UTA Iyer lab ChIP-Seq protocol

From EncodeDCC

Iyer lab ChIP-Seq Protocol

ChIP protocol (1):

1. Cell culture: 225 x 225 squre plate/1~2 ChIP (80 ml media, 90% confluence)
2. Formaldehyde cross linking: directly add to the plates at RT to final 1% formaldehyde (2.16 ml). Incubate for 7 min at RT w/ shaking.

3. Add 2.5 M glycine to make final 125 mM (4 ml) for 5 min at RT w/ shaking.

4. Rinse the cells w/ PMSF containing PBS for 1 time. Scrape the cells and rinse the cells two times w/ PBS. Spin the cells at 3500 rpm for 4 min.

5. Resuspend the cell pellet w/ SDS lysis buffer.

6. Sonication with Bioruptor:

- Cell No.: 10^7 cells
- Working volumes: 200ul of samples in SDS lysis buffer with protease inhibitor cocktail.
- Conditions: 30 sec on and 1 min off with high power, for 10min X 4 times. (The size of sheared DNA fragment is checked on 1% gel by loading 5ul of 5min heated samples) The size of sheared DNA fragments is around 300 to 500bp.

7. Spin the extract at maximum speed for 7 min. Transfer supernatant to new tubes (you may store your sample at -70ºC here). And add 800ul of ChIP dilution buffer.

8. Pre-clear extract w/ pre-washed 50 ul of 50% protein A or G beads w/ ChIP dilution buffer for 3 hour at 4ºC using nutator.

9. Add antibody (1 : 100 dilution usually) and incubate O/N at 4ºC.

10. Apply 50 ul of pre-washed protein A or G beads and incubate for 3 hours

at 4ºC.

11. Wash the beads at RT: low salt x2, high salt x1, LiCl x1, TE x2 for 5 min each.

12. Add 100 ul of SDS elution buffer. Incubate at 65ºC for 20 min. Spin down (3000 rpm, microcentrifuge) and transfer 80 ul of supernatant to new tube and add additional 50 ul elution buffer to the original tube. Incubate again and transfer 70 ul to a new tube.

13. Seal well (or use screw cap tube) and incubate at 70ºC 6 hours to O/N.

14. Add 3 ul of 0.5 ug/ul RNaseA, incubate at 37ºC for 30min.

15. Add 150 ul of TE and 2 ul of 20 mg/ml glycogen and 3 ul of 20 mg/ml proteaseK. Incubate at 37ºC for 2

hours.

16. Add 300 ul of PCI mix and get the upper phase after applying Phase Lock Gel.

17. Add 1/10 volume of 3 M sodium acetate(pH 5.2) and 2.5 volume of 100% EtOH. Keep at -20ºC for 2 hours and do precipitation.

18. Apply 70% EtOH for washing, dry the pellet and resuspend in 20 ul of TE.

ChIP-Seq DNA library construction:

According to Illumina manufacture protocols:

Preparing Samples for ChIP Sequencing of DNA (11257047 Rev A)

Illumina ChIP-Seq library prep protocol

Further purification with Agencourt AMPure Kit to remove primer dimmers:

1. Add 1.8 volumes of Agencourt AMPure beads to the sample and incubate for 5 minutes at room temperature on a rotator.

2. Place the tube of beads in the magnetic rack to separate the beads from solutions. Wait for the solution to clear before proceeding to the next step.

3. Remove the supernatant and discard.

4. Dispense 200 ul of freshly prepared 70% ethanol, vortex the tube, and incubate for 30 seconds at room temperature.

5. If needed, pool the beads from both tubes into one 1.5 ml tube.

6. Place the tube of beads in the magnetic rack to separate the bead from solution. Wait for the solution to clear before proceeding to the next step.

7. Aspirate the ethanol and discard.

8. Repeat step 4 to 7 two more time.

> Note: Using freshly prepared 70% ethanol is critical because a higher percentage will result in an in efficient washing of smaller-sized molecules while wusing lower than 70% ethanol could cause loss of sample.

9. Place the tube of beads in the magnetic rack and remove the supernatant 2-3 more times to remove the residual ethanol

10. Dry the beads at room temperature.

11. Prepare a 10 mM Tris, pH 8 solution.

12. Elute the DNA by adding 20 ul, 10 mM Tris pH 8, vortexing for 10 seconds and ensuring homogeneity by piptting the solution up and down several time.

13. Place the tube of beads in the magnetic rack to separate beads form solution. Wait for the solution to clear

before proceeding to the next step

14. Save the eluted sample in a 1.5 tube.

15. Place the tube of eluted sample in the magnetic rack again to separate any remaining beads from solution. Wait for the solution to clear before proceeding to the next step.

16. Save the eluted sample in a new 1.5 ml tube.

17. Repeat steps 15, 16 once.

18. Store the DNA in a 1.5ml tube.

ChIP protocol (1) Solutions				
			Stock conc.	**For 10 ml**
SDS lysis buffer				
	SDS	1%	10%	1 ml
	EDTA	10 mM	0.5 M	200 ul
	TrisCl(8.1)	50 mM	1 M	500 ul
	*Adding protease Inhibitor before using			
ChIP dilution buffer				
	SDS	0.10%	10%	100 ul
	Triton X-100	1%	10%	1 ml
	EDTA	2 mM	0.5 M	40 ul
	TrisCl(8.1)	20 mM	1 M	200 ul
	NaCl	150 mM	5 M	300 ul
	*Adding protease Inhibitor before using			
Low salt wash buffer				
	Deoxycholate	0.10%	10%	100 ul
	Triton X-100	1%	10%	1 ml
	EDTA	1 mM	0.5 M	20 ul
	HEPES(7.5)	50 mM	1 M	500 ul
	NaCl	150 mM	5 M	300 ul
High salt wash buffer				

	Deoxycholate	0.10%	10%	100 ul
	Triton X-100	1%	10%	1 ml
	EDTA	1m M	0.5 M	20 ul
	HEPES(7.5)	50 mM	1 M	500 ul
	NaCl	500 mM	5 M	1 ml
LiCl wash buffer				
	LiCl	250 mM	3 M	833 ul
	NP40	0.50%	20%	250 ul
	Deoxycholate	0.50%	10%	500 ul
	EDTA	1 mM	0.5 M	20 ul
	TrisCl(8.1)	10 mM	1 M	100 ul
SDS elution buffer				
	SDS	1%	10%	1 ml
	EDTA	10 mM	0.5 M	200 ul
	TrisCl(8.1)	50 mM	1 M	500 ul

Category: Uncategorized