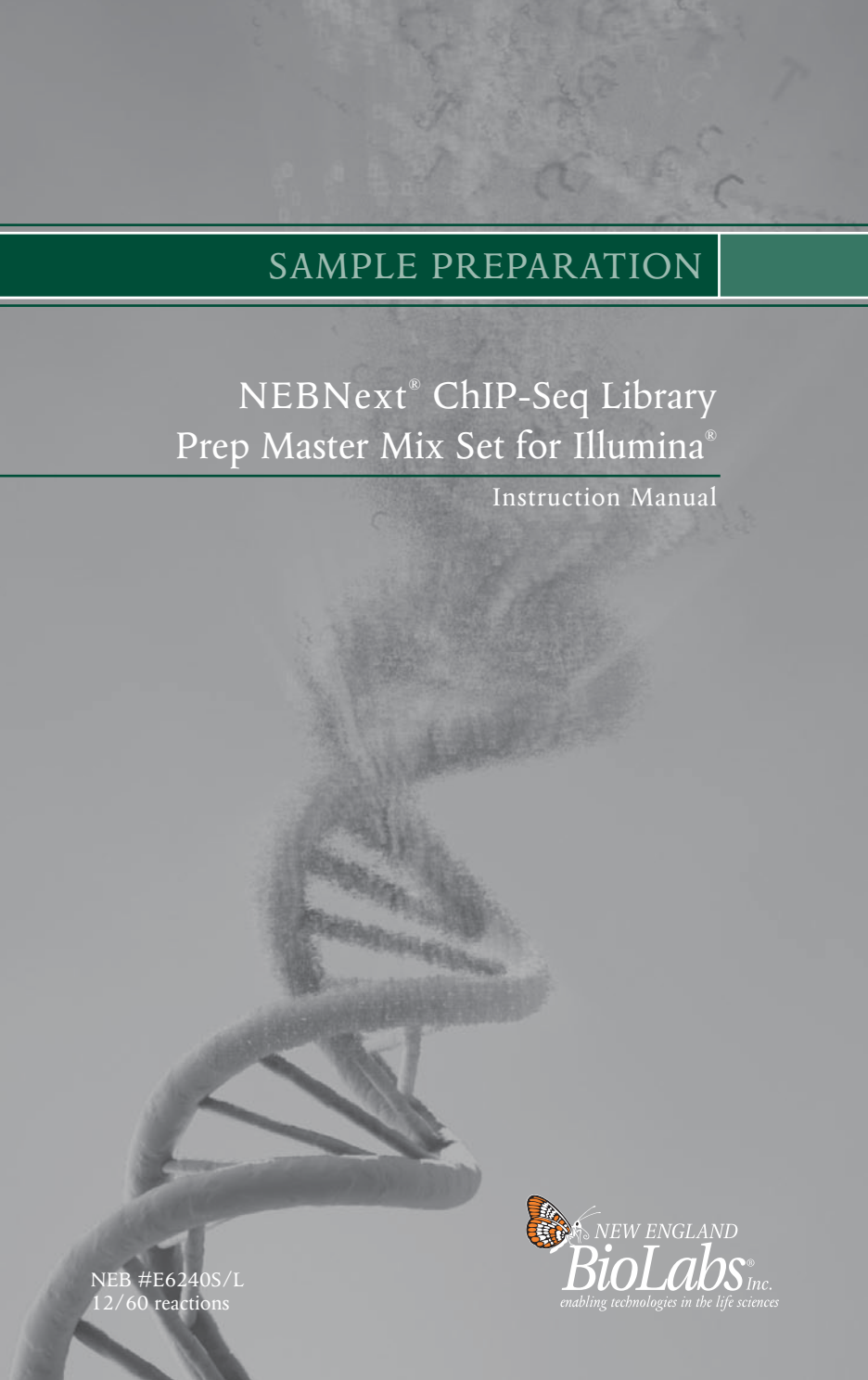
SAMPLE PREPARATION

NEBNext® ChIP-Seq Library Prep Master Mix Set for Illumina®

Instruction Manual



NEB #E6240S/L
12/60 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 | RR Recombinant Enzyme | NEB2 Optimum Buffer | BSA Requires BSA | Yes Heat Inactivation

Table of Contents:

The Master Mix Set Includes:

The volumes provided are sufficient for preparation of up to 12 reactions (NEB #E6240S) and 60 reactions (NEB #E6240L). (All reagents should be stored at –20°C):

NEBNext End Repair Enzyme Mix

NEBNext End Repair Reaction Buffer (10X)

Klenow Fragment (3´→5´ exo⁻) RR

NEBNext dA-Tailing Reaction Buffer (10X)

Quick T4 DNA Ligase RR

NEBNext Quick Ligation Reaction Buffer (5X) RR

Phusion High-Fidelity PCR Master Mix with HF Buffer

NEBNext High-Fidelity 2X PCR Master Mix (SAMPLE)

Applications:

The NEBNext ChIP-Seq Library Prep Master Mix Set for Illumina contains enzymes and buffers in convenient master mix formulations that are ideally suited for sample preparation for next-generation sequencing of Chromatin Immunoprecipitated DNA. 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 ChIP-Seq Library Prep Master Mix Set for Illumina 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 ChIP-Seq DNA library on an Illumina Sequencer (Illumina, Inc.).

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.

Protocol for Use With NEBNext Singleplex (#E7350) or Multiplex (#E7335, #E7500) Oligos for Illumina

Starting Material: 10 ng of chromatin-immunoprecipitated (ChIP) qPCR verified or control DNA, in ≤ 40 µl of water or elution buffer

End Repair of ChIP DNA

1. In a sterile microfuge tube mix the following components:

ChIP DNA	1–40 µl
NEBNext End Repair Reaction Buffer	5 µl
NEBNext End Repair Enzyme Mix	1 µl
Sterile H_2O	variable
Total volume	50 µl

2. Incubate in a thermal cycler for 30 minutes at 20°C.

Clean Up Using AMPure® XP Beads (Beckman Coulter, Inc.)

1. Vortex beads to resuspend.
2. Add 90 µl (1.8X) of resuspended AMPure XP beads to the reaction (~ 50 µl). Mix well on a vortex mixer or by pipetting up and down at least 10 times.
3. Incubate for 5 minutes at room temperature.
4. Put the tube/PCR plate on an appropriate magnetic stand to separate beads from supernatant. After the solution is clear (about 5 minutes), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets.
5. Add 200 µl of 80% ethanol to the tube/PCR plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.
6. Repeat Step 5 once.
7. Air dry beads for 10 minutes while the tube/PCR plate is on the magnetic stand with the lid open.
8. Elute DNA target from beads into 46 µl of 0.1X TE. Mix well on a vortex mixer or by pipetting up and down, and put the tube/PCR plate in the magnetic stand until the solution is clear.
9. Transfer 44 µl of the supernatant to a clean LoBind® tube (Eppendorf AG), and store at –20°C.

Alternatively, purify DNA sample on one purification column and elute in 44 µl of sterile dH_2O or elution buffer.

dA-Tailing of End Repaired DNA

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

End Repaired DNA	44 µl
NEBNext dA-Tailing Reaction Buffer (10X)	5 µl
Klenow Fragment (3´→5´ exo⁻)	1 µl
Total volume	50 µl

2. Incubate at 37°C for 30 minutes.

Clean up Using AMPure XP Beads

1. Vortex beads to resuspend.
2. Add 90 µl (1.8X) of resuspended AMPure XP beads to the dA tailing reaction(~ 50 µl). Mix well on a vortex mixer or by pipetting up and down at least 10 times.
3. Incubate for 5 minutes at room temperature.
4. Put the tube/PCR plate on an appropriate magnetic stand to separate beads from supernatant. After the solution is clear (about 5 minutes), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets.
5. Add 200 µl of 80% ethanol to the tube/PCR plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.
6. Repeat Step 5 once.
7. Air dry beads for 10 minutes while the tube/PCR plate is on the magnetic stand with the lid open.
8. Elute DNA target from beads into 21 µl of 0.1X TE. Mix well on a vortex mixer or by pipetting up and down, and put the tube/PCR plate in the magnetic stand until the solution is clear.
9. Transfer 19 µl of the supernatant to a clean LoBind tube, and store at –20°C.

Alternatively, purify DNA sample on one purification column and elute in 19 µl of sterile dH_2O or elution buffer.

Adaptor Ligation of dA-Tailed DNA

Note: Dilute the NEBNext Adaptor (15 µM) to 1.5 µM in Nuclease Free Water for immediate use.

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

End Repaired, dA-Tailed DNA	19 µl
Quick Ligation Reaction Buffer (5X)	6 µl
Diluted NEBNext Adaptor (1.5 µM)	1 µl
Quick T4 DNA Ligase	4 µl
Total volume	30 µl

2. Incubate at 20°C for 15 minutes.
3. Add 3 µl of USER enzyme, mix by pipetting up and down, and incubate at 37°C for 15 minutes.

Clean Up using AMPure XP Beads

1. Vortex AMPure XP beads to resuspend
2. Add 54 µl of resuspended AMPure XP beads to the ligation reaction (~ 30 µl). Mix well on a vortex mixer or by pipetting up and down at least 10 times.
3. Incubate for 5 minutes at room temperature.
4. Put the tube/PCR plate on an appropriate magnetic stand to separate beads from supernatant. After the solution is clear (about 5 minutes), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets.
5. Add 200 µl of 80% ethanol to the tube/PCR plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.
6. Repeat Step 5) once.
7. Air dry beads for 10 minutes while the tube/PCR plate is on the magnetic stand with the lid open.
8. Elute DNA target from beads into 105 µl of dH_2O. Mix well on a vortex mixer or by pipetting up and down, and put the tube/PCR plate in the magnetic stand until the solution is clear.
9. Transfer 100 µl of the supernatant to a clean tube/PCR plate.

Alternatively, purify DNA sample on one purification column and elute in 20 µl of sterile dH_2O or elution buffer.

Size Selection of Adaptor Ligated DNA Using AMPure XP Beads

Note: (X) refers to original sample volume of 100 µl.

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/PCR plate on an appropriate magnetic stand to separate beads from supernatant. After the solution is clear (about 5 minutes), carefully transfer the supernatant to a new tube/well **(Caution: do not discard the supernatant).** Discard beads that contain the large fragments.
4. Add 20 µl (0.2X) resuspended AMPure XP beads to the supernatant, mix well and incubate for 5 minutes at room temperature.
5. Put the tube/PCR plate on an appropriate magnetic stand to separate beads from supernatant. After the solution is clear (about 5 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 200 µl of 80% freshly prepared ethanol to the tube/PCR plate 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. Air dry beads for 10 minutes while the tube/PCR plate is on the magnetic stand with lid open.
9. Elute DNA target from beads into 25 µl water or 0.1X TE buffer. Mix well on a vortex mixer or by pipetting up and down, and put the tube/PCR plate in the magnetic stand until the solution is clear.
10. Transfer 23 µl of the supernatant to a clean PCR tube and proceed to enrichment.

Alternatively, size selection can be performed using a number of methods including E-Gel® (Life Technologies, Inc.) size select gels or standard 2% agarose gels. NEB's 100 bp ladder (NEB #N3231) can be used to determine the size of the fragments. Isolate library fragments in the 175–225 base pair range. Purify the DNA on one purification column and elute in 25 µl of sterile water or elution buffer.

PCR Enrichment of Adaptor Ligated DNA

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

Adaptor ligated DNA	23 µl
NEBNext High-Fidelity 2X PCR Master Mix**	25 µl
Universal PCR Primer (25 µM)	1 µl
Index 1 Primer* (25 µM)	1 µl
Total volume	50 µl

* If you are using the NEBNext Multiplex Oligos for Illumina (#E7335, #E7500), for each reaction, only one of the 12 PCR primer indices is used during the PCR step.

** NEBNext High-Fidelity 2X PCR Master Mix will be replacing Phusion High-Fidelity PCR Master Mix. Both vials will be supplied for a limited time only.

2. PCR cycling conditions;

CYCLE STEP	TEMP	TIME	CYCLES
Initial Denaturation	98°C	30 sec	1
Denaturation Annealing Extension	98°C 65°C 72°C	10 sec 30 sec 30 sec	15
Final Extension	72°C 4°C	5 min hold	1

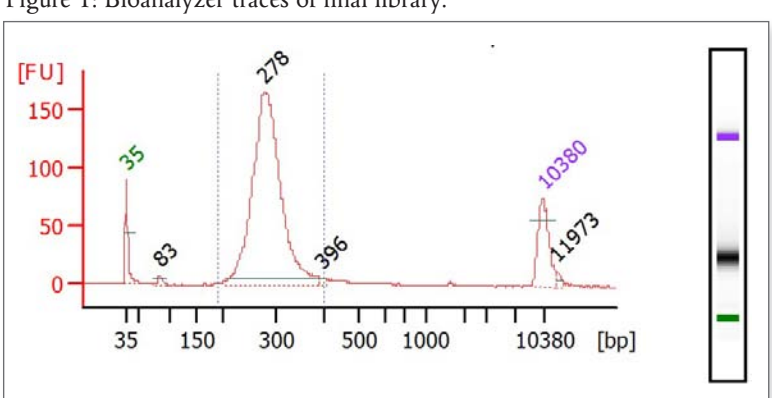
Clean Up Using AMPure XP Beads

1. Vortex beads to resuspend.
2. Add 50 µl (1X) of resuspended AMPure XP beads to the PCR reactions (~ 50 µl). Mix well on a vortex mixer or by pipetting up and down at least 10 times.
3. Incubate for 5 minutes at room temperature.
4. Put the tube/PCR plate on an appropriate magnetic stand to separate beads from supernatant. After the solution is clear (about 5 minutes), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets.
5. Add 200 µl of 80% ethanol to the tube/PCR plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.
6. Repeat Step 5 once.
7. Air dry beads for 10 minutes while the tube/PCR plate is on the magnetic stand with the lid open.
8. Elute DNA target from beads into 17 µl of 0.1X TE. Mix well on a vortex mixer or by pipetting up and down, and put the tube/PCR plate in the magnetic stand until the solution is clear.
9. Transfer 15 µl of the supernatant to a clean LoBind tube, and store at –20°C.

Alternatively, purify sample on one purification column and elute in 15 µl of sterile water or elution buffer.

Dilute the library 20 fold with nuclease free water, and assess the library quality on a Bioanalyzer® (Agilent high sensitivity chip) (Agilent Technologies, Inc.). Check that the electropherogram shows a narrow distribution with a peak size around 275 bp is expected (an example is shown below).

Figure 1: Bioanalyzer traces of final library.



Protocol for Use with End User Supplied Primers and Adaptors:

Starting Material: 10 ng of chromatin-immunoprecipitated (ChIP) qPCR verified or control DNA, in ≥ 40 µl of water or elution buffer

End Repair of ChIP DNA

1. In a sterile microfuge tube mix the following components:

ChIP DNA	1–40 µl
NEBNext End Repair Reaction Buffer	5 µl
NEBNext End Repair Enzyme Mix	1 µl
Sterile H_2O	variable
Total volume	50 µl

2. Incubate in a thermal cycler for 30 minutes at 20°C.

Clean Up Using AMPure XP Beads

1. Vortex beads to resuspend.
2. Add 90 µl (1.8X) of resuspended AMPure XP beads to the reaction (~ 50 µl). Mix well on a vortex mixer or by pipetting up and down at least 10 times.
3. Incubate for 5 minutes at room temperature.
4. Put the tube/PCR plate on an appropriate magnetic stand to separate beads from supernatant. After the solution is clear (about 5 minutes), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets.
5. Add 200 µl of 80% ethanol to the tube/PCR plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.
6. Repeat Step 5 once.
7. Air dry beads for 10 minutes while the tube/PCR plate is on the magnetic stand with the lid open.
8. Elute DNA target from beads into 46 µl of 0.1X TE. Mix well on a vortex mixer or by pipetting up and down, and put the tube/PCR plate in the magnetic stand until the solution is clear.
9. Transfer 44 µl of the supernatant to a clean LoBind tube, and store at –20°C.

Alternatively, purify DNA sample on one purification column and elute in 44 µl of sterile dH_2O or elution buffer.

dA-Tailing of End Repaired DNA

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

End Repaired DNA	44 µl
NEBNext dA-Tailing Reaction Buffer (10X)	5 µl
Klenow Fragment (3´→5´ exo⁻)	1 µl
Total volume	50 µl

2. Incubate at 37°C for 30 minutes.

Clean up using AMPure XP Beads

1. Vortex beads to resuspend.
2. Add 90 µl (1.8X) of resuspended AMPure XP beads to the dA tailing reaction(~ 50 µl). Mix well on a vortex mixer or by pipetting up and down at least 10 times.
3. Incubate for 5 minutes at room temperature.
4. Put the tube/PCR plate on an appropriate magnetic stand to separate beads from supernatant. After the solution is clear (about 5 minutes), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets.
5. Add 200 µl of 80% ethanol to the tube/PCR plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.
6. Repeat Step 5 once.
7. Air dry beads for 10 minutes while the tube/PCR plate is on the magnetic stand with the lid open.
8. Elute DNA target from beads into 21 µl of 0.1X TE. Mix well on a vortex mixer or by pipetting up and down, and put the tube/PCR plate in the magnetic stand until the solution is clear.
9. Transfer 19 µl of the supernatant to a clean LoBind tube, and store at –20°C.

Alternatively, purify DNA sample on one purification column and elute in 19 µl of sterile dH_2O or elution buffer.

Adaptor Ligation of dA-Tailed DNA

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

End Repaired, dA-Tailed DNA	19 µl
Quick Ligation Reaction Buffer (5X)	6 µl
Adaptor (1.5 µM)	1 µl
Quick T4 DNA Ligase	4 µl
Total volume	30 µl

* Adaptors are not included, use adaptors appropriate to specific application. If necessary adjust the adaptor concentration to obtain a final adaptor to DNA molar ratio of 10:1.

2. Incubate at 20°C for 15 minutes.

Clean Up Using AMPure XP Beads

1. Vortex AMPure XP beads to resuspend
2. Add 54 µl (1.8X) of resuspended AMPure XP beads to the ligation reaction (~ 30 µl). Mix well on a vortex mixer or by pipetting up and down at least 10 times.
3. Incubate for 5 minutes at room temperature.
4. Put the tube/PCR plate on an appropriate magnetic stand to separate beads from supernatant. After the solution is clear (about 5 minutes), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets.
5. Add 200 µl of 80% ethanol to the tube/PCR plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.
6. Repeat Step 5) once.
7. Air dry beads for 10 minutes while the tube/PCR plate is on the magnetic stand with the lid open.
8. Elute DNA target from beads into 105 µl of dH_2O. Mix well on a vortex mixer or by pipetting up and down, and put the tube/PCR plate in the magnetic stand until the solution is clear.
9. Transfer 100 µl of the supernatant to a clean tube/PCR plate.

Alternatively, purify DNA sample on a single purification column and elute in the appropriate volume of sterile dH_2O or elution buffer for desired size selection.

Size Selection of Adaptor Ligated DNA Using AMPure XP Beads

Note: (X) refers to original sample volume of 100 µl.

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/PCR plate on an appropriate magnetic stand to separate beads from supernatant. After the solution is clear (about 5 minutes), carefully transfer the supernatant to a new tube/well **(Caution: do not discard the supernatant)**. Discard beads that contain the large fragments.
4. Add 20 µl (0.2X) resuspended AMPure XP beads to the supernatant, mix well and incubate for 5 minutes at room temperature.
5. Put the tube/PCR plate on an appropriate magnetic stand to separate beads from supernatant. After the solution is clear (about 5 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 200 µl of 80% freshly prepared ethanol to the tube/PCR plate 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. Air dry beads for 10 minutes while the tube/PCR plate is on the magnetic stand with lid open.
9. Elute DNA target from beads into 25 µl water or 0.1X TE buffer. Mix well on a vortex mixer or by pipetting up and down, and put the tube/PCR plate in the magnetic stand until the solution is clear.
10. Transfer 23 µl of the supernatant to a clean PCR tube and proceed to enrichment.

Alternatively, size selection can be performed using a number of methods including E-Gel size select gels or standard 2% agarose gels. NEB's 100 bp ladder (NEB #N3231) can be used to determine the size of the fragments. Isolate library fragments in the 175–225 base pair range. Purify the DNA on one purification column and elute in 25 µl of sterile water or elution buffer.

PCR Enrichment of Adaptor Ligated DNA

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

Adaptor ligated DNA	23 µl
NEBNext High-Fidelity 2X PCR Master Mix**	25 µl
Primer 1* (25 µM)	1 µl
Primer 2* (25 µM)	1 µl
Total volume	50 µl

* Primers are not included, use primers appropriate to specific application.

** NEBNext High-Fidelity 2X PCR Master Mix will be replacing Phusion High-Fidelity PCR Master Mix. Both vials will be supplied for a limited time only.

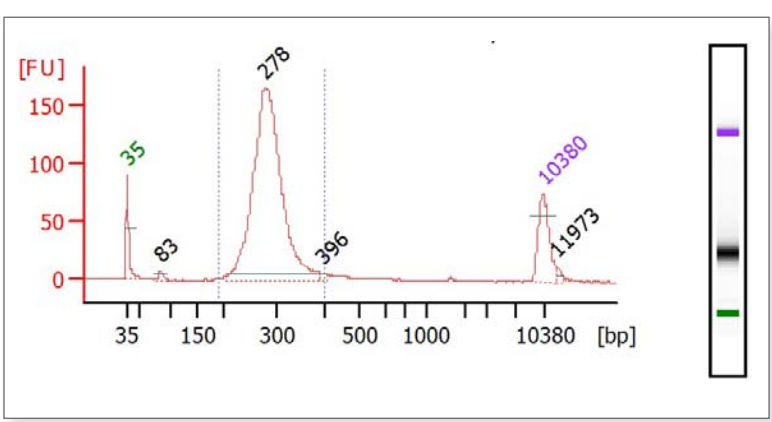
Clean up using AMPure XP Beads

1. Add 50 µl (1X) of resuspended AMPure XP beads to the PCR reactions (~ 50 µl). 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. Put the tube/PCR plate on an appropriate magnetic stand to separate beads from supernatant. After the solution is clear (about 5 minutes), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets.
4. Add 200 µl of 80% ethanol to the tube/PCR plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.
5. Repeat Step 4) once.
6. Air dry beads for 10 minutes while the tube/PCR plate is on the magnetic stand with the lid open.
7. Elute DNA target from beads into 17 µl of buffer EB. Mix well on a vortex mixer or by pipetting up and down, and put the tube/PCR plate in the magnetic stand until the solution is clear.
8. Transfer 15 µl of the supernatant to a clean LoBind tube, and store at –20°C.

Alternatively, purify sample on one purification column and elute in 15 µl of sterile water or elution buffer.

Dilute the library 20 fold with nuclease free water, and assess the library quality on a Bioanalyzer (Agilent high sensitivity chip). Check that the electropherogram shows a narrow distribution with a peak size around 275 bp is expected (an example is shown below).

Figure 2: Bioanalyzer traces of final library.



NEBNext End Repair Enzyme Mix

#E6241A: 0.015 ml
#E6241AA: 0.06 ml

Store at –20°C

Description: NEBNext End Repair Enzyme Mix is optimized to convert fragmented to repaired DNA having 5´-phosphorylated, blunt ends.

NEBNext End Repair Enzyme Mix:
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
0.1% Triton X-100
pH 7.4 @ 25°C

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

#E6242A: 0.06 ml Concentration: 10X
#E6242AA: 0.3 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 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

Klenow Fragment (3´→5´ exo⁻)

#E6207A: 0.015 ml
#E6207AA: 0.06 ml

Store at –20°C

Description: Klenow Fragment (3´→5´ exo⁻) is an N-terminal truncation of DNA Polymerase I which retains polymerase activity, but lacks 5´→3´ exonuclease activity. Mutations (D355A, E357A) abolish the 3´→5´ exonuclease activity (1). Klenow Fragment (3´→5´ exo⁻) with dA-Tailing buffer can be used to add a dAMP to the 3´ end at a blunt DNA fragment (2).

Source: An *E. coli* strain containing a plasmid with a fragment of the *E. coli* polA (D355A, E357A) gene starting at codon 324.

Supplied in: 25 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT 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 5 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 5 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 5 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 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.

3´→5´ Exonuclease Activity: Incubation of a minimum of 50 units of enzyme in 20 µl of a 10 nM solution of a fluorescent 5´-FAM labeled oligonucleotide for 30 minutes at 37°C yields no detectable 3´→5´ degradation as determined by capillary 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. Derbyshire, V. et al. (1988) *Science,* 240, 199–201.
2. Clark, J.M. et al. (1987) *J. Mol. Biol.* 198(1); 123–127.

NEBNext dA-Tailing Reaction Buffer

#E6045A: 0.06 ml **Concentration: 10X**
#E6045AA: 0.3 ml

Store at –20°C

1X NEBNext dA-Tailing Reaction Buffer:
10 mM Tris-HCl
10 mM $MgCl_2$
50 mM NaCl
1 mM DTT
0.2 mM dATP
pH 7.9 @ 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 (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

#E6209A: 0.048 ml
#E6209AA: 0.240 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 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 Quick Ligation Reaction Buffer

#E6243A: 0.072 ml **Concentration: 5X**
#E6243AA: 0.36 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 (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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Phusion High-Fidelity PCR Master Mix with HF Buffer

E6012A: **0.3 ml** **Concentration: 2X**
E6012AA: **1.5 ml**

Store at –20°C

Storage and Stability: Store at –20°C. After thawing the mix can be refrozen or optionally stored at 4°C for three months.

Component Specifications: 2X Phusion High-Fidelity Master Mix with HF Buffer contains 0.04 units/µl Phusion DNA Polymerase, 2X Phusion HF Buffer (provides 1.5 mM $MgCl_2$ in final reaction concentration), and 400 µM of each dNTP.

Manufactured and quality controlled by New England Biolabs, Inc., Thermo Scientific Phusion® High-Fidelity DNA Polymerase is purified from an *E. coli* strain expressing the cloned Phusion DNA Polymerase gene. This DNA polymerase possesses the following activities: 5´→3´ DNA polymerase activity and 3´→5´ exonuclease activity. Phusion DNA Polymerase is purified free of contaminating endo- and exonucleases.

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing 1X Phusion Master Mix 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 1X Phusion Master Mix 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.

Phosphatase Activity: Incubation of 1X Phusion 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.

7.5 kb Genomic and 20 kb Lambda PCR: 30 cycles of PCR amplification in a 50 µl reaction containing 50 ng genomic DNA with 1X Phusion High-Fidelity PCR Master Mix with HF Buffer and 1.0 µM primers results in the expected 7.5 kb product.

20 kb Lambda DNA PCR: 22 cycles of PCR amplification in a 50 µl reaction containing 10 ng Lambda DNA with 1X Phusion High-Fidelity PCR Master Mix with HF Buffer and 1.0 µM primers results in the expected 20 kb product.

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Notice to Customer: Phusion® DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific.

NEBNext High-Fidelity 2X PCR Master Mix

E6013A: 0.3 ml **Concentration: 2X**
E6013AA: 0.75 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.

GC-Rich Genomic 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.

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