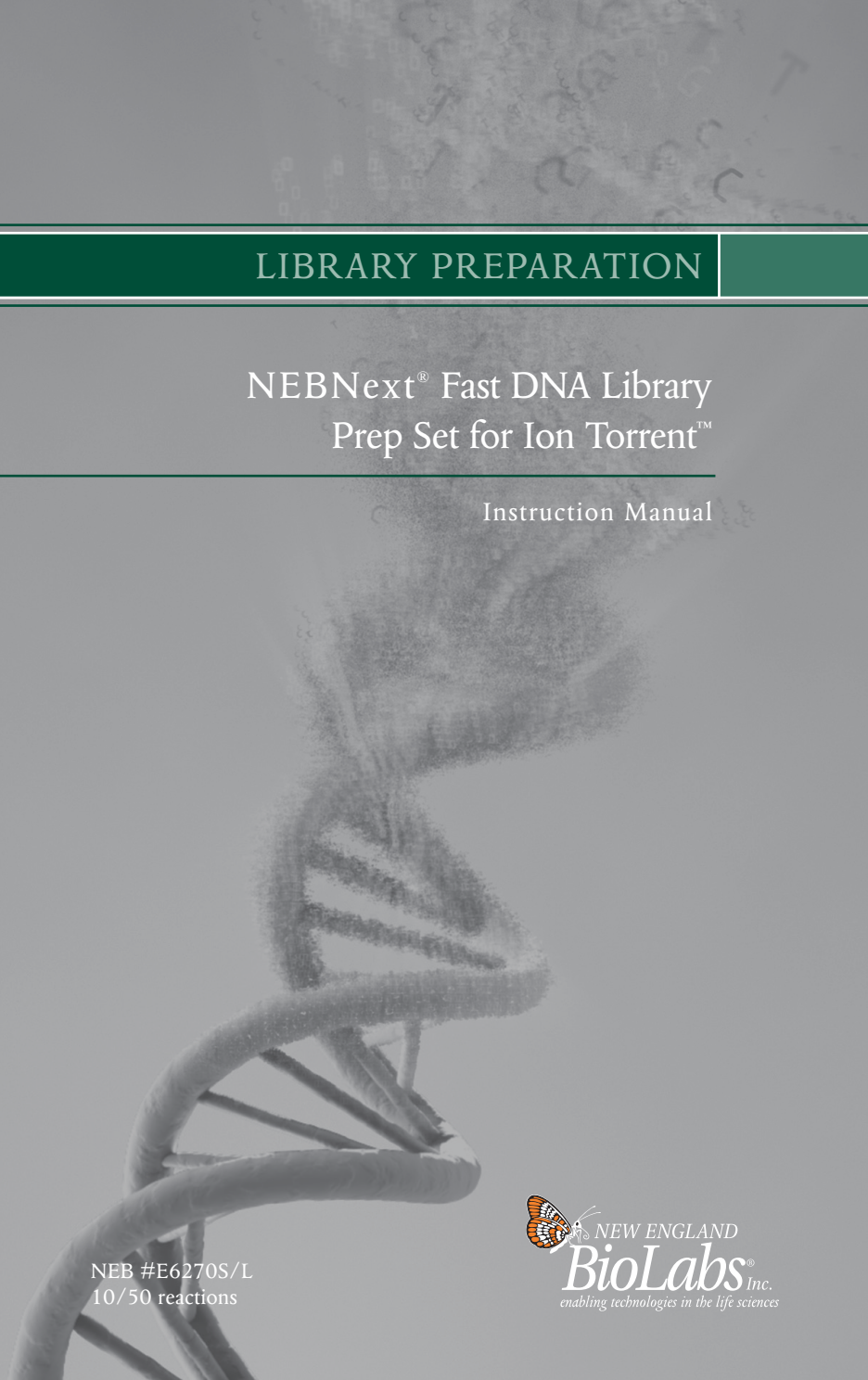
LIBRARY PREPARATION

NEBNext® Fast DNA Library Prep Set for Ion Torrent™

Instruction Manual



NEB #E6270S/L
10/50 reactions

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enabling technologies in the life sciences

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nqa ISO 9001 Registered Quality Management

nqa ISO 14001 Registered Environmental Management

nqa ISO 13485 Registered Medical Devices

Cloned at NEBiolabs | Recombinant Enzyme | NEB2 Optimum Buffer | BSA Requires BSA | Yes Heat Inactivation

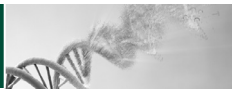


Table of Contents:

The Library Set Includes:

The volumes provided are sufficient for preparation of up to 10 reactions (NEB #E6270S) and 50 reactions (NEB #E6270L). (All reagents should be stored at –20°C).

- (green) NEBNext End Repair Enzyme Mix
- (green) NEBNext End Repair Reaction Buffer
- (red) T4 DNA Ligase
- (red) *Bst* 2.0 WarmStart DNA Polymerase
- (red) T4 DNA Ligase Buffer for Ion Torrent
- (blue) NEBNext DNA Library Primers for Ion Torrent
- (red) NEBNext DNA Library Adaptors for Ion Torrent
- (white) TE Buffer (0.1X)
- (blue) NEBNext High-Fidelity 2X PCR Master Mix

Required Materials Not Included:

PCR Column Purification Kit (Qiagen or other) or AMPure® XP Beads (Beckman Coulter, Inc.)

80% Ethanol

Size selection materials [E-Gel® (Life Technologies, Inc., etc.) or AMPure XP Beads]

Applications:

The NEBNext Fast DNA Library Prep Set for Ion Torrent contains enzymes and buffers in convenient master mix formulations that are ideally suited for sample preparation for next-generation sequencing on the Ion Torrent Sequencer (Life Technologies, Inc.). 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 Fast DNA Library Prep Set for Ion Torrent 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 Ion Torrent PGM.

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 with multiple options*

● *Colored bullets indicate a reagent to be added and the color of it's cap.*

Starting Material: 10 ng –1 µg of Fragmented DNA.

Note: For use with the Ion Xpress™ Barcode Adapters 1-16 Kit, a minimum of 100 ng starting material is recommended. Lower amounts may cause adaptor concatamerization.

End Repair of DNA Protocol

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

Fragmented DNA	1–51 µl
● (green) NEBNext End Repair Reaction Buffer	6 µl
● (green) NEBNext End Repair Enzyme Mix	3 µl
Sterile H_2O	variable
Total volume	60 µl

2. Incubate in a thermal cycler for 20 minutes at 25°C, followed by 10 minutes at 70°C, hold at 4°C.

3. Pulse spin the microfuge tube and return to ice.

Preparation of Adaptor Ligated DNA

1. Add the following to the microfuge tube:

For 10 ng		**For 100 ng – 1 µg**	
Sterile H_2O	18 µl	Sterile H_2O	3 µl
● (red) T4 DNA Ligase Buffer for Ion Torrent	10 µl	● (red) T4 DNA Ligase Buffer for Ion Torrent	10 µl
● (red) NEBNext DNA Library Adaptors for Ion Torrent	5 µl	● (red) NEBNext DNA Library Adaptors for Ion Torrent	20 µl
● (red) *Bst* 2.0 WarmStart DNA Polymerase	1 µl	● (red) *Bst* 2.0 WarmStart DNA Polymerase	1 µl
● (red) T4 DNA Ligase	6 µl	● (red) T4 DNA Ligase	6 µl
Total volume	40 µl	Total volume	40 µl

2. The total volume in the microfuge tube should be 100 µl. Mix the contents by pipetting up and down several times.
3. Incubate in a thermal cycler for 15 minutes at 25°C, followed by 5 minutes at 65°C, hold at 4°C.

⚠ If performing size selection with beads, proceed directly to size selection using AMPure XP Beads. If using E-gel or agarose gel for size selection, cleanup adaptor ligated DNA before proceeding to size selection.

Cleanup of Adaptor Ligated DNA

1. Add 180 µl (1.8X volume) of AMPure XP Beads to the sample and mix by pipetting up and down.
2. Incubate for 5 minutes at room temperature.
3. Pulse spin the tube and place in a magnetic rack for approximately 2–3 minutes until the beads have collected to the side of the tube and the solution is clear.
4. Carefully remove and discard the supernatant without disturbing the beads.
5. Keep the tube on the magnet and add 500 µl freshly prepared 80% ethanol. Incubate at room temperature, for 30 seconds, and carefully remove and discard the supernatant.
6. Repeat step 5.
7. Pulse-spin the tube, return to the magnet, and remove any residual ethanol with a pipet.
8. Keeping the tube in the magnetic rack, with the cap open, air dry the beads for 5 minutes at room temperature.
9. Resuspend the beads in 25 µl of sterile 0.1X TE (volume may be adjusted for specific size selection protocol.)
10. Pulse-spin the tube and return to the magnet, until the beads have collected to the side of the tube and the solution is clear.
11. Transfer approximately 20 µl of the supernatant to a clean tube, being careful not to transfer any beads.

Alternatively, adaptor ligated DNA can be purified on one column.

Size Selection

Size distribution and library yields will vary depending on the size selection method. Size selection can be performed using a number of methods including E-Gel size select gels, standard 2% agarose gels, or AMPure XP Beads (see protocol below). For E-Gel size select gels, select adaptor ligated DNA in the 190–230 bp range for 100 bp libraries and 290–330 bp range for 200 bp read lengths. For AMPure XP bead size selection, use the protocol below. Expect size distributions in the range of 230–270 for 100 bp reads and 310–370 for 200 bp reads.

Table 1: Recommended Conditions for Dual Bead-based Size Selection

	INSERT SIZE (bp)	
BEAD:DNA RATIO*	100 bp	200 bp
1st Bead Selection	0.9X	0.7X
2nd Bead Selection	0.15X	0.15X

*Bead:DNA ratio is calculated based on the original volume of DNA solution.

AMPure XP Bead-based Dual Bead Size Selection for 100 bp Inserts

Caution: The following protocol is for size selecting libraries with a 100 bp insert from a **100 µl volume**. For libraries with a 200 bp insert please use the bead:DNA ratio listed in the chart above.

1st Bead Selection to Remove Large Fragments: This step is used to bind the large, unwanted fragments to the beads. The supernatant will contain the desired fragments.

1. Add 90 µl (0.9X) 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 on a magnetic rack to separate the beads from the supernatant. After the solution is clear (about 5 minutes), carefully transfer the supernatant to a new tube **(Caution: do not discard the supernatant).** Discard beads that contain the large fragments.

2nd Bead Selection to Remove Small Fragments and to Bind DNA Target: This step will bind the desired fragment sizes (contained in the supernatant from Step 3) to the beads. Unwanted smaller fragment sizes will not bind to the beads.

4. Add 15 µl (0.15X) resuspended AMPure XP beads to the supernatant, mix well and incubate for 5 minutes at room temperature.
5. Put the tube on a magnetic rack to separate beads from supernatant. After the solution is clear (approximately 3 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 500 µl of 80% freshly prepared ethanol to the tube 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. Keeping the tube on the magnetic rack, with the cap open, air dry the beads for 5 minutes.
9. Elute DNA target from beads into 45 µl water. Mix well on a vortex mixer or by pipetting up and down, and put the tube in a magnetic rack until the solution is clear, approximately 3 minutes.
10. Transfer approximately 40 µl of the supernatant to a clean tube, being careful not to transfer any beads.

Note: Be sure not to transfer any beads. Trace amounts of bead carry over may affect the optimal performance of the polymerase used in the NEBNext High-Fidelity 2X PCR Master Mix in the subsequent PCR step.

PCR Amplification of Adaptor Ligated DNA

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

For 10 ng – 100 ng	
Adaptor Ligated DNA	1–40 µl
(blue) Primers	4 µl
Sterile H_2O	variable
(blue) NEBNext High-Fidelity 2X PCR Master Mix	50 µl
Total volume	100 µl

For 1 µg	
Adaptor Ligated DNA	1–40 µl
(blue) Primers	10 µl
Sterile H_2O	variable
(blue) NEBNext High-Fidelity 2X PCR Master Mix	50 µl
Total volume	100 µl

PCR cycling conditions

STEP	TEMPERATURE	TIME
Initial Denaturation	98°C	30 seconds
4–12 Cycles	98°C	10 seconds
	58°C	30 seconds
	72°C	30 seconds
1 Cycle	72°C	5 minutes
Hold	4°C	∞

Cycling Suggestions:

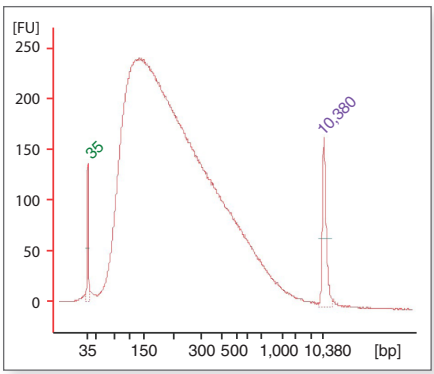
DNA	CYCLES
10 ng	10–12
100 ng	6–8
1 µg	4–6

Clean Up of Amplified Library

1. Add 100 µl (1.0X volume) of AMPure XP Reagent to the sample and mix by pipetting up and down.
2. Incubate for 5 minutes at room temperature.
3. Pulse-spin the tube and place in a magnetic rack for approximately 3 minutes until the beads have collected to the wall of the tube and the solution is clear.
4. Carefully remove and discard the supernatant without disturbing the beads.
5. Keep the tube on the magnet and add 500 µl freshly prepared 80% ethanol. Incubate 30 seconds, and carefully remove and discard the supernatant.
6. Repeat step 5.
7. Pulse-spin the tube, return to the magnet and remove any residual ethanol with a pipet.
8. Keeping the tube in the magnetic rack, with the cap open, air dry the beads for 5 minutes at room temperature.
9. Resuspend the beads in 35 µl of 0.1 x TE.
10. Pulse-spin the tube, return to the magnet until the beads have collected to the wall of the tube and solution is clear.
11. Transfer approximately 30 µl of supernatant to a fresh tube. Be careful not to transfer any beads.
12. Assess the library quality on a Bioanalyzer

Alternatively, adaptor ligated DNA can be purified on one column, elute in 30 µl of 0.1X TE.

Figure 1: Relative size distribution of Fragmented End Repaired DNA as seen using the Bioanalyzer® 2100 (Agilent Technologies, Inc.).



1 µg of E. coli DNA was fragmented and end repaired for 20 minutes at 25°C, followed by 10 minutes at 70°C.

Figure 2: Final Library Size distribution using E-Gel Size Selection.

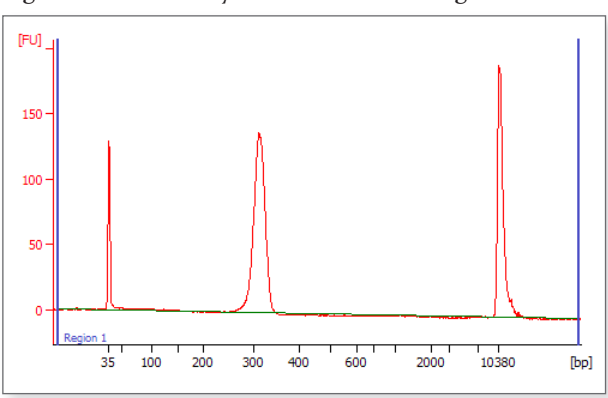
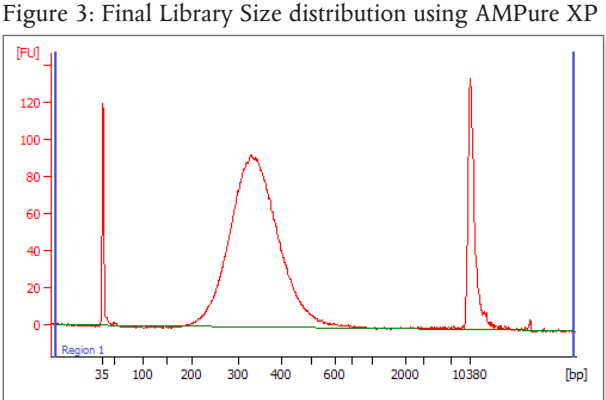


Figure 3: Final Library Size distribution using AMPure XP Beads.



NEBNext End Repair Enzyme Mix

#E6271A: 0.030 ml
#E6271AA: 0.150 ml

Store at –20°C

Description: NEBNext End Repair Enzyme Mix is optimized to convert 10 ng–1 µg of fragmented DNA to repaired DNA having 5´-phosphorylated, blunt ends.

Quality Control Assays

SDS-PAGE Purity: SDS-PAGE analyses 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): 0.2 µl of this enzyme mix 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.

Functional Activity (Nucleotide Incorporation and Phosphorylation): 5 µl of this enzyme mix repairs and phosphorylates the ends of > 95% of 10 µg of DNA fragments containing both 3´ and 5´ overhangs within 30 minutes at 20°C in 1X End Repair Buffer, as determined by capillary electrophoresis.

Lot Controlled

NEBNext End Repair Reaction Buffer

#E6272A: 0.060 ml **Concentration: 10X**
#E6272AA: 0.300 ml

Store at –20°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

T4 DNA Ligase

#E6275A: 0.060 ml
#E6275AA: 0.300 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 $MgCl_2$) 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 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 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 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.

Bst 2.0 WarmStart DNA Polymerase

#E6277A: 0.015 ml
#E6277AA: 0.05 ml

Store at –20°C

Description: *Bst* 2.0 WarmStart DNA Polymerase is an *in silico* designed homologue of *Bacillus stearothermophilus* DNA Polymerase I, Large Fragment (*Bst* DNA Polymerase, Large Fragment) with a reversibly-bound aptamer, which inhibits polymerase activity at temperatures below 45°C. The aptamer rapidly releases the *Bst* 2.0 WarmStart DNA Polymerase above 45°C and therefore no special activation step is needed to activate the polymerase. *Bst* 2.0 WarmStart DNA Polymerase contains 5´→3´ DNA polymerase activity and strong strand-displacement activity but lacks 5´→3´ exonuclease activity. *Bst* 2.0 WarmStart DNA Polymerase displays improved amplification speed, yield, salt tolerance, and thermostability compared to wild-type *Bst* DNA polymerase, Large Fragment.

Source: *Bst* 2.0 WarmStart DNA Polymerase is prepared from an *E. coli* strain that expresses the *Bst* 2.0 DNA Polymerase protein from an inducible promoter.

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

Exonuclease Assay: Incubation of a 50 µl reaction in 1X ThermoPol™ Reaction Buffer containing a minimum of 500 units of Bst 2.0 DNA Polymerase with 1 µg of a mixture of single and double-stranded [^{3}H] E. coli DNA (10^6 cpm/µg) for 4 hours at 65°C releases < 0.1% of the total radioactivity.

Endonuclease Assay: Incubation of a 50 µl reaction in 1X ThermoPol Reaction Buffer containing a minimum of 500 units of *Bst* 2.0 DNA Polymerase with 1 µg of supercoiled ϕX174 DNA for 4 hours at 65°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.

Physical Purity: Purified to > 99% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

Phosphatase Assay: Incubation of a 200 µl reaction in 1 M Diethanolamine (pH 9.8) and 0.5 mM $MgCl_2$ containing 2.5 mM *p*-Nitrophenol Phosphate and a minimum of 100 units of *Bst* 2.0 DNA Polymerase incubated for 4 hours at 37°C yields no detectable phosphatase activity as determined by spectrophotometric analysis of released *p*-nitrophenylene anion at 405 nm.

RNase Activity: Incubation of a 10 µl reaction in 1X NEBuffer 4 containing a minimum of 1 µl of *Bst* 2.0 WarmStart DNA Polymerase and 40 ng of F-300 RNA transcript incubated for 16 hours at 37°C results in < 10% substrate degradation as determined by gel electrophoresis using fluorescent detection.

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Notice to Purchaser: Nucleic acid-based aptamers for use with thermophilic DNA polymerases are licensed exclusively by New England Biolabs, Inc. from SomaLogic, Inc. (See Patent Nos. 5,475,096; 5,670,637; 5,696,249; 5,874,557; and 5,693,502). New England Biolabs, Inc. gives the Buyer/User a non-exclusive license to use the aptamer-based *Bst* 2.0 WarmStart™ DNA Polymerase for RESEARCH PURPOSES ONLY. Commercial use of the aptamer-based *Bst* 2.0 WarmStart™ DNA Polymerase requires a license from New England Biolabs, Inc. Please contact busdev@neb.com for more information.

T4 DNA Ligase Buffer for Ion Torrent

#E6276A: **0.1 ml** **Concentration: 10X**
#E6276AA: **0.5 ml**

Store at –20°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 a minimum of 10 µl of this reaction buffer at a 1X concentration with 1 µg of ϕX174 RF 1 DNA in assay buffer for 4 hours at 37°C in 50 µl reactions results in < 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 a minimum of 10 µl 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.

Lot Controlled

NEBNext DNA Library Adaptors for Ion Torrent

#E6274A: 0.2 ml
#E6274AA: 1 ml

Store at –20°C

Quality Control Assays

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

RNase Activity: Incubation of adaptors 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 a minimum of 10 µl of adaptors 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.

HPLC: NEBNext DNA Library Adaptors for Ion Torrent purity is determined by HPLC to be > 99%.

Lot Controlled

NEBNext DNA Library Primers for Ion Torrent

#E6291A: 0.100 ml
#E6291AA: 0.500 ml

Store at –20°C

Quality Control Assays

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

RNase Activity: Incubation of primers 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 a minimum of 10 µl of primers 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.

HPLC: NEBNext DNA Library Primers for Ion Torrent purity is determined by HPLC to be > 99%.

Lot Controlled

TE Buffer

#E6293A: 0.6 ml **Concentration: 0.1X**
#E6293AA: 3.0 ml

Store at –20°C or 4°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 Buffer at 0.1X 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 TE 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 a minimum of 10 µl of TE Buffer at a 0.1X concentration with 1 µg of ϕX174 RF I DNA in assay buffer for 4 hours at 37°C in 50 µl reactions results in < 10% conversion to RF II as determined by agarose gel electrophoresis.

RNase Activity: Incubation of TE Buffer at a 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 a minimum of 10 µl of TE Buffer at a 0.1X 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

NEBNext High-Fidelity 2X PCR Master Mix

E6298A: 0.5 ml **Concentration: 2X**
E6298AA: 2.5 ml

Store at –20°C

Description: The NEBNext High-Fidelity 2X 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 High-Fidelity DNA Polymerase also has an ultra-low error rate (> 50-fold lower than that of *Taq* DNA Polymerase and 6-fold lower than that of *Pyrococcus furiosus* (Pfu) DNA Polymerase)

Quality Control Assays

16-Hour Incubation: A 50 µl reactions containing NEBNext High-Fidelity 2X PCR Master Mix 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 100 units of NEBNext High-Fidelity 2X 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 High-Fidelity 2X 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 PCR: 30 cycles of PCR amplification of 20 ng genomic DNA in a 50 µl reaction containing 0.5 µM primers and 1X NEBNext High-Fidelity PCR Master Mix result in the expected 737 bp product.

Lot Controlled

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) 665-4632
Toll Free: 1-800-387-1095
Fax: (905) 665-4635
Fax Toll Free: 1-800-563-3789
e-mail: info.ca@neb.com
www.neb.ca

CHINA, PEOPLE'S REPUBLIC
New England Biolabs (Beijing), Ltd.
Telephone: 010-82378265/82378266
Fax: 010-82378262
e-mail: info@neb-china.com
www.neb-china.com

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

GERMANY & AUSTRIA
New England Biolabs GmbH
Telephone: +49/(0)69/305 23140
Free Call: 0800/246 5227 (Germany)
Free Call: 00800/246 52277 (Austria)
Fax: +49/(0)69/305 23149
Free Fax: 0800/246 5229 (Germany)
e-mail: info.de@neb.com
www.neb-online.de

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

SINGAPORE
New England Biolabs Pte. Ltd.
Telephone: +65 6776 0903
Fax: +65 6778 9228
e-mail: sales.sg@neb.com
www.neb.sg

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
www.neb.uk.com

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