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Seq-Star™ DNA Size Selection Kit

Cat#: AS-MB-008

Instruction Manual version 1.0

Product summary

Product description

Seq-Star™ DNA Size Selection kit provides a magnetic beads-based technology for simple and rapid DNA size selection for next generation sequencing library preparation. The kit is suited for almost all sequencing platforms, including Illumina, Life Technologies SOLiD, Ion Torrent and Roche 454 systems.

With the Seq-Star DNA Size Selection Kit, undesired adapter dimers are efficiently removed by the adaptor dimer removal protocol (Fig. 1A). The desired size selection range for the library is tuned according to Table 1 in the user guide (Fig. 1B).

Starting materials

- 1~500 ng DNA fragments at various lengths in a standard 50 µL solution for optimal result.
- Double stranded DNA fragments, such as genomic DNA, ChIP enriched DNA, MeDIP/hMeDIP DNA, exon capture DNA, or cDNA synthesized from RNA.

Kit components

Components	24-Reactions Cat#AS-MB-008-01	96- Reactions Cat#AS-MB-008-02	Storage
Seq-Star™ DNASelect Beads	2.4 mL	9.6 mL	4°C
Elution Buffer	1 mL	4 mL	4°C
User Manual	√	√	

Additional required materials

- Magnetic stand (tube compatible)

- Pipettors and tips
- Fresh 80% ethanol

Protocols

Protocol I	To remove DNA fragments < 150 bp, such as adapters, adapter dimers.
Protocol II	To obtain a desired size range of DNA fragments, with lower and upper size cutoffs.

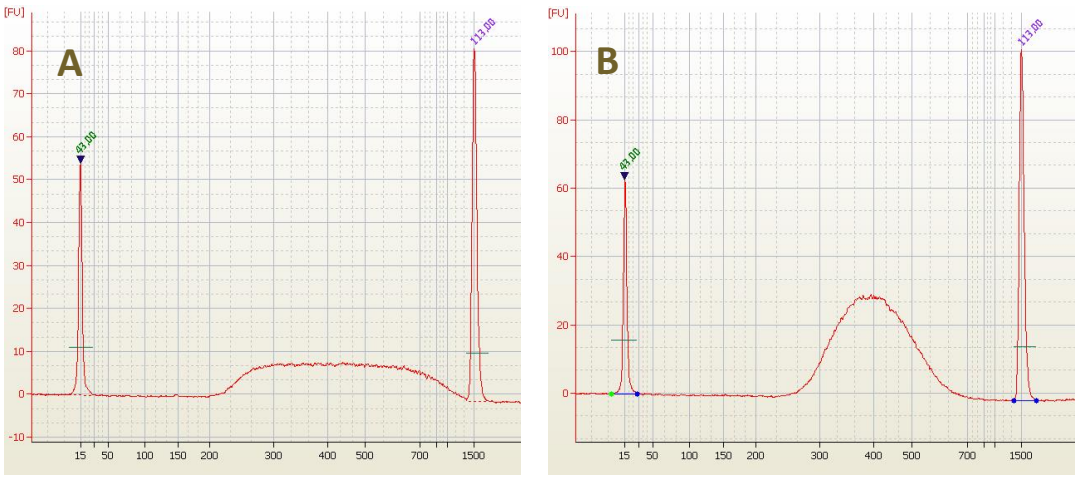

Figure 1. NGS libraries prepared by using Seq-Star™ DNA Size Selection Kit. (A) DNA-seq library using Protocol I, showing the absence of adapter dimers in the < 150 bp range. The library has a size range of 200~1500 bp. (B) DNA-seq library size selected at the peak size of 400 bp by using Protocol II. The prepared library size range is between 250~700 bp. Protocol I and Protocol II were performed successively on 100 ng adapter ligated sonicated genomic DNA isolated from MCF-7 cells.

Protocol I

This protocol removes DNA fragments < 150 bp, such as adapters and adapter dimers, by using the Bead:DNA volume ratio at 1.0X.

1. Gently shake the Seq-Star™ DNASelect Beads vial to resuspend the beads particles.
2. Add 50 µL bead suspension to the 50 µL DNA solution (Bead:DNA ratio at 1.0X). The DNA could be, for example, adapter ligated DNA for gDNA-seq, ChIP-seq, RNA-seq, or MeDIP/hMeDIP-seq library preparation. Mix thoroughly by pipetting up and down 10 times or by vortexing briefly for 15 seconds.

Note: If the DNA solution volume is less than 50 µL, add water to a total volume of 50 µL.

3. Incubate the mix at room temperature for 5 min.
4. Place the mix tube at a magnetic stand until the solution becomes completely clear (about 1~2 minutes). Carefully aspirate and discard the supernatant.
5. 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 procedure.
6. Repeat Step 5 once for a total of 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 remove the residual ethanol. However, do not over dry the beads which may significantly decrease the DNA elution efficiency.
7. Remove the tube off the magnetic stand and resuspend the beads in 15~20 µL Elution Buffer.
8. Incubate at room temperate for 2 minutes; Place the tube on magnetic stand until the supernatant is completely clear from the beads.
9. The supernatant contains the size selected DNA. Transfer the supernatant to a new tube for downstream use.

Protocol II

Protocol II selects DNA fragments around a peak size. As PEG and Mg^{2+} carried over from the prior adaptor ligation reaction can significantly affect the selection size, **we strongly recommend** performing Protocol I first before Protocol II for consistent size range.

The size selected DNA is suitable for most types of sequencing libraries, including gDNA-seq, ChIP-seq, MeDIP/hMeDIP-seq, and RNA-seq. The size selection condition is calibrated with dsDNA fragments. For standard dsDNA library preparation, the size selection procedure should be performed after the adapter ligation but before the library PCR amplification. For sequencing library involving DNA denaturation, such as MeDIP-seq where the DNA is denatured for immunoprecipitation, the size selection should be performed after the library PCR amplification.

Protocol II consists of two parts: the First round for removing DNA fragments larger than the upper cutoff and the Second round for removing DNA fragments smaller than the lower cutoff.

Part I: First round selection

1. Resuspend Seq-Star™ DNASelect Beads by shaking. Add the bead suspension to 50 µL DNA solution at the exact volume ratio according to **Table 1,** "Bead volume for Part I". For example, for a desired 400 bp peak size, you should choose the bead suspension volume of 30 µL for Part I and 10 µL for Part II.
2. Mix thoroughly by pipetting up and down 10 times or by vortexing for 15 seconds. Incubate the mix at room temperature for 5min.
3. Place the mix tube at magnetic stand until the solution becomes completely clear (about 1~2 minutes).
4. The supernatant contains the DNA fragments below the upper cutoff. Carefully transfer the supernatant to a new tube and discard the beads containing the unwanted DNA fragments larger than the upper cutoff.

   Caution: Do not discard the supernatant in this step.

Part II: Second round selection

5. Add Seq-Star™ DNASelect Beads to the supernatant from Part I according to **Table 1,** "Bead volume for Part II". For example, select the bead volume of 10 µL for the library peak size of 400 bp.
6. Mix thoroughly by pipetting up and down 10 times or by vortexing for 15 seconds. Incubate the mix at room temperature for 5min.
7. Place the mix tube at magnetic stand until the solution becomes completely clear (about 1~2 minutes). Aspirate and discard the supernatant.
8. 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 procedure!
9. Repeat Step 8 once for a total of 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 remove the residual ethanol. However, do not over dry the beads which may significantly decrease the DNA elution efficiency.
10. Remove tube off the magnetic stand and resuspend the beads in 15~20 µL Elution Buffer.
11. Incubate at room temperate for 2 minutes; Place the tube on the magnetic stand until the supernatant is completely clear from the beads.
12. The supernatant contains the size selected DNA. Transfer supernatant to a new tube for downstream library PCR amplification or other use.

Table 1. The amount of beads to add in a standard 50 µL DNA solution starting at Protocol II, Part I.

Library peak size	300 bp	350 bp	400 bp	500 bp	600 bp	700 bp
Bead volume for Part I (μL)	40	35	30	27.5	25	22.5
Bead volume for Part II (μL)	10	10	10	7.5	7.5	7.5

Troubleshooting

Problem	Possible cause	Suggestion
Low yield	Starting DNA amount too low	1~500 ng starting DNA is recommended for optimal result.
	Starting DNA does not contain DNA fragments around the selection size.	Check the DNA size distribution of the starting DNA.
Adapter dimer in final NGS libraries	Trace amount of adapter dimer was amplified by PCR after the size selection.	• Reduce the library amplification PCR cycles. • Size select the PCR product again using **Protocol I.**
Unexpected DNA size range after **Protocol II**	Too much PEG or Mg^{2+} carried over in the starting DNA solution.	Perform the **Protocol I** first.
	Single-stranded starting DNA.	Do size selection after PCR amplification (e.g. MeDIP/hMeDIP-seq)