# Lab Methods

*Daniel Padfield*

## Outline

This page is designed to summarise some key methods. To find what you are looking for use `Ctrl-F` (`Cmd-F` on a mac) and search for what you are looking for.

### Xgal addition to KB agar

Xgal need to be added to KB agar before plating to be able to characterise lacz mutants of *Pseudomonas fluorescens* which turn blue on the plates.

Xgal is dissolved in DMSO before addition to the agar. DMSO is super toxic so this needs to be done under the fume hood.

#### Concentration

300 mg of Xgal is added to 15 mL of DMSO. 4.5 mL of this solution is added to 1 L of agar.

Some common concentrations:

- 1 L of agar : 100 mg Xgal in 5 mL DMSO.
- 2 L of agar : 200 mg Xgal in 10 mL DMSO.
- 3 L of agar : 300 mg Xgal in 15 mL DMSO.

#### Method

- Get the Xgal out of the AB freezer (top shelf is the communal shelf).
- Put foil round a 50 mL falcon tube (Xgal is light sensitive).
- Measure out the required amount of Xgal on the sensitive scales and place it in the falcon tube.
- Get the DMSO from the chemicals cabinet (underneath the fume hood) and add the desired amount to the falcon tube using a serological pipette.
- Add this to agar (not too hot).
- Shake agar
- Plate

### Serial dilution for plating

Serial dilution is done get an appropriate concentration at which to plate bacteria

#### Method

- Get the M9 10x concentrate and autoclaved DI water and create a working solution of M9 in a 50 mL falcon tube (5 mL of M9 10x and 45 mL of water)
- Put 180 µL of M9 into each well of a 96 well plate as necessary
- Put 20 µl of bacterial culture in each well
- Mix the culture in the well then transfer to the next row (representing another ten fold dilution)
- With new pipette tips mix the culture in the next row and then transfer to the next row
- Dispense of pipette tips
- Repeat as necessary until all rows have been mixed and desired concentration for plating is achieved

- **MAKE SURE EACH ROW HAS BEEN TRANSFERRED OTHERWISE THE PLATES WILL HAVE NOTHING ON THEM**