

## Freezing Baculovirus-infected insect cells (BIIC) stocks

### Freezing BIICs (100mL culture for 10 aliquots of 1mL BIIC)

1. Grow 100 mL culture of SF21 cells to  $1 \times 10^6$  cells/mL
2. Infect cells with Virus (dep on strength, usually 5ml)
3. Maintain a concentration of  $1 \times 10^6$  cells/mL until Day after Proliferation Arrest (DPA)  
You will be able to just barely detect YFP on the fluorimeter
4. Centrifuge 800rpm for 5-10 mins. Use falcon tubes to split the volume
5. Prepare a 50mL solution containing (this is enough for 50BIICs but has to be made fresh) :  
45mL = 90% of medium  
0.5g = 10g/L of BSA  
5mL = 10% of DMSO – already sterile and for insect cells only  
} Sterile Filtered (0.22  $\mu$ m)
6. After spinning, remove supernatant
7. Resuspend cells gently to a final density of  $1 \times 10^7$  cells/mL (10x concentration)
8. Aliquot 1mL into each cryovial
9. Place at  $-20^\circ\text{C}$  for 1 hr (or directly into  $-80^\circ\text{C}$  using Mr Frosty)
10. Store at  $-80^\circ\text{C}$  for 24-48 hrs
11. Store in liquid nitrogen.

### Infection after thawing a BIIC (1mL of BIIC for 800mL culture)

1. Pre-adapt 375 mL cultures of uninfected SF21 cells at  $\sim 1 \times 10^6$  cells/mL
2. Quickly thaw one vial in your hands (use paper towels to protect your skin)
3. Dilute the vial in 50 mL of medium  
*You obtain 50 mL at  $2 \times 10^5$  cells/mL*
4. Add 25 mL of this solution in each of the 375 mL flasks  
*You should obtain 400 mL of cells at slightly more than  $1 \times 10^6$  cells/mL (the concentration increases by 2.7% only)*
5. Maintain the cells at  $1 \times 10^6$  cells/mL until you observe the proliferation arrest
6. Monitor the YFP until it reaches a plateau
7. Harvest the cells (10 minutes 1200 rpm)

It is **eminently important to test the BIICs immediately after preparation**. Occasionally BIIC production fails because harvesting too early (not enough virus yet) or too late (virus budded off). We always sacrifice one BIIC aliquot directly after freezing to test the production capacity of the BIICs and prepare a report which we archive with the BIICs. We found that expression from BIICs is stable for several years (longest tested in our lab was  $\sim 5$  years).