Before You Begin

Read through this entire protocol sheet carefully before you start your experiment and prepare any materials you may need.

Cell measurement protocol

Materials:

Competent cells (ideally *Escherichia coli* strain DH5α or TOP10)
Terrific broth (at half strength: 0.5x TB) or can use LB (Luria Bertani) media as an alternative
1 mL Chloramphenicol (1x stock concentration: 25 mg/mL dissolved in EtOH)
50 ml Falcon tube (or equivalent)
Incubator at 37°C
1.5 ml eppendorf tubes for sample storage
Ice bucket with ice
Pipettes

Devices (from InterLab Measurement Kit):

- Positive control
- Negative control
- Device 1: J23101+I13504
- Device 2: J23106+I13504
- Device 3: J23117+I13504

Method:

Day 1: transform Escherichia coli DH5a or TOP10 with these following plasmids:

- Positive control
- Negative control
- Device 1: J23101+I13504
- Device 2: J23106+I13504
- Device 3: J23117+I13504

Hint: If the plate reader protocol has been followed, you can also re-streak an existing colony on a new plate, if it is not fresh anymore.

Day 2: Cell growth

- ☐ Pick 3 colonies from each plate and inoculate it in 5-10 mL LB Medium + Chloramphenicol (25mg/mL).
- ☐ Grow the cells overnight (16-18 hours) at 37°C and 220 rpm.

Day 3: Cell growth, sampling, and assay

- ☐ Set your instrument to read OD600 (as OD calibration setting)
- ☐ Measure OD600 of the overnight cultures
- ☐ Dilute the cultures to a target OD600 of 0.02 (see the volume of preloading culture and media in the Excel 'normalization' sheet) in 10 ml 0.5x TB (or LB) medium + Chloramphenicol (25mg/mL) in 50 mL falcon tube.
- ☐ Incubate the cultures at 37°C and 220 rpm.
- ☐ Take 100 µL of the cultures at 6 hours of incubation, transfer to Eppendorf tube.
- ☐ Take 1 mL of the cultures, measure OD
- ☐ Spin cell sample down (10'000g, 2 min, 4°C)
- ☐ Re-suspend pellet in 1 mL cooled, sterile filtered 1xPBS
- ☐ Adjust sample with PBS to the appropriate density for your flow cytometer. If unknown, check with the person who maintains the instrument. Usually it is somewhere around OD 0.01. Keep all samples on ice.
- ☐ Measure samples using flow cytometer.

Acquire Data with Flow Cytometer

- ☐ Adjust side-scatter (SSC) and forward scatter (FSC) PMT voltages using bacteria from your negative control, until the distribution of each is centred on the scale.
- ☐ Adjust FITC/GFP PMT voltage using bacteria from your positive control, until the upper edge of the "bell curve" from the fluorescent population is one order of magnitude below the upper end of the scale.
- ☐ Acquire at least 10'000 events from a sample of calibration beads (recommended: SpheroTech RCP-30-5A Rainbow Calibration Particles: 1 drop of beads into 1 mL PBS is sufficient for 10'000 events). Transfer the Number of Events to the Flow Cytometry Workbook cell B5.
- ☐ Acquire at least 10'000 events for each biological sample.

Calibration to Standard Units

- ☐ Examine a histogram of the used calibration particles and find the geometric means of the observed centers of at least the three most strongly fluorescent large peaks. See the provided example file. Transfer these values to the Flow Cytometry Workbook cell B8 and following.
- ☐ The values of each peak are divided by the MEFL-values to produce a mean conversion factor in cell B11.
- ☐ Enter the number of events of each device and replica in cells B16:P16.
- ☐ Compute the geometric mean of fluorescence for each biological sample, excluding all events with values below 10 (many flow cytometers can do this directly). Fill out cells B17:P17.
- ☐ In cells B18:P18 the geometric mean fluorescence for each sample will be multiplied by the mean conversion ratio, to produce a value in MEFL.