Preparation:

1. Pour media on level surface (without stacking plates) so that media solidifies level (essential for even spreading of liquid culture). I’ve found that a very slight grade is OK just don’t stack plates.
2. Using a standard single punch hole puncher, cut discs using whatman #1 filter paper and autoclave in a container, let cool and save for later.

Zone Plating

1. *Chlamydomonas* WT controls and other strains to be tested were grown to exponential phase in liquid culture and cell density checked by OD750. Cells were then counted via hemocytometer and normalized to 1-1.5x107 cells/ml (extrapolate out for amount of plates needed per strain with 2ml of cell culture/100mm plate).
   1. Alternatively, can normalize samples for OD750 equivalent to 0. 5-1.0 Abs but this method is less accurate because Abs is actually measuring turbidity which doesn’t account for changes in individual cell size between strains.
2. Plate 2 mL of normalized liquid culture on each plate and swirl to spread evenly so that entire plate is covered. Pour a few extras just in case several plates aren’t perfectly level. For these sized plates, 2ml is more than enough liquid (takes ~2.5-3 hours to dry with lids tilted). Less could be used with a glass spreader but cells may spread unevenly with streaking (I haven’t tried this).
3. Calculate out # of discs needed and scale up chemical dilutions accordingly, use sterile microcentrifuge tubes to prepare dilutions in cell culture hood. Chemicals to be loaded onto disks should be filter sterilized when applicable (I do for antibiotics but depends; diluting from 50% stabilized hydrogen peroxide I already consider it “sterile” same goes with sodium hypochlorite/bleach).
4. Once cell suspensions are dry, perform dilutions of drug/antibiotic and preload sterile whatman discs. Use metal tweezers, sterilized with 70% ethanol and flame to prevent contamination. In my hands, with the right range of dilutions, 2-3 discs of drug + 1 vehicle (i.e. whatever solvent was used in diluting drug) can be place on each 100mm plate.
5. Place in incubator with lid side up so discs don’t drop (vents well and allows faster growth). Watch for development of condensation on the lid, if this appears, clear condensation and return to incubator.
   1. Can wrap plates if necessary – depends on goals of experiment.
6. This setup works well for 5-7 days of constant light and 24 deg C. However, our incubators have lower light intensity of ~35 umol photons so shorter incubation times may be necessary in higher light conditions.

Example:

