This is the protocol for library prep. The sample library’s generated at the end of this protocol will be sent for genome skimming using a NovaSeq.

We will use the TrueSeq DNA PCR-Free Library Prep Kit by Illumina.

Fragment the DNA

Fragmenting DNA will use NEB Fragmentase and will use the combined TrueSeq and Fragmentase protocol in Vancampenhout et al 2014.

1. Add 500ng-1 µg of DNA was added to 2 µl of 10x Fragmentase reaction buffer and 0.2 µl of 100x BSA, add diH20 to a final volume of 18 ul to a PCR tube.
2. Place tube on ice for 5 min.
3. Add 2 µl of NEBNext dsDNA Fragmentase.
4. Incubate at 37°C for 30 min to create fragments of 200 bps.
5. Stop the reaction by adding 5 µl 0.5M EDTA solution (pH 8) on ice.
6. Add 40 µl of Sample Purification Beads (SPB) to the stopped reaction.
7. Incubate at room temperature for 5 min.
8. Place onto the magnetic stand until liquid is clear.
9. Carefully remove and discard all supernatant.
10. Wash beads twice with 100 µl 80% EtOH on the magnetic stand.
11. Use a 20 µl pipette to remove and discard the EtOH on the magnetic stand.
12. Air dry for 5 min on the Magnetic stand
13. Add 52.5 µl Resuspension buffer (RSB) on the magnetic stand (Takes long time to thaw)
14. Remove the tube from the magnetic stand and mix.
15. Incubate at room temperature for 2 min.
16. Centrifuge at 280 g for 1 min
17. Place on magnetic stand until liquid is clear.
18. Remove 50 µl of liquid to a new tube.

Repair ends and Select Library Size (running the HS protocol)

1. Add 10µl End Repair Control (CTE). Takes long time to thaw
2. Add 40 µl End Pair Mix 2/ End Pair Mix 3 (ERP2/ERP3) and mix.
3. Centrifuge at 280 g for 1 min.
4. Incubate the reaction at 30°C for 30 min and then place on ice.
5. Dilute Sample Purification Beads (SPB) with PCR grade water 160 µl per 100 µl of sample.
6. Vortex Sample Purification Beads (SPB) until well dispersed.
7. Add 160 µl of diluted Sample Purification Beads (SPB) and mix.
   1. Dilute by adding 109.25 µl of SPB / # of samples to 74.75 µl PCR grade water /# of samples
8. Incubate at room temperature for 5 min.
9. Centrifuge at 280 g for 1 min.
10. Place the tube on the magnetic stand until the liquid becomes clear.
11. Transfer 250 µl of the supernatant to a new tube.
12. Add 30 µl of undiluted Sample Purification Beads (SPB) to the tube and mix.
13. Incubate at room temperature for 5 min.
14. Centrifuge at 280 g for 1 min.
15. Place in magnetic stand until liquid becomes clear.
16. Remove and discard the supernatant.
17. Wash twice with 200 µl 80% EtOH.
18. Use a 20 µl pipette to remove residual EtOH.
19. Airdry for 5 min.
20. Add 17.5 µl Resuspension Buffer (RSB).
21. Remove from magnetic stand and mix.
22. Incubate at room temperature for 5 min.
23. Centrifuge at 280 g for 1 min.
24. Place tube in the magnetic stand until liquid becomes clear.
25. Transfer 15 µl of supernatant to new tube (make sure now it’s a PCR tube).

Safe stopping point for 7 days at 20C

Adenylate 3’ Ends

1. Add 2.5 µl A-Tailing Control (CTA).
2. Add 12.5 µl A-Tailing Mix 1/ A-Tailing Mix 2 (ATL/ATL2) and mix.
3. Place tube at 37°C for 30 min followed by 70°C for 5 min and finally place on ice for 5 min.
4. Place in thermocycler and the ATAIL70 program.
   1. ATAIL70 Program:
      1. 37 °C for 30 min
      2. 70 °C for 5 min
      3. 4 °C for ever

Ligate Adapters

1. Transfer sample to a new 1.5ml tube. Add the following to the tube:
   1. Ligation Control (CTL): 2.5 µl,
   2. Ligation Mix 2 (LIG2): 2.5 µl,
   3. DNA adapters: 2.5 µl
2. Centrifuge at 280 g for 1 min.
3. Incubate at 30°C for 10 min and then place the tube on ice.
4. Add 5 µl of Stop Ligation Buffer (STL) and mix.
5. Centrifuge at 280 g for 1 min.
6. Conduct the following to the tube:
   1. Add 42.5 µl Sample Purification Beads (SPB).
   2. Incubate at room temperature for 5 min.
   3. Centrifuge at 280 g for 1 min.
   4. Place the tube on the magnetic stand until liquid becomes clear.
   5. Remove and discard the supernatant.
   6. Wash twice with 200 µl 80% EtOH.
   7. Use a 20 µl pipette to remove excess EtOH.
   8. Air dry for 5 min.
   9. Add 52.5 µl Resuspension Buffer (RSB).
   10. Remove from the magnetic stand and mix.
   11. Incubate at room temperature for 2 min.
   12. Centrifuge at 280 g for 1 min.
   13. Place onto the magnetic stand until liquid becomes clear.
7. Transfer 50 µl supernatant to new tube.
8. Redo step 6 but change the volume of Sample Purification Buffer (SPB) to 50 µl and Resuspension Buffer (RSB) to 22.5 µl.
9. Transfer 20 µl of the supernatant to a new tube.
10. Store tube at -20°C for up to 7 days.

Validate Libraries

1. Quantify the libraries using qPCR with the following modifications:
   1. Use at least 2 µl of the original library stock.
   2. Perform 2 additional dilutions.
   3. Determine the concentration of the diluted library.
   4. Perform a size adjustment calculation.
   5. Calculate the concentration of the undiluted library.
2. Verify the fragment size by checking the library size distribution.
   1. Dilute the DNA library 1:5 with water.
   2. Run 1 µl of the diluted library on a high sensitivity chip.