***Library Preparation***

***Hi-C library***

1. Dilute each sample in 96-well plate to 5 ng/uL.
2. Prepare Transposition Mix (318 uL per plate)
   1. 106 uL 5X TTBL Buffer (Vazyme)
   2. 26.5 uL TTE Mix V50
   3. 185.5 uL water
3. Transfer 39 uL Transposition Mix to each tube of an 8-strip tube.
4. Transfer 3 uL Transposition Mix to a 96-well plate with a multi-channel pipette.
5. Add 2 uL 5 ng /uL MALBAC products with a multi-channel pipette, mix and run:
   1. 55 C for 10 min
   2. 4 C forever
6. Prepare 200 uL 0.2 % SDS, transfer 25 uL 0.2 % SDS to each tube of an 8-strip tube.
7. Transfer 1.25 uL 0.2 % SDS to a 96-well plate with a multi-channel pipette, mix well and incubate at RT for 10 min.
8. Add 2 uL 5 uM i5 index primer with a multi-channel pipette.
9. Add 2 uL 5 uM i7 index primer with a multi-channel pipette
10. Prepare PCR Mix (994.5 uL per plate)
    1. 408 uL 5X KAPA HiFi GC Buffer
    2. 61.2 uL dNTP Mix (10 mM each) (NEB)
    3. 40.8 uL KAPA HiFi DNA Polymerase (1 U/uL)
    4. 484.5 uL water
11. Transfer 124 uL PCR Mix to each tube of an 8-strip tube.
12. Transfer 9.75 uL PCR Mix to each well of the 96-well plate with a multi-channel pipette.
13. Amplify by running (20 uL volume):
14. 4 C for 3 min (to allow the lid to pre-heat)
15. 72 C for 3 min
16. 98 C for 30 s
17. 98 C for 15 s
18. 60 C for 30 s
19. 72 C for 2 min
20. Goto 4 for 9 cycles
21. 72 C for 5 min
22. 4 C forever

***Enriched library***

1. Prepare Transposition Mix (318 uL per plate)
   1. 106 uL 5X TTBL Buffer (Vazyme)
   2. 26.5 uL TTE Mix V50
   3. 185.5 uL water
2. Transfer 39 uL Transposition Mix to each tube of an 8-strip tube.
3. Transfer 3 uL Transposition Mix to a 96-well plate with a multi-channel pipette.
4. Add 2 uL 5 ng /uL MALBAC products with a multi-channel pipette, mix and run:
   1. 55 C for 10 min
   2. 4 C forever
5. Prepare 200 uL 0.2 % SDS, transfer 25 uL 0.2 % SDS to each tube of an 8-strip tube.
6. Transfer 1.25 uL 0.2 % SDS to a 96-well plate with a multi-channel pipette, mix well and incubate at RT for 10 min.
7. Add 2 uL 5 uM i5 index primer with a multi-channel pipette.
8. Prepare PCR Mix (1198.5 uL per plate)
   1. 408 uL 5X KAPA HiFi GC Buffer
   2. 61.2 uL dNTP Mix (10 mM each) (NEB)
   3. 6.8 uL 50 uM RNA-P7 primer
   4. 6.8 uL 50 uM RNA-P7 primer
   5. 6.8 uL 50 uM RNA-P7 primer
   6. 40.8 uL KAPA HiFi DNA Polymerase (1 U/uL)
   7. 668.1 uL water
9. Transfer 149 uL PCR Mix to each tube of an 8-strip tube.
10. Transfer 11.75 uL PCR Mix to each well of the 96-well plate with a multi-channel pipette.
11. Amplify by running (20 uL volume):
12. 4 C for 3 min (to allow the lid to pre-heat)
13. 72 C for 3 min
14. 98 C for 30 s
15. 98 C for 15 s
16. 60 C for 30 s
17. 72 C for 2 min
18. Goto 4 for 11 cycles
19. 4 C forever
20. Take the 96-well plate from Thermocycler and add 2 uL 5 uM i7 index primer with a multi-channel pipette.
21. Go running (22 uL volume):
22. 4 C for 3 min
23. 98 C for 30 s
24. 98 C for 15 s
25. 60 C for 30 s
26. 72 C for 2 min
27. Goto 3 for 3 cycles
28. 72 C for 5 min
29. 4 C forever

***Reagents***

**2X SC Lysis Buffer HS**: 100mM NaCl, 40 mM Tris pH8.0, 0.3% Triton X100, 2 mM EDTA, 50 mM DTT. Mix 200 uL 1M Tris pH8.0, 100 uL 5 M NaCl, 150 uL 10 % Triton X 100, 20 uL 500 mM EDTA, 250 uL 1 M DTT and 4280 uL water for 5 mL.

**60 mg/mL Qiagen Protease**: To each vial of 7.5 AU Qiagen Protease (Qiagen 19155), add 2.78 mL water. Mix well and filter through a 0.2-um filter. Aliquot and store at 4 C.

**GAT5-RT:** GTAGGTGTGAGTGATGGT TGAGGTAGT ATTGCGCAATG NNNNNNNN TTTTTTTTTTTTTTTVN

**GAT5-7N:** GTAGGTGTGAGTGATGGT TGAGGTAGT NNNNNNN

**GAT5:** GTAGGTGTGAGTGATGGT TGAGGTAGT

**RNA-P7**: **GTCTCGTGGGCTCGG** AGATGTGTATAAGAGACAGGGT TGAGGTAGT ATTGCGCAATG

**Nextera i5 Primers :**

N501: AATGATACGGCGACCACCGAGATCTACACTAGATCGCTCGTCGGCAGCGTC

N502: AATGATACGGCGACCACCGAGATCTACACCTCTCTATTCGTCGGCAGCGTC

N503: AATGATACGGCGACCACCGAGATCTACACTATCCTCTTCGTCGGCAGCGTC

N504: AATGATACGGCGACCACCGAGATCTACACAGAGTAGATCGTCGGCAGCGTC

N505: AATGATACGGCGACCACCGAGATCTACACGTAAGGAGTCGTCGGCAGCGTC

N506: AATGATACGGCGACCACCGAGATCTACACACTGCATATCGTCGGCAGCGTC

N507: AATGATACGGCGACCACCGAGATCTACACAAGGAGTATCGTCGGCAGCGTC

N508: AATGATACGGCGACCACCGAGATCTACACCTAAGCCTTCGTCGGCAGCGTC

**Nextera i7 Primers :**

N701: **CAAGCAGAAGACGGCATACGAGAT**TCGCCTTAGTCTCGTGGGCTCGG

N702: CAAGCAGAAGACGGCATACGAGATCTAGTACGGTCTCGTGGGCTCGG

N703: CAAGCAGAAGACGGCATACGAGATTTCTGCCTGTCTCGTGGGCTCGG

N704: CAAGCAGAAGACGGCATACGAGATGCTCAGGAGTCTCGTGGGCTCGG

N705: CAAGCAGAAGACGGCATACGAGATAGGAGTCCGTCTCGTGGGCTCGG

N706: CAAGCAGAAGACGGCATACGAGATCATGCCTAGTCTCGTGGGCTCGG

N707: CAAGCAGAAGACGGCATACGAGATGTAGAGAGGTCTCGTGGGCTCGG

N708: CAAGCAGAAGACGGCATACGAGATCCTCTCTGGTCTCGTGGGCTCGG

N709: CAAGCAGAAGACGGCATACGAGATAGCGTAGCGTCTCGTGGGCTCGG

N710: CAAGCAGAAGACGGCATACGAGATCAGCCTCGGTCTCGTGGGCTCGG

N711: CAAGCAGAAGACGGCATACGAGATTGCCTCTTGTCTCGTGGGCTCGG

N712: CAAGCAGAAGACGGCATACGAGATTCCTCTACGTCTCGTGGGCTCGG