Clean hood/wipe down with 70% EtOH, and assemble reagents:

* Sterile tips (1 ml, 200 ul, and multi-channel)
* 1 ml, 20-200, and multi-channel pipetmans
* microfuge tube rack
* Sterile microcentrifuge tubes and wooden sticks
* TARG liquid media or sterile ddH2O
* Autoclaved trough
* 96 well cell culture rectangular plate
* Solid media for drops
* Permanent marker
* Waste beaker
* timer
* Plastic cuvettes

Once supplies are assembled, annotate in notebook your samples for drops, the OD measured, 20X OD, volume of cells to be diluted, and amount of water/TARG to dilute with.

**NOTE:** drops should be performed on recently refreshed cultures (i.e. within the past 5 days). However, old cultures may take 2 or more rounds of refreshing so all cultures are actively growing. Alternatively, can use liquid cultures of cells in exponential phase.

**Procedure**

1. Label microcentrifuge tubes in rack – I usually set up two rows (undiluted and diluted). Add 500 ul of sterile ddH2o to each tube (or if using same media among all plates can use liquid media).
2. Using sterile technique, transfer culture from solid media to appropriately labeled tube. Repeat for all samples.
3. Cap tightly and vortex on high for 30-120 seconds. If cells clump together, adding an incubation step here in ddH2O prior to vortexing can help de-clump culture. Take care when vortexing cell wall deficient lines. Return microcentrifuge tubes to hood (pulse vortex for 10 seconds and pipet up/down to mix prior to transfer).
4. Add 950 ul of ddH2O and 50ul of re-suspended culture for 20x dilution then measure absorbance at 750nm. For more accurate readings, Abs for diluted samples should be between 0.1 - 0.5 Abs (also depending on the spectrophotometer).
5. Normalize cultures to the same OD (I typically normalize to 20xOD750 of 5.0 every time). Add calculated ddH2O to each microcentrifuge tube before adding amount of cells. An example of calculations is below:
   1. (A 20xOD)(200 ul)/(B 20xOD) = vol cells where A is lowest OD value or set value
   2. 200 – vol cells = vol ddH2O
6. Using multi-channel pipetman, transfer 90 ul of sterile ddH2O from trough into 5 adjacent columns of 96 well culture plate (for 10-5, 10‑4, 10-3, 10-2, and 10-1 dilutions).
7. Mix by pulse vortex (~5 seconds) or pipet up/down 15x before transferring 90 ul to the 6th column for each sample. Adjust volume on multi-channel to 10 ul. Mixing thoroughly each time, transfer 10 ul from cells to the adjacent row and so on. It’s critical to check volume in each pipet tip before transferring. If there’s not a good seal on each pipet tip of the multichannel loading will be uneven.
8. Adjust multi-channel or p-20 to 5-10 ul and carefully perform drops (choose drops vol). Be careful not to let drops bleed together. Best is to not dry out plate so long as there’s no water droplets on agar. Place drops ~1 cm away from edges if possible. Also, take care not to stab gel (can take practice coordinating through 3-dimensional space for spotting on plate).
9. Repeat for each dilution and sample.
10. Carefully tilt lid to allow drops to dry. Depending on how wet plate is, this can take 5-15 minutes.
11. Be sure to label plates and store in conditions of interest. Decide on your intervals for imaging; 5, 10, and 15 days is usually good although much longer than 2 weeks and media can start drying out. Parafilm can help limit contamination but may also affect gas exchange.

Example:

**Dilution:**

**10o 10-1 10-2 10-3 10-4 10-5**

