Advanced Technologies for 3D Genome Research

COLD SPRING HARBOR ASIA

Organized by:

Yijun Ruan, Zhejiang University

Minji Kim, University of Michigan

Wei Xie, Tsinghua University

Suzhou, China

July 15 – July 22, 2024

Advanced Technologies for 3D Genome Research

COLD SPRING HARBOR ASIA

Yijuan Ruan *Minji Kim* *Wei Xie*

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Welcome to

Advanced Technologies for 3D Genome Research summer school

Greetings from Dr. Yijun Ruan,

I am thrilled to welcome you to Cold Spring Harbor Asia for our inaugural summer course on 3D Genome Biology, happening from July 15 to July 22, 2024. This marks a significant milestone as it is the first time that we are offering this course in China, and I am excited about the opportunities it presents in this extraordinary facility.

We have planned an exceptional week, providing hands-on technical training, in-depth discussions, and featuring enlightening lectures on the latest developments in the field aimed to reinforce central ideas and inspire new ones. The lectures will provide you with valuable insights into cutting-edge research and advancements, helping to enhance your basic knowledge and broaden your perspectives in 3D genome biology.

I am grateful for your interest in the advanced methods to study long-range chromatin interactions and map the 3D genome organization. We will be covering both ChIATAC and Hi-C techniques, offering details of experimental protocols, data processing pipeline and analysis strategies. This course aims to equip you with the skills and knowledge to expand your research capability to explore unresolved biological questions.

Thank you for attending. I am confident you will have an enriching experience and look forward to the discoveries made through your participation in this course.

Best regards,

Dr. Yijun Ruan

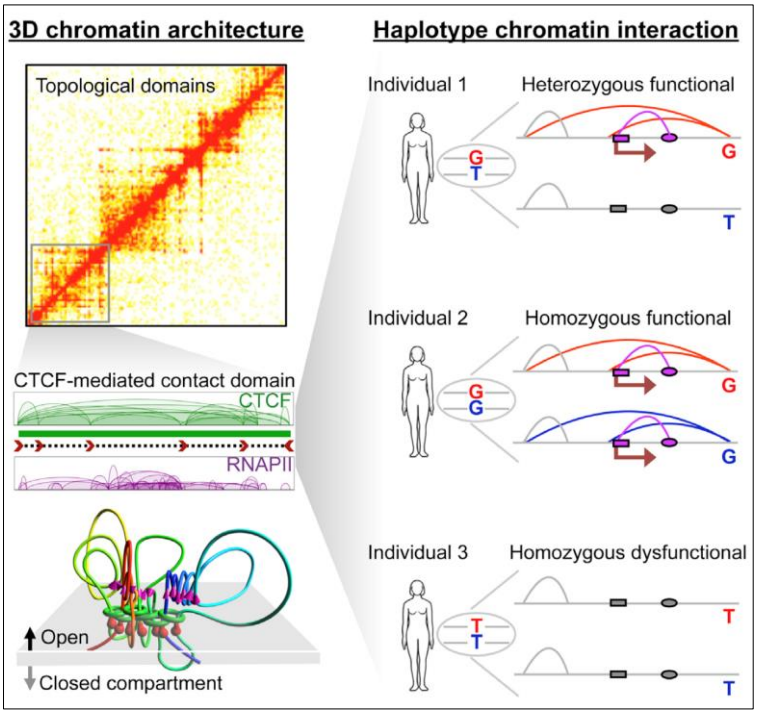
Life Sciences Institute, Zhejiang University

Cold Spring Harbor Asia

Introduction to

3D Genome Research Methods:

ChIATAC and Hi-C



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Schedule

DAY ONE: 16th July 2024

09:00-09:30	**OPENING REMARK** Drs. Yijun Ruan, Minji Kim
09:30-10:30	**LECTURE** | 3D Genome: Past, Present and Future Dr. Yijun Ruan
10:30-10:40	——— *Coffee break*
10:40-11:40	**LECTURE** | Molecular communications among cohesin, CTCF, and RNA Polymerase II Dr. Minji Kim
12:00-13:00	——— *Lunch*
13:30-15:30	**LECTURE** | 3D genome mapping 101: A brief introduction Dr. Haoxi Chai
15:30-17:00	**LAB WORK DAY 1** | Cell lysis, permeabilization, and restriction digestion
18:00-19:00	——— *Dinner*
19:00-20:00	**LECTURE** | Nuclear architecture through biomolecular condensates Dr. Jurian Schuijers
20:00-21:00	**LECTURE** | Condensates in transcriptional regulation Dr. Jurian Schuijers

DAY TWO: 17th July 2024

09:00-10:00	**LECTURE** | 3D genome in development Dr. Cheng Li
10:30-10:40	——— *Coffee break*
10:40-11:40	**LECTURE** | 3D genome, transcription factor and chromatin accessibility Dr. Cheng Li
12:00-13:00	——— *Lunch*
13:00-15:30	**LAB WORK DAY 2** | A tailing, quenching, and proximity ligation
18:00-19:00	——— *Dinner*

19:00-20:00	**LECTURE** | 3D mapping technologies Dr. Dong Xing
20:00-21:00	**LECTURE** |Single-cell 3D mapping technologies Dr. Dong Xing

DAY THREE: 18th July 2024

09:00-12:00	**LAB WORK DAY 3** | *In situ* transposition for ChIATAC/DNA purification for Hi-C
12:00-13:00	——— *Lunch*
13:00-18:00	**LAB WORK DAY 3** | Library preparation, QC
18:00-19:00	——— *Dinner*
19:00-20:00	**LAB WORK DAY 3** | Q&A

DAY FOUR: 19th July 2024

09:00-10:15	**LECTURE** | Algorithms to analyze single-cell and bulk-cell 3D genomics data Dr. Minji Kim
10:15-10:30	——— *Coffee break*
10:30-11:10	**LECTURE** | Loop extrusion: the one-sided model Dr. Minji Kim
12:00-13:00	——— *Lunch*
13:00-14:00	**LECTURE** | 3D genome in DNA replication Dr. Jiazhi Hu
14:00-14:10	——— *Coffee break*
14:10-15:10	**LECTURE** | 3D genome in antibody development Dr. Jiazhi Hu
18:00-19:00	——— *Dinner*
19:00-20:00	**LECTURE** | 3D genome mapping by imaging Dr. Haifeng Wang
20:00-21:00	**LECTURE** | 3D genome and CRISPR-Cas9 Dr. Haifeng Wang

DAY FIVE: 20th July 2024

09:00-10:00	**LECTURE** | Polymer modeling of chromatin Dr. Dariusz Plewczynski
10:00-10:10	——— *Coffee break*
10:10-11:10	**LECTURE** | Artificial intelligence methods to predict 3D interactions and epigenomic features & expression Dr. Dariusz Plewczynski
12:00-13:00	——— *Lunch*
13:00-14:00	**LECTURE** | 3D genome in biomedical application Dr. Yanfen Zhu
14:00-14:10	——— *Coffee break*
14:10-15:10	**LECTURE** | Trans and multi-way chromatin interactions Dr. Yanfen Zhu
15:30-17:30	**LECTURE** | ChIA-PET data analysis Dr. Guoliang Li
18:00-19:00	——— *Dinner*

DAY SIX: 21th July 2024

09:00-12:00	**LECTURE** | RNA-chromatin interactions in 3D genome Dr. Xiang-Dong Fu
12:00-13:00	——— *Lunch*
13:30-14:30	**LECTURE** | Hi-C data analysis Dr. Guoliang Li
15:00-16:00	**LECTURE** | 3D genome in embryo development Dr. Wei Xie
16:00-16:10	——— *Coffee break*

16:10-17:10	**LECTURE** | 3D genome in transcription regulation Dr. Wei Xie
18:00-19:00	——— *Dinner*
19:00-21:00	**LECTURE** | Integrative data analysis Dr. Guoliang Li

DAY SEVEN: 22th July 2024

09:00-9:30	**CONCLUDING REMARK** Dr. Wei Xie
10:00-15:00	——— *Tour*
18:00-19:00	——— *Dinner*
19:00-21:00	——— *Social hours*

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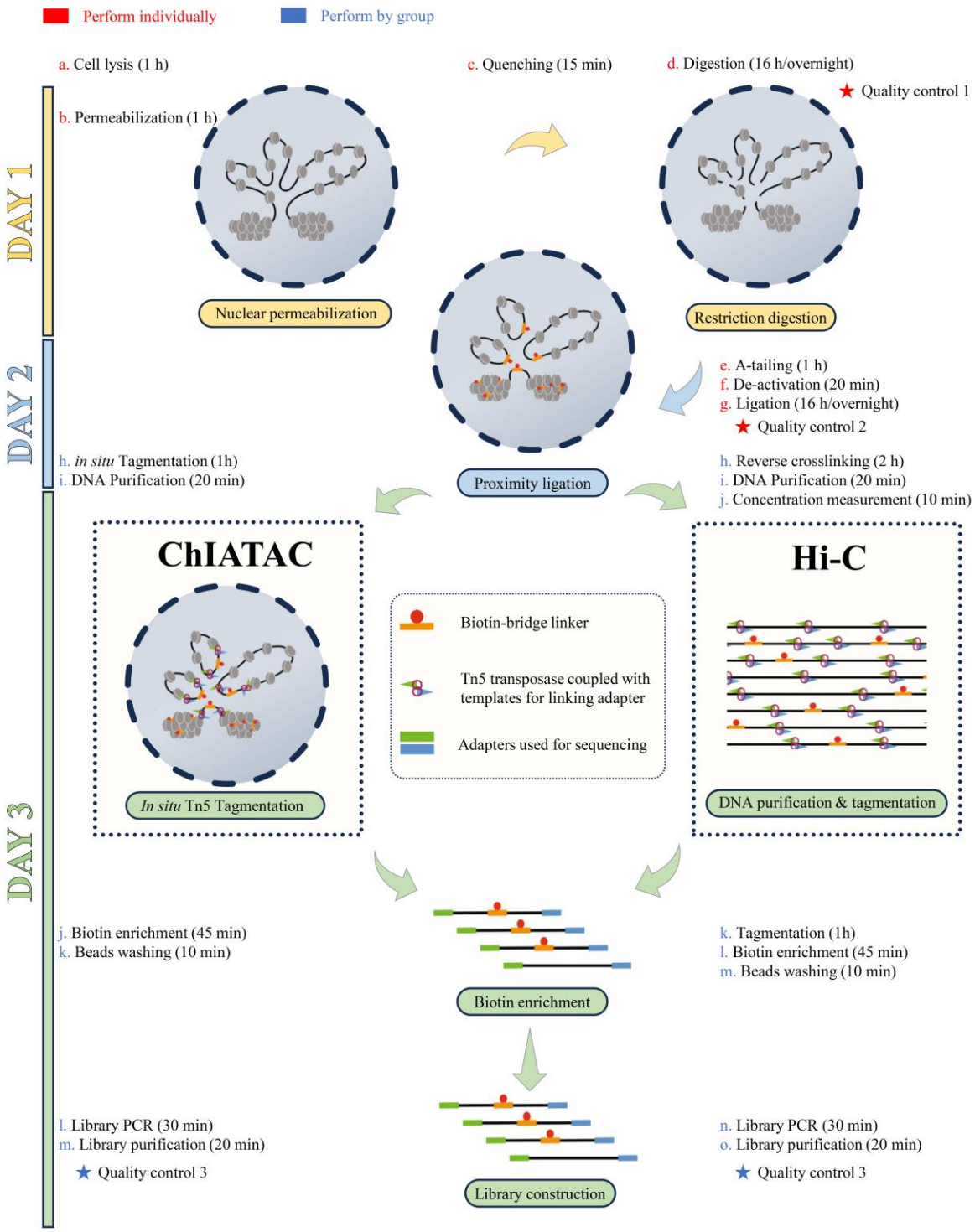
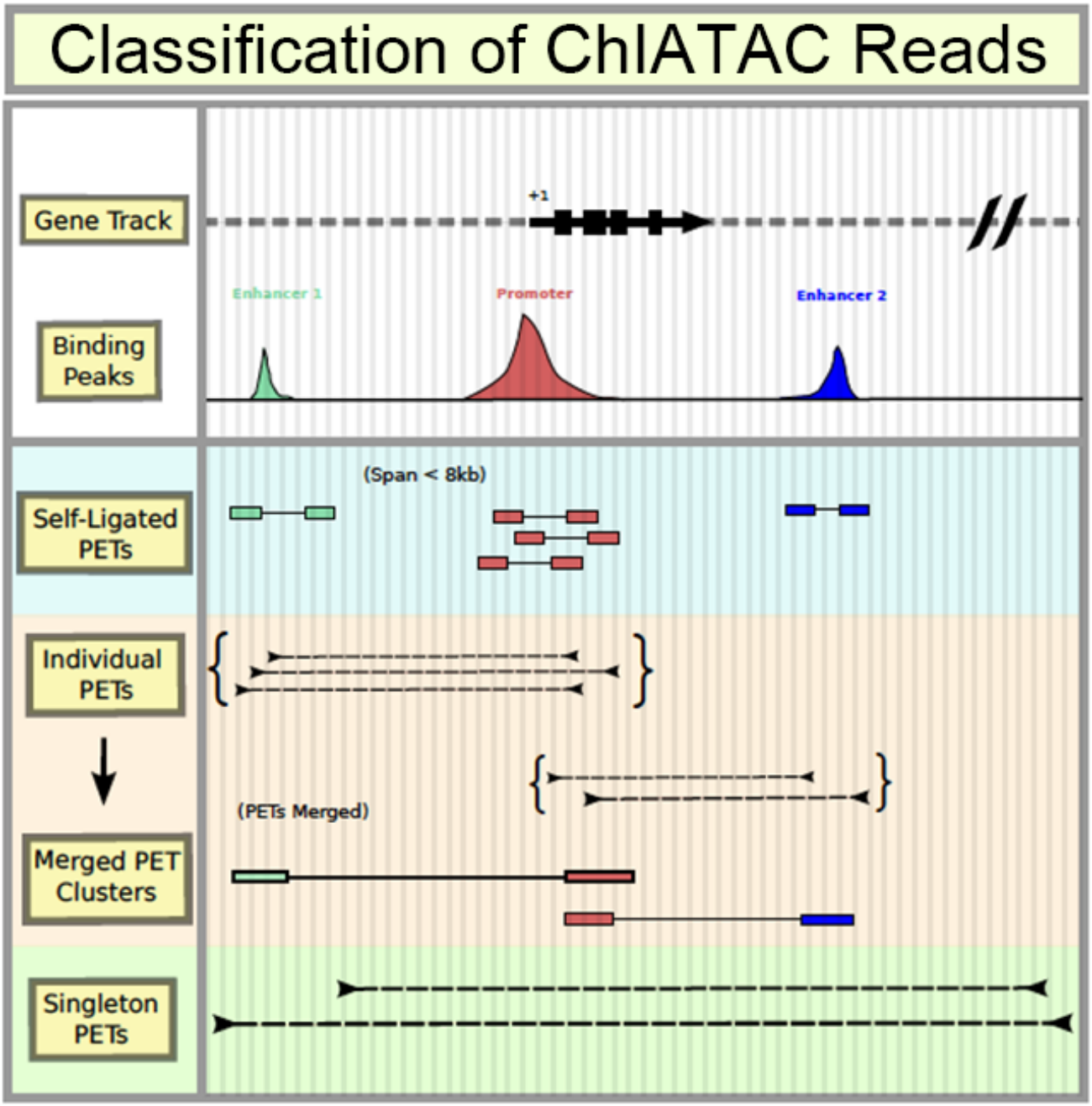
ChIATAC and Hi-C:
Workshop Protocol Overview

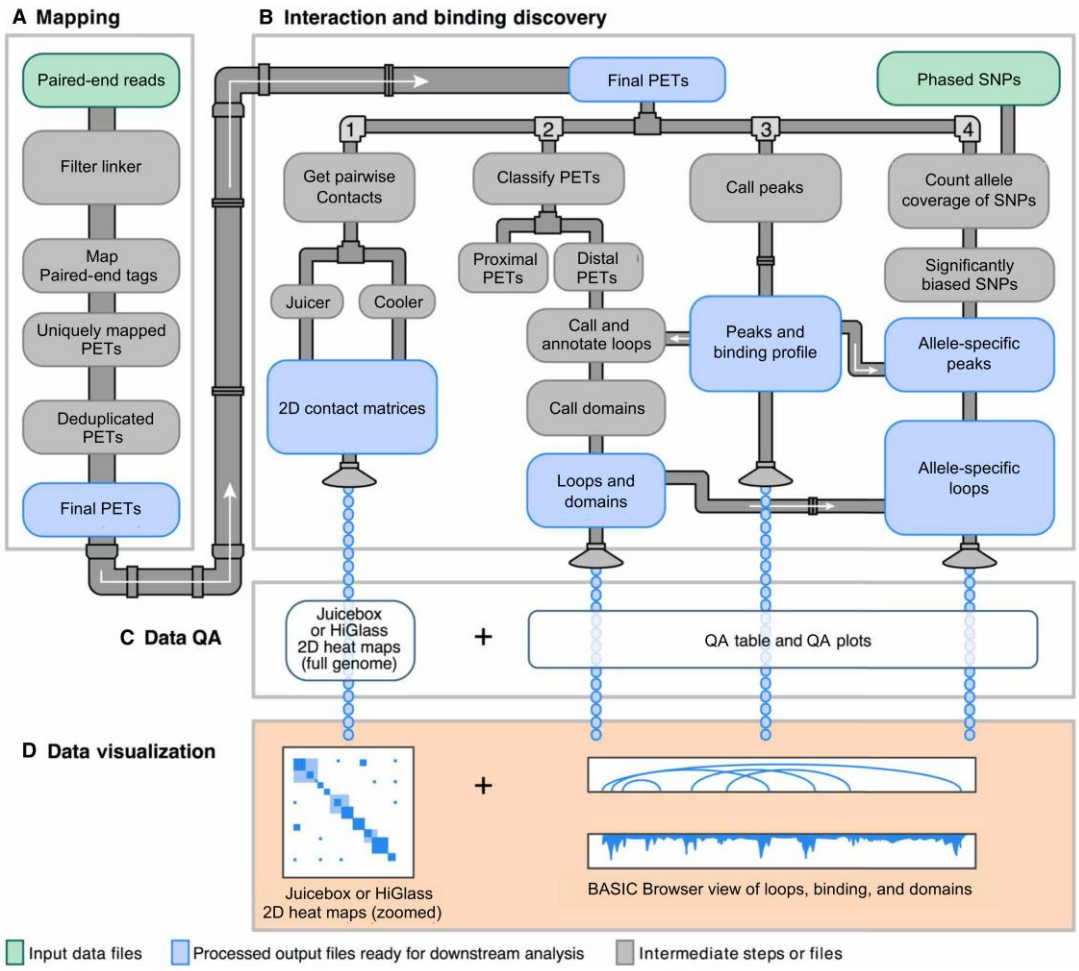


ChIATAC and Hi-C:
Data Processing Overview

Features and categories of ChIATAC tag sequence data, and mapping outcomes.



Features and categories of ChIATAC tag sequence data, and mapping outcomes.



Software Used in Data Processing Pipeline

1. *Linker filtering*: cutadapt (*https://code.google.com/p/cutadapt/*)
2. *Mapping*: BWA (*http://bio-bwa.sourceforge.net/bwa.shtml*)
3. *PCR Duplication Removal*: Picard (*http://broadinstitute.github.io/picard/*)
4. *Clustering*: In-house Perl script utilizing Samtools and Bedtools
5. ChIA-PET protocol; ChIATAC protocol; ChIA-PIPE (*http://www.ruanlab.org/?about_6/*)

Glossary of terms

PET: Paired-End Tag, a set of short sequences at either end of a DNA fragment that are unique enough such that the fragment can be mapped to the reference genome and the distance between them can be determined.

Self-Ligation PET: A PET derived from a single ChIP fragment. To qualify, both tags of the PET are mapped to the same chromosome with a span of less than 8kb, expected self-ligation orientation, and on opposite strands.

Inter-Ligation PET: When the criteria of self-ligation PET cannot be met, the PET is interpreted as being derived from two ChIP fragments.

Intrachromsomal: A term used to describe genomic interactions that take place within a single chromosome.

Intrachromsomal Inter-Ligation PETs: PETs that are classified as intrachromosomal and more than 8kb from each other (in any orientation).

Interchromosomal: A term used to describe genomic interactions that take place between two different chromosomes.

Resources

Ruan Lab Website: http://www.ruanlab.org

Kim Lab Website: https://www.minjilab.com/group

Xie Lab Website: https://www.xielab.org.cn/

Agilent Bioanalizer Manual: http://gcf.pbrc.edu/docs/Agilent/Agilent%20Manual.pdf

Illumina Manual: http://supportres.illumina.com/documents/documentation/system_documentation/miseq/miseq_system_user_guide_15027617_l.pdf

Qubit Manual: http://www.ebc.uu.se/digitalAssets/176/176882_3qubit2fluorometerusermanual.pdf

Protocol of both ChIATAC and Hi-C

Day1: cell lysis, permeabilization and restriction digestion

Cell lysis

1. Take out a 50,000 dual crosslinked cell pellet from -80 °C freezer, leave it on ice for 20 min to thaw the cells, centrifuge the tube at 2,500 × g, 4°C, 5min and carefully discard the supernatant.
2. Resuspend cells with 100 µl 0.1% SDS cell lysis buffer (+ Proteinase inhibitor (1x PI)) and keep on ice for 1 h.

*To prepare PI: dissolve 1 tablet in 500 ul H_2O to make 100x stock, store at -20 °C.

3. Centrifuge the tube at 2,500 × g, 4 °C for 5 min and then carefully discard supernatant (leave~ 5µl).

Cell permeabilization

4. Resuspend the cell pellet with 10 µl 0.0125% SDS solution (+1x PI).

The condition for nuclei permeabilization (incubation time and SDS concentration) has to be optimized for different cells used. For GM12878, incubate cells with 0.1% SDS + PI for 1.5 h at RT with agitation (UU25).

5. Incubate the tube at RT for 1 h.
6. Add 2.5 µl 20% Triton X-100 solution to the tube.
7. Incubate the tube at 37 °C for 15 min with agitation to quench SDS (UU25).

Restriction enzyme digestion

8. Prepare the restriction enzyme digestion

Component	Volume per reaction
Sample (from step 7)	17.5 µl
10× NEB CutSmart buffer	5 µl
AluI	1 µl
HpyCH4V	1 µl
ddH_2O	25.5 µl
Total volume per reaction	**50 µl**

9. Incubate the tube at 37 °C for at least 2 h or overnight with agitation (UU25).

Day 2: A-tailing & *in situ* Proximity ligation

*Perform the first quality control test (QC #1):

a. Prepare decrosslinking reaction

Note: Pipette mix thoroughly before transferring digestion sample.

Component	Volume per reaction
Digestion sample (step 9)	1 µl
TE buffer (pH 8.0)	39 µl
Recombinant Proteinase K solution (20 mg/ ml)	10 µl
Total volume per reaction	**50 µl**

b. Pipette mix and incubate for 2 h at 65 °C, 900rpm

c. Purify the DNA with Zymo DNA Clean and Concentrator-5 Kit.

A-tailing

10. Set up A-Tailing reaction on ice

Component	Volume per reaction
Restriction digested sample (step 9)	50 µl
BSA (2 mg/ml)	1 µl
1 mM dATP	0.6 µl (1 µl for K562)
Klenow Large fragment (3'→5' exo-)	1 µl
Total volume per reaction	**52.6 µl**

11. Mix well, incubate the tube at 37 °C for 1 h with agitation (UU25).
12. Incubate the tube at 65 °C for 20 min without agitation.

in situ Proximity ligation

13. Prepare the proximity ligation reaction at RT.

Component	Volume per reaction
A-tailed sample (from step 12)	52.6 µl
Bridge linker (2 ng/µl)	3 µl
T4 DNA ligase	1 µl
Nuclease-free water	33.4 µl
NEB 10× buffer for T4 ligase	10 µl
Total volume per reaction	**100 µl**

14. Mix well and incubate the tube at RT for at least 4 h or at 16 °C for overnight ligation with agitation (UU25).

Day3: ChIATAC

*Transfer **1 µl of the ligation sample** to a new tube. Perform the second quality control test same as QC #1

***In situ* tagmentation**

15. Centrifuge the tube at 2,500 × g, 4°C, for 5 min, carefully remove the supernatant (leave ~10 µl).
16. Wash with 100 µl ATAC washing buffer once.

Component	Volume per reaction
Tris-HCl (1 M pH7.5)	10 µl
NaCl (5 M)	2 µl
$MgCl_2$ (1 M)	3 µl
Tween-20 (10%)	10 µl
ddH_2O	975 µl
Total volume per reaction	**1000 µl**

17. Centrifuge the tube at 2,500 × g, 4 °C, carefully remove the supernatant (leave ~5 µl).
18. Resuspend cells in 14 µl 1x DPBS.
19. Add 25 µl 2× TDB buffer, and 6 µl TDE enzyme.

Component	Volume per reaction
ATAC-washed sample	5 µl
1x DPBS	14 µl
2× TDB (buffer)	25 µl
TDE (enzyme)	6 µl
Total volume per reaction	**50 µl**

20. Incubate at 37 °C for 1 h, 1000 rpm.
21. Purify the DNA by using Zymo DNA Clean and Concentrator-5 Kit, elute with 50 µl EB buffer.

Biotin enrichment

Preparation of iBlocked beads

22. Equilibrate M-280 streptavidin dynabeads to RT for 30 min, fully resuspend and transfer 30 µl beads into a new tube.
23. Place tube on magnetic stand, discard supernatant and wash beads with 150 µl 2× Binding & Washing buffer twice.
24. Resuspend beads in 100 µl iBlock Buffer, mix and incubate at RT for 45 min with agitation (UU, 50rpm)
25. Discard iBlock buffer with the help of magnetic stand, wash beads with 200 µl of 1× Binding and Washing buffer twice.
26. Discard wash buffer, then add the 100 µl blocking mixture (500 ng genomic DNA in 50 µl nuclease-free water + 50 µl 2× Binding and Washing buffer) to reduce non-specific DNA with no biotin labelling binding to the M-280 beads (The genomic DNA can be prepared from any species).
27. Mix well with the iBlocked beads, then incubate for 30 min with agitation at RT. (UU, 50rpm)
28. Discard supernatant, wash beads with 200 µl of 1× Binding and Washing buffer twice.

Enrichment of biotin labeled DNA fragments

29. Add purified DNA (50 µl) to the tube, add 50 µl 2× Binding and Washing buffer, mix well, incubate at RT for 45 min with agitation. (UU, 50rpm)
30. Short spin the tube, place tube on magnetic stand, discard supernatant, wash beads with 500 µl 0.5% SDS/ 2× SSC buffer five times.

    Keep M280 beads on the magnetic stand all times during wash.

31. Wash the M-280 beads with 500 µl 1× Binding and Washing buffer twice.

    Keep M280 beads on the magnetic stand all times during wash.

32. Discard supernatant, resuspend beads in 45 µl EB buffer.

Library construction

33. Prepare the Illumina library PCR reaction on ice.

Component	Volume per reaction
DNA Library-coated beads	15 µl
NEBNext® High-Fidelity 2× PCR Master Mix	25 µl
Index Primer 1 (i5)	5 µl
Index Primer 2 (i7)	5 µl
Total volume per reaction	**50 µl**

34. Amplify *in situ* ChIATAC libraries using the follow PCR conditions:

Temperature	Time	Cycles
72 °C	3 min	1
98 °C	30 s	1
98 °C	10 s	12
63 °C	30 s	
72 °C	40 s	
72 °C	5 min	1
4 °C	∞	1

Library purification

35. Transfer 50 µl PCR product supernatant from the reaction tube to a new tube with the help of magnetic stand.

36. Purify the DNA by using 1x (50 µl) SPRI beads.

37. Perform PCR reactions using the rest DNA Library-coated beads as mentioned above.

38. Combine all PCR products (3 times), remove primer dimers and short fragments lacking contact information by using 0.8x SPRI beads.

39. Measure the concentration of the PCR product with Qubit and check the DNA profile with a Bioanalyzer2100 HS DNA chip.

Day3: Hi-C

*Transfer **1 µl of the ligation sample** to a new tube. Perform the second quality control test same as QC #1

Reverse crosslinking and DNA purification

1. Centrifuge the tube at 2,500 × *g*, 4°C, for 5 min, carefully remove the supernatant (leave ~10 µl).
2. Add 70 µl TE buffer (pH 8.0), 10 µl Proteinase K, and 10 µl 10% SDS. Mix well, incubate at 65 °C with 900 rpm shaking for 2 h.
3. Purify the DNA by using Zymo DNA Clean and Concentrator-5 Kit, elute with 50 µl EB buffer.

***In vitro* tagmentation**

4. Prepare the *in vitro* tagmentation reaction on ice.

Component	Mass / Volume per reaction
The purified DNA	50 ng
2× TDB (buffer)	25 µl
TDE (enzyme)	8.5 µl
ddH_2O	Up to 50 µl
Total volume per reaction	**50 µl**

5. Set up the thermal cycling conditions for tagmentation.

Temperature	Time
4 °C	∞
55 °C	10 min
4 °C	∞

Load the tubes into the thermal cycler, then start to run.

6. Purify the DNA with Zymo DNA Clean and Concentrator-5 Kit, elute with 50 µl EB buffer.

Biotin enrichment

Preparation of iBlocked beads

7. Equilibrate M-280 streptavidin dynabeads to RT for 30 min, fully resuspend and transfer 30 µl beads into a new tube.
8. Place tube on magnetic stand, discard supernatant and wash beads with 150 µl 2× Binding & Washing buffer twice.
9. Resuspend beads in 100 µl iBlock Buffer, mix and incubate at RT for 45 min with agitation (UU, 50rpm)
10. Discard iBlock buffer with the help of magnetic stand, wash beads with 200 µl of 1× Binding and Washing buffer twice.
11. Discard wash buffer, then add the 100 µl blocking mixture (500 ng genomic DNA in 50 µl nuclease-free water + 50 µl 2× Binding and Washing buffer) to reduce non-specific DNA with no biotin labelling binding to the M-280 beads.
    The genomic DNA can be prepared from any species.
12. Mix well with the iBlocked beads, then incubate for 30 min with agitation at RT. (UU, 50rpm)
13. Discard supernatant, wash beads with 200 µl of 1× Binding and Washing buffer twice.

Enrichment of biotin labeled DNA fragments

14. Add purified DNA (50 µl) to the tube, add 50 µl 2× Binding and Washing buffer, mix well, incubate at RT for 45 min with agitation. (UU, 50rpm)
15. Short spin the tube, place tube on magnetic stand, discard supernatant, wash beads with 500 µl 0.5% SDS/ 2× SSC buffer five times.
    Keep M280 beads on the magnetic stand all times during wash.
16. Wash the M-280 beads with 500 µl 1× Binding and Washing buffer twice.
    Keep M280 beads on the magnetic stand all times during wash.
17. Discard supernatant, resuspend beads in 45 µl EB buffer.

Library construction

18. Prepare the Illumina library PCR reaction on ice.

Component	Volume per reaction
DNA Library-coated beads	15 µl
NEBNext® High-Fidelity 2× PCR Master Mix	25 µl
Index Primer 1 (i5)	5 µl
Index Primer 2 (i7)	5 µl
Total volume per reaction	**50 µl**

19. Amplify Hi-C libraries using the follow PCR conditions:

Temperature	Time	Cycles
72 °C	3 min	1
98 °C	30 s	1
98 °C	10 s	12
63 °C	30 s	
72 °C	40 s	
72 °C	5 min	1
4 °C	∞	1

Library purification

20. Transfer 50 µl PCR product supernatant from the reaction tube to a new tube with the help of magnetic stand.
21. Purify the DNA by using 1x (50 µl) SPRI beads.
22. Perform PCR reactions using the rest DNA Library-coated beads as mentioned above.
23. Combine all PCR products (3 times), remove primer dimers and short fragments lacking contact information by using 0.8x SPRI beads.
24. Measure the concentration of the PCR product with Qubit and check the DNA profile with a Bioanalyzer2100 HS DNA chip.

Appendix

Materials

Dulbecco's phosphate-buffered saline (DPBS), calcium- and magnesium-free (Gibco, cat. no. 14190-250)
0.1% SDS cell lysis buffer (see recipe)
cOmpleteTM Mini EDTA-free Protease Inhibitor Cocktail (PI; Roche, cat. no. 11836170001)
10% Triton X-100, molecular biology grade (Sigma-Aldrich, cat. no. 648464)
10× CutSmart buffer (New England Biolabs)
AluI restriction enzyme (NEB, cat. no. R0137L)
HpyCH4V restriction enzyme (NEB, cat. no. R0620L)
TE buffer, pH 8.0, RNase-free (Thermo Fisher Scientific, cat. no. AM9858)
10 mM dATP solution (NEB, cat. no. N0400S)
Agilent DNA High-Sensitivity Kit (Agilent Technologies, cat. no. 5067-4626)
Bovine serum albumin (BSA, molecular-biology grade, 20 mg/ml; NEB, cat. no. B9000S)
Nuclease-free water (Thermo Fisher Scientific, cat. no. AM9932)
Klenow Fragment (3'→5' exo-) (NEB, cat. no. M0212L)
NEB 10× buffer for T4 ligase (NEB, cat. no. B0202S)
Bridge linker, 2 ng/µl
T4 DNA ligase (NEB, cat. no. M0202L)
Dynabeads Protein G beads for immunoprecipitation (Thermo Fisher Scientific, cat. no. 10009D)
Buffer EB (Qiagen, cat. no. 19086)
Qubit® dsDNA HS Assay Kit (Thermo Fisher Scientific, cat. no. Q32854)
Nextera XT Index Kit v2 Set A (Illumina, cat. no. FC-131-2001)
Illumina Tagment DNA Enzyme and Buffer Large Kit (Illumina, cat. no. 20034198)
Dynabeads M-280 Streptavidin (Thermo Fisher Scientific, cat. no.11205D)
2x Binding & Wash buffer (see recipe)
iBlock buffer (see recipe)
Sheared genomic DNA mixture (see recipe)
2× SSC/0.5% (w/v) SDS (see recipe)
NEBNext® High-Fidelity 2x PCR Master Mix (NEB, cat. no. M0541S)
SPRI beads

80% ethanol

DNA LoBind Tubes (1.5 ml, Eppendorf, cat. no. 022431021)

DNA LoBind Tubes (0.5 ml, Eppendorf, cat. no. 022431005)

DynaMag-2 Magnet (magnetic stand; Thermo Fisher Scientific, cat. no. 12321D)

Qubit® Assay tubes (Thermo Fisher Scientific, cat. no. Q32856)

0.2-ml PCR tubes PCR machine (BioRad, C1000 Touch Thermal Cycler)

DNA Clean & Concentrator-5 kit (Zymo Research, cat. no. D4014)

RM-2M Intelli-Mixer, Medium (ELMI, cat. no. IMIX-02)

Buffers and Reagents:

0.1% SDS cell lysis buffer

Final concentration of chemicals	stock	For 50ml
50 mM HEPES-KOH pH 7.5	1M	2.5ml
150 mM NaCl	5M	1.5ml
1 mM EDTA	0.5M	100ul
1% (w/v) Triton X-100	100%	500ul
0.1% (w/v) sodium deoxycholate	10%	500ul
0.1% (w/v) SDS	10%	500ul
ddH_2O	-	44.4ml

Store at 4 °C for several months.

10% (wt/vol) sodium deoxycholate (100 ml) Add 10 g of sodium deoxycholate to 80 ml of water and mix it well until the solution is clear; then bring the final solution volume to 100 ml with water. The buffer can be stored at room temperature (18–22 °C) for up to 6 months, prevent from light.

0.1% SDS solution

Final concentration of chemicals	stock	For 50ml
0.1% SDS	10%	500ul
ddH_2O	-	49.5ml

Store at 4°C.

20% Triton X-100

Final concentration of chemicals	stock	For 50ml
20% Triton X-100	100%	10ml
ddH_2O	-	40ml

Store at RT.

ATAC wash buffer

Final concentration of chemicals	stock	For 50ml	For 1ml
10 mM Tris-HCl pH 7.4	1M	500ul	10ul
10 mM NaCl	5M	100ul	2ul
3 mM MgCl2	1M	150ul	3ul
0.1% Tween-20	10%	500ul	10ul
ddH_2O	-	48.75ml	975ul

Make fresh and keep on ice.

iBlock buffer

Chemicals	stock	For 50ml
iBlock protein	-	1g
SDS	10%	2.5ml
ddH_2O	-	47.5ml

Dissolve 2 g of iBlock Protein-Based Blocking Reagent (Thermo Fisher Scientific, cat. no. T2015) in 90 ml of ddH2O in a 65 °C water bath, add 5 ml of 10% (w/v) SDS, and bring volume to 100 ml with ddH2O.

Stored at room temperature for several months. Prevent from light

Sheared blocking genomic DNA

The sheared genomic DNA can be prepared from any species. Usually, genomic DNA is sheared to an approximate size range of 200-1000 bp. Measure the sheared DNA concentration with Qubit assay. Use 500 ng of sheared DNA for each reaction.

Stored at -20 °C for several months.

Binding & Wash buffer, 1×

Final concentration of chemicals	stock	For 50ml
5 mM Tris-HCl pH 7.5	1M	250ul
0.5 mM EDTA	0.5M	50ul
1 M NaCl	5M	10ml
ddH_2O	-	39.7ml

Store at room temperature for several months.

Binding & Wash buffer, 2×

Final concentration of chemicals	stock	For 50ml
10mM Tris-HCl pH 7.5	1M	500ul
1mM EDTA	0.5M	100ul
2M NaCl	5M	20ml
ddH_2O	-	29.4ml

Store at room temperature for several months.

2× SSC/0.5% (w/v) SDS

Chemicals	Stock	For 50ml
SSC	20x	5ml
SDS	10%	2.5ml
ddH_2O	-	42.5ml

Add 85 ml of ddH2O to a 100-ml beaker first, and then add sequentially 10 ml of 20× SSC and 5 ml of SDS (10% (wt/vol)) and mix them well.

Store at room temperature for several months.

Notes