Original ChIP protocol

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1 Introduction

This protocol from Faith *et.al.*, PLoS Biology 2007 is a mixture of four protocols from abcam, upstate, upstate Tips/Protocol, and Lin and Grossman, Cell 1998, and Herring *et.al.* Journal of Bacteriology 2007. It is provided in case anyone wants to replicate our ChIP optimization results or compare the optimized protocols with the original. The optimized protocols have increased signal-to-noise, higher throughput, and are *much* faster than this protocol. In general, the optimized protocols should be used instead of this one.

2 Recipes

2.1 Phosphate Buffered Saline (PBS)

Reagent	Amount per Liter
KCl	0.2g
NaCl	8g
KH_2PO_4	0.240g
Na_2HPO_4	1.44g

Adjust pH to 7.4 with HCl, I always check with the pH meter and it never has needed adjusting. Autoclave to sterilize.

2.2 100 mM PMSF

17.4 mg/ml in isopropanol. Store at -20C. Has a tendency to precipitate over time in the freezer, so I typically make it fresh.

2.3 lysis buffer

Reagent	Concentration	Stock Conc.	Amount for 50ml
Tris-HCl	10mM Tris	1M	500 μl
NaCl	50mM	5M	500 μl
EDTA	10mM	0.5M	1 ml
Sucrose	20%	-	10 g
Ready-lyse lysozyme	0.2 μl per 500 add fresh	-	

Recipe taken from Herring *et.al.* Journal of Bacteriology 2006.

2.4 2xIP buffer

Reagent	Concentration	Stock Conc.	Amount for 50ml
Tris-HCl	200mM Tris	1M	10ml
NaCl	600mM	5M	6ml
Triton X-100	4%	-	2ml
1mM PMSF	added just prior to use	100mM	500 μl

2.5 Dilution Buffer

Reagent	Concentration	Stock Conc.	Amount for 50ml
Triton X-100	1%	-	500 μl
EDTA	2 mM	0.5 M	200 μl
NaCl	150 mM	5 M	1.5 ml
Tris-HCl pH 8	20 mM	1 M	1 ml
PMSF	1 mM added just prior to use	100 mM	500 μl

Taken from www.abcam.com

2.6 Low Salt Immune Complex Wash Buffer

Reagent	Concentration	Stock Conc.	Amount for 50ml
SDS	0.1%	-	250 μl (of 20%)
Triton X-100	1%	-	500 μl
EDTA	2 mM	0.5 M	200 μl
Tris-HCl pH 8	20 mM	1 M	1 ml
NaCl	150 mM	5 M	1.5 ml

2.7 High Salt Immune Complex Wash Buffer

Reagent	Concentration	Stock Conc.	Amount for 50ml
SDS	0.1%	-	250 μl (of 20%)
Triton X-100	1%	-	500 μl
EDTA	2 mM	0.5 M	200 μl
Tris-HCl pH 8	20 mM	1 M	1 ml
NaCl	500 mM	5 M	5 ml

2.8 LiCl Immune Complex Wash Buffer

Reagent	Concentration	Stock Conc.	Amount for 50ml
LiCi	0.25 M	5 M	2.5 ml
NP40	1%	-	500 μl
deoxycholate	1%	solid powder	500 mg
EDTA	1 mM	0.5 M	100 μl
Tris-HCl pH 8	10 mM	1 M	500 μl

2.9 TE

Reagent	Concentration
Tris pH 8	10 mM
EDTA pH 8	1 mM

2.10 Elution buffer

Reagent	Concentration
SDS	1%
NaHCO3	0.1 M

make this solution fresh

3 Supplies

Many bio-supply companies sell the reagents listed in the supplies section above. Here we list the part numbers for the reagents we commonly use. Most of the dry chemicals were purchased from Fisher Scientific.

Solution	Supplier	Part Number
EDTA pH 8.0	Ambion	AM9260G
LiCl 7.5 M	Ambion	AM9480
NaCl 5 M	Ambion	AM9760G
NP40	US Biological	N3500
protein G agarose beads	Upstate	16201MI
proteinase K	Ambion	AM2546
Ready-Lyse	Epicenter	R1804M
RNAse cocktail	Ambion	AM2286
SDS 20%	Fisher	BP1311-200
TE pH 8.0	Ambion	AM9849
Tris 1M pH 8.0	Ambion	AM9856
Triton X-100	MP Biomedicals	8074

4 Protocol

4.1 Day 1: Lyse, Crosslink, Shear

4.1.1 Cell Growth

1. Dilute an overnite culture of your strain(s) 1:100 dilution in 50 ml of liquid media in a 250 ml flask with appropriate antibiotics

2. after 1 hr add IPTG or other inducer as needed for induction of any cloned constructs (if you have a specific non-cloned gene or tagged gene in the genome, this step is not necessary)

3. After your cells reach sufficient density (OD600 = 0.5) pipette a 15 ml sample into a 15 ml centrifuge tube [Corning Part No: 430053].

4.1.2 Crosslinking

1. add 400 μl of 37% formaldehyde[1] (1% final concentration)
2. invert tube 10 times to mix
3. incubate 10 min at room temp. Do NOT crosslink too long!
4. pellet cells by centrifugation for 15 min at 3200g
5. decant supernatant into a formaldehyde/media hazardous waste bin
6. wash the cells 2x in 10 ml of ice-cold PBS (spin 3200g for 10 min in between washes to pellet cells). From here on, keep samples on ice to limit the effect of proteases.
7. prior to lysis remove any remaining drops of PBS that wouldn't go away by decanting with a P1000 pipettor (alternatively you can placing the centrifuge tube upside down on a stack of paper towels should do the trick as well)

4.1.3 Lysis

1. add 0.4 μl Ready-Lyse per ml of lysis buffer into a ChIP lysis buffer master mix (just before using)
2. begin lysis by adding 500 μl ChIP lysis buffer and incubate for 37C for 30 min (no shaking)
3. add 10 μl of 100mM PMSF and 2 μl RNAse cocktail per ml of 2x IP buffer (just before using)
4. add 500 μl 2xIP buffer with 1 μl fresh RNAse cocktail [Ambion] and incubate at 37C with shaking at 300rpm
5. lysate should be clear like slightly-soapy water
6. transfer the 1ml lysate to a 1.5ml eppy tube with a P1000

[1] formaldehyde is typically supplied as a 37% solution

4.1.4 Shearing

1. sonicate samples using a Branson 250 Sonifier for 30 secs at 20% power (more consistent results can be obtained by using a digital Branson where the exact time can be set. also, it helps to rig up the stand holding the sonifier tip to also hold the eppy tube so the tip stays in the same part of every eppy tube sample.)
2. keep samples on ice for at least 1 min between sonifications
3. sonicate each sample 4 times for shearing range of between 1000bp and 100bp with an average around 350bp (See Figure 1 for an example)

12 samples takes around an hour; dreadfully slow and boring; I play my ipod under the protective sonicator earphones. After half-an-hour this becomes painful if you have the ipod earbuds, so adjust them (in between sonications) until it doesn't hurt anymore. Shearing is much less tedious with some tunes.

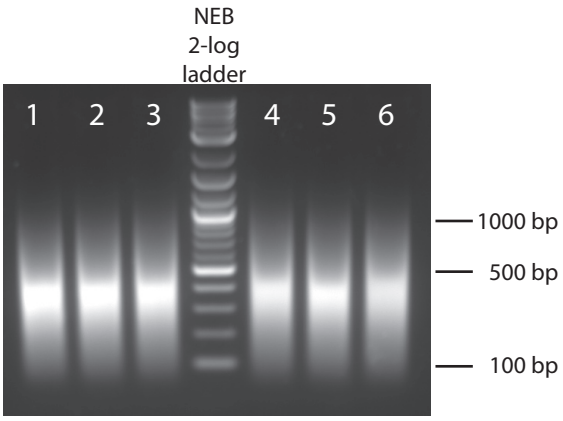

Figure 1: Chromatin samples sheared using a Branson 250 Sonifier – 4x30 sec at 20% power with a 1 minute break on ice in between shearings – have a shearing distribution from 1000bp-100bp with most of the DNA concentrated in the 300-500bp range.

4.1.5 Quantification of starting DNA

We prefer to start with the same amount of chromatin for each precipitation. We estimate the starting amount by cleaning up and quantifying a portion of the sheared chromatin. We can than start all of our precipitations with equal micrograms of DNA.

1. remove 100 μl from each sample to quantify the amount of DNA in each lysate and to verify the shearing range
2. place the remaining 900 μl in the -20C freezer for use in immunoprecipitation (it should be more than enough)
3. add 350 μl of water to the 100 μl sample (450 μl total volume); add 5 μl of 20mg/ml proteinase K added;
4. reverse crosslinks overnite at 65C

4.2 Day 2: Sheared DNA yield / Beginning of immunoprecipitation

This protocol also takes a day but with lots of breaks.

4.2.1 Quantification of starting DNA part 2

Although the DNA yield from this step is pretty high, I can never see a substantial DNA pellet (even though when doing precipitations with much smaller amounts I have seen one; perhaps because the DNA is sheared to such small fragments?). This used to worry me; now I just trust that it'll work.

1. separate DNA from proteins by phenol:chloroform extraction using gel phase lock tubes; I use the gel-phase tube only for the second half (the chloroform only step) and I get cleaner DNA than when I use the gel phase lock for both steps. Plus, it seems to smell less like phenol this way.
2. add 1/10th volume NaAcetate and 1 μl of glycoblue [Ambion] to help see the pellet
3. add 1 ml ethanol
4. place in -80C for 30min
5. spin at 0C at maximum rpm for 20 min
6. remove supernatant with vacuum at 50 mbar, place tip on/near side opposite the g-force (i.e. the side the DNA is not stuck to)
7. add 1 ml of cold 70% ethanol (some people say to resuspend DNA pellet here by vortexing, I don't).

8. spin at 4C for 5 min
9. remove supernatant with vacuum at 50 mbar, place tip on/near side opposite the g-force (i.e. the side the DNA is not stuck to)
10. air dry in fume-hood to allow ethanol to evaporate
11. resuspend DNA in 100 μl of TE
12. quantify the DNA using spectrophotometer (e.g. a Nanodrop)

The cleaned up DNA should be frozen at -20C and saved. It can serve as the positive control for downstream qPCR reactions. 500- 600ng of each sheared DNA sample should be run an a 1.5% agarose gel to verify shearing range.

4.2.2 Immunoprecipitation

Immunoprecipitations are begun with equal starting DNA (10 μg). So DNA is first quantified using the protocol above. The following steps I do in 2 ml eppy tubes.

1. take two 10 μg samples from the 900 μl left of the sheared chromatin. Label these **A**, **B**.
2. dilute all samples 1:10 in dilution buffer (if your DNA isn't concentrated enough for 1:10 to fit in the 2 ml eppy tube, dilute as much as possible, should still work).
3. preclear solution by adding 40μl of agarose beads and rotate at 4C for 90 minutes
4. spin at 1000g for 1 min and transfer supernatant to a new tube
5. add 2ug of the correct antibody to sample **A** (e.g. if your tag is myc put anti-myc here)
6. add 2ug of an incorrect antibody to **B** (a negative control) (e.g. if your tag is myc put anti-Xpress here)
7. **C** is a negative control with no antibody

8. rotate all samples overnite at 4C, preferably in 2 ml eppy tubes (these allow better mixing during rotation because the liquid doesn't get stuck as poorly in the bottom; they do however make the later bead washing more difficult because it is hard to see the beads in the 2ml tube)

5 Day 3: Sheared DNA yield / Beginning of immunoprecipitation

5.1 Add beads to capture antibody

1. add 60μl of salmon sperm / agarose beads. Rotate at 4C for 2hr.

5.2 Bead Washing

1. wash beads 1x with low salt wash
2. wash beads 1x with high salt wash
3. wash beads 1x with LiCl wash
4. wash beads 2x with TE (steps at room temp from now on)
5. elute by adding 225 μl fresh elution buffer and rotating for 15 min. Transfer the supernant to a 1.5 ml eppy tube.
6. repeat elution with 225 μl more elution buffer and combine supernant with previous supernant.

5.3 Crosslink reversal

1. add 10 μl 5M NaCl solution to the 450 μl elution and incubate overnite in a 65C heat block

6 Day 4: Final DNA cleanup

I proceed similar to the section **Quantification of starting DNA part 2** above, except I add an extra 10 min to each centrifuge time, just to be safe.

1. add 10 μl EDTA, 20 μl Tris [pH 8] then add 1 μl of proteinase K and incubate 1hr at 45C.

2. separate DNA from proteins by phenol:chloroform extraction using gel phase lock tubes

3. add 1/10th volume NaAcetate and 2 μl of N mg/ml glycogen as a DNA carrier and ethanol precipitate

4. resuspend DNA in 100 μl of TE

Quantification of the product from the above protocol doesn't seem to be useful and just wastes a little sample. There's not enough DNA to quantify.