TACIT step by step Protocol

From the He lab @PKU

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Buffers:

Reagents	Composition
Wash Buffer	1 mL 1 M HEPES-KOH pH 7.5, 1.5 mL 5 M NaCl, 12.5 μL 2 M spermidine, 500 μL sodium butyrate, 100 μL 5% digitonin, 125 μL 20% Triton X-100, 1 × cocktail, and the final volume to 50 mL with ddH_2O
High-salt Wash Buffer	1 mL 1 M HEPES-KOH pH 7.5, 3 mL 5 M NaCl, 12.5 μL 2 M spermidine, 500 μL sodium butyrate, 100 μL 5% digitonin, 125 μL 20% Triton X-100, 1 × cocktail, and the final volume to 50 mL with ddH_2O
Antibody Buffer	Mix 4 μL 0.5 M EDTA with 1 mL Wash-buffer
Tagmentation Buffer	20 mM TAPS-NaOH pH 8.3, 20 mM $MgCl_2$, 1 × cocktail, 20 mM sodium butyrate, and 2 mM PMSF
Lysis Buffer	10 mM Tris-HCl pH 8.5, 0.05% SDS and 0.1 mg/mL proteinase K

Notes: The hyperactivity of protein A-Tn5 (PAT) is most critical in this protocol to achieve extremely high genome coverage in single cells. Most commercially available PAT products are not so good in our hands. He lab is happy to provide a reasonable amount of in-house PAT upon request.

1. Take out methanol-fixed cells from -80°C freezer and put on ice for at least 15 minutes for rehydration.
2. Wash cells twice with 180 μL Wash Buffer (or 0.1% BSA/PBS) to remove residual methanol. For each time, cells were collected at an appropriate speed for 3 minutes at 4°C and 10-20 μL Wash Buffer was retained to reduce cell loss.
3. Resuspend cells with 100 μl Antibody Buffer containing 0.5 μg primary antibodies.
4. Put the tube on a rotator and incubate at 4°C for at least 4 hours.
5. Collect cells at an appropriate speed for 3 minutes at 4°C.

6. Resuspend cells with 180 μL Wash Buffer and rotate cells at 4°C for 5 minutes to wash out free antibodies.
7. Collect cells at an appropriate speed for 3 minutes at 4°C.
8. Resuspend cells with 100 μl Antibody Buffer containing 0.4 μg secondary antibodies.
9. Put the tube on a rotator and incubate at 4°C for 30 minutes.
10. Collect cells at an appropriate speed for 3 minutes at 4°C.
11. Wash cells twice with 180 μL Wash Buffer and rotate cells at 4°C for 5 minutes to wash out free antibodies.
12. Collect cells at an appropriate speed for 3 minutes at 4°C.
13. Resuspend cells with 100 μl High-salt Wash Buffer containing 3 μg/ml PAT-MEA/B enzyme.
14. Put the tube on a rotator and incubate at 4°C for 60 minutes.
15. Wash cells twice with 180 μL High-salt Wash Buffer and rotate cells at 4°C for 5 minutes to wash out free PAT-MEA/B.
16. Collect cells at an appropriate speed for 3 minutes at 4°C. Aspirate the supernatant and retain about 10 μL High-salt Wash Buffer.
17. Resuspend cells with 10 μl Tagmentation Buffer and incubate at 37°C for 60 minutes.
18. Add 20 μl 40 mM EDTA and incubate at room temperature for 15 minutes to stop the reaction.
19. Wash cells twice with 0.1% BSA/PBS.
20. Pre-rinse the 96-well plates with 0.1% BSA/PBS to avoid loss of DNA fragments.
21. And add 2 μl Lysis Buffer to each well of the pre-rinsed plates.
22. Pick single cell into a well of a 96-well plate with a mouth pipette under a microscope.
23. Add 5 μL mineral oil (Sigma) to each well and incubate the plates at 55°C for 15 minutes to release DNA fragments.
24. Add 0.5 μL of 10 mM PMSF and 1 μL of 0.9% Triton X-100 to each well to deactivate protease K and quench SDS.
25. Add 17 μL PCR mix (0.2 μL KAPA HiFi HotStart DNA polymerase, 4 μL 5× KAPA High-GC buffer, 0.5 μL 10 mM dNTP mix, 0.5 μL 25 mM $MgCl_2$ and 10.8 μL H_2O) to each well with 0.5 μL 10 mM Nextera i5 index primer and 0.5 μL 10 mM i7 index primer.
26. Perform PCR with the following program: 1 cycle of 72°C for 5 minutes; 1 cycle of 95°C for 3 minutes; 22 cycles of 98°C for 20 seconds, 65°C for 30 seconds, 72°C for 1 minutes; 1 cycle of 72°C for 5 minutes; and hold at 4 °C.
27. Purify the DNA fragments with 1× AMPure XP beads (Beckman) once, and

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200-1,000 bp fragments were selected with 0.5 × + 0.5 × AMPure XP beads.

28. The libraries were sequenced with paired-end 150-bp reads on a NovaSeq 6000 platform (Illumina) or BGI T7 or MGI2000 platform.

Table 1. The sequences of PAT-MEAB used in TACIT.

Primer	Sequence
Mosaic-A_primer (MEA)	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG
Mosaic-B_primer (MEB)	CACCGTCTCCGCCTCAGATGTGTATAAGAGACAG
Common annealing primer	5Phos/CTGTCTCTTATACACATCT

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Table 2. The sequences of Nextera i5 and Nextera i7 used in TACIT.

Primer	Sequence
i5-N501	AATGATACGGCGACCACCGAGATCTACACTAGATCGCTCGTCGGCAGCGTC
i5-N502	AATGATACGGCGACCACCGAGATCTACACCTCTCTATTCGTCGGCAGCGTC
i5-N503	AATGATACGGCGACCACCGAGATCTACACTATCCTCTTCGTCGGCAGCGTC
i5-N504	AATGATACGGCGACCACCGAGATCTACACAGAGTAGATCGTCGGCAGCGTC
i5-N505	AATGATACGGCGACCACCGAGATCTACACGTAAGGAGTCGTCGGCAGCGTC
i5-N506	AATGATACGGCGACCACCGAGATCTACACACTGCATATCGTCGGCAGCGTC
i5-N507	AATGATACGGCGACCACCGAGATCTACACAAGGAGTATCGTCGGCAGCGTC
i5-N508	AATGATACGGCGACCACCGAGATCTACACCTAAGCCTTCGTCGGCAGCGTC
i5-N511	AATGATACGGCGACCACCGAGATCTACACTCTCTCCGTCGTCGGCAGCGTC
i5-N512	AATGATACGGCGACCACCGAGATCTACACTCGACTAGTCGTCGGCAGCGTC
i5-N513	AATGATACGGCGACCACCGAGATCTACACTTCTAGCTTCGTCGGCAGCGTC
i5-N514	AATGATACGGCGACCACCGAGATCTACACCCTAGAGTTCGTCGGCAGCGTC
i5-N515	AATGATACGGCGACCACCGAGATCTACACGCGTAAGATCGTCGGCAGCGTC
i5-N516	AATGATACGGCGACCACCGAGATCTACACAAGGCTATTCGTCGGCAGCGTC
i5-N517	AATGATACGGCGACCACCGAGATCTACACGAGCCTTATCGTCGGCAGCGTC
i5-N518	AATGATACGGCGACCACCGAGATCTACACTTATGCGATCGTCGGCAGCGTC
i5-N519	AATGATACGGCGACCACCGAGATCTACACATCTGAGTTCGTCGGCAGCGTC
i5-N520	AATGATACGGCGACCACCGAGATCTACACGGATACTATCGTCGGCAGCGTC
i5-N521	AATGATACGGCGACCACCGAGATCTACACTAAGATCCTCGTCGGCAGCGTC
i5-N522	AATGATACGGCGACCACCGAGATCTACACAAGAGATGTCGTCGGCAGCGTC
i5-N523	AATGATACGGCGACCACCGAGATCTACACAATGACGTTCGTCGGCAGCGTC
i5-N524	AATGATACGGCGACCACCGAGATCTACACGAAGTATGTCGTCGGCAGCGTC
i5-N525	AATGATACGGCGACCACCGAGATCTACACATAGCCTTTCGTCGGCAGCGTC
i5-N526	AATGATACGGCGACCACCGAGATCTACACTTGGAAGTTCGTCGGCAGCGTC
i5-N701	CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTCTCGTGGGCTCGG
i5-N702	CAAGCAGAAGACGGCATACGAGATCTAGTACGGTCTCGTGGGCTCGG

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Primer	Sequence
i5-N703	CAAGCAGAAGACGGCATACGAGATTTCTGCCTGTCTCGTGGGCTCGG
i5-N704	CAAGCAGAAGACGGCATACGAGATGCTCAGGAGTCTCGTGGGCTCGG
i5-N705	CAAGCAGAAGACGGCATACGAGATAGGAGTCCGTCTCGTGGGCTCGG
i5-N706	CAAGCAGAAGACGGCATACGAGATCATGCCTAGTCTCGTGGGCTCGG
i5-N707	CAAGCAGAAGACGGCATACGAGATGTAGAGAGGTCTCGTGGGCTCGG
i5-N708	CAAGCAGAAGACGGCATACGAGATCCTCTCTGGTCTCGTGGGCTCGG
i5-N709	CAAGCAGAAGACGGCATACGAGATAGCGTAGCGTCTCGTGGGCTCGG
i5-N710	CAAGCAGAAGACGGCATACGAGATCAGCCTCGGTCTCGTGGGCTCGG
i5-N711	CAAGCAGAAGACGGCATACGAGATTGCCTCTTGTCTCGTGGGCTCGG
i5-N712	CAAGCAGAAGACGGCATACGAGATTCCTCTACGTCTCGTGGGCTCGG
i5-N731	CAAGCAGAAGACGGCATACGAGATGGATGGAAGTCTCGTGGGCTCGG
i5-N732	CAAGCAGAAGACGGCATACGAGATATTGAGGCGTCTCGTGGGCTCGG
i5-N733	CAAGCAGAAGACGGCATACGAGATCGGATAGAGTCTCGTGGGCTCGG
i5-N734	CAAGCAGAAGACGGCATACGAGATTGGTAGACGTCTCGTGGGCTCGG
i5-N735	CAAGCAGAAGACGGCATACGAGATACCTGGTTGTCTCGTGGGCTCGG
i5-N736	CAAGCAGAAGACGGCATACGAGATCAGTTCTGGTCTCGTGGGCTCGG
i5-N737	CAAGCAGAAGACGGCATACGAGATTCGAACGTGTCTCGTGGGCTCGG
i5-N738	CAAGCAGAAGACGGCATACGAGATCGTTGCTTGTCTCGTGGGCTCGG
i5-N739	CAAGCAGAAGACGGCATACGAGATTACCGTTCGTCTCGTGGGCTCGG
i5-N740	CAAGCAGAAGACGGCATACGAGATTAGGTTGCGTCTCGTGGGCTCGG
i5-N741	CAAGCAGAAGACGGCATACGAGATGAGGCTAAGTCTCGTGGGCTCGG
i5-N742	CAAGCAGAAGACGGCATACGAGATCGACCATAGTCTCGTGGGCTCGG

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