

Chromatin Preparation from Small Cell Populations

Cross-linking

1. Spin down cells at max speed for 10s at RT then aspirate supe.
2. Re-suspend cell pellet in 500 μ l PBS and chop with fine scissors if necessary.
3. Add 500 μ l 2.22% formaldehyde/PBS.
4. Rotate 10 min at RT.
5. Stop cross-linking by adding 59 μ l 2.5 M glycine to final conc 0.14 M.
6. Rotate 5 min at RT.
7. Spin down cells at max speed for 10s at RT then aspirate supe.
8. Add 1 mL PBS to wash, spin down cells, and aspirate supe.
9. Bring up pellet in 100-200 μ l cold **ChIP Whole Cell Lysis Buffer**.
10. Homogenize with small plastic pestle by hand and incubate on ice for 10 min.

Sonication and Input Prep.

11. Sonicate using BioRuptor:
 - a. High setting, 3 x 5min, cycling 30s on – 30s off
12. Spin in microfuge at max RPM at 4°C for 15 minutes.
13. Take 10 μ l as Input, add 90 μ l PBS and 3.5 μ l 5M NaCl and incubate at 65°C overnight.
14. Freeze remainder in liquid N₂.
15. The next day, add 4 μ l 1M Tris-HCl (pH 7.5), 2 μ l 500mM EDTA, and 1 μ l 10mg/mL Proteinase K, to the Input sample.
16. Incubate 1 hour at 45°C
17. Column purify (PCR cleanup kit) and elute in 50 μ l EB.
18. Run ~1 μ l on Bioanalyzer to check for appropriate shearing.

Buffers

1. 2.22% formaldehyde/PBS (make fresh)
 - a. for 1 mL: 60 μ l 37% formaldehyde + 940 μ l PBS
 - b. for 5 mL: 300 μ l 37% formaldehyde + 4700 μ l PBS
2. 2.5 M glycine (make fresh)
 - a. 190 mg in 1ml H₂O
3. **ChIP Whole Cell Lysis Buffer:**
(10 mM Tris-Hcl, pH 8.0, 10 mM NaCl, 3 mM MgCl₂, 1% NP-40, 1% SDS, 0.5% DOC)

1M Tris-HCl (pH 8.1)	10 μ l
5M NaCl	2 μ l
1M MgCl ₂	3 μ l
10% NP-40	100 μ l
10% SDS	100 μ l
10% DOC	50 μ l
50X Prot Inhib.	20 μ l
100X Phos'tase Inhib.	10 μ l
H ₂ O	<u>705μl</u>
	1mL

Chromatin Immunoprecipitation Assay

1. Thaw chromatin quickly at 37°C and place on ice
2. Add appropriate amount (1-10 µg) to 1 mL of **ChIP Dilution Buffer + 20 µL 50X Protease Inhibitor**

ChIP Dilution Buffer (16.7 mM Tris-HCl pH 8.1, 167 mM NaCl, 0.01% SDS, 1.1% Triton-X 100)

1M Tris-HCl, pH 8.1	835 µL
5M NaCl	1.67 mL
10% SDS	50 µL
10% Triton-X 100	5.5 mL
H ₂ O	<u>41.9 mL</u>
	50 mL

3. Add antibody (~2 µg) and rotate in cold room overnight

Block Agarose:

4. Wash protein-A or -G agarose 3X with 1 mL cold **ChIP Dilution Buffer**
- resuspend in appropriate amount of cold **ChIP Dilution Buffer + BSA** (see below)

For 5 samples (500 µL final volume) for 1 sample (100 µL final volume):

protein-A/G agarose slurry	200 µl (100 µL agarose)	40 uL (20 µL)
10mg/ml BSA	50 µL	10 µL
50X Protease Inhibitor	10 µL	2 µL
ChIP Dilution Buffer	<u>340 µL</u>	<u>68 µL</u>
	500 µL	100 µL

5. Rotate in cold room overnight

Immunoprecipitate:

6. Add 100 µL of blocked protein-A or -G agarose to each chromatin sample
- rotate in cold room for 1 hour
7. Spin down beads 30s at minimum speed and aspirate supe

Wash:

8. Add 1 mL of appropriate room temp. **Wash Buffer** to agarose pellet (see below)
- rotate at room temp. for 5 min
- spin down agarose 30s at minimum speed and aspirate supe.
9. Perform wash 1X with each of the following **Wash Buffers** in order: **TSE I, TSE II, ChIP Buffer III, TE**

TSE I: 20 mM Tris-HCl (pH 8.1), 150 mM NaCl, 2mM EDTA, 0.1% SDS, 1% Triton X-100

1M Tris-HC, pH 8.1	1 mL
5M NaCl	1.5 mL
0.5M EDTA	200 µL
10% SDS	500 µL
10% Triton X-100	5 mL
H ₂ O	41.8 mL

TSE II: 20 mM Tris-HCl (pH 8.1), 500 mM NaCl, 2mM EDTA, 0.1% SDS, 1% Triton X-100

1M Tris-HCL, pH 8.1	1 mL
5M NaCl	5 mL
0.5M EDTA	200 μ L
10% SDS	500 μ L
10% Triton X-100	5 mL
H ₂ O	38.3 mL

ChIP Buffer III: 10 mM Tris-HCl (pH 8.1), 0.25M LiCl, 1mM EDTA, 1% NP-40, 1% deoxycholate

1M Tris-HCl, pH 8.1	500 μ L
5M LiCl	2.5 ml
0.5M EDTA	100 μ L
10% NP-40	5 mL
10% deoxycholic acid	5 mL
H ₂ O	36.9 mL

TE: 10 mM Tris-HCl (pH 8.1), 1mM EDTA

1M Tris-HCl, pH 8.1	500 μ L
0.5M EDTA	100 μ L
H ₂ O	49.4 mL

Elute/Reverse Crosslinks:

10. Add 100 μ L freshly made **Elution Buffer** to final pellet and rotate 15 min at room temp

Elution Buffer: (1% SDS, 0.1 M NaHCO₃)

10% SDS	100 μ L	
1M NaHCO ₃	100 μ L	(84 mg in 1 mL H ₂ O made fresh)
H ₂ O	800 μ L	

11. Pellet agarose and transfer supe to new tube

12. Repeat elution and combine eluates

13. Add 8 μ L 5M NaCl per 200 μ L eluate (192 mM NaCl) and incubate at 65°C overnight

14. Add 8 μ L 1 M Tris-Hcl, pH 7.5, 4 μ L 0.5 M EDTA, and 1 μ L 10 mg/ml proteinase K
- incubate 1 hour at 45°

15. Purify un-crosslinked chromatin using Qiagen PCR purification kit. Elute in 50 μ L EB.

FGC's ChIP-Seq Sample Prep Suggestions

One can order the kit directly from Illumina and follow their protocol:

ChIP Seq DNA sample prep kit is: IP-102-1001

To lower cost, one can order the same reagents separately from NEB and follow Illumina's protocol (with some minor changes below):

Quick Ligation Kit: M2200S

T4 DNA Polymerase: M0203S

Klenow(3' -> 5' exo): M0212S

T4 Polynucleotide Kinase: M0201S

Phusion High Fidelity Polymerase: F-530S

DNA Polymerase I, Large (Klenow) Fragment: M0210S

T4 DNA Ligase Buffer with 10mM ATP: B0202S

In addition one would have to order primers from Illumina: FC-102-1003 ~ \$5,300

- 100 RXN Genomic DNA Sample Prep Oligo Only Kit
Kitted oligos and adapters for Genomic DNA Sample Prep of 100 DNA samples

Minor protocol adjustments, if buy reagents from NEB:

Add 'A' Bases to the 3' End of the DNA Fragments:

- Use NEBuffer 2 supplied with the Klenow, instead Klenow Buffer

Ligate Adaptors to DNA Fragments:

- Use 1ul of DNA Ligase and 3ul of Water, instead of 4ul of DNA Ligase. The Illumina Kit's DNA Ligase is more dilute than the NEB DNA Ligase.

GEL size selection step:

Invitrogen's SYBR GOLD: S11494

BioRad's Low Range Ultra Agarose: 161-3106

- Ethidium bromide and UV light can degrade and damage your DNA library, if taking too long to cut the bands out. We like to use Sybr Gold instead of EtBr and use the Dark Reader Transilluminator instead of a Gel dock.
- Dilute the Sybr Gold to 1:1000.
- Add 1000ng of Ladder, instead of 500ng.
- The Ladder Mix is 2ul of 1000ng Ladder, 8ul Diluted Sybr Gold, 3ul Loading Buffer, and 1ul of Salt Buffer (for example Roche Restriction Enzyme Buffer H).
- Add 1ul of Salt Buffer in addition to the 3ul of loading buffer to the DNA.

DNA purification steps:

Each time DNA is purified through a column, we generally elute in 2ul more volume and pipette around the plastic rim to remove as much ethanol as possible.

For more info refer to [Quail, M.A. et al. A large genome center's improvements to the Illumina sequencing system. *Nature Methods*. **12**, 1005-1010 \(2008\).](#)