Protocol : Chromatin-ImmunoPrecipitation

Wash

- Collect the worms in M9
- Wash 3x in M9 to discard the maximum of bacteria
- Let the worm incubate 30 min in M9 (bacteria in intestnes)
- Centrifuge and discard the maximum of s/n

Fixation

- Fixation with ***2% formaldehyde in M9*** (v/v)
- Add ~20 x vol of formaldehyde (1ml of worm = 20ml of formaldehyde)
- Let incubate at RT during 30 min on a wheel. Be carefu the temperature must be 20-26°C. If higher, incubate a shorter time. This step is very important and sensitive! It depends also of the stage of worm (larvae or adult, bigger cuticle)

Wash

- Centrifuge and remove the formaldehyde
- Wash 2x (3min) with ***stop solution*** (= 0.1M Tris-HCl pH = 7.5)
- Remove the solution, then wash 3x with M9 (3min)
- Remove max of M9

Lysis

- Add 1 vol of freshly made ***lysis solution*** (50mM HEPES-KOH, pH 7.6, 1mM EDTA, 140mM KCl, 0.5% NP-40, 10% glycerol, 5mM DTT, protease inhibitor cocktail). 1 tablet for 6-8ml of solution (1/2 life = 30min). Keep the solution on ice
- We can stop at this step: Add the lysis solution, then snap-freeze into EtOH/ CO_2 ice before storing @ -70°C

Sonication

- Thaw out the worm gently on ice. During this time, pre re ¼ of complete mini tablet (make powder with a tip, or if there is less worms, break in “big” part, it can help for sonication, like beads). Add 1ml of fixed worm into sterile ED containing the tablet. Keep on ice

- Prepare ice + EtOH for cooling. Sonication: Put the so tor nearly the bottom of the tube (touch the bottom and go up for 1-2 mm). Use program 2: 20s sonication, 1min pause (8x). Put the temperature t rmometer into ice. Sonication depends principally of:

    - Temperature
    - Position of the tip
    - Foaming, bubbles (if present, bigger bands)

- Once done, centrifuge directly @ 4°C at 14'000 rpm during 20 min.

- Remove s/n (=SONICATED LYSATE). Be sure to take no waste. The s/n should be clear. Once done, snap-freeze and keep @ -70°C

- Recentrifuge the waste for 5 min. Take s/n in a new ED (= SONICATION CONTROL). Add 5M NaCl (20µl for 500µl solution) and let incubate @ 65°C for at least 6h à o/n (=reversion of cross-linking) (can do that in PCR machine if the volume is small, better, no evaporation)

Sonication control

- Mix 20µl sonication control + 1µl SDS 10% +0.5µl Prot K. Incubate 1 hour @ 37°C followed by 2 hours @ 65°C (digestion of prot)

- Extract with phenol/chloroform

- Then 2 possibilities:

    - Rapid: Use PCR purification kit. Let migrate the DNA on a 1% agarose gel. Important ! Load always the same among of DNA (8µl first) and run the gel for same period

    - More sensitive:
        - precipitation in EtOH with 10µg/ml glycogen (final conc)
        - resuspend DNA in 20µl TE
        - treat with 40µg/ml RNAse for 1 hour @ 37°C
        - run on agarose gel with appropriated ladder

- If the shearing is ok, continue. If the bands are too g, can sonicate once more (2x 20s, test after that). If there is only smire, restart with new batch of worms

Pre-cleared

- Defreeze and thaw the sonicated lysate (pool the 2 lot . Add the protease inhibitor tablet (dissolve in ChIP buffer)

- Centrifuge the sonicated lysates once at top speed @ 4°C for 20 min. Take

the s/n

- Divide them in 6 unique tubes (special with “nose bott ”) equally by volume
- Dilute them to 1ml by adding ***ChIP buffer***
- Add 30µl of Prot G beads (wash before 2x with ChIP buffer) pe tube
- Add tablet (in solution), rotate the tubes @ 4°C for 1 hou (it’s the minimum, can be longer)
- Centrifuge down the beads. Take the s/n carefully
- Repeat the incubation with beads
- Centrifuge once more at top speed @ 4°C for 20min
- Take s/n carefully. Pool all s/n (= PRE-CLEARED LYSATE)

Ab incubation

- Take 1ml of pre-cleared lysate per tube for sample Ab / no Ab. Snap fr ze the rest (about 1ml) @ -70°C
- Add 4µg Ab per tube (AcH3: 2µg). Use tips with white filter
- Rotate the tubes @ 4°C O/N

Beads binding

- Wash bead 2x with ChIP buffer. Add 40µl beads per tube
- Add ¼ of complete mini tablet per tube
- Rotate @ 4°C for 2 hours (min 1 hour, best 2)

Wash

- Centrifuge the beads (2000 rmp, 30’’, rotate 180°, 30’’)
- Take s/n to new labeled tubes (keep for snap-freeze later)
- Wash the beads:
    - 2x 1ml of ***ChIP buffer I (100mM KCl)*** for 3-5min on wheel @ 4°C
    - 2x 1ml of ***ChIP buffer II (1M KCl)***
    - 2x 1ml of ***TE***. Last wash: Discard the maximum of s/n (important !!!)

- Be careful with the beads: Use special long tip to avoid discard beads

Elution

- Add once 200µl ***elution buffer*** (Tris-HCl 10mM pH8, 1% (w/v) SDS)
- Vortex for 30" and rotate 30 min @ RT on wheel. Centrfuge, take s/n (= ELUATES) in a new ED
- Redo the elution once (20% of recuperation). Add to the ED (final vol 400µl of eluates)
- Keep the eluates @ -20°C. Snap-freeze the beads (not necessary)

Reverse of cross-linking

- Defreeze the snap-frozen pre-cleared lysate, take 19µl of lysate (snap-freeze & keep the rest)
- Add 400µl ***elution buffer*** (as INPUT control). Glean all samples (400µl of eluates) plus INPUT control
- Add 5M NaCl (20µl for 500µl of lysate à 16µl) Incubate @ 65°C for 6 hours (min 4 hours) Possibility to freeze @ -20°C after this step
- Add 8µl EDTA 0.5M, 16µl Tris-HCl pH7 and 1.6µl Prot K (1µl for 250µl lysate). Incubate 1 hour @ 45°C

Purification

- 2 possibilities for purify: as before (precipitation o kit)
- Finally, elute DNA into 30µl H_2O. Use 2µl per 25µl PCR reaction

PCR amplification

- PCR procedure:

95°C	2'	
95°C	30''	21 cycles
57°C	30''	
72°C	1'	
72°C	3'	

- If no signal, 4 cycles added, etc
- Primer (1µM): 20-24bp, 50% of CG, producing a 200-500 bp fragment

Detection

- Detect the PCR products (10-15µl) separated on 2% agarose gles or 5-6% non denaturing PAA gels