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Top2 Chromatin Accessibility by Etoposide Cross-linking

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1 Works for me dx.doi.org/10.17504/protocols.io.2rngd5e

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ABSTRACT

The best way to determine TOP2 specific accessibility to chromatin is via etoposide treatment, which leads to a covalent cross-link between TOP2 proteins and DNA, that can only occur where TOP2 proteins have access to DNA. TOP2 cross-linking to DNA has previously been shown to require DNA accessibility and chromatin regulators that alter DNA accessibility (Dykhuizen and Hargreaves et al. Nature 2013, Miller et al. NSMB 2017).

GUIDELINES

Buffer A
- 25mM HEPES pH 7.0
- 25mM KCl
- 0.05mM EDTA
- 5mM MgCl2
- 10% Glycerol
- 0.1% NP-40

High Salt RIPA
- 50mM TRIS pH 7.8
- 500mM NaCl2
- 1% NP-40
- 0.1% DOC
- 0.1% SDS

1% SDS RIPA
- 50mM TRIS pH 7.8
- 150mM NaCl2
- 1% NP-40
- 0.1% DOC
- 1% SDS

Protease Inhibitors (1000x stock in DMSO all from Calbiochem)
- Chymostatin #230790 (10mg/ml)
- Leupeptin, Hemisulfate #108975 (10mg/ml)
- Pepstatin A, Synthetic #516481 (10mg/ml)

15m

1 Cell Culture:
Trypsinize and count cells
Transfer 0.75e6-1e6 cells to (4) different eppendorfs
Save (1) eppendorf for your input sample for step 4
Bring volumes to 1ml with media (If volume is already more than 1ml you can transfer to a 15ml conical and bring volume up to known amount where all cell lines are at the same volume)

1h

2 Etoposide Cross-linking
Add Etoposide (1:1000; final concentration of 100uM) for 0, 15 and 60 minutes
Add to 60' sample first, and then 15' sample 45' later
Rotate at RT for 60'

10m

3 Washes
Spin at 500g for 4 minutes at 4°C
Wash with PBS
Spin at 500g for 4 minutes at 4°C
Remove suppernatant

15m

4 Buffer A -- Making Nuclei
For all samples + Input
Resuspend in 1 ml Buffer A + Protease Inhibitors (1:1000) + DTT (1:1000; final concentration:)
Incubate on ice for 10'
Spin at 300g for 4 minutes
Aspirate out suppernatant
Spin breifly and remove remaining suppernatant

25m

5 High salt RIPA extraction
Resuspend nuclei in 50-100ul High salt RIPA with 500mM NaCl
Incubate on ice for 20'
Spin 12,000g for 5' at 4°C
Remove suppernatant (can save if you want)
Spin breifly and remove remaining suppernatant

33m

6 Solubalize chromatin pellet
Resuspend in 50ul 1% SDS RIPA + PIs + DTT + Benzonase (1:200)
Be sure to fully resuspend pellet by pipetting up and down ~20 times
Incubate at room temp for 10'
Incubate on ice for 20'
Spin 12,000g for 3 minutes and transfer suppernatant to a new tube (there should not be much if any of a visible chromatin pellet after spinning. If there is then try resuspending in a larger volume)

7 Proceed to Western Blot adding equal volumes of each sample