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

Cat#: AS-MB-013 (Set A); (Set B)

Instruction Manual version 1.0

Product summary

Product description

The Seq-Star™ Rapid RNA-seq Kit (Illumina) uses a template-switching method for rapid RNA-seq library preparation from as little as 100 pg rRNA-depleted or oligo(dT)-enriched RNA. Adapter sequences are incorporated into the cDNA ends in a single reverse transcription reaction, without the need for 2nd strand cDNA synthesis or adapter-ligation. The entire library construction process can be completed in 3.5 hours (Fig. 1). The directionality of the RNA strand is preserved to make the library strand-specific, which is excellent for both mRNA and lncRNA classes.

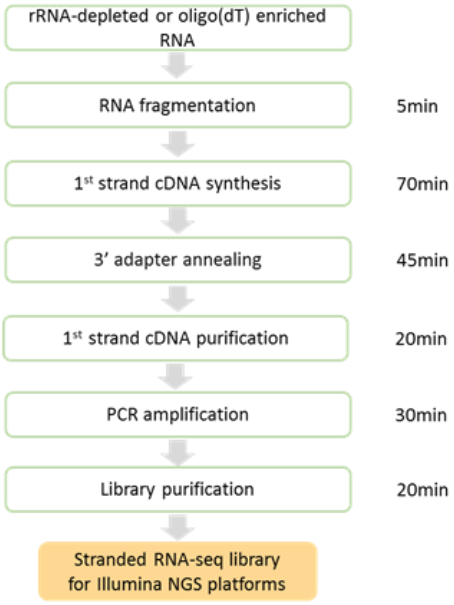

Figure 1. Workflow of Seq-Star™ Rapid RNA-seq Kit (Illumina).

Starting materials

- 100 pg - 100 ng rRNA-depleted or oligo(dT)-enriched RNA

Kit components

Components	24-reactions Cat#	Storage
Fragmentation Buffer	96 µL	-20°C
RT primer	24 µL	-20°C
Seq-Star™ RTase I	12 µL	-20°C
DTT (100 mM)	12 µL	-20°C
RNase inhibitor	12 µL	-20°C
dNTP (10 mM)	48 µL	-20°C
3' Annealing Mix	12 µL	-20°C
3' Adapter	24 µL	-20°C
Universal PCR Primer	24 µL	-20°C
2× PCR Mix	600 µL	-20°C
Index Primer Set (A)	10 µL	-20°C
Index Primer Set (B)	10 µL	-20°C

Additional required materials

- Seq-Star™ DNAClean Beads (#AS-MB-007) or Agencourt AmPure XP beads (Beckman Coulter)
- Magnetic stand (tube compatible)
- Pipettors and tips
- Thermal cycler
- Agilent 2100 Bioanalyzer (optional)
- Fresh 80% ethanol
- Nuclease-free water

Protocol

Part I: RNA fragmentation

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

100 pg ~ 100 ng rRNA-depleted or oligo(dT)-enriched RNA	10.0 µL
RT Primer	1.0 µL
Fragmentation Buffer	4.0 µL
Total volume	**15.0 µL**

2. Mix thoroughly by pipetting the reaction up and down several times. Spin down briefly.
3. Perform fragmentation using the parameters below:

Input RNA	Desired size	Fragmentation
Intact	200 - 300 bp	5 min@94°C
	300 - 400 bp	3min@94°C
Partially degraded	100 - 300 bp	5 min@85°C
Degraded	100 - 200 bp	5 min@65°C

4. Chill the tube on ice for 2 min and proceed immediately to the next step.

Part II: 1st Strand cDNA synthesis

5. Add the following Mix into the fragmented RNA from Step 4:

DTT (100 mM)	0.5 µL
dNTP (10mM)	2.0 µL
3’ Annealing Mix	0.5 µL
RNase Inhibitor	0.5 µL
Seq-Star™ RTase I	0.5 µL
Total volume	**4.0 µL**

6. Mix thoroughly by gently pipetting the reaction up and down 10 times.
7. Incubate the tube in a thermal cycler at 25°C for 10 min and 42°C for 60 min.
8. Pause the reaction at 42°C in the thermal cycler.

Part III: 3’ Adapter annealing

9. Heat denature 1 µL 3’ Adapter per sample at 70°C for 5 min, then chill on ice for 2 min.
10. Add 1 µL denatured 3’ Adapter to reaction from Step 8.
11. Mix thoroughly by pipetting up and down several times. Spin down briefly.
12. Incubate the tube at 42°C for 30 min, 70°C for 15min.

Part IV: 1st Strand cDNA purification

13. Add 20 µL Seq-Star™ DNAClean Beads to the reaction from Step 12.
14. Mix thoroughly by pipetting up and down 10 times. Spin down briefly.
15. Incubate at room temperature for 5 - 15 min for the cDNA binding to the beads.
16. Use a magnetic stand to separate the beads until the solution becomes completely clear (about 1-2 minutes). Carefully aspirate and discard the supernatant.

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

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

18. Repeat Step 17 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 helps to remove residual ethanol. However over-drying the beads may result in dramatic yield loss.

19. Remove the tube off the magnetic stand and resuspend the beads in 24.5 µL nuclease-free water.

20. Incubate at room temperature for 2 min to elute the DNA. Place the tube on the magnetic stand until the supernatant is completely clear from the beads.

21. Transfer 23 µL supernatant to a new 200 µL PCR tube.

Part V: Library PCR amplification and purification

22. Add the following mix to the 23 µL purified cDNA from Step 21.

2× PCR Mix	25.0µL
Universal PCR primer	1.0 µL
Index Primer	1.0 µL
Total volume	**27.0 µL**

23. Mix thoroughly by pipetting up and down 10 times.

24. Run the PCR program:

Cycle	Temperature	Time
1×	98°C	45 sec
9 ~ 18× (See Note)	98°C	15 sec
	55°C	30 sec
	72°C	30 sec
1×	72°C	5 min
	4°C	hold

    Note: The number of PCR cycles depends on the input RNA amount: 16 - 18 cycles for 0.1 - 1 ng, 12 - 14 cycles for 5 - 10 ng and 9 cycles for 100 ng input RNA.

25. Add 50 µL Seq-Star™ DNAClean Beads to the 50 µL PCR reaction.

26. Mix thoroughly by pipetting up and down 10 times.

27. Incubate at room temperature for 5 - 15 min for the DNA binding to the beads.

28. Use a magnetic stand to separate the beads until the solution becomes completely clear (about 1 - 2 minutes). Carefully aspirate and discard the supernatant.

29. 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 operation!

30. Repeat Step 29 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 helps to remove the residual ethanol. However over-drying the beads may result in dramatic yield loss.

31. Remove the tube off the magnetic stand and resuspend the beads in 21.5 µL nuclease-free water.

32. Incubate at room temperature for 2 min for elution. Place the tube on the magnetic stand until the supernatant is completely clear from the beads.

33. Transfer 20 µL supernatant to a new tube as the final product of RNA-seq library.

34. The library can be validated using Agilent 2100 Bioanalyzer (Fig. 2).

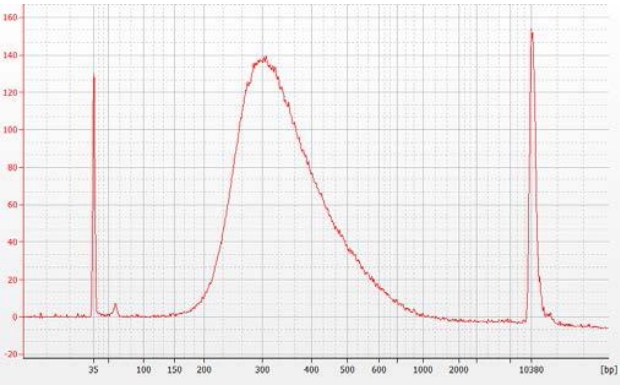

Figure 2. Agilent 2100 Bioanalyzer fragment analysis of a RNA-seq library constructed with Seq-Star Rapid RNA Kit. The input RNA was 10 ng oligo(dT)-enriched mRNA isolated from 1 µg total RNA of MCF-7 cells.

Troubleshooting

Problem	Possible causes	Suggestion
Low library yield	Insufficient amount of starting RNA	Use more sensitive method to measure low RNA concentration (*e.g.* Agilent 2100 Bioanalyzer). Use qPCR to evaluate the efficiency of oligo(dT) enrichment or rRNA removal.
	Contamination of enzymatic inhibitors in RNA samples	Make sure no residual RNA-binding inhibitors or organics in your input RNA samples!

Appendix

Index sequences for Illumina multiplexing barcodes

```
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
```

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