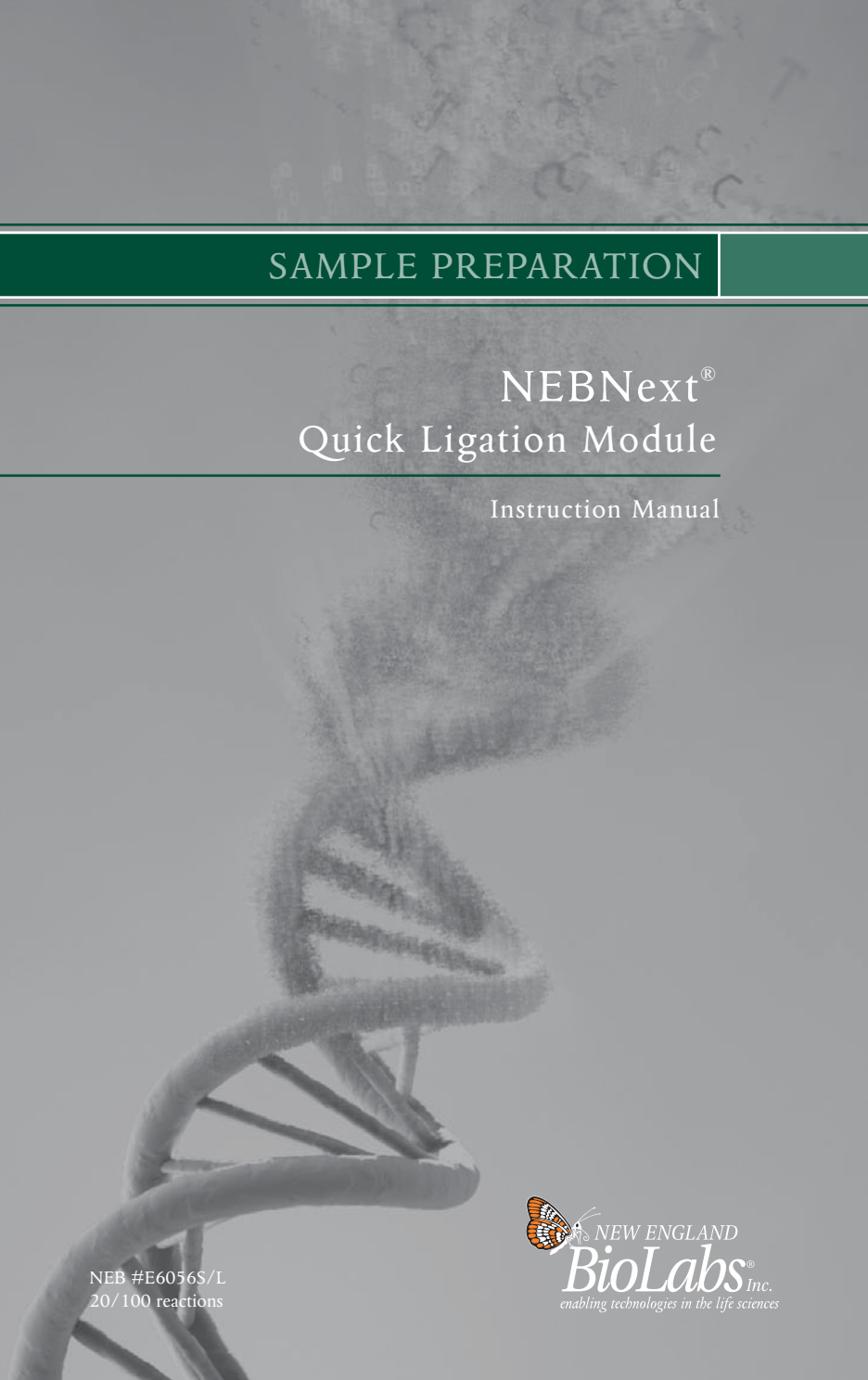
SAMPLE PREPARATION

NEBNext®
Quick Ligation Module

Instruction Manual



NEB #E6056S/L
20/100 reactions

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

Phusion® DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific. Phusion DNA Polymerase is manufactured by New England Biolabs, Inc. under agreement with, and under the performance specifications of Thermo Fisher Scientific.

Cloned at NEBiolabs

Recombinant Enzyme

Table of Contents:

The Module Includes:

The volumes provided are sufficient for preparation of up to 20 reactions (NEB #E6056S) and 100 reactions (NEB #E6056L). (All reagents should be stored at –20°C):

Quick T4 DNA Ligase

NEBNext Quick Ligation Reaction Buffer (5X)

Description:

The NEBNext Quick Ligation Module has been optimized to efficiently ligate DNA adaptors to blunt or dA-Tailed DNA fragments. The NEBNext Quick Ligation Module is provided as a master mix to maximize efficiency and convenience in DNA sample preparation workflows.

The NEBNext Quick Ligation Module has been validated by sequencing with the Illumina Genome Analyzer II (Illumina, Inc.) in conjunction with the NEBNext End Repair Module, NEBNext dA-Tailing Module and Phusion® High-Fidelity PCR Master Mix.

The NEBNext Quick Ligation Module has also been validated by sequencing with 454™ GS FLX Titanium™ (Roche) in conjunction with the NEBNext End Repair Module and the NEBNext Adapter Fill-in and ssDNA Isolation Module.

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.

Applications:

DNA Sample Preparation

Ligation of DNA adaptors to blunt or dA-Tailed DNA

Ligation of blunt or dA-Tailed DNA and cloning vectors

Advantages:

- Efficient – Ligates blunt DNA or dA-Tailed DNA
- Convenient – Reactions are provided in master mix format to reduce steps during DNA sample prep workflows
- Automation Friendly

NEBNext Quick Ligation Module Protocol

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

End Repaired, Blunt or dA-Tailed DNA	variable
NEBNext Quick Ligation Reaction Buffer (5X)	10 µl
DNA Adapters (not provided please use adaptors appropriate to specific application)	variable
Quick T4 DNA Ligase	5 µl
Sterile H_2O	variable
total volume	50 µl

2. Incubate in a thermal cycler for 15 minutes at 20°C.
3. Purify DNA sample on one column.

Quick T4 DNA Ligase

#E6057A: 0.1 ml
#E6057AA: 0.5 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 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 $MgCl_2$) 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.

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 Quick Ligation Reaction Buffer

#E6058A: 0.2 ml **Concentration: 5X**
#E6058AA: 1.0 ml

Store at –20°C

1X NEBNext Quick Ligation Reaction Buffer:
66 mM Tris-HCl
10 mM $MgCl_2$
1 mM dithiothreitol
1 mM ATP
6% 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 $MgCl_2$) 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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