PCHi-C Time course Nutlin 3A

TUESDAY, 7/20/2021

HCT116 WT cultivated with Nutlin 3A BR2 (detailed in 210720 Nultin3a timecourse for Hi-C) , harvested and fixed for PCHi-C

1. Cells were trypsnized
2. Cells were fixed in suspension with DMEM 10%FBS 2% formaldehyde rotating 10min RT
3. Add ice cold Glycine to 0.125M and rotate 5min RT
4. Incubate 15min on ice with occasional mixing by inversion
5. Centrifuge 800g 10min 4ºC
6. Remove supernatant, wash with ice cold 1x PBS
7. Centrifuge 800g 10min 4ºC
8. Remove supernatant, flash freeze

TUESDAY, 11/30/2021

Start 1st week Hi-C

Prepare lysis buffer:

- 4690ul ddH2O
- 50ul 1M Tris
- 50ul 20% NP-40/IGEPAL
- 10ul NaCl 5M
- 200ul cOmplete protease inhibitor 25X

1. **Resuspend** cells **in 1ml lysis buffer**
2. **Incubate 30min on ice** with slight vortex every 5min
3. **Centrifuge** them **1000g 10min 4ºC**
4. **Remove supernatant**
5. **Resuspend** nuclei **in 500ul 1.25X NEB2 buffer**
6. **Centrifuge** them **1000g 10min 4ºC**
7. **Remove supernatant** leaving behind 20ul
8. **Resuspend** nuclei **in 358ul 1.25X NEB2 Buffer**
9. **Add 11ul SDS 10%** (removes proteins that were not directly crosslinked to the DNA)
10. **Incubate 1h 37ºC 950rpm** in a thermomixer
11. **Add 75ul Triton X-100 10%** to quench SDS
12. **Incubate 1h 37ºC 950rpm** in a thermomixer
13. **Add 15ul of HindIII** (NEB 100U/ul, R0104T) to digest chromatin
14. **Incubate overnight 37ºC 950rpm** in a thermomixer

WEDNESDAY, 12/1/2021

1. **Add an extra 5ul of HindIII** (NEB 100U/ul R0104T) to further digest chromatin
2. **Incubate 1h 37ºC 950rpm** in a thermomixer
3. **Prepare** the following **mastermix** (30ul per sample) **and add it**:
    a. 6ul 10x NEB2 Buffer
    b. 2ul ddH2O
    c. 1.5ul 10mM dCTP
    d. 1.5ul 10mM dTTP

   e. 1.5ul 10mM dGTP
   f. 37.5ul 0.4mM biotin-14-dATP (Invitrogen 19524-016)
   g. 10ul Klenow DNA pol I large fragment (NEB 5U/ul M0210L)
4. Mix without creating bubbles and **incubate 75min at 37ºC** in a thermomixer. Mix by inversion a couple of times every few minutes.
5. Cool thermomixer to 16ºC. **Prepare** the following **mastermix and add it**:
   a. 100ul T4 DNA Ligase Buffer 10x (NEB B0202S)
   b. 5ul BSA 20mg/ml (NEB B9000S)
   c. 25ul T4 DNA Ligase (Invitrogen 1U/ul 15224-025)
   d. 346ul ddH2O
6. **Inucbate samples 4h 16ºC** in a thermomixer. Every now and then mix by inversion.
7. **Incubate samples 30min RT**
8. **Add 10ul RNaseA** 10mg/ml and **incubate 30min 37ºC**
9. **Add 60ul Proteinase K 10mg/ml** and **incubate 30min 37ºC and overnight 65ºC 950rpm** in a thermomixer

THURSDAY, 12/2/2021

1. **Add an extra 30ul of Proteinase K 10mg/ml** to further decrosslink
2. **Incubate 2h 37ºC** in a thermomixer
3. **Freeze -20ºC** due to time constraints. Phenol-cloroform will be performed in the next days

THURSDAY, 12/9/2021

1. **Add** to the sample **1 volume** (545ul) **of Phenol:Chloroform**
2. **Vortex** vigurously and **spin 12000g 5min** in a tabletop centrifuge
3. **Transfer the aqueous phase** to a new 2ml tube
4. To precipitate DNA **add the following to the sample** (aprox 545ul):
   a. 1362.5ul of ice cold EtOH 100%
   b. 54.5ul sodium acetate 3M pH 5.2
   c. 2ul Glycoblue
5. **Incubate 1h -80ºC**
6. **Spin** sample at **maximum speed 30min 4ºC** in a tabletop centrifuge
7. **Discard supernatant** and **add 1.5ml** of freshly made **EtOH 70%**
8. **Spin** sample at **maximum speed 10min RT**
9. **Discard supernatant and air dry pellet**
10. **Resuspend in 83ul TLE**
11. **Quantify** by using Qubit dsDNA HS

Table1

	A	B	C
1	Sample	ng/ul	ng TOTAL
2	HCT116 1h	1370	113710
3	HCT116 4h	1340	111220
4	HCT116 7h	1200	99600
5	HCT116 24h	620	51460

1h 0'5ul Dekker // 1ul Roger, 03, Hist

4h 0'5ul Dekker // 1ul Roger, 03, Hist
7h 0'5ul Dekker // 1ul Roger, 03, Hist
24h 1ul Dekker // 2ul Roger, 03, Hist

Tempase Hotstart Pol VWR

95ºC	15'	
94ºC	30''	|
60ºC	1'	| 34 cycles
72ºC	1'	|
72ºC	10'	
4ºC	Hold	

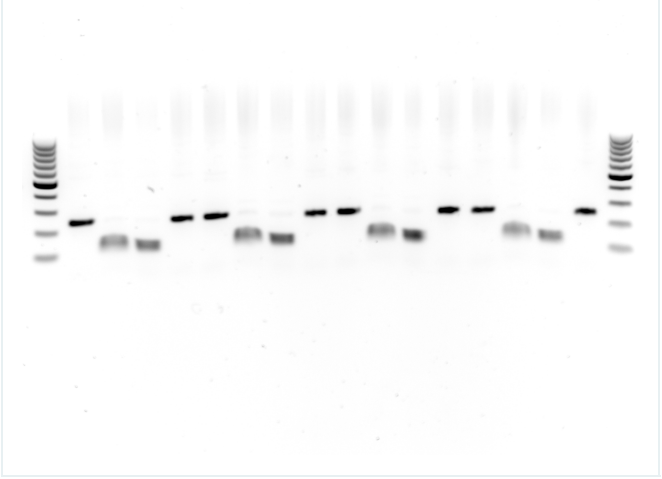
[1h] Dekker, Roger, 03, Hist // [4h] Dekker, Roger, 03, Hist // [7h] Dekker, Roger, 03, Hist // [24h] Dekker, Roger, 03, Hist // [NTC] Dekker, Roger, 03, Hist

211212 p53 time course nutlin interaction controls.tif

Nice. Reamplify 5 cycles 2.5ul PCR product from Dekker
Digest with HindIII, NheI, x2 or nothing

[1h] HindIII, NheI, x2, none // [4h] HindIII, NheI, x2, none // [7h] HindIII, NheI, x2, none // [24h] HindIII, NheI, x2, none

211213 p53 time course nutlin ligation controls.tif



Nice

WEDNESDAY, 12/15/2021

Get 10ug from each sample and top up with ddH2O to 164ul

Table2

	A	B	C
1	Sample	ul library to 10ug	ul ddH2O
2	1h	7.3	156.7
3	4h	7.46	156.54
4	7h	8.33	155.67
5	24h	16.13	147.87

1. Add to each 164ul the following mastermix (total volume 200ul)
    a. 1ul BSA 20mg/ml
    b. 1ul ddH2O
    c. 20ul NEB2 10x
    d. 4ul dATP
    e. 10ul T4 DNA Pol (M0203L)
2. Incubate 4h at 20ºC no rotation
3. Add 4ul EDTA 0.5M and mix
4. Top up to 300ul with TLE and perform a Phenol-Chloroform extraction
5. Add to the 300ul aqueous phase:
    a. 750ul 100% EtOH ice cold
    b. 30ul sodium acetate 3M pH 5.2
    c. 2ul Glycoblue
6. Incubate 1h -80ºC
7. Centrifuge 4ºC 30min max speed
8. Discard supernatant
9. Resuspend and wash in 1ml EtOH 70%
10. Centrifuge RT 10min max speed
11. Discard supernatant and resuspend in 130ul

THURSDAY, 12/16/2021

1. Transfer to a Covaris 130ul cuvette
2. Sonicate in Covaris M220
    a. DF 20%
    b. CPB 200
    c. PIP 50
    d. 65sec
3. Transfer to a new 1.5ml low-bind tube
4. Add the following mastermix to each sample:
    a. 18ul 10x NEB ligation buffer
    b. 18ul dNTPs 2.5mM each
    c. 6.5ul T4 DNA Pol (NEB M0203)
    d. 6.5ul T4 PNK (NEB M0201)
    e. 1.3ul Klenow (NEB M0210)
5. Incubate 20ºC 30min
6. Add 130ul SPRI beads (1vol) and perform standard DNA cleanup
7. Resuspend in 35.7ul TLE

FRIDAY, 12/17/2021