ChIP on BJ infected by HSV-1

Version 1

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ABSTRACT

ChIP-seq and ChIP-qPCR are powerful tools that allows the specific matching of proteins or histone modifications to regions of the genome. Chromatin is isolated and antibodies to the antigen of interest are used to determine whether the target binds to a specific DNA sequence or to map the distribution across the genome . The output DNA produced using this protocol can either be analyzed using qPCR in ChIP-qPCR, or with sequencing in ChIP-seq.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Promyelocytic leukemia (PML) nuclear bodies (NBs) induce latent/quiescent HSV-1 genomes chromatinization through a PML NB/Histone H3.3/H3.3 Chaperone Axis.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS TEXT

Lysis Buffer 5X

50% Glycerol
HEPES pH7,5 250mM
NaCl 700mM
NP40 3,77%
Triton 1,25%
EDTA 5mM

Wash Buffer 10X

NaCl 2M
Tris pH8 200mM
EGTA 5mM
EDTA 10mM

Shearing Buffer 10X

Tris HCl pH 7,6 100mM
EDTA 10mM
1% SDS

IP Buffer 2X

NaCl 300mM
Tris pH 8 10mM
EDTA 1mM
SDS 0,1%
Triton 2%

Low Salt Wash Buffer

0,1% SDS
1% Triton
2 mM EDTA ph8
20 mM Tris HCl ph 8
150 mM NaCl

High Salt Wash Buffer
0,1% SDS
1% triton
2 mM EDTA
20mM Tris HCl ph8
500mM NaCl

LiCl Wash Buffer
0,25M LiCl
1% NP40
1% NaDOC
1mM EDTA
10mM Tris HCl ph 8

TE Buffer
10mM Tris ph8
1mM EDTA

Elution Buffer
1%SDS
100mM NaHCO3

SAFETY WARNINGS

A. Fixation and Sonication

1
- Crosslink cells by adding in the media 16% formaldehyde methanol free solution to a final concentration of 1% during 5 min at room temperature. Place cells on a shaking platform
- Quench the crosslonking reaction by adding the appropriate volume of Glycine 2,5M to fixed cells for an additional 5min at room temperature
- Completely aspirate the solution from the plate
- Wash the plate with cold PBS
- Add 10ml cold PBS to each dish and scrape cells.
- Wash the plate with an additional volume of cold PBS to collect any remaining cells
- Collect cells at 300g for 5 min , 4°C

NOTE
The speed can be adjusted according to cells

SAFETY INFORMATION
Add 1X PIC in each buffer

- Wash cells twice by resuspending in cold PBS and collecting by centrifugation at 300g 4°C 5 min.
- Add Lysis Buffer 1X containing 1X PIC to cells, gently resuspend by aspirating and incubate for 10min on a rocker at 4°C
- Collect intact nuclei by centrifugation at 1700g for 5min 4°C and discard the supernatnant
- Resuspend pellet in Wash buffer 1X containing 1X PIC to cells and incubate for 10min on a rocker at 4°C

- Add Shearing Buffer 1X containing 1X PIC to cells, resuspend by aspirating
- Collect intact nuclei by centrifugation at 1700g for 5min 4°C and discard the supernatnant
- Repeat steps 12 and 13 an additional time. Remove and discard the supernatant.

NOTE
The pellet becomes translucent

- Resuspend nuclei pellet in the Shearing Buffer 1 X and transfer to 1ml AFA tube(s)
- Shear chromatin with S220 Focused ultrasonicator (140W, Duty Off de 10% ,Cycles Burst à 200).

B. Immuno-precipitation

2
- Add 1 volume IP Buffer 2X to ssonicated sample
- Centrifuge chromatin for 10 min, 4°C, 16000g to remove cell debris.
- Transfer supernatant to a new tube and take chromatin for IP and input (10%)

NOTE
Chromatin can be snap frozen and stored at -80°C

- Add primary antibody or IgG as control to all samples and rotate at 4°C overnight
- Add 50µl protein G or A agarose beads (Salmon Sperm DNA/Protein A or G Agarose Millipore) and incubate 2h on a rocker at 4°C.
- Centrifuge the IP samples for 30 sec at 100g 4°C and remove the supernatant.
- Pellet agarose by gentle centrifugation (700 to 1000 rpm at 4°C, 1min). Carefully remove the supernatant that contains unbound, non-specific DNA.
- Wash the protein A agarose/antibody/histone complex for 3-5 minutes on a rotating platform with 1ml of each of the buffers listed in the order as given below: 1X Low Salt Buffer , 1X High Salt Buffer, one wash, 2X LiCl Buffer, one wash, 1X TE
- Elute the protein-DNA complex from the antibody by adding 200µl elution buffer. Incubate at 65°C for 30 min with rotation.
- Spin agarose and carefully transfer the supernatant fraction to another tube

C. Decrosslinking and DNA extraction

3
- Add 2.5µl RNaseA (stock 20mg/ml, finale concentration 0,2mg/mL) et 2.5µl PK (Stock 20mg/ml, final concentr ation 0,2mg/ml) in IP and input
- Incubate the samples at 65°C overnight.
- Recover DNA by phenol/chloform purification or with a PCR Clean UP kit