Xielab sisHiC protocol

Crosslink

1. Add 100ul PBS to the cells
2. 1% formaldehyde, RT 10min
3. Add 5.56ul 2.5M Glycine, RT 10min
4. Wash the cells with 1XPBS

Cell lysis & RE digestion

5. 50ul lysis buffer (10mM Tris-HCl pH7.4, 10mM NaCl, 0.1mM EDTA, 0.5% NP-40 and 1Xproteinase inhibitor) + <100000 cells on ice, 30-50 min
6. 3000rpm, 5min, remove the supernatant
7. 10ul 0.5% SDS, 62℃ 10min
8. Add 25ul ddH2O, 5ul 10% Triton X-100, mix well, incubate at 37℃ 15min
9. Add 5ul 10XNEBuffer 2 and 2ul (50U) MboI, and digest overnight at 37℃

Biotin end repair & ligation

10. Incubate at 62℃ for 20min to inactivate MboI
11. Add the following mixture

1mM dATP	1.5ul
1mM dGTP	1.5ul
1mM dTTP	1.5ul
0.4Mm Biotin-14-dCTP	3.75ul
5U/ul Klenow	2ul

37℃, 90min

12. Things on the list were added to the nuclei suspension and incubated at **RT for 5.5h** with rotation.

10x T4 DNA ligase reaction buffer	12ul
10% Triton X-100	7ul
100x BSA （10mg/ml）	1.2ul
400U/ul T4 DNA ligase	1ul
Water	39ul

Reverse crosslink and DNA purification

13. Add 5ul 20mg/ml PK, 12ul 10% SDS, 55℃, 30min
14. Add 13ul 5M NaCl, and incubate at 68℃ overnight
15. Cool tubes to RT (10min)
16. Add 15ul 3M NaAc, 1ul Glycogen, mix well. And add 240ul ethanol, mix by inverting and -80℃ 15min
17. 4℃,max speed 20min
18. Wash with 500ul ice cold 75% ethanol
19. Max speed for 5min at 4℃
20. 50ul ddH2O, 37℃ 15min.

Shear DNA & Biotin Pull down & End repair

21. Shear DNA with a Covaris M220 SONICATOR.

22. Take 10ul C1 Streptavidin beads and wash twice in 100µL of Tween Wash Buffer (5mM Tris-HCl pH 8.0, 1M NaCl, 0.5mM EDTA and 0.05% Tween).

23. Re-suspend beads in 50ul 2XBinding Buffer (10mM Tris-HCl pH 8.0, 2M NaCl and 1mM EDTA) and add to the reaction. Incubate at RT 50min.

24. Wash the beads twice in 100µL of TWB.

25. Wash beads in 100ul 1x NEB T4 DNA ligase buffer

26. Repair the ends of DNA by adding the following:

Stock	Volume
10X Ligation Buffer (NEB)	5µL
2.5mM dNTPs	10µL
T4 DNA Polymerase (NEB)	2µL
T4 Polynucleotide Kinase (NEB)	2.5µL
Klenow (NEB)	0.5µL
ddH2O	30µL

27. Incubate sample at room temperature for 50 minutes

Add A & add adaptor

28. Wash the beads twice in 100µL of TWB.

29. Wash beads once in 100ul 1X NEB buffer 2

30. Add 'A' base

10X NEB 2	2.5ul
10mM dATP	0.5ul
Klenow exo-(5U/ul)	1.5ul
ddH2O	20.5ul

37℃, 50min

31. Wash the beads twice in 100µL of TWB

32. Wash beads once in 100ul 1X NEB Quick ligation buffer

33. Add adaptor

2X Quick ligase buffer	12.5ul
1.5uM adaptor	1ul
Quick ligase (NEB)	1ul
ddH2O	10.5ul

4℃， 16h with rotation and then room temperature for 10min, rotating.

Final PCR & size selection

34. Wash the beads twice in 100µL of TWB

35. Wash beads in 100ul TE

36. Resuspend in 32 ul ddH2O, 66℃ 30min.

37. PCR for 12-15 cycles.

DNA template	31.5ul
dNTPs(2.5mM)	5ul
ExTaq-HS buffer (10X)	5ul
Truseq Primer-F (10uM)	4ul
Truseq Primer-R (10uM)	4ul
ExTaq-HS	0.5ul
Total	50ul

38. Size selection with AMPure beads (0.48:1 and then 1:1)
39. Elute with 50ul ddH2O.