nature protocols

https://doi.org/10.1038/s41596-024-01058-z

Protocol

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Multiplexed chromatin immunoprecipitation sequencing for quantitative study of histone modifications and chromatin factors

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Multiplexed chromatin immunoprecipitation-sequencing for quantitative study of histone modifications and chromatin factors

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Supplementary information

Protocol for fragmentation optimization

Fragmentation optimization

Native - Micrococcal nuclease digestion

1. Add 10 µL of 100X protease inhibitor to 490 µL of 2X Ly buffer. The 2X Ly buffer should be pipetted carefully to avoid creating excess bubbles.

2. Dilute stock MNase (2000 U/µL) to 200 U/µL in 2X Ly buffer in a total volume of 45 µL (mix 4.5 µL of MNase + 40.5 µL 2X Ly buffer).

3. Prepare different MNase dilutions on ice as described. We recommend the range of MNase concentrations given below as a starting point for most human cell lines and mouse embryonic stem cells.

200 U/µL MNase (µL)	2x Ly buffer (µL)	2x Stock MNase conc. (U/µL)	MNase (U) per 1 Mio cells
8	42	**32**	**800**
4	46	**16**	**400**
2	48	**8**	**200**
1	49	**4**	**100**
0.5	49.5	**2**	**50**
0.25	49.75	**1**	**25**

4. Resuspend six freshly collected (or flash frozen) cell pellets (1 million cells each) in 25 µL of ice-cold PBS. Cell pellets must be free of residual medium, trypsin, and excess wash buffer.

5. Mix 25 µL of cells with corresponding 25 µL MNase-2X Ly buffer dilution to a final volume of 50 µL.

6. Lyse cells on ice for 20 mins.

7. Move samples to a pre-heated 37°C heat block/waterbath. Digest at 37°C for exactly 10 mins, with 800 rpm shaking.

8. Quickly move samples to ice and stop the reaction with 5 µL of 0.5 M EGTA.

9. Digest RNA and protein by adding 0.5 µL of RNase A and 1 µL of Prot K and incubating at 37ºC for 15 min and 63ºC for 1 h respectively.

10. Purify DNA using AMPure XP beads as detailed below (bring to RT 30 min before use):

    - To 56.5 µL of sample add 113 µL of AMPure XP beads (2X).
    - Mix by pipetting until the solution looks homogenous, wait 10 min.
    - Magnetize for 5 min.
    - Remove supernatant.
    - Wash 2× with 200 µL 70% (v/v) ethanol.
    - Air-dry at RT for 3 min or until the beads look dry.
    - Elute DNA in 10 µL of 1xTE, transfer eluted DNA to new tube.
    - Use 1 µL to measure DNA concentration with Qubit.

11. Run products on Bioanalyzer or agarose gel to check fragment size.

⚠CRITICAL STEP

For the main protocol, pick the MNase concentration that results in mono-, di-nucleosomal fragments of the chromatin as seen in lane 3 of Figure 1c.

Fixed - Sonication

1. Add 5 µL of 100X protease inhibitor to 490 µL of ice-cold 1X Son buffer.

2. Resuspend six freshly collected (or flash frozen) formaldehyde crosslinked cell pellets (1 million cells each) in 50 µL of above buffer. Cell pellets must be free of residual medium, trypsin, and excess wash buffer.

⚠CRITICAL STEP

We recommend crosslinking with 1 % PFA (v/v) *for 10 min at room temperature and quenching with 750 mM Tris-HCl (pH 8.0). Ensure that the samples are fixed under precise and comparable conditions to enable quantitative comparison between samples (with the same aliquot of formaldehyde, same temperature and time, ideally in the same batch).*

3. Transfer to 0.5 mL Bioruptor tubes and lyse cells on ice for 10 mins.

⚠CRITICAL STEP

Using hard plastic tubes designed for sonication greatly improves the efficiency of fragmentation and reduces the cycle number needed.

4. Sonicate samples for different durations on the sonicator to select the optimal condition to shear the chromatin to mono-, di-nucleosomal fragments. We tested 5 min, 10 min, and 20 min on Diagenode Bioruptor™ with high power and 30 sec on/off cycles as shown in Figure 4a.

5. Digest RNA and protein by adding 0.5 µL of RNase A and 1 µL of Prot K and incubating at 37ºC for 1 h and 63ºC overnight (to also reverse crosslink) respectively.

6. Purify DNA using AMPure XP beads as detailed below (bring to RT 30 min before use):

   - To 56.5 µL of sample add 113 µL of AMPure XP beads (2X).
   - Mix by pipetting until the solution looks homogenous, wait 10 min.
   - Magnetize for 5 min.
   - Remove supernatant.
   - Wash 2× with 200 µL 70% (v/v) ethanol.
   - Air-dry at RT for 3 min or until the beads look dry.
   - Elute DNA in 10 µL of 1xTE, transfer eluted DNA to new tube.
   - Use 1 µL to measure DNA concentration with Qubit.

7. Run products on Bioanalyzer or agarose gel to check fragment size.