DNA Library Preparation

Shear DNA

- **Nanodrop** sample to obtain initial concentration (ng/µl)
- **Calculate** sample volume to start with 2µg DNA, add EB to final total 40µl volume (can start with less DNA, but stick with 40µl)
- **Shear DNA** on Bio-Ruptor to 200 bp – 500 bp
  - 4°C, Time interval 30” on / 30” off, 15’ = 15 cycles
  - On Covaris, select the 500 bp module
- **Run 1.5% agarose gel** with 3µl sample to ensure sizes between 200 bp-500 bp*

*If sizes are correct, proceed to End Repair. If too high, carry out additional cycles.

End Repair

- **Set up reaction:**
  - 34µl sheared DNA template (up to 2µg in 34µl final volume). For ChIP libraries, start with 34µl ChIP material
  - 5µl 10x end repair buffer *Epicenter Biotechnologies End-Repair Kit*  
  - 5µl dNTP
  - 5µl ATP
  - 1µl end repair enzyme
    - 50µl reaction
  - **Incubate 45’ RT to repair ends**
  - **Incubate 10’ @ 70°C** to deactivate enzyme
  - **Clean-up with magnetic beads***
  - **Elute DNA in 42µl diH₂O,** pipette supernatant into PCR tube

Ligate A-tail

- **Set up reaction:**
  - 42µl eluted end-repaired template *NEBNext dA-tailing Module*
  - 5µl NEB Next d-A buffer *NEB #E6023S (20 rxn’s)*
  - 3µl Klenow
  - 50µl reaction
  - **Incubate at 37°C for 30’**
  - **Clean-up with magnetic beads***
  - **Elute in 9µl diH₂O,** pipet supernatant into clean PCR tube
Ligate Adapters

- **Set up reaction:**
  - 8µl A-tailed template
  - 10µl 2x rapid ligation buffer
  - 1µl adapters (2-50 µM stock, depending on application)
  - 1µl T₄ ligase enzyme
  - 20µl reaction

- **Incubate** RT for 10'
- **Clean-up with magnetic beads***
- **Elute in 16µl EB**, pipet supernatant into clean 0.6 ml tube, label as “adapter-ligation”
- **Nanodrop** & calculate % recovery

This material is ready for PCR with PE 2.0 and 1.0, or indexed adapters.

***Associated Protocol: Magnetic Bead Cleanup

- Thoroughly resuspend beads
- Add 1.5x volume of beads to sample (eg, to a 50µl reaction add 75µl beads), pipet up and down or flick to mix
- Incubate RT 3-5’
- Place tube by magnet until beads migrate and solution clears—3-4’
- Draw off supernatant & discard, leave tube on magnet and do not disturb pellet
- Add 200µl 70% EtOH to tube, withdraw
- Repeat for a total of two washes (tube on magnet throughout)
- Remove as much EtOH as possible, can do a quick spin and draw off last of liquid, but work fast. Tube is now off the magnet.
- Once EtOH is gone (but before the beads dry out) add appropriate amount of water or EB, resuspend
- Place tube back on magnet, draw off eluate when beads clear

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