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rev-ChIP V.2

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

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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.

Troubleshooting

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 °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 °C and then 00:30:00 at 65 °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 °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 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.

27 Air-dry until cracks appear on bead pellet.

Note

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

28 Elute in 25 µL 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.

Qubit

35 Qubit library prep using HS DNA buffer and standards.