* [ESF 921](https://expressionsystems.com/product/esf-921-insect-cell-culture-medium-protein-free/?gad_source=1&gclid=Cj0KCQiA1p28BhCBARIsADP9HrNf4NWKJZiCQnU_vO-iIJnzxWFeSbQj_qK4VWntXq8-nAmVufQeWZgaAmhQEALw)
* [Grace's Media](https://www.thermofisher.com/order/catalog/product/11605094)

1. Validate you have fully recombined BacMid DNA via PCR (using M13F-M13R primers). Ensure that there are NO contaminating bands, especially those with low KB values as those are WT Baculovirus.
2. Transfect BacMid DNA into SF9 cells. Dose response of 1-3ug of BacMid DNA into the adhered SF9 cells.
3. After collecting the supernatant to generate P1, add the same volume of media back to P0 plate to generate a second P1 in a few days. The cells are continuing to package virus over that time and sometimes that second P1 is more potent than the first.
4. P1: use the entire supernatant from P0 plate. Drop that into 50 mL of SF9 cells at 1Million Cells/mL. incubate for 4-5 days. Pellet the cells, filter the supernatant and supplement with 5-10% FBS for long term storage at 4 degrees Celsius.
5. After P1 generation I do extensive viral tittering to optimize P2 generation for protein production. I start with a dose response ranging from 0.1-1uL/mL of P1 into SF9 cells and monitor cell replication over the next 4-5 days. In our hands, the optimal viral titer for protein production will roughly have 1.5 doublings before arrestment (going from 1Million Cells/mL to ~3.5 Milllion cells/mL). This P2 is the one which I then use for protein production optimization. Depending on whether its going into mammalian or insect cell driven production will then change how we do that optimization, as in our hands, Expi293 infection with baculovirus above ~1% v/v leads to massive clumping.
6. As for fluorescent production, our EMBacY cells have an insect cell driven GFP promoter so we monitor that fluorescence. As for timing, we typically see a little bit of fluorescence at the end of the P0 stage (maybe 1-5%) and more at the end of the P1 stage (~20%). At the P2 stage nearly all cells should be fluorescing. To monitor this we always use a fluorescent microscope, but even without that you should be able to assess infection via cell morphology in a suspension culture (P1 onward). The cells will swell A LOT (~100-200% increase in size).