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ChIP-seq library generation

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We use this protocol and it's working

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Abstract

ChIP-seq library generation

Guidelines

Protocol summary (as presented on this page):
1. Collect 10 million “806” cells and fix with 1% formaldehyde in DMEM medium at room temperature for 8 min; quench with glycine (final concentration 0.125 M) for 5 min at room temperature.
2. Wash cells with 1 × PBS and resuspend in cold cell lysis buffer (50 mM HEPES pH 7.5, 140 mM NaCl, 0.1% sodium deoxycholate, 1% Triton X-100, 1 mM EDTA, 0.1% SDS, 0.25% sarkosyl, 1 × complete protease inhibitor cocktail).
3. Sonicate cells with 10 sec on / 20 sec off cycles for a total of 15 min.
4. Centrifuge chromatin (10 min, 13,000 × g, 4 °C) and dilute supernatants with dilution buffer (50 mM HEPES pH 7.5, 140 mM NaCl, 1% Triton X-100, 1 mM EDTA, 1 × complete protease inhibitor cocktail) for immunoprecipitation.
5. Incubate Dynabeads (Invitrogen) with antibody in high salt buffer (20 mM Tris-HCl pH 7.9, 500 mM NaCl, 2 mM EDTA, 1% Triton X-100, 0.1% SDS, 1 × complete protease inhibitor cocktail) for 3 hr at 4 °C with rotation.
6. Add antibody/beads to chromatin and incubate overnight at 4 °C with rotation.
7. Wash beads 7 times with high salt buffer and twice with TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA).
8. Elute DNA/protein complexes twice for 15 min at 65 °C in 100 µL elution buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA, 200 mM NaCl, 1% SDS).
9. Incubate supernatants with RNase A and proteinase K and reverse crosslink at 65 °C for 6 h or overnight.
10. Purify DNA by phenol/chloroform extraction and ethanol precipitation.
11. Resuspend DNA in water and repair to 5' phosphorylated and 3' dA-tailed DNA using NEBNext® Ultra™ II End Repair/dA-Tailing Module (NEB, E7546S).
12. Ligate barcodes (BIOO Scientific, 514153) using Quick Ligation kit (NEB, M2200).
13. After size selection, amplify library with 16 cycles of PCR.
14. Purify PCR-amplified libraries using KAPA beads (Kapa Biosystems) and send for sequencing.

Materials

- 1% formaldehyde (in DMEM medium)
- Glycine (final concentration 0.125 M)
- 1 × PBS
- Cold cell lysis buffer (50 mM HEPES pH 7.5, 140 mM NaCl, 0.1% sodium deoxycholate, 1% Triton X-100, 1 mM EDTA, 0.1% SDS, 0.25% sarkosyl, 1 × complete protease inhibitor cocktail)
- Sonicator (capable of 10 sec on/20 sec off cycles)
- Centrifuge (able to reach 13,000 × g at 4 °C)
- Dilution buffer (50 mM HEPES pH 7.5, 140 mM NaCl, 1% Triton X-100, 1 mM EDTA, 1 × complete protease inhibitor cocktail)
- High salt buffer (20 mM Tris-HCl pH 7.9, 500 mM NaCl, 2 mM EDTA, 1% Triton X-100, 0.1% SDS, 1 × complete protease inhibitor cocktail)
- Dynabeads (Invitrogen)
- Antibodies (appropriate for target protein A or protein G IP)
- TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA)
- Elution buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA, 200 mM NaCl, 1% SDS)
- RNase A
- Proteinase K
- Phenol/chloroform for DNA purification
- Ethanol (for precipitation)
- Nuclease-free water (for resuspension)
- NEBNext® Ultra™ II End Repair/dA-Tailing Module (NEB, E7546S)
- Barcodes (BIOO Scientific, 514153)
- Quick Ligation kit (NEB, M2200)
- KAPA beads (Kapa Biosystems)
- PCR reagents and thermocycler (used for 16 cycles after size selection)
- Consumables: tubes, rotator, magnetic rack for beads

Before start

- Starting material: 10 million “806” cells (collect and proceed to fixation as described).
- Prepare fixation (1% formaldehyde in DMEM) and quench (glycine final 0.125 M) solutions prior to use.
- Prepare all buffers (cold cell lysis buffer, dilution buffer, high salt buffer, TE buffer, elution buffer) and have protease inhibitors on ice.

1 Collect 10 million “806” cells and fix with 1% formaldehyde in DMEM medium at room temperature for 8 min; quench with glycine (final concentration 0.125 M) for 5 min at room temperature.

2 Wash cells with 1 × PBS and resuspend in cold cell lysis buffer (50 mM HEPES pH 7.5, 140 mM NaCl, 0.1% sodium deoxycholate, 1% Triton X-100, 1 mM EDTA, 0.1% SDS, 0.25% sarkosyl, 1 × complete protease inhibitor cocktail).

3 Sonicate the cells with 10 sec on / 20 sec off cycles for a total of 15 min.

4 Centrifuge the chromatin (10 min, 13,000 × g, 4 °C) and dilute the supernatants with dilution buffer (50 mM HEPES pH 7.5, 140 mM NaCl, 1% Triton X-100, 1 mM EDTA, 1 × complete protease inhibitor cocktail) for immunoprecipitation.

5 Prepare Dynabeads (protein A or protein G) by incubating with antibody in high salt buffer (20 mM Tris-HCl pH 7.9, 500 mM NaCl, 2 mM EDTA, 1% Triton X-100, 0.1% SDS, 1 × complete protease inhibitor cocktail) for 3 hr at 4 °C with rotation.

6 Add the antibody-bound beads to the chromatin and incubate overnight at 4 °C with rotation.

7 Wash the beads 7 times with high salt buffer and then wash twice with TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA).

8 Elute the DNA/protein complexes twice for 15 min at 65 °C in 100 µL elution buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA, 200 mM NaCl, 1% SDS).

9 Incubate the combined supernatants with RNase A and proteinase K and reverse crosslink at 65 °C for 6 h or overnight.

10 Purify DNA by phenol/chloroform extraction and ethanol precipitation.

11 Resuspend DNA in nuclease-free water and perform end repair/dA-tailing to generate 5' phosphorylated and 3' dA-tailed DNA using NEBNext® Ultra™ II End Repair/dA-Tailing Module (NEB, E7546S).

12 Ligate barcodes (BIOO Scientific, 514153) using the Quick Ligation kit (NEB, M2200).

13 After size selection, amplify the library by PCR (16 cycles).

14 Purify the PCR-amplified libraries using KAPA beads (Kapa Biosystems) and submit the libraries for sequencing.