Chromatin immunoprecipitation from *C. elegans* embryos

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Sevinc Ercan
University of North Carolina at Chapel Hill

Christina Whittle
University of North Carolina at Chapel Hill

Jason Lieb
University of North Carolina at Chapel Hill

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Introduction

Chromatin immunoprecipitation followed by microarray analysis (ChIP-chip) is a widely used technique ("*Nature Protocols* **1**, - 729 - 748 (2006) doi:10.1038/nprot.2006.98":http://www.natureprotocols.com/2006/07/10/chromatin_immunoprecipitation.php) for several model organisms. In this protocol, we describe the experimental methods we used to perform ChIP in *C. elegans* (worm) embryos. We describe fixation of embryos, preparation of embryo extracts, immunoprecipitation and amplification of recovered DNA.

Reagents

S Medium (1 L)

0.1 M NaCl (20 ml of 5M NaCl)

0.05 M Potassium phosphate pH 6 (50 ml of 1M Potassium phosphate pH 6)

1ml cholesterol (5mg/ml in EtOH)

H2O to 954 ml

Autoclave, then add

10 ml 1M Potassium Citrate pH 6

10 ml Trace Metals Solution

3 ml 1M $CaCl_2$

3 ml 1M $MgSO_4$

10 mL 100X PSN antibiotic (Gibco/Invitrogen cat#15640-055)

10 mL 100X 4mg/ml Nystatin (Sigma#N1638-100mL)

Trace Metals Solution (1 L)

5 mM Disodium EDTA

2.5 mM $FeSO_4.7H_2O$

1 mM $MnCl_2.4H_2O$

1 mM $ZnSO_4.7H_2O$

0.1 mM $CuSO_4.5H_2O$

H_2O to 1 liter

M9 buffer (1 L)

3 g KH_2PO_4

6 g Na_2HPO_4

5 g NaCl

1 ml 1M $MgSO_4$

H_2O to 1 liter

Autoclave for 20 min.

Bleaching solution

Prepare fresh before use

19 ml H_2O

1.25 ml 5 M NaOH

4.75 ml Household bleach (5% solution of sodium hypochlorite)

FA buffer:

50 mM HEPES/KOH pH 7.5, 1 mM EDTA, 1% Triton X-100, 0.1 % sodium deoxycholate; 150 mM NaCl

FA-1 M NaCl buffer:

50 mM HEPES/KOH pH 7.5, 1 mM EDTA, 1% Triton X-100, 0.1 % sodium deoxycholate; 1 M NaCl

FA-500 mM NaCl buffer:

50 mM HEPES/KOH pH 7.5, 1 mM EDTA, 1% Triton X-100, 0.1 % sodium deoxycholate; 500 mM NaCl

TEL buffer:

0.25 M LiCl, 1% NP-40, 1% sodium deoxycholate, 1 mM EDTA, 10 mM Tris-HCl, pH 8.0

Prepare 250 ml of FA and TEL buffers, filter sterilize and store at 4°C.

Elution buffer:

1% SDS in TE with 250 mM NaCl

Preparation of annealed linkers

Mix 250 ul 1M Tris-HCl pH 8 with 375 ul 40 uM oligo Long and 375 ul 40 uM oligo Short. Make 50-ul aliquots in microfuge tubes and place them in a 95°C heat block for 5 min. Transfer tubes to a 70°C heat block for 5 min, remove the heat block and place on the bench. Let them cool to room

temperature, then transfer to 4°C for 12-20 hours. Store the annealed linker at -20°C. When in use keep the linker on ice to avoid melting.

Oligo Long: GCGGTGACCCGGGAGATCTGAATTC

Oligo Short: GAATTCAGATC

Procedure

Embryo collection and fixing

Standard worm growing techniques (1) should be used.

1 Grow worms on 10-20 10 cm plates. After starving on plates, add concentrated *E. Coli* (*E. Coli* (HB101 or OP50) grown to saturation, then resuspended in M9+20%glycerol at equal weight:volume) to obtain as many adults with embryos. Bleach to obtain embryos as described previously (1).

2 Transfer embryos to 500 ml liquid media (S medium+1X PSN antibiotics and nystatin) and grow at 20 ºC in an incubator shaking at 100 rpm. Add concentrated *E. Coli* for food as necessary during growth. It takes around 4-5 days to obtain adults with embryos.

3 Collect adults (by filtration over 35uM nitex membrane)and transfer to 50 ml conical tubes. To 10 ml of worms add 40 ml of bleaching solution and mix for approximately 8-12 minutes while monitoring bleaching as described (1).

4 Spin down embryos at 3000 g for 5 minutes. Resuspend pellet (embryos) in 50 ml M9 and spin 3000 g for 5 minutes. Repeat this wash 2 more times.

5 Resuspend embryos in 47 ml M9 and add 2.8 ml 37% formaldehyde solution. Place on a shaker (50-100 rpm) and incubate for 30 minutes at room temperature (RT, between 22-26 ºC).

6 Wash embryos with once with 50 ml 100 mM Tris pH 7.5 (to quench excess formaldehyde), 2 times with 50 ml M9, and once with 10 ml FA buffer supplemented with protease inhibitors (Calbiochem Cat# 539131, Protease Inhibitor Cocktail Set I). Collect embryos in microfuge tubes by centrifugation at 13,000 g for 30 s. Discard supernatant and store the embryo pellet at -80 ºC.

Extract preparation

Extracts should be prepared as described (2) with minor changes.

7 Resuspend 500 ul of packed embryos in 1.5 ml FA buffer+ protease inhibitors. Dounce on ice (30 strokes) using a

glass dounce homogenizer pestle B. Transfer to a 15 ml tube and bring the volume to 2 ml with with FA buffer+ protease inhibitors.

8 Using a Branson sonifier microtip, sonicate samples on ice at the following settings: 30% amplitude, 0.9 sec on, 0.1 sec off, 12 pulses for 7 times. After each time, cool samples in dry ice/ethanol bath for 2 seconds. Make sure that the samples are not overheated.

9 Transfer samples to microfuge tubes and spin at 13,000 g for 15 minutes at 4ºC. Take the supernatant and discard pellet. Determine the protein concentration of the supernatant by the Bradford method. Continue with the next step or aliquot and snap freeze in liquid nitrogen and store at -80 ºC.

10 Add extract corresponding to ~3.3 mg of protein to a microfuge tube and bring the volume to 500 ul with FA buffer + protease inhibitors . Add 25 ul 20 % sarkosyl solution and spin at 13,000 g for 5 minutes at 4 ºC. Transfer the supernatant to a new tube. Remove 10 % of the material and store it at -20 ºC until the following day, when it will be used to prepare input DNA. Add 20-30 ug protein A-purified antibody or 3-5 ug affinity-purified antibody to the extract and rotate at 4 ºC for 16-20 h.

Collection of the immunocomplexes and washes

11 Take 20 ul of proteinA-sepharose beads (Amersham Biosciences) per ChIP sample and wash with 4 times with 1 ml FA buffer. Spin at 2500 g for 2 min to collect the beads. After the washes, suspend the beads in one bed volume of FA buffer.

12 Add 40 ul of the bead slurry to each ChIP sample and continue to rotate at 4 ºC for 2 h.

13 Wash beads at room temperature by adding 1 ml of each of the following buffers and incubating on a nutator (or a rotator). Collect beads by spinning for 2 minutes at 2500 g.

2 times FA buffer for 5 minutes

1 time FA-1 M NaCl for 5 minutes. After this wash, transfer beads to new tubes with the next wash buffer.

1 time FA-500 mM NaCl for 10 minutes

1 time TEL buffer for 10 min

2 times TE for 5 min

14 To elute the immunocomplexes, add 150 ul Elution Buffer and place the tube in a 65 ºC heat block for 15 min. Vortex briefly every 5 min. Spin down the beads at 2500 g for 2 min and transfer the supernatant to a new tube. Repeat elution and combine supernatants.

15 Thaw the input samples set aside the previous day and add 250 ul Elution Buffer. Add 2 ul of 10 mg/ml proteinase K (stock in water) to each sample and input. Incubate for 1-2 hours at 55 ºC , then transfer to 65 ºC for 12-20 h to reverse crosslinks.

16 Purify the DNA with Qiaquick PCR purification kit (Qiagen). Elute with 50 ul H2O. Run 5 ul of the input DNA on a 1.5% agarose gel to check the extent of shearing. Most of the DNA fragments should be 200-800 bp.

LM PCR amplification

The amplification method was derived from (3).

17 For blunting DNA ends, add 25 ul ChIP DNA or 2.5 ul input DNA (diluted to 25 ul with dH2O) to a PCR tube. Include a negative control by preparing a reaction with no DNA.

Blunting mix (for 1 reaction):

H_2O: 73 ul

10X NEB 2: 11 ul

BSA (10 mg/ml): 0.5 ul

dNTP mix (25 mM each): 0.4 ul

T4 DNA polymerase (3U/ul): 0.2 ul

Add and mix by pipetting. Incubate at 12°C for 20 min in a PCR machine. Purify DNA on a Zymo column (DNA clean up and concentrator, http://www.zymoresearch.com).

Elute in 25 ul H_2O and place on ice.

18 For ligation of the linkers, prepare ligation mix .

Ligation Mix (for 1 reaction):

H_2O: 13 ul

10X ligase buffer: 5 ul

annealed linkers (15 uM): 6.7 ul

T4 DNA ligase: 0.5 ul

Add 25 ul to each sample and incubate 12-20 hours at 16°C.

19 For amplification, purify the ligated DNA with Zymo column and elute in 15 ul H_2O. Transfer to PCR tube, and place on ice.

PCR Mix (for 1 reaction):

H_2O: 62.7 ul

10X PCR buffer (no MgCl2) : 10 ul

$MgCl_2$ (25 mM): 8 ul

dNTPs (25 mM each): 0.8 ul

oligo Long (40 uM): 2.5 ul

Taq DNA Polymerase (5u/ul): 1 ul

20 Add 85 ul PCR Mix and run the following program in a PCR machine.

Step 1: 55° C for 4 min

Step 2: 72°C for 5 min

Step 3: 95°C for 2 min

Step 4: 95°C for 30 s

Step 5: 55°C for 30 s

Step 6: 72°C for 1 min

Go to step 4 for 24-28 times

Step 7: 72° C for 4 min

Step 8: 4° C

21 Purify the amplified DNA with the Qiaquick PCR purification kit and elute in 50 ul dH2O. Load 5 ul of the eluate on a 1.5% agarose gel to check amplification. The size of the amplified DNA should be 200-600 bp.

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