Polysome profile (SQZ10 cell)

Buffer & Reagents:

- 100mg/ml Ampicillin & 50mg/ml chloramphenicol (in ethanol)
- Lysozyme (10mg/ml H_2O solution, sigma, L6876)
- 10% Deoxycholate
- 10% sucrose (103.8mg/ml) 4.2g in 40ml buffer (final volume)
- 40% sucrose (470.6mg/ml) 18.8g in 40ml buffer (final volume)
- 50% sucrose (614.8mg/ml) 46.1g in 75ml H_2O (final volume)

Lysis buffer		5ml
20mM Tris-HCl,pH7.5	1M	100ul
15mM $MgCl_2$	1M	75ul
400ug/ml chloramphenicol	50mg/ml	40ul
water		4.785ml

Sucrose buffer		40ml
20mM Tris-HCl,pH7.5	1M	0.8ml
15mM $MgCl_2$	1M	0.4ml
100mM NH_4Cl	5M	0.8ml
BME	14.4M	6ul
Sucrose		4.2g/18.8g

1. Prepare 2ml O/N culture.
2. 1:100 dilute O/N culture in 100ml LB with 100ug/ml ampicillin and grow at 37℃ until the absorbance reached A_{600} 0.5.
3. Add chloramphenicol (final concentration 100-400ug/ml) to the culture 5 min before harvesting. Place on ice immediately. Spin at 5000g for 10min (in the cold room).
4. Wash pellet with 1ml lysis buffer. Spin as above.
5. Resuspend cell in 0.5ml chilled lysis buffer contain 20mM Tris (pH7.5) and 15mM $MgCl_2$, 400ug/ml CM.
6. Lyse cell with 1mg/ml lysozyme (use Calbiochem lysozyme, Sigma's lysozyme contain too much RNase) and two freeze-thaw cycles in dry-ice ethanol bath.
7. Store at -80℃ until the polysome analysis could conveniently be preformed.

8. Thaw carefully in ice water until the last bit of ice melted.
9. Add 25ul of 10% sodium deoxycholate (~0.5%), (freshly prepared). Incubate on ice for 20min.
10. Centrifuge at 16000g for 10min.
11. Measure the absorbance of the extracts at 260nm.
12. Load 18 -12A_{260} onto 11ml 10-40% sucrose gradients in buffer containing 20mM Tris-HCl (pH7.5), 10mM $MgCl_2$, 100mM NH_4Cl and 2mM β-ME.
13. Centrifuge in a WS-41 rotor for 3.0hr at 39000rpm at 4℃.
14. Analyze gradients at 254nm by using an UA detector (ISCO) (sensitivity=1.0abs, chart speed=150 cm/hr).