

# Chromatin immunoprecipitation (CHIP)

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## < 準備 >

1. TE

2. 10% formaldehyde - PBS      10 ml of 37% formaldehyde // 27 ml of PBS    (用事調製)

3. 2 M glycine (MW: 75.07)      75.07 g // final 500 ml

4. Cell lysis buffer

|                     |                              |
|---------------------|------------------------------|
| 5 mM PIPES (pH 8.0) | 2.5 ml of 1 M PIPES (pH 8.0) |
| 0.5% NP-40          | 25 ml of 10% NP-40           |
| 85 mM KCl           | 42.5 ml of 1 M KCl           |
| // final 500 ml     |                              |

5. Nuclei lysis buffer

|                         |                                |
|-------------------------|--------------------------------|
| 1% SDS                  | 25 ml of 20% SDS               |
| 50 mM Tris-HCl (pH 8.1) | 25 ml of 1 M Tris-HCl (pH 8.0) |
| 10 mM EDTA              | 10 ml of 0.5 M EDTA            |
| // final 500 ml         |                                |

6. IP dilution buffer

|                           |                                  |
|---------------------------|----------------------------------|
| 16.7 mM Tris-HCl (pH 8.1) | 8.35 ml of 1 M Tris-HCl (pH 8.0) |
| 1.2 mM EDTA               | 1.2 ml of 0.5 M EDTA             |
| 167 mM NaCl               | 16.7 ml of 5 M NaCl              |
| 0.01 % SDS                | 0.5 ml of 10% SDS                |
| 1.1% Triton X-100         | 5.5 ml of Triton X-100           |
| // final 500 ml           |                                  |

4, 5, 6には使用直前に1mM PMSF (1 /100)、  
Aprotinin (5 / 1000) 、 1  $\mu$ g/ml Leupeptin (2 / 1000)を加える

7. 2 x RIPA

|                         |                                |
|-------------------------|--------------------------------|
| 40 mM Tris-HCl (pH 8.0) | 20 ml of 1 M Tris-HCl (pH 8.0) |
| 4 mM EDTA               | 4 ml of 0.5 M EDTA             |
| 300 mM NaCl             | 30 ml of 5 M NaCl              |
| 0.02% SDS               | 1 ml of 10% SDS                |
| 2% Triton X-100         | 10 ml of Triton X-100          |
| // final 500 ml         |                                |

8. Low salt wash buffer

|          |       |
|----------|-------|
| 2 x RIPA | 25 ml |
| MQ       | 25 ml |

9. High salt wash buffer

|             |                   |
|-------------|-------------------|
| 2 x RIPA    | 25 ml             |
| 500 mM NaCl | 3.5 ml of 5M NaCl |
| MQ          | 21.5 ml           |

10. LiCl wash buffer

|                         |                                  |
|-------------------------|----------------------------------|
| 10 mM Tris-HCl (pH 8.1) | 5 ml of 1 M Tris-HCl (pH 8.0)    |
| 1% Sodium deoxycholate  | 50 ml of 10% Sodium deoxycholate |
| 1% NP-40                | 50ml of 10% NP-40                |
| 1 mM EDTA               | 1 ml of 0.5 M EDTA               |
| 250 mM LiCl             | 25 ml of 5 M LiCl (MW; 42.39)    |
|                         | // final 500 ml                  |

11. Elution beffer

|                          |                   |
|--------------------------|-------------------|
| 1% SDS                   | 2.5 ml of 20% SDS |
| 0.1 M NaHCO <sub>3</sub> | 420 mg            |
| (MW: 84.01)              | // final 50 ml    |
| 使用直前にDTTを10 mMになるように加える  |                   |

12. Protein A or G agarose

|                                                        |                  |
|--------------------------------------------------------|------------------|
| 1 ml of protein A or G (50% v/v)に                      |                  |
| 400 $\mu$ g/ml ssDNA (salmon sperm DNAをsonicationしたもの) |                  |
| 1.5 mg/ ml BSA                                         | を加えて一晩以上blocking |

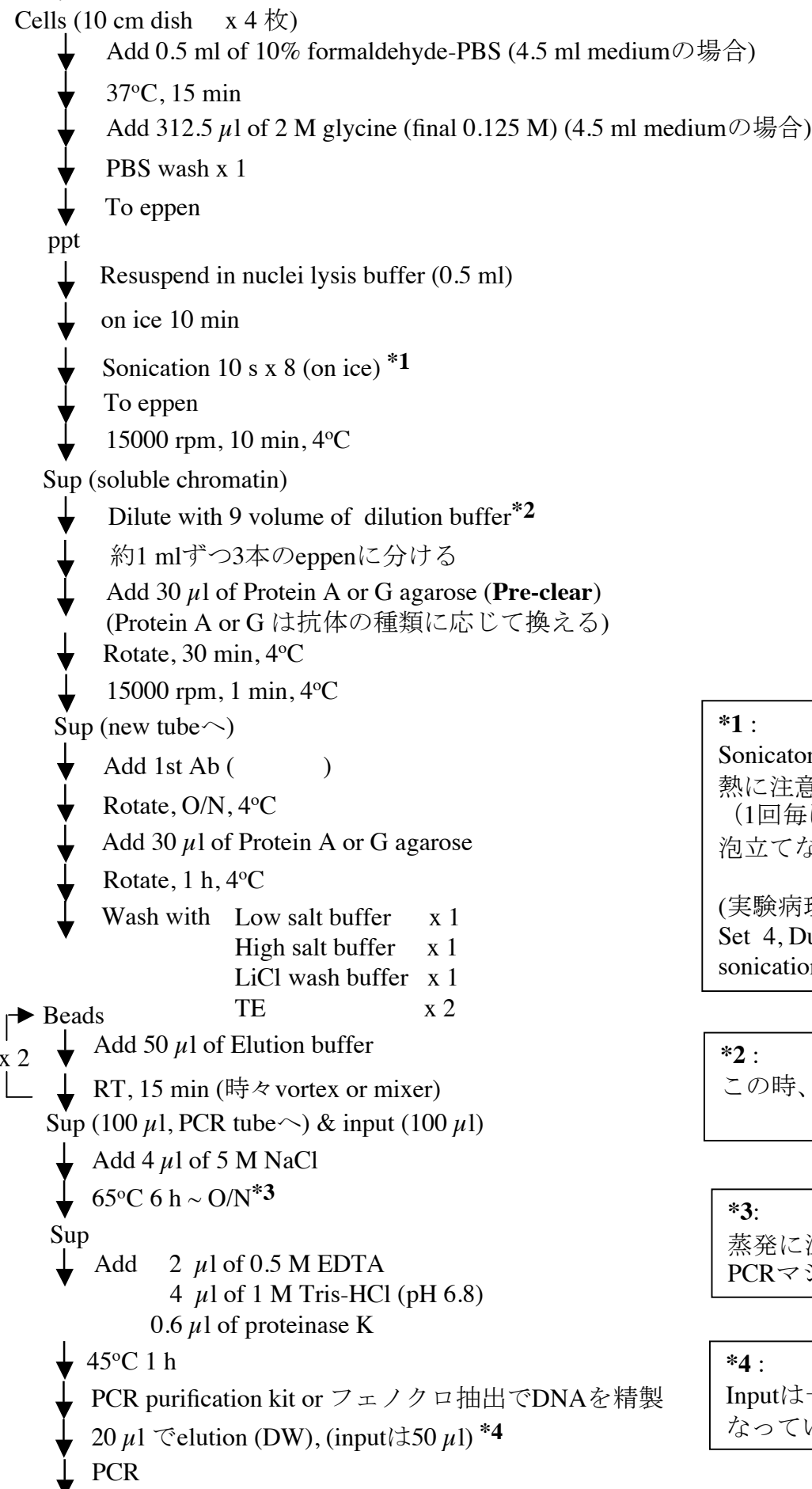
13. Proteinase K (6 mg/ml), 5M NaCl, 0.5 M EDTA, 1 M Tris-HCl (pH 6.8)

14. PCR purification kit (Qiagen)

15. Sonicator

16. 抗体

## <方法>



### \*1 :

Sonicatorによって条件異なる  
熱に注意  
(1回毎に電源切り、50 s 待つ)  
泡立っていない

(実験病理 (TOMY DU-201)の場合)  
Set 4, Duty 60  
sonication 10s, switch off 50s, 8 times

### \*2 :

この時、input用に100  $\mu$ l 別にとる

### \*3:

蒸発に注意  
PCRマシーンがbetter

### \*4 :

Inputは一部を流し、500bp前後に  
なっていることを確認する。