Bulldog Bio
One New Hampshire Avenue
Suite 125
Portsmouth, NH 03801

# Guidelines and Sample Protocol
# for
# Fragment Size Selection with CleanNGS SPRI Beads

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

| Ratio (*LEFT-RIGHT*) | 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 uL = 28 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 uL) – 28 uL = 14.5 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