Chromatin Immunoprecipitation (ChIP) Protocol using Dynabeads

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Adaptation from the original protocol using protein A beads.

Modified by Junji Lin (07/02/2010)

<Prior to Day1>

Coating protein A beads

Take enough dynabeads slurry (30ul-50ul each sample) (Invitrogen #100-01D)

Separate beads and buffer with Magnetic Separation Stand (promega # Z5332)

Remove the sup. with loading tip

Wash three times with 1 mL IP dilution buffer

Resuspend beads in 1mL pre-blocking buffer

Incubate at 4°C, greater than 2hr or O/N

Separate beads and buffer with Magnetic Separation Stand and remove the sup.

Wash twice with 1 mL IP dilution buffer

Add IP dilution buffer back to original volume

(Comments: This protocol is modified specifically for ChIP-Seq. Many protocols suggested that dynabeads don't need blocking. My own experience is no blocking will lead to non-specific binding and give a relatively stronger background especially you don't have a very clean antibody. Be sure to use non-DNA blocking reagent, otherwise, Solexa will be very sensitive to also pick up your carrier DNA sequence. And your true DNA reading will be much lower.

For separation of beads and sup, I first briefly centrifuge it and then put the tube to the magnetic stand. Remove the sup. by loading tips)

<Day 1>

Cross-linking of protein and DNA

Dechorinate 100 embryos in 1xER (put dechorinated embryos in cold HBSS (Invitrogen #14170-112) on ice)

Add 1% formaldehyde in 1xPBS in 1.5 mL tube

Rotate 15 min at RT, remove sup.

Add immediately 143uL 1M glycine and spin down. Remove the sup.

Add 0.125 M glycine to soak embryos, rotate 10 min at RT

Rinse embryos with 1 mL cold 1xPBS twice, centrifuge at 4 °C

(Comments: The amount of embryos needed for ChIP need to be optimized by specific sample. It also depends on your antibody specificity. As far as I know, I can see difference between negative control and sample with only 10 embryos with dynabeads.

Cross-linking condition are based on100 embryos at 38hpf. Time will vary if using younger or older embryos; 2.2% of formaldehyde can be used if necessary. Insufficient cross-linking will lose true binding signals and over cross-linking will bring non-specific bindings.)

Cell lysis and chromatin DNA extraction

Add 600 uL Cell lysis buffer (for 100 embryos), on ice 10–20 min

Pipet up and down every 5-10 min until no clear tissue can be visible (bone structure might be still there, but most of the tissue should be broken and dissolved)

Cfg: 3.5k rpm, 5 min at 4 °C, remove the sup.

Rinse once more with 600 uL Cell lysis buffer and spin down, remove the sup.

Re-suspend the nuclear pellet (white) in 200 uL nuclei lysis buffer

Pipet up and down to disrupt clumps

On ice 10–20 min (lay the tube on ice to avoid precipitation of SDS in the nuclei lysis buffer)
Add 400 uL IP dilution buffer + proteinase inhibitors

Freeze at -70 or -80 °C

- **Sonication**

  Thaw the sample and add 100 mg glass beads (Sigma, acid washed, 212-300 microns, Sigma #G-1277)

  Sonication machine (Branson Sonifer 250 with microtip)

  Set up: constant duty cycle, output on 2, value 20

  6 pulses of 20 sec each with 3 min interval on ice

  Take 10 uL for agarose gel electrophoresis to check the size of fragmented DNA after decross-linking

  *(Comments: Over sonication will lead to DNA degradation, insufficient sonication will lead to non-specific binding. Average fragment size should be between 100bp-600bp. Avoid introducing bubbles to the sample or generating too much heat since it will lead to protein degradation. Keep on ice during sonication and leave the sample on ice for enough time to cool down before next round of sonication.)*

  Cfg: 14k rpm, 15 min at 4°C

  Take the sup. Add IP dilution buffer to 600 uL per tube.

  Divide equal proportionally for ChIP and total input control. (For example: total amount after sonication is 600ul, using 500ul for ChIP assay and 100ul for input control for each sample) Store input sample at -20 °C.

- **Antibody binding**

  Pre-clear:

  Add 20-30 uL blocked dynabeads (in pre-Day1) to the ChIP sample

  Rotate at 4°C, > 2hrs

  Antibody binding:

  Separate beads and buffer with Magnetic Separation Stand.

  Take the sup. (Do not contaminate with any beads) and divide it equally as antibody and no-antibody control

  Add antibody 5ug to the sample (Keep the no-antibody control sample at 4 °C without adding any antibody) rotate O/N at 4°C

  **<Day 2>**

  - **Dynabeads binding, washing, elution, and decross-linking**

    Add 30uL blocked dynabeads to each sample (also to no-antibody control tube)

    Incubate on a rotating wheel/platform at RT 60-90min

    Separate beads and buffer with Magnetic Separation Stand.

    Remove sup. (Beads from no-antibody tube could serve as “no Ab” negative control after washing and elution)

    Wash the beads twice with 1 mL 1x dialysis buffer

    - Add 1 mL buffer

    - Rotate 15 min at RT

    - Separate beads and buffer with Magnetic Separation Stand.

    - Remove the sup. as much as possible with loading tip

    Wash the beads twice with 1 mL IP wash buffer (same as above)

    *(Comment: Washing step could be held at 4 °C with longer time; If non-specific binding still occur, wash 3 times for each buffer followed by 1-3 times TE wash)*

- **Elution and decross-linking**

  Add 150 uL elution buffer to the beads

  Incubate in 65 °C water bath for 10-15min (vortex every 2-3 min)

  Separate beads and buffer with Magnetic Separation Stand.
Transfer the sup. to a new tube

Repeat elution steps again and combine both elution (300 uL)

Add 30 uL 3 M NaCl and 1 uL 10 mg/mL RNaseA (For input sample, add right amount of NaCl and RNaseA)

Incubate 65 °C, 4-5 hr or O/N (input control samples also need to be decross-linking at the time)

Add 2 uL 5 mg/mL glycogen and 2.5V absolute EtOH, ·80°C O/N

<Day 3>

- **Proteinase K treatment**

Separate beads and buffer with Magnetic Separation Stand.

Discard sup.

Dissolve pellet in TE, and mix with 5x PK buffer PK(10 mg/mL)

| For ChIP sample | 100 uL | 25 uL | 2 uL |
| For Input sample | 200 uL | 50 uL | 2 uL |

45°C; 1-2 hr

Add TE to 300 uL

Add 300 uL phenol/CHCl₃, vortex, 14k rpm, 5 min at RT, take the sup.

Add 300 uL CHCl₃, vortex, 14k rpm, 5 min at RT, take the sup.

Add 3 M NaCl 54 uL, 5 mg/mL glycogen 2 uL, 2.5V absolute EtOH

-80°C, O/N

<Day4>

- **Precipitation and PCR**

14k rpm, 20 min at 4°C

Dissolve pellet in 10-30 uL TE or ddH₂O

Further DNA purification can use **QIAquick PCR purification Kit** if necessary. But it will also lose quite a bit amount of DNA.

DNA can be stored at -20 °C

qPCR with ChIP sample, no-Ab control and total input.

- **Recipes for solution and material information**

1 M glycine (FW = 75.07)

3.75 g / 50 mL dH₂O

filtrate and store at RT

100x ER

6 g instant ocean / 1 L dH₂O

1% formaldehyde in 1x PBS (freshly prepared)

Total 25ml

37% formaldehyde 676 uL

1x PBS 24.324uL

0.125 M glycine in 1x PBS (freshly prepared)

| 10 mL | 30 mL |
| 1 M glycine | 1.25 mL | 3.75 mL |
| 1x PBS | 8.75 mL | 26.25 mL |
Proteinase inhibitors

(final conc.) (per 1 mL solution/buffer)

100 uM PMSF 1 uL of 100 mM
100 ug/mL benzamideine 10 uL of 10 mg/mL
5 ug/mL leupeptin/pepstatin 1 uL of 5 mg/mL

Cell lysis buffer (at 4°C) (40 mL)
10 mM Tris-Cl (pH 8.1) 400 uL of 1 M (Using Tris Base)
10 mM NaCl 133 uL of 3 M
0.5% NP-40 2 mL of 10%
proteinase inhibitors (freshly add)
dH₂O 37.47 mL

Nuclei lysis buffer (20 mL)
50 mM Tris-Cl (pH 8.1) 1 mL of 1 M
10 mM EDTA 400 uL of 0.5 M
1% SDS (Sodium Dodecyl Sulfate) 2 mL of 10%
proteinase inhibitors (freshly add)
dH₂O 16.6 mL

IP dilution buffer (at 4°C) (40 mL)
16.7 mM Tris-Cl (pH 8.1) 668 uL of 1 M
167 mM NaCl 2227 uL of 3 M
1.2 mM EDTA 96 uL of 0.5 M
1.1% Triton X-100 2.2 mL of 20%
0.01% SDS 40 uL of 10%
proteinase inhibitors (freshly add)
dH₂O 34.77 mL

Pre-Blocking buffer for dynabeads: 1mL
glycogen 10uL (20mg/mL),
BSA 40uL (5mg/mL)
Yeast-tRNA 10uL (20mg/mL)
in 1mL IP dilution buffer

500 mM NaHCO₃ (filtrate, store at RT)
NaHCO₃ (FW = 84.01) 4.2 g
H₂O up to 100 mL

1x dialysis buffer (store at 4°C) (100 mL)
50 mM Tris-Cl (pH 8.1) 5 mL of 1 M
2 mM EDTA 400 uL of 0.5 M
0.2% sarkosyl (omit for monoclonal Ab) 1 mL of 20%
The other name is N-lauroylsarcosin solution (SIGMA L7414-10ML)
dH₂O 92.6 mL

IP wash buffer (stored at 4°C) (100 mL)
100 mM Tris-Cl (pH 9.0) (pH 8.0 for monoclonal Ab) 10 mL of 1 M
500 mM LiCl 10 mL of 5 M
1% NP-40 10 mL of 10%
1% deoxycholic acid (Sodium deoxycholate Sigma D6750-25G) 5 mL of 20%
dH₂O 65 mL

Elution buffer (40 mL)
50 mM NaHCO₃ 4 mL of 500 mM
1% SDS 4 mL of 10%
dH₂O 32 mL of dH₂O

5x PK buffer
50 mM Tris-Cl (pH 7.5) 5 mL of 1 M
25 mM EDTA 5 mL of 0.5 M
1.25% SDS 12.5 mL of 10%
dH₂O 77.5 mL

Glass beads, acid-washed, Sigma G1277-100G
10 mg/mL proteinase K
10 mg/mL glycogen
3 M NaCl
Phenol (saturated with TE)
CHCl₃ (+1/25 vol. iso-amyl alcohol)
EtOH (-20°C)