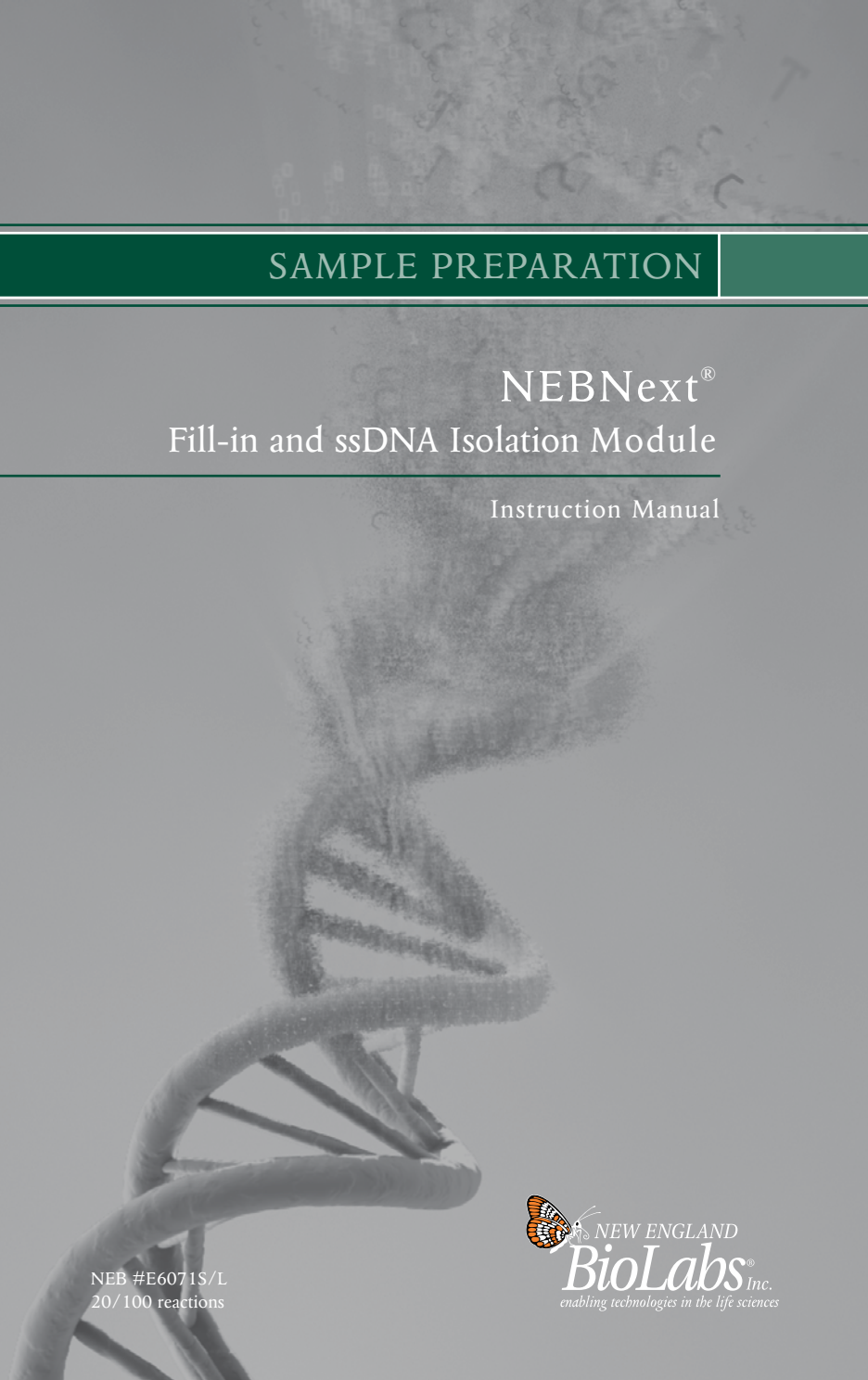
SAMPLE PREPARATION

NEBNext®
Fill-in and ssDNA Isolation Module

Instruction Manual



NEB #E6071S/L
20/100 reactions

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


Cloned at
NEBiolabs

Recombinant
Enzyme

Yes
Heat
Inactivation

Table of Contents:

The Module Includes:

The volumes provided are sufficient for preparation of up to 20 reactions (NEB #E6071S) and 100 reactions (NEB #E6071L).

Box 1: Store at –20°C

Bst DNA Polymerase, Large Fragment RR

NEBNext Adaptor Fill-in Reaction Buffer (10X) RR

Box 2: Store at 4°C

Hydrophilic Streptavidin Magnetic Beads (4 mg/ml)

NEBNext Bead Binding Buffer (2X)

NEBNext Bead Wash Buffer (1X)

Description:

The NEBNext® Fill-in and ssDNA Isolation Module has been optimized to fill in adapter sequence from a nick generated by ligation of adaptors lacking a 5´-phosphate to a DNA template coupled with ssDNA isolation. *Bst* DNA Polymerase, Large Fragment, recognizes the nick resulting from the ligation of unphosphorylated adapters, displaces the nicked strand and extends the 3´ end of the DNA template and fills in the adapter sequence to generate full length dsDNA. Hydrophilic Streptavidin Magnetic Beads and buffers are provided to allow binding of dsDNA fragments bearing one biotinylated adapter prior to adapter fill-in and elution of the full length, unbiotinylated ssDNA strands after adapter fill-in.

The NEBNext Fill-in and ssDNA Isolation Module is provided as a master mix to maximize efficiency and convenience in DNA sample preparation workflows (1,2,3). The NEBNext Fill-in and ssDNA Isolation Module has been validated by sequencing with the Roche 454 GS FLX Titanium in conjunction with the NEBNext End Repar Module, and the NEBNext Quick Ligation Module.

Applications:

DNA sample preparation

Adapter fill-in and ssDNA isolation of 1–5 µg fragmented DNA adapter ligand

Advantages:

- Efficient – Enables capture of biotinylated dsDNA or Streptavidin beads. Removes nicks generated by ligation of unphosphorylated primers on 1–5 µg DNA, and enables isolation of full length ssDNA from beads.
- Convenient – Reactions are provided in master mix format to reduce steps during DNA sample prep workflows
- Automation Friendly

References:

1. Maricic, T. and S. Paabo (2009). "Optimization of 454 sequencing library preparation from small amounts of DNA permits sequence determination of both DNA strands." *Biotechniques,* 46, 51–52, 54–57.
2. Straus, D. and F.M. Ausubel (1990). "Genomic subtraction for cloning DNA corresponding to deletion mutations." *Proc. Natl. Acad. Sci. USA* 87, 1889–1893.
3. Zhou, X. and D.T. Wong (2007). "Single nucleotide polymorphism mapping array assay." *Methods Mol. Biol.* 396, 295–314.

NEBNext®
Fill-in and ssDNA Isolation Module Protocol:

Recommended: *Removal of small fragments using Agencourt AMPure Beads or gel size selection.*

1. Transfer 50 µl of Hydrophilic Streptavidin Magnetic Beads to a 1.5 ml tube.
2. Using a magnet, pellet the beads and remove the buffer
3. Wash beads twice with 100 µl of 2X Bead Binding Buffer, pelleting the beads with a magnet to remove the buffer after each wash.
4. Resuspend beads in 25 µl of 2X Bead Binding Buffer.
5. Add 25 µl adapter-ligated DNA fragments to the beads.
6. Vortex and place on a tube rotator at room temperature for 20 minutes.
7. Using a magnet, wash the beads twice with 100 µl (1X) Bead Wash Buffer, pelleting the beads with a magnet to remove the buffer after each wash.
8. Mix the following components in a separate sterile microfuge tube:

Molecular Biology Grade Water	42 µl
Adapter Fill-in Reaction Buffer	5 µl
Bst DNA Polymerase, Large Fragment	3 µl

9. Transfer the 50 µl Fill-in Reaction Mix to the beads.
10. Vortex lightly and incubate at 37°C for 20 minutes.
11. Wash beads twice with 100 µl of 1X Bead Wash Buffer, pelleting the beads with a magnet to remove the buffer after each wash.
12. Prepare Melt Solution:

10 N NaOH	125 µl
Water	9.875 ml

13. Prepare Neutralization Solution in a 1.5 ml tube.

3 M sodium acetate, pH 5.2	10 µl
Column binding buffer with pH indicator	500 µl

14. Add 50 µl of Melt Solution to the beads.
15. Vortex well, pellet the beads with a magnet.
16. Carefully transfer the Melt Solution containing the ssDNA Fragment Library to 1.5 ml tube containing the Neutralization Solution.
17. Repeat steps 14–16, adding the second round of Melt Solution containing the ssDNA Fragment Library to the same 1.5 ml tube containing the Neutralization Solution and the first round of Melt Solution and ssDNA Fragments. Adjust pH if necessary by adding an additional 5 µl of 3 M sodium acetate.
18. Purify DNA on one column without adding any additional column binding buffer. Wash column twice with column wash buffer to remove all residual salts. Elute in 25 µl of sterile dH_2O or elution buffer.

Bst DNA Polymerase, Large Fragment

#E6076A: 0.06 ml
#E6076AA: 0.3 ml

Store at –20°C

Description: *Bst* DNA Polymerase, Large Fragment is the portion of the *Bacillus stearothermophilus* DNA Polymerase protein that contains the 5´→ 3´ polymerase activity, but lacks 5´→ 3´ exonuclease activity.

Source: *Bst* Polymerase, Large Fragment is prepared from an *E. coli* strain containing a genetic fusion of the *Bacillus stearothermophilus* DNA Polymerase gene, lacking the 5´→ 3´ exonuclease domain, and the gene coding for *E. coli* maltose binding protein (MBP). The fusion protein is purified to near homogeneity and the MBP portion of the fusion is cleaved off *in vitro.* The remaining polymerase is purified free of MBP (1).

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM dithiothreitol, 0.1% Triton X-100 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 8 units of this enzyme and 1 µg of HaeIII 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 8 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 $MgCl_2$) 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 8 units of this enzyme with 40 ng of 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 [^{3}H]-dTTP, 70 µg/ml denatured herring sperm DNA and 50 µg/ml BSA.

References:

1. Kong, H., Aliotta, J. and Pelletier, J.J., New England Biolabs, unpublished results.

NEBNext® Adapter Fill-in Reaction Buffer

#E6077A: 0.10 ml **Concentration: 10X**
#E6077AA: 0.5 ml

Store at –20°C

1X NEBNext Adapter Fill-in Reaction Buffer:
20 mM Tris-HCl
10 mM $(NH_4)_2SO_4$
10 mM KCl
2.0 mM $MgSO_4$
0.1% Triton X-100
0.4 mM dATP
0.4 mM dCTP
0.4 mM dGTP
0.4 mM dTTP
pH 8.8 @ 25°C

Description: New England Biolabs supplies a unique 10X reaction buffer for *Bst* DNA Polymerase, Large Fragment

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing this reaction buffer at 1X concentration and 1 µg of HaeIII 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.

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.

Hydrophilic Streptavidin Magnetic Beads

#E6072A: 1 ml **Concentration: 4 mg/ml**
#E6072AA: 5 ml

Store at 4°C

Description: Hydrophilic Streptavidin Magnetic Beads are 2 µm supermagnetic particles covalently coupled to a highly pure form of streptavidin. The beads can be used to capture biotin labeled DNA.

Supplied in: 4 mg/ml suspension in phosphate buffer (PBS) (pH 7.4) containing 0.1% BSA and 0.02% NaN_3.

Support Matrix: 2 µm non-porous magnetic microparticle.

Binding Capacity: The beads will bind greater than 800 pmol of free biotin per mg and greater than 400 pmol of single-stranded 20 bp biotinylated oligonucleotide per mg.

NEBNext® Bead Binding Buffer

#E6074A: 4.5 ml **Concentration: 2X**
#E6074AA: 22.5 ml

Store at 4°C

1X NEBNext Bead Binding Buffer:
5 mM Tris-HCl
0.5 mM EDTA
1 M NaCl
pH 7.5 @ 25°C

Description: NEBNext Bead Binding Buffer has been optimized for binding biotin-labeled DNA to Magnetic Streptavidin Beads.

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing this reaction buffer at 0.2X concentration and 1 µg of HaeIII 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 0.2X 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 0.2X 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.

NEBNext® Bead Wash Buffer

#E6073A: 8 ml **Concentration: 1X**
#E6073AA: 40 ml

Store at 4°C

1X NEBNext Bead Wash Buffer:
5 mM Tris-HCl
0.5 mM EDTA
1 M NaCl
pH 7.5 @ 25°C

Description: NEBNext Bead Wash Buffer has been optimized to remove non-specific binding to Magnetic Streptavidin Beads.

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing this reaction buffer at 0.1X concentration and 1 µg of HaeIII 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 0.1X 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 0.1X 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 0.1X 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 0.5X 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.

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) 837-2234
Toll Free: 1-800-387-1095
Fax: (905) 837-2994
Fax Toll Free: 1-800-563-3789
e-mail: info@ca.neb.com

China, People's Republic
New England Biolabs (Beijing), Ltd.
Telephone: 010-82378265/82378266
Fax: 010-82378262
e-mail: info@neb-china.com

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

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

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

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

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