ULI-NChIP assay protocol

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Method Article

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Abstract

This protocol presents ULI-NChIP-seq (ultra-low-input micrococcal nuclease-based native ChIP-seq) assay to generate high quality and complexity genome-wide histone mark profiles from rare oocytes andembryos populations. The procedure of ULI-NChIP-seq assay typically consists of five parts including Binding antibodies to magnatic beads, Chromatin shearing and nuclear membrane solubilization, Magnetic immunoprecipitation, Washes and DNA isolation. Sample preparation involves to remove the zona Pellucida of oocyte and polar body to avoid the genomic contamination of polar bodies.

Introduction

By optimizing the traditional chromatin immunoprecipitation (ChIP) technology, combined with the new DNA library technology (TELP), scientists have developed a new set of high-sensitivity research on histone modification technology suitable for very low cell mass, making One can detect histone modification patterns in the whole genome[1-6]. According to the existing research results, people's understanding of histone modifications has become clearer, and they have gradually realized that the distribution of various histone modifications on the genome is not uniform: the trimethylation of lysine is mainly enriched in the CDS region of genes , Acetylation is mainly distributed in the promoter region of specific genes and the 5'end of CDS. Generally speaking, each modification of histone has its own special modification map (landscape) on the genome. At the evolutionary level, by comparing the sequencing data of yeast, mouse and human, it is found that the distribution pattern of histone modifications is relatively conservative. At the same time, due to the limitations of research methods, there are still few studies on the establishment and regulation of specific histone modifications. If a mutation study is performed on histones, it is difficult to carry out because of the multiple copies of histone coding genes in mammalian cells. Generally, when a gene is knocked out or knocked down, a certain histone modification is increased or decreased to speculate that the gene is involved in the regulation of the histone modification. At the same time, there are obvious differences in the distribution pattern of a histone modification on the genome in different developmental stages and different tissues, and the same histone modification may have multiple modification enzymes to modify it. Thanks to the accumulation of various research methods and the establishment of the new micro-sequencing technology ULI-NChIP (ultra-low-input micrococcal nuclease-based native ChIP), we can study the genetic and reprogramming patterns and molecular regulation of histone modifications before and after fertilization mechanism.

Reagents

1. Micrococcal Nuclease (MNase) Cat:NEB M0247S

2. 100mM DTT INVITROGEN # 18080044

SuperScript III Reverse Transcriptase Kit 100mM DTT

3. Poly ethylene glycol (PEG6000)

SIGMA CAS: 25322-68-3 81255-250G

4. Dynabeads® Protein A for Immunoprecipitation (DPA beads)

LIFE Cat: 10001D-100ml

5. Ethylenediaminetetraacetic acid solution (EDTA)

SIGMA CAS: 60-00-4 03690-100ml

6. TritonTM X-100 solution

SIGMA CAS: 9002-93-1 93443-100ml

7. Sodium deoxycholate (DOC)

SIGMA CAS: 302-95-4 d6750-10g

8. MaXtractTM High Density (PhaseLock tube)

QIAGEN Cat.No. 129046

9. phenol:chloroform:isoamyl alcohol

SOLARBIO Cat.No. P1012

10. Sodium acetate (NaOAc)

SIGMA CAS: 127-09-3 v900212-500g

11. Glycogen

Roche Product No. 10901393001

12. Isopropyl alcohol

CAS: 67-63-0

13. Ethanol

CAS: 64-17-5

14. Protease Inhibitor Cocktail Tablets in EASYpacks (PIC)

ROCHE Product No. 04693116001

15. Sodium dodecyl sulfate solution (SDS)

SIGMA CAS: 151-21-3 71736-100ML

16. Sodium bicarbonate (NaHCO3)

SIGMA CAS:144-55-8 v900182-500g

17. UltraPureTM Distilled Water

INVITROGEN 10977-015

18. Magnesium chloride hexahydrate (MgCl2)

SIGMA CAS:7791-18-6 M2670-100G

19. Nonidet P40 Substitute (NP40)

ROCHE Product No. 11332473001

20. Phenylmethanesulfonyl fluoride (PMSF)

SIGMA CAS: 329-98-6 p7626-1g

21. Sodium chloride (NaCl)

SIGMA CAS: 7647-14-5 V900058-500G

22. Agencourt AMPure XP 60 mL Kit

Beckman A63880/A63881

Equipment

1. DiaMag 0.2ml - magnetic rack

DiagenodeCat: B04000001

2. DynaMag™-2 Magnet

Fischer Scientific Cat: 12321D

3. DNA mixer Beyotime E1676,DH-II

4. Centrifuge 5424R (refrigerated) Eppendorf Cat: 5404000090

Procedure

Binding antibodies to magnatic beads

1、 For each IP , use 10ul stock solution of beads, wash twice the protein A/G coated magnetic beads with ice cold ChIP Buffer. Prepare same amount of beads for each IP if pre-clearing is desired.

2、 After washing, resuepend the beads in ChIP Buffer to the same concentration as stock.

3、 Aliquot 90ul of ChIP Buffer per Ip to a mewly prepared tube

4、 Add 10ul of pre-washed Protein A/G-beads per IP tube made up 100ul total per IP

5、 Add specific antibody and control antibodies. The amount of antibody varies depends on the antibody used, the binding capacity of 10 ul magnetic beads is 3ug

6、 Incubate the tubes at 40rpm on a rotating wheel for at least 2hours at 4℃.

Chromatin shearing and nuclear membrane solubilization

1、 Seeding cells in 20ul Nuclei Extraction Buffer, invert tubes several times gently, then centrifuge for 5 minutes in 4000rpm in 4℃. Discard 10ul supernatant and keep another 10ul with nucleus.

2、 Prepare MNase Master mix 、

3、 Add 40ul MNase Master Mix to each sample ,mix well by pipetting or gentle vertex

4、 Proceed at room temperature (25℃) for 10min

5、 Add 5.5ul MNase Stop Solution,mix well by gentle vertex

6、 Add 5.5ul Nuclear Break Buffer into the tube , mix well by gentle vertex. These are sheared chromatin ready to be ChIPed

7、 Add Complete ChIP Buffer to make 100ul sheared chromatin for each IP and 10ul for input

Magnetic immunoprecipitation

1、 Briefly spin the tube containing the antibody-coated beads to bring down liquid caught inside the lid.

2、 Place tubes in the ice-cold magnetic rack , wait for 1min. discard the supernatant. Keep the antibody-coated beads

3、 Add 100ul of diluted sheared chromatin to each IP tube, and keep 10ul of diluted chromatin as input at 4 ℃ or store at -20℃

4、 Invert the tubes several times make sure beads are resuspended

5⃣ Incubate samples at 4℃ under constant rotation on a rotator at 40 rpm for 2 hours up to overnight

Washes

1⃣ Brief spin the tubes to bring down the liquid caught on the lid

2⃣ Place the tubes into the magnetic rack, wait 1 min and discard the buffer

3⃣ Wash twice using 100ul of ice-cold Low Salt Wash Buffer and twice using High Salt Wash Buffer

4⃣ Add 100ul Hot Elution Buffer to the beads and 90ul Elution buffer to the input and transfer them to a new tubes

5⃣ Put the strips in a PCR block at 65 for 1.5-2h

6⃣ Place the strips on a magnetic rack, let sit for 1min, transfer them to a PhaseLock tube

DNA isolation

1⃣ Brief spin the PhaseLock tube to bring down the agrose

2⃣ Add 100ul DNA and equal volume(100ul) phenol:chloroform:isoamyl alcohol into the PhaseLock tube. Mix well by vertex

3⃣ Spin at 13000g for 5min at room temperature

4⃣ Transfer the supernatant to a new 1.5Ml tube

5⃣ Add 1/10 of the vol.NaOAc and LPA

6⃣ Add 2.5X Vol. ice-cold 100%EtOH. Mix well by vertex

7⃣ Put the tubes at -20℃ to precipate for 30min up to overnight

8⃣ Centrifuge DNA at 13000g for 30min at 4℃

9⃣ Remove the supernatant and add 1ML 70% EtOH , let the tube sit for 5min to allow the salt to dissolve. Then centrifuge at 13000g for 5min at 4℃ to attach the pellet to the bottom.

10⃣Remove the EtOH and let the pellet to dry for 5min at room temperature

11⃣ Add 30ul DNA elution Buffer

Troubleshooting

1. Step1: If you are in a hurry, Binding antibodies to magnatic beads should be at least 3h;

2. Regarding antibody selection: a variety of antibodies can be selected for screening

Time Taken

Day -1: Binding antibodies to magnatic beads

Day 0: Chromatin shearing and nuclear membrane solubilization and Magnetic immunoprecipitation

Day2: Washes and DNA isolation

Anticipated Results

Glycoblue Coprecipitant gathered at the bottom of the tube and the subsequent library was successfully built

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Figures

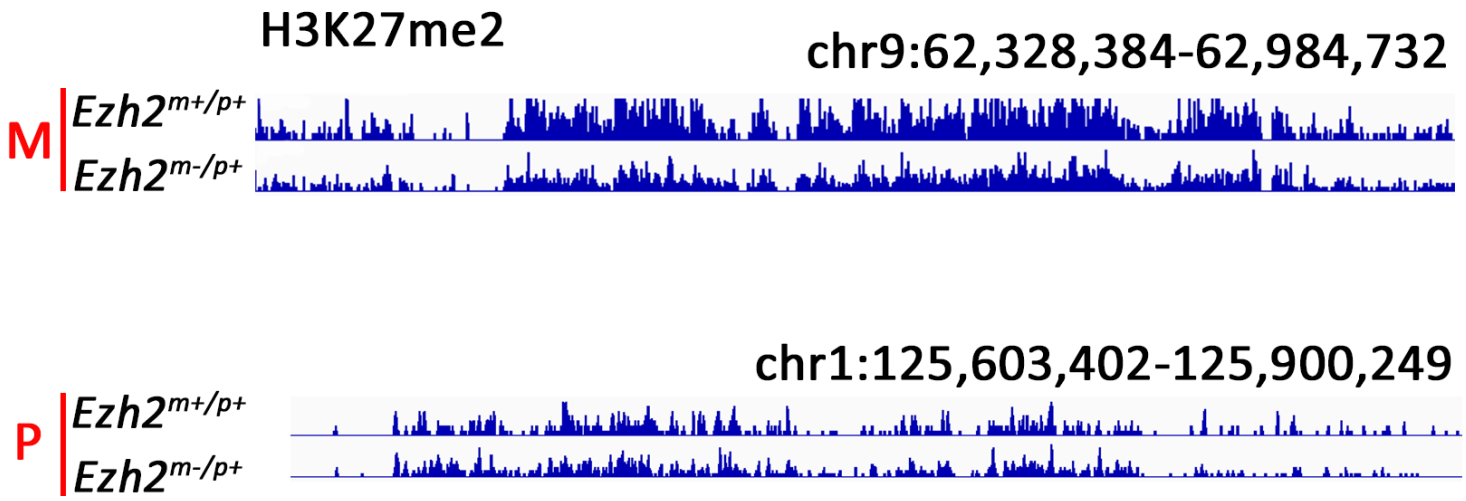

Figure 1

Global view of H3K27me2 distribution on the maternal and paternal genomes in Control and Ezh2m-/p+ zygotes.

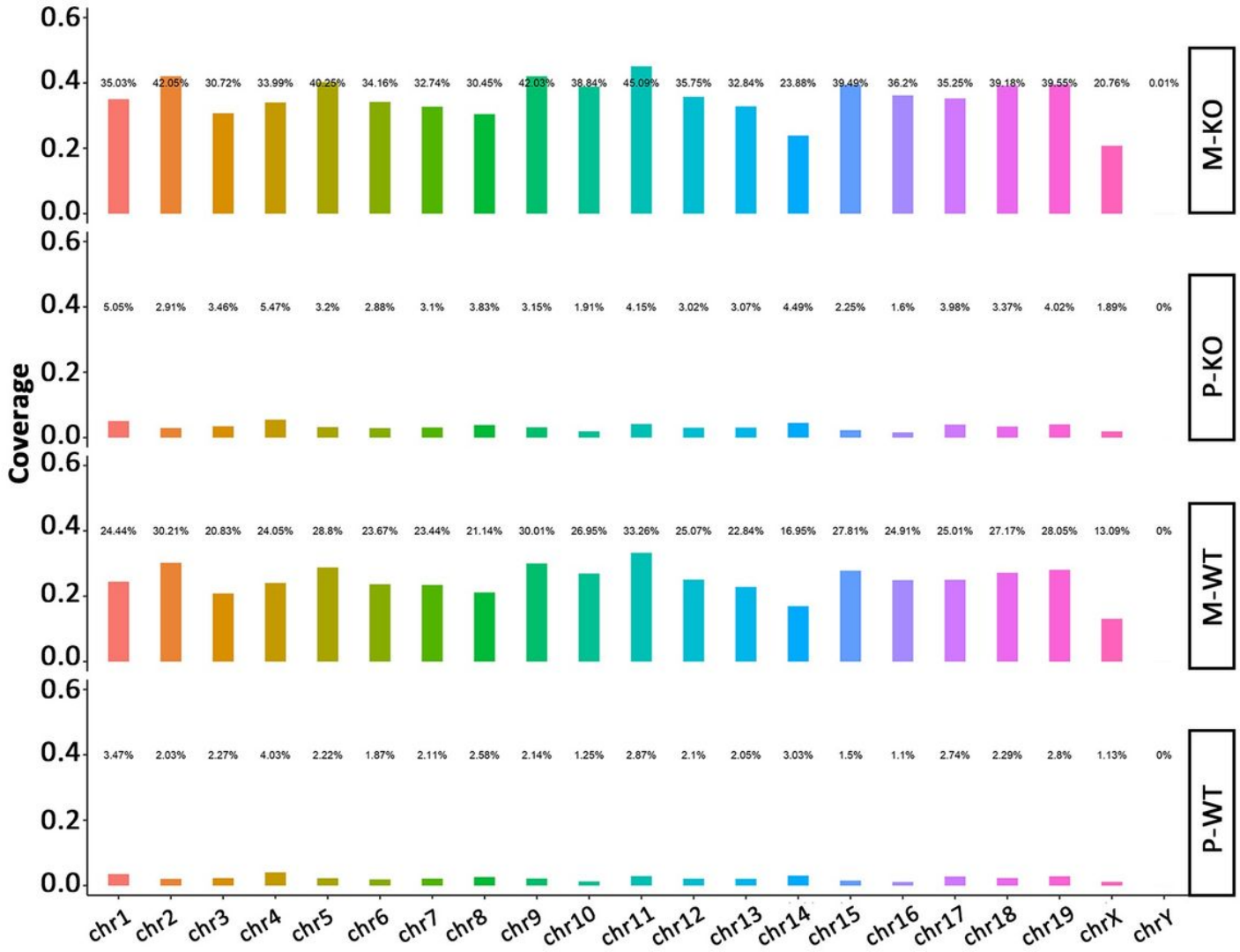

Figure 2

The level of de novo H3K27me2 in the paternal pronucleus and in the maternal pronucleus. The coverage of H3K27me2 in Ezh2m-/p+ and Ezh2m+/p+ parental genomes.