Passaging Sf9 Cells in Solution

Sf9 cells should be passaged once they reach 8e6 cells/mL, though there is some leeway in how long after that point you can leave them without passaging them. When you do passage them, try to end up with a final concentration of 1e6 cells/mL. Insect cells typically double in number every 24 hours, so the cells typically need to be passaged every 3-4 days. Their growth speed may vary depending on the temperature they're grown at and whether a baffled flask is employed however.

Before passaging:

1. Make sure you have enough sf9 medium with antibiotics added.
2. If you don't, thaw out an aliquot of antibiotics, and while it's thawing, sterilize a hood for the subsequent work. (To sterilize a hood, first turn on the fan and turn off the UV light. **Never open a hood for sterile work unless its fan is on**. Then, while wearing ethanol cleaned gloves and a clean lab coat, spray the interior of the hood with 70% ethanol in water, and wipe it down with kim wipes or paper towels.)
3. Bring a container of sf9 medium and the thawed antibiotic to the sterilized hood. Spray both containers with 70% ethanol and wipe them with paper towels/kim wipes before adding them to the sterile hood. **Any object which enters a hood for sterile work should be thoroughly sprayed with 70% ethanol and wiped down.** The gloves that you wear should be ethanol sprayed as well, but don't bother wiping them with paper towels.
4. Pour the antibiotic into the sf9 medium. While pouring it and at any other point in which you open a container of medium, **do not have your arm or hand positioned above the open container** (particulate contaminants are constantly falling off of us, and these contaminants could ruin the cell medium even in the presence of antibiotics)
5. Close the sf9 medium container, and treat its contents as sterile from there onwards. **Any container whose interior must remain sterile cannot be opened outside of a sterilized hood whose fan is on.**
6. Store at 4 C until needed.

Passaging Sf9 cells:

1. Retrieve your sf9 medium + antibiotic and place it in a room temperature water bath.
2. Sterilize a hood. Sterilize any materials needed for cell counting. I typically bring a p10 pipette, sterile p10 pipette tips (sterilized in an autoclave), a counting slide, some small tubes whose interiors don't need to be sterile,

and some trypan blue (trypan blue is a dye that stains the exteriors of living cells, and stains the interiors and exteriors of dead cells, making them easy to tell apart.)

3. With all of those materials ready in the hood, retrieve your flask of insect cells and bring it to the hood.
4. Loosen the cap of the cell flask, hold the flask at a 45 degree angle, then remove the cap, but hold it slightly above the open flask so as to shield it from any contaminants (this should all be done with one hand). With the other hand, use the p10 pipette to extract 5 uL of cells. **Remember not to have your hands above the open flask**. That's why we tilt the flask by 45 degrees before pipetting, otherwise the hand holding the pipette would be above the open flask.
5. Close the cell flask and add the 5 uL of cells to the non-sterile tube, then add 5 uL of trypan blue as well.
6. Mix and pipette into the cell counting slide
7. Return the cell flask to the incubator, and bring the counting slide out of the hood for quantification (My lab uses a Countess 3 Automatic Cell Counter from Invitrogen and the corresponding Countess slides). The insect cells don't like being out of the incubator for too long, which is why we return them to the incubator while we quantify the cells.
8. Use the $C_1 \times V_1 = C_2 \times V_2$ equation to calculate how much cell solution you should carry over to the next passage to make the new concentration 1e6 cells/mL (for example, if your flask has a concentration of 8e6 cells/mL, and your flask's typical growing volume is 50 mL, then (8e6 cells/mL) x V_1 = (1e6 cells/mL) x 50 mL. V_1 = 6.25 mL)
9. Sterilize a pippetter, the exterior of a 50 mL flacon tube whose interior doesn't need to be sterile, and the sf9 medium + antibiotic (which should be at room temperature by now), and add them to the hood. Have 25 mL serological pipettes near the hood, but they don't have to be sterilized and placed inside.
10. Retrieve the flask of cells again. Open it using the same precautions mentioned above, and pipette out the cell solution. Deposit it into the 50 mL falcon tube. Leave behind an amount of solution in the flask equal to what you calculated for V_1
11. Fill the flask to 50 mL again by adding fresh medium.
12. Seal the flask and return it to the incubator. Seal the medium and return it to 4 C.
13. Add some 10% bleach to the falcon tube containing the excess cells, mix it, and let it sit for a few minutes to kill the cells inside (we don't want to

release transgenic cells into nature.). Afterwards, its safe to pour down the drain, and the 50 mL tube can be reused as a cell disposal tube.

14. Remove all of the other items from the hood.
15. Wipe the hood down again with ethanol.
16. Close it, turn its fan off, and turn on its UV light.

Note that the same flask can be used over many passages, and if you see some kind of "gunk ring" building up from where the cells are swirled, don't worry: it's not contamination. You can tell a flask is contaminated when it goes from an opacity comparable to apple cider, to completely opaque. You can also check for the presence of prokaryotes under a microscope (they look kind of like a writhing ocean of ripples alongside the much larger insect cells). If a flask ever contains contaminated cell solution, don't bother trying to reuse it after cleaning it out. Simply throw it away in the biohazard waste bin instead.