Atsu’s Membrane Lysate Prep

This protocol is used to isolate membranes from cultured Sf9 cells, all steps are performed on ice. You will need to prepare the following solutions:

**Buffer**: 1mM NaHCO3  
1mM PMSF

40mM NaOH  Start with 4N NaOH and do a 1:100 dilution with Buffer

20mM NaOH  Start with 4N NaOH and do a 1:200 dilution with Buffer

**Procedure**

- 1200mL of Sf9 cells are used for one membrane prep, these are spun down 4000rpm, 15min, 12°C and the supernatant is removed by slowly decanting  
  - The bottles used for this centrifugation do not need to be autoclaved
- Divide the pellets into 6 x 80mL centrifuge tubes using cold PBS
- Add ~10mL of PBS to the 400mL tube, resuspend and divide into the 80mL tubes
- Add about 20mL more of PBS resuspend again to get every last little bit of pellet
- Repeat again with more PBS (total not to exceed 80mL in each 80mL tube)
- Centrifuge again, 4000rpm, 15min, 12°C  
  - The pellet is too viscous by itself so you can dilute it with **Buffer**
- Here we have 6 pellets, add 5mL buffer to bring up to 10mL
- Combine the pellets so there are 2 pellets in 3x 80mL tubes
- Add an equal volume of 40mM NaOH to each tube  
  - Since we have combined into 3 tubes, add 10ml to each new tube  
  - This gives us a final concentration of 20mM NaOH  
  - From now on you will only use the 20mM NaOH, you will NOT need the 40mM NaOH anymore
• Wash the empty tube that used to contain the pellet with 20mM NaOH to remove all the cells that are stuck to the walls
• Fill each of the centrifuge tubes to the 80mL line with 20mM NaOH
• The cells need to be broken down, sonicate each tube until mixture is homogenous (amplitude 80, for about 30 seconds)
  o If you do not sonicate enough you will have a bad recovery
• Fast Cool the Eppendorf Centrifuge
• Spin 12,000rpm, 7°C (if you set this particular centrifuge at 4°C while spinning at max rpm an error can happen) 25-30min
• Decant supernatant
• Take the *Buffer* use 12mL to suspend all 3 of the pellets together
  o f you started with 1200mL, final will be 100mL of cells concentrated into 12x 1.5mL tubes
• Mixture is still not homogenous so you need to sonicate it to mix
• Sonicate at a weaker amplitude ~ 30amp for 30 seconds
• Aliquot 1mL in to 12 x 1.5mL tubes

Now you can purify protein or freeze in Liquid Nitrogen and store at –80°C, but try to use it within 3months