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RAT-ChIP - Restriction Assisted Tagmentation Chromatin Immunoprecipitation V.1

PLOS One

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ABSTRACT

Chromatin immunoprecipitation coupled with next-generation sequencing (ChIP-seq) has revolutionized our understanding of chromatin-related biological processes. The method, however, requires thousands of cells and has therefore limited applications in situations where cell numbers are limited. Here we describe a novel method called Restriction Assisted Tagmentation Chromatin Immunoprecipitation (RAT-ChIP) that enables global histone modification profiling from as few as 100 cells. The method is simple, cost-effective and takes a single day to complete.

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GUIDELINES

This protocol has been tested with 100 and 1000 K562 and H1299 cells and pooled material from 3 blastocyst stage bovine embryos laser-dissected into inner cell mass and trophectoderm. Replication experiments showed that RAT-ChIP can constantly obtain data from 100 cells and less, however, there is more experimental variation with such a low number of cells and thus replicates are especially important to make biologically valid conclusions.

MATERIALS

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- ⊗ NEBNext High-Fidelity 2X PCR Master Mix - 250 rxns **New England Biolabs Catalog #M0541L**
- ⊗ Nextera DNA library preparation kit 24 samples **Contributed by users Catalog #FC-121-1030**
- ⊗ Agencourt RNAClean XP Magnetic Beads **Beckman Coulter Catalog #A6398**
- ⊗ cOmplete™ Protease Inhibitor Cocktail **Roche Catalog #4693116001**
- ⊗ Dynabeads™ Protein G for Immunoprecipitation **Thermo Fisher Catalog #10004D**
- ⊗ FastDigest Buffer (10X) **Thermo Fisher Catalog #B64**
- ⊗ FastDigest AluI **Thermo Fisher Catalog #FD0014**
- ⊗ FastDigest MvaI **Thermo Fisher Catalog #FD0554**
- ⊗ FastDigest HinfI **Thermo Fisher Catalog #FD0804**
- ⊗ FastDigest SqaI **Thermo Fisher Catalog #FD2174**

2x Nuclei preparation buffer:

- 20 mM Tris HCl pH 7.4
- 20 mM NaCl
- 6 mM MgCl₂
- 0.2% IGEPAL CA-630 (NP-40)

Complete Immunoprecipitation buffer (IP)

- 20 mM Tris-HCl pH 8.0
- 2 mM EDTA
- 150 mM NaCl
- 0.1% Triton X-100
- 1x Protease inhibitor cocktail (Roche)

Low Salt Wash buffer

- 20 mM Tris-HCl pH 8.0
- 2 mM EDTA

- 150 mM NaCl
- 1% Triton X-100

High Salt Wash buffer

- 20 mM Tris-HCl pH 8.0
- 2 mM EDTA
- 500 mM NaCl
- 1% Triton X-100
- 0.1% SDS

TE buffer

- 10 mM Tris-HCl pH 8.0
- 1 mM EDTA

PCR primer sequences for sequencing library generation:

Ad1_noMX:	AATG ATAC GGCG ACCA CCGA GATC TACA CTCG TCGG CAGC GTCA GATG TG
Ad2.1_TAAGGCGA	CAAG CAGA AGAC GGCA TACG AGAT TCGC CTTA GTCT CGTG GGCT CGGA GATG T

Ad2.2_CGTACTAG	CAAG CAGA AGAC GGCA TACG AGAT CTAG TACG GTCT CGTG GGCT CGGA GATG T
Ad2.3_AGGCAGAA	CAAG CAGA AGAC GGCA TACG AGAT TTCT GCCT GTCT CGTG GGCT CGGA GATG T
Ad2.4_TCCTGAGC	CAAG CAGA AGAC GGCA TACG AGAT GCTC AGGA GTCT CGTG GGCT CGGA GATG T
Ad2.5_GGACTCCT	CAAG CAGA AGAC GGCA TACG AGAT AGGA GTCC GTCT CGTG GGCT CGGA GATG T

Ad2.6_TAGGCATG	CAAG CAGA AGAC GGCA TACG AGAT CATG CCTA GTCT CGTG GGCT CGGA GATG T
Ad2.7_CTCTCTAC	CAAG CAGA AGAC GGCA TACG AGAT GTAG AGAG GTCT CGTG GGCT CGGA GATG T
Ad2.8_CAGAGAGG	CAAG CAGA AGAC GGCA TACG AGAT CCTC TCTG GTCT CGTG GGCT CGGA GATG T
Ad2.9_GCTACGCT	CAAG CAGA AGAC GGCA TACG AGAT AGCG TAGC GTCT CGTG GGCT CGGA GATG T

Ad2.10_CGAGGCTG	CAAG CAGA AGAC GGCA TACG AGAT CAGC CTCG GTCT CGTG GGCT CGGA GATG T
Ad2.11_AAGAGGCA	CAAG CAGA AGAC GGCA TACG AGAT TGCC TCTT GTCT CGTG GGCT CGGA GATG T
Ad2.12_GTAGAGGA	CAAG CAGA AGAC GGCA TACG AGAT TCCT CTAC GTCT CGTG GGCT CGGA GATG T
Ad2.13_GTCGTGAT	CAAG CAGA AGAC GGCA TACG AGAT ATCA CGAC GTCT CGTG GGCT CGGA GATG T

Ad2.14_ACCACTGT	CAAG CAGA AGAC GGCA TACG AGAT ACAG TGGT GTCT CGTG GGCT CGGA GATG T
Ad2.15_TGGATCTG	CAAG CAGA AGAC GGCA TACG AGAT CAGA TCCA GTCT CGTG GGCT CGGA GATG T
Ad2.16_CCGTTTGT	CAAG CAGA AGAC GGCA TACG AGAT ACAA ACGG GTCT CGTG GGCT CGGA GATG T
Ad2.17_TGCTGGGT	CAAG CAGA AGAC GGCA TACG AGAT ACCC AGCA GTCT CGTG GGCT CGGA GATG T

Ad2.18_GAGGGGTT	CAAG CAGA AGAC GGCA TACG AGAT AACC CCTC GTCT CGTG GGCT CGGA GATG T
Ad2.19_AGGTTGGG	CAAG CAGA AGAC GGCA TACG AGAT CCCA ACCT GTCT CGTG GGCT CGGA GATG T
Ad2.20_GTGTGGTG	CAAG CAGA AGAC GGCA TACG AGAT CACC ACAC GTCT CGTG GGCT CGGA GATG T
Ad2.21_TGGGTTTC	CAAG CAGA AGAC GGCA TACG AGAT GAAA CCCA GTCT CGTG GGCT CGGA GATG T

Ad2.22_TGGTCACA	CAAG CAGA AGAC GGCA TACG AGAT TGTG ACCA GTCT CGTG GGCT CGGA GATG T
Ad2.23_TTGACCCT	CAAG CAGA AGAC GGCA TACG AGAT AGGG TCAA GTCT CGTG GGCT CGGA GATG T
Ad2.24_CCACTCCT	CAAG CAGA AGAC GGCA TACG AGAT AGGA GTGG GTCT CGTG GGCT CGGA GATG T

SAFETY WARNINGS

Please refer to the SDS (Safety Data Sheet) for safety warning and hazard information.

Bind antibodies to protein G Dynabeads

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Note

The amounts given in the protocol below are for a single immunoprecipitation (IP). When working with multiple samples, please scale the volumes accordingly. For simultaneous processing of multiple samples, please use multichannel pipets and 0.2ml 8-strip PCR tubes.

- Pipet 1 μ l of protein G Dynabeads per IP into standard 0.2ml eppendorf tube containing 50 μ l of IP buffer at RT.
- Capture the magnetic beads by 1 min incubation on a magnetic stand (Diagenode) and careful removal of the buffer.
- Take beads up in, 2 times the volume of beads (2 μ l per IP), IP buffer. Add 0.25 μ g of corresponding antibody per IP and incubate 2 h at RT with end-over end mixing (30 rpm).
- Wash the beads 2 times with 50 μ l of IP buffer at RT by gently pipetting the beads 10 times up and down.
- Suspend the beads in the original amount of IP buffer (1 μ l per IP).

Note

We recommend preparing one sample for IP with general histone H3 antibody, that can be used as an input. Process the H3 sample identical to the other samples.

Cell preparation for IP

- 2
- Collect your cells of interest and count density using hemocytometer.
 - Centrifuge 1000 g for 5 min, remove supernatant by pipeting and take the pellet up in PBS at desired density (for example 100 cells or 1000 cells) per 0.5 μ l.
 - Prepare 10 μ l of nuclear lysis/restriction buffer by combining:
 - 0.5 μ l of PIC (protease inhibitor cocktail) (Roche)
 - 1 μ l 10x FastDigest buffer (Thermo Fisher Scientific)
 - 0.5 μ l of restriction enzyme mix (AluI, SmaI, MvaI, HinfI - mixed in equal volumes)
 - 8 μ l of 2x nuclear lysis buffer
 - Mix 0.5 μ l cells with 0.5 μ l of nuclear lysis/restriction buffer and incubate 10 min on ice and 5 min at 37°C.

- Put samples back to ice and add:
1µl of 0.2% TritonX-100/0.2% NaDOC solution
- Incubate 10 min on ice and vortex for 30 sec.

Chromatin immunoprecipitation

- 3** - To prepare the samples for immunoprecipitation add:

8µl of IP buffer
1µl of antibody bound Dynabeads

- Perform IP 4h at 4°C with end-over end mixing (30 rpm).
- Wash 3 times with 100ul of low salt buffer, 3 times with 100ul of high salt buffer, 1 time with 100µl of IP buffer and 1 time with Tris HCl pH7.4 by gently pipetting the beads 10 times up and down. Capture the magnetic beads by 1min incubation on a magnetic stand (Diagenode).
- After adding Tris HCl pH7.4, suspend the beads and carry them over to a new 0.2ml tube to reduce background.

Note

Be careful when removing Tris HCl pH7.4 buffer during the last wash as the beads tend to come loose from the magnet!

On beads tagmentation

- 4** - Prepare tagging mix by combining (scale according to number of reactions):

2.5µl 2x Tagmentation buffer from Nextera DNA Library preparation kit
2.5µl mQ
0.5µl of Tn5 transposase

- Add 5µl of the mix to the magnetic beads, resuspend by pipetting and perform tagmentation 1min at 37°C.
- Wash once with 50µl of low salt buffer and once with TE.

PCR for sequencing library construction

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Note

Please choose appropriate primers for the samples that you want to sequence simultaneously. Ad1_noMX primer is always the same. Ad2 primers have different barcodes that during data processing will be used to assign the reads to a correct sample.

- Prepare PCR mix (scale according to the number of reactions):

10µl of NEBNext High-Fidelity 2X PCR Master Mix

5µl mQ

2.5µl Ad1_noMX primer (5uM)

- Add 17.5µl of mix directly to washed beads and finally add 2.5µl of corresponding Ad2.X primer (5µM) and do 16 cycles of PCR using the following program:

1. 72°C 5 min,
2. 98°C 2 min,
3. 98°C 10 sec,
4. 63°C 10 sec,
5. 72°C 1 min,
6. repeat steps 3-5 15 times
7. hold at 4°C

PCR purification

- 6
- After PCR put samples to magnetic rack and take 20µl of supernatant to a clean 0.2ml tube.
 - Add 20µl of mQ to and 40µl of Agencourt RNA XP magnetic beads (1:1).
 - Incubate 10 min at RT.
 - Wash 2 times with 100ul of 70% EtOH .
 - Dry beads 5 min at RT.
 - Elute with 10µl of Tris HCl pH7.4 by pipetting and remove supernatant after 1 min incubation on magnetic rack.

Library quality control and sequencing

Quantify the library (by Nanodrop, Qubit, TapeStation etc.) and perform sequencing using Illumina platform.