Cat. # NN0003
NN0004

For Research Use

TaKaRa

ssDNA-Seq Kit

Product Manual

v202504Da

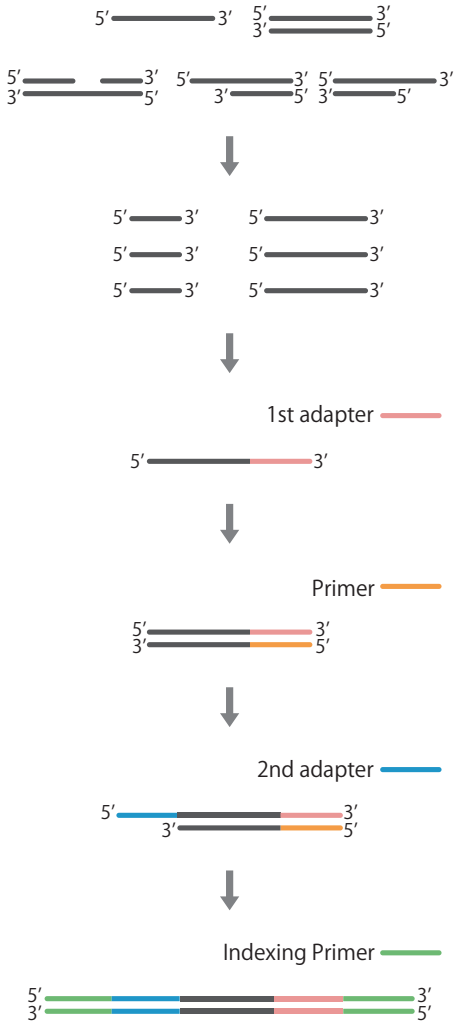
Table of Contents

I. Description

While many DNA library preparation kits are limited to double-stranded DNA (dsDNA), this kit offers the flexibility to prepare high-quality sequencing libraries from both single-stranded (ssDNA) and dsDNA inputs, producing high-quality libraries compatible with Illumina sequencers. Using single-strand DNA ligase (SDL), which can bind ssDNA together, allows a stable library yield can be obtained from ssDNA that would be lost in conventional NGS library preparation of dsDNA, dsDNA that has become damaged or degraded, and even DNA that has undergone advanced fragmentation.

Various DNA samples can be used as input, such as cell-free DNA (cfDNA), DNA derived from formalin-fixed paraffin-embedded (FFPE) specimen, ssDNA, combinations of ssDNA and dsDNA, environmental DNA, viral genome, short DNA, DNA aptamer, and synthetic oligo. Moreover, analysis is possible using trace amounts of DNA (10 pg) or ultra-short ssDNA (50 bases).

The library preparation process consists of the following 5 steps.



Input DNA

(1) Pretreatment & denaturation
Input DNA is pretreated and denatures to become ssDNA.

(2) 1st adapter ligation reaction
The adapter is added to the 3′ end of the denatured ssDNA by SDL.

(3) Extension reaction
A primer aligns with the 1st adapter and generates a second strand complementary to the input DNA.

(4) 2nd adapter ligation reaction
A 2nd adapter is selectively added to the 5′ end of the dsDNA.

(5) PCR amplification of DNA library
Indexing primers incorporate indexing sequences needed for Illumina sequencing.

The sequencing library is completed within 2 hours.

Fig. 1. ssDNA-Seq Kit protocol overview

II. Components [24 reactions (NN0003) / 96 reactions (NN0004)]

	Cat. #NN0003	NN0004
① Preparation enzyme	24 µl	96 µl
② Preparation buffer	48 µl	192 µl
(*) TE buffer	1 ml x 3	6 ml x 2
③ SDL enzyme	48 µl	192 µl
④ SDL buffer	600 µl	1.2 ml x 2
⑤ SDL adapter	24 µl	96 µl
⑥ Extension polymerase Premix	600 µl x 2	1.2 ml x 4
⑦ Extension primer	24 µl	96 µl
⑧ VDC enzyme	24 µl	96 µl
⑨ VDC buffer	150 µl	600 µl
⑩ PCR polymerase Premix	600 µl	1.2 ml x 2

* Component colors of the TE buffer are as follows.
NN0003: ○ (white)
NN0004: (no component color)

III. Storage — 20℃

IV. Materials Required but not Provided

Note: The following reagents and materials are required but not supplied. The specified brands have been validated to work with this protocol.

- Unique Dual Index Kit (Cat. #634752 - 634756)
- NucleoMag NGS Clean-up and Size Select (Cat. #744970.5/.50/.500)*
- Thermal Cycler
  - Clontech PCR Thermal Cycler GP (Cat. #WN400)*, etc.
- 80% (v/v) ethanol (freshly made for each experiment from molecular-biology-grade 100% ethanol)
- Microcentrifuge (capable of spin-down)
- Vortex mixer
- Magnetic stand
- 0.2 ml PCR tube
  - 0.2 ml 8-strip tube, individual Flat Caps (Cat. #NJ600)*
  - 0.2 ml Hi-8-Tube (Cat. #NJ300)*, etc.
- 96well PCR plate
  - 0.2 ml 96 well-plate for Real Time (Frosted) (Cat. #NJ401)*
  - 96 well snap plate (Cat. #NJ710)*, etc.
- PCR plate seal (for when using plate)
- 1.5 ml DNA Low-Bind tube
- Bioanalyzer or TapeStation (Agilent)
- Qubit Fluorometer (Thermo Fisher Scientific)

* Not available in all geographic locations. Check for availability in your area.

V. Sample Requirements

V-1. Input DNA requirements

Item	Requirements
Configuration	• ssDNA • dsDNA • Combination of ssDNA and dsDNA
Type	• Genomic DNA (gDNA) • cfDNA • DNA derived from FFPE specimen • Ancient DNA • Environmental DNA • Viral genome • Short DNA • Synthetic oligo • DNA aptamer
DNA size	50 to 600 bp (50 to 600 bases)
Amount of input	10 pg to 250 ng
Input liquid volume	Maximum 18 μl
Buffer for use in preparation	TE buffer (10 mM Tris, 0.1 mM EDTA)

Notes:

- This kit is not suitable for RNA.
- When using synthetic oligo as input, both ends cannot be modified. Synthetic oligo with a phosphate group at the end can be used.
- This kit cannot be used with intact cells.

V-2. Measurement of input DNA concentration and size

Sample type	Recommended quantitation method
ssDNA	Qubit ssDNA assay kit
dsDNA	Qubit dsDNA HS assay kit
Combination of ssDNA and dsDNA	Qubit dsDNA HS assay Kit (Refer to results of dsDNA quantitation)

- To quantify the concentration of input DNA, use Qubit Fluorometer or equivalent reagent and equipment.
- Measure the size of dsDNA with Agilent Bioanalyzer or TapeStation. Confirm the ssDNA size with polyacrylamide electrophoresis in the presence of a denaturant (urea or formamide).
- Performing the measurement may not be necessary if the size of the synthetic oligo is known.

V-3. Fragmentation of input DNA

If the size of the input DNA exceeds 600 bp (as confirmed by Bioanalyzer or TapeStation), perform mechanical fragmentation, enzymatic fragmentation, etc.

Perform fragmentation according to the instruction manual for the device or enzyme, taking care to ensure that the resulting size of the main peak is 50 to 600 bp.

< Evaluation of fragmentation size using TapeStation for human dsDNA fragmented by sonication >

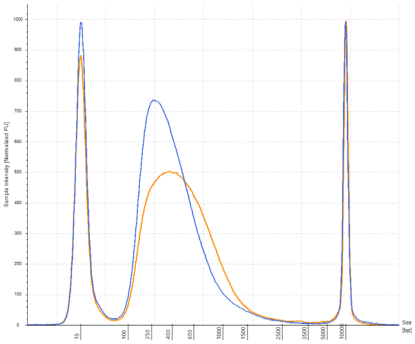


Fig. 2. Example in which the main peak size was in the range of 50 to 600 bp after fragmentation

Blue : input DNA exhibiting main peak size of approx. 250 bp
Orange: input DNA exhibiting main peak size of approx. 400 bp

< Evaluation of fragmentation size using TapeStation for DNA extracted from FFPE specimen >

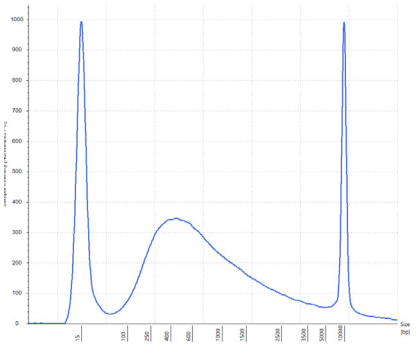


Fig. 3. Example in which the main peak size was already in the range of 50 to 600 bp and fragmentation was not necessary

DIN value : 1.6 (TapeStation; Agilent)

Long/short ratio: 0.02 (Takara FFPE DNA QC All-in-One Kit; Cat. #NN0001)

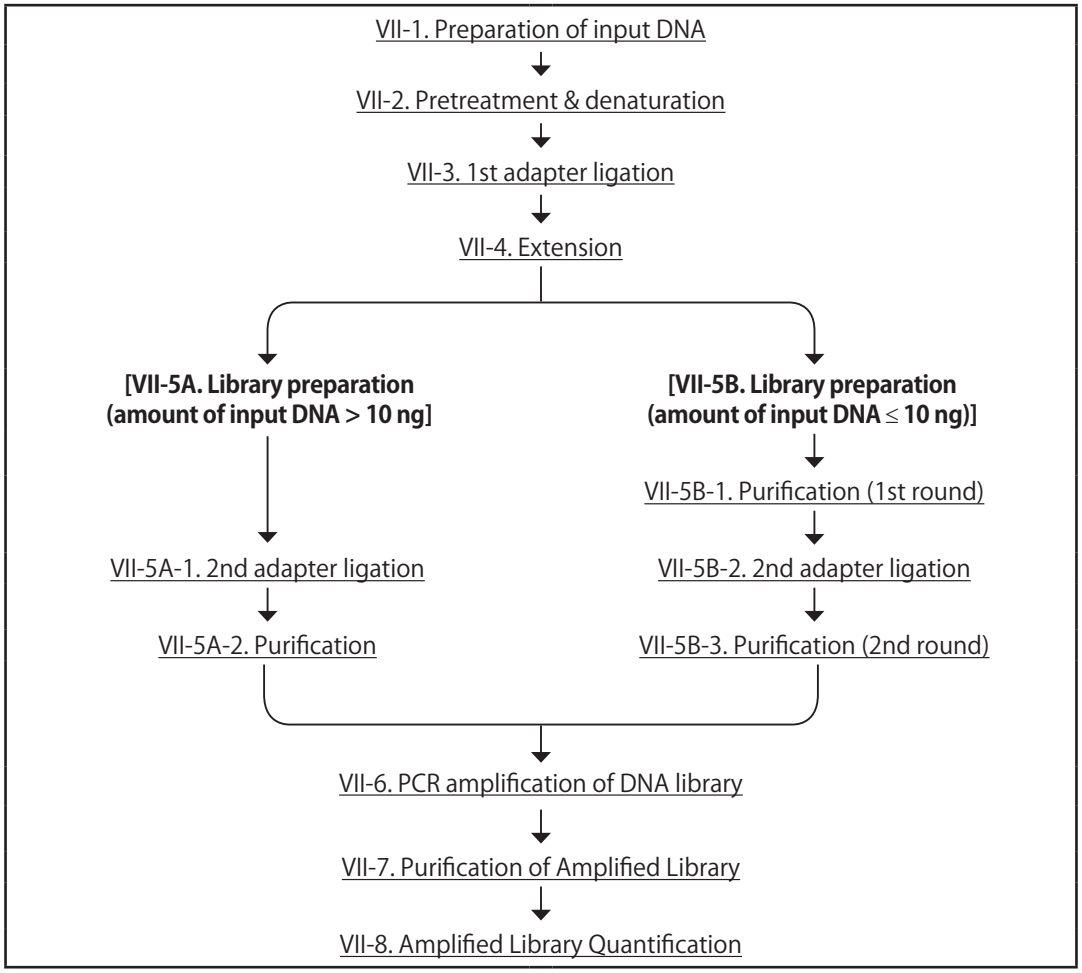
VI. Precautions for Use

Read these precautions before use and follow them when using this kit.

1. This kit is sensitive to the pipetting precision at the time of preparation. Be sure to use a correctly calibrated pipette.
2. Thaw the enzyme (enzyme and polymerase Premix) over ice before use and spin down. Then mix thoroughly by pipetting or tapping and spin down.
3. For components other than the enzyme, thaw at room temperature before using and spin down. Spin down again after thorough mixing.
4. When preparing each reaction solution, it is recommended that you prepare a little more than the necessary amount to account for loss from pipetting. Add the enzyme last and mix well.
5. All reagents should be stored and worked with in a nucleic acid- and nuclease-free cabinet up until the 1st adapter ligation reaction. Moreover, to reduce the risk of contamination of input DNA, DNA library, and Indexing Primer, perform steps from PCR amplification onward in an area for handling PCR products.
6. Avoid repeated freezing and thawing.
7. Perform all steps of the protocol on ice.

VII. Protocol

Overview



Notes:

- The protocol in "VII-5. Library preparation" varies depending on the amount of input DNA. The other steps in the protocol are unaffected by input volume.
- The method described uses 0.2 ml PCR tubes, but protocol can also be performed in a 96-well plate.
- It is recommended that you use positive and negative controls to confirm that the kit is functioning properly.
  - Positive control
    Human genomic DNA fragmented to approximately 200 to 400 bp by mechanical fragmentation or enzymatic fragmentation, or synthetic 160-base ssDNA oligo
  - Negative control
    TE buffer 18 μl (component of this kit)

VII-1. Preparation of input DNA

Prepare input DNA up to a total volume of 18 μl.

Reagent	Volume
Input DNA	x μl
(*1) TE buffer	Up to18 μl

*1 Component colors of the TE buffer are as follows.
NN0003: ○ (white), NN0004: (no component color)

VII-2. Pretreatment & denaturation

1) Prepare pretreatment reaction solution with the following composition for the total number of reactions to be processed, plus 10% of the total volume.

[Per reaction]

Reagent	Volume
① Preparation enzyme	1 μl
② Preparation buffer	2 μl
Total	3 μl

2) Gently mix by pipetting or gentle vortexing then spin down.
3) Add 3 μl of pretreatment reaction solution (from Step 1) to 18 μl of input DNA (total of 21 μl).
4) Gently mix by pipetting or gentle vortexing then spin down.
5) Place the tubes in a preheated thermal cycler and run the following program:

[Reaction conditions]
37℃ 15 min
95℃ 5 min
4℃ Hold

6) At the end of the program, spin down the tubes and immediately proceed to the next step using the same tube.

VII-3. 1st adapter ligation reaction

1) Prepare ligation reaction solution with the following composition for the total number of reactions to be processed, plus 10% of the total volume.

[Per reaction]

Reagent	Volume
③ SDL enzyme	2 μl
④ SDL buffer	25 μl
⑤ SDL adapter	1 μl
Total	28 μl

2) Gently mix by pipetting or gentle vortexing then spin down.
3) Add 28 μl of ligation reaction solution to 21 μl of pretreatment product (total of 49 μl).
4) Gently mix by pipetting or gentle vortexing then spin down.
5) Place the tubes in a preheated thermal cycler and run the following program:

[Reaction conditions]
37℃ 15 min
95℃ 5 min
4℃ Hold

6) At the end of the program, spin down the tubes and immediately proceed to the next step using the same tube.

VII-4. Extension reaction

1) Prepare the extension reaction solution with the following composition for the total number of reactions to be processed, plus 10% of the total volume.

[Per reaction]

Reagent	Volume
⑥ Extension polymerase Premix	49 μl
⑦ Extension primer	1 μl
Total	50 μl

2) Gently mix by pipetting or gentle vortexing then spin down.
3) Add 50 μl of extension reaction solution to 49 μl of 1st adapter ligation product (total of 99 μl).
4) Gently mix by pipetting or gentle vortexing then spin down.
5) Place the tubes in a preheated thermal cycler and run the following program:

[Reaction conditions]
95℃ 30 sec
60℃ 30 sec
68℃ 5 min
4℃ Hold

6) At the end of the program, spin down and immediately proceed to the next step in the same tube.

VII-5. Library preparation

Amount of input DNA	Protocol
＞10 ng	Proceed to VII-5A
≦ 10 ng	Proceed to VII-5B

- If the amount of input exceeds 10 ng, perform the procedure described in [VII-5A. Library preparation (amount of input DNA > 10 ng)].
- If the amount of input DNA does not exceed 10 ng or if short DNA (50 to 160 bp) will be analyzed, perform the procedure described in [VII-5B. Library preparation (amount of input DNA ≤ 10 ng)].

  If additional bead purification is performed with this protocol, it is possible to obtain a higher library yield. By changing the bead ratio, it is possible to prevent the loss of short DNA and obtain a stable library yield from short DNA.

[VII-5A. Library preparation (amount of input DNA > 10 ng)]

VII-5A-1. 2nd adapter ligation reaction

1) Add 1 μl of ⑧ VDC enzyme to 99 μl of extension product (total of 100 μl).
2) Gently mix by pipetting or gentle vortexing then spin down.
3) Place the tubes in a preheated thermal cycler and run the following program:

[Reaction conditions]
25℃ 15 min
4℃ Hold

4) At the end of the program, spin down and immediately proceed to the next step in the same tube.

VII-5A-2. Purification

Using NucleoMag NGS Clean-up and Size Select (Cat. #744970.5/.50/.500), perform purification according to the following procedure.

Notes:
- Prepare fresh 80% ethanol for each experiment.
- When evaporating ethanol from beads, proceed only until the beads no longer appear moist. Slight cracks are acceptable, but a heavily cracked pellet indicates the beads are overly dry. Overdrying may decrease the recovery rate for the target DNA.

1) Let NucleoMag NGS Clean-up and Size Select stand at room temperature for approximately 30 minutes to bring the bead solution to room temperature.
2) Mix the bead solution well by vortexing until it appears homogenous.
3) Add 100 µl of bead solution to 100 µl of 2nd adapter ligation product.
4) Vortex to mix then incubate at room temperature for 5 minutes.
5) Spin down the tubes.
6) Place the tubes on the magnetic stand and let stand 5 minutes or longer until the liquid appears completely clear and there are no beads left in the supernatant.
7) Remove the supernatant with a pipette being careful not to disturb the beads.
8) Leave the tubes on the magnetic stand and add 200 µl of 80% ethanol without disturbing the beads.
9) Leave the tubes on the magnetic stand and incubate for 30 seconds.
10) Leave the tubes on the magnetic stand and remove the ethanol with a pipette being careful not to disturb the beads.
11) Repeat steps 8) through 10) and then spin down the tubes.
12) Place the tubes on the magnetic stand and remove the residual ethanol with a pipette without disturbing the beads.
13) Leave the tubes on the magnetic stand with the lids open to allow the ethanol to evaporate (see note at the beginning of this section).
14) After sufficiently drying the beads, leave the tubes on the magnetic stand and add 21 µl of TE buffer. Close the tube lids.
15) Remove the tubes from the magnetic stand, suspend with a vortex, and incubate for 2 minutes at room temperature to rehydrate the beads.
16) Spin down the tubes.
17) Leave the tubes on the magnetic stand for 1 minute or longer until the solution turns completely clear.
18) Transfer 20 µl of the clear supernatant to a fresh 0.2 ml PCR tube.

Safe Stopping Point: If you need to pause the protocol, store the purified product at -20℃ or below for up to one week. When ready to continue, thaw the tube on ice and spin down before proceeding to the next step.

Note: For the remainder of the workflow, follow the instructions under "VII-6. PCR amplification of DNA library."

[VII-5B. Library preparation (amount of input ≤ 10 ng)]

VII-5B-1. Purification (1st round)

Using NucleoMag NGS Clean-up and Size Select (Cat. #744970.5/.50/.500), perform purification according to the following procedure.

Notes:
- Prepare fresh 80% ethanol for each experiment.
- When evaporating ethanol from beads, proceed only until the beads no longer appear moist. Slight cracks are acceptable, but a heavily cracked pellet indicates the beads are overly dry. Overdrying may decrease the recovery rate for the target DNA.

1) Let NucleoMag NGS Clean-up and Size Select stand at room temperature for approximately 30 minutes to bring the bead solution to room temperature.
2) Mix the bead solution well by vortexing until it appears homogenous.
3) Add 99 μl of bead solution to 99 μl of extension product.
4) Vortex to mix then incubate at room temperature for 5 minutes.
5) Spin down the tubes.
6) Place the tubes on the magnetic stand and let stand 5 minutes or longer until the liquid appears completely clear and there are no beads left in the supernatant.
7) Remove the supernatant with a pipette being careful not to disturb the beads.
8) Leave the tubes on the magnetic stand and add 200 μl of 80% ethanol without disturbing the beads.
9) Leave the tubes on the magnetic stand and incubate for 30 seconds.
10) Leave the tubes on the magnetic stand and remove the ethanol with a pipette being careful not to disturb the beads.
11) Repeat steps 8) through 10) and then spin down the tubes.
12) Place the tubes on the magnetic stand and remove the residual ethanol with a pipette without disturbing the beads.
13) Leave the tubes on the magnetic stand with the lids open to allow the ethanol to evaporate (see note at the beginning of this section).
14) After sufficiently drying the beads, leave the tubes on the magnetic stand and add 24 μl of TE buffer. Close the tube lids.
15) Remove the tubes from the magnetic stand, suspend with a vortex, and incubate for 2 minutes at room temperature to rehydrate the beads.
16) Spin down the tubes.
17) Leave the tubes on the magnetic stand for 1 minute or longer until the solution turns completely clear.
18) Transfer 23 μl of the clear supernatant to a fresh 0.2 ml PCR tube.

Safe stopping point: If you need to pause the protocol, store the purified product at -20℃ or below for up to one week. When ready to continue, thaw the tube on ice and spin down before proceeding to the next step.

VII-5B-2. 2nd adapter ligation reaction

1) Prepare ligation reaction solution with the following composition for the total number of reactions to be processed, plus 10% of the total volume.

[Per reaction]

Reagent	Volume
⑧ VDC enzyme	1 μl
⑨ VDC buffer	6 μl
Total	7 μl

2) Gently mix by pipetting or gentle vortexing then spin down.
3) Add 7 μl of ligation reaction solution to 23 μl of the first purification product (total of 30 μl).
4) Gently mix by pipetting or gentle vortexing then spin down.
5) Place the tubes in a preheated thermal cycler and run the following program:

[Reaction conditions]
25℃ 15 min
4℃ Hold

6) At the end of the program, spin down and immediately proceed to the next step in the same tube.

VII-5B-3. Purification (2nd round)

Using NucleoMag NGS Clean-up and Size Select (Cat. #744970.5/.50/.500), perform purification according to the following procedure.

Notes:
- Prepare fresh 80% ethanol for each experiment.
- When evaporating ethanol from beads, proceed only until the beads no longer appear moist. Slight cracks are acceptable, but a heavily cracked pellet indicates the beads are overly dry. Overdrying may decrease the recovery rate for the target DNA.

Note: See below for input DNA size and recommended conditions for added bead solution.

Input DNA size	Added bead solution
50 - 160 bp	60 μl
160 bp ≦	30 μl

When preparing a library including both of the sizes shown above, add 60 μl of bead solution.

1) Let NucleoMag NGS Clean-up and Size Select stand at room temperature for approximately 30 minutes to bring the bead solution to room temperature.
2) Mix the bead solution well by vortexing until it appears homogenous.
3) Add bead solution to the 30 μl of 2nd adapter ligation product, at a volume based on the size of the input DNA and referring to the table above.
4) Vortex to mix then incubate at room temperature for 5 minutes.
5) Spin down the tubes.
6) Place the tubes on the magnetic stand and let stand 5 minutes or longer until the liquid appears completely clear and there are no beads left in the supernatant.
7) Remove the supernatant with a pipette, being careful not to disturb the beads.

8) Leave the tubes on the magnetic stand and add 200 µl of 80% ethanol without disturbing the beads.
9) Leave the tubes on the magnetic stand and incubate for 30 seconds.
10) Leave the tubes on the magnetic stand and remove the ethanol with a pipette being careful not to disturb the beads.
11) Repeat steps 8) through 10) and then spin down the tubes.
12) Place the tubes on the magnetic stand and remove the residual ethanol with a pipette without disturbing the beads.
13) Leave the tubes on the magnetic stand with the lids open to allow the ethanol to evaporate (see note at the beginning of this section).
14) After sufficiently drying the beads, leave the tubes on the magnetic stand and add 21 µl of TE buffer. Close the tube lids.
15) Remove the tubes from the magnetic stand, suspend with a vortex, and incubate for 2 minutes at room temperature to rehydrate the beads.
16) Spin down the tubes.
17) Leave the tubes on the magnetic stand for 1 minute or longer until the solution turns completely clear.
18) Transfer 20 µl of the clear supernatant to a fresh 0.2 ml PCR tube.

Safe stopping point: If you need to pause the protocol, store the purified product at -20℃ or below for up to one week. When ready to continue, thaw the tube on ice and spin down before proceeding to the next step.

Note: For the remainder of the process, follow the instructions under "VII-6. PCR amplification of DNA library."

VII-6. PCR amplification of DNA library

Use in combination with Unique Dual Index Kit (Cat. #634752 - 634756) including dual index PCR primer and assign an index sequence specific to each sample in the DNA library PCR amplification step.

Notes:
- Be sure to use Unique Dual Index Kit (Cat. #634752 - 634756). Do not substitute with another index reagent.
- When sequencing multiple samples at the same time, assign a different index for each sample.
- After thawing Unique Dual Index Kit (UDI kit) at room temperature, vortex and spin down.
- Do not prepare a master mix combining the UDIs and PCR polymerase Premix.
- To avoid contamination, be sure to change the pipette tip each time you add a new Indexing Primer from the UDI kit.
- To reduce the risk of contamination of input DNA, DNA library, and Indexing Primer, perform the remaining steps of the protocol in an area for handling PCR products.

1) To 20 μl of the purified ligation product, add 5 μl of the appropriate assigned Indexing Primer from the UDI kit.
2) Add 25 μl of ⑩ PCR polymerase Premix. Mix gently by pipetting up and down a few times or gently vortexing then spin down.
3) Place the tubes in a preheated thermal cycler and run the following program:

[Reaction conditions]

98℃ 2 min
↓
95℃ 20 sec ┐
65℃ 30 sec ┘ x cycles*2
↓
68℃ 5 min
4℃ Hold

*2 The number of cycles is dependent on the amount of input DNA. Determine the number of cycles for your experiment using the table below.

Amount of input DNA	No. of cycles
10 pg	19 - 20
100 pg	15 - 16
1 ng	12 - 13
10 ng	10 - 11
100 ng	6 - 7
250 ng	4 - 5

Notes:
- It is necessary to optimize the number of cycles not only according to the amount of input DNA but also the quality and type of sample.
- If too many cycles are performed relative to the amount of input DNA, a PCR artifact (a peak other than the target peak, as seen on a Bioanalyzer or TapeStation) may occur.

VII-7. Purification of Amplified Library

Using NucleoMag NGS Clean-up and Size Select (Cat. #744970.5/.50/.500), perform purification according to the following procedure.

Notes:
- Prepare fresh 80% ethanol for each experiment.
- When evaporating ethanol from beads, proceed only until the beads no longer appear moist. Slight cracks are acceptable, but a heavily cracked pellet indicates the beads are overly dry. Overdrying may decrease the recovery rate for the target DNA.

1) Let NucleoMag NGS Clean-up and Size Select stand at room temperature for approximately 30 minutes to bring the bead solution to room temperature.
2) Mix the bead solution well by vortexing until it appears homogenous.
3) Add 50 μl of bead solution to 50 μl of the amplified product.
4) Vortex to mix then incubate at room temperature for 5 minutes.
5) Spin down the tubes.
6) Place the tubes on the magnetic stand and let stand 5 minutes or longer until the liquid appears completely clear and there are no beads left in the supernatant
7) Remove the supernatant with a pipette being careful not to disturb the beads.
8) Leave the tubes on the magnetic stand and add 200 μl of 80% ethanol without disturbing the beads.
9) Leave the tubes on the magnetic stand and incubate for 30 seconds.
10) Leave the tubes on the magnetic stand and remove the ethanol with a pipette being careful not to disturb the beads.
11) Repeat steps 8) through 10) and then spin down the tubes.
12) Place the tubes on the magnetic stand and remove the residual ethanol with a pipette without disturbing the beads.
13) Leave the tubes on the magnetic stand with the lids open to allow the ethanol to evaporate (see note at the beginning of this section).
14) After sufficiently drying the beads, leave the tubes on the magnetic stand and add 31 μl of TE buffer. Close the tube lids.
15) Remove the tubes from the magnetic stand, suspend with a vortex, and incubate for 2 minutes at room temperature to rehydrate the beads.
16) Spin down the tubes.
17) Leave the tubes on the magnetic stand for 1 minute or longer until the solution turns completely clear.
18) Transfer 30 μl of the clear supernatant to a fresh 0.2 ml PCR tube.

Note: The purified DNA library can be stored at -20℃ or below.

VII-8. Amplified Library Quantification

Measure the concentration and size of the DNA library with Bioanalyzer or TapeStation. Because the measured concentration of the DNA library will be within the measurement range of the instrument, the DNA library should be diluted with TE buffer based on the DNA concentration quantified with Qubit Fluorometer or equivalent equipment and reagent kit.

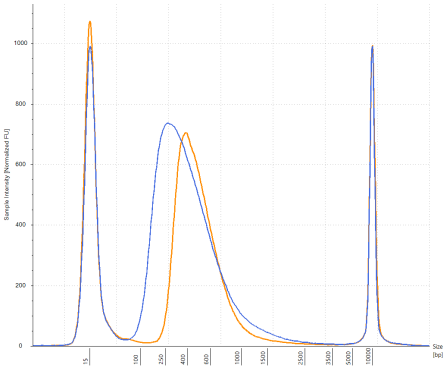


Fig. 5. Example of TapeStation results of DNA library prepared from fragmented human DNA. Blue: input DNA, Orange: DNA library of fragmented DNA.

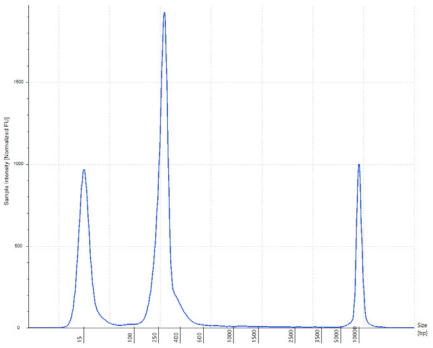


Fig. 6. Example of TapeStation results of DNA library prepared from synthetic 160-base ssDNA oligo.

VIII. Sequencing

Amplified DNA libraries prepared with this kit have a library structure that can be analyzed with Illumina NGS sequencers. The library prepared from each sample contains the specific index information selected at the time of PCR amplification. When pooling amplified DNA libraries before sequencing, confirm that there is no redundancy of indices.

To sequence, refer to the instruction manual of the instrument.

Notes:

- Unique Dual Index Kits (Cat. #634752 - 634756) are compatible with Illumina's 4-color and 2-color SBS Chemistry sequencers. Compatibility with Illumina's 1-color SBS Chemistry sequencer has not been verified.
- When sequencing samples with low diversity of PCR products, etc and decreased Q-score (sequence quality score) has been confirmed, adding PhiX Control (Illumina) to the prepared DNA library before sequencing can improve the Q-score.

IX. Data analysis

A 5-base specific sequence (CCCTT) is added to the 5' end of the insert DNA in the DNA library. For analysis, trim the 5 bases at the start of Read 2.

X. Troubleshooting

Problem	Cause	Solution
No library yield	No addition of input DNA	Use positive and negative controls in parallel to preparing the sample libraries.
Low library yield	Quality of input DNA is low	Increase amount of input DNA or number of PCR cycles according to the table in the PCR protocol.
	Poor DNA elution due to excessive drying of beads	Pipette or vortex until purification beads are completely dissolved in TE buffer. Take care not to under- or overdry the bead pellets
Adapter dimers (approx. 120 bp) are generated	Substances contained in input DNA that impede the reaction	Dilute the input DNA with TE buffer.
	Amount of input DNA is small	Increase the amount of input DNA.
There are multiple peaks in library	DNA of differing lengths are contained in the input DNA	This is not a problem, so simply continue the analysis.
	A PCR artifact (a peak other than the target peak, confirmed by Bioanalyzer or TapeStation) was generated	Reduce the amount of input DNA or reduce the number of PCR cycles.
Mapping rate is low	Contamination with DNA other than target DNA	Reprepare the input DNA.

XI. References

Miura, Fumihito *et al*. Identification of an enzyme with strong single-stranded DNA ligation activity and its application for sequencing. *Nucleic acids research.* (2025) **53**: 3.

XII. Related Products

Unique Dual Index Kit (Cat. #634752 - 634756)
NucleoMag NGS Clean-up and Size Select (Cat. #744970.5/.50/.500)*
Takara FFPE DNA QC All-in-One Kit (Cat. #NN0001)*
Clontech PCR Thermal Cycler GP (Cat. #WN400)*
0.2 ml 8-strip tube, individual Flat Caps (Cat. #NJ600)*
0.2 ml Hi-8-Tube (Cat. #NJ300)*
0.2 ml 96 well-plate for Real Time (Frosted) (Cat. #NJ401)*
96 well snap plate (Cat. #NJ710)*

* Not available in all geographic locations. Check for availability in your area.