ChIP protocol – all tissues

Version 3.2 – CIGENE/NMBU

Day 3 – Wash beads, decrosslinking and DNA recovery

Preparations

- ☐ Equipment:
  - a 1.5/2ml centrifuge set-up at 4˚C
  - a magnetic rack
  - a mini-centrifuge
  - an incubator set-up at 65˚C/68˚C
- ☐ Solutions (see **Buffers for ChIP**):
  - IP buffer completed with PIC, PMSF and sodium butyrate (named complete IP buffer in the protocol)
  - Low salt wash buffer completed with PIC, PMSF and sodium butyrate (named low salt wash buffer in the protocol)
  - High salt wash buffer completed with PIC, PMSF and sodium butyrate (named high salt wash buffer in the protocol)
  - ChIP elution buffer and elution buffer

Wash of the beads

1 Pulse spin the tubes, put on magnet and discard supernatant
2 Resuspend the beads in 200 µl of complete IP buffer
3 Pulse spin the tubes, put on magnet and discard supernatant
4 Resuspend the beads in 200 µl of low salt wash buffer, put in the magnet, discard supernatant
5 Repeat step 4
6 Resuspend the beads in 200 µl of high salt wash buffer, put in the magnet, discard supernatant
7 Repeat step 6
8 Resuspend the beads in 70 µl of ChIP elution buffer
9 Elute the DNA from the beads for 1H30 at 65˚C, under agitation [1]
10 Pulse spin the tubes and place on magnetic rack
11 Transfer the eluted chromatin to new clean LoBind 1.5-ml Eppendorf tubes
12 Wash the beads with 30 µl of ChIP elution buffer. Mix and pulse spin the tubes
13 Place the tubes on magnetic rack. Transfer this volume to the eluted chromatin from step 11 (100 µl final)
14 Take out the "input control" tube stocked in the fridge
15 Complete the volume of the input to 100 µl final
16 Add 5 µl of SDS 20% (1% final of SDS) into the input tube, mix well

The input tube and the eluted chromatin from IP tubes are ready for DNA extraction

One-step decrosslinking

17 To each tube, add 2 µl of RNase A (100 mg/ml), 5 µl of proteinase K (20 mg/ml) and 6 µl of NaCl 5M
18 Incubate 1H30, 68˚C, under agitation [2]
19 Take out from the fridge the Qubit HS kit

Purification and quantification

20 Purify the samples with MinElute PCR purification kit. Elute from the column with 32 µl of elution buffer
21 Quantify 2 µl of the samples with Qubit HS kit

The DNA from the input and immunoprecipitated chromatin can be stored at – 20˚C until qPCR and library preparation

[1] 1000 rpm into an Eppendorf Thermomixer
[2] 500 rpm into an Eppendorf Thermomixer

Version history

Version 3.2 – 01.10.2020	Add of a version history section