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DNA Size Select Beads

Description

The DNA Size Select Beads are composed of solid-phase-reversible immobilization (SPRI) beads and is formulated for size selection of DNA fragments greater than 50bp. The protocol consists of binding, washing and elution steps that can be easily automated for high-throughput applications. Applications include gDNA and PCR product size selection, PCR inhibitor removal, adapter dimer removal from NGS library preparation and DNA precipitation. By adjusting the volumetric ratio of the DNA Size Select beads to the sample, you can recover or remove fragments of different sizes.

Store at 2-8ºC away from the light.

Materials

Reagents required:

- DNA Size Select Beads
- 70% ethanol freshly prepared from 200 proof non-denatured ethanol
- Nuclease free water or 10mM Tris-Acetate pH8.0

Consumables and Hardware required:

- Automation compatible 96-well reaction plates, or PCR tubes, or 1.5ml tubes
- Magnetic separation devices. For 96 well plates: ALPAQUA Magnum FLX Enhanced Universal Magnet Plate (SKU:A000400). For 0.2ml 8-strip PCR tubes and 1.5ml tubes: Permagen Magnetic Separation Rack (PN:MSR1224B)
- Liquid Handling Instruments for Automation or multi-channel hand pipette

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General Procedure for DNA fragment size selection

Please see Figure 1 Round 1 size selection, lane B1 to A2, to use as a guide to select appropriate ratios of the beads to use to recover specific size range of DNA fragments.

1. Bring the DNA Size Select Beads to room temperature and mix thoroughly by gentle shaking.

2. Add desired ratio/volume of the DNA size select, i.e. 18µl of the beads to 10µl DNA solution.

3. Mix the DNA Size Select beads and DNA solution thoroughly by vortexing for a few seconds.

4. Incubate at room temperature for 10 minutes or mix for 10 minutes on a roller mixer.

5. Centrifuge the mixture briefly and place the tube on a magnetic separation rack to separate beads with bound DNA from solution for 5 minutes or until solution becomes clear. Larger sample volumes may require more time for separation.

6. Retain the tube on magnet and aspirate the cleared solution from the tube and discard. Do not disturb beads while removing supernatant.

7. Dispense 200µl of 70% ethanol to each sample and incubate for 30-60 seconds at room temperature. Remove the ethanol and discard. Repeat for a total of two washes. Samples must remain on magnet during these washes.

8. Place the reaction plate on benchtop and air dry about 10 minutes. Do not over dry the beads as this will reduce elution efficiency.

9. Add desired volume of elution buffer of your choice (10mM Tris-Acetate pH8.0, nuclease free H_2O, etc.) to each sample and vortex 30 seconds. Centrifuge briefly and incubate the tube at 60°C for 10 minutes or at room temperature for overnight for maximal recovery of DNA fragments from the beads.

10. Place samples on magnet for 3 minutes or until solution is clear and transfer supernatant to a fresh tube.

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Applications

Single Size Selection of DNA fragments and recovery of unbound fragments example:

Note: Size of DNA as well as concentration and volume of the sample will affect the yield.

Input DNA		Round 1		Round 2		Combined Total	
Tube#	Vol. of sample	Ratio	Bead Volume	Ratio	Bead Volume	Ratio	Volume
1	20µl	0.5X	10µl	2.0X	40µl	2.5X	50µl
2	20µl	0.6X	12µl	1.9X	38µl	2.5X	50µl
3	20µl	0.7X	14µl	1.8X	36µl	2.5X	50µl
4	20µl	0.8X	16µl	1.7X	34µl	2.5X	50µl
5	20µl	1.0X	20µl	1.5X	30µl	2.5X	50µl
6	20µl	1.5X	30µl	1.0X	20µl	2.5X	50µl
7	20µl	2.0X	40µl	0.5X	10µl	2.5X	50µl

Table 1. Round 1 size selection: Lower ratio will select for larger fragments. Round 2 will recover all fragments that did not bind to the beads during round 1 size selection.

Round 1 size selection:

Input DNA = 20µl of GeneRuler 100bp DNA ladder (FERSM0243, **not Ready To Use**) diluted 1:4 in nuclease free water.

Step 1. Add desired ratio/volume of the DNA size select, i.e. if the ratio of 1.0X is desired, then add 20µl of the beads to 20µl DNA solution. Ratio used in this example: 0.5X – 2.0X.

Follow the steps 2-5 of General Procedure for DNA fragment size selection.

Step 6. While retaining the tubes on magnet, removed the cleared solution from the reaction tubes and transfer to fresh tubes and **save for recovery of unbound DNA fragments**. Do not disturb beads while removing supernatant.

Follow step 7-10 for washing and elution of the bound DNA fragment. DNA was eluted in 20µl of nuclease free water.

Round 2-recovery of unbound DNA fragments from Round 1 size selection.

Add a fresh aliquot of the DNA Size Select Beads to the supernatant saved from step 6 of Round 1 size selection (see Table 1 above) at combined total ratio = 2.5X starting sample volume.

Follow General Procedure for DNA fragment size selection for binding, washing, and elution. DNA was eluted in 20µl of nuclease free water.

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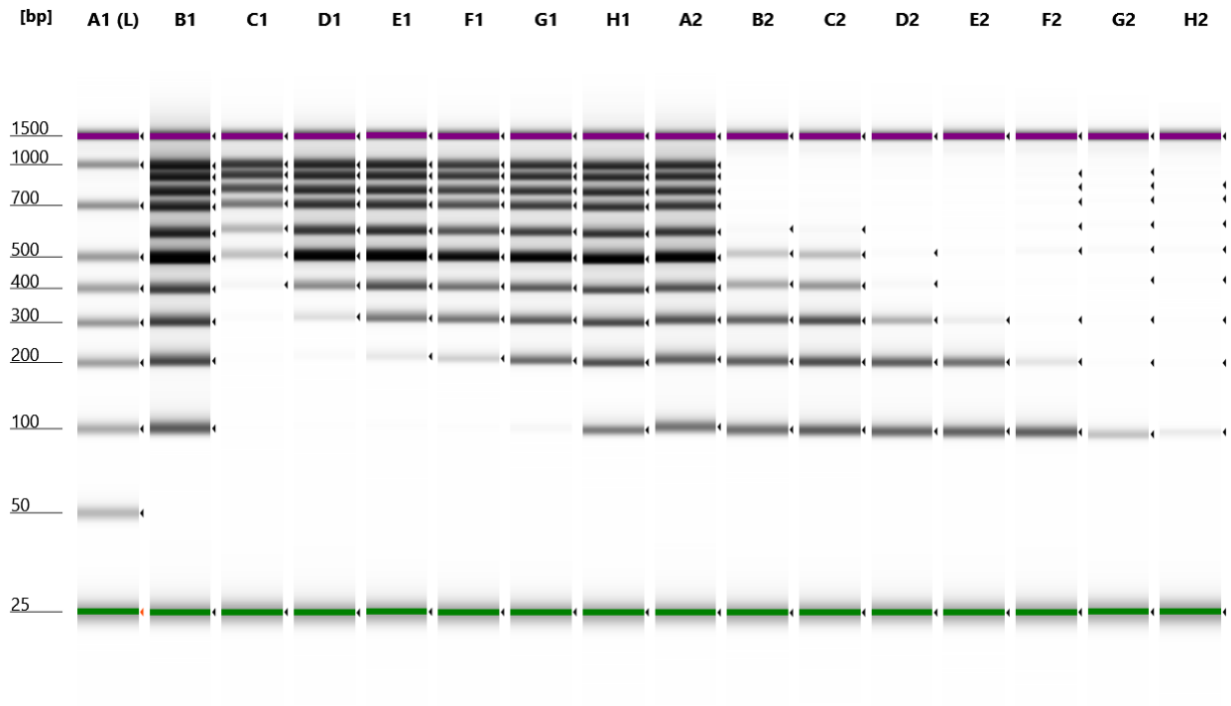

Fig. 1 TapeStation D1000 Tape data of Single Size Selection.
A1: Ladder
B1: Input GeneRuler 100bp ladder
C1: Round 1 - 0.5X
D1: Round 1 - 0.6X
E1: Round 1 - 0.7X
F1: Round 1 - 0.8X
G1: Round 1 - 1.0X
H1: Round 1 - 1.5X
A2: Round 1 - 2.0X
B2: Round 2 - 2.0X
C2: Round 2 - 1.9X
D2: Round 2 - 1.8X
E2: Round 2 - 1.7X
F2: Round 2 - 1.5X
G2: Round 2 - 1.0X
H2: Round 2 - 0.5X

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Dual Size Selection of DNA fragment example protocol:

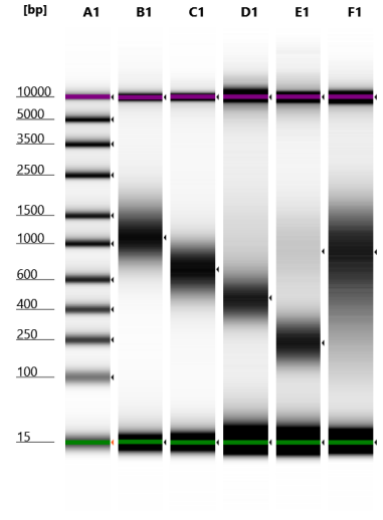
This protocol combines both Round 1 and Round 2 size selections to purify DNA fragments of desired size range. Round 1 of dual size selection is the same as Round 1 of single size selection. In dual size selection, fragments selected in Round 1 size selection are discarded and the supernatants are subjected to Round 2 size selection.

In Round 2 of dual size selection, the amount of the DNA Size Selection Beads defines the lower size limit. The amount of the beads needs to be added = Total ratio (desired end ratio) – The Round 1 ratio.

Input DNA = sonicated insect cell genomic DNA at 10ng/µl

Sample #	Sample Volume	Round 1 Ratio/ Bead Volume	Round 2 Ratio/ Bead Volume	Total Ratio/Volume
B	50µl	0.4X/20µl	+1X/5µl	0.5X/25µl
C	50µl	0.5X/25µl	+1X/5µl	0.6X/30µl
D	50µl	0.6X/30µl	+1X/5µl	0.7X/35µl
E	50µl	0.7X/35µl	+3.5X/17.5µl	1.5X/52.5µl

Table 2. 50µl of sonicated insect gDNA was subjected to dual size selection: (0.4X/0.5X), (0.5X/0.6X), (0.6X/0.7X), and (0.7X/10.5X).



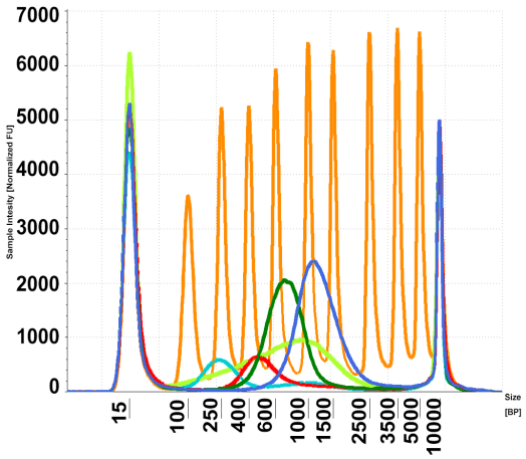


Fig.2 TapeStation D5000 Tape scaled gel picture of gDNA Dual Size Selection.
Lane A1: DNA Ladder
Lane B1: 0.4X/0.5X Peak DNA size: 1084bp
Lane C1: 0.5X/0.6X Peak DNA size: 696bp
Lane D1: 0.6X/0.7X Peak DNA size: 469bp
Lane E1: 0.7X/1.5X Peak DNA size: 250bp
Lane F1: gDNA Peak DNA size: 950bp

Fig.3 TapeStation trace shows gDNA size distribution after dual size selections described above.
Orange line DNA ladder
Purple line 0.4X-0.5X
Green line 0.5X-0.6X
Pink line 0.7X-0.8X
Blue line 0.7X-1.5X
Lime line gDNA