CHIP-Seq protocol

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(30 min) Day 1

Preparation of magnetic beads

1. Add 100 μl IgG coated magnetic beads to 1.5 ml microcentrifuge tube.
2. Add 1 ml of *block solution* to beads.
3. Collect beads using magnet and remove supernatant with pipette.
    1. Repeat wash 2x.
4. Re-suspend beads in 250 μl *bock solution,* add 10 μg of antibody and incubate at 4°C over night on a rotor.

(4 hours) Day 2

Preparation of magnetic beads (continuation)

5. Wash beads 3x in 1 ml *block solution*
6. Resuspend beads in 100 μl *block solution*.

Formaldehyde cross-linking cells

Note: use 1.0 x10^8 cells per IP (1.0 × 10^6 cells/ml BF or 10 ×10^6 cell/ml PF)

7. Bring cells to 1.0 x 10^8 cells/40 ml of medium
    PF: add 30 ml of SDM-79
    BF: concentrate by centrifugation, 10 min/1800 *g*/RT.
8. To 40 ml of cells add 4 ml of *formaldehyde solution*, mix thoroughly and incubate at RT for 20 min. Invert tubes every several minutes.
9. Add 2.5 ml of 2.0 M glycine and spin down 20 min/4000 *g*/4°C.
10. Wash cells with 30 ml cold PBS and spin down 20 min/4000 *g*/4°C. Do not use pipette to resuspend cells. Cells are very sticky and will stick to anything. White "clumps" will be visible in suspension. Discard supernatant.

Cell lysis and sonication

11. Resuspend pellet in 10 ml *lysis buffer 1*. Vortex thoroughly. No pipetting.
12. Rock at 4°C on platform rocker for 10 min.
13. Spin down cells 20 min/4000 *g*/4°C and discard supernatant.
14. Resuspend pellet in 10 ml of *lysis buffer 2*. Vortex thoroughly and transfer cells to 15 ml falcon tube.
15. Rock at RT for 10 min.

16. Spin down cells 20 min/4000 *g*/4°C and discard supernatant.
17. Resuspend pellet in 2.0 ml *lysis buffer 3*. Vortex thoroughly until all cells as suspended.
18. Sonicate for 10 cycle of 30 sec on / 30 sec off each, setting high. (We used a Bioruptor form Wolf Laboratories Limited but optimal sonication conditions will vary among sonicators and should be optimized.)
19. To 2 ml of sonicated cell lysates add 220 µl of 10% Triton X-100
20. Split sample into two 1.5 ml microcentrifuge tubes and spin 10 min/16,100 *g*/4°C to pellet debris.
21. Move supernatant to a 15mL Falcon tube
    a. Take 50 µl 'input sample' and 100 µl 'size sample' and store both at -20°C.
22. Mix 0.9 ml *lysis buffer 3*, 100 µl 10% Triton X-100 and 1x protease inhibitors and add it to the 2 ml of sonicated cell lysates to bring the total volume to 3 ml.
23. Split between two 2.0 ml microcentrifuge tubes.
24. To each microcentrifuge tube add 50 µl of beads (of 100 µl total) and NaCl (for a final of 100–500 mM NaCl depending on antibody).
25. Incubate overnight on rotator at 4°C.

(2 hours + 9 hours incubation) Day 3

From this point forward, I recommend using non-stick microcentrifuge tubes.

Wash (washes are done at 4–8° C)

26. Place IP samples on magnets and remove liquid
27. Add 1.0 ml cold *RIPA buffer* to each tube, re-suspend beads, collect beads and discard supernatant.
    b. Repeat wash step 7 times. (Move beads to a fresh tube after wash #3)
28. Wash once with 1 ml TE + 50 mM NaCl and remove buffer
29. Spin 3min/1000 *g*/4°C and remove any residual TE buffer.

Elution

30. Add 200 µl of *Elution buffer* to each tube and incubate in a 65°C water bath for 30 min. Vortex every 2-5 min.
31. Spin down beads 1 min/16,100 *g*/RT.
32. Remove 200 µl of supernatant and transfer to new microcentrifuge tube.

Crosslink reversal

33. To prepare 'input sample', add 50 µl of 'input sample' to a microcentrifuge tube and add 150 µl (3 volumes) of Qiagen elution buffer and mix. From here, the 'input sample' is treated like the IP fractions.
34. To prepare 'size sample', add 100 µl of 'size sample' to a microcentrifuge tube and add 300 µl (3 volumes) of Qiagen elution buffer and mix. From here, the 'size sample' is treated like the IP fractions.
35. Reverse crosslink the immunoprecipitation by incubating at 65°C for ~ 9 hours (not more than 15 hours).

36. Material can be stored at -20°C overnight.

(4.5 hours) Day 4

Purification of DNA

37. Add 8 µl of RNaseA (10 mg/ml) to each sample and mix by inverting tube several times and incubate at 37°C for 2 hours.
38. Add 4 µl of Proteinase K (20 mg/ml) to each sample and mix by inverting tube several times and incubate at 55°C for 2 hours.
39. Purify DNA using a Qiagen gel extraction kit, elute sample with 60 µl EB. For "size sample" use a MinElute Spin column and elute with only 15µL EB and load everything on a 1.5% agarose gel.

Day 5

Real Time PCR set up

Perform real time PCR to check quality of DNA

Day 6

DNA repair

57 µl purified DNA
7.5 µl 10x blunting buffer
7.5 µl 1 mM dNTPs mix
3 µl blunt enzyme mix

- Incubate at RT for 45 min
- Purify DNA using the QIAquick PCR Purification Kit and elute with 41 µl EB

Add 'A' bases to the 3' end of DNA fragments

41 µl blunted DNA
1 µl of 10mM dATP
5 µl NEB Buffer 2
3 µl Klenow exo

- Incubate at 37°C for 45 min
- Purify DNA using the QIAquick PCR Purification Kit, a MinElute Spin column and elute with 16 µl EB

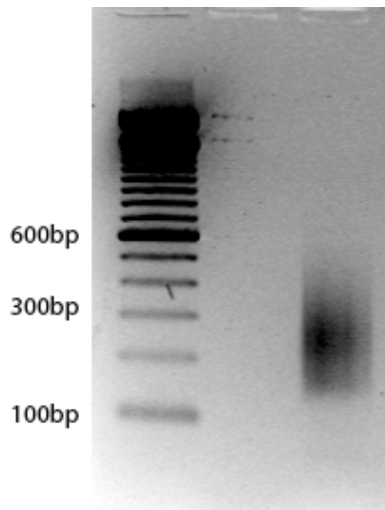
Ligate Adapter to DNA fragments

16 µl DNA
2 µl 10x DNA ligase buffer
1 µl Adapter (the amount of adapter depends on the amount of DNA and should to be titrated)
1 µl T4 ligase

- Incubate RT for 6 hours and purify DNA. Ligation time can probably be shortened.
- Purify DNA using the QIAquick PCR Purification Kit, a MinElute Spin column and elute with 10–15 μl EB

DNA (all)	10–15 μl
dNTPs (10 mM each)	1 μl
Phusion hot start polymerase	0.5 μl
5x Phusion HF buffer	10 μl
PCR primer 1.1 (Illumina)	0.75 μl
PCR primer 1.2 (Illumina)	0.75 μl
DMSO	1.5 μl
Add H_2O to a total volume of 50 μl	
Total	50 μl

Run PCR for 18–20 cycles depending on amount of IPed DNA.
Program:
1x 98°C, 30sec
20x 98°C, 10 sec / 65°C, 30 sec / 72°C, 30 sec
1x 72°C, 5 min



- Run 5 μl of PCR product on a 1.5 % agarose gel
- Purify DNA using the QIAquick PCR Purification Kit, with a MinElute Spin column and elute with 12 μl EB
- Quant-iT or Bioanalyzer. NanoDrop is not optimal as is also measures single stranded DNA, e.g. unused primers.

Reagents

Block Solution:
1x PBS, 0.5% bovine serum albumin (BSA)

Formaldehyde Solution:
Should be made fresh.
50 mM HEPES-KOH, pH 7.5, 100 mM NaCl, 1 mM EDTA, 0.5 mM EGTA and 11% formaldehyde.

Lysis Buffer 1:
Add protease inhibitors just before use, filter and keep cold.
50 mM HEPES-KOH, pH 7.5, 140 mM NaCl, 1 mM EDTA, 10% glycerol, 0.5% NP-40, 0.25% Triton X-100, 1x protease inhibitors

Lysis Buffer 2:
Add protease inhibitors just before use, filter and keep cold.
10 mM Tris-HCl, pH 8.0, 200 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 1x protease inhibitors

Lysis Buffer 3:
Add protease inhibitors just before use, filter and keep cold.
10 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 0.1% Na-Deoxycholate, 0.5% N-lauroylsarcosine, 1x protease inhibitors.

Wash Buffer (RIPA):
Keep cold.
50 mM HEPES-KOH, pKa 7.55, 500 mM LiCl, 1 mM EDTA, 1.0% NP-40, 0.7% Na-Deoxycholate.

Elution Buffer:
50 mM Tris-HCl, pH 8.0, 10 mM EDTA, 1.0% SDS

Klenow Fragment (3´→5´ exo–):
NEB, # M0212S

Quick blunting kit
NEB, # E1201S

Phusion™ Hot Start High-Fidelity DNA Polymerase
NEB, # F-540S

Non-stick microcentrifuge tubes:
E.g. Ambion 1.5 ml #AM12450 and 2.0 ml #AM12475

Magnetic beads:
Dynabeads® (2.8 μm) coupled to sheep anti-mouse or anti-rabbit IgG antibodies

Quant-iT™ DNA Assay Kit
Invitrogen, High Sensitivity *0.2 - 100 ng*, # Q-33120

Qiagen Kits:

QIAquick PCR Purification Kit, # 28104
MinElute Gel Extraction Kit, # 28604.
Whenever an elution with less than 30 μl is required use the MinElute columns.

Protease Inhibitor Cocktail
Sigma, P8340. Other protease inhibitors should work as well.

Adapters and PCR primers
Illumina, ChIP sequencing kit or genomic DNA sequencing kit. We usually only purchase the adapters and PCR primers from Illumina but Illumina offers complete kits including DNA blunting mix, ligases etc.