# ChIP-seq 実験にベリタス取扱製品を使用　研究者の声【16】

東京大学　山中 総一郎 先生

プロトコル紹介

【実際に行っている Dynabeads Protein G を利用した ChIP-seq のプロトコル】

## 1.　細胞の固定

↓FACS で生殖細胞を 10000 細胞分取する（in low-binding tube）

↓remove supernatant

↓suspend with 1ml DMEM (10% FBS)

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

↓RT, 10min, rotation

↓add 62.5µl 2M glycine (final 125mM)

↓RT, 5min, rotation

↓centrifuge, 6000rpm, 1min x2

↓take supernatant (~900µl)

↓centrifuge, 6000rpm, 1min

↓take supernatant completely > stock in -80°C freezer

## 2.　Dynabeads のブロッキング

↓take 20µl Dynabeads Protein G into two tubes per sample (one for IP and one for pre-clear)

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

↓suspend with 1ml, 0.5% BSA in PBS

↓4°C, rotation, 1h

## 3.　IP 用 Dynabeads の調製

↓add 2µg anti-body (H3K27me3)

↓4°C, rotation, o/n

## 4.　ソニケーション

Frozen cells

↓add 1ml Swelling Buffer, 4°C, rotation, 20min

↓centrifuge, 8000rpm, 1min x2

↓take supernatant (~900µl)

↓ centrifuge, 8000rpm, 1min
↓ take supernatant completely
↓ suspend with 100µl 1x Sonication buffer (Covaris)
↓ sonication [ Branson: 30%, total ON time=2min, ON/OFF=2sec/3sec ]
↓ centrifuge, 13500rpm, 5min
↓ save supernatant to low-binding tube
↓ centrifuge, 13500rpm, 10min
↓ save supernatant to low-binding tube
↓ dilute with 1ml 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 20µl Abcam RIPA buffer
↓ add to cell extract
↓ 4℃, rotation, 1h
↓ save supernatant
↓ aliquot 100µl for [WCE] > the rest of the cell extract(~1ml) is for IP

## 6. IP

３で調製した“beads conjugated with anti-body”
↓ wash with 1ml, Abcam RIPA buffer x4 回
↓ suspend with 20µl Abcam RIPA buffer
↓ add beads to 1ml cell extract
↓ 4℃, rotation, o/n

## 7. Beads wash > elution

IP を行った beads
↓ wash with 1ml, LOW buffer x3 回
↓ wash with 1ml, HIGH buffer x3 回
↓ add 200µl direct elution buffer
↓ incubate at 65℃, 15min, with constant vortexing
↓ save supernatant

WCE

100µl of WCE

↓ add 100µl direct elution buffer

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

## 8. ProK treatment > reverse crosslinking

IPed sample and WCE sample

↓ transfer to screw tube

↓ add 5µl proteinase K (Roche)

↓ incubate 37 ℃, >6h

↓ 65℃, >6h

## 9. DNA 回収

↓ add 20µl 3M NaOAc, 200µl Phenol:Chroloform (Thermo)

↓ centrifuge, 13500rpm, 10min

↓ save supernatant to low-binding tube

↓ +1µl pellet paint (Merck)

↓ -80℃, 15min

↓ centrifuge, 14000rpm, 30min

↓ remove supernatant

↓ add 150µl, 70% ethanol

↓ centrifuge, 14000rpm, 5min

↓ remove supernatant

↓ dry up RT, 10min

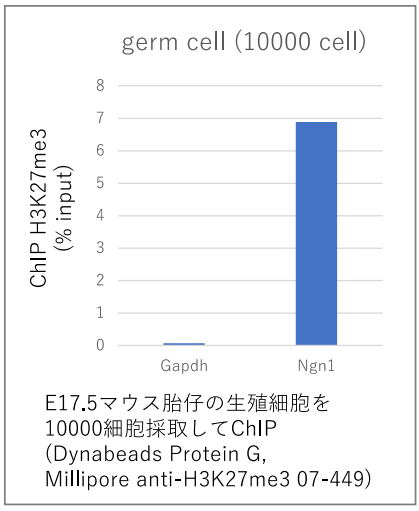
suspend with 35µl 0.1M Tris-HCl.

## 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1-10-14 住友東新橋ビル3号館5F
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 techservice@veritastk.co.jp

RDBM-19-0848
(19.12.25)