Analysis of chromatin interaction and accessibility by Trac-looping

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Abstract

Spatial organization of the genome modulates pivotal biological processes. The emerging new technologies have provided novel insights into genome structure and its role in regulating cell activities. To examine the genome-wide chromatin interactions at accessible chromatin regions, we developed a DNA transposase-mediated analysis of chromatin looping (Trac-looping) method for simultaneously detecting chromatin interactions and chromatin accessibility. Here, we describe a detailed protocol of generating Trac-looping libraries.

Key words

Genome structure, Tn5, Trac-looping, Chromatin looping, Chromatin accessibility

1 Introduction

Genome architecture plays important regulatory role in orchestrating spatial and temporal gene expression[1-4]. Our understanding of genome organization has been revolutionized by the development of cutting-edge technologies for probing chromatin structures[5-10 11-22] .The spatial organization of the genome has a hierarchical pattern[5,23]. Each chromosome resides preferentially in separated territory[11]. Based on the openness status of chromatin regions, they are separated into 'compartment A' (open) and 'compartment B' (closed)[11,12,24]. At the scale of hundreds of kilobases to several megabases, chromatins fold into topologically associating domains (TADs), which are enriched of chromatin interactions within the domain[25-27]. Spatial chromatin interactions are realized by the formation of chromatin loops[28-32].

The temporospatial gene expression essential for cellular differentiation is controlled by key transcription regulatory elements such as enhancers that form chromatin loops with target promoters for their functions. To specifically examine the genome-wide chromatin looping among promoters and enhancers, we developed the Trac-looping method [33] by utilizing the DNA transposase Tn5 that preferentially targets accessible chromatin regions[34]. Briefly, Trac-looping first uses Tn5 to insert a bivalent linker to two spatially proximal accessible chromatin regions, which covalently joints the two regions together. After digesting the genomic DNA with restriction enzymes, DNA fragments with the inserted linker are circularized and subjected to rolling circle amplification. The library is further amplified and indexed by PCR, then sequenced on NGS platforms (Fig. 1). With relatively low sequencing depth, Trac-looping can produce high resolution genome-wide chromatin interaction maps together with chromatin accessibility information. In this chapter, we will introduce the detailed experimental procedure of Trac-looping.

2 Materials

2.1 Reagents

1. Competent cells BL21 Gold (DE3) (Agilent cat# 230132)
2. pET15b-His6Tnp (Addgene plasmid #79807)
3. Nuclease-free water (Life Technologies, cat#. AM9930)
4. 1M Tris-HCl (pH 7.4; Quality Biological, cat. # 351-006-101)
5. 1M Tris-HCl (pH 8.0; KD medical, cat#: RGF-3360)
6. 1x PBS (Corning Incorporated - Life Sciences, cat# 21-040-CV)
7. 0.5M EDTA (Quality Biological, cat # 351-027-721)
8. Ethyl alcohol (Warner-Graham, cat # 64-17-5) **Caution**: Highly flammable.
9. Isopropanol (MG Scientific cat# 6810008227637)**. Caution**: Highly flammable.
10. 16% Formaldehyde (w/v), Methanol-free (Thermo Fisher Scientific, cat# 28906 or 28908)
11. Phenol-Chloroform. (Amresco, cat# 0883-100ML). **Caution**:Use in chemical hood.

12. Ni-NTA agarose bead slurry (Qiagen cat#1018244)
13. Imidazole (Sigma cat# I5513-25G)
14. ATP (Sigma cat# A7699-1G)
15. 100% Glycerol (Invitrogen cat# 15514-011)
16. 1 mg/mL Tn5 transposase (home made, describe in the methods)
17. 5M Sodium Chloride, Molecular Biology Grade (Promega, cat # V4221)
18. 10% SDS. (KD medical cat# RGE-3230)
19. 20 mg/mL Protease K (Sigma, cat# 3115828001).
20. 20 mg/mL RNase A. (Fisher Scientific, cat# 12091021)
21. 20 mg/mL Glycogen. (Millipore Sigma, cat# 10901393001)
22. 3 M sodium acetate (pH 5.2). (Quality Biological, cat# 351-035-721)
23. dNTP Mix (10 mM ea, Thermo Fisher Scientific, cat. # 18427089)
24. EB buffer (Qiagen, cat. # 19086)
25. AMPure XP beads. (Beckman Coulter, cat# A63880)
26. NlaIII (10 U/µL, New England BioLabs cat# R0125L)).
27. MluCI (10 U/µL New England BioLabs cat# R0538L).
28. Dynabeads™ MyOne™ Streptavidin C1 beads. (Fisher Scientific, cat# 65001)
29. 100% Triton X-100 . (Sigma cat# T8787-250ML)
30. 10% Nonidet P40 (Sigma cat# 11332473001)
31. 100% Tween 20. (Sigma cat# P9416-100ML)
32. T7 DNA ligase with 2 x T7 DNA ligase buffer (3,000 U/µL NEB cat# M0318L).
33. 15 mg/mL GlycoBlue. (Thermo Fisher cat#: AM9515)
34. TempliPhi Amplification Kit. (Sigma cat# GE25-6400-10)
35. Qubit dsDNA HS Assay Kit. (Fisher Scientific cat# Q32851)
36. E-Gel® EX Gel, 2%, 20-Pak (Thermo fisher scientific, cat. # G402002)
37. Phusion® High-Fidelity PCR Master Mix with HF Buffer (New England BioLabs, cat #M0531S)
38. NEBuffer 2 (New England BioLabs, cat #B7002S)
39. FBS (fetal bovine serum, heat inactivated. Sigma-Aldrich. Cat# F4135-500ML)
40. 1 Kb Plus DNA Ladder (Invitrogen, cat #10488090)
41. MinElute Gel Extraction Kit (Qiagen, cat. #28604)

2.2 Buffers

1. Bacterial lysis buffer (50mM Tris-HCl pH8.0, 300 mM NaCl, 20mM Imidazole, 0.1% Triton X-100, 10 µg/ml Pepstatin A (Calbiochem cat# 516481), 10 µg/ml Leupeptin Hemisulfate (Calbiochem cat# 108975), 10 µg/ml Chymostatin (Calbiochem cat# 230790), 6 µg/ml Antipain Dihydrochloride (Sigma cat# A6191), 1mg/ml lysozyme
2. Ni-NTA beads wash buffer (50mM Tris-HCl pH8.0, 1M NaCl, 20mM Imidazole, 0.1% Triton® X-100).
3. Tnp elution buffer (50mM Tris-HCl pH8.0, 1M NaCl, 250mM Imidazole, 0.1% Triton® X-100)
4. 10x annealing buffer (0.5 M Tris-acetate pH 7.5, 1.5 M potassium acetate, 40 mM spermidine).
5. 1.25 M glycine: dissolve 4.68 g glycine in 50 mL ddH_2O and filter (Millipore cat# SLHA033SS) sterilize it.
6. Lysis buffer: 10 mM Tris-HCl pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$, 0.5% NP-40.
7. 10 x Tn5 reaction buffer: 0.5 M Tris-acetate (pH 7.5), 1.5 M potassium acetate, 100 mM magnesium acetate, 40 mM spermidine.
8. EB: 10 mM Tris-HCl pH 8.0.
9. 1x B/W/T buffer: 5 mM Tris-HCl pH 7.5, 0.5 mM EDTA, 1 M NaCl, 0.1% Triton X-100.
10. 2x B/W buffer: 10 mM Tris-HCl pH 7.5, 1 mM EDTA, 2 M NaCl
11. E-Gel® EX Agarose Gels, 2% (Invitrogen, cat #G401002)
12. 2xT7 DNA ligase reaction buffer: prepared according to New England BioLabs (NEB) 1x buffer. 1X T7 DNA Ligase Reaction Buffer: 66 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM ATP (Sigma cat# A7699-1G), 1 mM DTT, 7.5% Polyethylene glycol (PEG 6000), pH 7.6 @ 25°C

2.3 Equipment

1. Thermal mixer (Eppendorf thermal mixer R).
2. Rotating mixer or Tube revolver (Thermo Scientific, model N0. 88881001)
3. Vortex mixer (Benchmark Scientific Inc. cat# BV1003).
4. Microcentrifuge (Eppendorf centrifuge 5424).
5. Sonicator (Misonix4000)
6. Magnetic Rack for microcentrifuge tubes (Invitrogen cat# 123210).
7. Nanodrop One spectrophotometer (Thermo Scientific, Model: nanodrop One)
8. Qubit fluorometer (Life Technologies, cat. #Q32866)
9. MJ Research PTC-200 Thermal Cycler (MJ Research, cat. # 8252-30-0001)
10. ProFlex™ 3 x 32-well PCR System (Thermo Fisher Scientific, **cat#:** 4484073)

2.4 Oligos

1. Half_adapter_top: /5phos/CTGTCTCTTATACACATCTCTGATGGCGCGAGGGA/3ddCTP/.
2. Half_adapter_bottom: /5AmC6/TCCCTCGCGCCATCAGAGATGTGTATAAGAGACAG.
3. Bivalent_linker_top: /5Phos/CTGTCTCTTATACACATCTCCGAGCCCACGAGAC/iBiodT/CGTCGGCAG CGTCAGATGTGTATAAGAGACAG.
4. Bivalent_linker_bottom: /5Phos/CTGTCTCTTATACACATCTGACGCTGCCGACGAGTCTCGTGGGCTCGG AGATGTGTATAAGAGACAG.
5. Illumina_Nextera_PE_PCR_primer_F: AATGATACGGCGACCACCGAGATCTACAC [8 bp i5 barcode] TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG. Such as:
   N501: **AATGATACGGCGACCACCGAGATCTACACTAGATCGCTCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**
   N502: AATGATACGGCGACCACCGAGATCTACAC CTCTCTAT TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG
6. Illumina_Nextera_PE_PCR_primer_R: CAAGCAGAAGACGGCATACGAGAT [8 bp i7 barcode] GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG. Such as:

N701: CAAGCAGAAGACGGCATACGAGAT TCGCCTTA GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG

N702: CAAGCAGAAGACGGCATACGAGAT CTAGTACGGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG

3 Methods

3.1 *Expression and purification of hyperactive Tn5 and annealing of adapters*

1. Transform competent cells BL21 Gold (DE3) (Agilent cat# 230132) with pET15b-His6Tnp (Addgene plasmid #79807). Plate the transformed bacterial cells on LB agar plates containing 100 µg/ml Carbenicillin and incubate the plates at 37°C overnight.
2. Inoculate 60 ml LB containing Carbenicillin (100 µg/ml) with one single colony and incubate at 37°C with shaking at 200 rpm overnight.
3. Dilute 10 ml of the above culture to 0.6 liter of the same media in a 2L flask and continue to grow until OD_{600} reaches 0.8 at 37°C while shaking at 200 rpm. Use two flasks for a total of 1.2 L media.
4. When the OD_{600} reaches 0.8, transfer the flasks to ice water bath and cool them down for 10 min. Add IPTG to 0.5mM (final concentration) and shake the culture at 200rpm at room temperature for 4 hours.
5. Cool down the culture in ice water, pellet the cells by spinning at 3700 rpm at 4°C for 15 min. Remove the supernatant and resuspend the cell pellets in 30 ml bacterial lysis buffer (50mM Tris-HCl pH8.0, 300 mM NaCl, 20mM Imidazole, 0.1% Triton X-100, 10 µg/ml Pepstatin A (Calbiochem cat# 516481), 10 µg/ml Leupeptin Hemisulfate (Calbiochem cat# 108975), 10 µg/ml Chymostatin (Calbiochem cat# 230790), 6 µg/ml Antipain Dihydrochloride (Sigma cat# A6191), 1mg/ml lysozyme; incubate on ice for 30 minutes. Split into six 15 ml tubes.
6. Lyse the cells in each tube by sonication with a microtip on Misonix4000 (total process time of 105” with 15” on, 30” off, 90% for amplitude). Pool the lysates into one centrifuge tube.
7. Spin the lysates at 15,000 rpm for 10 min at 4°C; transfer supernatant to a new tube and add 2ME (2-mecapotoethenal, final concentration 5mM), PMSF (1 µM) and NaCl (1M). Note: it is important to maintain the 1M NaCl concentration to reduce the DNA contamination.

8. Wash 2 ml 50% Ni-NTA agarose bead slurry (Qiagen cat#1018244) with 30 ml of 50mM Tris-HCl pH8.0, 300 mM NaCl and 20mM Imidazole. Spin at 1320 rpm at 4°C for 5min. Remove supernatant.
9. Add the cleared lysates to the Ni-NTA agarose beads and rotate at 4°C for one hour. Spin the beads at 1320rpm for 5min at 4°C and remove supernatant. Wash the beads once with 15ml Ni-NTA beads wash buffer (50mM Tris-HCl pH8.0, 1M NaCl, 20mM Imidazole, 0.1% Triton® X-100).
10. Transfer the beads to a 1ml syringe with glass wool on the bottom. Wash the beads with 20ml wash buffer. Elute the bound Tnp using 3 ml of Tnp elution buffer (50mM Tris-HCl pH8.0, 1M NaCl, 250mM Imidazole, 0.1% Triton® X-100) by loading 0.5ml elution buffer for 6 times and collect the eluates into 6 Eppendorf tubes. To each tube of eluates, add dithiothreitol (final concentration 1 mM) and glycerol (final concentration 50%). Check the purity of eluted Tn5 proteins by SDS-PAGE. The purified Tnp is stored at -80°C freezer.

11. To anneal the adapters, dissolve all the primers at 100 μM with EB (10mM Tris-HCl, pH8.0). Mix 250μl 20μM Bivalent_linker_top with 250 μl 20 μM linker_bottom and 55 μl 10x annealing buffer (0.5 M Tris-acetate pH 7.5, 1.5 M potassium acetate, 40 mM spermidine). Heat at 98°C for 5min followed by slow cooling to room temperature to form the 9 μM bivalent linker. Mix 250μl 100μM Half_adapter_top and 250μl 100μM Half_adapter_bottom. Heat at 98°C for 5min followed by slow cooling to room temperature to form the 50 μM half adapter. The annealed adaptors are stored at -20°C freezer.

3.2 Cell fixation

1. Harvest 5 x 10^7 cells and resuspend in 50 mL culture medium containing 10% FBS in a 50ml tube.
2. Add 3.33 mL of 16% formaldehyde to the cell suspension (1% final concentration) and mix by inverting the tube gently. Incubate at room temperature for 10 min with rotation on a tube revolver.

3. Split the cells equally into two 50 ml tubes. Quench the cross-linking reaction by adding 2.65 mL 1.25 M glycine (0.125 M final concentration) into each tube. Mix well and incubate at room temperature for 5 min by rotation on a tube revolver.
4. Collect cells by centrifugation at 370 x g for 10 min at 4 °C. Discard the supernatant by aspiration and resuspend the cells with 25 mL ice cold 1 x PBS. Pool the cells into one tube. Pellet the cells at 370 x g for 10 min at 4 °C. Repeat PBS washing once. The fixed cells can be resuspended in 10M cells per 100 µl 1xPBS and stored at -80°C freezer.

3.3 Assemble the Tn5 complex and DNA transposition reaction

1. Prepare the Tn5 transposase complex in a 1.5 mL tube by mixing 16 µL 100% glycerol, 4.5 µL 50 µM annealed half adapter and 12.5 µL 9 µM annealed bivalent linker. Mix well before adding Tn5. Add 30 µL 1 mg/mL Tn5 transposase into the mixture, then mix well gently by pipetting up and down several times. Incubate at room temperature for 20 min to assemble the transposase complex. Store the complex on ice.
2. Thaw the fixed 5 x 10^7 cells on ice-water. Spin down the cells at 1500 rpm for 3 min in a microfuge and remove supernatant. Resuspend cell pellet with 50 mL lysis buffer, and incubate on ice for 15 min to permeabilize the cells.
3. Centrifuge at 370 x g for 10 min at 4 °C, then remove supernatant.
4. Resuspend the cell pellet with 1.8 mL lysis buffer, then add 200 µL 10 x Tn5 reaction buffer. Mix well by pipetting up and down several times, then dispense the cell suspension into 100 µL aliquots in 20 1.5 mL tubes (2.5 x 10^6 cells/tube).
5. Add 1.6 µL Tn5 complex into 100 µL cell suspension and mix well. Incubate on a thermal mixer at 37 °C for 2 hours with interval shaking (shaking at 800rpm 30 sec ON, 5 min OFF). Then add 1.5 µL of Tn5 complex again into the reaction and continue with the shaking/incubation overnight.

3.4 Reverse cross-linking and purify genomic DNA

1. Stop the reaction by adding 5 μL 0.5 M EDTA (25 mM final concentration). Pool the reaction mixtures from every 2 tubes into one tube (208 μL total volume in each of the final 10 tubes).
2. To each tube, add 6 μL 10% SDS (0.3% final concentration) and 5 μL 20 mg/mL Protease K (0.5 mg/mL final concentration). Incubate on a thermal mixer at 55 °C for 2 hours with shaking, then incubate at 65 °C overnight to reverse cross-linking.
3. Add 5 μL 20 mg/mL RNase A (0.5 mg/mL final concentration) into each tube, and incubate at 37 °C for 30 min.
4. Add 250 μL Phenol-Chloroform into each tube, then vortex vigorously for 30 sec. Spin at 12,000 rpm in a microcentrifuge at 4°C for 10 min.
5. Transfer the upper aqueous phase into a new tube. Add 1 μL 20 mg/mL glycogen, 25 μL 3 M Sodium Acetate (pH 5.2) and 650 μL ethanol into each tube and mix well. Keep the tubes on dry ice for 30 min, then spin at 12,000 rpm for 15 min at 4 °C.
6. Aspirate to remove the supernatant, then wash the DNA pellet twice with 70% ethanol. Remove the supernatant after final wash, then air dry the pellet for 3 min.
7. Add 50 μL EB into each tube to resuspend the DNA pellet.

3.5 Repair DNA gaps between the bivalent adapter and genomic DNA

1. To each tube, add 10 μL 10 x NEBuffer 2.1, 34 μL ddH_2O, 2 μL 10 mM dNTPs and 4 μL T4 DNA Polymerase (3 units/μL). Mix well and incubate at room temperature for 1 hour. Then add 5 μL 0.5 M EDTA to each tube to stop the reaction.
2. Add 61 μL AMPure XP beads to each tube (volume ratio of beads to DNA is 0.6). Mix well and incubate at room temperature for 30 min. DNA fragments over 200 bp are captured and free linkers are removed at this step.
3. Collect beads on a magnet and remove the supernatant. Wash beads with 1 mL 70% ethanol twice. Air dry the beads briefly after final wash.
4. Elute bound DNA from beads with 188 μL 1 x CutSmart buffer.

3.6 DNA restriction enzyme digestion and enrichment via the biotinylated bivalent adapter

1. Half of the eluted DNA (five tubes) are subjected to NlaIII digestion, and the other half (five tubes) are subjected to MluCI digestion. Add 12 μL restriction enzyme separately (10 units/μL) to each tube. Mix well and incubate at 37 °C for 3 hours. Note: there is no NlaIII nor MluCI site in the adapter. Only the genomic DNA will be cut.
2. Prepare 40 μL Streptavidin C1 beads for each tube (400 μL for 10 tubes). Wash beads twice with 500 μL 1 x B/W buffer, then resuspend beads with 400 μL 2 x B/W buffer.
3. Add 40 μL washed Streptavidin C1 beads to each restriction digestion tube. Incubate at room temperature for 30 min with gentle rotation.
4. Collect beads on a magnet, and remove supernatant. Wash beads five times with 1 mL 1 x B/W buffer plus 0.1% Triton X-100 by rotating at room temperature for 5 mins. At the final wash step, transfer the beads in wash buffer to a new tube to avoid carry-over contamination. Note: it is important to get rid of the unbound DNA as much as possible.
5. Rinse beads once with 1 mL EB. Collect beads and remove supernatant.
6. Prepare Streptavidin C1 beads elution buffer by mixing 870 μL EB, 30 μL 10% SDS and 100 μL 20 mg/mL Protease K. Add 100 μL Streptavidin C1 beads elution buffer to each tube and incubate at 55 °C for 4 hours with 800 rpm shaking.
7. Collect beads on a magnet, and transfer eluates to a new tube. Wash beads with 100 μL EB plus 0.5 M NaCl. Pool the eluates from the same tube.
8. Add 200 μL Phenol-Chloroform to each tube, then vortex vigorously for 30 sec. Spin at 12,000 rpm in a microcentrifuge at 4 °C for 10 min. Transfer the upper phase to a new tube.
9. Add 1 μL 20 mg/mL glycogen and 0.5 mL ethanol to each tube. Mix well and keep on dry ice for 30 min. Then spin at 12,000 rpm for 15 min at 4 °C. Remove the supernatant and wash the DNA pellets with 1 mL 70% ethanol twice. Air dry the pellets briefly, then resuspend with 200 μL EB.

3.7 Self-circularization of genomic DNA fragments in a large volume

1. Pool the DNA from all of the 10 tubes (2 mL in total) into a 50 mL tube. Add 10 mL 2 X T7 DNA ligase buffer, 8 mL ddH_2O. mix well. Add 40 μL T7 DNA ligase (3,000 units/μL) to the tube. Mix well and incubate at room temperature overnight.
2. Add 20 mL Phenol-Chloroform to the ligation reaction. Vortex vigorously for 30 seconds then spin at 4,200 rpm in a centrifuge for 30 min.
3. Transfer the upper phase to 24 x 1.5 mL tubes (about 0.83 mL each tube). Spin at 12,000 rpm in a microcentrifuge for 10 min. Transfer the upper phase to a new tube. Add 0.2 μL GlycoBlue (15 mg/mL), 80 μL 3 M Sodium Acetate (pH 5.2) and 0.8 mL isopropanol to each tube. Mix well and keep on dry ice for 30 min.
4. Spin at 12,000 rpm for 30 min at 4 °C. Remove the supernatant and wash the pellets twice with 70% ethanol. Air dry the pellets briefly after final wash. Note: do not over dry the pellets before adding the sampling buffer in the next step.

3.8 RCA reaction in a small volume

1. Resuspend the pellet from each tube with 11 μL sampling buffer from TempliPhi Amplification Kit, then split into two PCR tubes in an 8-tube strip (5.5 μL each tube). Now the samples are in 6 x 8-tube PCR strips.
2. Heat at 97 °C for 3 min to denature DNA on a thermocycler, then chill on ice.
3. Prepare RCA reaction master mix by mixing 5 μL RCA reaction buffer and 0.2 μL enzyme from the TempliPhi amplification kit for each tube. Add 5.2 μL master mix to denatured DNA. Put the tubes on a thermocycler and run the following cycle: 30 °C 16 hours, 65 °C 15 min and 4 °C hold.

3.9 Library indexing and amplification

1. Pool RCA reactions from eight PCR tubes in one strip into one 1.5 mL tube. Rinse the PCR tubes with 10 μL ddH_2O and pool with RCA reactions (6 tubes, 160 μL total for each tube. Only use 20 μL in the next step).

2. Transfer 20 μL from each tube to a new 1.5 mL tube. Add 20 μL AMPure XP beads to each tube. Mix well and incubate at room temperature for 30 min.
3. Collect beads on a magnet, then wash beads with 500 μL 70% ethanol twice. Air dry beads briefly after final wash, then add 40 μL EB to elute DNA from beads.
4. Measure DNA concentration of eluates using NanoDrop Spectrophotometer. Usually the concentration is 30-40 ng/μL.
5. Prepare PCR reaction by mixing 0.5 μL purified RCA product as template (about 20 ng), 25 μL 2 x NEB Phusion HF master mix, 22.5 μL ddH$_2$O, 1 μL 10 μM Illumina_Nextera_PE_PCR_primer_F (such as N501) and 1 μL 10 μM Illumina_Nextera_PE_PCR_primer_R (such as N701). Run the following PCR program: 98 °C 30 sec; 11 cycles of 98 °C 10 sec, 65 °C 30 sec and 72 °C 8 sec; 72 °C 5 min; 4 °C hold.
6. After gel electrophoresis, excise the gel slices containing DNA fragments between 220 and 700 bp. Purify DNA using MinElute Gel Extraction Kit.
7. Measure the DNA concentration of the library using Qubit dsDNA HS Kit following manufacturer's instructions.
8. Proceed to Illumina Hiseq or Novaseq Paired-End 50-8-8-50 format sequencing.
9. Map the sequencing reads to the expected genomes and analyze chromatin accessibility and interaction as described previously [33].

4 Notes

1. Different types of cells may require different fixation conditions. We use the protocol for fixing mammalian suspension cells. For other cell types, the condition may need to be optimized.
2. To achieve optimal complexity of Trac-looping libraries, we started with 5 x 10^7 cells. It would also work by using fewer cells. The amount of Tn5 transposase complex should be adjusted accordingly.
3. It may increase the diversity of libraries by performing Tn5 mediated linker integration and RCA reactions in multiple separate tubes. If a centrifuge is available to hold large tubes, there is no need to split into small tubes in step3 in section 3.7.

4. When using AMPure XP beads to purify genomic DNA, due to the large size of DNA, beads will aggregate and stick to tubes. Vortex can increase the efficiency of eluting DNA from beads.
5. Check the size of RCA products by gel electrophoresis. RCA products should be over 10 kb.

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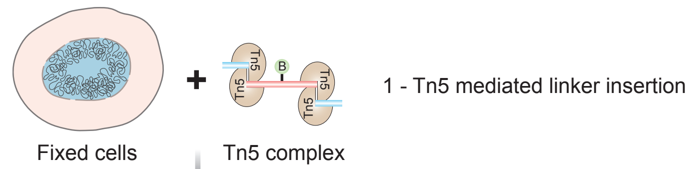
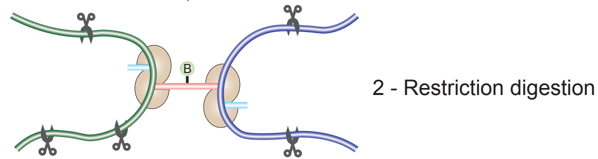
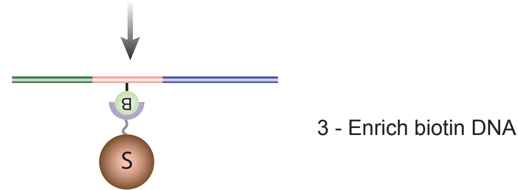
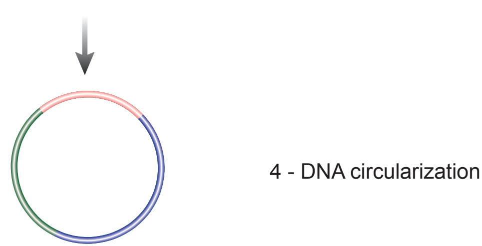
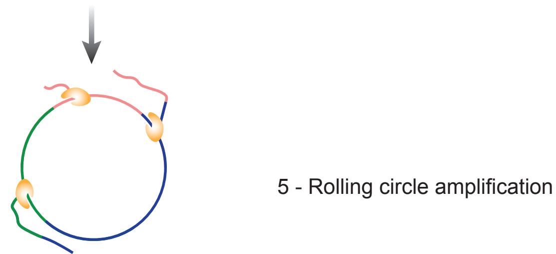
Figure 1: Schematic representation of the main steps of Trac-looping.

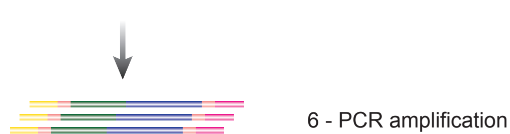

Tn5
B
Tn5
Tn5
Tn5
Fixed cells
Tn5 complex
1 - Tn5 mediated linker insertion

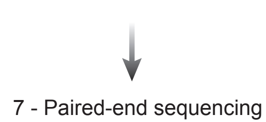

B
2 - Restriction digestion

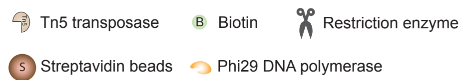

B
S
3 - Enrich biotin DNA


4 - DNA circularization


5 - Rolling circle amplification


6 - PCR amplification
7 - Paired-end sequencing


Tn5 transposase
B Biotin
Restriction enzyme


S Streptavidin beads
Phi29 DNA polymerase