コストパフォーマンスに優れた次世代磁気ビーズをChIP-seqで使用　研究者の声【43】

東京大学 山中 総一郎 先生

プロトコル紹介

>> ChIP-seq 実験にベリタス取扱製品を使用 研究者の声【16】 にて紹介した既存プロトコルをDynaGreen版に一部編集（2024/09/18）

【実際に行ったDynaGreenを利用した ChIP-seq のプロトコル】

1. 細胞の固定

↓**マウス胚性幹細胞を 40,000 細胞分取する** (in low-binding tube)

↓remove supernatant

↓suspend with 1 ml DMEM (10% FBS)

↓add 67 µl, 16% Formaldehyde (Pierce) (final 1%)

↓RT, 10min, rotation

↓add 62.5 µl 2 M glycine (final 125 mM)

↓RT, 5 min, rotation

↓centrifuge, 6,000 rpm, 1 min x2

↓take supernatant (~900 µl)

↓centrifuge, 6,000 rpm, 1 min

↓take supernatant completely > stock in -80℃ freezer

2. DynaGreen のブロッキング

↓take **25 µl DynaGreen beads** into two tubes per sample (one for IP and one for pre-clear)

↓wash with 800 µl, 0.5% BSA in PBS, twice

↓suspend with 1 ml, 0.5% BSA in PBS

↓4℃, rotation, 1 h

3. IP 用 DynaGreen の調製

↓add 2 µg anti-body (H3K9me3)

↓4℃, rotation, o/n

4. ソニケーション

Frozen cells

↓add 1 ml Swelling Buffer, 4℃, rotation, 20 min

↓centrifuge, 8,000 rpm, 1 min x2

↓take supernatant (~900 µl)

↓centrifuge, 8,000 rpm, 1 min

↓take supernatant completely

↓suspend with 100 µl 1x Sonication buffer (Covaris)

↓sonication [Branson: 30%, total ON time=2 min, ON/OFF=2 sec/3 sec]

↓centrifuge, 13,500 rpm, 5 min

↓save supernatant to low-binding tube

↓centrifuge, 13,500 rpm, 10 min

↓save supernatant to low-binding tube

↓dilute with 1 ml of Abcam RIPA buffer

5. pre-clear

２で調製した“pre-clear beads”

↓wash with 1ml, Abcam RIPA buffer x2 回

↓suspend with 20 µl Abcam RIPA buffer

↓add to cell extract

↓4℃, rotation, 1 h

↓save supernatant

↓aliquot 100 µl for [WCE] > the rest of the cell extract(~1 ml) is for IP

6. IP

３で調製した“beads conjugated with anti-body”

↓wash with 1 ml, Abcam RIPA buffer x4 回

↓suspend with 20 µl Abcam RIPA buffer

↓add beads to 1 ml cell extract

↓4℃, rotation, o/n

7. Beads wash > elution

IP を行った beads

↓wash with 1 ml, LOW buffer x3 回

↓wash with 1 ml, HIGH buffer x3 回

↓add 200 µl direct elution buffer

↓incubate at 65℃, 15 min, with constant vortexing

↓save supernatant

WCE_100 µl of WCE

↓add 100 µl direct elution buffer

↓incubate at 65℃, 15 min, with constant vortexing

8. ProK treatment > reverse crosslinking

IPed sample and WCE sample

↓transfer to screw tube

↓add 5 µl proteinase K (Roche)

↓incubate 37 ℃, >6 h

↓65℃, >6 h

9. DNA 回収

↓add 20 µl 3 M NaOAc, 200 µl Phenol:Chroloform (Thermo)
↓centrifuge, 13,500 rpm, 10 min
↓save supernatant to low-binding tube
↓+1 µl pellet paint (Merck)
↓-80℃, 15 min
↓centrifuge, 14,000 rpm, 30 min
↓remove supernatant
↓add 150 µl, 70% ethanol
↓centrifuge, 14,000rpm, 5 min
↓remove supernatant
↓dry up RT, 10 min
suspend with 35 µl 0.1M Tris-HCl.

10. ChIP-seq library preparation

Check the quality of IPed sample with qPCR
↓prepare the DNA library for sequencing with QIAGEN QIAseq Ultralow Input library Kit
According to the manufacturer's instruction
↓check the DNA size with Bioanalyzer

使用した Buffer の組成

- Swelling buffer

20 mM Hepes(pH7.9)
1.5 mM MgCl2
10 mM KCl
0.1% NP-40
1mM DTT (add before use)

- Abcam RIPA buffer

(RIPA buffer in Abcam protocol)
50 mM Tris-HCl, pH8.0
150 mM NaCl
2 mM EDTA pH8
1% NP-40
0.5% Sodium Deoxycholate (DOC)
0.1% SDS

- Low buffer
(Wash buffer in Abcam protocol)
0.1% SDS
1% Triton X-100
2 mM EDTA pH8
150 mM NaCl
20 mM Tris-HCl, pH8.0

- High buffer
(Final wash buffer in Abcam protocol)
0.1% SDS
1% Triton X-100
2 mM EDTA pH8
500 mM NaCl
20 mM Tris-HCl, pH8.0
- Direct elution buffer
10 mM Tris-HCl, pH8.0
5 mM EDTA
300 mM NaCl
0.5% SDS

株式会社ベリタス 〒105-0013 東京都港区浜松町1-18-16 住友浜松町ビル6階
TEL 03-5776--0078 FAX 03-5776-0076
技術的なお問い合わせは：TEL 03-5776-0040 E-mail Tech_support@veritastk.co.jp

RPDBM-24-1428
(2024.9.18)