210720 Nutlin3a timecourse for Hi-C

MONDAY, 7/19/2021

HCT116 wild type (2 biological replicates)
10uM Nutlin3a/vehicule (DMSO) timecourse (1h,4h,7h, 24h)
p100 (1.4M/p100)
We don't include time 0 and time 10h because they were included in a previous experiment

Seed 4*p100 per replicate at 1.4M each

TUESDAY, 7/20/2021

Add 10uM Nutlin3a (stock 10mM. at -80ºC) to 24h plates-10ul per plate

WEDNESDAY, 7/21/2021

Add 10uM Nutlin3a (stock 10mM. at -80ºC) to 7h plates-10ul per plate
Add 10uM Nutlin3a (stock 10mM. at -80ºC) to 4h plates-10ul per plate
Add 10uM Nutlin3a (stock 10mM. at -80ºC) to 1h plates-10ul per plate
Harvest cells

Table1

	A	B
1	Cond	Cells
2	WT BR1 1h	7.11M
3	WT BR1 4h	6.75M
4	WT BR1 7h	4.38M
5	WT BR1 24h	1M
6	WT BR2 1h	10.88M
7	WT BR2 4h	8.8M
8	WT BR2 7h	8.18M
9	WT BR2 24h	2.36M

Cell count and fix for HiC:

1. Re-suspend cells in 1148ul of DMEM/10% FBS RT by pipetting in order to get individual cells.
2. Add 164.1ul of RT fresh formaldehyde (16% stock solution, R1026, Agar Scientifics) to a final concentration of 2% and fix for exactly 10 min at room temperature (RT) with gentle mixing on a rocker.
3. Quench reaction by adding 187.5ul of cold 1 M glycine (0.125 M final).
4. Incubate for 5 min at RT with gentle mixing, followed by 15 min on ice mixing eventually.
5. Centrifuge at 1000g (rcf) for 10 min at 4°C.
6. Discard supernatant by pipetting (leave 50ul behind), re-suspend pellet carefully in 1ml cold 1x PBS by pipetting
7. Centrifuge at 1000g (rcf) for 10 min at 4°C, discard supernatant (leave 150ul behind).
8. Flash freeze the pellet in liquid N2 and store pelleted cells at -80°C

BR1 was processed by Biola/Lucía & Blanca Valero in sept 2021 but library preparation failed (due to problems with old truseq adaptors??)
BR2 was processed by Llorenç in Dec 2021 (see PCHi-C Time course Nutlin3A entry)