Ultra-Low Input Native Chromatin Immunoprecipitation coupled with Sequencing

The protocol for Ultra-Low Input Native Chromatin Immunoprecipitation coupled with next generation sequencing (ULI-ChIP-Seq) is adapted from Brind'Amour et al., 2015.

Native ChIP-Seq has several advantages compared to classical cross-linked ChIP-Seq methods that make it ideally suited to assess histone modifications within small cell populations. Omitting formaldehyde fixation can circumvent epitope masking by chemical alteration and weakly bound protein partners, which can be washed away during the chromatin isolation. Additionally, MNase fragmentation has been shown to provide both higher resolution and better signal-to-noise ratio when compared with fixation followed by sonication (Kasinathan et al., 2014; Brind'Amour et al., 2015).

Make the chromatin

Snap frozen cell pellets of 100,000 CD14+, CD16- monocytes were thawed on ice for 5 minutes. To lyse the cell membranes, the cells were resuspended in 20 μL Nuclear Isolation Buffer and incubated an additional 5 minutes on ice.

A final concentration of 2 Units/μL MNase was added in 20 μL MNase Digestion Buffer and the cells were incubated at 37 °C for 5 minutes to digest the chromatin down to mononucleosomes.

The MNase reaction was stopped by the addition of 10% volume 100 mM EDTA. To ensure that the chromatin was completely solubilized prior to immunoprecipitation, 10% volume of 1% Triton / 1% Deoxycholate was added. The chromatin was incubated on ice for 15 minutes, and then vortexed at a medium setting for 30 seconds. The volume was adjusted to 200 μL with Complete IP Buffer and the chromatin was rotated 1 hour at 4 °C before vortexing again for 30 seconds.

10% of the total chromatin was removed to assess the digestion efficiency and to use as an input control. This input chromatin was extracted with phenol-chloroform, ethanol precipitated, and assessed for size distribution and yield.

Chromatin Immunoprecipitation

Protein A beads for pre-clearing and for immunoprecipitation were washed with Complete IP Buffer. The chromatin was pre-cleared for 1 – 4 hours at 4 °C to remove any non-specific interactions with the beads. Simultaneously, a second set of beads was bound to an antibody specific for the target of interest in order to form antibody-bead complexes.

Chromatin Modification	**Antibody**	**Amount Used for IP**
H3K27me3	Millipore 07-449	0.3 μg
H3K27Ac	Abcam ab4729	0.05 μg
H3K4me3	Abcam ab8580	0.03 μg

H3K4me1	Abcam ab8895	0.2 μg
H3K36me3	Abcam ab9050	0.1 μg

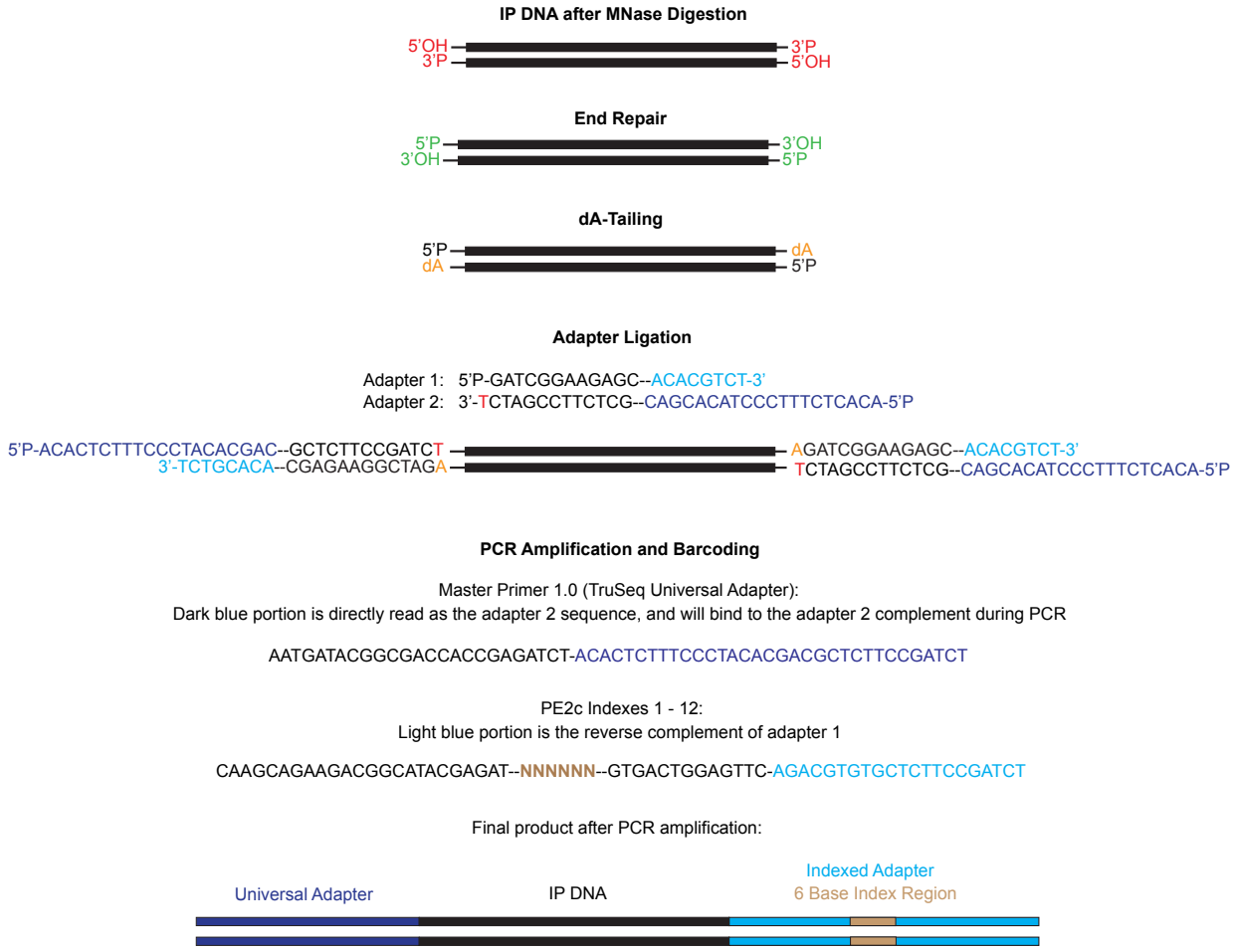
The chromatin was removed from the pre-clearing beads and added to the antibody-bound beads for immunoprecipitation overnight at 4 °C.

The following day, the beads containing bead-antibody-chromatin complexes were washed twice with a low salt wash buffer (150 mM NaCl) and twice with a high salt wash buffer (500 mM NaCl) to remove non-specific binding interactions. The chromatin was eluted from the beads by resuspending in Elution Buffer and shaking 1.5 hours at 65 °C.

The eluted DNA was separated from the proteinaceous chromatin components by phenol-chloroform extraction and ethanol precipitation. In order to remove any residual salts or contaminants, the immunoprecipitated DNA was cleaned with a 1.8x SPRI bead cleanup prior to library preparation.

Illumina Library Preparation

The immunoprecipitated DNA was prepared for sequencing on the Illumina platform using the NEBNext ChIP-Seq Library Prep Master Mix Set for Illumina (E6240L) using modified Illumina TruSeq adapters as in the schematic below.



Post library construction, the fold-enrichment of target DNA over input DNA was assessed by qRT-PCR with positive and negative primers for each mark. Successful IPs were sent for sequencing at The Centre for Applied Genomics on the Illumina platform using the NEBNext ChIP-Seq Library Prep Master Mix Set using modified Illumina TruSeq adapters.

Adapter 1: 5' P-GATCGGAAGAGCACACGTCT
Adapter 2: 5' ACACTCTTTCCCTACACGACGCTCTTCCGATCT

MP1.0 (Master Primer 1.0)
AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT

(PE2c1) Index1: ATCACG

CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCAGACGTGTGCTC
TTCCGATCT

(PE2c2) Index2: CGATGT
CAAGCAGAAGACGGCATACGAGATACATCGGTGACTGGAGTTCAGACGTGTGCTC
TTCCGATCT

(PEc3) Index3: TTAGGC
CAAGCAGAAGACGGCATACGAGATGCCTAAGTGACTGGAGTTCAGACGTGTGCTC
TTCCGATCT

(PE2c4) Index4: TGACCA
CAAGCAGAAGACGGCATACGAGATTGGTCAGTGACTGGAGTTCAGACGTGTGCTC
TTCCGATCT

(PE2c5) Index5: ACAGTG
CAAGCAGAAGACGGCATACGAGATCACTGTGTGACTGGAGTTCAGACGTGTGCTC
TTCCGATCT

(PE2c6) Index6: GCCAAT
CAAGCAGAAGACGGCATACGAGATATTGGCGTGACTGGAGTTCAGACGTGTGCTC
TTCCGATCT

(PE2c7) Index7: CAGATC
CAAGCAGAAGACGGCATACGAGATGATCTGGTGACTGGAGTTCAGACGTGTGCTC
TTCCGATCT

(PE2c8) Index8: ACTTGA
CAAGCAGAAGACGGCATACGAGATTCAAGTGTGACTGGAGTTCAGACGTGTGCTC
TTCCGATCT

(PE2c9) Index9: GATCAG
CAAGCAGAAGACGGCATACGAGATCTGATCGTGACTGGAGTTCAGACGTGTGCTC
TTCCGATCT

(PE2c10) Index10: TAGCTT
CAAGCAGAAGACGGCATACGAGATAAGCTAGTGACTGGAGTTCAGACGTGTGCTC
TTCCGATCT

(PE2c11) Index11: GGCTAC
CAAGCAGAAGACGGCATACGAGATGTAGCCGTGACTGGAGTTCAGACGTGTGCTC
TTCCGATCT

(PE2c12) Index12: CTTGTA
CAAGCAGAAGACGGCATACGAGATTACAAGGTGACTGGAGTTCAGACGTGTGCTC
TTCCGATCT

Buffers

Nuclear Isolation Buffer

0.1% Triton
0.1% deoxycholate
1x Protease Inhibitor Cocktail
10 mM Sodium Butyrate

MNase Dilution Buffer

0.01 M Tris, pH 7.5
0.01 M NaCl
0.001 M EDTA
50% Glycerol

Complete Immunoprecipitation Buffer

20 mM Tris-HCl pH 8.0
2 mM EDTA
150 mM NaCl
0.1% Triton X-100
1x Protease Inhibitor Cocktail
1 mM PMSF
10 mM Sodium Butyrate

Triton-Deoxycholate Solution (in H2O)

1% (w/v) Triton X-100
1% (w/v) sodium deoxycholate

Low Salt Wash Solution

20 mM Tris-HCl pH 8.0
2 mM EDTA
150 mM NaCl
1% Triton X-100
0.1% SDS
1x Protease Inhibitor Cocktail
10 mM Sodium Butyrate

High Salt Wash Solution

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

1% Triton X-100
0.1% SDS
1x Protease Inhibitor Cocktail
10 mM Sodium Butyrate

Elution Buffer

100 mM NaHCO3
1% SDS

REFERENCES

1. Brind'Amour, J., Liu, S., Hudson, M., Chen, C., Karimi, M. M., Lorincz, M.C. (2015) An ultra-low input native ChIP-seq protocol for genome-wide profiling of rare cell populations. *Nature Communications, 6.* DOI: 10.1038/ncomms7033
2. Kasinathan, S., Orsi, G. A., Zentner, G. E., Ahmad, K., Henikoff, S. (2014) High-resolution mapping of transcription factor binding sites on native chromatin. *Nature Methods, 11*. DOI: 10.1038/nmeth.2766