Amplifying Virus

P1 Stock

1. Plate two 100 mm Tissue Culture dishes of SF-9 cells at $7x10^6$ each with 10 ml of complete medium (ESF 921 + 2%FBS + 1% PSF).
2. Add 500 ul viral stock to the medium in one dish. Keep the other as a control.
3. Harvest supernatant after 3-5 days (longer is usually better). Ask Alison whether your cells look infected (swollen and beginning to lyse).
4. Spin this P1 stock as before and save in a box in fridge. Freeze 1ml aliquots with 2%FBS for making p2 virus in the future.
5. You can scrape cells at this point into PBS and if you're lucky you'll see protein band.

P2 Stock

1. Add 1 ml of P1 virus stock to 100 ml sf-9 in 250ml shaker flask.
2. Optional: Harvest 1 ml culture every 24 hr to look at protein expression by spinning down cells and freezing cell pellet on liquid nitrogen, then resuspending in SB and boiling.
3. Harvest virus-containing supernatant after 4-6 days (when cells are starting to lyse - drop some on a slide and examine them). The supernatant should be filter-sterilized and kept at 4^oC. This stock will deteriorate in 4-6 months so you might want to amplify from bacmid every 3 months.