AS 4/6/06
Version 2

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 $NaHCO_3$
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, 12ºC 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, 12ºC
    - 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)
    - If you do not sonicate enough you will have a bad recovery
- Fast Cool the Eppendorf Centrifuge
- Spin 12,000rpm, 7ºC (if you set this particular centrifuge at 4oC 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
    - 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 –80ºC, but try to use it within 3months