FREEZE DRIED RFP MEASUREMENT PROTOCOL

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).

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- 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: