Determine Osmolarity Activity Using RFP under PompC

Materials and Equipment:

- *E. coli* :
  - Top10 containing RFP under Plac
  - Top10 (blank)

  Isogenic strains containing mRFP under PompC:
  - BW21153
  - JW3367-3 – ΔenvZ

  Isogenic strains (for blank):
  - BW21153
  - JW3367-3 – ΔenvZ (kan resistance)
- 50ml Falcons
- LB medium
- Antibiotics (CM, Amp, Kan)
- Bioassay medium
- 4x 500ml Erlenmeyer flasks
- 2x 48 well plate **– label A-F and 1-8**
- 5M NaCl solution
- several 96 well plates for plate reader
- Plate reader
- Multi-Pippetor, Pippettes, pippettors, tips (from Roee's lab)

Method:

1. Grow the cells in LB medium + appropriate antibiotics at 37°C overnight (in a falcon)
2. **Tester strains**: transfer 1ml aliquot to 100ml LB containing appropriate antibiotics (in 500ml Erlenmeyer flask)
   Blank strains: transfer 100µl aliquot to 10ml LB containing appropriate antibiotics (in 50ml falcon)
3. Grow at 37°C until OD_{600} of 0.15-0.2. (about an hour)
4. Transfer to 50ml Falcon tubes, mark the volume on the falcon itself. (should be around 50ml for the tester strains that grew in Erlenmeyer flasks)
5. Pellet cells (centrifuge 10 min at 4000rpm) and then pour off the supernatant.
6. Vortex to break the pellet.
7. Add bioassay medium to falcons up to the volume marked. Re-suspend the cells in this bioassay.
8. Prepare the two 48plates as follows (see Figure 1 and Table 1 below):
   For x3 dilutions (use multipipettor)
   a. Add 4ml culture (bioassay + cells) to the appropriate sample wells in column A.
   b. Add 2ml culture (bioassay + cells) to the appropriate sample wells in column B-F
   c. Add 100µl 5M NaCl solution to the wells in column A
   d. Perform x3 dilutions as follows:
      Transfer 1ml from sample wells in column A to column B. Transfer 1ml from sample wells in column B to column C. Continue in this fashion until column E.
   e. Take 1ml from well E and discard so well E will contain 2ml of bioassay +cells+NaCl suspension solution

   For x10 dilutions (use multipipettor)
   a. Add 2ml culture (bioassay + cells) to the appropriate sample wells in column A-F.

b. Add 100µl 5M NaCl solution to the wells in column A
c. Perform x10 dilutions as follows:
   Transfer 100µl from sample wells in column A to column B. Transfer 100µl from sample wells in column B to column C. Continue in this fashion until column E.
d. Take 100µl from well E and discard so well E will contain 2ml of bioassay +cells+NaCl suspension

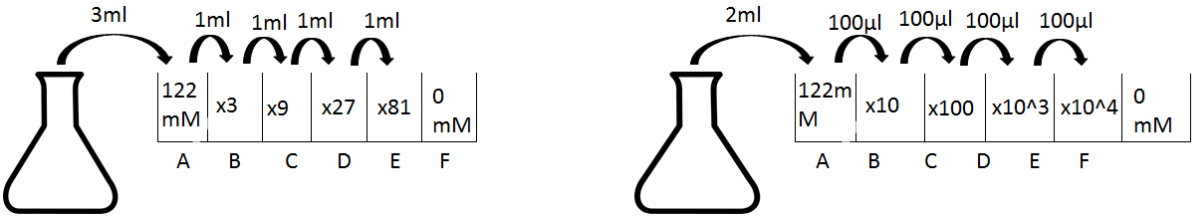

Figure 1: dilution diagram

Blank preparation:
Add 2ml bioassay+cells (without plasmids) to the "blank" wells.

9. After 1hr remove 200µl from each well and transfer to the plate-reader plate and read absorbance (OD600 – for cell concentration) and fluorescence (excitation peak: 584nm, emission peak: 608nm) with plate reader. Repeat every 1 hour for 5 hours. Use each 96 well plate for two readings. Use half for one reading and the other half for another reading. (see Table 2)
10. Calculate relative expression on graph:

$$Expression = \frac{F}{OD_{600}} - \left(\frac{F}{OD_{600}}\right)_{blank}$$

Table 1: 48 well plate for x3 dilution **– write the numbers and letters on the plate**

	A	B	C	D	E	F
1	BW+ 100µL 5M NaCl	BW dilution x3	BW dilution x9	BW dilution x27	BW dilution x81	BW+0mM NaCl
2	BW+ 100µL 5M NaCl	BW dilution x3	BW dilution x9	BW dilution x27	BW dilution x81	BW+0mM NaCl
3	JW+ 100µL 5M NaCl	JW dilution x3	JW dilution x9	JW dilution x27	JW dilution x81	JW+0mM NaCl
4	JW+ 100µL 5M NaCl	JW dilution x3	JW dilution x9	JW dilution x27	JW dilution x81	JW+0mM NaCl
5	Top10+ 100µL 5M NaCl	Top10 dilution x3	Top10 dilution x9	Top10 dilution x27	Top10 dilution x81	Top10+0mM NaCl
6	Top10+ 100µL 5M NaCl	Top10 dilution x3	Top10 dilution x9	Top10 dilution x27	Top10 dilution x81	Top10+0mM NaCl
7						
8	**Blank BW**	**Blank BW**	**Blank JW**	**Blank JW**	**Blank top10**	**Blank top10**

Table 2: 96 well plate for x3 dilution

	1	2	3	4	5	6	7	8	9	10	11	12
A	BW+ 100µL 5M NaCl	BW dilution x3	BW dilution x9	BW dilution x27	BW dilution x81	BW+0mM NaCl	BW+ 100µL 5M NaCl	BW dilution x3	BW dilution x9	BW dilution x27	BW dilution x81	BW+0mM NaCl
B	BW+ 100µL 5M NaCl	BW dilution x3	BW dilution x9	BW dilution x27	BW dilution x81	BW+0mM NaCl	BW+ 100µL 5M NaCl	BW dilution x3	BW dilution x9	BW dilution x27	BW dilution x81	BW+0mM NaCl
C	JW+ 100µL 5M NaCl	JW dilution x3	JW dilution x9	JW dilution x27	JW dilution x81	JW+0mM NaCl	JW+ 100µL 5M NaCl	JW dilution x3	JW dilution x9	JW dilution x27	JW dilution x81	JW+0mM NaCl
D	JW+ 100µL 5M NaCl	JW dilution x3	JW dilution x9	JW dilution x27	JW dilution x81	JW+0mM NaCl	JW+ 100µL 5M NaCl	JW dilution x3	JW dilution x9	JW dilution x27	JW dilution x81	JW+0mM NaCl
E	Top10+ 100µL 5M NaCl	Top10 dilution x3	Top10 dilution x9	Top10 dilution x27	Top10 dilution x81	Top10+0mM NaCl	Top10+ 100µL 5M NaCl	Top10 dilution x3	Top10 dilution x9	Top10 dilution x27	Top10 dilution x81	Top10+0mM NaCl
F	Top10+ 100µL 5M NaCl	Top10 dilution x3	Top10 dilution x9	Top10 dilution x27	Top10 dilution x81	Top10+0mM NaCl	Top10+ 100µL 5M NaCl	Top10 dilution x3	Top10 dilution x9	Top10 dilution x27	Top10 dilution x81	Top10+0mM NaCl
G												
H	**Blank BW**	**Blank BW**	**Blank JW**	**Blank JW**	**Blank top10**	**Blank top10**	**Blank BW**	**Blank BW**	**Blank JW**	**Blank JW**	**Blank top10**	**Blank top10**

Table 1: 48 well plate – for x10 dilution **– write the numbers and letters on the plate**

	A	B	C	D	E	F
1	BW+ 100µL 5M NaCl	BW dilution x10	BW dilution x100	BW dilution x1000	BW dilution x1000	BW+0mM NaCl
2	BW+ 100µL 5M NaCl	BW dilution x10	BW dilution x100	BW dilution x1000	BW dilution x1000	BW+0mM NaCl
3	JW+ 100µL 5M NaCl	JW dilution x10	JW dilution x100	JW dilution x1000	JW dilution x1000	JW+0mM NaCl
4	JW+ 100µL 5M NaCl	JW dilution x10	JW dilution x100	JW dilution x1000	JW dilution x1000	JW+0mM NaCl
5	Top10+ 100µL 5M NaCl	Top10 dilution x10	Top10 dilution x100	Top10 dilution x1000	Top10 dilution x1000	Top10+0mM NaCl
6	Top10+ 100µL 5M NaCl	Top10 dilution x10	Top10 dilution x100	Top10 dilution x1000	Top10 dilution x1000	Top10+0mM NaCl
7						
8	**Blank BW**	**Blank BW**	**Blank JW**	**Blank JW**	**Blank top10**	**Blank top10**

Table 2: 96 well plate – for x10 dilution

	1	2	3	4	5	6	7	8	9	10	11	12
A	BW+ 100µL 5M NaCl	BW dilution x10	BW dilution x100	BW dilution x1000	BW dilution x1000	BW+0mM NaCl	BW+ 100µL 5M NaCl	BW dilution x10	BW dilution x100	BW dilution x1000	BW dilution x1000	BW+0mM NaCl
B	BW+ 100µL 5M NaCl	BW dilution x10	BW dilution x100	BW dilution x1000	BW dilution x1000	BW+0mM NaCl	BW+ 100µL 5M NaCl	BW dilution x10	BW dilution x100	BW dilution x1000	BW dilution x1000	BW+0mM NaCl
C	JW+ 100µL 5M NaCl	JW dilution x10	JW dilution x100	JW dilution x1000	JW dilution x1000	JW+0mM NaCl	JW+ 100µL 5M NaCl	JW dilution x10	JW dilution x100	JW dilution x1000	JW dilution x1000	JW+0mM NaCl
D	JW+ 100µL 5M NaCl	JW dilution x10	JW dilution x100	JW dilution x1000	JW dilution x1000	JW+0mM NaCl	JW+ 100µL 5M NaCl	JW dilution x10	JW dilution x100	JW dilution x1000	JW dilution x1000	JW+0mM NaCl
E	Top10+ 100µL 5M NaCl	Top10 dilution x10	Top10 dilution x100	Top10 dilution x1000	Top10 dilution x1000	Top10+0mM NaCl	Top10+ 100µL 5M NaCl	Top10 dilution x10	Top10 dilution x100	Top10 dilution x1000	Top10 dilution x1000	Top10+0mM NaCl
F	Top10+ 100µL 5M NaCl	Top10 dilution x10	Top10 dilution x100	Top10 dilution x1000	Top10 dilution x1000	Top10+0mM NaCl	Top10+ 100µL 5M NaCl	Top10 dilution x10	Top10 dilution x100	Top10 dilution x1000	Top10 dilution x1000	Top10+0mM NaCl
G												
H	**Blank BW**	**Blank BW**	**Blank JW**	**Blank JW**	**Blank top10**	**Blank top10**	**Blank BW**	**Blank BW**	**Blank JW**	**Blank JW**	**Blank top10**	**Blank top10**