RADtag generation > **RADtag isolation** > Illumina library prep

Dynabeads bind our RADtags at the biotinylated adaptor. Other fragments wash away.

Setup

- Move frozen Bioruptor tubes and CutSmart to fridge
- Bring Dynabeads out of fridge to warm up.
- Pull 2X Bind and Wash Buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0, 2 M NaCl).
  - 650 ul per library for 2X wash, same to make up 1X.
- Make up 1X BW. Aliquot 1/2 of 1X BW to warm in thermomixer at 56C.

A. prepare Dynabead M-280 streptavidin magnetic beads

Dynabead wash steps are done with tubes removed from magnet.

1. Gently pulse-vortex Dynabead bottle > 30 s.
2. For each Bioruptor tube, transfer 30 ul Dynabeads to a 1.7 ml tube.
3. Place tubes in magnetic rack and remove supernatant when clear.
4. Wash the beads with 100 ul 2X BW buffer: add, aspirate, wait 30 s.
5. Quick spin as needed, place on magnet, remove sup.
6. Repeat the 2X BW wash.
7. Resuspend beads in 100 ul 2X BW buffer.

B. Dynabead binding

1. Pool Bioruptor tube contents in a 1.7 ml tube, place on magnet.
2. Pull 100 ul eluate for each tube of Dynabeads.
3. Aspirate.
4. Incubate at RT for 20 min with gentle aspiration every 2 minutes.
5. Quick spin, place on magnet.
6. Remove sup.
7. Resuspend in 150 ul 1X BW by aspiration.
8. Replace on magnet, remove sup.
9. Repeat wash step in 1X BW.
10. Repeat wash step with 150 ul 56C 1X BW buffer.
11. Repeat 56C wash step.

C. liberate DNA from Dynabeads

SbfI cuts the biotynilated section of the BestRAD adaptor, releasing the RADtags from the beads.

Setup: dilute CutSmart to 1X before beginning these washes and setting up SbfI reaction.
Per library, make 550 ul 1X: 495 H2O, 55 ul 10X Cutsmart.

1. Resuspend washed beads in 100 ul 1X CutSmart, move to new 1.7 ml tubes.
2. Place on magnet, remove sup.
3. Repeat 1X CutSmart wash for a second rinse.
4. Make a master mix of 80 ul 1X Cutsmart and 4 ul SbfI
5. Resuspend beads and bound DNA in 42 ul MM per tube.
6. Aspirate as pipette to 0.2 ml pcr tubes.
7. Thermocycle, slight change from first Sbf1 digest. Aspirate gently every 12 min.

4-BIOTIN-DIG:

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- Lid 105C, TSP heated lid on, switch off lid at low block temp
- Temp mode: standard
- Volume 40 ul
1. 37C 60:00 (1 hour)
2. 12C infinity
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Could start 2nd library now.

D. bead cleanup of biotin digest

Setup

- Bring cleanup beads out of fridge to warm up.
- Make 80% ethanol, 1 ml for 2 libraries: 800 ul 100% EtOH, 200 ul H2O.

1. Combine biotin digests in 1.7 ml tube, place on magnet.
2. **Save eluate** to new 1.7 ml tube. Measure volume.
3. Transfer equal volume resuspended cleanup beads to tube. Aspirate.
4. Incubate at RT 5 minutes.
5. Place on magnet for $\leq$ 2 minutes; aspirate beads away from bottom of tube.
6. **Remove and discard supernatant.** Cleanup beads now bind the DNA.
7. Add 195 ul 80% ethanol (cover beads); leave in for 30 s. Remove and discard ethanol.
8. Repeat ethanol wash.
9. Quick spin. Remove trace ethanol.
10. Dry with cap open 2 min.
11. Resuspend beads in 57 ul 0.1X TE by aspiration.
12. Incubate 5 min.
13. Place on magnet for $\leq$ 5 min.
14. Take 1 ul for quantifying by Qubit. Expect low concentrations (0.1–0.6 ng/ul).
15. Resuspend, leave beads in for next step.

RFU _____ fluor _____ RFU _____ fluor _____
Stopping point, freeze overnight.