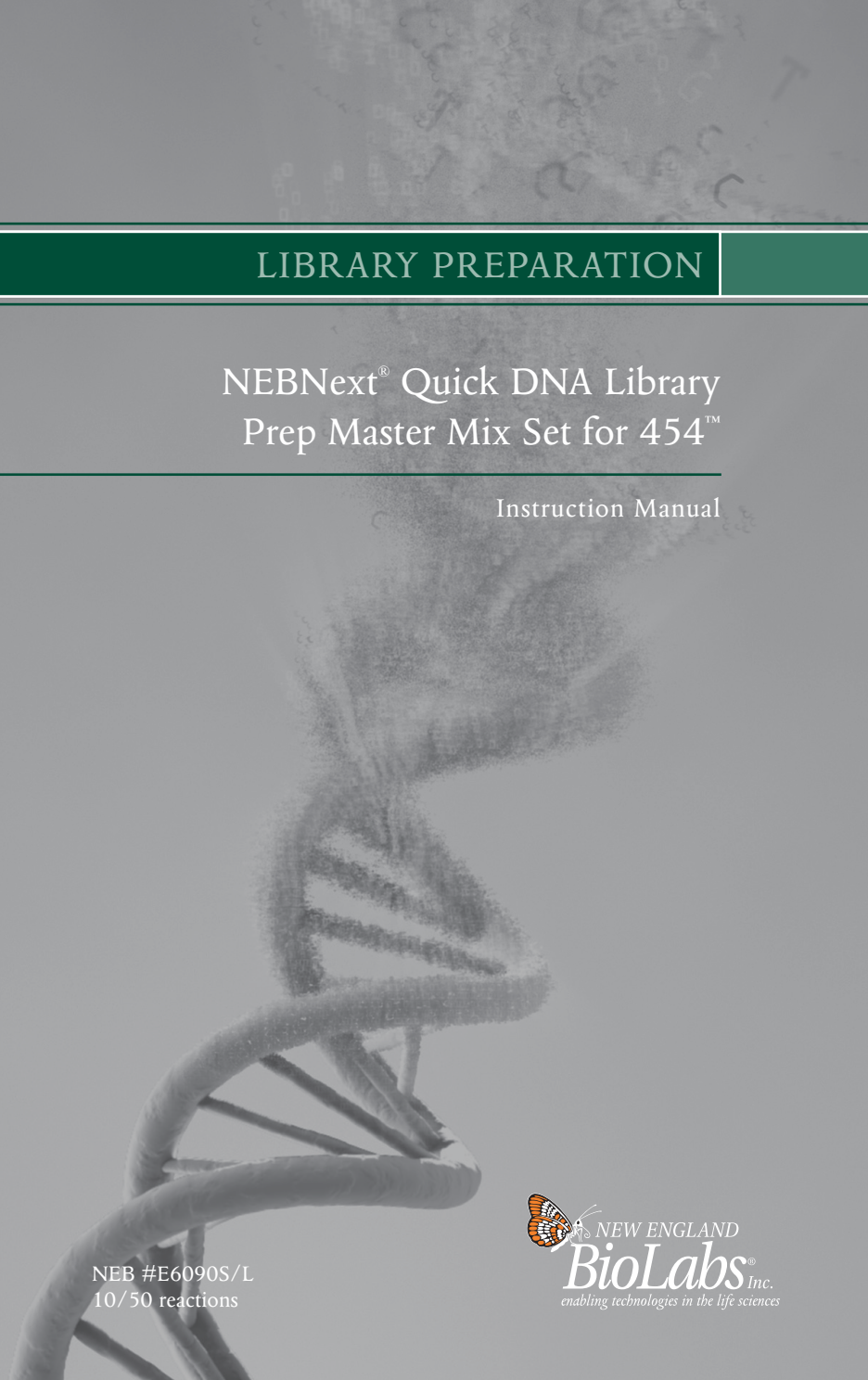
LIBRARY PREPARATION

NEBNext® Quick DNA Library Prep Master Mix Set for 454™

Instruction Manual



NEB #E6090S/L
10/50 reactions

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

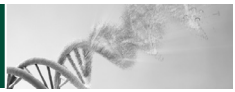

Cloned at NEBiolabs


Recombinant Enzyme


Optimum Buffer


Requires BSA


Heat Inactivation



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The Quick Master Mix Set Includes:

The volumes provided are sufficient for preparation of up to 10 reactions (NEB #E6090S) and 50 reactions (NEB #E6090L). (All reagents should be stored at –20°C).

NEBNext End Prep Enzyme Mix
NEBNext End Repair Reaction Buffer (10X)
Quick T4 DNA Ligase RR
NEBNext Sizing Buffer
TE Buffer
Nuclease-free Water

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 Master Mix Set for 454 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 Quick DNA Library Prep Master Mix 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.

Functional Validation: Each set of reagents is functionally validated together through construction and sequencing of a genomic DNA library by 454 GS FLX Titanium™ (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:

End Prep Enzyme Mix	1.0 µl
End Repair Reaction Buffer (10X)	2.5 µl
Nuclease -free Water	5.5 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 Beads 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 side 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.

NEBNext End Prep Enzyme Mix

#E6091A: 0.015 ml
#E6091AA: 0.075 ml

Store at –20°C

Description: NEBNext End Prep Enzyme Mix is optimized to convert 0.5 µg of fragmented DNA to repaired DNA having 5´-phosphorylated, dA-tailed ends.

NEBNext Quick Master Mix:
1,250 units/ml *Taq* DNA Polymerase
10,000 units/ml T4 Polynucleotide Kinase
3,000 units/ml T4 DNA Polymerase

Storage Conditions:
10 mM Tris-HCl
100 mM KCl
1 mM DTT
0.1 mM EDTA
50% Glycerol
0.5% Tween-20
0.5% NP-40
pH 7.4 @ 25°C

Quality Control Assays

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

Endonuclease Activity: Incubation of a minimum of 10 µl of this enzyme mix 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 10 µl of this enzyme 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 (Nucleotide Incorporation, Phosphorylation and dA-Tailing): 1 µl of this enzyme mix repairs and phosphorylates the ends of > 95% of 0.5 µg of DNA fragments containing both 3´ and 5´ overhangs within 20 minutes at 25°C, in 1X End Repair Reaction Buffer, as determined by capillary electrophoresis.

1 µl of this enzyme mixture adds a single nucleotide to the 3´ end of 0.5 µg of repaired DNA fragments within 20 mintues at 72°C in 1X End Repair Reaction Buffer, as determined by capillary electrophoresis.

Lot Controlled

NEBNext End Repair Reaction Buffer

#E6092A: 0.025 ml **Concentration: 10X**
#E6092AA: 0.125 ml

Store at –20°C

1X NEBNext End Repair Reaction Buffer:
50 mM Tris-HCl
10 mM $MgCl_2$
10 mM DTT
1 mM ATP
0.4 mM dATP
0.4 mM dCTP
0.4 mM dGTP
0.4 mM dTTP
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 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.

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

Quick T4 DNA Ligase

#E6087A: 0.015 ml
#E6087AA: 0.075 ml

Store at –20°C

Source: Purified from *E. coli* C600 pcI857 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 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 (Adaptor Ligation): 50 µl reactions containing 0.125 µl Quick T4 DNA Ligase, 8 nmol 12 bp adaptor, and 1X T4 DNA Ligase Buffer incubated at 16°C overnight results in no detectable unligated adaptor 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.

Lot Controlled

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.

Lot Controlled

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 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.

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

Nuclease-free Water

#E6093A: 0.5 ml
#E6093AA: 1.0 ml

Store at –20°C or 4°C

Description: Nuclease-free Water is free of detectable DNA and RNA nucleases and phosphatases and is suitable for use in DNA and RNA applications.

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing Nuclease-free Water, with 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 Nuclease-free Water, with 1X NEBuffer 2 (500 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, pH 7.9) 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 10 µl reaction containing Nuclease-free Water, with 1X NEBuffer 2 (500 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, pH 7.9) and 300 ng supercoiled plasmid for 4 hours at 37°C produced no nicked molecules as determined by gel electrophoresis.

RNase Activity: Incubation of a 10 µl reaction containing Nuclease-free Water, with 1X NEBuffer 2 (500 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, pH 7.9) and 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 1X Nuclease-free Water 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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Version 2.1
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