ChIP protocol – preparation from head kidney

Version 3.3 – NMBU/CIGENE

Day 1 – Tissue disruption, crosslinking and sonication

Preparations

- ☐ Equipment:
  - microscope, cell counting chamber, Trypan Blue/Hoescht
  - tweezers
  - a dounce homogenizer with pestles [1]
  - a probe sonicator / sonication device
  - an ice bucket
  - a 15-ml centrifuge set-up at 4˚C
  - a 1.5/2-ml centrifuge set-up at 4˚C
  - a metallic eppendorf tubes holder
  - an incubator set-up at 68˚C
- ☐ Solutions (see **Buffers for ChIP**):
  - PBS with PIC
  - formaldehyde solution 1% (keep in fridge until use)
  - sonication buffer completed with PIC, PMSF and sodium butyrate (named complete sonication buffer in the protocol)
  - IP buffer completed with PIC, PMSF and sodium butyrate (named complete IP buffer in the protocol)
- ☐ To do:
  - refrigerate the metallic Eppendorf tubes holder in the fridge at 4˚C
  - Pour 4.5 ml of PBS with PIC in the dounce homogenizer, and place it in the ice bucket
  - take the appropriate volume of formaldehyde solution 1% from the fridge and place it at room temperature before use

Tissue disruption

The procedure (step 1 to 3) should take less than 15 min and the samples should stay in cold environment as much as possible. Glass homogenizers must be used in priority to plastic ones, due to better conductivity of cold

1. Transfer the piece of head kidney (20 mg recommended for head kidney) into the dounce homogenizer containing 4.5 ml of PBS with PIC
2. Use pestles A and B for the homogenization, 5 strokes each or until obtaining a homogenized solution or feel no more strong resistance
3. Transfer the homogenate into a 5-ml eppendorf tube [2]. You can cut the bottom of the pipet tip to facilitate the aspiration
4. Rinse the douncer and the pestles with 0.5 ml of PBS with PIC. Add it to the previous solution. You reach 5ml
5. After homogenization of the tube, take an aliquot of 10 µl to assess the number of nuclei (dilute 10x, add trypan blue or Hoescht for microscopy observation)
   Counting: **nuclei/ml** **5ml =** **million nuclei total** (expected: 20 million)
6. Centrifuge 3 min, 2 500 g, 4˚C
7. Remove and discard supernatant

Crosslinking

8. Add to the pellet 2.5 ml* of formaldehyde solution 1% at room temperature (RT) and resuspend the pellet by pipetting

**if more than 24 million of nuclei, resuspend in 5 ml of formaldehyde solution 1% and split in two tubes*

9. Incubate under constant agitation [3] at room temperature, 5 min
10. Quench the reaction with 360 µl of glycine 1M (0.125M final). Incubate 10 mins, room temperature, under constant agitation [2]
11. Centrifuge 5 min, 1 000 g, 4˚C
12. Remove and eliminate in appropriate trash the supernatant containing formaldehyde
13. Resuspend with 4 ml* of PBS with PIC by pipetting (quick vortex if necessary)

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[1] 2-ml or 15-ml dounce

[2] or a 15-ml canonical Falcon tube

[3] Place the tube in the Hula Mixer, 360˚ rotation, 40 rpm. Other options : in horizontal position in a box on the top of an orbital shaker/rocker (60 rpm) or directly between two tubes of a tube roller mixer (60 rpm)

**if more than 24 million of nuclei were crosslinked in two tubes, resuspend each pellet in 2 ml of PBS with PIC, and merge them*

14 Centrifuge 5 min, 1 000 g, 4˚C
15 Remove the supernatant
16 Resuspend in 5 ml of PBS with PIC by pipetting (quick vortex if necessary)
17 Centrifuge 5 min, 1 000 g, 4˚C
18 Remove the supernatant
19 Resuspend with 1 ml of PBS with PIC and transfer in a clean 1.5-ml Eppendorf tube
20 Centrifuge 10 min, 3 000 g, 4˚C
21 Remove the supernatant

Sonication

22 Resuspend the pellet in a ratio of 400 µl of complete sonication buffer for 6 million of nuclei. Pipet up and down until the pellet is completely dissolved. Keep the solution in ice 5 min before the start of the sonication
23 The pellet of nuclei from head kidney clump; to recover the maximum of material, break the pellet with the pipet it and keep these clumps for sonication
24 Take out from the fridge the Qubit BR kit and Bioanalyzer DNA 1000 kit or Tape Station material, and set-up the incubator at 68˚C
25 Place the metal Eppendorf holder from the fridge on ice. Aliquot your solution by 400 µl
26 Keep the tubes on the holder all along the process
27 Sonicate the chromatin with the following parameters [4]

Cycles	**14**
Intensity	55%
On/Off	1 sec/1 sec [5]
Cycle time	30 sec (total of 15 sec ON/15 sec OFF)

28 Centrifuge the tubes of sonicated chromatin 10 min, 8 000 g, 4˚C
29 Transfer 350 µl or more of the supernatant into a new LoBind 1.5-ml Eppendorf tube. Note the volume
Volume of sonicated chromatin before dilution: µl
If sonication done in multiple tubes, keep them separated until the quality control of the sonication for each tube
30 Take out an aliquot of 20 µl for sonication control by tube of transferred supernatant
31 Dilute the remaining chromatin by adding 3 volumes of complete IP buffer
32 SAFE STOP POINT: Place the tubes of diluted sonicated chromatin for storage in -80 ˚C

Test of the sonication

33 To the 20 µl for sonication control from step 29, add 80 µl of elution buffer and 2 µl of RNase A (100 mg/ml), 5 µl of proteinase K (20 mg/ml) and 6 µl fo NaCl 5M
34 Incubate 1H30, 68˚C, under agitation [6]
35 Purify with a Qiagen DNA Minelute PCR purification kit. Elute with 20 µl of elution buffer
36 Quantify by Qubit the chromatin concentration of 1 µl of eluate. Report the value
Qubit concentration: ng/µl
37 Run 1 µl of the eluate in the Bioanalyzer DNA 1000 ChIP or Tape Station to assess the size profile

To be qualified as good for ChIP, minimum 60% of the sonicated chromatin should have a size distribution of 200-700 bp, centered around 350-400 bp
Later, you can pool together the tubes with this expected distribution of size of fragmented chromatin to run the immunoprecipitation

[4] These parameters have been established for the device "EpiShear Probe Sonicator" from Active Motif
[5] 1 cycle is 30 sec total, composed by a repetition of 1 sec ON/ 1 sec OFF. The total ON period is 15 sec
[6] 500 rpm for a Thermomixer from Eppendorf

Version history

Version 3.3 – 01.10.2020	Comment about the sonication change
Version 3.2 – 01.10.2020	Add of a version history section Change of the safe stop point explanation Change of number of sonication cycle Change of number of nuclei by volume for sonication Add of explanation for special clumping for head kidney nuclei Validation of 1 000 g instead of 800 g