(10/10/11)

# Chromatin Immunoprecipitation Assay (ChIP Assay)

## Day 1

**Prep:**

- ChIP Dilution Buffer (CDB) (@ 4°C)
- liquid nitrogen in container
- rotator @ 4°C
- tubes (1 for blocked beads, 1 for each ChIP sample)
- heat block/water bath @37°C
- BSA (NEB) (@-20°C)
- Protease Inhibitor (PI) (@-20°C)
- Protein G agarose beads (@4°C)
- Antibody

**Solutions:**

1.) ChIP Dilution Buffer (CDB) (16.7 mM Tris-HCl pH 8.1, 167 mM NaCl, 0.01% SDS, 1.1% Triton-X 100)

| | | |
|---|---|---|
| 1M Tris-HCl, pH 8.1 | 835 ul | |
| 5M NaCl | 1.67 ml | |
| 10% SDS | 50 ul | Keep on ice at all times. |
| 10% Triton-X 100 | 5.5 ml | |
| $H_2O$ | 41.9 ml | |
| Total | 50 ml | |

2.) Protease Inhibitor (PI) (50X)

- Disolve 1 tablet in 1000 ul dd$H_2O$.

**Directions:**

Preclear Chromatin

1.) Gently shake and slightly warm agarose beads @ rm. temp. until well resuspended.
2.) In tube on ice, add: 1 ml CDB + 80 ul agarose beads slurry.
3.) Quickly thaw chromatin (@-80°C) in 37°C heatblock/waterbath. Place on ice.
4.) Add at least 5 ug chromatin to CDB and agarose beads slurry.
5.) Rotate @ 4°C for 30-60 minutes.

Block Agarose Beads (do at same time as Preclearing Chromatin)

1.) In tube, add: 1 ml CDB + 40 ul agarose beads slurry.
   - For each additional ChIP, add another 40 ul agarose beads slurry.

2.) Rotate @4°C for 5 minutes.
3.) Spin @ 3.4 x 1000 rpm (1.1 rcf) for 30 sec.
4.) Aspirate supernatant.
   - Be careful to not remove beads!

5.) Add 1 ml CDB.
6.) Repeat steps 2-5 for 2 more washes, but after 3rd wash, do not add CDB.
   - During 2nd wash step, thaw PI and BSA @ rm. temp.

7.) After last aspiration, add:

| | 1X | |
|---|---|---|
| (agarose beads only) | (20 ul) | (washed) |
| CDB | 68 ul | |
| 10 mg/ml BSA | 10 ul | |
| 50X PI | 2 ul | |
| Total | 100 ul | |

8.) Rotate blocked beads overnight @ 4°C.

Antibody Binding

1.) After 30-60 min. rotation @ 4°C, on ice, carefully remove precleared chromatin supernatant (~1 ml) from beads and put into new 1.5 microcentrifuge tube.

2.) Add:
- 20 ul PI (50X)
- ~2 ug antibody

3.) Rotate overnight @ 4°C.

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## Day 2

**Prep:**

- ChIP Buffer III (@ rm. temp)
- incubator/oven @ 65°C
- TSE I (@ rm. temp)
- TSE II (@ rm. temp)
- TE (@ rm. temp)
- Elution Buffer (fresh, @ rm. temp)

**Solutions:**

1.) **TSE I**: 20 mM Tris-HCl (pH 8.1), 150 mM NaCl, 2 mM EDTA, 0.1% SDS, 1% Triton X-100

| | |
|---|---|
| 1 M Tris-HCl, pH 8.1 | 1 ml |
| 5 M NaCl | 1.5 ml |
| 0.5 M EDTA | 200 ul |
| 10% SDS | 500 ul |
| 10% Triton X-100 | 5 ml |
| $ddH_2O$ | 41.8 ml |
| Total | 50 ml |

2.) **TSE II:** 20 mM Tris-HCl (pH. 8.1), 500 mM NaCl, 2 mM EDTA, 0.1% SDS, 1% Triton X-100

| | |
|---|---|
| 1 M Tris-HCl, pH 8.1 | 1 ml |
| 5 M NaCl | 5 ml |
| 0.5 M EDTA | 200 ul |
| 10% SDS | 500 ul |
| 10% Triton X-100 | 5 ml |
| $ddH_2O$ | 38.3 ml |
| Total | 50 ml |

3.) **ChIP Buffer III:** 10 mM Tris-HCl (pH 8.1), 0.25 M LiCl, 1 mM EDTA, 1% NP-40, 1% deoxycholate

| | |
|---|---|
| 1 M Tris-HCl, pH 8.1 | 500 ul |
| 5 M LiCl | 2.5 ml |
| 0.5 M EDTA | 100 ul |
| 10% NP-40 | 5 ml |
| 10% deoxycholic acid | 5 ml |
| $ddH_2O$ | 36.9 ml |
| Total | 50 ml |

4.) **TE:** 10 mM Tris-HCl (pH 8.1), 1 mM EDTA

| | |
|---|---|
| 1 M Tris-HCl, pH 8.1 | 500 ul |
| 0.5 M EDTA | 100 ul |
| $ddH_2O$ | 49.4 ml |

| | |
|---|---|
| Total | 50 ml |

5.) **Elution Buffer (make fresh):** 1% SDS, 0.1 M $NaHCO_3$

| | | |
|---|---|---|
| 10% SDS | 100 ul | |
| 1 M $NaHCO_3$ | 100 ul | (84 mg sodium bicarbonate in 1 ml $ddH_2O$, fresh) |
| ddH2O | 800 ul | |
| Total | 1 ml | |

**Directions:**

Immunoprecipitate (do all steps @ 4°C)

1.) Add 100 ul cold, blocked agarose bead slurry to each chromatin sample (do @4°C).
2.) Rotate @ 4°C for 45 min.
3.) Spin down beads @3.4 x 1000 rpm (1.1 rcf), 30 sec, rm. temp.
4.) Aspirate supernatant.

Wash (do all steps @ rm. temp)

Wash Buffer order: **TSE I, TSE II, ChIP Buffer III, TE**

1.) Add 1 ml of appropriate Wash Buffer to agarose beads.
2.) Rotate @ rm. temp. for 5 min.
3.) Spin down @ 3.4 x 1000 rpm (1.1 rcf), 30 sec, rm. temp.
4.) Aspirate supernatant.
5.) Repeat steps 1-4 for each Wash Buffer.

Elute/Reverse Crosslinks

1.) Add 100 ul **fresh** Elution Buffer.
2.) Rotate 15 min. @ rm. temp.
3.) Spin down @ 3.4 x 1000 rpm (1.1 rcf), 30 sec, rm. temp.
4.) Transfer supernatant to new tube.
5.) Repeat steps 1-4 combining eluates into 1 tube.
6.) Add 8 ul 5M NaCl.
    - per 200 ul eluate (192 mM NaCl)
7.) Incubate @65°C overnight.

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## Day 3

**Prep:**

- Heat block @ 45°C
- 1 M Tris-HCl, pH 7.5
- 0.5 M EDTA
- 10 mg/ml proteinase K
- Qiagen Qiaquick PCR Purification Kit

**Directions:**

1.) Remove tube from 65°C oven and add:

| | |
|---|---|
| 8 ul | 1M Tris-HCl, pH7.5 |
| 4 ul | 0.5 M EDTA |
| 1 ul | 10 mg/ml proteinase K |

2.) Incubate @ 45°C for 1 hour.

3.) Follow directions as written in Qiagen's Qiaquick PCR Purification Kit to purify un-crosslinked chromatin.
- Elute into 50 ul Qiagen Elution Buffer