# Blueprint: Histone ChIP

To obtain the highest DNA yield, with the least amount of background from $5x10^5$ cells, the most optimal experiment is described below. Chromatin is sonicated such that the final concentration is $15*10^6$ cells/ml, therefore take 33µl chromatin per ChIP.

## 1. ChIP

1. Mix the following reagents together.

| | **ChIP (µl)** |
|---|---|
| Chromatin | 33 |
| Dilution buffer (No SDS) | 255 (-µl Ab) |
| Protease Inhibitor Cocktail (25x) | 12 |
| Ab | * |
| Total volume | 300 |

* Use the antibody amount written in the table below.

| Mark | Use per ChIP |
|---|---|
| H3K4me3 | 1 µg |
| H3K36me3 | 0,5 µg |
| H3K27ac | 1 µg |
| H3K4me1 | 0,5 µg |
| H3K27me3 | 1 µg |
| H3K9me3 | 2 µg |

2. Rotate overnight at 4°C.

## 2. Preparation protein A/G magnetic beads:

1. For 1 ChIP sample take 10 µl protein A beads and 10 µl protein G beads.
   (E.g. for 10 ChIPs take 100 µl protein A beads and 100 µl protein G beads)

2. Wash the beads with 500 µl dilution buffer (+0.15% SDS) + 0,1% BSA.
   (for example: 2ml dilution buffer with 20µl 10% BSA stock)

3. Short spin down the beads and place them in a magnetic rack for 1 minute, remove supernatant.

4. Repeat step 2 and 3.

5. Dissolve the beads in 20 µl dilution buffer(+0.15% SDS) + 0,1% BSA per ChIP.

6. Add 20 µl washed beads to the chromatin + Ab mix.

7. Rotate 60 minutes at 4°C.

8. Wash the beads with 400 µl buffer for 5 minutes at 4°C with the following buffers:
   - ChIP wash 1 1x
   - ChIP wash 2 2x
   - ChIP wash 3 2x

(**Short spin down in between the wash steps and place in a magnetic rack for 1 minute, remove supernatant.**)

9. Transfer samples to a new tube after washing, remove last wash buffer and add 200 μl **freshly made** elution buffer each.
10. Rotate 20 minutes at RT.
11. Short spin down the beads and place them in a magnetic rack for 1 minute.
12. Collect supernatant.
13. Add 8 μl 5M NaCl and 2 μl proteinase K (10mg/ml) to each 200 μl of supernatant.
14. As control an input sample will be made as follows:
    Take 33,33 μl chromatin and add 366,67 μl elution buffer, 16 μl 5M NaCl and 4 μl proteinase K (10mg/ml).
15. Shake the samples at 1000 rpm on 65°C for at least 4 hours. (decrosslinking)

**<u>3. Column purification (Qiagen; QIAquick MinElute PCR Purification Kit (Cat: 28006):</u>**

1. Add 5 volumes PB buffer (i.e. 1ml) to each decrosslinked sample and add 40 µl 3M NaAc pH 5,2.
2. Load 700 μl of your decrosslinked sample onto one MinElute column.
3. Centrifuge 1 minute at 13.000 rpm, remove flow through.
4. Repeat step 2 and 3 until all sample has been loaded onto the MinElute column.
5. Wash with 700 μl PE buffer.
6. Centrifuge 1 minute at 13.000 rpm, remove flow through.
7. Centrifuge again 1 minute at 13.000 rpm, to dry the column.
8. Put the column in a new tube.
9. Add 10 μl EB buffer to elute.
10. Put the tube 1 minute on 37 °C.
11. Centrifuge 1 minute at 13.000 rpm.
12. Repeat step 9, 11, 12.
13. Pool the two eluted samples together.

# Buffers:

ChIP dilution buffer (100 ml):

| Stock | Final concentration | Volume stocksolution (ml) |
|---|---|---|
| 10 % | 0,15 % SDS | 1,5 |
| 20 % | 1,0 % Triton | 5 |
| 0,5 M | 1,2 mM EDTA | 0,24 |
| 1 M | 16,7 mM Tris (pH 8,0) | 1,67 |
| 5 M | 167 mM NaCl | 3,34 |
| MilliQ | | Fill up to 100 ml |

ChIP wash 1 (400 ml):

| Stock | Final concentration | Volume stocksolution (ml) |
|---|---|---|
| 0,5 M | 2 mM EDTA | 1,6 |
| 1 M | 20 mM Tris (pH 8,0) | 8 |
| 20 % | 1 % Triton | 20 |
| 10 % | 0,1 % SDS | 4 |
| 5 M | 150 mM NaCl | 12 |
| MilliQ | | Fill up to 400 ml |

ChIP wash 2 (400ml):

| Stock | Final concentration | Volume stocksolution (ml) |
|---|---|---|
| 0,5 M | 2 mM EDTA | 1,6 |
| 1 M | 20 mM Tris (pH 8,0) | 8 |
| 20 % | 1 % Triton | 20 |
| 10 % | 0,1 % SDS | 4 |
| 5 M | 500 mM NaCl | 40 |
| MilliQ | | Fill up to 400 ml |

ChIP wash 3 (TE) (1000 ml):

| Stock | Final concentration | Volume stocksolution (ml) |
|---|---|---|
| 0,5 M | 1 mM EDTA | 2 |
| 1 M | 10 mM Tris (pH 8,0) | 10 |
| MilliQ | | Fill up to 1000 ml |

Elution buffer (50 ml)

| Stock | Final concentration | Volume stocksolution (ml) |
|---|---|---|
| 10% | 1% SDS | 5 |
| 1 M | 0,1 M $NaHCO_3$ | 5 |
| MilliQ | | Fill up to 50 ml |

**Questions and/or remarks:**

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