

Testing the effectiveness of UV Protocol

- Grow liquid cultures of *E.coli* Top10
- Measure OD of the culture and apply a correction factor so that the OD is 1.
({OD you want /OD you have} x volume you are inoculating = Volume of LB to add)
- Reduce the OD of the culture (so that colonies are distinguishable/countable when grown on a plate) by making two 1 in 1000 dilutions with LB so that the resultant culture is a 10ml 1 in 1,000,000 dilution.
- Place 200 μ l of the culture in 3 wells of a 96 well plate. Keep the remaining amount of the liquid culture for the control and repeats
- Pipette 200 μ l of the culture and spread onto a LB plate, label it control
- Irradiate the samples in the 96 well plate for 1 minute in the UV Trans illuminator operating at 254nm (This was part of our uvp Bio Doc it² Imager).
- Plate up on an Agar plate each of three 200 μ l samples from the 96 well plate
- Incubate overnight
- Using the same culture repeat this but irradiate for 1 minute, 2.5 minutes, 5 minutes and 10 minutes using the same culture
- Count the colonies on each plate (Note: should be done in the morning so the colonies do not grow too large)