Cell Cycle analysis by Flow Cytometry (DNA content by PI staining)

1. Grow cells in log phase. (0.3-0.8 at OD660)
2. Spin down and resuspend in 3ml of fresh 70% EtOH. Rotate them at room temp over night. (Can stop here)
3. Add 5ml dH2O, sonicate for 10 sec at 4 channel.
4. Spin, and add 0.5ml of 50mM Tris, pH 7.8. Transfer to eppendorf tubes.
5. Wash with 1.0ml of 50mM Tris, pH 7.8
6. Resuspend in 0.4ml 50mM Tris pH 7.8 and add 100ul of Rnase (stock 10mg/ml, Sigma R6513)
7. Incubate O/N at 37 degree. (Rotate them in the incubator)
8. ~~Spin and resuspend in 0.5ml of 5mg/ml pepsin (Sigma P7000) freshly dissolved in 55mM HCl (To make 55mM HCl, put 460 micro liter HCl per 100ml). Incubate for 30 min at 37 degree in water bath.~~
9. (or) Add 50ul of Proteinase K (20mg/ml stock) and incubate at 55 degrees for 1 hour
10. Spin down and resuspend in 1.0ml of 200mM Tris pH7.5/211mM NaCl/78mM MgCl2 (50ml 1M Tris/19.5ml of 1M MgCl2/10.55ml 5M NaCl for 250ml)
11. Spin and resuspend in 0.5ml of 200mM Tris pH7.5/211mM NaCl/78mM MgCl2. (Can stop here)
12. Add 50ul of 750ug/ml propidium iodide (Sigma 4170) to step 11.
13. When you read in FACS, dilute 50ul from step 12 in 1ml 50mM Tris pH 7.8

RNase A (10mg/mL stock)

1g RNAse A (Sigma R6513)

1mL Tris (1M stock, pH 7.5)

0.3mL NaCl (5M stock)

99mL H2O

--100mL total

Resuspend RNAse A and aliquot into 15mL conicals. (DO NOT BOIL)

Propidium Iodide

Sigma 4170 37.5ml/50ml H2O (750ug/ml stock)

Pepsin: Sigma 7000

Proteinase K 20mg/ml solution : Fisher AM2548