Required reagents

1. Nextera XT DNA sample prep kit (Illumina)
2. Nextera XT Index Kit (Illumina)
3. Ampure XP beads (Beckman Coulter)
4. 80% Ethanol – Make fresh before use (Major laboratory suppliers)
5. EB buffer (10mM Tris-HCl, pH 8.5) or TE buffer (10 mM Tris-HCl, 1mM EDTA, pH 8.0)

Nextera XT C1 Library Prep and Cleanup

6. Dilute samples in Harvest mix to 0.10 – 0.16 ng/uL in separate tubes or in a plate. Label the plate "diluted samples".
7. In a 1.5 mL tube, create a master mix. Add 2.5 uL Tagment DNA Buffer and 1.25 uL amplification Tagment Mix for each reaction. For 96 samples mix 270 uL Tagment DNA Buffer and 135 uL Amplification Tagment Mix.
8. Prepare an 8-tube strip with 50 uL of the master mix for aliquoting into an empty 96 well plate.
9. Aliquot 3.75 uL of the master mix into each well of an empty 96 well plate.
10. Transfer- 1.25 uL of each diluted DNA sample from the diluted samples plate to the corresponding well of the plate containing the master mix.
11. Seal, vortex and spin down for 20 sec
12. Perform incubation: 10 min at 55C and hold at 10 C
13. After reaching 10C, add 1.25 uL NT buffer to each library. (total volume now is 6.25 uL)
14. Cover, vortex and spin down
15. Add 3.75 PCR Master Mix (total to 10 uL)
16. Add 1.25 uL of the appropriate Primer 1 (starts with N). Each primer will correspond to one of the 12 column of the plate
17. Add 1.25 uL of the appropriate Primer 2 (starts with S). Each primer will correspond to one of the 8 rows of a plate.
18. Cover, vortex and spin down
19. Perform thermal cycling:

Temperature	Time	Cycles
72 °C	3 min	1
95 °C	30 sec	1
95 °C	10 sec	12
55 °C	30 sec	
72 °C	60 sec	
72 °C	5 min	1
10 °C	Hold	1

20. Store at -20C for storage before cleanup
21. Total volume is 12.5 uL

Library Fragment Clean Up

1. Warm up AMPure XP beads to room temperature.
2. Pool 1 – 3 uL of each library into one tube
3. Estimate volume of pooled libraries.
4. Add 0.4x volume of beads (if library pool is 100 uL, add 40 uL beads), agitate for 10 min at room temperature, then place on magnetic stand for 2 min
5. Transfer supernatant into a new tube. Keep the supernatant. (This step gets rid of libraries with sizes larger than 1.2 kbp)
6. Add beads solution equivalent to 0.3x of the original pooled libraries volume (if original library pool is 100 uL, add another 30 uL beads at this step), agitate for 10 min at RT, then place on magnetic stand for 2 min. (This step retains >300bp libraries)
7. Prepare fresh 80% ethanol in a tube.
8. Carefully remove and discard supernatant without disturbing beads.
9. Add 180 uL of freshly prepared 80% ethanol and incubate for 30 sec on magnetic stand.
10. Remove ethanol; Repeat one more time
11. Let tube sit on magnetic stand and air dry for 10-15 min. Rotate tubes occasionally to speed up drying.
12. Elute by adding 30-50 uL EB or TE buffer. Pipette to ensure homogeneous mixing of beads and EB.
13. Vortex and incubate for 5 min at RT; Place on magnetic stand for 2 min
14. Transfer supernatant to another PCR tube.

- If a large amount of primers are expected in the final library pool, repeat the bead purification protocol with a single step purification using 0.7x beads
- This protocol removes all libraries smaller than 300 bp including adapters. If shorter libraries are desired, increase the amount of beads added during step 6 to 0.35x or 0.4x.