

Simultaneous Capture of Chromatin-Associated RNA and Global RNA–RNA Interactions With Reduced Input Requirements

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Abstract

Chromatin-associated RNAs (caRNAs) have been increasingly recognized as key regulators of gene expression and genome architecture. A few technologies, such as ChRD-PET and RedChIP, have emerged to assess protein-mediated RNA–chromatin interactions, but each has limitations. Here, we describe the TaDRIM-seq (targeted DNA-associated RNA and RNA–RNA interaction mapping by sequencing) technique, which combines Protein G (PG)-Tn5-targeted DNA tagmentation with in situ proximity ligation to simultaneously profile caRNAs across genomic regions and capture global RNA–RNA interactions within intact nuclei. This approach reduces the required cell input, shortens the experimental duration compared to existing protocols, and is applicable to both mammalian and plant systems.

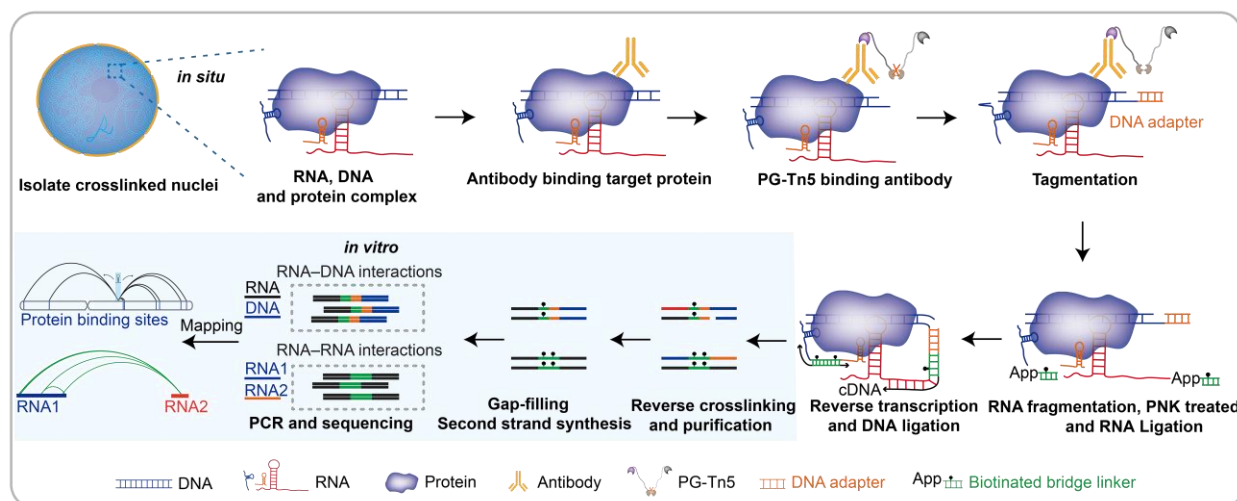
Key features

- A multi-omics sequencing strategy.
- Compatible with mammalian and plant systems.
- Profiling of epigenome, RNA–DNA, and RNA–RNA interactomes.

Keywords: Chromatin-associated RNAs (caRNAs), Multi-omics, RNA–DNA interactomes, RNA–RNA interactomes, DNA element, TaDRIM-seq, Low-input requirement

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Graphical overview



Targeted DNA-associated RNA and RNA–RNA interaction mapping by sequencing (TaDRIM-seq) technique schematic

Background

Chromatin-associated RNAs (caRNAs) have been proposed to represent an additional regulatory layer of the epigenome [1]. Some caRNAs act in trans, being recruited to distant genomic loci (trans-acting RNAs), while others function in cis, remaining enriched at their transcription sites (cis-acting RNAs) [2]. Increasing evidence has demonstrated that caRNAs play crucial roles in regulating gene transcription [3–5], X-chromosome inactivation [6], and the three-dimensional organization of the genome [7,8].

Current methods, such as ChRD-PET [9] and RedChIP [10], enable the identification of DNA elements targeted by specific caRNAs. However, these approaches are resource-intensive, time-consuming, and inefficient due to in vitro ligation limitations, and they are unable to capture RNA–RNA interactions. To overcome these limitations, we recently developed a method named TaDRIM-seq [11]. In this protocol, we provide a step-by-step guide for performing TaDRIM-seq in rice and human cells. The assay is also compatible with other cell lines, as well as plant and animal tissues, making it broadly applicable to the study of caRNAs-mediated chromatin regulation.

Materials and reagents

Biological materials

1. Human K562 (ATCC, catalog number: CCL-243)
2. Rice leaf tissue [Xian/indica cv. Minghui 63 (MH63)]

Reagents

1. Formaldehyde (37%) (Sigma-Aldrich, catalog number: F8775)
2. Glycine (Sigma-Aldrich, catalog number: G7126-1kg)
3. PBS (10×) (Invitrogen, catalog number: AM9625)
4. HEPES (1 M) (Coolaber, catalog number: SL6056-500mL)
5. NaCl (5 M) (Invitrogen, catalog number: AM9759)
6. Spermidine (Sigma-Aldrich, catalog number: S2626-1g)
7. Protease inhibitor cocktail tablets (Roche, catalog number: 58914000)
8. RiboLock RNase inhibitor (40 U/μL) (Invitrogen, catalog number: EO0381)

9. Nuclease-free water (NF-H₂O) (Thermo Fisher Scientific, catalog number: EF0651)
10. Digitonin (5%) (Thermo Fisher Scientific, catalog number: BN2006)
11. EDTA (0.5 M) (Invitrogen, catalog number: AM9260G)
12. BSA (20 mg/mL) (NEB, catalog number: B9000S)
13. MgCl₂ (1 M) (Invitrogen, catalog number: AM9530G)
14. SDS (10%) (Invitrogen, catalog number: AM9822)
15. dNTPs (10 mM) (Invitrogen, catalog number: 18427088)
16. Triton X-100 (Sigma-Aldrich, catalog number: 9036-19-5)
17. H3K4me3 antibody (50 µg) (Abcam, catalog number: ab8580)
18. H3K27me3 antibody (100 µg) (Abcam, catalog number: ab6002)
19. Tris-HCl (1 M, pH 7.5) (Invitrogen, catalog number: 15567027)
20. rCutSmart™ buffer (10×) (NEB, catalog number: B6004V)
21. T4 polynucleotide kinase (T4 PNK, 10 U/µL) (Thermo Fisher Scientific, catalog number: EK0032)
22. T4 polynucleotide kinase reaction buffer (10×) (Thermo Fisher Scientific, catalog number: EK0032)
23. 5' DNA Adenylation kit (NEB, catalog number: E2610L)
24. T4 RNA ligase 2, truncated KQ (200 U/µL) (NEB, catalog number: M0373L)
25. T4 RNA ligase reaction buffer (10×) (NEB, catalog number: B0216S)
26. PEG 8000, 50% (w/v) (NEB, catalog number: B1004S)
27. T4 DNA Ligase (400 U/µL) (NEB, catalog number: M0202S)
28. DNA ligase reaction buffer (10×) (NEB, catalog number: B0202S)
29. Phenol/chloroform/isoamyl alcohol (phenol/ChCl₃/IAA, 25:24:1, pH 7.9) (Ambion, catalog number: AM9730)
30. Sodium acetate (3 M, pH 5.2) (VWR, catalog number: E521-100mL)
31. Ethanol absolute (pure) (Sangon Biotech, catalog number: A500737)
32. T4 DNA polymerase (3 U/µL) (NEB, catalog number: M0203L)
33. NEBuffer™ r2.1 (10×) (NEB, catalog number: B6002S)
34. Tween 20 (Sigma-Aldrich, catalog number: P9416-100ml)
35. Isopropanol (Sigma-Aldrich, catalog number: I-9516-500ml)
36. Buffer EB (QIAGEN, catalog number: 19086-250ml)
37. ATP (10 mM) (NEB, catalog number: P0756L)
38. RNase remover and nucleic acid decontamination reagent (Vazyme, catalog number: R504)
39. Hyperactive pG-Tn5 Transposase (500 ng/µL) (Vazyme, catalog number: S602)
40. TruePrep DNA Library Prep Kit V2 for Illumina (Vazyme, catalog number: TD501)
41. DNA Clean Beads (Vazyme, catalog number: N411-03)
42. Dynabeads™ M-280 (10 mg/mL) (Invitrogen, catalog number: 11205D)
43. I-Block protein-based blocking reagent (Thermo Fisher, catalog number: T2015)
44. M-MuLV reverse transcriptase (200 U/µL) (NEB, catalog number: M0253L)
45. M-MuLV reverse transcriptase reaction buffer (10×) (NEB, catalog number: B0253L)
46. RNase IF (NEB, catalog number: M0243S)
47. NEBNext® second strand synthesis enzyme mix (NEB, catalog number: E6111L)
48. DNA Clean & Concentrator-5 (Zymo, catalog number: D4013)
49. Oligo Clean & Concentrator (Zymo, catalog number: D4060)
50. Primers (synthesized by Sangon Biotech with HPLC purification)

Solutions

1. Buffer S (see Recipes)
2. Buffer F (see Recipes)
3. 1× wash buffer A (see Recipes)
4. Antibody buffer (see Recipes)
5. 1× transposase incubation buffer (see Recipes)
6. 1× wash buffer B (see Recipes)
7. Lysis buffer (see Recipes)
8. Proteinase K solution buffer (see Recipes)
9. iBlock buffer (see Recipes)
10. 2× binding and washing buffer (see Recipes)

11. 1× binding and washing buffer (see Recipes)
12. 2× SSC/0.5% (w/v) SDS (see Recipes)
13. Sheared DNA solution (see Recipes)

Recipes

1. Buffer S

Reagent	Final concentration	Quantity or Volume
1 M HEPES	50 mM	0.5 mL
5 M NaCl	150 mM	0.3 mL
0.5M EDTA	1 mM	0.02 mL
Triton X-100	1%	0.1 mL
10% sodium deoxycholate	0.1%	0.1 mL
10% SDS	1%	1 mL
50× protease inhibitors	1×	0.2 mL
RiboLock RNase (40 U/μL)	0.2 U/μL	0.05 mL
H ₂ O	n/a	7.73 mL
Total	n/a	10 mL

Note: This buffer should be prepared fresh before use.

2. Buffer F

Reagent	Final concentration	Quantity or Volume
1 M HEPES	50 mM	0.5 mL
5 M NaCl	150 mM	0.3 mL
2 M Spermidine	1 mM	0.005 mL
Triton X-100	1%	0.1 mL
10% sodium deoxycholate	0.1%	0.1 mL
50× protease inhibitors	1×	0.2 mL
RiboLock RNase (40 U/μL)	0.2 U/μL	0.05 mL
H ₂ O	n/a	8.745 mL
Total	n/a	10 mL

Note: This buffer should be prepared fresh before use.

3. 1× wash buffer A

Reagent	Final concentration	Quantity or Volume
1 M HEPES (pH 7.6)	20 mM	100 μL
5 M NaCl	150 mM	150 μL
2 M Spermidine	0.5 mM	1.25 μL
50× protease inhibitors	1×	100 μL
RiboLock RNase (40 U/μL)	0.2 U/μL	25 μL
H ₂ O	n/a	4.623 mL
Total	n/a	5 mL

Note: This buffer should be prepared fresh before use.

4. Antibody buffer

Reagent	Final concentration	Quantity or Volume
1× wash buffer A	n/a	495 μL
0.5M EDTA	2 mM	2 μL
30% BSA	0.1%	1.6 μL
5% Digitonin	0.05%	5 μL
Total	n/a	503.6 μL

Note: This buffer should be prepared fresh before use.

5. 1× transposase incubation buffer

Reagent	Final concentration	Quantity or Volume
1 M HEPES (pH 7.6)	20 mM	100 µL
5 M NaCl	300 mM	300 µL
2 M Spermidine	0.5 mM	1.25 µL
5% Digitonin	0.01%	10 µL
50× protease inhibitors	1×	100 µL
RiboLock RNase (40 U/µL)	0.2 U/µL	25 µL
H ₂ O	n/a	4.467 mL
Total	n/a	5 mL

Note: This buffer should be prepared fresh before use.

6. 1× wash buffer B

Reagent	Final concentration	Quantity or Volume
1 M Tris-HCl (pH 7.5)	20 mM	1 mL
1 M MgCl ₂	10 mM	0.5 mL
10% Tween 20	0.2%	1 mL
RiboLock RNase (40 U/µL)	0.2 U/µL	0.25 mL
H ₂ O	n/a	47.25 mL
Total	n/a	50 mL

Note: This buffer should be prepared fresh before use.

7. Lysis buffer

Reagent	Final concentration	Quantity or Volume
1 M Tris-HCl (pH 7.5)	10 mM	10 µL
5 M NaCl	10 mM	2 µL
10% NP40	0.2%	20 µL
50× protease inhibitors	1×	20 µL
RiboLock RNase (40 U/µL)	0.2 U/µL	5 µL
H ₂ O	n/a	943 µL
Total	n/a	1 mL

Note: This buffer should be prepared fresh before use.

8. Proteinase K solution buffer

Reagent	Final concentration	Quantity or Volume
1 M Tris-HCl (pH 7.5)	50 mM	50 µL
10% SDS	1%	100 µL
5 M NaCl	100 mM	20 µL
0.5 M EDTA	1 mM	2 µL
Proteinase K (20mg/mL)	1 mg/mL	50 µL
H ₂ O	n/a	778 µL
Total	n/a	1 mL

Note: This buffer should be prepared fresh before use.

9. iBlock buffer

Reagent	Final concentration	Quantity or Volume
iBlock	2% (w/v)	0.2 g
10% SDS	0.5%	0.5 mL
H ₂ O	n/a	9.5 mL
Total	n/a	10 mL

Note: Incubate the reagent in a 65 °C water bath to facilitate dissolution. The buffer is stable for up to 6 months when stored at room temperature.

10. 2× binding and washing buffer

Reagent	Final concentration	Quantity or Volume
1 M Tris-HCl (pH 8)	10 mM	0.5 mL
5 M NaCl	2 M	20 mL
0.5 M EDTA	1 mM	0.1 mL
H ₂ O	n/a	29.4 mL
Total	n/a	50 mL

Note: This buffer is stable for up to 6 months when stored at 4 °C.

11. 1× binding and washing buffer

Reagent	Final concentration	Quantity or Volume
1 M Tris-HCl (pH 8)	10 mM	0.5 mL
5 M NaCl	1 M	10 mL
0.5 M EDTA	1 mM	0.1 mL
H ₂ O	n/a	39.4 mL
Total	n/a	50 mL

Note: This buffer is stable for up to 6 months when stored at 4 °C.

12. 2× SSC/0.5% (w/v) SDS

Reagent	Final concentration	Quantity or Volume
20× SSC	2×	5 mL
10% SDS	0.5%	2.5 mL
H ₂ O	n/a	42.5 mL
Total	n/a	50 mL

Note: This buffer can be stored at room temperature for up to 3 months; however, lower room temperatures may cause SDS to precipitate. If this occurs, place the buffer in a 25 °C water bath to fully dissolve the SDS before use.

13. Sheared DNA solution

Reagent	Final concentration	Quantity or Volume
Sheared DNA	0.5 ng/μL	x μL
2× binding and washing buffer	1×	50 μL
H ₂ O	n/a	50 – x μL
Total	n/a	100 μL

Note: The genomic DNA isolated from plant or animal was fragmented into 300–500 bp fragments by sonication using a Bioruptor (Diagenode) set to high intensity with 30 cycles (30 s ON, 50 s OFF) at 4 °C. The sheared DNA solution is stable for up to 6 months when stored at -20 °C.

Equipment

1. Eppendorf Thermo Mixer® C (Eppendorf, model: ThermoMixer C)
2. Centrifuge (Eppendorf, model: 5425R)
3. DynaMag-2 Magnet (Life Technologies, catalog number: 12321D)
4. Thermal Cycler (Bio-Rad, model: T100™)
5. Vortex-Genie Mixers (VWR, catalog number: 58816-121)
6. Low-temperature incubator (Beig, catalog number: BC-60L)
7. Qubit 2.0 Fluorometer (Invitrogen, catalog number: Q32866)
8. Intelli-mixer RM-2L (Rose Scientific, catalog number: MX1000)
9. Bioruptor® Plus sonication device (Diagenode)

Software and datasets

1. All data and code have been deposited to GitHub: <https://github.com/304243504/HiR>

Procedure

A. Crosslinking (1–1.5 h)

1. Cells

- a. Prepare the cell suspension and wash the cells twice with 1× PBS. Pin down the cells by centrifugation at 1,000× *g* for 5 min.
- b. Resuspend the cells with 1 mL of 1% formaldehyde in 1× PBS for 10 min with rotation at room temperature.
Critical step: Using a formaldehyde concentration lower than 1% can result in a low signal-to-noise ratio.
- c. Add glycine to a final concentration of 0.2 M for 5 min at room temperature with rotation to quench the crosslinking reaction. Wash cells three times with 1× PBS and store at -80 °C.

2. Plant tissue

- a. Cross-link mature rice leaves with 1% formaldehyde (add 4 mL of 37% formaldehyde to 144 mL of 1× PBS) and apply vacuum for 20 min.
- b. Quench formaldehyde by adding 13.2 mL of 2.5 M glycine to the media to a final concentration of 0.2 M and vacuum for 5 min.
- c. Wash the leaves three times with NF-H₂O and store at -80 °C.

B. pG-Tn5 transposase assembly (timing: 2.5 h)

1. Dissolve primer A (5'-phos-CTGTCTCTTATACACATCTT-3') and primer B (5'-phos-AAGATGTGTATAAGAGACAG-3') to 100 μM in annealing buffer.
2. Mix the equimolar mixture of primer A and B (primer mix) and perform the PCR program as described in Table 1.

Table 1. PCR program for primer annealing

Temperature (°C)	Time (min)
75	15
60	10
50	10
40	10
25	30

3. Prepare the assembled mixture as in Table 2.

Table 2. Assembly of pG-Tn5 transposase reaction mix (TTE mix)

Reagent	Volume (μL)
Hyperactive pG-Tn5 Transposase (500 ng/μL)	20
Primer mix	3.5
Coupling buffer	14

4. Thoroughly mix the components and incubate for 1 h at 30 °C using a thermal cycler. Label as TTE Mix.

C. Preparation of bridge linker (timing: ~2 h)

1. Dissolve the bridge linker forward and reverse oligonucleotides in NF-H₂O to a final concentration of 100 μM.
The forward strand: 5'-[5Phos]CCTCGATAC/iBIOdT/TGAGCTGAC-3'
Reverse strand: 5'-[5Phos]GTCAGC/iBIOdT/CAAGTATCGAG-3'
Note: The iBIOdT-modified primers were custom-synthesized by Sangon Biotech with HPLC purification.

2. Prepare the adenylation reaction mixture (Table 3).

Table 3. Adenylation reaction mix for bridge linker preparation

Reagent	Volume (μL)
10× 5' DNA adenylation reaction buffer	12
1 mM ATP	12
Forward strand oligo (100 μM)	6
Mth RNA ligase	12
Nuclease-free water	78

3. Incubate the mixture at 65 °C for 1 h to allow annealing, then heat at 85 °C for 5 min to inactivate enzymatic activity.

4. Add 6 μL of the reverse strand oligo to the adenylation reaction mixture and incubate by PCR using the following program: 95 °C for 2 min; ramp from 95 °C to 75 °C at 0.1 °C/s, hold at 75 °C for 2 min; ramp from 75 °C to 65 °C at 0.1 °C/s, hold at 65 °C for 2 min; ramp from 65 °C to 50 °C at 0.1 °C/s, hold at 50 °C for 2 min; ramp from 50 °C to 37 °C at 0.1 °C/s, hold at 37 °C for 2 min; ramp from 37 °C to 20 °C at 0.1 °C/s, hold at 20 °C for 2 min and hold at 4 °C.

5. Purify the annealed linker using the Oligo Clean & Concentrator kit (Zymo) and elute in 100 μL of nuclease-free water. Label as the bridge linker.

D. Nuclei preparation and tagmentation (timing: ~1 h)

1. Plant nuclei isolation

a. Grind the crosslinked tissue (0.3–0.5 g) into a fine powder using liquid nitrogen.

b. Add 300 μL of Buffer S to the powdered tissue and incubate at 4 °C for 30 min with gentle rotation.

c. Add 1.2 mL of Buffer F and continue incubation at 4 °C for 15 min with rotation. Centrifuge at 1,000× g for 10 min at 4 °C to collect the nuclei.

Note: After nuclei isolation, the experiment should be performed immediately. The extraction from mature rice leaves is shown in Figure 1.

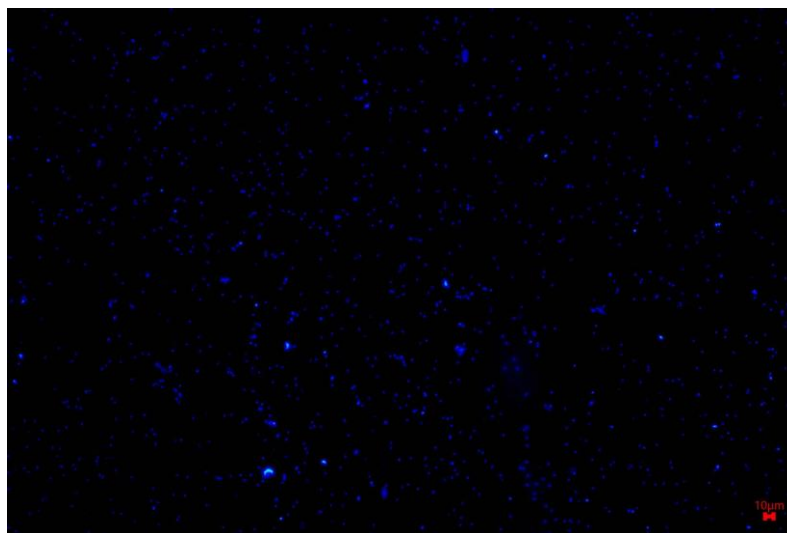


Figure 1. Isolated nuclei are stained with DAPI. Crosslinked rice tissue was ground into a fine powder in liquid nitrogen. The powder was suspended in buffer S/F, and the nuclei were isolated by centrifugation. The nuclei were stained with DAPI and visualized using a fluorescence microscope. Scale bar: 10 μm.

2. Cell nuclei isolation

a. Resuspend crosslinked cells (1×10^6 to 1×10^7) in 1 mL of lysis buffer and incubate for 10 min on ice followed by centrifugation at 2,500× g for 3 min at 4 °C.

3. pG-Tn5 transposase binding to the antibody and tagmentation

- Wash cells or plant nuclei twice with 300 μ L of 1 $\times$ wash buffer A. Collect nuclei by centrifugation at 2,500 $\times$ g for 3 min at 4 $^{\circ}$ C.
- Resuspend nuclei in 500 μ L of antibody buffer, add 5–10 μ g of antibody, and incubate at 4 $^{\circ}$ C for 2 h with rotation.
- Collect nuclei by centrifugation at 2,500 $\times$ g for 3 min at 4 $^{\circ}$ C. Wash the nuclei with 300 μ L of 1 $\times$ wash buffer A and perform 3–5 washing steps.
- Resuspend nuclei in 500 μ L of 1 $\times$ transposase incubation buffer. Add 5 μ L of TTE Mix and incubate at 4 $^{\circ}$ C for 1 h with rotation.
- Collect the nuclei by centrifugation at 2,500 $\times$ g for 3 min at 4 $^{\circ}$ C and wash 3–5 times with 300 μ L of 1 $\times$ transposase incubation buffer.
- Resuspend nuclei in 500 μ L of 1 $\times$ transposase incubation buffer, add 5 μ L of 1 M $MgCl_2$, and incubate at 37 $^{\circ}$ C for 1 h.
- Add 500 μ L of 40 mM EDTA to stop the reaction.
- Collect nuclei by centrifugation at 2,500 $\times$ g for 3 min at 4 $^{\circ}$ C. Wash the nuclei pellet twice with 500 μ L of 1 $\times$ PBS and then incubate in 200 μ L of 0.5% SDS at 62 $^{\circ}$ C for 10 min.
- Immediately quench the SDS by adding 580 μ L of nuclease-free water and 100 μ L of 10% (v/v) Triton X-100. Incubate at 37 $^{\circ}$ C for 15 min with rotation.

E. RNA fragmentation and PNK treated (timing: ~1.5 h)

- Collect nuclei by centrifugation at 2,500 $\times$ g for 3 min and wash twice with 500 μ L of 1 $\times$ wash buffer B.
- Resuspend nuclei in 500 μ L of 1 $\times$ CutSmart buffer, add 1.6 μ L of RNase IF (previously diluted 10-fold in 1 $\times$ PBS), and incubate at 37 $^{\circ}$ C for 3 min, followed by an additional 10-min incubation on ice.
- Prepare the PNK mixture as in Table 4.

Table 4. Reaction mixture for T4 polynucleotide kinase

Reagent	Volume (μ L)
10 $\times$ T4 PNK reaction buffer	40
100 mM ATP	4
RiboLock RNase	10
T4 PNK	20
Nuclease-free water	326

- Resuspend nuclei and incubate at 37 $^{\circ}$ C for 40 min in a thermo mixer with shaking at 500 rpm.

F. In situ ligation DNA and RNA (timing: overnight reaction)

- Wash the isolated nuclei twice with 500 μ L of 1 $\times$ wash buffer B, then perform in situ RNA ligation using the prepared ligation solution (Table 5).

Table 5. T4 RNA polymerase master mix

Reagent	Volume (μ L)
10 $\times$ T4 RNA ligase reaction buffer	40
Bridge linker (from section C)	50
RiboLock RNase	10
50% (w/v) PEG 8000	120
10% Triton X-100	4
T4 RNA Ligase 2 truncated KQ	20
Nuclease-free water	156

- Resuspend nuclei and incubate overnight at 16 $^{\circ}$ C using a Intelli-mixer RM-2L with rotation.
- Collect the nuclei by centrifugation at 2,500 $\times$ g for 3 min at 4 $^{\circ}$ C. Wash the pellet three times with 500 μ L of 1 $\times$ wash buffer B and add the reverse transcription solution. Perform reverse transcription as in Table 6.

Table 6. Reverse transcription mixture

Reagent	Volume (μL)
10× MMLV buffer	40
10mM dNTP	20
RiboLock RNase	10
MMLV (RNase H-)	20
Nuclease-free water	310

4. Resuspend nuclei and then incubate at 42 °C for 1 h in a ThermoMixer with shaking at 500 rpm.
5. Wash isolated nuclei three times with 500 μL of 1× wash buffer B and perform in situ DNA ligation solution (Table 7).

Table 7. T4 DNA polymerase master mix

Reagent	Volume (μL)
10× DNA ligase reaction buffer with 10 mM ATP	120
BSA (20 mg/mL)	6
10% Triton X-100	12
T4 DNA ligase	12
Nuclease-free water	1050

6. Resuspend the nuclei and incubate overnight at 16 °C with rotation.

G. Reversal crosslinking and RNA/DNA purification (timing: ~4.5 h)

1. Centrifuge at 2,500× g for 3 min at 4 °C and resuspend the nuclei in 500 μL of proteinase K solution buffer at 65 °C for 3 h with shaking at 800 rpm using a ThermoMixer.
2. Add an equivalent volume of phenol:chloroform:IAA (25:24:1) (pH 7.9).
3. Centrifuge a MaxTract high density tube at 13,000× g for 1 min and add the mixture from step G1 to the tube. Then centrifuge the tube at 13,000× g for 5 min.
4. Transfer the upper aqueous phase into a new 1.5 mL tube and add 2 μL of glycoblue, 1/10th volume of 3 M sodium acetate (pH 5.2), and an equivalent volume of ice-cold 100% (v/v) isopropanol. Mix well and incubate at -80 °C for 30 min.
5. Centrifuge at 13,000× g for 15 min at 4 °C to precipitate the nucleic acid pellet and wash twice with 75% cold ethanol. Air-dry the pellet and dissolve the nucleic acid pellet in 50 μL of nuclease-free water.

H. Gap-filling and second-strand cDNA synthesis (timing: ~3 h)

1. Prepare the Gap-filling mixture as in Table 8.

Table 8. Gap-filling reaction mixture

Reagent	Volume (μL)
10× NEBuffer r2.1	10
10 mM dNTP	8
DNA (from section G)	50
T4 DNA polymerase	6
Nuclease-free water	26

2. Mix and incubate at room temperature for 40 min, and then purify nucleic acid using AMPure XP beads. Dissolve the nucleic acid in 50 μL of nuclease-free water.
3. Prepare second-strand cDNA synthesis mixture (Table 9).

Table 9. Second-strand cDNA synthesis mixture

Reagent	Volume(μL)
NEBNext second strand synthesis reaction buffer	8
NEBNext second strand synthesis enzyme mix	4
DNA (from step H2)	50

Nuclease-free water

18

4. Mix and incubate at 16 °C for 1 h in a ThermalCycler and purify nucleic acid using AMPure XP beads. Dissolve the nucleic acid in 50 µL of QIAGEN Buffer EB. Measure the concentration of the product with Qubit.

I. DNA library preparation, PCR enrichment, and sequencing (timing: ~6 h)

1. Fragment the proximity-ligated nucleic acid (from section H) using the Tn5 transposome (from the TruePrep DNA Library Prep Kit for Illumina) according to the manufacturer's instructions (Table 10).

Table 10. Proximity ligation nucleic acid fragmentation mixture with Tn5 transposome

Reagent	Volume (µL)
50 ng proximity ligation nucleic acid	x
5× TTBL	10
Tn5 Tagmentation enzyme	5
Nuclease-free water	35 – x

2. Incubate at 55 °C for 10 min in a ThermalCycler and purify nucleic acid using DNA Clean & Concentrator-5. Dissolve the proximity ligation nucleic acid in 50 µL of nuclease-free water.

3. Mix the M280 Streptavidin–Dynabeads suspension thoroughly and transfer 30 µL of the M280 beads to a 1.5-mL LoBind tube.

4. Place the tube on the magnetic stand, discard the supernatant, and wash the beads twice with 200 µL of 2× B&W buffer.

5. Resuspend the M280 beads in 100 µL of iBlock buffer and incubate at room temperature for 45 min with shaking at 50 rpm on an Intelli-Mixer (UU, 50 rpm).

6. Place the tube on the magnetic rack, discard the iBlock buffer, and wash the M280 beads twice with 200 µL of 1× B&W buffer.

7. Resuspend the M280 beads in 100 µL of sheared DNA solution and incubate at room temperature for 30 min with shaking at 50 rpm on an Intelli-Mixer (UU, 50 rpm).

8. Place the tube on the magnetic rack, discard the sheared DNA solution, and wash the M280 beads twice with 200 µL of 1× B&W buffer.

9. Resuspend the M280 beads in 100 µL of proximity ligation nucleic acid and incubate at room temperature for 45 min with shaking at 50 rpm on an Intelli-Mixer (UU, 50 rpm).

10. Place the tube on the magnetic rack, discard the supernatant, and wash the M280 beads five times using 500 µL of 2× SSC/0.5% SDS. Then, wash the M280 beads two times using 500 µL of 1× B&W buffer.

11. Resuspend the M280 beads in 24 µL of nuclease-free water. Use the resuspended beads as the template for PCR amplification of the library, as in Table 11. Run the PCR amplification with the program described in Table 12.

Table 11. M280 beads as a template for PCR amplification

Reagent	Volume (µL)
Bead solution (from step I11)	24
5× TAB	10
Index primer (i7)	5
Index primer (i5)	5
PPM	5
TAE	1

Table 12. Thermal cycling conditions for library amplification

Temperature (°C)	Time (s)	
72	180	
98	30	
98	10	
60	30	10–12 cycles
72	180	
72	300	

12. Perform double-sided size selection using DNA Clean Beads as follows:

- Add 27.5 μ L (0.55 $\times$) of DNA clean beads to the 50 μ L sample. Mix thoroughly by pipetting and incubate at room temperature for 5 min.
- Place the tube on a magnetic rack and carefully transfer the supernatant to a new tube.
- Add 10 μ L (0.2 $\times$) of DNA Clean Beads to the supernatant. Mix well and incubate at room temperature for 5 min. Place the tube on the magnetic rack and discard the supernatant.
- Wash the beads twice with 80% ethanol (freshly prepared), keeping the tube on the magnetic rack during washing.
- Carefully remove all residual ethanol. Air-dry the beads at room temperature, avoiding over-drying.
- Resuspend the beads in 16 μ L of EB buffer and incubate for 5 min.
- Place the tube on the magnetic rack and transfer the supernatant to a new tube. This is the final TaDRIM-seq library (as shown in Figure 2).

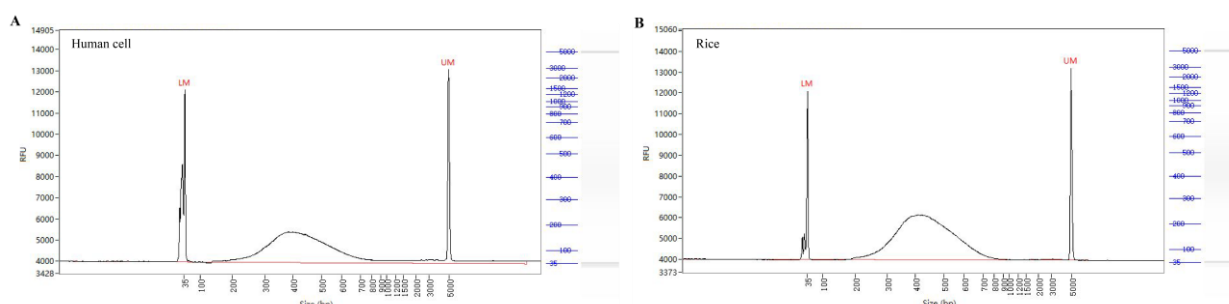


Figure 2. Size distribution of final TaDRIM-seq libraries. (A) Human cell. (B) Rice.

Data analysis

1. Use flash (version 1.2.11) and cutadapt (version 2.3) to split data into combined or independent reads and find the linker.

```
• flash --threads 20 -M 150 -O --output-prefix PREFIX.flash --output-directory PREFIX_R1.fq.gz PREFIX_R1.fq.gz
• cutadapt -b file:$Linker_file -n 14 --no-indels -o PREFIX.combine.noLinker --info-file PREFIX.combine.Linker_info --discard -O 35 PREFIX.flash.extendedFrgs.fastq > PREFIX.combine.stat
• cutadapt -b file:$Linker_file -n 8 --no-indels -o PREFIX.notCombined.1.noLinker --info-file PREFIX.notCombined.1.Linker_info --discard -O 35 PREFIX.flash.notCombined_1.fastq > PREFIX.notCombined.1.stat
• cutadapt -b file:$Linker_file -n 8 --no-indels -o PREFIX.notCombined.2.noLinker --info-file PREFIX.notCombined.2.Linker_info --discard -O 35 PREFIX.flash.notCombined_2.fastq > PREFIX.notCombined.2.stat
```

The Linker_file file should be in the following format:

A. CCTCGATACTTGAGCTGACAAGATGTGTATAAGAGACAG
B. CTGTCTCTTATACACATCTTGTCTCAGCTCAAGTATCGAGG

```
• perl ts_cutadapt2online.pl PREFIX.combine.Linker_info PREFIX.combine.cut.info > PREFIX.combine.cut.info.log
• perl ts_cutadapt2online.pl PREFIX.notCombined.1.Linker_info PREFIX.notCombined.1.cut.info > PREFIX.notCombined.1.cut.info.log
• perl ts_cutadapt2online.pl PREFIX.notCombined.2.Linker_info PREFIX.notCombined.2.cut.info > PREFIX.notCombined.2.cut.info.log
```

2. Separate RNA and DNA sequences based on the linker sequence.

```
• perl split.combine.pl PREFIX.combine.cut.info PREFIX 1> PREFIX.combine.fq.stat
2 > PREFIX.combine.fq.stat.log
• perl split.only.pl PREFIX.notCombined.1.cut.info PREFIX.notCombined.2.cut.info
PREFIX.only 1> PREFIX.only.fq.stat 2>PREFIX.only.fq.stat.log
• cat PREFIX.combine.RNA.fq PREFIX.only.RNA.fq > PREFIX.RNA.fastq
• cat PREFIX.combine.DNA.fq PREFIX.only.DNA.fq > PREFIX.DNA.fastq
```

3. Use bwa (version 0.7.15-r1140), hisat2 (version 2.1.0), and bowtie2 (version 2.3.5.1) to map reads to a reference genome, and make multi-align to only match. Use samtools (version 1.9) to convert a sm file to a bam file.

```
• perl split.pl PREFIX.DNA.fastq $OUTPUT_PREFIX 70
• bwa aln -t 20 -f PREFIX.DNA.short.sai $BWA_GENOME_INDEX PREFIX.DNA.short.fastq &&
bwa samse -f PREFIX.DNA.short.sam $BWA_GENOME_INDEX PREFIX.DNA.short.sai
PREFIX.DNA.short.fastq
• bwa mem -t 20 $BWA_GENOME_INDEX PREFIX.DNA.long.fastq > PREFIX.DNA.long.sam
• hisat2 --known-splicesite-infile $knownspl -p 20 -x $HISAT_GENOME_INDEX --rna-
strandness F -U PREFIX.RNA.fastq -S PREFIX.RNA.sam && python flt_bam.py
PREFIX.RNA.sam PREFIX.RNA && samtools sort -@ 20 -o PREFIX.RNA.hisat.bam
PREFIX.RNA.uniqmap.bam
• samtools view -Sb -F 256 PREFIX.RNA.multimap.bam | bamToFastq -i - -fq
PREFIX.tmp.multi.fq
• bamToFastq -i PREFIX.RNA.unmap.bam -fq PREFIX.tmp.unmap.fq
• cat PREFIX.tmp.multi.fq PREFIX.tmp.unmap.fq > PREFIX.remap.fq
• bowtie2 -p 20 --local --very-sensitive-local -x $BOWTIE2_GENOME_INDEX -U
PREFIX.remap.fq -S PREFIX.remap.sam && samtools view -Sb -q 2 PREFIX.remap.sam |
samtools sort -@ 20 -o PREFIX.remap.bam
• samtools merge -f PREFIX.RNA.bam PREFIX.RNA.hisat.bam PREFIX.remap.bam
• samtools index PREFIX.RNA.bam
```

4. Use samtools (version 1.9) to get the unique mapped reads.

```
• java -cp LGL.jar LGL.util.UniqueSam PREFIX.DNA.long.sam PREFIX.DNA.long.rmdup.sam
• python combine_DNA_file.py PREFIX.DNA.short.sam PREFIX.DNA.long.rmdup.sam
PREFIX.DNA.sam
• samtools view -Sb PREFIX.DNA.sam | samtools sort -@ 20 -o PREFIX.DNA.bam
• samtools index PREFIX.DNA.bam
```

5. Use samtools and deeptools (version 3.2.1) to convert the bam file to a BigWig file.

```
• samtools index PREFIX.DNA.bam
• samtools index PREFIX.RNA.bam
• bamCoverage -o PREFIX.DNA.bw --binSize 5 -b PREFIX.DNA.bam --numberOfProcessors
20 --minMappingQuality 20
• bamCoverage -o PREFIX.RNA.bw --binSize 5 -b PREFIX.RNA.bam --numberOfProcessors
20 --minMappingQuality 20
```

6. Merge DNA–RNA interaction according to read ID and extraction of high-confidence DNA–RNA interactions using the hypergeometric distribution test.

```
• bamToBed -cigar -i PREFIX.DNA.bam | awk -v OFS="\t" '{if($6=="+") {print
$1,$2,$2+500,$4,$5,$6,$7}else{start=$3-500;if(start<0){start=0};print
$1,start,$3,$4,$5,$6,$7}}' | intersectBed -a - -b $DNA_ANCHOR -wao | python
uniq_DNA.py > PREFIX.DNA.bed &
```

```

• htseq-count -o PREFIX.RNA.yes.gene.sam -f bam -r name -s yes -t gene -i ID -m
intersection-nonempty PREFIX.RNA.bam $gene_file > PREFIX.RNA.yes.gene.count
• samtools view -H PREFIX.RNA.bam | cat - PREFIX.RNA.yes.gene.sam >
PREFIX.RNA.yes.gene.cg.sam
• htseq-count -o PREFIX.RNA.yes.exon.sam -f sam -r name -s yes -t exon -i ID -m
intersection-nonempty PREFIX.RNA.yes.gene.cg.sam $exon_file >
PREFIX.RNA.yes.exon.count
• samtools view -H PREFIX.RNA.bam | cat - PREFIX.RNA.yes.exon.sam | perl -lane
'if (/XF:Z/) {$_ =~ s/XF:Z/GE:Z/; print $_} else {print $_}' > PREFIX.RNA.yes.sam
• python sam2bed2.py PREFIX.RNA.yes.sam 1> PREFIX.RNA.yes.bed
2>PREFIX.RNA.yes.bed.log
• python combineDNAandRNA_withanchor.py PREFIX.DNA.bed PREFIX.RNA.yes.bed
PREFIX.DNARNA.bedpe
• awk -v qu=$MAPPING_CUTOFF '$8>=qu' PREFIX.DNARNA.bedpe | sort --parallel=$NTHREADS
-k14,14 -k13,13 -k1,1 -k2,2n -k3,3n -k4,4 -k5,5n -k6,6n -k8,8n -k9,9 -k10,10 -k11,11
-k12,12 > PREFIX.DNARNA.sorted.bedpe
• python uniqread.py PREFIX.DNARNA.sorted.bedpe > PREFIX.DNARNA.uniq.bedpe
• python PetClusterWithGivenAnchors.py $DNA_ANCHOR $BED_file
PREFIX.DNARNA.uniq.bedpe | sort --parallel=$NTHREADS -k1,1 -k4,4 -k2,2n -k5,5n -
k3,3n -k6,6n > PREFIX.DNARNA.givenanchor.cluster
• awk '$9>1' PREFIX.DNARNA.givenanchor.cluster >
PREFIX.DNARNA.givenanchor.gt1.cluster
tmp=`wc -l PREFIX.DNARNA.uniq.bedpe | awk '{print $1}'`
• Rscript $PROGRAM_DIRECTORY/hypergeometric5.r
PREFIX.DNARNA.givenanchor.gt1.cluster
PREFIX.DNARNA.givenanchor.gt1.cluster.withpvalue
• awk '$13+0.0<=0.05' PREFIX.DNARNA.givenanchor.gt1.cluster.withpvalue >
PREFIX.DNARNA.givenanchor.FDRfiltered.txt
• awk '$9>2' PREFIX.DNARNA.givenanchor.FDRfiltered.txt >
PREFIX.DNARNA.givenanchor.FDRfiltered.gt2.txt
• cat PREFIX.DNARNA.givenanchor.FDRfiltered.gt2.txt | awk '{if ($1==$4 &&
($5+$6)>=($2+$3)) print $1"\t"$2"\t"$3"\t"$4"\t"$5"\t"$6"\t"$9"\t"$12"\t"$13}' >
PREFIX.givenanchor.cluster.intra.DNA-RNA.curve
• cat PREFIX.DNARNA.givenanchor.FDRfiltered.gt2.txt | awk '{if ($1==$4 &&
($5+$6)<($2+$3)) print $4"\t"$5"\t"$6"\t"$1"\t"$2"\t"$3"\t"$9"\t"$12"\t"$13}' >
PREFIX.givenanchor.cluster.intra.RNA-DNA.curve

```

The main code files used in the process of RNA–DNA data processing are as follows:

<https://github.com/fengchuiguo1994/ChRDPETpipeline/blob/main/run.sh>

<https://github.com/fengchuiguo1994/ChRDPETpipeline/blob/main/run.coverage.sh>

<https://github.com/fengchuiguo1994/ChRDPETpipeline/blob/main/run.interaction.sh>

TaDRIM data process pipeline to get RNA–RNA interaction information

1. First, merge fragments shorter than or equal to 290 bp in sequenced files (*.fq.gz) with FLASH (version 1.2.11).

```

• flash -M 145 -O --output-prefix PREFIX.flash --output-directory PREFIX_R1.fq
PREFIX_R1.fq

```

2. Use cutadapt (version 2.3) to identify the RNA–RNA bridge linker for merged (≤ 290 bp) and unmerged (> 290 bp) fragments.

```

• Use cutadapt to identify the RNA-RNA bridge linker for merged ( $\leq 290$  bp) and
unmerged ( $> 290$  bp) fragments.

```

```
• cutadapt -b file:$Linker_file -n 8 -e 0.2 --no-indels -o PREFIX.combine.noLinker
--info-file PREFIX.combine.Linker_info --discard -O 34
PREFIX.flash.extendedFrgs.fastq > PREFIX.combine.stat
• cutadapt -b file:$Linker_file -n 8 -e 0.2 --no-indels -o
PREFIX.notCombined.1.noLinker --info-file PREFIX.notCombined.1.Linker_info --
discard -O 34 PREFIX.flash.notCombined_1.fastq > PREFIX.notCombined.1.stat
• cutadapt -b file:$Linker_file -n 8 -e 0.2 --no-indels -o
PREFIX.notCombined.2.noLinker --info-file PREFIX.notCombined.2.Linker_info --
discard -O 34 PREFIX.flash.notCombined_2.fastq > PREFIX.notCombined.2.stat
```

The Linker_file file should be in the following format:

A. CCTCGATACTTGAGCTGACGTCAGCTCAAGTATCGAGG

B. CCTCGATACTTGAGCTGACGTCAGCTCAAGTATCGAGG

3. Quantitative profiling of RNA-RNA linker distributions in merged and non-merged paired-end reads

```
• perl linker_info_sta.pl PREFIX.combine.Linker_info PREFIX.combine.cut.info >
PREFIX.combine.cut.info.log
• perl linker_info_sta.pl PREFIX.notCombined.1.Linker_info
PREFIX.notCombined.1.cut.info > PREFIX.notCombined.1.cut.info.log
• perl linker_info_sta.pl PREFIX.notCombined.2.Linker_info
PREFIX.notCombined.2.cut.info > PREFIX.notCombined.2.cut.info.log
```

4. Extract tags from dual-end sequencing results with only one linker, where the lengths of both ends of the linker are greater than the threshold (13 bp).

```
• sh scriptdir/Extract_valid_data.sh PREFIX 13
```

5. Reverse complementary sequencing of the 3' end of the linker to obtain sequence-specific interaction information:

```
• python sequence_RC.py PREFIX.total-PET_SE_3nd.txt PREFIX.total-
PET_SE_3nd_FXHBcorrected.fq
```

6. Remove PCR replicates with identical PETs.

```
• python remove_duplication.py -r1 PREFIX.total-PET_SE_5nd.fq.gz -r2 PREFIX.total-
PET_SE_3nd_FXHBcorrected.fq.gz -o1 PREFIX.part1.fq -o2 PREFIX.part2.fq
```

7. Use hisat2 (version 2.1.0) to align the sequences interacting at both ends of the fragments to the reference genome indexed in advance.

```
• hisat2 -x ${HISAT_GENOME_INDEX} -U PREFIX.part1.fq -S PREFIX.part1.sam --summary-
file PREFIX.part1_hisat2_aln_out.txt
• hisat2 -x ${HISAT_GENOME_INDEX} -U PREFIX.part2.fq -S PREFIX.part2.sam --summary-
file PREFIX.part2_hisat2_aln_out.txt
```

8. Filter the interaction information: only sequencing records with high-quality ($Q > 20$) and uniquely mapped pair-end tags are retained.

```
• cat PREFIX.part1.sam | grep 'NH:i:1$' > PREFIX.part1_unique.sam
• cat PREFIX.part2.sam | grep 'NH:i:1$' > PREFIX.part2_unique.sam
• awk '{if($5>=20) print $0}' PREFIX.part1_unique.sam > PREFIX.part1_unique.q20.sam
• awk '{if($5>=20) print $0}' PREFIX.part2_unique.sam > PREFIX.part2_unique.q20.sam
• awk '{print $1}' PREFIX.part1_unique.q20.sam > IDtags1.txt
• awk '{print $1}' PREFIX.part2_unique.q20.sam > IDtags2.txt
• grep -Fwf IDtags2.txt PREFIX.part1_unique.sam > PREFIX.part1_PET_unique_aln.sam
• grep -Fwf IDtags1.txt PREFIX.part2_unique.sam > PREFIX.part2_PET_unique_aln.sam
```

9. Convert sam files to a bam format and perform name-based sorting.

```
• samtools view -H PREFIX.part1.sam > PREFIX.header.sam
• cat PREFIX.part1_PET_unique_aln.sam PREFIX.part2_PET_unique_aln.sam >
PREFIX.part_PET_unique_aln.sam
• cat PREFIX.header.sam PREFIX.part_PET_unique_aln.sam > PREFIX.PET_unique_aln.sam
• samtools view -bS PREFIX.PET_unique_aln.sam > PREFIX.PET_unique_aln.bam
• samtools sort -n -o PREFIX.PET_unique_aln_sort.bam PREFIX.PET_unique_aln.bam
```

10. Distinguish the interaction information between intra- and intermolecules.

```
• python class_bam_PairTag.py -g genome.element.bed -i
PREFIX.PET_unique_aln_sort.bam -o PREFIX.genome > PREFIX.genome.class.log
```

11. Convert the format of interaction information (from .bam to .bedpe).

```
• python IntraSam2bedpe.py -i PREFIX.genome.interaction.intraMolecular.bam -o
PREFIX.genome.interaction.intraMolecular.bedpe
• LANG=C sort -k1,1 -k8,8 -k2,2n -k3,3n -k5,5n -k6,6
PREFIX.genome.interaction.intraMolecular.bedpe >
PREFIX.genome.interaction.intraMolecular.sorted.bedpe
• python Interbam2bedpe.py -i PREFIX.genome.interaction.interMolecular.bam -o
PREFIX.genome.interaction.interMolecular.bedpe
```

12. Calculate and obtain intramolecular RNA–RNA interaction clusters.

```
• java -jar ClusterCalling.jar call
PREFIX.genome.interaction.intraMolecular.sorted.bedpe
PREFIX.genome.interaction.intraMolecular.cluster
```

13. Calculate the strength and significance of intramolecular RNA–RNA interaction clusters.

```
• java -jar ClusterCalling.jar count
PREFIX.genome.interaction.intraMolecular.sorted.bedpe
PREFIX.genome.interaction.intraMolecular.cluster
PREFIX.genome.interaction.intraMolecular.cluster.count
• Rscript Hypergeometric.r PREFIX.genome.interaction.intraMolecular.cluster.count
PREFIX.genome.interaction.intraMolecular.cluster.count.pvalue
```

14. Calculate and obtain intermolecular RNA–RNA interaction clusters.

```
• python getInteraction.py -i PREFIX.genome.interaction.interMolecular.bedpe.gz -o
PREFIX.total.bedpe.interaction.txt -l genome.element.bed
```

15. Randomly simulate intermolecular interaction information and use the randomly simulated interaction information to calculate the randomly simulated intermolecular RNA–RNA clusters as described previously in Cai et al. [12].

```
• python generateSim.py -i PREFIX.genome.interaction.interMolecular.bedpe.gz -o
sim/PREFIX.total.sim > PREFIX.total.sim.log
• for pp in $(seq ${Nsimu}); do pigz sim/PREFIX.total.sim.sim.${pp}.tmp.bedpe; done
• for pp in $(seq ${Nsimu}); do
python getInteraction.py -i sim/PREFIX.total.sim.sim.${pp}.tmp.bedpe.gz -o
sim/PREFIX.total.sim.sim.${pp}.tmp.bedpe.interaction.txt -l genome.element.bed
done
```

16. Calculate the significance of real intermolecular interaction clusters based on randomly simulated intermolecular interaction clusters.

```
python calpvalue.py -i PREFIX.total.bedpe.interaction.txt.gz -ip
sim/PREFIX.total.sim.sim -is tmp.bedpe.interaction.txt.gz -o PREFIX.total.rna-
rna.interaction.withpvalue.txt
```

The main code files used in the process of RNA–RNA data processing are as follows:

<https://github.com/304243504/HiR/tree/main/scriptdir>

<https://github.com/304243504/HiR/blob/main/getGeneElement.py>

<https://github.com/304243504/HiR/blob/main/Run.sh>

Validation of protocol

This protocol or parts of it has been used and validated in the following research article(s):

- Ding et al. [11]. Simultaneous profiling of chromatin-associated RNA at targeted DNA loci and RNA–RNA Interactions through TaDRIM-seq.

General notes and troubleshooting

General notes

1. Before starting the experiment, wipe down the bench surface thoroughly with RNase remover and nucleic acid decontamination reagent to ensure an RNase-free environment.
2. For TaDRIM-seq library preparation, it is recommended to perform 4–6 tagmentation reactions, each using 200–300 ng of proximity-ligated nucleic acids.
3. The 50% (w/v) PEG-8000 solution is highly viscous. Mix the bridge linker and enzyme thoroughly first and add PEG-8000 last to ensure proper reagent distribution and reaction efficiency.
4. If the experiment focuses on RNA–DNA interactions, the primer and bridge linker can be pre-designed as follows:

Bridge linker:

Forward strand: 5'-[5Phos]CCTCGATAC/iBIOdT/TGAGCTGACT-3'

Reverse strand: 5'-[5Phos]GTCAGC/iBIOdT/CAAGTATCGAG-3'

Primer: primer A (5'-phos-CTGTCTCTTATACACATCTA-3')

primer B (5'-phos-AGATGTGTATAAGAGACAG-3')

5. The ratio of RNA–DNA to DNA–DNA interactions may vary significantly across different species and tissue types.
6. RNA and DNA end specificity are two important characteristics for evaluating the library and the data. The DNA fragment data should align with the corresponding protein ChIP-seq or CUT&Tag sites, while RNA can reflect the transcriptional direction (as shown in Figures 3 and 4).

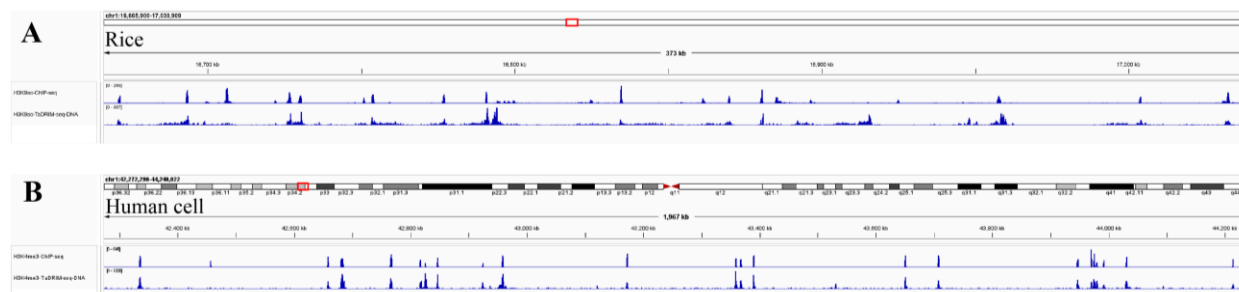


Figure 3. Genome browser tracks showing the distribution of DNA from H3K4me3 and H3K9ac TaDRIM-seq data in (A) rice and (B) human cells

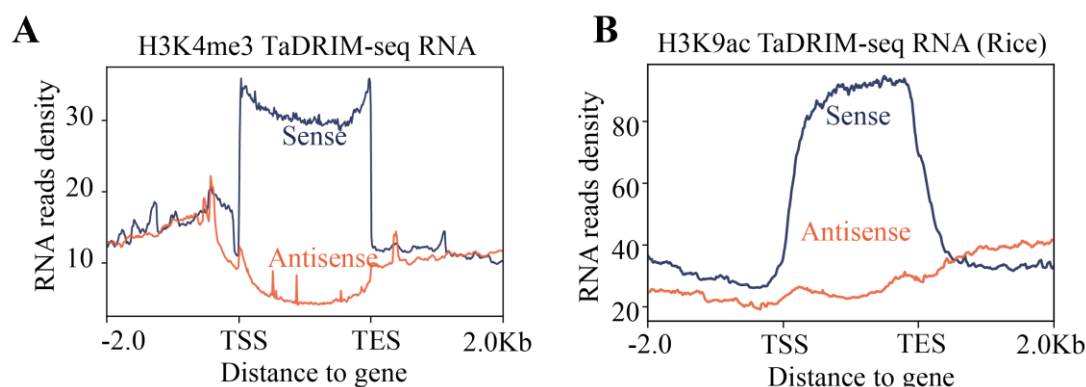


Figure 4. Strand orientation of TaDRIM-seq sense and antisense RNA. (A) H3K4me3 TaDRIM-seq RNA in human cell. (B) H3K9ac TaDRIM-seq RNA in rice.

Troubleshooting

1. Low complexity of the library

Potential solution: Reduce the number of PCR cycles and increase the amount of input DNA during library preparation.

2. Poor DNA-end specificity

Solution: Replace the antibody if necessary or increase the number of washes in step D3 during pG-Tn5 transposase binding and tagmentation to improve specificity.

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Authors' contribution

Conceptualization, C.D., X.L.; Investigation, G.Y., C.G., C.Y., Z.X., J.D.; Writing—Original Draft, C.D., G.C., S.L.; Writing—Review & Editing, C.D., G.C., M.F.F.; Funding acquisition, J.Y., X.L.; Supervision, J.Y., X.L.

Competing interests

Authors declare that they have no competing interests.

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