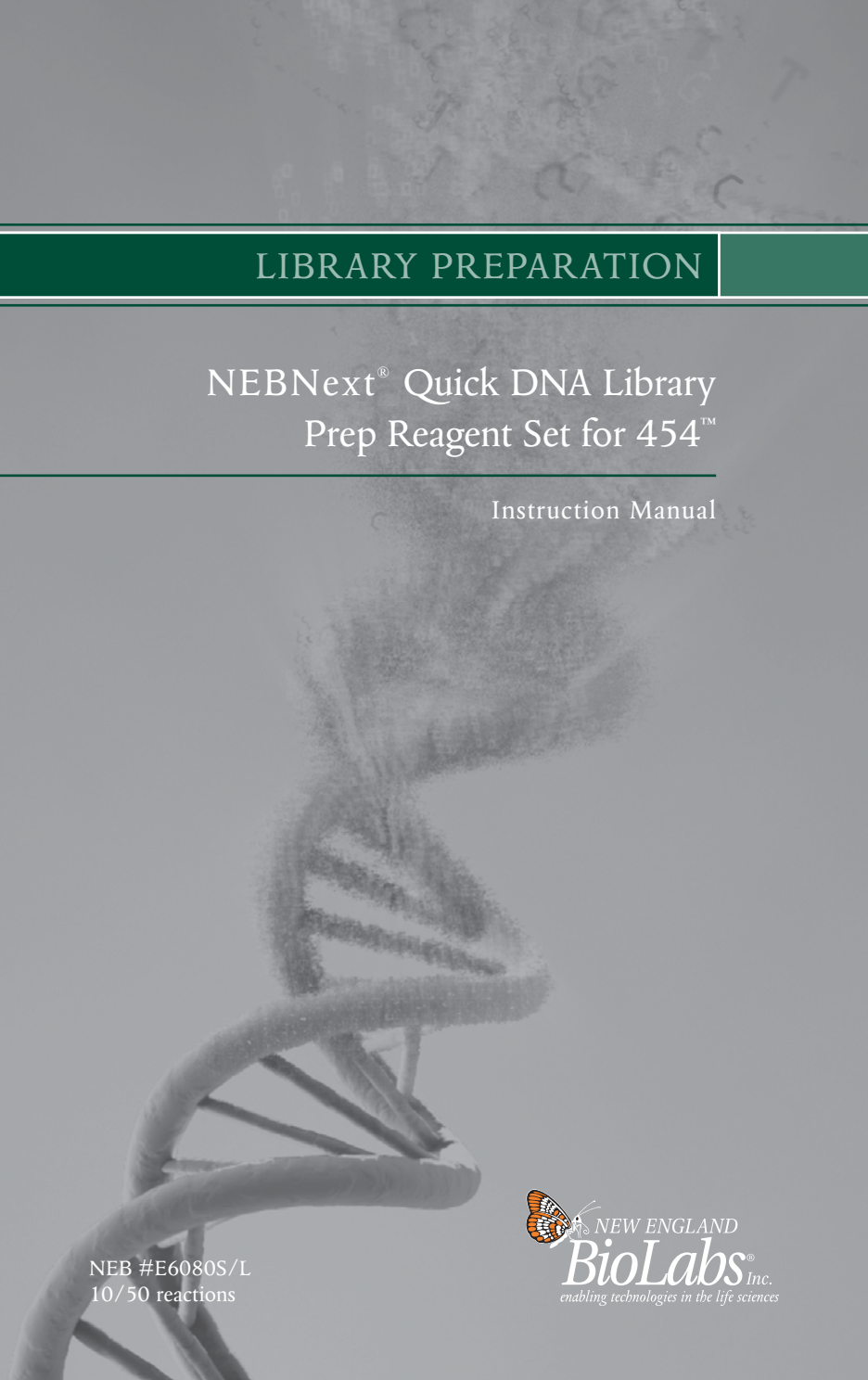
LIBRARY PREPARATION

NEBNext® Quick DNA Library Prep Reagent Set for 454™

Instruction Manual



NEB #E6080S/L
10/50 reactions

NEW ENGLAND BioLabs® Inc.
enabling technologies in the life sciences


Cloned at NEBiolabs


Recombinant Enzyme


Optimum Buffer


Requires BSA


Heat Inactivation

Table of Contents:

The Reagent Set Includes:

The volumes provided are sufficient for preparation of up to 10 reactions (NEB #E6080S) and 50 reactions (NEB #E6080L). (All reagents should be stored at –20°C).

NEBuffer 2 (10X)

Deoxynucleotide Solution Mix (10 mM each dNTP)

T4 DNA Polymerase RR

Adenosine 5´- Triphosphate (10 mM)

T4 Polynucleotide Kinase RR

Taq DNA Polymerase RR

Quick T4 DNA Ligase RR

NEBNext Sizing Buffer

TE Buffer

Required Materials Not Included:

Agencourt AMPure® Beads (Beckman Coulter, Inc.)

DNA Adaptors or Vectors

Magnetic Separation Rack (NEB #S1510)

Applications:

The NEBNext Quick DNA Library Prep Reagent Set for 454 contains enzymes and buffers that are ideally suited for library 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 Quick DNA Library Prep Reagent Set for 454 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 a 454 GS FLX Titanium™ System (Roche), and by construction of an expression library.

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:

NEBNext End Repair and dA-Tailing

Starting Material: 0.5 µg of DNA Fragmented to 100–1000 bp in 16 µl of TE.

1. In a 1.7 ml micro-centrifuge tube add:

NEBuffer 2 (10X)	2.5 µl
ATP	2.5 µl
dNTP Mix	1.0 µl
T4 DNA Polymerase	1.0 µl
PNK	1.0 µl
Taq DNA Polymerase	1.0 µl
	9.0 µl

2. Mix by pipetting and add to the 16 µl fragmented DNA sample.
3. Vortex briefly to mix, followed by a quick spin to collect all liquid from the sides of the tube.
4. In a thermocycler, with the heated lid on, run the following program:
   20 minutes @ 25°C
   20 minutes @ 72°C
   Hold at 4°C

Agencourt AMPure Bead Preparation

1. Vortex Ampure beads to re-suspend.
2. Transfer 125 µl of Ampure beads to a 1.7 ml micro-centrifuge tube.
3. Place the tube on a Magnetic Separation Rack. After the beads have collected to the side of the tube and the solution is clear, carefully remove and discard the supernatant. Be careful not to disturb the beads.
4. Add 73 µl of TE to the beads and vortex until the beads are completely re-suspended.
5. Add 500 µl of Sizing Solution to the beads, vortex to mix and spin briefly to collect all liquid from the sides of the tube.

NEBNext Adaptor Ligation

1. After completion of the End Repair/dA-Tailing program add 1.0 µl of adaptor (or vector if generating an expression library), to the reaction tube. (Adaptors/vectors are not provided. Please use adaptors/vectors appropriate to specific application).
2. Add 1.0 µl of Quick T4 DNA Ligase. Vortex briefly to mix, followed by a quick spin.
3. Incubate for 10 minutes at 25°C.

NEBNext Small Fragment Removal

1. Add the End Repaired/dA-Tailed/Adaptor ligated DNA sample directly to the previously prepared Ampure beads. Vortex briefly to mix, followed by a quick spin to collect liquid from the sides of the tube.
2. Incubate at room temperature for 5 minutes.
3. Place the tube on a Magnetic Separator.
4. When the beads have collected to the wall of the tube and the solution is clear, remove and discard the supernatant. Be careful not to disturb the beads.
5. Add 100 µl of TE and vortex until the beads are completely re-suspended.
6. Add 500 µl of NEBNext Sizing Buffer and briefly vortex to mix.
7. Incubate at room temperature for 5 minutes.
8. Place the tube on a Magnetic Separator.
9. When the beads are collected to the wall of the tube and the solution is clear, remove and discard the supernatant. Be careful not to disturb the beads.
10. Repeat steps 5-9 one time.
11. Keep the tube on the magnet and wash the beads twice with 1 ml of 70% ethanol.
12. Keep the tube on the magnet, uncapped, and let the pellet air dry until there is no visible liquid on the sides of the tube. This typically takes 5 minutes.
13. Remove the tube from the magnet, add 53 µl of TE, vortex to re-suspend the beads and spin briefly.
14. Place the tube on the magnet, when the beads are collected to the wall of the tube, transfer 50 µl of the supernatant (library), to a new 1.7 ml micro-centrifuge tube. Be careful not to transfer any beads.

NEBuffer 2

#E6081A: 0.025 ml **Concentration: 10X**
#E6081AA: 0.125 ml

Store at –20°C

1X NEBuffer 2:
10 mM Tris-HCl
50 mM NaCl
10 mM $MgCl_2$
1 mM DTT
pH 7.9 @ 25°C

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing 1X NEBuffer 2 and 1 µg of HindIII digested ϕX174 RF I 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 1X NEBuffer 2 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 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 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.

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

Adenosine 5´-Triphosphate (ATP)

#E6082A: 0.025 ml **Concentration: 10 mM**
#E6082AA: 0.125 ml

Store at –20°C

Description: Adenosine 5´-Triphosphate (ATP) is a substrate for ATP-dependent enzyme systems.

Supplied in: Sterile purified water adjusted to pH 7.0 with NaOH

Molecular Weight: 551.2 daltons (disodium salt)

Quality Control Assays

Phosphatase Activity: Incubation of a minimum of 1 mM ATP 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.

16-Hour Incubation: 50 µl reactions containing a minimum of 0.2 mM ATP and 1 µg of HindIII digested ϕX174 RF I 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 0.2 mM ATP 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 a minimum of 0.1 mM ATP 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.

Lot Controlled

Deoxynucleotide Solution Mix

#E6083A: 0.015 ml 10 mM each dNTP
#E6083AA: 0.075 ml

Store at –20°C

Description: Deoxynucleotide Solution Mix is an equimolar solution of ultrapure dATP, dCTP, dGTP and dTTP, provide for the PCR enrichment reaction.

Supplied in: Milli-Q® 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 ϕX174 RF I 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 $MgCl_2$) 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.

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.

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T4 DNA Polymerase

#E6084A: 0.015 ml
#E6084AA: 0.075 ml

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 $KP0_4$ (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 $MgCl_2$) 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 (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 [^{3}H]-dTTP, 70 µg/ml denatured herring sperm DNA and 50 µg/ml BSA.

Lot Controlled

References:

1. Tabor, S. and Struhl, K. (1989). DNA-Dependent DNA Polymerases. In F. M. Ausebel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith and K. Struhl (Eds.), *Current Protocols in Molecular Biology* (pp. 3.5.10–3.5.12). New York: John Wiley & Sons Inc.
2. Sambrook, J. et al. (1989). *Molecular Cloning: A Laboratory Manual,* (2nd ed.), (pp. 5.44–5.47). Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

T4 Polynucleotide Kinase

#E6085A: 0.015 ml
#E6085AA: 0.075 ml

Store at –20°C

Description: Catalyzes the transfer and exchange of P_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 [^{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 ϕX174 RF I 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 $MgCl_2$) 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 [^{3}H]DNA (10^5 cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

Functional Activity (Labeling): ^{32}P end labeling of 5´-hydroxyl terminated $d(T)_8$ 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:

1. Richardson, C.C. (1981). In P.D. Boyer (Ed.), *The Enzymes* Vol. 14, (pp. 299–314). San Diego: Academic Press.
2. Sambrook, J. et al. (1989) *Molecular Cloning: A Laboratory Manual*, (2nd ed.), (pp. 10.59–10.67, 11.31–11.33). Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Taq DNA Polymerase

#E6086A: 0.015 ml
#E6086AA: 0.075 ml

Store at –20°C

Description: *Taq* DNA Polymerase is a thermostable DNA polymerase that possesses a 5´→3´ polymerase activity and a double-strand specific 5´→3´ exonuclease activity.

Source: Purified from an *E. coli* strain that carries the *Taq* DNA polymerase gene from *Thermus aquaticus* YT-1.

Supplied in: 10 mM Tris-HCl, 100 mM KCl, 1 mM Dithiothreitol, 0.1 mM EDTA, 50% Glycerol, 0.5% Tween-20 and 0.5% NP-40, pH 7.4 @ 25°C.

Quality Control Assays

Endonuclease Activity: Incubation of a 50 µl reaction in ThermoPol Reaction Buffer containing a minimum of 20 units of *Taq* DNA Polymerase with 1 µg of supercoiled ϕX174 DNA for 4 hours at 75°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.

3´→5´ Exonuclease Activity: Incubation of a 20 µl reaction in ThermoPol Reaction Buffer containing a minimum of 20 units of *Taq* DNA Polymerase with 10 nM fluorescent internally labeled oligonucleotide for 30 minutes at either 37°C or 75°C yields no detectable 3´→5´ degradation as determined by capillary electrophoresis.

Functional Activity (Nucleotide Incorporation): 25 cycles of PCR amplification of 5 ng Lambda DNA with 1.25 units of *Taq* DNA Polymerase, in the presence of 200 µM dNTPS and 0.2 µM primers in ThermoPol Reaction Buffer, results in the expected 5 kb product.

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Quick T4 DNA Ligase

#E6087A: 0.015 ml
#E6087AA: 0.075 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 ϕX174 RF I 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 $MgCl_2$) 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 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 [^{3}H] DNA (10^5 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 10^6 recombinant transformants per µg plasmid DNA.

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References:

1. Engler, M. J. and Richardson, C. C. (1982). In P. D. Boyer (Ed.), *The Enzymes* Vol. 5, (p. 3). San Diego: Academic Press.
2. Remaut, E., Tsao, H. and Fiers, W. (1983) *Gene* 22, 103–113.

NEBNext Sizing Buffer

#E6088A: 15 ml **Concentration: 1.2X**
#E6088AA: 75 ml

Store at –20°C

1X NEBNext Sizing Buffer:
7% Polyethylene Glycol 8000
1 M NaCl

Description: NEBNext Sizing Buffer has been optimized for use with AMPure beads to select DNA fragments > 300 bp from a sample of mixed sized DNA fragments.

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing this reaction buffer at 1X concentration and 1 µg of HindIII digested ϕX174 RF I 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.

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.

Functional Assay (Size Selection): Two rounds of selection, using 500 µl of this buffer and 125 µl of Agencourt AMPure beads, results in the selection of DNA Fragments > 300 bp from 0.5 µg of 100 kb DNA ladder (NEB# N3231), as determined by Bioanalyzer (Agilent Technologies, Inc.) analysis.

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TE Buffer

#E6089A: 5 ml **Concentration: 1X**
#E6089AA: 25 ml

Store at –20°C

Description: TE Buffer is free of detectable DNA and RNA nucleases and is suitable for use in molecular biology applications.

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing TE, 1X NEBuffer 2 (500 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, pH 7.9) and 1 µg of HindIII digested ϕX174 RF I 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 TE, 1X NEBuffer 2 (500 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, pH 7.9) 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 TE, 1X NEBuffer 2 (500 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, pH 7.9) and 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 TE, 1X NEBuffer 2 (500 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, pH 7.9) and 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 TE 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.

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