



rev-ChIP

Version 2

Lorane Texari¹, Carlos Guzman¹, Sven Heinz¹

¹University of California, San Diego

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Carlos Guzman



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.

PROTOCOL STATUS

Working

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

Lysis and Sonication

Thaw cell pellet on ice and resuspend cells in □500 ul of lysis buffer.

NOTE □ 500 µl of lysis buffer or tip sonication when sonicating less than 500K cells. In this case We do not recommend using we suggest using Covaris ($\square 130 \mu I$) or PIXUL ($\square 60 \mu I$).

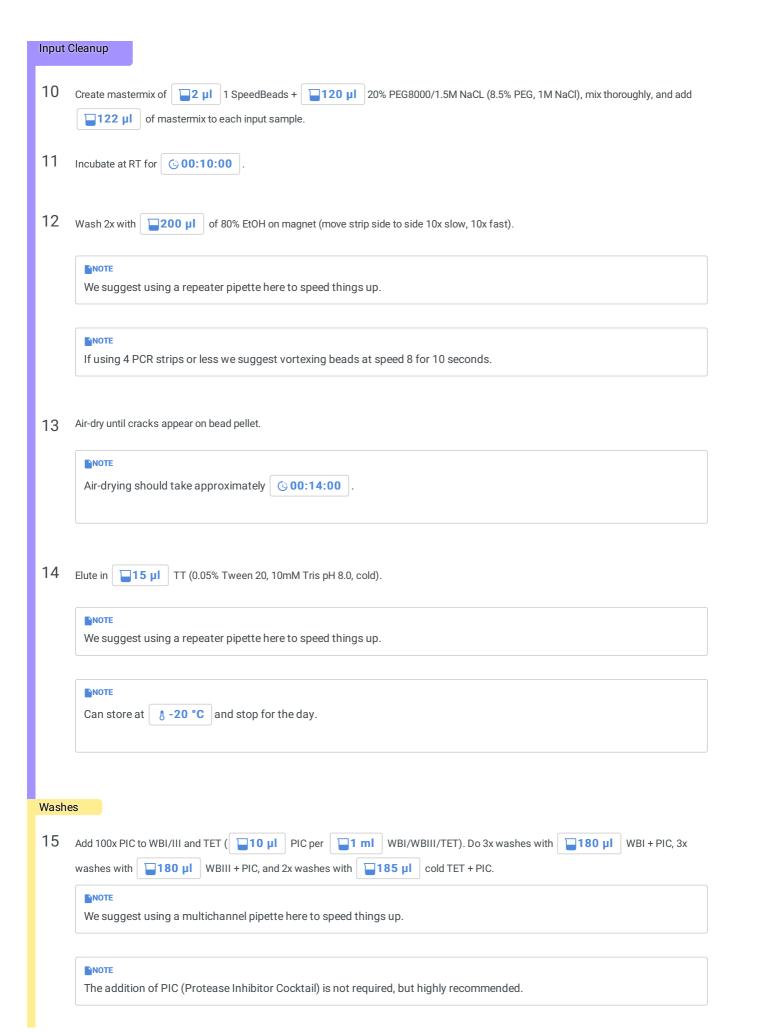
Sonicate samples for 7 cycles.

■NOTE

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

NOTE

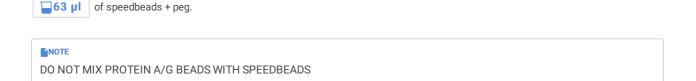
Double crosslinked DNA is harder to sonicate and requires more rounds of sonication. NOTE Make sure not to oversonicate your samples and keep them constantly cold. **EXPECTED RESULT** Chromatin size range of 200-500. Input Cleanup 3 10 μl of each sample (for input) and put them into new PCR strip. Dilute 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 of Elution Buffer (10mM Tris pH8, 0.5% SDS, 5mM EDTA, 280mM NaCl) + RNase A to each input sample and **७00:15:00** at **₹37 °C** . incubate for 6 □1 µl 55 of Proteinase K to each input sample and incubate at 3.55 °C **© 01:00:00** and then at **© 00:30:00** Immunoprecipitation 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 1 μg of antibody + 10 μl of dynabeads if using 100,000 cells or more. We recommend using **□**0.1 µg We recommend using of antibody + 1 μl of dynabeads if using 10,000 cells or less. 9 Incubate IP overnight on wheel at 84°C (rotating at 8rpm). (01:00:00 in most cases and for most antibodies for with minimal Optionally, you can incubate IP at | § 4 °C | for loss in data quality.



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 to each input taken. 18 Create a mastermix of 15 1.5 µl of Enzyme Mix End Prep + 23.5 µl of End Prep Reaction Buffer per sample, mix well and add of mastermix to each sample. Incubate for 6 00:30:00 at 8 20 °C and then 6 00:30:00 at 8 65 °C 19 Add 11 ul of Bioo ChIP Adaptors (10.625uM) to each sample. 20 Create a mastermix of | 15 \mu I | Ligation Master Mix + 0.5 \mu I | of Ligation Enhancer per sample, mix well, and add | 15.5 \mu I of mastermix to each sample and incubate for \(\infty 00:15:00 \) at \(\bar{\bar{\chi}} 20 \cdot C \) 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 ■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 of Proteinase K to each sample using multichannel and incubate for \(\infty 01:00:00 \) A 55 °C **© 00:30:00** at ₫ 65 °C **■**NOTE Alternatively, FA-fixed can be incubated for \(\oscite{00:30:00} \) § 55 NOTE Can leave at 4 °C overnight. Cleanup 24 □2 μl SpeedBeads + □61 μl 20% PEG8000/1.5M NaCL (8.5% PEG, 1M NaCl), mix thoroughly, and add of mastermix into new tube strips, then collect and transfer the supernatant of samples into these new tubestrips with

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- 25 Incubate at RT for **© 00:10:00**
- 26 Wash 2x with $200 \, \mu l$ 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.



Air-drying should take approximately 6 00:14:00

28 Elute in 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🛚	30 seconds
R	98🛚	10 seconds
60⊠	15 seconds	
72 🛚	30 seconds	
	72 🛚	1 minute
	4⊠	ON

Final Cleanup

