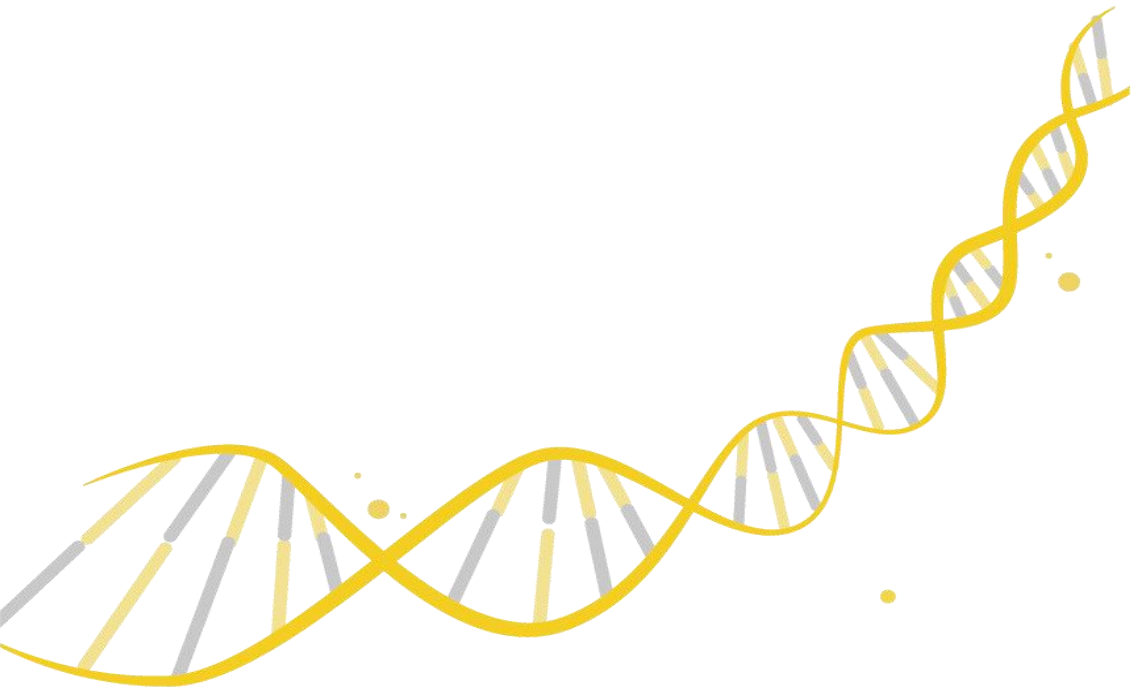
One-step DNA Lib Prep Kit for Illumina V2 (50 ng Input DNA)

RK20239



www.abclonal.com.cn

version: N16D20v2.5

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1. Introduction

The One-step DNA Lib Prep Kit for Illumina V2 enables users to prepare sequencing libraries with a fairly fast way. Compared with traditional DNA library construction methods, one-step library preparation can reduce the requirement for template investment, and significantly shorten the overall sequencing workflow time.

The One-step DNA Lib Prep Kit for Illumina V2 uses tagmentation to fragment DNA and add partial adapter sequences. This kit requires only 50 ng input DNA (Genomes of humans, animals, plants, microorganisms, and purified PCR amplicons (> 300 bp)).

2. Component

	Component	8 RXN	24 RXN	96 RXN
●	Tagment Enzyme T50	40 μL	120 μL	480 μL
●	5X Tagment Buffer	32 μL	96 μL	384 μL
●	Termination Buffer*	20 μL	60 μL	240 μL
●	PCR Mix	184 μL	552 μL	2208 μL
●	Control DNA (25 ng/μL)	20 μL	20 μL	20 μL

*, Note: This component should be dissolved at room temperature before use. If precipitation occurs in this component, it is a normal phenomenon. Please dissolve in a water bath at 37 ℃ and mix thoroughly before use.

3. Storage

All components should be stored at-20°C. The shelf life of each reagent is one year when stored properly.

4. Additional Materials Required

- ✧ 100% ethanol (80% ethanol needs to be prepared for immediate use)
- ✧ Nuclease-free water
- ✧ PCR strips, tubes, or plates
- ✧ Magnetic stand
- ✧ Pipettetips with filters
- ✧ Thermocycler
- ✧ Microcentrifuge
- ✧ Vortex mixer
- ✧ Pipettes and multichannel pipettes
- ✧ Agilent Bioanalyzer, or comparable method to assess the quality of DNA library
- ✧ AFTMag NGS DNA Clean Beads (ABclonal, cat. no. RK20257)
- ✧ Dual DNA Adapter 96 Kit for One-step DNA Lib Prep (ABclonal, cat. no. RK20290)
- ✧ ABQubit dsDNA Quantitation Kit（ABclonal, Cat.NO. RK30140）

5. Tips and Techniques

Input DNA

✧ It is recommended to use Qubit for concentration determination of DNA samples.

✧ For other template DNA input amounts (1 ng, 5 ng), please refer to the corresponding product manual (ABclonal, Cat. no. RK20237; ABclonal, Cat. no. RK20238).

✧ This kit provides with a control DNA (25 ng/μL).

Clean Up Libraries

✧Magnetic beads should be thoroughly mixed and balanced to room temperature before use.

✧Do not absorb the magnetic beads when transferring the supernatant.

✧80% ethanol used for magnetic bead rinsing should be prepared on site.

6. Library structure

5'-AATGATACGGCGACCACCGAGATCTACAC(i5-index)TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-NNNNN-CTGTCTCTTATACACATCTCCGAGCCCACGAGAC(i7-index)ATCTCGTATGCCGTCTTCTGCTTG-3'

7. Experimental flowchart

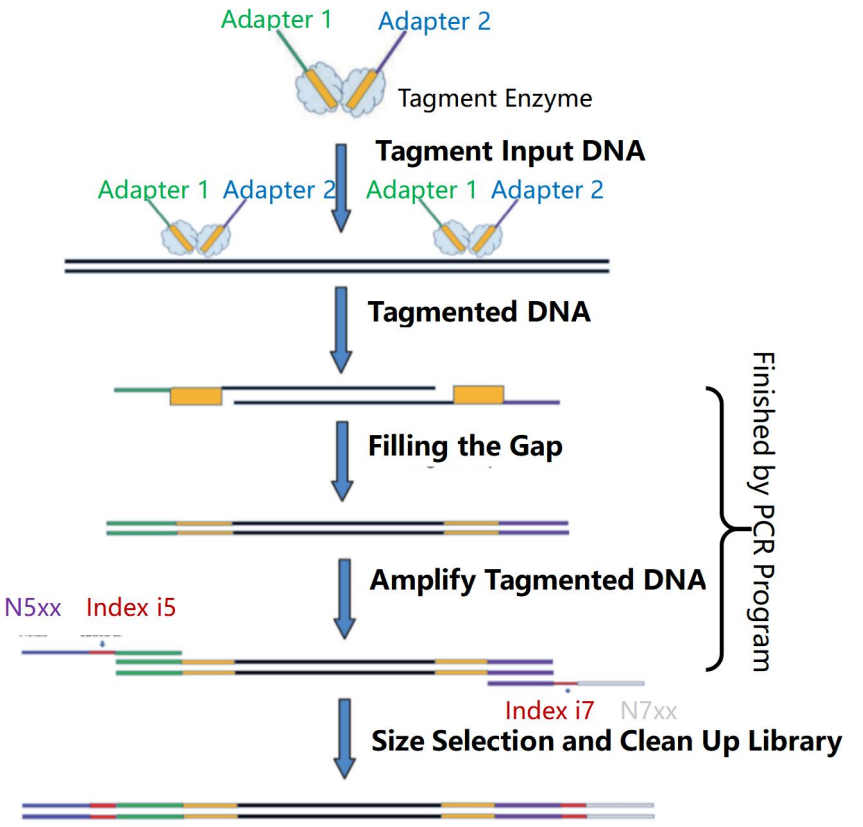


Figure 1. Experimental flowchart

8. Protocol

Preparing input DNA

DNA samples should be dissolved in nuclease free water. It is recommend -ed to use Qubit for accurate quantification of DNA samples.

Tagment DNA

1. Prepare the following consumables:

	Item	Storage	Instructions
●	5X Tagment Buffer	-20℃	Thaw on ice. Invert the thawed tubes 3-5 times, and then centrifuge briefly.
	DNA	-20℃	Thaw on ice. Invert the thawed tubes 3-5 times, and then centrifuge briefly.
●	Tagment Enzyme T50	-20℃	Thaw on ice. Invert the thawed tubes 3-5 times, and then centrifuge briefly.
	Nuclease free water	Room temperature	Use directly
●	Termination Buffer	-20℃	Check for precipitates. If present, vortex until all particulates are resuspended.
	DNA Clean Beads	4℃	Thoroughly mixed and balanced to room temperature before use.
	80% EtOH	Room temperature	Use directly

2. Prepare the following reaction mix in a new PCR tube:

	Reagents	Volume
	5X Tagment Buffer	4 μL
	Input DNA	50 ng
	Tagment Enzyme T50 *	5 μL
	Nuclease free water	Up to 20 μL
	Total	20 μL

*, Note: If there are multiple samples, please pay attention to replacing the gun head to avoid cross contamination between samples; When adding components, ddH2O can be added first, followed by other components, and finally, Tagment Enzyme T50 can be added.

3. Pipette 10 times to mix, and then centrifuge briefly.

4. Place on the thermal cycler and run the following program. When the program reaches 12℃, immediately proceed to step 5 because the transposome is still active.

Temperature	Time
Heating lid temperature(75℃)	---
55℃	5 min
12℃	hold

5. Add 2 μL Termination Buffer to each PCR tube.

6. Pipette 10 times to mix, and then centrifuge briefly.

7. Incubate at room temperature for 5 minutes.

Amplify Libraries

1. Prepare the following consumables:

	Item	Storage	Instructions
●	PCR Mix	-20℃	Thaw on ice. Invert the thawed tubes 3 -5 times, and then centrifuge briefly.
	Index Primers	-20℃	Thaw on ice. Invert the thawed tubes 3 -5 times, and then centrifuge briefly.
	Tagment Products	On ice	Use directly

2. Prepare the following reaction mix in a new PCR tube:

	Reagents	Volume
●	PCR Mix	23 μL
	Tagment Products	22 μL
●	N5xx (Index Primers)	2.5 μL
●	N7xx (Index Primers)	2.5 μL
	Total	50 μL

3. Pipette 10 times to mix, and then centrifuge briefly.

4. Place on the preprogr -ammed thermal cycler and run the following program.

Temperature	Time	Cycles
Heating lid temperature(105℃)	---	---
72℃	3 min	1
98℃	30 s	1
98℃	15 s	7
60℃	30 s	
72℃	1 min	
72℃	5 min	1
4℃	hold	---

5. **SAFE STOPPING POINT**: If you are stopping, seal the tube and store at 2°C to 8°C for up to 2 days. Alternatively, leave on the thermal cycler overnight.

6. If you are planning to use single-sided bead purification to purify amplified libraries, follow step **Clean Up Libraries (Optional).**

7. If you are planning to use size selection to purify amplified libraries, follow step **Size Selection Libraries (Optional).**

Clean Up Libraries (Optional)

1. Centrifuge briefly to collect contents at the bottom of the PCR tube.
2. add 50 μL AFTMag NGS DNA Clean Beads to each tube. Pipette 10 times to mix, and then centrifuge briefly.
3. Incubate at room temperature for 5 minutes.
4. Place on the magnetic stand and wait until the liquid is clear. Remove and discard all supernatant without disturbing the beads.
5. With the plate on the magnetic stand, add 200 μl fresh 80% EtOH without mixing. Incubate for 30 seconds, then remove and discard all supernat -ant without disturbing the beads.
6. Repeat Step 5.
7. Use a 10 μl pipette to remove and discard residual EtOH. Air-dry on the magnetic stand until there is no residual ethanol.
8. Remove from the magnetic stand and add 32 μl nuclease free water to the beads. Pipette 10 times to mix, and then centrifuge briefly.

9. Incubate at room temperature for 2 minutes.

10. Place on the magnetic stand and wait until the liquid is clear. Transfer 30 µl supernatant to a new PCR tube.

11. **SAFE STOPPING POINT**: If you are stopping, seal the tube and store at -25°C to -15°C for up to 7 days.

Size Selection Libraries (Optional)

1. Centrifuge briefly to collect contents at the bottom of the PCR tube. The following table shows the volume of the two rounds of beads for different library size.

Library average size	350 bp	450 bp	550 bp
Volume of 1st round Beads	35 µL (0.70X)	30 µL (0.60X)	25 µL (0.50X)
Volume of 2nd round Beads	10 µL (0.20X)	7.5 µL (0.15X)	7.5 µL (0.15X)

2. Add 1st round Beads to each tube. Pipette 10 times to mix, and then centrifuge briefly.

3. Incubate at room temperature for 5 minutes.

4. Place on the magnetic stand and wait until the liquid is clear. Transfer the supernatant into a new PCR tube. Add 2nd round Beads to each tube. Pipette 10 times to mix, and then centrifuge briefly.

5. Incubate at room temperature for 5 minutes.

6. Place on the magnetic stand and wait until the liquid is clear. Remove and discard all supernatant without disturbing the beads.

7. With the plate on the magnetic stand, add 200 µl fresh 80% EtOH without

mixing. Incubate for 30 seconds, then remove and discard all supernat -ant without disturbing the beads.

8. Repeat Step 7.

9. Use a 10 µl pipette to remove and discard residual EtOH. Air-dry on the magnetic stand until there is no residual ethanol.

10. Remove from the magnetic stand and add 22 µl nuclease free water to the beads. Pipette 10 times to mix, and then centrifuge briefly.

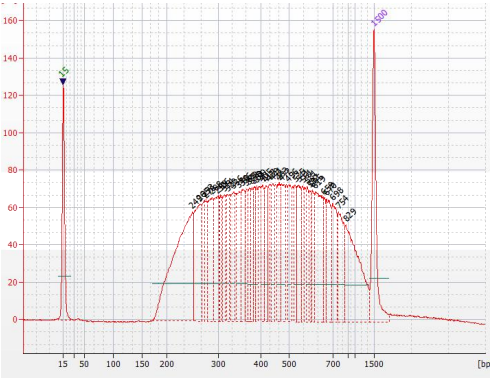
11. Incubate at room temperature for 2 minutes.

12. Place on the magnetic stand and wait until the liquid is clear. Transfer 20 µl supernatant to a new PCR tube.

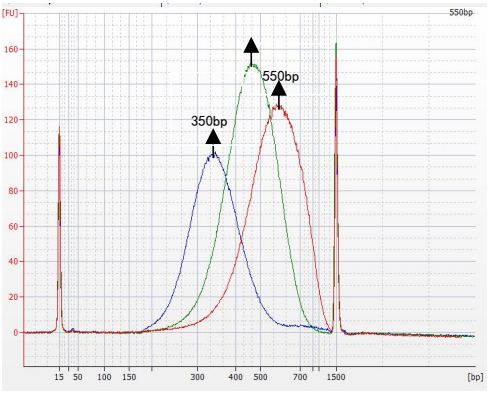
9. appendix

Check Library Quality

1. Run 1 µl **Clean Up Library** on an Agilent Technology 2100 Bioanalyzer using a High Sensitivity DNA kit.



2. Run 1 µl **Size Selection Library** on an Agilent Technology 2100 Bioanalyzer using a High Sensitivity DNA kit.



Q & A

Question: Why is my library size larger than expected?

Answer: The possible reason for the large fragments interrupted by the library is that there is too much Input DNA, and reducing the amount of Input DNA will reduce the production of large fragments; It is also possible that there are inhibitors present in Input DNA. It is recommended to accurately measure the concentration based on fluorescence method and add according to the recommended amount of the reagent kit. Using reliable DNA purification methods to remove chemicals that may interfere with the operation of transposase and preserve high-quality DNA.

Question: Why is my library size smaller than expected?

Answer: The main reason for the small fragments interrupted by the library is due to the low amount of Input DNA or the use of degraded DNA samples, such as FFPE samples. Suggest increasing the amount of Input DNA, using high-quality DNA samples, or reducing DNA fragmentation time.

Question: How many index combinations can the One step kits offer?

Answer: Dual DNA Adapter 96 Kit for One-step DNA Lib Prep（ABclonal, Cat.NO. RK20290）includes 8 N5 primer and 12 N7 primer options, offering 96 index combinations.

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