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.

This product is covered by one or more patents, trademarks and/or copyrights owned or controlled by New England Biolabs, Inc. For more information, please contact NEB’s Global Business Development team at gbd@neb.com.

AMPURE® is a registered trademark of Beckman Coulter, Inc.
BIOANALYZER® is a registered trademark of Agilent Technologies, Inc.
ILLUMINA® and GENOME ANALYZER II® are registered trademarks of Illumina, Inc.
E-GEL® is a registered trademark of Life Technologies, Inc.
LOBIND® is a registered trademark of Eppendorf AG.
MILLI-Q® is a registered trademark of Millipore Corporation.
QIAQUICK® and MINELUTE® are registered trademarks of Qiagen.
Table of Contents:

The Reagent Set Includes ............................................................ 2
Applications ........................................................................... 3
Protocols ................................................................................. 4
Phosphorylation Reaction Buffer ........................................... 12
Deoxynucleotide Solution Mix .................................................. 13
T4 DNA Polymerase ................................................................. 14
DNA Polymerase I, Large (Klenow) Fragment ......................... 15
T4 Polynucleotide Kinase .......................................................... 16
Deoxyadenosine 5´- Triphosphate (dATP) ............................... 17
Klenow Fragment (3´→5´ exo⁻) ............................................... 18
NEBuffer 2 for Klenow Fragment (3´→5´ exo⁻) ....................... 19
Quick T4 DNA Ligase ............................................................... 20
Quick Ligation Reaction Buffer ............................................. 21
NEBNext Q5 Hot Start HiFi PCR Master Mix ....................... 22
Revision History ................................................................. 23
The Reagent Set Includes:

*The volumes provided are sufficient for preparation of up to 12 reactions (NEB #E6000S) and 60 reactions (NEB #E6000L). (All reagents should be stored at −20°C).

- (green) Phosphorylation Reaction Buffer (10X)
- (green) Deoxynucleotide Solution Mix (10 mM each dNTP)
- (green) T4 DNA Polymerase
- (green) DNA Polymerase I, Large (Klenow) Fragment
- (green) T4 Polynucleotide Kinase
- (yellow) Deoxyadenosine 5´- Triphosphate (dATP) (1.0 mM)
- (yellow) Klenow Fragment (3´→5´ exo⁻)
- (yellow) NEBuffer 2 for Klenow Fragment (3´→5´ exo⁻) (10X)
- (red) Quick T4 DNA Ligase
- (red) Quick Ligation Reaction Buffer (2X)
- (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 (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 DNA Library Prep Reagent Set for Illumina contains enzymes and buffers 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 DNA Library Prep Reagent 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.

Functionally Validated: Each set of reagents is functionally validated together through construction and sequencing of a genomic DNA library on an Illumina sequencing 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.
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: 1–5 µg of Fragmented DNA

1.1 End Repair of Fragmented DNA
1. Mix the following components in a sterile microfuge tube:

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fragmented DNA</td>
<td>1–75 µl</td>
</tr>
<tr>
<td>(green) Phosphorylation Reaction Buffer (10X)</td>
<td>10 µl</td>
</tr>
<tr>
<td>(green) T4 DNA Polymerase</td>
<td>5 µl</td>
</tr>
<tr>
<td>(green) T4 Polynucleotide Kinase</td>
<td>5 µl</td>
</tr>
<tr>
<td>(green) dNTPs</td>
<td>4 µl</td>
</tr>
<tr>
<td>(green) DNA Polymerase I, Large (Klenow)</td>
<td>1 µl</td>
</tr>
<tr>
<td>Sterile H₂O</td>
<td>variable</td>
</tr>
<tr>
<td><strong>Total volume</strong></td>
<td><strong>100 µl</strong></td>
</tr>
</tbody>
</table>

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 160 µl (1.6X) 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 40 µl of 10 mM Tris-HCl or 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 32 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:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>End Repaired, Blunt DNA</td>
<td>32 µl</td>
</tr>
<tr>
<td>(yellow) NEBuffer 2 (10X)</td>
<td>5 µl</td>
</tr>
<tr>
<td>(yellow) Deoxyadenosine 5’-Triphosphate</td>
<td>10 µl</td>
</tr>
<tr>
<td>(yellow) Klenow Fragment (3’→5’ exo–)</td>
<td>3 µl</td>
</tr>
</tbody>
</table>

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 15 μl of 10 mM Tris-HCl or 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 10 µl of the supernatant to a fresh, sterile microfuge tube.

1.5 Adaptor Ligation of dA-Tailed DNA

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

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>dA-Tailed DNA</td>
<td>10 µl</td>
</tr>
<tr>
<td>(red) Quick Ligation Reaction Buffer (2X)</td>
<td>25 µl</td>
</tr>
<tr>
<td>(red) NEBNext Adaptor*</td>
<td>10 µl</td>
</tr>
<tr>
<td>(red) Quick T4 DNA Ligase</td>
<td>5 µl</td>
</tr>
<tr>
<td><strong>Total volume</strong></td>
<td><strong>50 µl</strong></td>
</tr>
</tbody>
</table>

*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.

4. Proceed to Cleanup Using AMPure XP Beads in Section 1.6

   **Warning:** 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 90 µl of resuspended AMPure XP Beads to the ligation reaction (~53 µl). 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.

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

Table 1.1: Recommended conditions for dual bead-based size selection.
1.7 Size Select Adaptor Ligated DNA Using AMPure XP Beads

The following size selection protocol is for libraries with 200 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 80 µl (0.8X) 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 17 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 15 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:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adaptor Ligated DNA Fragments</td>
<td>15 µl</td>
</tr>
<tr>
<td>(blue) Index Primer/i7 Primer*,**</td>
<td>5 µl</td>
</tr>
<tr>
<td>(blue) Universal PCR Primer/i5 Primer*,,,***</td>
<td>5 µl</td>
</tr>
<tr>
<td>(blue) NEBNext Q5 Hot Start HiFi PCR Master Mix</td>
<td>25 µl</td>
</tr>
<tr>
<td><strong>Total volume</strong></td>
<td><strong>50 µl</strong></td>
</tr>
</tbody>
</table>

* The primers are provided in NEBNext Singleplex (NEB #E7350) or Multiplex (NEB #E7335, #E7500, #E7710, #E7730, #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:

<table>
<thead>
<tr>
<th>CYCLE STEP</th>
<th>TEMP</th>
<th>TIME</th>
<th>CYCLES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Denaturation</td>
<td>98°C</td>
<td>30 seconds</td>
<td>1</td>
</tr>
<tr>
<td>Denaturation</td>
<td>98°C</td>
<td>10 seconds</td>
<td>75 seconds</td>
</tr>
<tr>
<td>Annealing/Extension</td>
<td>65°C</td>
<td>5 minutes</td>
<td>1</td>
</tr>
<tr>
<td>Final Extension</td>
<td>65°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hold</td>
<td>4°C</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*If library construction was performed with 5 µg of starting material, use 2–3 cycles of amplification. If starting material was 1 µg, use 4 cycles of amplification. However, optimization of PCR cycle number may be required to avoid over-amplification.

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:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adaptor Ligated DNA Fragments</td>
<td>15 µl</td>
</tr>
<tr>
<td>(blue) Index/ Universal Primer Mix*</td>
<td>10 µl</td>
</tr>
<tr>
<td>(blue) NEBNext Q5 Hot Start HiFi PCR Master Mix</td>
<td>25 µl</td>
</tr>
<tr>
<td><strong>Total volume</strong></td>
<td><strong>50 µl</strong></td>
</tr>
</tbody>
</table>

* 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:

<table>
<thead>
<tr>
<th>CYCLE STEP</th>
<th>TEMP</th>
<th>TIME</th>
<th>CYCLES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Denaturation</td>
<td>98°C</td>
<td>30 seconds</td>
<td>1</td>
</tr>
<tr>
<td>Denaturation</td>
<td>98°C</td>
<td>10 seconds</td>
<td>2–4*</td>
</tr>
<tr>
<td>Annealing/Extension</td>
<td>65°C</td>
<td>75 seconds</td>
<td></td>
</tr>
<tr>
<td>Final Extension</td>
<td>65°C</td>
<td>5 minutes</td>
<td>1</td>
</tr>
<tr>
<td>Hold</td>
<td>4°C</td>
<td>∞</td>
<td></td>
</tr>
</tbody>
</table>

*If library construction was performed with 5 µg of starting material, use 2-3 cycles of amplification. If starting material was 1 µg, use 4 cycles of amplification. However, optimization of PCR cycle number may be required to avoid over-amplification.

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/ PC 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/PC 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 300–320 bp.

**Figure 1.1: Example of DNA library size distribution on a Bioanalyzer.**
Phosphorylation Reaction Buffer

#E6001A: 0.12 ml  Concentration: 10X
#E6001AA: 0.6 ml

Store at –20°C

1X Phosphorylation Reaction Buffer:
50 mM Tris-HCl
10 mM MgCl$_2$
10 mM DTT
1 mM ATP
pH 7.5 @ 25°C

Quality Control Assays

16-Hour Incubation: 50 μl reactions containing this reaction buffer 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 this reaction buffer at a 1X concentration with 1 μg of φX174 RF I DNA for 4 hours at 37°C in 50 μl reactions results in less than 10% conversion to RF II as determined by agarose gel electrophoresis.

RNase Activity: Incubation of this reaction buffer at 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.

Phosphatase Activity: Incubation of this reaction buffer 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
Deoxynucleotide Solution Mix

#E6002A: 0.048 ml 10 mM each dNTP
#E6002AA: 0.24 ml

Store at –20°C

**Description:** Deoxynucleotide Solution Mix is an equimolar solution of ultrapure dATP, dCTP, dGTP and dTTP.

**Supplied in:** Milli-Q® (Millipore Corporation) water as a sodium salt at pH 7.5.

**Concentration:** Each nucleotide is supplied at a concentration of 10 mM. (40 mM total nucleotide concentration).

**Quality Assurance:** Nucleotide solutions are certified free of nucleases and phosphatases.

**Notes:** Storing nucleotide triphosphates in solutions containing magnesium promotes triphosphate degradation.

**Quality Control Assays**

**16-Hour Incubation:** 50 μl reactions containing a minimum of 2 mM dNTPs 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 a minimum of 2 mM dNTPs 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.

**RNase Activity:** Incubation of 1 mM dNTPs 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.

**Phosphatase Activity:** Incubation of a minimum of 5 mM dNTPs in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM MgCl₂) 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.

**HPLC:** dNTP purity is determined by HPLC to be > 99%.

**Functional Activity (PCR):** The dNTPs are tested in 25 cycles of PCR amplification generating 0.5 kb, 2 kb, and 5kb amplicons from lambda DNA.

**Lot Controlled**
T4 DNA Polymerase

Store at −20°C

**Description:** T4 DNA Polymerase catalyzes the synthesis of DNA in the 5´ → 3´ direction and requires the presence of template and primer. This enzyme has a 3´ → 5´ exonuclease activity which is much more active than that found in DNA Polymerase I. Unlike *E. coli* DNA Polymerase I, T4 DNA Polymerase does not have a 5´ → 3´ exonuclease function.

**Source:** Purified from a strain of *E. coli* that carries a T4 DNA Polymerase overproducing plasmid.

**Supplied in:** 100 mM KPO₄ (pH 6.5), 1 mM DTT and 50% glycerol.

**Quality Control Assays**

**SDS-PAGE Purity:** SDS-PAGE analysis of this enzyme indicates > 95% enzyme purity.

**Endonuclease Activity:** Incubation of a minimum of 50 units of this enzyme with 1 μg of φX174 RF I DNA in assay buffer for 4 hours at 37°C in 50 μl reactions results in less than 10% conversion to RF II as determined by agarose gel electrophoresis.

**Phosphatase Activity:** Incubation of a minimum of 30 units of this enzyme in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM MgCl₂) 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.

**Functional Activity (Nucleotide Incorporation):** One unit of this enzyme incorporates 10 nmol of dNTP into acid-precipitable material in a total reaction volume of 50 μl in 30 minutes at 37°C in 1X T4 DNA Polymerase Reaction Buffer with 33 μM dNTPs including [³H]-dTTP, 70 μg/ml denatured herring sperm DNA and 50 μg/ml BSA.

**Lot Controlled**

**References:**


DNA Polymerase I, Large (Klenow) Fragment

#E6004A: 0.018 ml
#E6004AA: 0.06 ml

Store at –20°C

Description: DNA Polymerase I, Large (Klenow) Fragment is a proteolytic product of *E. coli* DNA Polymerase I which retains polymerization and 3′→5′ exonuclease activity, but has lost 5′→3′ exonuclease activity. Klenow retains the polymerization fidelity of the holoenzyme without degrading 5′ termini.

Source: A genetic fusion of the *E. coli* polA gene, that has its 5′→3′ exonuclease domain genetically replaced by maltose binding protein (MBP). Klenow Fragment is cleaved from the fusion and purified away from MBP. The resulting Klenow fragment has the identical amino and carboxy termini as the conventionally prepared Klenow fragment.

Supplied in: 25 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol and 50% glycerol.

Quality Control Assays

SDS-PAGE Purity: SDS-PAGE analysis of this enzyme indicates > 95% enzyme purity.

16-Hour Incubation: 50 μl reactions containing a minimum of 5 units of this enzyme 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 a minimum of 5 units of this enzyme 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 minimum of 50 units of this enzyme with 1 μg of φX174 RF I DNA in assay buffer for 4 hours at 37°C in 50 μl reactions results in less than 10% conversion to RF II as determined by agarose gel electrophoresis.

Phosphatase Activity: Incubation of a minimum of 50 units of this enzyme in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM MgCl₂) 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 a minimum of 5 units of this enzyme 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.

Functional Activity (Nucleotide Incorporation): One unit of this enzyme incorporates 10 nmol of dNTP into acid-precipitable material in a total reaction volume of 50 μl in 30 minutes at 37°C in 1X NEBuffer 2 with 33 μM dNTPs including [³²H]-dTTP, 70 μg/ml denatured herring sperm DNA and 50 μg/ml BSA.

Lot Controlled

References:

T4 Polynucleotide Kinase

#E6005A: 0.06 ml
#E6005AA: 0.3 ml

Store at –20°C

Description: Catalyzes the transfer and exchange of P\textsubscript{i} from the γ position of ATP to the 5’-hydroxyl terminus of polynucleotides (double- and single-stranded DNA and RNA) and nucleoside 3’-monophosphates. Polynucleotide Kinase also catalyzes the removal of 3’-phosphoryl groups from 3’-phosphoryl polynucleotides, deoxynucleoside 3’-monophosphates and deoxynucleoside 3’-diphosphates (1).

Source: An E. coli strain that carries the cloned T4 Polynucleotide Kinase gene. T4 Polynucleotide Kinase is purified by a modification of the method of Richardson (1).

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 0.1 µM ATP and 50% glycerol.

Quality Assurance: Free of exonuclease, phosphatase, endonuclease and RNase activities. Each lot is tested under 5’-end-labeling conditions to assure maximal transfer of [\textsuperscript{32}P].

Quality Control Assays

SDS-PAGE Purity: SDS-PAGE analysis of this enzyme indicates > 95% enzyme purity.

16-Hour Incubation: 50 µl reactions containing a minimum of 10 units of this enzyme 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 a minimum of 10 units of this enzyme 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 minimum of 200 units of this enzyme with 1 µg of φX174 RF I DNA in assay buffer for 4 hours at 37°C in 50 µl reactions results in less than 10% conversion to RF II as determined by agarose gel electrophoresis.

Phosphatase Activity: Incubation of a minimum of 100 units of this enzyme in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM MgCl\textsubscript{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 a minimum of 100 units of this enzyme with 2 µg MS2 phage RNA for 1 hour at 37°C in 50 µl 1X T4 Polynucleotide Kinase Reaction Buffer followed by agarose gel electrophoresis shows no degradation. Incubation of 10 units of this enzyme 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.

Exonuclease Activity: Incubation of 300 units of enzyme with 1 µg sonicated [\textsuperscript{3}H]DNA (10\textsuperscript{6} cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

Functional Activity (Labeling): \textsuperscript{32}P end labeling of 5’-hydroxyl terminated d(T)\textsubscript{8} with a minimum of 50 units of this enzyme for 30 minutes at 37°C in 50 µl 1X T4 Polynucleotide Kinase Buffer followed by 20% acrylamide gel electrophoresis reveals that less than 1% of the product has been degraded by exonuclease or phosphatase activities.

Lot Controlled

References:
Deoxyadenosine 5’- Triphosphate (dATP)

**#E6006A:** 0.12 ml  
**Concentration:** 1.0 mM  
**#E6006AA:** 0.6 ml

Store at –20°C

**Supplied in:** Milli-Q water as a sodium salt at pH 7.5.

**Concentration:** dATP is supplied at a concentration of 1mM.

**Quality Assurance:** Nucleotide solutions are certified free of nucleases and phosphatases.

**Notes:** Storing nucleotide triphosphates in solutions containing magnesium promotes triphosphate degradation. Nucleotide concentrations are determined by measurements of absorbance.

**Quality Control Assays**

**Phosphatase Activity:** Incubation of a minimum of 1 mM dATP in protein phosphatase assay buffer (1M diethanolamine @ pH 9.8 and 0.5 mM MgCl₂) 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.

**16-Hour Incubation:** 50 μl reactions containing a minimum of 0.2 mM dATP and 1 μg of HindIII digested Lambda DNA incubated for 16 hours results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis. 50 μl reactions containing 0.2 mM dATP and 1 μg T3 DNA incubated for 16 hours also results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis.

**RNase Activity:** Incubation of a minimum of 0.1 mM dATP 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.

**HPLC:** dATP purity is determined by HPLC to be > 99%.

**Functional Activity (PCR):** This dATP in a pool of dNTPs is tested in 25 cycles of PCR amplification generating 0.5 kb, 2 kb, and 5kb amplicons from lambda DNA.

**Lot Controlled**
Klenow Fragment (3´→ 5´ exo⁻)

Store at –20°C

**Description:** Klenow Fragment (3´→ 5´ exo⁻) is an N-terminal truncation of DNA Polymerase I which retains polymerase activity, but lacks the 5´→ 3´ exonuclease activity and contains mutations (D355A, E357A), which abolish the 3´→ 5´ exonuclease activity (1).

**Source:** An *E. coli* strain containing a plasmid with a fragment of the *E. coli* polA (D355A, E357A) gene starting at codon 324.

**Supplied in:** 25 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT and 50% glycerol.

**Quality Control Assays**

**SDS-PAGE Purity:** SDS-PAGE analysis of this enzyme indicates > 95% enzyme purity.

**16-Hour Incubation:** 50 μl reactions containing a minimum of 5 units of this enzyme 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 a minimum of 5 units of this enzyme 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 minimum of 50 units of this enzyme with 1 μg of φX174 RF I DNA in assay buffer for 4 hours at 37°C in 50 μl reactions results in less than 10% conversion to RF II as determined by agarose gel electrophoresis.

**Phosphatase Activity:** Incubation of a minimum of 50 units of this enzyme in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM MgCl₂) 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 a minimum of 5 units of this enzyme 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.

**Exonuclease Activity:** Incubation of a minimum of 200 units of this enzyme with 1 μg sonicated [³H]DNA (10⁵ cpm/μg) for 4 hours at 37°C in 50 μl reaction buffer releases < 0.1% radioactivity.

**3´→ 5´ Exonuclease Activity:** Incubation of a minimum of 50 units of enzyme in 20 μl of a 10 mM solution of a fluorescent 5´-FAM labeled oligonucleotide for 30 minutes at 37°C yields no detectable 3´→5´ degradation as determined by capillary electrophoresis.

**Functional Activity (Nucleotide Incorporation):** One unit of this enzyme incorporates 10 nmol of dNTP into acid-precipitable material in a total reaction volume of 50 μl in 30 minutes at 37°C in 1X NEBuffer 2 with 33 μM dNTPs including [³H]-dTTP, 70 μg/ml denatured herring sperm DNA and 50 μg/ml BSA.

**References:**
NEBuffer 2 for Klenow Fragment (3’→5’exo–)

#E6008A: 0.06 ml  Concentration: 10X
#E6008AA: 0.3 ml

Store at –20°C

1X NEBuffer 2:
50 mM NaCl
10 mM Tris-HCl
10 mM MgCl₂
1 mM DTT
pH 7.9 @ 25°C

Quality Control Assays

16-Hour Incubation: 50 μl reactions containing this reaction buffer 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 this reaction buffer at a 1X concentration with 1 μg of φX174 RF I DNA for 4 hours at 37°C in 50 μl reactions results in less than 10% conversion to RF II as determined by agarose gel electrophoresis.

RNase Activity: Incubation of this reaction buffer at 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.

Phosphatase Activity: Incubation of this reaction buffer at a 1X concentration in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM MgCl₂) 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
Quick T4 DNA Ligase

#E6009A: 0.06 ml
#E6009AA: 0.3 ml

Store at –20°C

Source: Purified from *E. coli* C600 pcl857 pPLc28 lig8 (2).

**Quality Control Assays**

**SDS-PAGE Purity:** SDS-PAGE analysis of this enzyme indicates > 95% enzyme purity.

**16-Hour Incubation:** 50 μl reactions containing a minimum of 2,000 units of this enzyme 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 a minimum of 2,000 units of this enzyme 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 minimum of 3,200 units of this enzyme with 1 μg of φX174 RF I DNA in assay buffer for 4 hours at 37°C in 50 μl reactions results in less than 10% conversion to RF II as determined by agarose gel electrophoresis.

**Phosphatase Activity:** Incubation of a minimum of 20,000 units of this enzyme in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM MgCl₂) 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 a minimum of 2,000 units of this enzyme 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.

**Exonuclease Activity:** Incubation of a minimum of 3,200 units of this enzyme with 1 μg sonicated [³²H] DNA (10⁵ cpm/μg) for 4 hours at 37°C in 50 μl reaction buffer releases < 0.1% radioactivity.

**Functional Activity (Blunt End Ligation):** 50 μl reactions containing a 0.5 μl Quick T4 DNA Ligase, 18 μg HaeIII digested φX174 and 1X T4 DNA Ligase Buffer incubated at 16°C for 7.5 min results in > 95% of fragments ligated as determined by agarose gel electrophoresis.

**Functional Activity (Cohesive End Ligation):** 20 μl reactions containing 0.5 μl Quick T4 DNA Ligase, 12 μg HindIII digested lambda DNA and 1X T4 DNA Ligase Buffer incubated at 37°C overnight results in > 95% of fragments ligated as determined by agarose gel electrophoresis. Redigestion of the ligated products, 50 μl reactions containing 6 μg of the ligated fragments, 40 units HindIII, and 1X NEBuffer 2 incubated at 37°C for 2 hours, results in no detectable undigested fragments as determined by agarose gel electrophoresis.

**Functional Activity (Adapter Ligation):** 50 μl reactions containing 0.125 μl Quick T4 DNA Ligase, 8 nmol 12 bp adapter, and 1X T4 DNA Ligase Buffer incubated at 16°C overnight results in no detectable unligated adapter as determined by agarose gel electrophoresis.

**Functional Activity (Transformation):** After a five-minute ligation of linearized, dephosphorylated LITMUS™ 28 (containing either blunt [EcoRV] or cohesive [HindIII] ends) and a mixture of compatible insert fragments, transformation into chemically competent *E. coli* DH-5 alpha cells yields a minimum of 1 x 10⁶ recombinant transformants per μg plasmid DNA.

Lot Controlled

References:


Quick Ligation Reaction Buffer

#E6010A: 0.3 ml  Concentration: 2X
#E6010AA: 1.5 ml

Store at –20°C

1X Quick Ligation Reaction Buffer:
66 mM Tris-HCl
10 mM MgCl₂
1 mM dithiothreitol
1 mM ATP
7.5% Polyethylene glycol (PEG 6000)
pH 7.6 @ 25°C

Quality Control Assays

16-Hour Incubation: 50 μl reactions containing this reaction buffer 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 this reaction buffer at a 1X concentration with 1 μg of φX174 RF I DNA for 4 hours at 37°C in 50 μl reactions results in less than 10% conversion to RF II as determined by agarose gel electrophoresis.

RNase Activity: Incubation of this reaction buffer at 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.

Phosphatase Activity: Incubation of this reaction buffer at a 1X concentration in protein phosphatase assay buffer (1M diethanolamine @ pH 9.8 and 0.5 mM MgCl₂) 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 Q5 Hot Start HiFi PCR Master Mix

E6630A: 0.3 ml
E6630AA: 0.75 ml (2 vials provided)

Store at –20°C

Description: The NEBNext Q5 Hot Start HiFi PCR Master Mix is specifically optimized for robust, high-fidelity amplification of next-generation sequencing (NGS) libraries, regardless of GC content. The polymerase component of the master mix, Q5 High-Fidelity DNA Polymerase, is a novel thermostable DNA polymerase that possesses 3’→5’ exonuclease activity, and is fused to a processivity-enhancing Sso7d domain. Q5 also has an ultra-low error rate (> 100-fold lower than that of Taq DNA Polymerase and ~12-fold lower than that of Pyrococcus furiosus (Pfu) DNA Polymerase). The buffer component of the master mix has been optimized for robust amplification, even with GC-rich amplicons and offers enhanced compatibility with a variety of beads used in typical NGS workflows. These features make the NEBNext Q5 Hot Start HiFi PCR Master Mix ideal for NGS library construction. This convenient 2X master mix contains dNTPs, Mg^{++} and a proprietary buffer, and requires only the addition of primers and DNA template for robust amplification. The inclusion of the hot start aptamer allows convenient room temperature reaction set up.

Quality Control Assays

16-Hour Incubation: A 50 µl reaction containing NEBNext Q5 Hot Start HiFi PCR Master Mix and 1 µg of HindIII digested λ 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 Q5 Hot Start HiFi PCR Master Mix 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.

Phosphatase Activity: Incubation of NEBNext Q5 Hot Start HiFi PCR Master Mix 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.

Functional Activity (Multiplex PCR, Bead Inhibition): 30 cycles of PCR amplification of 20 ng genomic DNA with and without carboxylated magnetic beads in a 50 µl reaction containing 0.5 µM 4-plex primer mix and 1X NEBNext Q5 Hot Start HiFi PCR Master Mix result in the four expected amplicons and no inhibition of amplification in the presence of the beads.

Lot Controlled

This product is covered by one or more Patents. This product is licensed from Bio-Rad Laboratories, Inc. under U.S. Pat. Nos. 6,627,424; 7,541,170; 7,670,808; 7,666,645 and corresponding patents in other countries for use only in: (a) standard (non-real time) PCR in the research field only, but not real-time PCR or digital PCR; (b) any in vitro diagnostics application, except for applications using real-time or digital PCR; and (c) any non-PCR applications in DNA sequencing, isothermal amplification and the production of synthetic DNA.
<table>
<thead>
<tr>
<th>Revision #</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0</td>
<td>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. Changed PCR cycle number recommendations.</td>
</tr>
<tr>
<td>5.0</td>
<td>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)</td>
</tr>
<tr>
<td>6.0</td>
<td>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.</td>
</tr>
</tbody>
</table>