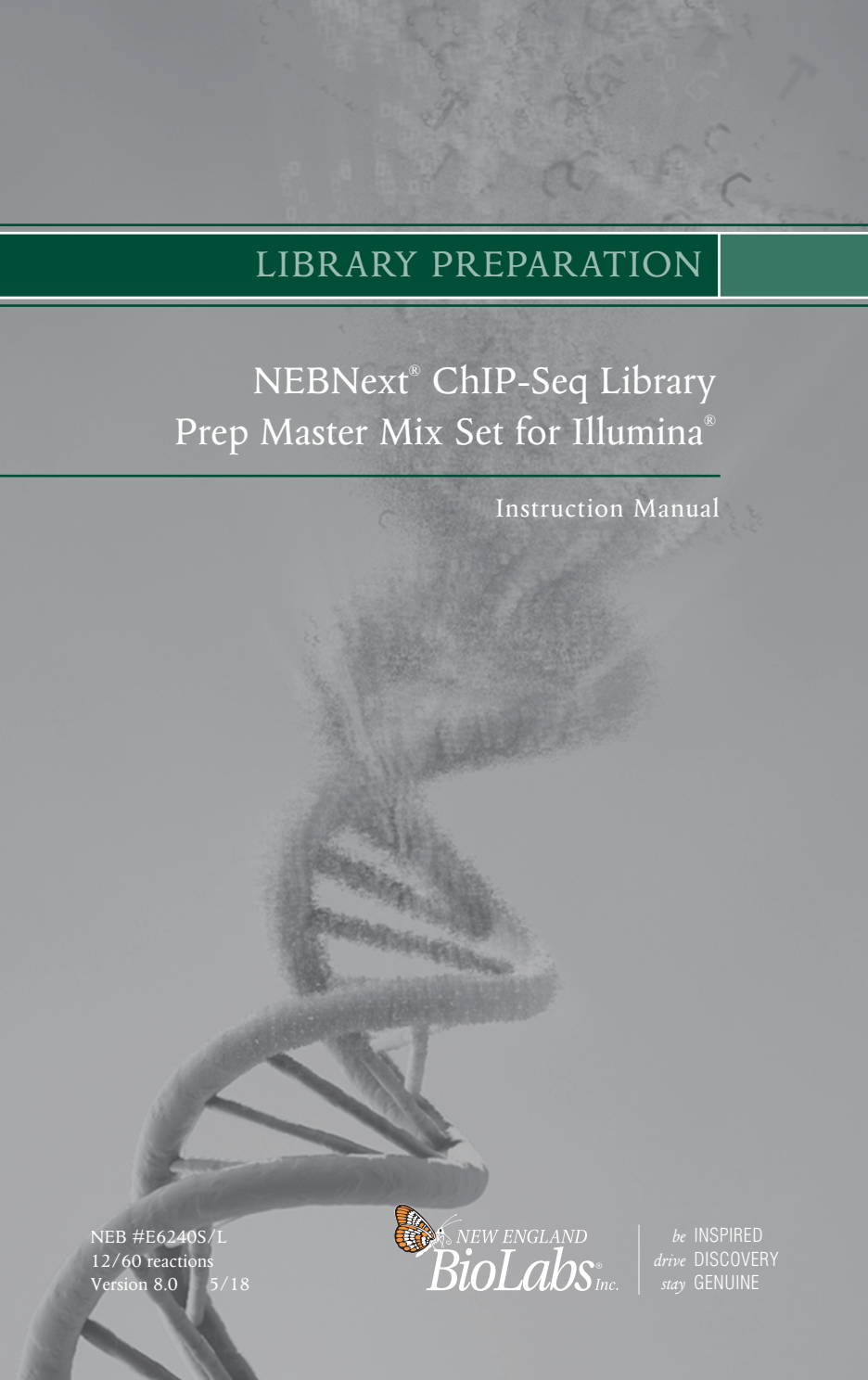
LIBRARY PREPARATION

NEBNext® ChIP-Seq Library Prep Master Mix Set for Illumina®

Instruction Manual



NEB #E6240S/L
12/60 reactions
Version 8.0 5/18

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drive DISCOVERY
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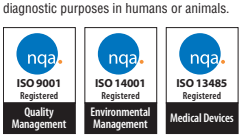
This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.



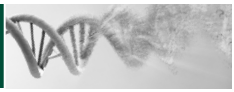


Table of Contents:

The Library Kit Includes:

The volumes provided are sufficient for preparation of up to 12 reactions (NEB #E6240S) and 60 reactions (NEB #E6240L). (All reagents should be stored at –20°C):

- (green) NEBNext End Repair Enzyme Mix
- (green) NEBNext End Repair Reaction Buffer (10X)
- (yellow) Klenow Fragment (3´→5´ exo$^-$)
- (yellow) NEBNext dA-Tailing Reaction Buffer (10X)
- (red) Quick T4 DNA Ligase
- (red) NEBNext Quick Ligation Reaction Buffer (5X)
- (blue) NEBNext Q5 Hot Start HiFi PCR Master Mix

Required Materials Not Included:

80% Ethanol (freshly prepared)

Nuclease-free Water

0.1X TE, pH 8.0

10 mM Tris-HCl, pH 7.5–8.0

10 mM NaCl (optional)

DNA LoBind Tubes (Eppendorf #022431021)

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

NEBNext Singleplex or Multiplex Oligos for Illumina (NEB #E7350, #E7335, #E7500, #E7710, #E7730, #E6609 or #E7600)

Magnetic rack/stand

PCR Machine

Applications:

The NEBNext ChIP-Seq Library Prep Master Mix Set for Illumina contains enzymes and buffers in convenient master mix formulations that are ideally suited for sample preparation for next-generation sequencing, and for preparation of expression libraries. 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 DNA Library Prep Master Mix Set for Illumina 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.

Functional Validation: Each set of reagents is functionally validated together through construction and sequencing of a genomic DNA library on an Illumina Sequencer (Illumina, Inc.).

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.

Protocols:

Symbols

⚠ *This caution sign signifies a step in the protocol that has multiple paths leading to the same end point but is dependent on a user variable, like the amount of input DNA.*

● *Colored bullets indicate the cap color of the reagent to be added to a reaction.*

Starting Material: 10 ng of chromatin-immunoprecipitated (ChIP) qPCR verified or control DNA, in ≤ 40 µl of water or elution buffer.

1.1 End Repair of ChIP DNA

1. Mix the following components in a sterile microfuge tube:

ChIP DNA	1–40 µl
● (green) NEBNext End Repair Reaction Buffer (10X)	5 µl
● (green) NEBNext End Repair Enzyme Mix	1 µl
Sterile H_2O	variable
Total volume	50 µl

2. Incubate in a thermal cycler for 30 minutes at 20°C.

1.2 Cleanup Using AMPure XP® Beads (Beckman Coulter, Inc.)

1. Vortex AMPure XP Beads to resuspend.
2. Add 90 µl (1.8X) of resuspended AMPure XP Beads to the reaction. Mix thoroughly on a vortex mixer or by pipetting up and down at least 10 times.
3. Incubate for 5 minutes at room temperature.
4. Put the tube/PCR plate on an appropriate magnetic stand to separate beads from supernatant. After the solution is clear (about 5 minutes), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain the DNA targets.
5. Add 200 µl of 80% freshly prepared ethanol to the tube/PCR plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.
6. Repeat Step 5 once.
7. Air dry beads for 5 minutes while the tube/PCR plate is on the magnetic stand with the lid open.

   Caution: Do not overdry the beads. This may result in lower recovery of DNA target.

8. Remove the tube/plate from the magnet. Elute the DNA target from the beads by adding 50 µl of 0.1X TE.

9. Mix well on a vortex mixer or by pipetting up and down and incubate for 2 minutes at room temperature.

10. Put the tube/PCR plate in the magnetic stand until the solution is clear. Without disturbing the bead pellet, carefully transfer 44 µl of the supernatant to a fresh, sterile microfuge tube.

1.3 dA-Tailing of End Repaired DNA

1. Mix the following components in a sterile microfuge tube:

End Repaired, Blunt DNA	44 µl
● (yellow) NEBNext dA-Tailing Reaction Buffer (10X)	5 µl
● (yellow) Klenow Fragment (3´→5´ exo⁻)	1 µl
Total volume	50 µl

2. Incubate in a thermal cycler for 30 minutes at 37°C.

1.4 Cleanup Using AMPure XP Beads

1. Vortex AMPure XP Beads to resuspend.

2. Add 90 µl (1.8X) of resuspended AMPure XP Beads to the reaction. Mix thoroughly on a vortex mixer or by pipetting up and down at least 10 times.

3. Incubate for 5 minutes at room temperature.

4. Put the tube/PCR plate on an appropriate magnetic stand to separate beads from supernatant. After the solution is clear (about 5 minutes), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain the DNA targets.

5. Add 200 µl of 80% freshly prepared ethanol to the tube/PCR plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.

6. Repeat Step 5 once.

7. Air dry beads for 5 minutes while the tube/PCR plate is on the magnetic stand with the lid open.

   Caution: Do not overdry the beads. This may result in lower recovery of DNA target.

8. Remove the tube/plate from the magnet. Elute the DNA target from the beads by adding 25 µl of 0.1X TE.

9. Mix well on a vortex mixer or by pipetting up and down and incubate for 2 minutes at room temperature.

10. Put the tube/PCR plate in the magnetic stand until the solution is clear. Without disturbing the bead pellet, carefully transfer 19 µl of the supernatant to a fresh, sterile microfuge tube. Sample can be stored at –20°C or proceed directly to Adaptor Ligation.

1.5 Adaptor Ligation of dA-Tailed DNA

Dilute the ● (red) NEBNext Adaptor for Illumina* (15 µM) 10-fold in 10 mM Tris-HCl or 10 mM Tris-HCl with 10 mM NaCl to a final concentration of 1.5 µM.

1. Mix the following components in a sterile microfuge tube:

dA-Tailed DNA	19 µl
● (red) Quick Ligation Reaction Buffer (5X)	6 µl
Diluted NEBNext Adaptor (1.5 µM)	1 µl
● (red) Quick T4 DNA Ligase	4 µl
Total volume	30 µl

* The NEBNext adaptor can be found in NEBNext Singleplex (NEB #E7350) or Multiplex (NEB #E7335, #E7500, #E7710, #E7730, #E6609 or #E7600) Oligos for Illumina.

2. Incubate in a thermal cycler for 15 minutes at 20°C.

3. Add 3 µl of ● (red) USER® Enzyme Mix by pipetting up and down, and incubate at 37°C for 15 minutes.

Note: This step is only required for use with NEBNext Adaptors. USER enzyme can be found in the NEBNext Singleplex (NEB #E7350) or Multiplex (NEB #E7335, #E7500, #E7710, #E7730, #E6609 and #E7600) Oligos for Illumina.

⚠ ***A precipitate can form upon thawing of the NEBNext Q5 Hot Start HiFi PCR Master Mix. To ensure optimal performance, place the master mix at room temperature while performing cleanup of adaptor-ligated DNA. Once thawed, gently mix by inverting the tube several times.***

1.6 Cleanup of Adaptor Ligated DNA

1. Vortex AMPure XP Beads to resuspend.

2. Add 54 µl of resuspended AMPure XP Beads to the ligation reaction. Mix thoroughly on a vortex mixer or by pipetting up and down at least 10 times.

3. Incubate for 5 minutes at room temperature.

4. Put the tube/PCR plate on an appropriate magnetic stand to separate beads from supernatant. After the solution is clear (about 5 minutes), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain the DNA targets.

5. Add 200 µl of 80% freshly prepared ethanol to the tube/PCR plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.

6. Repeat Step 5 once.

7. Air dry beads for 5 minutes while the tube/PCR plate is on the magnetic stand with the lid open.

   Caution: Do not overdry the beads. This may result in lower recovery of DNA target.

8. Remove the tube/plate from the magnet. Elute the DNA target by adding 105 µl of 10 mM Tris-HCl or 0.1 X TE to the beads for bead-based size selection.

   Note: For size selection using E-Gel size select gels or standard 2% agarose gels, elute the DNA target at desired volume.

9. Mix well on a vortex mixer or by pipetting up and down and incubate for 2 minutes at room temperature.

10. Put the tube/PCR plate in the magnetic stand until the solution is clear. Transfer 100 µl of supernatant (or desired volume) to a new tube/well, and proceed to bead based size selection.

1.7 Size Select Adaptor Ligated DNA Using AMPure XP Beads

Insert Size	150 bp	200 bp	250 bp	300 bp	400 bp	500 bp	700 bp
Total library size (insert + adaptor)	270 bp	320 bp	370 bp	420 bp	530 bp	660 bp	820 bp
Bead: DNA ratio* 1st bead selection	0.9X	0.8X	0.7X	0.6X	0.55X	0.5X	0.45X
Bead: DNA ratio* 2nd bead selection	0.2X	0.2X	0.2X	0.2X	0.15X	0.15X	0.15X

Table 1.1: Recommended conditions for dual bead-based size selection.

⚠ ***The following size selection protocol is for libraries with 150 bp inserts only. For libraries with different size fragment inserts, please optimize bead: DNA ratio according to Table 1.1 above.***

Note: (X) refers to the original sample volume of 100 µl

1. Add 90 µl (0.9X) resuspended AMPure XP Beads to 100 µl DNA solution. Mix well on a vortex mixer or by pipetting up and down at least 10 times.

2. Incubate for 5 minutes at room temperature.

3. Place the tube/PCR plate on an appropriate magnetic stand to separate beads from supernatant. After the solution is clear (about 5 minutes), carefully transfer the supernatant to a new tube/well (**Caution: do not discard the supernatant**). Discard beads that contain the large fragments.
4. Add 20 µl (0.2X) resuspended AMPure XP Beads to the supernatant, mix well and incubate for 5 minutes at room temperature.
5. Put the tube/PCR plate on an appropriate magnetic stand to separate beads from supernatant. After the solution is clear (about 5 minutes), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets (**Caution: do not discard beads**).
6. Add 200 µl of freshly prepared 80% ethanol to the tube/PCR plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.
7. Repeat Step 6 once.
8. Air dry beads for 5 minutes while the tube/PCR plate Is on the magnetic stand with the lid open.

   Caution: Do not overdry the beads. This may result in lower recovery of DNA target.
9. Remove the tube/plate from the magnet. Elute the DNA target from the beads by adding 22 µl of 10 mM Tris-HCl or 0.1X TE.
10. Mix well on a vortex mixer or by pipetting up and down and incubate for 2 minutes at room temperature.
11. Put the tube/PCR plate in the magnetic stand until the solution is clear. Without disturbing the bead pellet, carefully transfer 20 µl of the supernatant to a clean PCR tube and proceed to enrichment.

1.8 PCR Enrichment of Adaptor Ligated DNA

⚠ **Follow Section 1.8A if you are using the following oligos (10 µM primer):**

NEBNext Singleplex Oligos for Illumina (NEB #E7350)

NEBNext Multiplex Oligos for Illumina (Set 1, NEB #E7335)

NEBNext Multiplex Oligos for Illumina (Set 2, NEB #E7500)

NEBNext Multiplex Oligos for Illumina (Set 3, NEB #E7710)

NEBNext Multiplex Oligos for Illumina (Set 4, NEB #E7730)

NEBNext Multiplex Oligos for Illumina (Dual Index Primers, NEB #E7600)

Follow Section 1.8B if you are using NEBNext Multiplex Oligos for Illumina (96 Index Primers, NEB #E6609).

1.8A PCR Enrichment of Adaptor Ligated DNA

1. Mix the following components in sterile strip tubes:

Adaptor Ligated DNA Fragments	20 µl
(blue) Index Primer/i7 Primer*,**	2.5 µl
(blue) Universal PCR Primer/i5 Primer*,***	2.5 µl
(blue) NEBNext Q5 Hot Start HiFi PCR Master Mix	25 µl
Total volume	50 µl

* The primers are provided in NEBNext Singleplex (NEB #E7350) or Multiplex (NEB #E7335, #E7500, #E7710, #E7730 or #E7600) Oligos for Illumina. For use with Dual Index Primers (NEB #E7600), look at the NEB #E7600 manual for valid barcode combinations and tips for setting up PCR reactions.

** For use with NEBNext Multiplex Oligos (NEB #E7335, #E7500, #E7710 or #E7730) use only one Index Primer per PCR reaction. For use with Dual Index Primers (NEB #E7600) use only one i7 Primer per reaction.

*** For use with Dual Index Primers (NEB #E7600) use only one i5 Primer per reaction.

2. PCR cycling conditions:

CYCLE STEP	TEMP	TIME	CYCLES
Initial Denaturation	98°C	30 seconds	1
Denaturation Annealing/Extension	98°C 65°C	10 seconds 75 seconds	15
Final Extension	65°C	5 minutes	1
Hold	4°C	∞	

3. Proceed to Cleanup Using Ampure XP Beads in Section 1.9

1.8B PCR Enrichment of Adaptor Ligated DNA

1. Mix the following components in sterile strip tubes:

Adaptor Ligated DNA Fragments	15 µl
(blue) Index/ Universal Primer Mix*	10 µl
(blue) NEBNext Q5 Hot Start HiFi PCR Master Mix	25 µl
Total volume	50 µl

* The primers are provided in NEBNext Multiplex Oligos for Illumina, NEB #E6609. Please refer to the NEB #E6609 manual for valid barcode combinations and tips for setting up PCR reactions.

2. PCR cycling conditions:

CYCLE STEP	TEMP	TIME	CYCLES
Initial Denaturation	98°C	30 seconds	1
Denaturation Annealing/Extension	98°C 65°C	10 seconds 75 seconds	15
Final Extension	65°C	5 minutes	1
Hold	4°C	∞	

3. Proceed to Cleanup Using Ampure XP Beads in Section 1.9

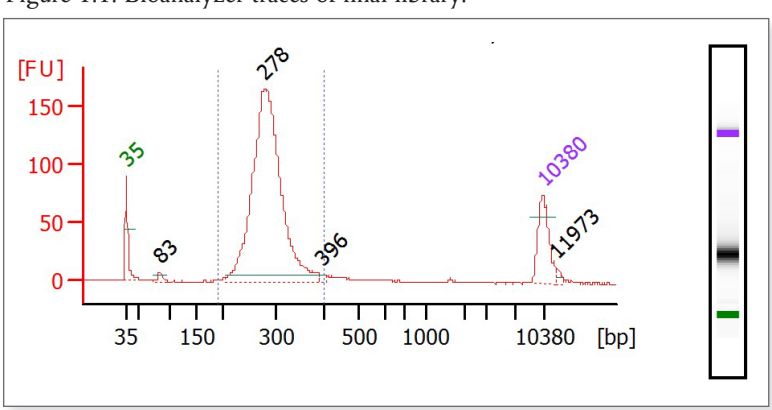
1.9 Cleanup Using AMPure XP Beads

1. Vortex AMPure XP Beads to resuspend.
2. Add 45 µl (0.9X) of resuspended AMPure XP Beads to the PCR reactions (~50 µl). Mix well on a vortex mixer or by pipetting up and down at least 10 times.
3. Incubate for 5 minutes at room temperature.

4. Put the tube/PCR plate on an appropriate magnetic stand to separate beads from supernatant. After the solution is clear (about 5 minutes), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain the DNA targets.
5. Add 200 µl of freshly prepared 80% ethanol to the tube/PCR plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.
6. Repeat Step 5 once.
7. Air dry the beads for 5 minutes while the tube/PCR plate is on the magnetic stand with the lid open.

   Caution: Do not overdry the beads. This may result in lower recovery of DNA target.
8. Remove the tube/plate from the magnet. Elute the DNA target from the beads by adding 30 µl of 0.1X TE.
9. Mix well on a vortex mixer or by pipetting up and down and incubate for 2 minutes at room temperature.
10. Put the tube/PCR plate in the magnetic stand until the solution is clear. Without disturbing the bead pellet, carefully transfer 25 µl of the supernatant to a clean LoBind® (Eppendorf AG) tube. Libraries can be stored at –20°C.
11. Dilute 2–3 µl of the library 20 fold with 10 mM Tris-HCl or 0.1X TE and assess the library quality on a Bioanalyzer® (Agilent Technologies, Inc.) high sensitivity chip. Check that the electropherogram shows a narrow distribution with a peak size approximately 275 bp.

Figure 1.1: Bioanalyzer traces of final library.



Kit Components

NEB #E6240S Table of Components

NEB #	PRODUCT	VOLUME
E6241A	NEBNext End Repair Enzyme Mix	0.015 ml
E6242A	NEBNext End Repair Reaction Buffer	0.06 ml
E6207A	Klenow Fragment (3´→5´ exo$^-$)	0.015 ml
E6045A	NEBNext dA-Tailing Reaction Buffer	0.06 ml
E6209A	Quick T4 DNA Ligase	0.048 ml
E6243A	NEBNext Quick Ligation Reaction Buffer	0.072 ml
E6630A	NEBNext Q5 Hot Start HiFi PCR Master Mix	0.3 ml

NEB #E6240L Table of Components

NEB #	PRODUCT	VOLUME
E6241AA	NEBNext End Repair Enzyme Mix	0.06 ml
E6242AA	NEBNext End Repair Reaction Buffer	0.3 ml
E6207AA	Klenow Fragment (3´→5´ exo$^-$)	0.06 ml
E6045AA	NEBNext dA-Tailing Reaction Buffer	0.3 ml
E6209AA	Quick T4 DNA Ligase	0.240 ml
E6243AA	NEBNext Quick Ligation Reaction Buffer	0.36 ml
E6630AA	NEBNext Q5 Hot Start HiFi PCR Master Mix	2 x 0.75 ml

Revision History:

REVISION #	DESCRIPTION	DATE
4.0	N/A	
5.0	Include protocol for use with NEBNext Q5 Hot Start HiFi PCR Master Mix. Include protocol for changes in concentration of NEBNext Singleplex and Multiplex Oligos for Illumina. Changed all AMPure Bead drying times after ethanol washes to 5 minutes. Changed all AMPure Bead elutions to 0.1X TE or 10 mM Tris-HCl. Changed ratio of AMPure Beads to 0.9X in final cleanup after PCR reaction. Added 2 minute incubation after eluting DNA from AMPure beads.	
6.0	Remove protocol for use with NEBNext High-Fidelity 2X PCR Master Mix. Include protocol for use with NEBNext Multiplex Oligos (96 Index Primers, NEB #E6609).	
7.0	Protocol updated to include NEB #E7710 and NEB #E7730. Section C in the PCR setup step was removed because all of the 25 µM primers are now expired.	6/16
8.0	Create "Kit Component – Table of Components" for small and large size kits. Delete individual component information pages.	5/18

USA
New England Biolabs, Inc.
240 County Road
Ipswich, MA 01938-2723
Telephone: (978) 927-5054
Toll Free: (USA Orders) 1-800-632-5227
Toll Free: (USA Tech) 1-800-632-7799
Fax: (978) 921-1350
e-mail: info@neb.com
www.neb.com

CANADA
New England Biolabs, Ltd.
Telephone: (905) 665-4632
Toll Free: 1-800-387-1095
Fax: (905) 665-4635
Fax Toll Free: 1-800-563-3789
e-mail: info.ca@neb.com
www.neb.ca

CHINA
New England Biolabs (Beijing), Ltd.
Telephone: 010-82378265/82378266
Fax: 010-82378262
e-mail: info@neb-china.com
www.neb-china.com

FRANCE
New England Biolabs France
Free Call: 0800-100-632
Free Fax: 0800-100-610
e-mail: info.fr@neb.com
www.neb-online.fr

GERMANY & AUSTRIA
New England Biolabs GmbH
Telephone: +49/(0)69/305 23140
Free Call: 0800/246 5227 (Germany)
Free Call: 00800/246 52277 (Austria)
Fax: +49/(0)69/305 23149
Free Fax: 0800/246 5229 (Germany)
e-mail: info.de@neb.com
www.neb-online.de

JAPAN
New England Biolabs Japan, Inc.
Telephone: +81 (0)3 5669 6191
Fax: +81 (0)3 5669 6192
e-mail: info.jp@neb.com
www.nebj.jp

SINGAPORE
New England Biolabs Pte. Ltd.
Telephone: +65 638 59623
Fax: +65 638 59617
e-mail: sales.sg@neb.com
www.neb.sg

UNITED KINGDOM
New England Biolabs (UK) Ltd.
Telephone: (01462) 420616
Call Free: 0800 318486
Fax: (01462) 421057
Fax Free: 0800 435682
e-mail: info.uk@neb.com
www.neb.uk.com

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