ChIP-seq with *N. furzeri* embryos

Materials

Proteinase K solution (Invitrogen 25530-049)
RNase A (Sigma R6513, dissolved in 10mM Tris-HCL, pH7.5, 15mM NaCL at concentration of 10 mg/mL)
Complete™, Mini, EDTA-free Protease Inhibitor Cocktail (Sigma 11836170001)
Dynabeads™ M-280 Sheep Anti-Rabbit IgG (Thermo Fisher 11203D)
Paraformaldehyde, 16% w/v aq. soln., methanol free, 10 x 10mL (Alfa Aesar 43368-9M)
Rabbit anti-H3K27me3 antibody (Millipore, 07-449 or Active motif 39155)
Rabbit anti-H3K4me3 antibody (Active Motif 39159)
DTT 1M (Thermo Fisher Scientific)
GlycoBlue 15 mg/mL (Ambion, AM9515)
16% Formaldehyde solution (w/v), Methanol-free (Thermo Scientific, 28906)

Recipes

All buffer should be filter-sterilized and store at 4°C.

Buffer A2 (250ml, w/o PI)	**Reagent**	**Volume**	**Final**
	1M HEPES pH 7.5	3.75 ml	15 mM
	5 M NaCl	7 ml	140 mM
	0.5M EDTA	0.5 ml	1 mM
	0.5M EGTA	250 ul	0.5 mM
	Triton X100	2.5 ml	1%
	10% sodium deoxycholate	2.5 ml	0.1%
	10% SDS	25 ml	1%
	10% N-lauroylsarcosine	12.5 ml	0.5%
	(Add DTT (final 0.5mM) and proteinase inhibitor before use)		

Low-salt buffer (500 ml)	**Reagent**	**Volume**	**Final**
	1M Tris-HCl pH 8.0	10 ml	20 mM
	5M NaCl	15 ml	150 mM
	0.5M EDTA	2 ml	2 mM
	10% Triton X-100	50 ml	1%

High-salt buffer (500 ml)	**Reagent**	**Volume**	**Final**
	1M Tris-HCl pH 8.0	10 ml	20 mM
	5M NaCl	50 ml	500 mM
	0.5M EDTA	2 ml	2 mM
	10% Triton X-100	50 ml	1%

LiCl buffer (500 ml)	Reagent	Volume	Final
	1M Tris-HCl pH 8.0	10 ml	20 mM
	5M LiCl	25 ml	250 mM
	0.5M EDTA	1 ml	1 mM
	10% Triton X-100	50 ml	1%
	10% NP-40	5 ml	0.1%
	10% sodium deoxycholate	25 ml	0.5%

Harvest cells [Day 1]

Wash 20-40 fin blastema with PBS twice @RT. Cut samples into small pieces on ice for 5 min in a well of 24 well plate.

Add 1 ml 1% FA in PBS and crosslink for 12 min. Quickly transfer crosslinked samples into 1.5 ml tubes and centrifuge for 1 min at 10000 rpm to quickly collect cells.

Remove fixation buffer and add 2.5M glycine to 0.125M final to quench crosslink.

Wash with ice-cold PBS/0.125M glycine twice and once with Buffer A2.
You can keep the pellet at -80°C after a snap freeze.

Prepare beads [Day 2, just before shear chromatin]

(Vortex the bottle of beads well before use, and below using ab4729 as an example)
Add 130µl Rabbit IgG Dynal beads to a 1.5ml microfuge safe-lock tube for each ChIP.
Wash beads with PBS twice.
Add 700µl PBS-0.5% BSA with 10µg ab4729 antibody, and incubate 2-6h @ 4°C.
(Please check the affinity of beads with the antibodies you use. For Protein A/G, 50µl beads for 10µg antibody)

Shear chromatin [Day 1-2]

Resuspend cells (≈0.5-1 × 10^7) with 700-800µl Buffer A2 per tube.
Sonicate 8-10 × 10s @ power 3 with Microson ultrasonic cell disrupter till the size of DNA from 150 to 1000 bp. (at power setting #5, 30 seconds sonication followed by 30 seconds interval, 15 rounds total.
Centrifuge 10 min @ max speed @ 4°C and, keep supernatant for ChIP.

ChIP [Day 2]

Wash beads with PBS-0.5% BSA twice.
Add 700µl sheared chromatin, and incubate 6h or O/N @ 4°C. (**Leave 30ul for input**)
Wash beads-chromatin twice with low salt buffer, twice with the high-salt buffer, and once with LiCl buffer @ cold room. (Keep the supernatant to check sonication efficiency)

Purify DNA [Day 3]

Add 150µl elution buffer, 150µl TE, and 4µl RNase, incubate at 37°C, with manual shaking every 10 minutes for 30 min total (For input, add 120µl elution buffer, 150µl TE, and 4µl RNase)
Add 2µl Proteinase K and incubate at 65°C with manual shaking every 20 minutes for 6h total.
Extract with 300µl phenol-ch-iso for (25:24:1), vortex 1 min then centrifuge 5 min at 20000g.
Take out 280-300µl upper phase and transfer to a new microfuge tube containing 12µl of 5M NaCl (final = 200mM) and 6µl glycogen (final = 30µg)
Mix well and add 750µl cold (4°C) 100% EtOH. Keep @ -80°C for 1h or @-20°C O/N then centrifuge at max speed for 30min at 4°C and wash once with 1ml 75% EtOH.
Air-dry 30min at RT, and then resuspend pellets in 40µl ddH_2O for 1h @ 4°C
Check sonication condition by running a 1.7% DNA gel.

ChIP elution buffer (50ml)

10 mM Tris, pH8.0
1 mM EDTA
1% SDS
250 mM NaCl

v1.0: Chi-Kuo Hu, 2021/09/01

Adapted from the working protocol of

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