VERSION 2

DEC 03, 2018

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.vrbe52n

Protocol Citation: Lorane Texari, Carlos Guzman, Sven Heinz 2018. rev-ChIP.

protocols.io

<https://dx.doi.org/10.17504/protocols.io.vrbe52n>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
We use this protocol in our group and it is working. Developments and edits occur as needed.

Created: Nov 19, 2018

Last Modified: Dec 03, 2018

PROTOCOL integer ID:
17923

rev-ChIP V.2

Lorane

Texari¹,

Carlos Guzman¹, Sven Heinz¹

¹University of California, San Diego

Carlos Guzman

ABSTRACT

Understanding the precise regulation of transcriptional programs in human health and disease requires the accurate identification and characterization of genomic regulatory networks. Next-generation sequencing (NGS) technologies are powerful, and widely applied tools to map the in vivo genome-wide location of transcription factors (TFs), histone modifications, chromatin accessibility, and nascent transcription that make up these regulatory networks. While chromatin immunoprecipitation followed by sequencing (ChIP-seq) is one of the oldest, and most-utilized experimental techniques to study the location and abundance of TFs, experiments still frequently require optimization to reproducibly yield good data with high signal-to-noise ratios due to the massive variability between possible antibody-antigen combinations and commercial reagents.

To overcome these obstacles, we systematically carried out well over 500 ChIP-seq experiments designed to test every aspect of typical ChIP-seq experiments and developed rev-ChIP, a novel ChIP-seq method that is optimized for scalability, robustness, low-input, speed, cost efficiency and data quality. We find that rev-ChIP can be scaled to work for cell numbers ranging from millions to under a thousand, and from a single sample to 500 samples a week in a non-automated fashion with minimal hands-on time. Additionally, rev-ChIP has been tested on a variety of sample types ranging from cell lines to sorted primary cells and solid tissues.

Lysis and Sonication

- 1 Thaw cell pellet on ice and resuspend cells in 500 μL of lysis buffer.

Note

We do not recommend using 500 μL of lysis buffer or tip sonication when sonicating less than 500K cells. In this case we suggest using Covaris (130 μL) or PIXUL (60 μL).

- 2 Sonicate samples for 7 cycles.

Note

This step is dependent on crosslinking method, and cell line or tissue type and should be optimized.

Note

Double crosslinked DNA is harder to sonicate and requires more rounds of sonication.

Note

Make sure not to oversonicate your samples and keep them constantly cold.

Expected result

Chromatin size range of 200-500.

Input Cleanup

- 3 Take 10 μL of each sample (for input) and put them into new PCR strip.
- 4 Dilute lysis buffer (LB3 - add 55 μL 10% Triton X-100, Metivier - add 750 μL Metivier Dilution Buffer, RIPA - None). If splitting lysates for IP, make sure diluted samples are well mixed. Split lysates as required if needed.
- 5 Add 68 μL of Elution Buffer (10mM Tris pH8, 0.5% SDS, 5mM EDTA, 280mM NaCl) + 1 μL RNase A to each input sample and incubate for 00:15:00 at 37 °C .
- 6 Add 1 μL 55 of Proteinase K to each input sample and incubate at 55 °C for 01:00:00 and then at 65 °C for 00:30:00 .

Immunoprecipitation

- 7 Prepare Dynabeads A/G: capture Dynabeads on magnet, remove supernatant and resuspend in equal volume of appropriate lysis buffer.

Note

For LB3 use LB3 + 1/9th volume of 10% Triton X-100, for Metivier use an equal volume of Metivier Dilution Buffer and for RIPA use an equal volume of RIPA buffer.

- 8 Add the appropriate volume of dynabeads beads + antibody to each ChIP sample.

Note

We recommend using 1 µg of antibody + 10 µL of dynabeads if using 100,000 cells or more.

We recommend using 0.1 µg of antibody + 1 µL of dynabeads if using 10,000 cells or less.

- 9 Incubate IP overnight on wheel at 4 °C (rotating at 8rpm).

Note

Optionally, you can incubate IP at 4 °C for 01:00:00 in most cases and for most antibodies for with minimal loss in data quality.

Input Cleanup

- 10 Create mastermix of 2 µL 1 SpeedBeads + 120 µL 20% PEG8000/1.5M NaCl (8.5% PEG, 1M NaCl), mix thoroughly, and add 122 µL of mastermix to each input sample.
- 11 Incubate at RT for 00:10:00 .
- 12 Wash 2x with 200 µL of 80% EtOH on magnet (move strip side to side 10x slow, 10x fast).

Note

We suggest using a repeater pipette here to speed things up.

Note

If using 4 PCR strips or less we suggest vortexing beads at speed 8 for 10 seconds.

- 13 Air-dry until cracks appear on bead pellet.

Note

Air-drying should take approximately 00:14:00 .

- 14 Elute in 15 μL TT (0.05% Tween 20, 10mM Tris pH 8.0, cold).

Note

We suggest using a repeater pipette here to speed things up.

Note

Can store at $-20\text{ }^{\circ}\text{C}$ and stop for the day.

Washes

- 15 Add 100x PIC to WBI/III and TET (10 μL PIC per 1 mL WBI/WBIII/TET). Do 3x washes with 180 μL WBI + PIC, 3x washes with 180 μL WBIII + PIC, and 2x washes with 185 μL cold TET + PIC.

Note

We suggest using a multichannel pipette here to speed things up.

Note

The addition of PIC (Protease Inhibitor Cocktail) is not required, but highly recommended.

- 16 Resuspend beads in 25 μL of cold TT using repeater pipette.

Library Preparation

- 17 Collect beads and take 2 μL of each input supernatant (1-2ul for 500K cells) that will be library prepped and add 23 μL of TT to each input taken.
- 18 Create a mastermix of 1.5 μL of Enzyme Mix End Prep + 3.5 μL of End Prep Reaction Buffer per sample, mix well and add 5 μL of mastermix to each sample. Incubate for 00:30:00 at 20 $^{\circ}\text{C}$ and then 00:30:00 at 65 $^{\circ}\text{C}$.
- 19 Add 1 μL of Bioo ChIP Adaptors (10.625uM) to each sample.
- 20 Create a mastermix of 15 μL Ligation Master Mix + 0.5 μL of Ligation Enhancer per sample, mix well, and add 15.5 μL of mastermix to each sample and incubate for 00:15:00 at 20 $^{\circ}\text{C}$.
- 21 Create a STOP solution mastermix of 4 μL 10% SDS + 3 μL 0.5M EDTA + 20 μL water per sample and add 27 μL of mastermix to each sample.
- 22 Add 4.5 μL of 5M NaCl to each sample.

Note

We recommend using a multichannel to speed things up.

Proteinase K and Reverse Crosslinking

- 23 Add 1 μL of Proteinase K to each sample using multichannel and incubate for 01:00:00 at 55 °C and then 00:30:00 at 65 °C .

Note

Alternatively, FA-fixed can be incubated for 00:30:00 at 55 °C .

Note

Can leave at 4 °C overnight.

Cleanup

- 24 Create mastermix of 2 μL SpeedBeads + 61 μL 20% PEG8000/1.5M NaCl (8.5% PEG, 1M NaCl), mix thoroughly, and add 63 μL of mastermix into new tube strips, then collect and transfer the supernatant of samples into these new tubestrips with 63 μL of speedbeads + peg.

Note

DO NOT MIX PROTEIN A/G BEADS WITH SPEEDBEADS

- 25 Incubate at RT for 00:10:00 .

- 26 Wash 2x with 200 μ L of 80% EtOH on magnet (move strip side to side 10x slow, 10x fast).

Note

We recommend using a repeater pipette to speed things up.

- 27 Air-dry until cracks appear on bead pellet.

Note

Air-drying should take approximately 00:14:00 .

- 28 Elute in 25 μ L TT (0.05% Tween 20, 10mM Tris pH 8.0, cold). Collect beads on magnet and transfer supernatant into new PCR strips.

Note

We recommend using a multichannel to speed things up.

Library Prep Amplification PCR

- 29 Do PCR.

Mastermix Library PCR		
25.5ul MM + 24.5ul sample	1x	20x
Sample	24.5	
(Blue Cap) NEBNext Ultra II Q5 2x MM	25	500
100uM Solexa 1GA	0.25	5
100uM Solexa 1GB	0.25	5

PCR Program		
-------------	--	--

	98°C	30 seconds
R	98°C	10 seconds
60°C	15 seconds	
72°C	30 seconds	
	72°C	1 minute
	4°C	ON

Final Cleanup

30 Make mastermix of 2 µL SpeedBeads + 38.5 µL of per sample and add 40.5 µL of mastermix to each sample.

31 Incubate at RT for 00:10:00 .

32 Wash 2x with 200 µL of 80% EtOH on magnet (move strip side to side 10x slow, 10x fast).

Note

We recommend using a repeater pipette to speed things up.

33 Air-dry until cracks appear on bead pellet.

Note

Air-drying should take approximately 00:14:00 .

34 Elute in 20 µL of TT.

35 Qubit library prep using HS DNA buffer and standards.