You will receive **6 samples**; 3 samples contain RFP under the control of a strong promoter, 3 constructs have RFP under the control of a weak promoter. Each sample has been stored under different conditions (room temperature, 4 °C, -80 °C).

**Filter Settings:**

**RFP**

Excitation – 584nm

Emission – 607nm

Path length correction should be off.

**OD600**

Use instrument’s regular OD600 protocol.

**Notes:**

All procedures should be performed under sterile conditions.

Specimens are Erythromycin (Erm) resistant, LB media requires an Erm concentration of 500µg/ml.

**Reviving Freeze Dried Bacteria:**

1. Warm 500µl of SOC medium per sample (6 in total) to 37°C ( ̴15 mins in an incubator).
2. Revive each freeze dried sample by resuspending the “powder” in 400µl of prewarmed SOC.
3. Inoculate the full 400µl in 10ml liquid media of LB – Erm (500µg/ml) in a 50 ml falcon tube.
4. Make up 10ml LB-Erm as a control (blank).
5. Cover tubes with aluminium foil to block light.
6. Take sample for T0.
7. Incubate at 37°C and 220 rpm.

**Sampling:**

* Take 500µl samples at 0, 1, 2, 4, and 6 hours and measure fluorescence at each time point. Place the samples on ice during processing.
* Pipette 100 µl per sample into each well, with three technical replicates per sample.
* Depending on the OD600, samples should be diluted to be in the detection range of the machine (usually the detection range is between 0 and 1).
* For each time point, at least 3 wells should contain 100 ul of the blank solution made up previously (step 4, see above).
* RFP and OD600 should be measured at each timepoint.
* Import Raw data to Excel Spreadsheet and please send them to team UNOTT :D

**Example Plate:** 