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ChIP-seq

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Crosslinking 30m

1 Fix cells adding formaldehyde to a final concentration of 1% and incubation at 37ºC for 10 min.

2 Quench fixation adding glycine to a final concentration of 125 mM at cell culture plates and incubate at RT for 5 min.

3 After two washes with cold PBS, in the presence of complete protease inhibitor cocktail (Roche) and PMSF, scrape cells and centrifuge at 4ºC for 5 min at 300 g.

Cell lysis and chromatin sonication 45m

4 Add 2,5 ml of 0.5% NP-40 lysis buffer to lyse cell pellet for nuclei isolation and incubate 10 min.

Centrifuge 5 min 3000g 4ºC

5

6 Resuspend cell pellet in 1 ml SDS 1% lysis buffer.

7 Sonicate cells in lysis buffer using Bioruptor (Diagenode, UCD-200) at high intensity for three cycles of 10 min with 30s sonication and 30s pause.

8 Clarify chromatin by centrifugation at 17000g for 10 min at 4ºC

IP 1d

9 Incubate 30 ug of chromatin with 4 ug of antibody overnight at 4ºC in IP buffer (0.1% SDS, 1% 161 TX-100, 2 mM EDTA, 20 mM TrisHCl pH8, 150 mM NaCl) with rotation.

10 Add 25 ul of pre-blocked (1 mg/ml BSA) Dynabeads protein A and Dynabeads protein G (ThermoFisher) for 4 hours.

11 Wash beads with IP buffer for 10 min.

12 Wash beads with IP buffer with increased salt concentration (500 mM NaCl) for 10 min.

13 Wash beads with LiCl buffer (0.25 M LiCL, 1% NP40, 1% NaDoc, 20 165 mM TrisHCl pH8 and 1 mM EDTA) for 10 min.

14 ChIPmentation was carried out as described in Schmidl, 2015 using Tagment DNA Enzyme provided by the Proteomic Service of CABD (Centro Andaluz de Biología del Desarrollo).

15 Elute DNA from beads by incubation at 50ºC in 100 ul elution buffer (1% SDS and 100 mM NaHCO3) for 30 min.

De-crosslinking and purification 1d

16 Incubate eluted samples with 200 mM NaCl and 100 ug/ml of Proteinase K (ThermoFisher) overnight at 65ºC to revert crosslinking.

17 Purify DNA using Qiagen PCR Purification columns.

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Library Preparation 1d

18 Amplify DNA for N-1 cycles (being N the optimum Cq determined by qPCR reaction) using standard protocol of NEBNext High-Fidelity Polymerase (M0541, New England Biolabs) in order to generate libraries.

19 Purify libraries and size-select using Sera-Mag Select (GE Healthcare). DNA not bound to 0,7x volumen of Sera-Mag beads were subsequentially incubated with 0,15x volumen of Sera-Mag beads. In this way, DNA fragments between 100-500 bp were isolated. Libraries were sequenced using Illumina NextSeq 500 in a single-end configuration.

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