**ProNex Beads: Dual Size-selection to target fragments of 100-700+ bp**

(Use Low-Retention Filter tips for everything except Washes – 03 June 2021)

1. Bring ProNex resin to room temperature for 30-60 min.
2. Edit Excel Worksheet Table (below) for sample volume (*for different size-selections, also edit ProNex:sample ratios*).
3. To aid in bead retention on the magnet, add 5 ul of dilute Tween-20 (0.\_\_\_% in TVLE [10 mM Tris, 0.005 mM EDTA) to bring sample to ~0.005% Tween-20 in Step 8 (2nd Size-Selection).
4. Transfer samples to 1.5-ml Lo-Bind Eppendorf tubes.
5. Resuspend ProNex resin (vortex hard, 10 s).
6. 1st Size-Selection: Remove large fragments (>700-1000 bp) using a 0.9X Bead:Sample ratio.
   1. For \_\_\_ ul sample (original volume + 5 ul dilute Tween-20), add \_\_\_ ul ProNex beads.
   2. Mix by pipetting (10X); if needed, gently flick tubes to gather liquid at bottom of tube.
   3. Incubate 10 min (RT).
   4. Touch-spin to settle contents; put on Magnetic stand (10 min – enhances bead removal vs. recommended 2 min).
7. Transfer supernatants to new Lo-Bind tubes.
   1. *Optional*: Store beads (add 20 ul TVLE; touch-spin) in case they kept smaller fragments in error.
   2. Supernatants should contain desired fragment population, plus fragments <100-bp.
8. 2nd Size-Selection: To retain >100-bp fragments on beads, add more ProNex to bring total ProNex:Sample ratio to ~3X.
   1. To each sample (now ~\_\_\_ ul), add \_\_\_ ul ProNex beads (2nd Size-Sel. Bead volume – Initial Bead volume).
   2. Mix by pipetting (10X); if needed, gently flick tubes to gather liquid at bottom of tube.
   3. incubate 10 min (RT).
   4. Touch-spin to settle contents; place on Magnetic stand (10 min).
9. Remove supernatant.
   1. *Optional*: In case desired fragments were not retained on beads, store supernatant in new Lo-Bind tubes.
   2. Desired fragment population (~100-bp to ~1000-bp) should be on beads.
10. Leaving sample on magnet,
    1. Add 200 ul Wash Buffer (can use 1 tip for all tubes).
    2. Incubate 1 min; then, slowly rotate tubes 2X to expose beads internal to ‘glob’.
    3. Incubate an additional 2 min to fully pellet beads on magnet.
    4. Remove and discard Wash Buffer.
11. Repeat Step 9 (*but, repeat Step 10 only if ‘small fragment’ population in sample is extremely high*).
12. Air dry sample (minimum, 5’; up to 60’ for maximum EtOH removal – w/o losing HMW DNA).
13. Remove samples from Magnet.
14. Add 20 ul EB (10 mM Tris [pH 8.5], 0.005% Tween-20).
    1. Resuspend resin by vortexing or pipetting.
    2. Incubate samples (RT, 2 min)
    3. Lightly vortex & touch-spin samples.
    4. Incubate samples (RT, 3 min).
    5. Put samples on Magnet (10 min;  *Note – If the beads do not separate well from the supernatant with 20 ul of EB, add 10 ul more of EB and repeat Steps 14a-e*).
    6. Transfer supernatants to new 1.5-ml Lo-Bind tubes.
15. Put samples back on magnet.
    1. After 15 min, examine samples for residual beads.
    2. If needed, repeat the transfer of supernatants to new Lo-Bind tubes.
16. Fragments should be roughly between 100 bp and 700+ bp in the final library.

