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Seq-Star™ Rapid DNA-seq Kit (Illumina)

Cat#: AS-MB-010-01 (Set A); AS-MB-010-02 (Set B)

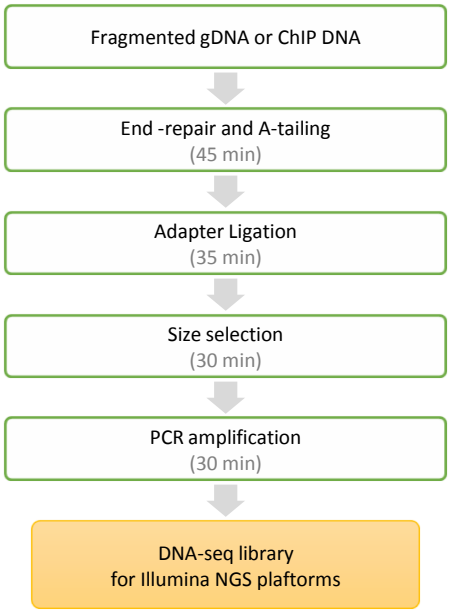
Instruction Manual version 1.0

Product summary

Product description

Seq-Star™ Rapid DNA-seq Kit (Illumina) contains specially optimized enzyme mix and buffers for rapid library preparation of chromatin immunoprecipitated (ChIP) or regular genomic DNA for Illumina next generation sequencing (NGS) platforms.

The kit features the simplest workflow for DNA-seq library preparation. The end-repair, A-tailing and adapter ligation steps can be completed in a one-tube system without cleanups in between. DNAClean™ beads are supplied for purification after the adapter ligation and library PCR amplification. The kit method has been successfully used to construct DNA-seq libraries from ChIP DNA as low as 1 ng.



Starting materials

- 1 ng~1 µg of fragmented, double-stranded genomic DNA or ChIP DNA
- High quality DNA is recommended for the kit. The OD260/280 ratio should be 1.8~2.0. DNA with heavy nicks or single stranded DNA contamination may cause DNA library preparation failure.

Kit components

Components	24 Reactions	Storage
Rapid-Prep Enzyme Mix	72 µL	-20°C
Rapid-Prep Buffer	144 µL	-20°C
Rapid-Prep Ligase	24 µL	-20°C
Multiplex Illumina Adapters (Set A or Set B: Tube 1-12)	10 µL	-20°C
2× Rapid-Prep PCR Mix	600 µL	-20°C
PCR Primer Mix	48 µL	-20°C
DNAClean Beads	2.4 mL	4°C

Additional required materials

- Magnetic stand (tube compatible)
- Pipettors and tips
- Thermal cycler
- Agilent 2100 Bioanalyzer (optional)
- Fresh 80% ethanol
- Nuclease-free water

Protocol

Part I: End-repair and A-tailing

1. Prepare the following Mix in a 200 µL PCR-tube for each sample:

1 ng ~1 µg gDNA or ChIP DNA	X µL
Nuclease-free water	Y µL
Rapid-Prep Enzyme Mix	6 µL
Rapid-Prep Buffer	3 µL
Total volume	25 µL

2. Incubate the tube in a thermal cycler for [25°C, 20min; 72°C, 20min; 4°C, hold].

Part II: Adapter ligation

3. Add the following components directly to the reaction from Step 2:

Multiplex Illumina Adapter	1 µL
Rapid-Prep Ligase	1 µL
Total volume	2 µL

Note: Multiplex Illumina Adapters should be diluted at 1/10 if the starting DNA amount is 1~10 ng.

4. Mix thoroughly. Incubate at 25°C for 30 mintutes and hold at 4°C in a thermal cycler.
5. Add 23 µL nuclease-free water to the 27 µL reaction and mix thoroughly.
6. Add 50 µL DNAClean Beads. Mix thoroughly by pipetting up and down 10 times.
7. Incubate at room temperature for 5~10 minutes.
8. Place the reaction tube at magnetic stand until the solution becomes completely clear (about 1~2 minutes). Carefully aspirate and discard the supernatant.
9. Keep the tube on the magnetic stand and add 200 µL freshly prepared 80% ethanol. Incubate at room temperature for 30 seconds and aspirate off the supernatant.

   Caution: Do not disturb the separated magnetic beads during the operation!

10. Repeat Step 9 once for total two washes. Make sure to remove all the remaining ethanol from the bottom of the tube.

    Optional: Air drying the beads for less than 5 minutes will ensure to remove all the ethanol. However over drying the beads may significantly decrease the elution efficiency.

11. Remove tube off the magnetic stand and resuspend the beads in 24 µL nuclease-free water.
12. Incubate at room temperate for 2 minutes. Place the tube on magnetic stand until the supernatant is completely clear from beads.
13. Transfer 23 µL supernatant to a new tube for downstream size selection or PCR amplification.

Part III: Library size selection (optional)

The purified adapter-ligated DNA from Step 13 can be directly used for PCR amplification (Step 17) without incurring adapter-dimer contamination in the final DNA-seq libraries.

If you want to obtain the sequencing library in a desired size range, the size selection can be performed by using recommended Seq-Star™ DNA Size Selection Kit (Cat: #####) or other similar kits (**Figure 1**).

14. Add 27 µL nuclease-free water to the purified 23 µL adapter-ligated DNA for a 50 µL total volume.
15. Perform the size selection procedure according to the Seq-Star™ DNA Size Selection Kit manual (or the manufacturer's instructions of the other similar kit used).

Table1. Bead amounts for size selection with Seq-Star™ DNA Size Selection Kit

Library peak size (bp)	300	350	400	500	600	700
Beads (µL) 1st round	40	35	30	27.5	25	22.5
Beads (µL) 2nd round	10	10	10	7.5	7.5	7.5

16. Elute the size selected DNA in 23 µL nuclease-free water.

Part IV: PCR amplification and purification

17. Prepare the following mix for each sample:

2× Rapid-Prep PCR Mix	25 µL
PCR Primer Mix	2 µL
DNA from Step 13 or Step 16	23 µL
Total volume	50 µL

18. Run the PCR reaction as following protocol [98°C, 30s; 10-18 cycles of (98°C, 10s; 60°C, 30s; 72°C, 30s); 72°C, 5min; 4°C, hold].

    Note: The number of PCR cycles should depend on the input DNA amount: 10 cycles for 100 ng, 12 cycles for 50 ng, 16 cycles for 5 ng, and 18 cycles for 1 ng input DNA.

19. Add 50 µL DNAClean Beads to the 50 µL PCR reaction. Mix thoroughly by pipetting up and down 10 times.

20. Incubate at room temperature for 5~10 minutes.

21. Place the tube at magnetic stand until the solution become completely clear (about 1~2 minutes). Carefully aspirate and discard the supernatant.

22. Keep the tube on magnetic stand and add 200 µL freshly prepared 80% ethanol. Incubate at room temperature for 30 seconds and aspirate off the supernatant.

    Caution: Do not disturb the separated magnetic beads during the operation!

23. Repeat Step 22 once more for total two washes. Make sure to remove all the remaining ethanol from the bottom of the tube.

    Optional: Air drying the beads for less than 5 minutes will ensure to remove the remaining ethanol. However, do not over dry the beads as the elution efficiency may be significantly decreased.

24. Remove tube off the magnetic stand and resuspend the beads in 21 µL nuclease-free water.

25. Incubate at room temperate for 2 minutes. Place the tube on the magnetic stand until the supernatant is completely clear from the beads.

26. Transfer 20 µL supernatant containing the final DNA-seq library to a new tube.

27. **OPTIONAL**: Assess the library quality using an Agilent 2100 Bioanalyzer (Fig. 1).

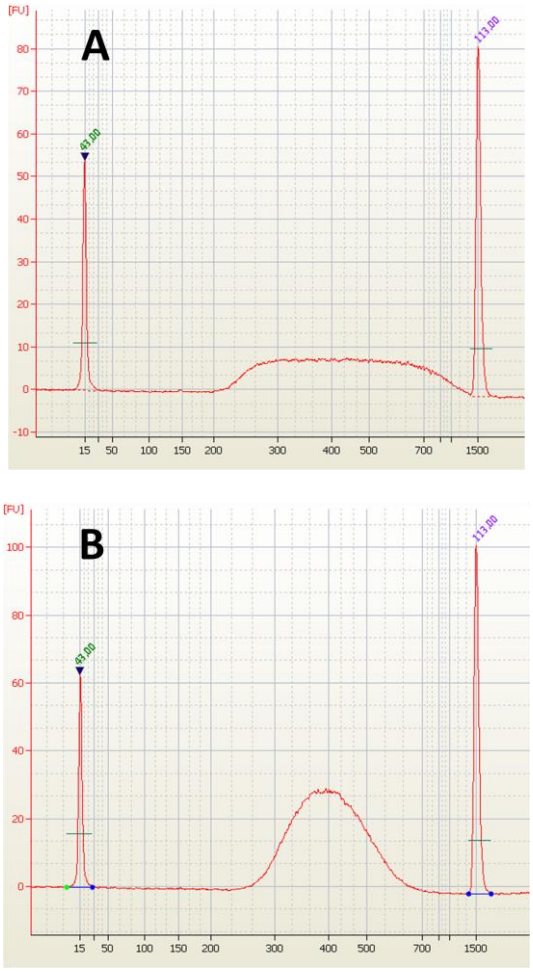


Figure 1. NGS libraries prepared with Seq-Star™ Rapid DNA-seq Kit (Illumina). **(A)** DNA-seq library without size selection appears as 200~1500 bp smear. Adapter dimer peak is absent; **(B)** DNA-seq library size selected with Seq-Star™ DNA Size Selection Kit (peak size ~400 bp). Both libraries were prepared from 1 ng of sonicated genomic DNA from MCF-7 cells.

Troubleshooting

Problem	Possible causes	Suggestion
Library yield too low	Insufficient amount of starting DNA	Sensitive method should be used to more accurately measure low sample amounts (*e.g.* Qubit fluorometer)
	Contaminants inhibiting enzyme activities in the DNA sample	Make sure no residual DNA-binding proteins or organics in the input DNA samples
	Too much single stranded DNA contamination	Denatured DNA samples cannot be used with this kit

Appendix

Illumina Multiplex Barcode sequences

Set A:

#01 CGATGT; #02 TGACCA; #03 ACAGTG; #04 GCCAAT;
#05 CAGATC; #06 CTTGTA; #07 AGTCAA; #08 AGTTCC;
#09 ATGTCA; #10 CCGTCC; #11 GTCCGC; #12 GTGAAA;

Set B:

#13 ATCACG; #14 TTAGGC; #15 ACTTGA; #16 GATCAG;
#17 TAGCTT; #18 GGCTAC; #19 GTGGCC; #20 GTTTCG;
#21 CGTACG; #22 GAGTGG; #23 ACTGAT; #24 ATTCCTTT;