Buffer Preparation for ChIP

Glycine (2.5M)
Glycine: 93.8g
ddH2O: 500ml
Gentle heating might be required

Lysis Buffer
1M HEPES-KOH (pH7.5): 10ml
5M NaCl: 5.6ml
0.5M EDTA (pH8): 0.4ml
Triton X-100: 2ml
10% SDS: 2ml
10% Sodium Deoxycholate: 2ml
ddH2O: 178ml
Total: 200ml
Protease inhibitors (add fresh)

Low Salt Wash Buffer
10% SDS: 0.5ml
Triton X-100: 5ml
0.5M EDTA (pH8): 2ml
1M Tris-HCl (pH8): 10ml
5M NaCl: 15ml
ddH2O: 467.5ml
Total: 500ml

High Salt Wash Buffer
10% SDS: 0.5ml
Triton X-100: 5ml
0.5M EDTA (pH8): 2ml
1M Tris-HCl (pH8): 10ml
5M NaCl: 50ml
ddH2O: 432.5ml
Total: 500ml

LiCl Wash Buffer
1M LiCl: 125ml
NP-40: 5ml
10% Sodium Deoxycholate: 50ml
0.5M EDTA (pH8): 1ml
1M Tris-HCl (pH8): 5ml
ddH2O: 314ml
Total: 500ml

TE Buffer
1M Tris-HCl (pH8):
10ml
0.5M EDTA (pH8): 1ml
ddH2O: 489ml
Total: 500ml

Elution Buffer
10% SDS: 10ml
0.5M EDTA (pH8): 10ml
1M Tris-HCl (pH8): 5ml
ddH2O: 75ml
Total: 100ml

ChIP General Protocols

Cross-linking and Chromatin Preparation

1. Add Formaldehyde to cell culture medium at a final concentration of 1%. (Start with 50-100 million cells per experiment)
2. Shake culture flasks for 10 minutes at RT.
3. Add glycine to quench the reaction at a final concentration of 125mM. Shake culture flasks for 5 minutes at RT.
4. Wash cells twice with 10-15ml of ice-cold PBS.
5. Add 5-10ml of cold PBS to flask, scrape cells and transfer to 50ml tube.
6. Centrifuge at 1000xg, 5 mins at 4°C.
7. Remove supernatant and resuspend pellet in lysis buffer (750µl for 10 million cells) (For suspension cultures, pellet cells after glycine treatment, wash twice with ice-cold PBS. Resuspend pellet in lysis buffer (750µl for 10 million cells))
8. Incubate pellet on ice for 10 minutes.
9. Proceed with sonication or MNase digestion to shear DNA.
10. Make sure that the sheared DNA give rise to fragment size of 200-1000bp. Optimization is needed for each cell types or tissues.
11. After sonication, centrifuge cell lysate at 8,000xg for 10 mins at 4˚C.
12. Transfer supernatant to new tube. Remove 50µl to determine fragment size and DNA concentration, keep the rest at -80°C freezer for storage.

Determination of fragment size and DNA concentration

1. Add 70µl of elution buffer to 50µl of sheared DNA. Add 1µg RNAse A and 4µg Proteinase K, and heat at 65˚C overnight to reverse crosslink.
2. Purify DNA by phenol-chloroform or using a kit.
3. Run 5µl of purified DNA sample on 1.5% agarose gel. Determine DNA concentration with spectrometer.

Immunoprecipitation and Washing

1. Use 25µg of DNA per IP as a start. (The optimal starting material need to be optimized according to each sample type and antibody)
2. Prepare 1 tube of chromatin for antibody IP, and another tube for control (beads only). Remove 50µl of chromatin as input sample, store temporarily at -20°C for later use.
3. To preclear the chromatin, add 50µl Protein-A agarose/salmon sperm DNA or Protein-G agarose/salmon sperm DNA beads to chromatin and rotate for 1-2h at 4 ° C. Centrifuge chromatin samples at 2000xg for 5 mins at 4°C. Transfer supernatant to new tube.
4. Add primary antibody to tube (except for bead-only tube). The amount of antibody used per IP can found on the datasheet or determined by user.
5. Rotate overnight at 4°C.
6. Add 50µl of blocked Protein A or G slurry to capture complexes.
7. Incubate for 2 hour at 4°C on a rotator.
8. Centrifuge the tube at 2000xg for 1min at 4°C.
9. Carefully discard the supernatant.
10. Wash beads once with low-salt buffer, once in high-salt buffer, once in LiCl wash buffer and once with TE buffer. Washing procedure: Resuspend beads in 1 ml wash buffer, rotate for 10 minutes at 4°C, centrifuge at 2000xg for 1 min at 4°C and remove supernatant.

Elution, reverse-crosslink and DNA purification

1. Elute DNA by adding 300µl of Elution buffer (supplemented with 1µl of Proteinase K (20µg/µl) to beads. Incubate samples for 2 hours at 55°C. Gently vortex tubes occasionally.
2. To reverse-crosslink, incubate overnight at 65 ° C. (Input should also be reverse cross-linked at the same time)
3. Centrifuge samples at 15,000xg for 5 mins at RT.
4. Transfer supernatant to a new tube.
5. Purify DNA using phenol-chloroform or DNA purification kit.
6. Resuspend or elute DNA in 50µl TE buffer and analyze by PCR or Real-Time PCR.

How Not to Fail a ChIP Experiment?

1. Cross-linking

Cross-linking is an important step to make sure that the protein is still bound to the DNA fragment during immunoprecipitation. However, it is a time-critical process and should be optimized for each sample type or antibody type. Excessive cross-linking might mask the antibody binding sites and reduce binding ability. Therefore, it is advisable to optimize the cross-linking steps by using different concentration of formaldehyde or changing cross-linking time.

2. Fragmentation method

Optimal sonication time course or concentration of micrococcus nucleus should be determined prior to performing a ChIP experiment. Make sure that the majority of fragmented DNA falls between 200-500bp. Load 5-10 μl of sample on agarose gel to analyze DNA fragment size after each sonication course.

3. Choice of Protein A or Protein G

Protein G is often considered a more universal IgG Binding Protein than Protein A, but different species, and subtypes of species, do vary in their binding to these proteins. Refer to the table at page 4 to find the best material that suit your antibody subclasses.

4. Include positive and negative antibody controls

Histone H3K4me3 antibody is a popular positive control to use when studying active gene, while Histone H3K9me3 antibody is a negative control for studying inactive gene. An antibody that do not recognize chromatin epitope such as GFP antibody or IgG isotype control antibody should also be included in ChIP experiments to make sure that results came out to be valid.

Species	Subclass	Protein A	Protein G	Protein L
Human	Total IgG	+++	+++	+++
	IgG1	+++	+++	+++
	IgG2	+++	+++	+++
	IgG3	-	+++	+++
	IgG4	+++	+++	+++
	IgA,M,D	-	-	+++
Mouse	Total IgG	+++	+++	+++
	IgG1	+++	+++	+++
	IgG2	+++	+++	+++
	IgG3	-	+++	+++
	IgG4	+++	+++	+++
	IgA,M,D	-	-	+++
Rat	Total IgG	-	++	+++
	IgG1	-	+	+++
	IgG2a	-	+++	+++
	IgG2b	-	+	+++
	IgG2c	+	+	+++
Chicken	IgY	-	-	-
Goat	Total IgG	-	++	-
Rabbit	Total IgG	+++	+++	-

+++ Strong binding ++ Medium binding + Weak binding - No binding

Protein A

Protein A is a cell wall component of Staphylococcus aureus which binds to the Fc region of immunoglobulins, especially IgG.

Protein G

Protein G is a cell wall component isolated from Group B streptococci which binds to most mammalian immunoglobulins through their Fc region.

Protein L

Protein L is a surface component of Peptostreptococcus magnus which binds immunoglobulins through their light chains. This protein is thus able to bind to antibody classes including IgA, IgD, IgE or IgM