detectable *p*-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

RNase Activity: Incubation of this adaptor at a 1X concentration with 40 ng of a FAM-labeled RNA transcript for 16 hours at 37°C results in no detectable RNase activity as determined by polyacrylamide gel electrophoresis.

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USER Enzyme

#E7602A: 0.288 ml

Store at –20°C

Supplied in: 50 mM KCl, 5 mM NaCl, 10 mM Tris-HCl (pH 7.4 @ 25°C), 0.1 mM EDTA, 1 mM DTT, 175 µg/ml BSA and 50% Glycerol

Quality Control Assays

Non-Specific DNase Activity (16 Hour): A 50 µl reaction in NEBuffer 1 containing 1 µg of Lambda DNA and a minimum of 50 units of Uracil DNA Glycosylase incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis. A 50 µl reaction in Endonuclease VIII Reaction Buffer containing 1 µg of Lambda-HindIII DNA and a minimum of 25 units of Endonuclease VIII incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

Exonuclease Activity (Radioactivity Release): A 50 µl reaction in NEBuffer 1 containing 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA and a minimum of 50 units of Uracil DNA Glycosylase incubated for 4 hours at 37°C releases < 0.1% of the total radioactivity. A 50 µl reaction in Endonuclease VIII Reaction Buffer containing 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA and a minimum of 10 units of Endonuclease VIII incubated for 4 hours at 37°C releases < 0.5% of the total radioactivity.

Endonuclease Activity (Nicking): A 50 µl reaction in UDG Reaction Buffer containing 1 µg of supercoiled ϕX174 DNA and a minimum of 50 units of Uracil DNA Glycosylase incubated for 4 hours at 37°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.

Phosphatase Activity: Incubation of a minimum of 10 µl of USER at a 1X concentration in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) containing 2.5 mM *p*-nitrophenyl phosphate at 37°C for 4 hours yields no detectable *p*-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

Lot Controlled

NEBNext i501 Primer–NEBNext i508 Primer

Description: 8 Index Primers are included for producing barcoded libraries.

NEB #	PRODUCT	INDEX PRIMER SEQUENCE	EXPECTED INDEX PRIMER SEQUENCE READ
#E7603A: 0.060 ml	**NEBNext i501 Primer**	5´-AATGATACGGCGACCACCGAGATC-TACACTATAGCCTACACTCTTTCCCTA-CACGACGCTCTTCCGATC*T-3´	TATAGCCT
#E7604A: 0.060 ml	**NEBNext i502 Primer**	5´-AATGATACGGCGACCACCGAGATC-TACACATAGAGGCACACTCTTTCCCTA-CACGACGCTCTTCCGATC*T-3´	ATAGAGGC
#E7605A: 0.060 ml	**NEBNext i503 Primer**	5´-AATGATACGGCGACCACCGAGATC-TACACCCTATCCTACACTCTTTCCCTA-CACGACGCTCTTCCGATC*T-3´	CCTATCCT
#E7606A: 0.060 ml	**NEBNext i504 Primer**	5´-AATGATACGGCGACCACCGAGATC-TACACGGCTCTGAACACTCTTTCCCTA-CACGACGCTCTTCCGATC*T-3´	GGCTCTGA
#E7607A: 0.060 ml	**NEBNext i505 Primer**	5´-AATGATACGGCGACCACCGAGATC-TACACAGGCGAAGACACTCTTTCCCTA-CACGACGCTCTTCCGATC*T-3´	AGGCGAAG
#E7608A: 0.060 ml	**NEBNext i506 Primer**	5´-AATGATACGGCGACCACCGAGATC-TACACTAATCTTAACACTCTTTCCCTA-CACGACGCTCTTCCGATC*T-3´	TAATCTTA
#E7609A: 0.060 ml	**NEBNext i507 Primer**	5´-AATGATACGGCGACCACCGAGATC-TACACCAGGACGTACACTCTTTCCCTA-CACGACGCTCTTCCGATC*T-3´	CAGGACGT
#E7610A: 0.060 ml	**NEBNext i508 Primer**	5´-AATGATACGGCGACCACCGAGATC-TACACGTACTGACACACTCTTTCCCTA-CACGACGCTCTTCCGATC*T-3´	GTACTGAC

NEBNext i701 Primer–NEBNext i712 Primer

Description: 12 Index Primers are included for producing barcoded libraries.

NEB #	PRODUCT	INDEX PRIMER SEQUENCE	EXPECTED INDEX PRIMER SEQUENCE READ
#E7611A: 0.040 ml	**NEBNext i701 Primer**	5´-CAAGCAGAAGACGGCATACGAGA-TCGAGTAATGTGACTGGAGTTCAGAC-GTGTGCTCTTCCGATC*T-3´	ATTACTCG
#E7612A: 0.040 ml	**NEBNext i702 Primer**	5´-CAAGCAGAAGACGGCATACGAGA-TTCTCCGGAGTGACTGGAGTTCAGAC-GTGTGCTCTTCCGATC*T-3´	TCCGGAGA
#E7613A: 0.040 ml	**NEBNext i703 Primer**	5´-CAAGCAGAAGACGGCATACGAGA-TAATGAGCGGTGACTGGAGTTCAGAC-GTGTGCTCTTCCGATC*T-3´	CGCTCATT
#E7614A: 0.040 ml	**NEBNext i704 Primer**	5´-CAAGCAGAAGACGGCATACGAGA-TGGAATCTCGTGACTGGAGTTCAGAC-GTGTGCTCTTCCGATC*T-3´	GAGATTCC
#E7615A: 0.040 ml	**NEBNext i705 Primer**	5´-CAAGCAGAAGACGGCATACGAGA-TTTCTGAATGTGACTGGAGTTCAGAC-GTGTGCTCTTCCGATC*T-3´	ATTCAGAA
#E7616A: 0.040 ml	**NEBNext i706 Primer**	5´-CAAGCAGAAGACGGCATACGAGA-TACGAATTCGTGACTGGAGTTCAGAC-GTGTGCTCTTCCGATC*T-3´	GAATTCGT
#E7617A: 0.040 ml	**NEBNext i707 Primer**	5´-CAAGCAGAAGACGGCATACGAGA-TAGCTTCAGGTGACTGGAGTTCAGAC-GTGTGCTCTTCCGATC*T-3´	CTGAAGCT
#E7618A: 0.040 ml	**NEBNext i708 Primer**	5´-CAAGCAGAAGACGGCATACGAGA-TGCGCATTAGTGACTGGAGTTCAGAC-GTGTGCTCTTCCGATC*T-3´	TAATGCGC
#E7619A: 0.040 ml	**NEBNext i709 Primer**	5´-CAAGCAGAAGACGGCATACGAGA-TCATAGCCGGTGACTGGAGTTCAGAC-GTGTGCTCTTCCGATC*T-3´	CGGCTATG
#E7620A: 0.040 ml	**NEBNext i710 Primer**	5´-CAAGCAGAAGACGGCATACGAGA-TTTCGCGGAGTGACTGGAGTTCAGAC-GTGTGCTCTTCCGATC*T-3´	TCCGCGAA
#E7621A: 0.040 ml	**NEBNext i711 Primer**	5´-CAAGCAGAAGACGGCATACGAGAT-GCGCGAGAGTGACTGGAGTTCAGAC-GTGTGCTCTTCCGATC*T-3´	TCTCGCGC
#E7622A: 0.040 ml	**NEBNext i712 Primer**	5´-CAAGCAGAAGACGGCATACGAGA-TCTATCGCTGTGACTGGAGTTCAGAC-GTGTGCTCTTCCGATC*T-3´	AGCGATAG

NEBNext i501–i508 and i701–i712 Primers (Cont.)

Store at –20°C **Concentration: 10 µM**

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing 1 µl NEBNext Index [X] Primer and 1 µg of HindIII digested Lambda DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis. 50 µl reactions containing NEBNext Index [X] Primer for Illumina and 1 µg of T3 DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis.

Endonuclease Activity: Incubation of a 50 µl reaction containing 1 µl NEBNext Index [X] Primer with 1 µg of ϕX174 RF I supercoiled DNA for 4 hours at 37°C results in less than 10% conversion to RF II (nicked molecules) as determined by agarose gel electrophoresis.

RNase Activity: Incubation of a 10 µl reaction containing 1 µl NEBNext Index [X] Primer with 40 ng of RNA transcript for 16 hours at 37°C resulted in no detectable degradation of RNA as determined by gel electrophoresis.

Phosphatase Activity: Incubation of NEBNext Index [X] Primer in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) containing 2.5 mM *p*-nitrophenyl phosphate at 37°C for 4 hours yields no detectable *p*-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

Lot Controlled

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	
2.0	Removed kit specific protocols.	
2.1	Update "Required Materials Not Included for DNA or ChIP Libraries" and "Required Materials Not Included for RNA Libraries". Update the list of kits using the NEBNext Multiplex Oligos for Illumina. Insert new step 1 to Chapter 1 section 1.1B.	3/16

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