Auto-Mag® NGS Clean-up and Size selection Kit

Cat # AMD-S013 Version 2.0

Protocol for DNA double-sided size selection (96-well Plate Format)

This protocol can be used to generate DNA fragment libraries with a certain size range or to narrow fragment-size distribution. Through a two times selections process, the both smaller and larger fragments can be removed and the expected size range of DNA fragments can be obtained. This method is called double-sided size selection. The following protocol exemplifies a size selection of DNA fragment libraries with a size range of 250–400bp from a 50ul DNA samples. The information provided is an approximate guide. By altering the volume ratios of DNA fragment libraries and PCR-Pure reagents, other size ranges can be obtained. The optimal ratio for other size ranges should be determined empirically.

Materials and Equipment to be supplied by User:

- 100% Ethanol
- For 96 well format: 96 well cycling plate
- Magnetic separation device compatible with 96-well PCR plate
- Well calibrated pipette and Disposable pipette tips
- 80% ethanol for additional DNA Wash.

Before Starting

- Prepare DNA Wash Buffer according to Preparation of Reagents Section.
- If kit was stored at 2-8°C, bring the Auto-Mag® PCR-Pure reagent to room temperature for at least 30 min before use.
- Suspension Auto-Mag® PCR-Pure reagent by vortexing.

Procedure

1. Completely float the Auto-Mag® PCR-Pure reagent until it appears homogeneous in color.

2. For using the 96-well plate, a starting sample volume of 50µl is recommended. For smaller samples, add nuclease-free water to adjust the volume.

3. For removing the large DNA fragments (>400bp), first add 35µl of well dispensed Auto-Mag® PCR-Pure reagent into the wells containing 50µl DNA sample. Mix thoroughly by pipetting up and down 10 times or until homogenous.

Note: The volume ratio of Auto-Mag® PCR-Pure reagent suspension to the sample is 0.7x.

4. Incubate the mixture for 5 minutes at room temperature to allow the large DNA fragments are bound to Auto-Mag® PCR-Pure beads.

5. Place the sample plate on the 96-well magnetic separation device and allow the magnetic beads to settle on the magnet for 5-10 minutes or until the supernatant turns completely clear.

6. Keep the sample plate on the magnet and transfer the ~85µl of supernatant into the well of a new plate and discard the beads that contain the unwanted large DNA fragments.

Note: Do not disturb the attracted magnetic beads while aspirating the supernatant.

7. Completely float the Auto-Mag® PCR-Pure reagent again until it appears homogeneous in color.

8. For the smaller DNA fragments cutoff (< 250bp), add 15µl of well dispersed Auto-Mag® PCR-Pure reagent into the well containing supernatants from step 6. Mix thoroughly by pipetting up and down 10 times or until homogenous.

Note: The total volume ratio of Auto-Mag® PCR-Pure reagent suspension to the original sample is 1.0 x now; (35µl and 15µl to 50µl).

9. Incubate the sample plate for 10 minutes at room temperature to allow the selected DNA fragment are bound to Auto-Mag® PCR-Pure beads.

10. Place the sample plate on the 96-well magnetic separation device and allow the magnetic beads to settle on the magnet for 5-10 minutes or until the supernatant turns completely clear. Carefully remove and discard the supernatant by pipetting.

11. Keep the sample plate on the magnet and add 200µl of DNA Wash Buffer or 80 % ethanol to each well and incubate for 30 seconds at room temperature. Carefully remove and discard the supernatant by pipetting.

Note: DNA Wash Buffer must be diluted with ethanol prior to use.

12. Repeat Steps 11 for second DNA wash.

13. Keep the sample plate on the magnetic separation device and air dry the magnetic beads by incubating sample tube at room temperature for 5 minutes.

Note: It is critical to completely remove all traces of liquid but take caution in not over drying the beads as this will reduce the yield.

14. Remove the sample plate from the magnetic separation device. Add an appropriate volume (20-50µl) of Elution Buffer to each sample and mix by pipetting up and down 10 times. Incubate the sample plate for 5 minutes at room temperature.

15. Place the sample plate back on the magnetic separation device and wait 2 minutes or until the magnetic beads clear from solution.

16. Transfer all of supernatant to a new 96-well plate or an appropriate storage vessel. The ultra-pure DNA fragments (250-400bp) are ready for next step of NGS library preparation process or store at -20°C.