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Guidelines and Sample Protocol for Fragment Size Selection with CleanNGS Beads

Refer to the following table to determine bead volume ratio for a fragment size range*:

Ratio (<i>LEFT-RIGHT</i>)	BP Region
Shear (no selection)	40 – 3000
0.9 – 0.5	175 – 1300
0.85 – 0.56	200 – 700
0.8 – 0.61	230 – 660
0.77 – 0.64	260 – 575
0.75 – 0.67	280 – 450

**Please note that some adjustment may be necessary, as the salt content of the samples themselves will have some effect on size selection results.*

Overview: Start with a lower CleanNGS ratio (*RIGHT*) to bind large fragments that fall outside the desired range to the 1st set of CleanNGS beads. Once the large fragments have been bound, you remove the beads with a magnet, and then transfer the supernatant (without beads) to a fresh tube. You then increase the CleanNGS concentration once (to bind the smaller target size) by adding more CleanNGS beads such that the *LEFT* ratio is achieved.

For example:

50 uL sample volume, selected ratios 0.85-0.56 (*LEFT - RIGHT*)

1. *First binding:* Add $0.56 * 50 \text{ uL} = 28 \text{ uL}$ of CleanNGS
2. Mix well and incubate for 5 minutes to bind the large DNA fragments to the beads
3. Separate the beads from solution using a magnet for 3 minutes
4. Transfer the cleared supernatant to a new clean well or tube
5. *Second binding:* Add the remaining volume of CleanNGS $(0.85 * 50 \text{ uL}) - 28 \text{ uL} = 14.5 \text{ uL}$
6. Mix well and incubate to bind the DNA fragments with the size of interest to the beads
7. Separate the beads from solution using a magnet for 3 minutes
8. Remove and discard the liquid
9. Wash the beads twice using 200 uL of 80% ethanol (freshly prepared)
10. Dry the beads for 5 minutes
11. Elute the DNA from the beads using water or a 10 mM Tris-HCl pH8.0 buffer
12. Separate the beads from solution using a magnet for 3 minutes
13. Transfer the clean eluate to a new plate/tube for downstream use