Sucrose gradient with long chromatin fragments (RNase free)

Day 1

Prepare the gradient:

- Prepare a 5% and a 30% sucrose solution containing 150 mM NaCl.
- Make a 5%-30% sucrose gradient using a gradient mixer (15 ml 5% and 15 ml 30%)
- Tubes: Beckman Polyallomer 1x3 ½ (25x89mm)

Chromatin isolation (or any other RNA sample)

- 8x Pellets Hela S3 cells (in 50% Glycerol/ 50% IT, stored at -80°C)
- wash 1x with **IT-buffer** containing RNase inhibitor
- centrigure 1200 rpm 4 min 4°C
- remove supernatant and repeat washing step
- combine 2 pellets in 5 ml **Solution A** containing RNase inhibitor
- incubate 10 min on ice
- use a douncer (tide pestle) up to 15x strikes
- centrifuge 2000 rpm 4 min 4°C
- resuspend pellet in 1 ml ice cold **sonication buffer**
- Sonicate using a covaris system (1 to 5 min, depending on the cell type; for about 5000 bp fragments) -> duty cycle 20%; Intensity 5; Cycle per burst 200; power mode frequency sweeping; degassing mode continuous; temperature 6-7°C (cool down 1 hour in advance). Tubes: covaris #520056.
- Centrifuge after sonication, 10 min, 13000rpm, 4°C
- Take supernatant and pipet carefully on top of the gradient

Sucrosegradient

- Equilibrate gradient and tara and start centrifuge: 16000 rpm 16h 4°C Accel: slow Deccel: no break
- Swinging bucket Rotor: Beckman SW 32Ti

Day 2

Fractionate the gradient

- With a needle, make a hole at the bottom of the tube and take 1 ml fractions.
- Add 1 µl RNase inhibitor in each fraction
- Take 50 µl of each fraction and store the rest at 4°C
- Proceed to DNase digestion and PtK digestion of the 100 µl samples
- Load the samples on an a 1%Agarose/TAE gel for analysis
- Purify RNA from the interesting fractions (the same can be done with DNA to analyze for example the length of the chromatin fragments in each fraction).

SolutionA
10mM Hepes pH7.5
10mM KCl
1.5mM $MgCl_2$

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0.5mM DTT

IT-Buffer

25mM Tris HCl pH 7.5
137mM NaCl
5mM KCl
0.5mM $MgCl_2$
0.7mM $CaCl_2$
0.3mM Na_2HPO_4

Sucrose buffer

Sucrose in
10mM TrisHCl pH7.5
1mM EDTA
100mM NaCl

Sonication buffer

50 mM Hepes pH 7.9
140 mM NaCl
1 mM EDTA
1% Triton X-100
0.1% Na-deoxycholate