Nov 26, 2019

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

PLOS One

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1 Works for me dx.doi.org/10.17504/protocols.io.69qhh5w

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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.

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0225801

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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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

NAME	CATALOG #	VENDOR
NEBNext High-Fidelity 2X PCR Master Mix - 250 rxns	M0541L	New England Biolabs
Nextera DNA library preparation kit 24 samples	FC-121-1030	
Agencourt RNAClean XP Magnetic Beads	A63987	Beckman Coulter
cOmplete™ Protease Inhibitor Cocktail	4693116001	Roche
Dynabeads™ Protein G for Immunoprecipitation	10004D	Thermo Fisher
FastDigest Buffer (10X)	B64	Thermo Fisher
FastDigest AluI	FD0014	Thermo Fisher

NAME	CATALOG #	VENDOR
FastDigest MvaI	FD0554	Thermo Fisher
FastDigest HinfI	FD0804	Thermo Fisher
FastDigest SaqAI	FD2174	Thermo Fisher

MATERIALS TEXT

2x Nuclei preparation buffer:
- 20 mM Tris HCl pH 7.4
- 20 mM NaCl
- 6 mM $MgCl_2$
- 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:	AATGATACGGCGACCACCGAG ATCTACACTCGTCGGCAGCGT CAGATGTG
Ad2.1_TAAGGCGA	CAAGCAGAAGACGGCATACGA GATTCGCCTTAGTCTCGTGGG CTCGGAGATGT
Ad2.2_CGTACTAG	CAAGCAGAAGACGGCATACGA GATCTAGTACGGTCTCGTGGG CTCGGAGATGT
Ad2.3_AGGCAGAA	CAAGCAGAAGACGGCATACGA GATTTCTGCCTGTCTCGTGGG CTCGGAGATGT
Ad2.4_TCCTGAGC	CAAGCAGAAGACGGCATACGA GATGCTCAGGAGTCTCGTGG GCTCGGAGATGT
Ad2.5_GGACTCCT	CAAGCAGAAGACGGCATACGA GATAGGAGTCCGTCTCGTGG GCTCGGAGATGT

Ad2.6_TAGGCATG	CAAGCAGAAGACGGCATACGA GATCATGCCTAGTCTCGTGGG CTCGGAGATGT
Ad2.7_CTCTCTAC	CAAGCAGAAGACGGCATACGA GATGTAGAGAGGTCTCGTGG GCTCGGAGATGT
Ad2.8_CAGAGAGG	CAAGCAGAAGACGGCATACGA GATCCTCTCTGGTCTCGTGGG CTCGGAGATGT
Ad2.9_GCTACGCT	CAAGCAGAAGACGGCATACGA GATAGCGTAGCGTCTCGTGG GCTCGGAGATGT
Ad2.10_CGAGGCTG	CAAGCAGAAGACGGCATACGA GATCAGCCTCGGTCTCGTGGG CTCGGAGATGT
Ad2.11_AAGAGGCA	CAAGCAGAAGACGGCATACGA GATTGCCTCTTGTCTCGTGGG CTCGGAGATGT
Ad2.12_GTAGAGGA	CAAGCAGAAGACGGCATACGA GATTCCTCTACGTCTCGTGGG CTCGGAGATGT
Ad2.13_GTCGTGAT	CAAGCAGAAGACGGCATACGA GATATCACGACGTCTCGTGGG CTCGGAGATGT
Ad2.14_ACCACTGT	CAAGCAGAAGACGGCATACGA GATACAGTGGTGTCTCGTGG GCTCGGAGATGT
Ad2.15_TGGATCTG	CAAGCAGAAGACGGCATACGA GATCAGATCCAGTCTCGTGGG CTCGGAGATGT
Ad2.16_CCGTTTGT	CAAGCAGAAGACGGCATACGA GATACAAACGGGTCTCGTGGG CTCGGAGATGT
Ad2.17_TGCTGGGT	CAAGCAGAAGACGGCATACGA GATACCCAGCAGTCTCGTGGG CTCGGAGATGT
Ad2.18_GAGGGGTT	CAAGCAGAAGACGGCATACGA GATAACCCCTCGTCTCGTGGG CTCGGAGATGT
Ad2.19_AGGTTGGG	CAAGCAGAAGACGGCATACGA GATCCCAACCTGTCTCGTGGG CTCGGAGATGT
Ad2.20_GTGTGGTG	CAAGCAGAAGACGGCATACGA GATCACCACACGTCTCGTGGG CTCGGAGATGT
Ad2.21_TGGGTTTC	CAAGCAGAAGACGGCATACGA GATGAAACCCAGTCTCGTGGG CTCGGAGATGT
Ad2.22_TGGTCACA	CAAGCAGAAGACGGCATACGA GATTGTGACCAGTCTCGTGGG CTCGGAGATGT
Ad2.23_TTGACCCT	CAAGCAGAAGACGGCATACGA GATAGGGTCAAGTCTCGTGG GCTCGGAGATGT

Ad2.24_CCACTCCT	CAAGCAGAAGACGGCATACGA GATAGGAGTGGGTCTCGTGG GCTCGGAGATGT

SAFETY WARNINGS

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

Bind antibodies to protein G Dynabeads

1

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 1µl of protein G Dynabeads per IP into standard 0.2ml eppendorf tube containing 50 µl 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 (2µl per IP), IP buffer. Add 0.25µg 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 µl 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 (1µl per IP).

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 heamocytometer.
- 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 0.5µl.
- Prepare 10µl of nuclear lysis/restriction buffer by combining:

0.5µl of PIC (protease inhibitor cocktail) (Roche)
1µl 10x FastDigest buffer (Thermo Fisher Scientific)
0.5µl of restriction enzyme mix (AluI, SaqAI, MvaI, HinfI - mixed in equal volumes)
8µl of 2x nuclear lysis buffer

- Mix 0.5µl cells with 0.5µl 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 wortex 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.

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

5

Please choose appropriate primers for the samples that you want to sequence simultaneously. Ad1_noMX primer is always the same. Ad2 primers have diffferent 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

7

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