8/1/19

Purpose: Run viability assay

- OD_{600} of ΔcrcB = 0.3
- OD_{600} of CHOP = 0.3
    - Lower than optimal (0.4-0.6)
- All had 180uL of LB, 5uL of Cam, 5uL of Fluoride, 2uL of cells
    - Different concentrations of fluoride for each
- Incubated at 37 degrees and sealed with parafilm

A CHOP (Δcrc B)	0F	1F	25F	50F	75F	100F	150F	250F	625F	XXX	Control 10uL water w/ LB and cells
B (Δcrc B)	0F	1F	25F	50F	75F	100F	150F	250F	625F	XXX	Control 10uL water w/ LB and cells
	1	2	3	4	5	6	7	8	9	10	11

8/2/19

Purpose: Run viability assay

- 198uL of LB into 1 - 10
- 2uL transferred from
    - A 1-9 to D 1-9,
    - B 1-9 to E 1-9,
    - B 11 - E10,
    - A 1-9 to F 1-9
- Rhese will read the plate from A - F

8/6/19

Purpose:

- Changing to 1-500 uL dilutions for 1st condition of culture
- 198 uL of kanamycin + LB in C&D 1-11
- 2uL transferred from A1-9 to C1-9 (inhibited condition to uninhibited condition)
- To prevent condensation, we let the plate come to room temperature before placing in plate reader.

08/8/19

- Discussed issue of condensation on lid of well plates which made the results from the plate reader inaccurate
- Came up with a solution: leave lid off!
- Initial OD wasn't high enough to plate (restricted)

8/9/19

- Having problems with condensation, we need to find a way to read the plate without a lid
- Changing F concentration 0, 2, 4, 8, 16, 32, 64, 128, 256, 625

8/13/19

- OD was .65
- Insufficient inhibited growth.
    - Some solutions included increasing the concentration to 1:100
    - Another solution was to lower the level of chloramphenicol in the wells
- Rhese ran the well plates with our various solutions to the condensation
    - The best solution was 30 °C without the lid because the evaporation was not consequential at the lower temperature
- Plated the new well plates
    - Inhibited - 188 µL LB, 5µL of fluoride (Range = 0-625mM), 5µL CAM (.2 or 2 mg/mL), 2µL bacteria

Row	Final Concentration CAM
A	50 mg/mL
B	50 mg/mL
C	5 mg/mL
D	5 mg/mL

Column 10 = 5 mL H_2O

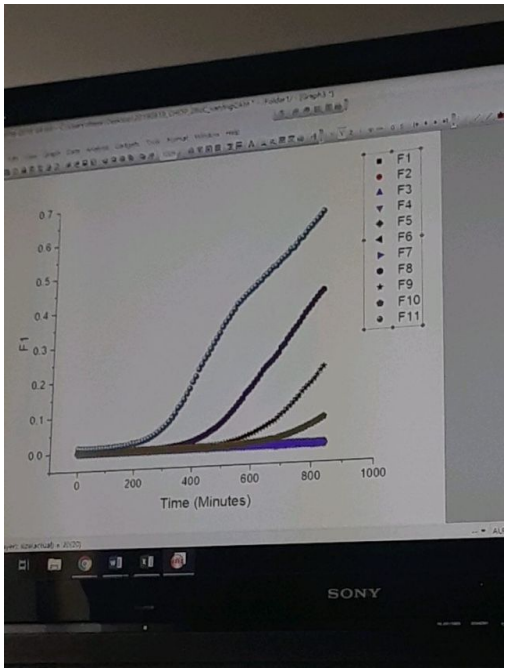
Column 11 = 10 mL H_2O

8/20/19

- We found from previous tests that 30 µg/mL CAM was ideal for growth
    - 40 µg/mL was toxic and there was no growth
    - 20 µg/mL resulted in indiscriminate growth
- We will try the well plate with different CAM levels
- Columns have different fluoride levels
- Columns 1-11 have 2µL bacteria OD = .5
- Columns 1- 10 have 5µL of varying [F] solution and rows A-D have 200µL LB
- Rows E-H have 195 µL LB and kan and 5µL of restricted growth bacteria

	0	2	4	8	16	32	64	128	256	650	B
A (40 µg)											
B (30 µg)											
C (20 µg)											
D (10 µg)											
E											
F											
G											
H											

- Run at 28 °C with no lid for 14 hours with a measurement every 10 minutes
- Results from the 30 µg/mL CAM



8/21/19

- Prepping another assay
- Columns 1- 10 have 5µL of varying [F] solution and rows A-C have 200µL LB

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
A	water	80µL	160µL	320µL	640µL	1.28 mM	2.56 mM	5.12 mM	10.24 mM	25mM NaF	5µL water
B											
C											

- Rows A-C and columns 1-10 have 30 CAM
- 5µL water in 11A-C
- Columns 1-11 and rows A-C have 2µL bacteria OD = .85
    - Change the pipette tip after 10C for 11A-C

8/22/19

- The assay made on 8/21/19 will need to be repeated as everything grew.
    - Everything most likely grew due to it not being put together properly (bad pipetting) or the antibiotics being old or something
- Rheese made more overnight cultures so it can be repeated tomorrow.