NEBNext® Ultra™ DNA Library Prep Protocol for Illumina® With Size Selection (E7370)

Isabel Gautreau

Abstract

This protocol is a group of the following 5 methods from the NEB website:
1. NEBNext End Prep
2. Adaptor Ligation
3. Size Selection of Adaptor-ligated DNA
4. PCR Amplification
5. Cleanup of PCR Amplification

Citation: Isabel Gautreau NEBNext® Ultra™ DNA Library Prep Protocol for Illumina® With Size Selection (E7370). **protocols.io**
dx.doi.org/10.17504/protocols.io.cg5ty5
Published: 10 Feb 2015

Guidelines

The NEBNext Ultra DNA Library Prep Kit for Illumina contains enzymes and buffers that are ideal to convert a small amount of DNA input into indexed libraries for next-generation sequencing on the Illumina platform (Illumina, Inc).

Adaptor Ligation:

If DNA input is < 100 ng, dilute the NEBNext Adaptor for Illumina* 1:10 in sterile water use immediately to a final concentration of 1.5 µM.

Size Selection:

This protocol is suitable for input amounts of 50ng or higher. For lower input amounts we recommend that the protocol without size selection be followed.

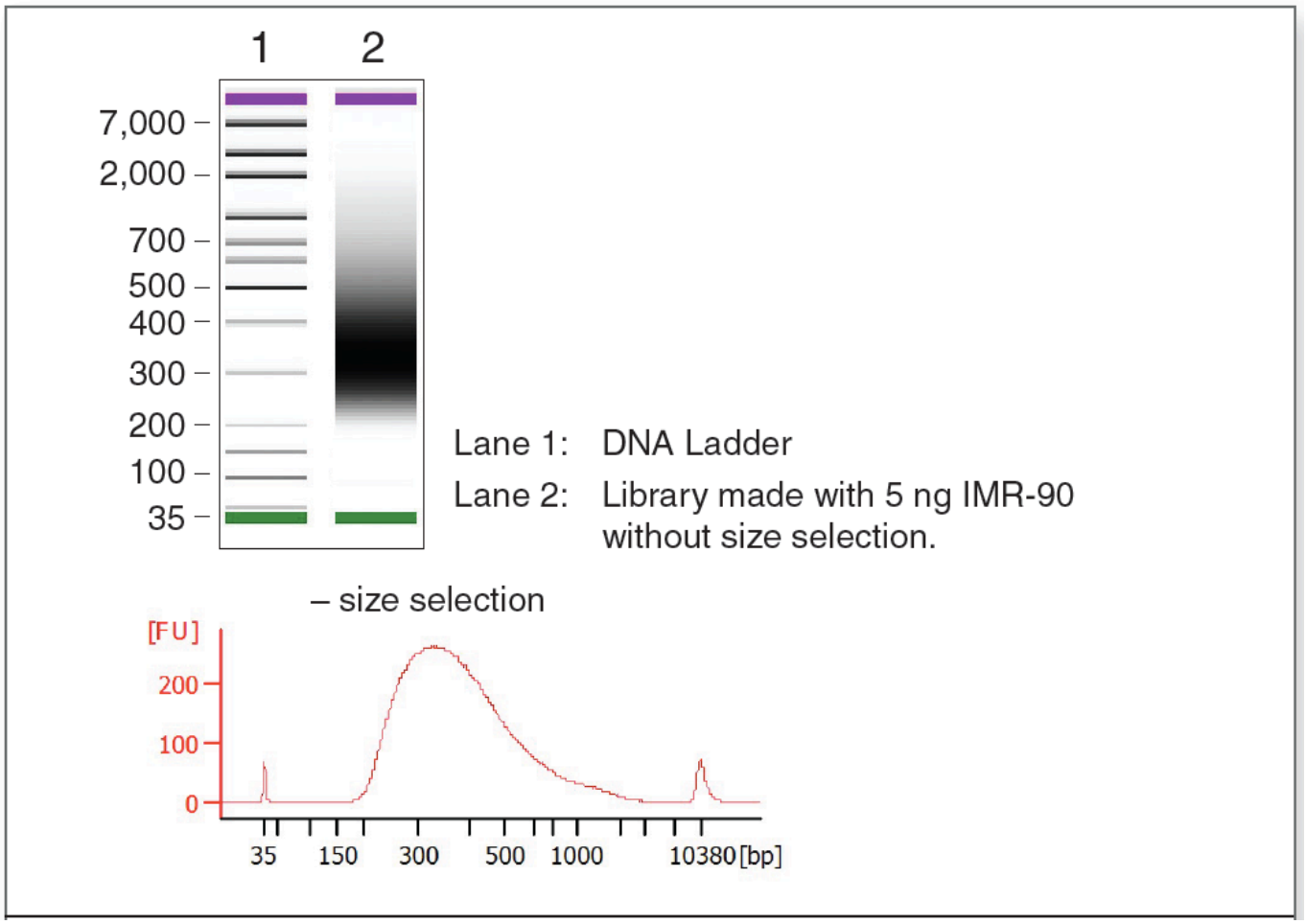
This size selection is for libraries with 200 bp inserts only and is based on a starting volume of 100 µl. For libraries with different size fragment inserts, refer to Table 1 for the appropriate volume of beads to be added.

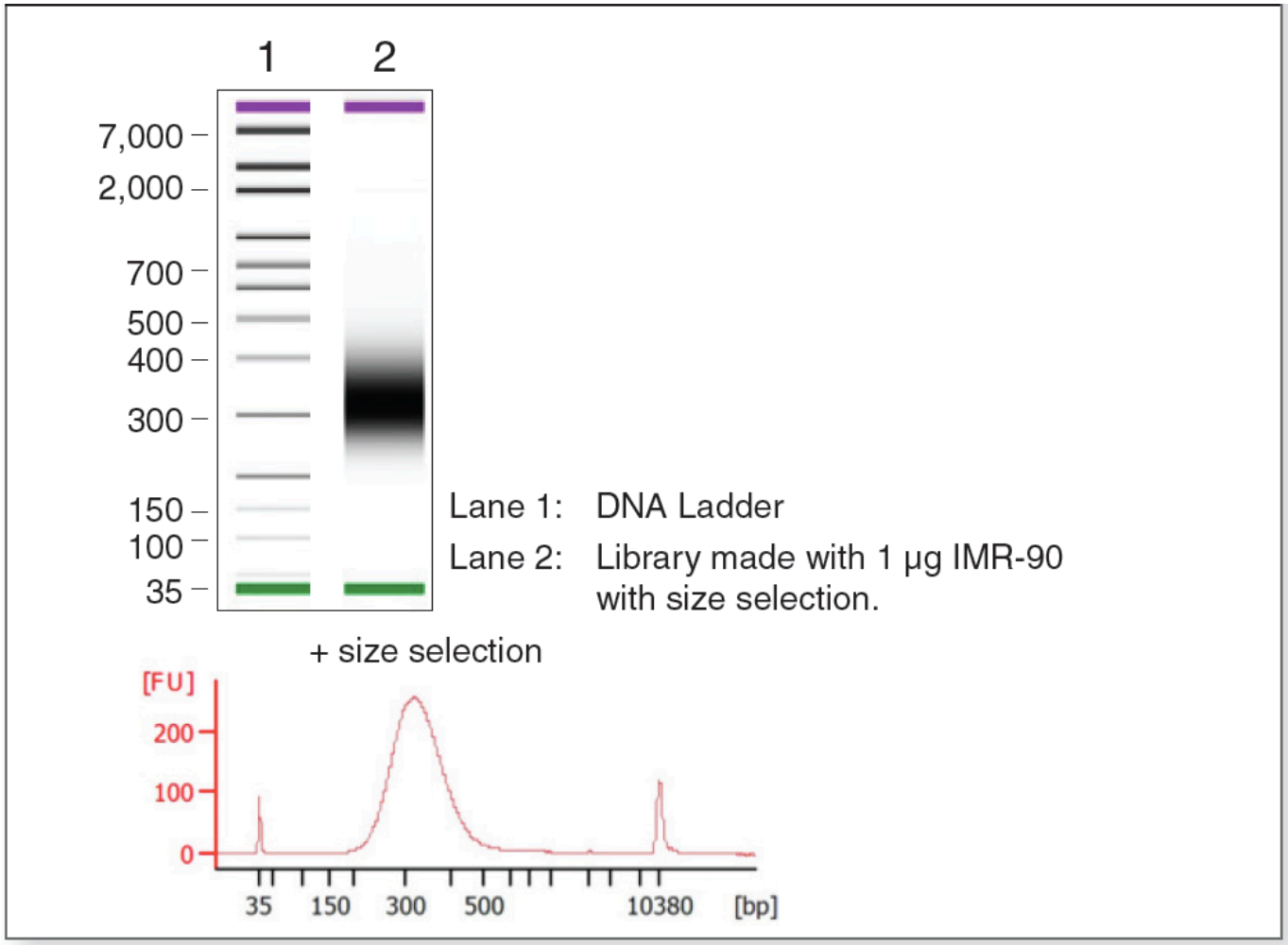
Table 1: Recommended conditions for bead based size selection.

LIBRARY PARAMETERS	APPROXIMATE INSERT SIZE	150 bp	200 bp	250 bp	300-400 bp	400-500 bp	500-700 bp
	Total Library Size (insert + adaptor)	270 bp	320 bp	400 bp	400-500 bp	500-600 bp	600-800 bp
VOLUME TO BE ADDED (µl)	1st Bead Selection	65	55	45	40	35	30
	2nd Bead Selection	25	25	25	20	15	15

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.

Figure 1: Examples of libraries prepared with human IMR-90 gDNA.





Materials

NEBNext Ultra DNA Library Prep Kit for Illumina - 24 rxns E7370S by New England Biolabs

Protocol

NEBNext End Prep

Step 1.

Mix the following components in a sterile nuclease-free tube (Total volume **65 µl)**:

PROTOCOL

. NEBNext End Prep Mixture

CONTACT: Isabel Gautreau

NOTES

Isabel Gautreau 20 Oct 2014

Starting Material: 5 ng–1 µg fragmented DNA.

Step 1.1.

End Prep Enzyme Mix 3.0 µl

ANNOTATIONS

Peter Hoyt 12 Oct 2015

Will be optimizing for long read instruments.

Step 1.2.

End Repair Reaction Buffer (10X) 6.5 µl

ANNOTATIONS

Peter Hoyt 12 Oct 2015

Might be in the wrong protocol as the UltraII End Prep says use 7ul of reaction buffer.

Step 1.3.

Fragmented DNA 55.5 µl

ANNOTATIONS

Peter Hoyt 12 Oct 2015

Initial experiment shooting for fragments greater than 20kb. Not sure why this protocol doesn't show the recommended 500pg - 1ug of fragmented DNA, but we will need approximately 4ug of HMW (High Molecular Weight) DNA to make of the molar difference in molecule ends.

NEBNext End Prep

Step 2.

Mix by pipetting

NEBNext End Prep

Step 3.

Quick spin to collect all liquid from the sides of the tube.

NEBNext End Prep

Step 4.

Place in a thermocycler, with the heated lid on, and run the following program:

Time	Temperature
30 minutes	20°C
30 minutes	65°C
Hold	4°C

Adaptor Ligation

Step 5.

Add the following components directly to the End Prep reaction mixture and mix well (Total volume 83.5 µl):

PROTOCOL

. Adaptor Ligation Mixture (E7370)

CONTACT: New England Biolabs

NOTES

Isabel Gautreau 26 Jan 2015

The NEBNext adaptor is provided in NEBNext Singleplex (NEB #E7350) or Multiplex (NEB #E7335, #E7500) Oligos for Illumina.

Step 5.1.

Blunt/TA Ligase Master Mix 15 µl

Step 5.2.
NEBNext Adaptor for Illumina 2.5 µl

NOTES

New England Biolabs 29 Sep 2014
The NEBNext adaptor is provided in NEBNext Singleplex (NEB #E7350) or Multiplex (NEB #E7335, #E7500) Oligos for Illumina.
New England Biolabs 20 Oct 2014
If DNA input is < 100 ng, dilute the NEBNext Adaptor for Illumina 1:10 in sterile water. Use immediately to a final concentration of 1.5 µM.

ANNOTATIONS

Peter Hoyt 12 Oct 2015
Not sure we need this step. Need to get back to this step to see if it helps create capped (ligated) ends.

Step 5.3.
Ligation Enhancer 1 µl

Adaptor Ligation

Step 6.
Mix by pipetting

Adaptor Ligation

Step 7.
Quick spin to collect all liquid from the sides of the tube.

Adaptor Ligation

Step 8.
Incubate at 20°C for 15 minutes in a thermal cycler.

DURATION

00:15:00

Adaptor Ligation

Step 9.
Add 3 µl of USER™ enzyme to the ligation mixture from step 8.

AMOUNT

3 µl Additional info:

REAGENTS

USER Enzyme - 50 units M5505S by New England Biolabs

Adaptor Ligation

Step 10.
Mix well and incubate at 37°C for 15 minutes.

DURATION

00:15:00

Size Selection

Step 11.
Vortex AMPure XP beads to resuspend.

NOTES

Isabel Gautreau 20 Oct 2014
The size selection protocol that follows is based on a starting volume of 100 µl.
Isabel Gautreau 20 Oct 2014

The following size selection protocol is for libraries with 200 bp inserts only. For libraries with different size fragment inserts, refer to Table 1 in the guidelines for the appropriate volume of beads to be added.

Size Selection

Step 12.
Add 13.5 µl dH2O to the ligation reaction for a 100 µl total volume.

Size Selection

Step 13.
Add 55 µl of resuspended AMPure XP beads to the 100 µl ligation reaction.

NOTES

Isabel Gautreau 20 Oct 2014
55 µl is the appropriate amount for libraries with 200 bp inserts only. For libraries with different size fragment inserts, refer to Table 1 in the guidelines for the appropriate volume of beads to be added.

Size Selection

Step 14.
Mix well by pipetting up and down at least 10 times.

Size Selection

Step 15.
Incubate for 5 minutes at room temperature.

DURATION
00:05:00

Size Selection

Step 16.
Quickly spin the tube.

Size Selection

Step 17.
Place the tube on an appropriate magnetic stand to separate the beads from the supernatant.

DURATION
00:05:00

Size Selection

Step 18.
After the solution is clear (about 5 minutes), carefully transfer the supernatant containing your DNA to a new tube (**Caution: do not discard the supernatant**).

Size Selection

Step 19.
Discard the beads that contain the unwanted large fragments.

Size Selection

Step 20.
Add 25 µl resuspended AMPure XP beads to the supernatant.

NOTES

Isabel Gautreau 20 Oct 2014
25 µl is the appropriate amount for libraries with 200 bp inserts only. For libraries with different size fragment inserts, refer to Table 1 in the guidelines for the appropriate volume of beads to be added.

Size Selection

Step 21.
Mix well and incubate for 5 minutes at room temperature.

DURATION
00:05:00

Size Selection

Step 22.
Quickly spin the tube.

Size Selection

Step 23.
Place it on an appropriate magnetic stand to separate the beads from the supernatant.

DURATION
00:05:00

Size Selection

Step 24.
After the solution is clear (about 5 minutes), carefully remove and discard the supernatant that contains unwanted DNA. Be careful not to disturb the beads that contain the desired DNA targets (**Caution: do not discard beads**).

Size Selection

Step 25.
Wash #1: Add 200 µl of 80% freshly prepared ethanol to the tube while in the magnetic stand.

Size Selection

Step 26.
Wash #1: Incubate at room temperature for 30 seconds

DURATION
00:00:30

Size Selection

Step 27.
Wash #1: Carefully remove and discard the supernatant.

Size Selection

Step 28.
Wash #2: Add 200 µl of 80% freshly prepared ethanol to the tube while in the magnetic stand.

Size Selection

Step 29.
Wash #2: Incubate at room temperature for 30 seconds

Size Selection

Step 30.
Wash #2: Carefully remove and discard the supernatant.

Size Selection

Step 31.
Wash #3: Add 200 µl of 80% freshly prepared ethanol to the tube while in the magnetic stand.

Size Selection

Step 32.
Wash #3: Incubate at room temperature for 30 seconds

Size Selection

Step 33.
Wash #3: Carefully remove and discard the supernatant.

Size Selection

Step 34.

Air the dry beads for 10 minutes while the tube is on the magnetic stand with the lid open.

Size Selection

Step 35.

Elute the DNA target from the beads into 28 µl of 10 mM Tris-HCl or 0.1 X TE, pH 8.0.

NOTES

Isabel Gautreau 26 Jan 2015

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.

Size Selection

Step 36.

Mix well on a vortex mixer or by pipetting up and down.

Size Selection

Step 37.

Quickly spin the tube and place it on a magnetic stand.

DURATION

00:05:00

NOTES

Isabel Gautreau 29 Sep 2014

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.

Size Selection

Step 38.

After the solution is clear (about 5 minutes), transfer 23 µl to a new PCR tube for amplification.

ANNOTATIONS

Lenny Teytelman 10 Nov 2015

According to the comments, this is a safe stopping point. After the ligated fragments have been purified, it's ok to freeze overnight and start the PCR amplification step the following day.

PCR Amplification

Step 39.

Mix the following components in sterile strip tubes (**Total volume 50 µl**):

AMOUNT

25 µl Additional info:

PROTOCOL

. Mixture for PCR Amplification (E7370)

CONTACT: New England Biolabs

NOTES

Isabel Gautreau 26 Jan 2015

The primers are provided in NEBNext Singleplex (NEB #E7350) or Multiplex (NEB #E7335, #E7500) Oligos for Illumina.

Step 39.1.
Adaptor Ligated DNA Fragments 23 µl

Step 39.2.
NEBNext High Fidelity 2X PCR Master Mix 25 µl

AMOUNT
25 µl Additional info:

REAGENTS
NEBNext High-Fidelity 2X PCR Master Mix - 50 rxns M0541S by New England Biolabs

Step 39.3.
Index Primer 1 µl

Step 39.4.
Universal PCR Primer 1 µl

PCR Amplification

Step 40.
PCR using the following cycling conditions:

CYCLE STEP	TEMP	TIME	CYCLES
Initial Denaturation	98°C	30 seconds	1
Denaturation	98°C	10 seconds	6-15*
Annealing	65°C	30 seconds	
Extension	72°C	30 seconds	
Final Extension	72°C	5 minutes	1
Hold	4°C	∞	

NOTES
Isabel Gautreau 26 Jan 2015
We suggest 6 PCR cycles for 1 µg DNA input 10 cycles for 50 ng, and 13–15 for 5 ng DNA input. Further optimization of PCR cycle number may be required.

Cleanup of PCR Amplification

Step 41.
Vortex AMPure XP beads to resuspend.

Cleanup of PCR Amplification

Step 42.
Add 50 µl of resuspended AMPure XP beads to the PCR reactions (50 µ l).

Cleanup of PCR Amplification

Step 43.
Mix well by pipetting up and down at least 10 times.

Cleanup of PCR Amplification

Step 44.
Incubate for 5 minutes at room temperature.

DURATION
00:05:00

Cleanup of PCR Amplification

Step 45.
Quickly spin the tube and place it on an appropriate magnetic stand to separate beads from supernatant.

DURATION
00:05:00

Cleanup of PCR Amplification

Step 46.

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**).

Cleanup of PCR Amplification

Step 47.

Wash #1: Add 200 µl of 80% ethanol to the PCR plate while in the magnetic stand.

Cleanup of PCR Amplification

Step 48.

Wash #1: Incubate at room temperature for 30 seconds.

DURATION

00:00:30

Cleanup of PCR Amplification

Step 49.

Wash #1: Carefully remove and discard the supernatant.

Cleanup of PCR Amplification

Step 50.

Wash #2: Add 200 µl of 80% ethanol to the PCR plate while in the magnetic stand.

Cleanup of PCR Amplification

Step 51.

Wash #2: Incubate at room temperature for 30 seconds.

DURATION

00:00:30

Cleanup of PCR Amplification

Step 52.

Wash #2: Carefully remove and discard the supernatant.

Cleanup of PCR Amplification

Step 53.

Air dry the beads for 10 minutes while the PCR plate is on the magnetic stand with the lid open.

DURATION

00:10:00

Cleanup of PCR Amplification

Step 54.

Elute DNA target from beads into 33 µl 10 mM Tris-HCl, pH 8.0 or 0.1X TE.

Cleanup of PCR Amplification

Step 55.

Mix well by pipetting up and down at least 10 times.

Cleanup of PCR Amplification

Step 56.

Quickly spin the tube and place it on an appropriate magnetic stand to separate beads from supernatant.

DURATION

00:05:00

Cleanup of PCR Amplification

Step 57.

After the solution is clear (about 5 minutes), carefully transfer 28 µl supernatant to a new PCR tube.

Cleanup of PCR Amplification

Step 58.

Dilute the library 5 fold with water, and check the size distribution on an Agilent high sensitivity chip.

Warnings

This protocol is suitable for input amounts of 50ng or higher. For lower input amounts we recommend that the protocol without size selection be followed.