ChIP from tissue samples

Day 1

Biopsy fixation

1. Use round-bottom Eppendorf. Add 940 µL 1% formaldehyde in cold PBS to 1mm^3 biopsies.
2. Place on rotator for 12 min.
3. Add 64 µL of 2M glycine. Rotate for 5 min.
4. Remove formaldehyde and glycine. Wash 5x with cold PBS. Be sure to wash inside the tube cap as well.
5. Remove the last wash and get all fluid out.
6. Proceed directly or snap-freeze for later.

Day 2

Preparation of beads

1. Prepare a slurry of Dynabeads protein A, 25-50 µL/ChIP in a 1.5 mL lo-bind eppendorf tube. Wash enough amount of beads for all the ChIP.
2. Capture beads using a magnet, remove buffer, remove magnet, add 500 µL RIPA buffer. Do 2x RIPA buffer washing.
3. Vortex, capture beads, remove buffer, add enough RIPA buffer to make 1:1 slurry.
4. Vortex, place tubes on ice.
5. Add 25 µL washed Dynabeads slurry to 100-abµL RIPA and 3µg (histone marks) or 6µg (proteins) antibody to tube. Prepare enough beads+ab mix for all ChIP reactions.
6. Incubate at 4°C, 4h on 40 rpm rotator or 1300 rpm on a shaker/cooler.
7. Capture beads using a magnet, remove buffer, remove magnet, add 500 µL RIPA buffer. Do 2x RIPA buffer washing. Remove supernatant and use beads in the IP.

Nuclear extraction from fixed biopsies

1. Add 1 mL of cold nuclear extraction buffer containding proteinase inhibitors (PMSF, aprotinin, leupeptin) to sample and thaw on ice.
2. Dounce 25 times using tissue 2 mL grind pestle (looser pestle), leave in ice for 5 min.

3. Dounce 25 times, remove samples using a plastic Pasteur pipette.
4. Spin 10 min at 2400g 4°C, carefully remove supernatant, go immediately for chromatine preparation.

Chromatin preparation

1. Add 50 µL RT lysis buffer to the nuclei, pipett to homogenize, leave on ice for 5 min and resuspend nuclei by pipetting.
2. Add 70 µL RIPA (total volume is 120-130 µL), mix by pipetting.
3. Sonication (optimized for 6 discs/tube): 6 cycles – 30s ON, 30s OFF, highest setting. Spin down briefly, after the first 3 cycles.
4. Spin down at 12000g for 10 min 4°C, transfer 100 µL of supernatant to fresh lo-bind tube.
5. Add 100 µL cold RIPA to the pellet and pull paired samples, mix by vortexing and centrifuge at 12000g for 10 min 4°C. Collect supernatant.
6. Take 20 µL of the sheared chromatin and test sonication and DNA concentration.
    a) Elute in 40 µL of complete elution buffer. Incubate 2h at 68°C.
    b) Do DNA purification using Qiagen PCR kit, elute in 25 µL
    c) Run 1% agarose: 20 µL + 5 µL loading dye
    d) Check DNA concentration in the nanodrop. Aim to use 8-10 µg of chromatin per ChIP.

Chromatine IP

(from now on work in the cold room until elution step)

1. Total volume per IP is 250 µL: Mix 10-100µL chromatin with X µL RIPA and add on top of washed bead-ab complex (no volume).
2. Take 20 µL of sheared chromatin aside for imput sample.
3. Leave IP reaction on 40 rpm overnight at 4°C.

Day 3

Wash ChIP

1. Put tubes in a magneti crack. Let it stand for 5 min.
2. Remove supernatant, wash beads3x with 500 μL RIPA. Incubate on a rotator at 40 rpm for 4 min at 4°C in between washes.
3. Remove supernatant and add 500 μL TE buffer. Incubate on a rotator at 40 rpm for 4 min at 4°C.
4. Transfer entire sample to a fresh tube.
5. Capture beads adn remove TE buffer.
6. Add 150 μL complete elution buffer to beads in RT. Reverse crosslink at overnight at 68°C while shaking 1300 rpm.
7. Don't forget also elute input salmples: add 40 μL complete elution buffer (to 20 μL chromatin)

Day 4

Purify ChIP-DNA

1. Briefly spin tubes, capture beads using magnet, collect supernatants and place in fresh tubes.
2. Purify samples on Qiagen PCR columns:

a) PB buffer: 150 μL samples add 750 μL; 60 μL input add 300 μL

b) Elute in 30 μL.

ChIP-qPCR:

- 10 μL of master mix primers mix + 2 μL per well and do triplicate for each sample (Vf=12 μL)

Buffers

Stock solution

Tris-HCl (pH 7.5)	1 M
500 mL	60,6 gr

NaCl	2 M
500 mL	58,4 gr

MgCl	0,5 M
50 mL	2,4 gr

Sucrose	3 M
100 mL	102,7 gr

EGTA	0,5 M
50 mL	9,5 gr

SDS	10%
100 mL	10 gr

EDTA	0,5 M
50 mL	9,3 gr

Formaldeide	10%
100 mL	10 gr

Glicina	2 M
100 mL	15,01 gr

Nuclear extraction buffer 500 mL

	Final concentration	Stock	Volume
Tris-HCl	10 mM	1 M	5 mL
NaCl	100 mM	2 M	2,5 mL
MgCl2	2 mM	0,5 M	2 mL
Sucrose	0,3 M	3 M	50 mL
Igepal CA-630	0,25%		1,25 mL

Add protease inhibitors just before use.

Lysis buffer 500 mL

	Final concentration	Stock	Volume
Tris- HCl (pH 8.0)	50 mM	1 M	25 mL
EDTA	10 mM	0,5 M	10 mL
SDS	1%	10%	50 mL

Add protease and phosphatase inhibitors just before use

RIPA buffer 500 mL

	Final concentration	Stock	Volume
Tris-HCl (pH 7.5)	10 mM	1 M	5 mL
NaCl	140 mM	2 M	35 mL
EDTA	1 mM	0,5 M	1 mL
EGTA	0,5 mM	0,5 M	500 µL
Triton-X-100	0,10%		500 µL
SDS	0,10%	10%	5 mL
Na-deoxycholate	0,10%		0,5 gr

Add protease and phosphatase inhibitor just before use

Completet elution buffer 500 mL

	Final concentration	Stock	Volume
Tris-HCl (pH 7.5)	20 mN	1 M	1o mL
EDTA	5 mM	0,5 M	5 mL
NaCl	50 mM	2 M	12,5 mL
SDS	1%		
Protease K and phosphatase inhibitor	50 µg/mL		

Protease inhibitors

PMSF		1 mM
Aprotinin		0,1 mM
Leupeptin		0,1 mM

Phosphatase inhibitors

Ocadaic acid		1:10000
Beta glicerophosphate		1:500
NaF		1:1000