Protocol (E6240) for Use With NEBNext Singleplex (#E7350) or Multiplex (#E7335, #E7500) Oligos for Illumina

Overview

Protocol

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 AMPure XP beads to resuspend.
2. Add 90 µl (1.8X) of resuspended AMPure XP beads to the ligation reaction (~50 µl). Mix thoroughly 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 the DNA targets.
5. 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.
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 50 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 AMPure XP beads to resuspend.
2. Add 90 µl (1.8X) of resuspended AMPure XP beads to the dA-tailing reaction (~ 50 µl). Mix thoroughly 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 the DNA targets.
5. 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.
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 25 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
   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 thoroughly 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 the DNA targets.
5. 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.
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 110 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 supernatant to a clean tube/PCR plate.

Alternatively, purify DNA sample on one purification column and elute in 20 µl of sterile H_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 30 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.

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 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 20 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).