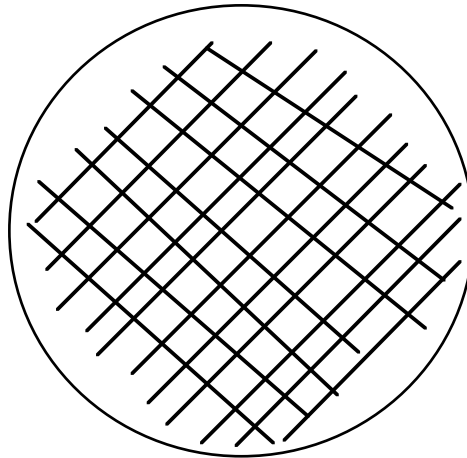


**Growth of *P. syringae* strains for Silwet L-77 Dip Inoculation  
Kunkel Lab, Washington University**

For inoculating *A. thaliana* you will need approximately 1 L of cells per flat of 3" pots.

1. The afternoon prior to inoculating your plants streak out the strains that you will be using in the following manner:
  - With a sterile inoculating stick or loop scoop up a large clump of *P. syringae* cells (approximately the size of a grain of rice) off of a relatively fresh KB or NYGA plate.
  - Streak out the bacteria evenly over the entire surface of the plate (either KB or NYGA is fine).



- You will need 5 to 6 plates full of bacteria to make up 1 L of cells at the appropriate cell density ( $2 - 4 \times 10^8$  cells/ml, or an OD600 of  $\sim 0.2 - 0.4$ )
2. Incubate the freshly patched out cells overnight at 28-30°C.
  3. When you are ready to start your inoculations, flood each plate with  $\sim 10$  ml 10mM MgCl<sub>2</sub>.
  4. Using a sterile spreading stick or glass pipette, scrape up the cells, so that they begin to resuspend in the MgCl<sub>2</sub>.
  5. Remove the cells from the plates by aspirating the cells into a sterile pipette.
  6. Add the cells from all 5-6 plates to 1 L of 10 mM MgCl<sub>2</sub>, and mix well.
  7. Check the cell density with a spectrophotometer, and adjust the suspension if needed by diluting with 10mM MgCl<sub>2</sub>, so that you end up with an OD600 of  $\sim 0.5$ .