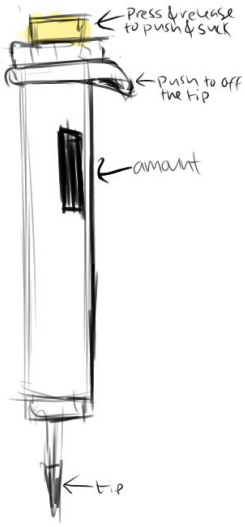
06/27/19

NOTES: Sophia

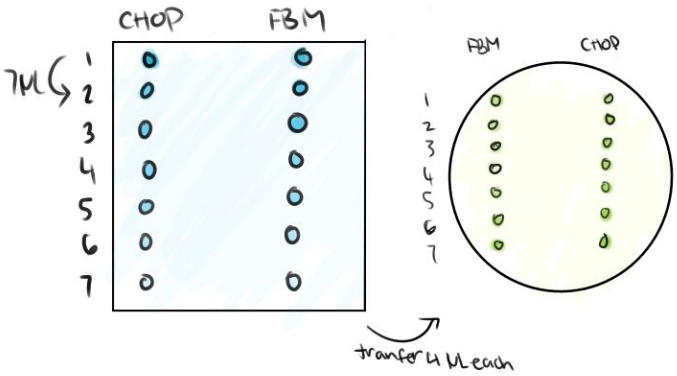


Reviewed how to use a micropipette

1) Put tip on it and calibrate it
2) Press down on top to the first stop
3) Insert in substance
4) Release top to bring up substance
5) Press down to the second stop to release substance
6) Eject the tip

Serial Dilutions

- ➔ Serial Dilution 10 fold: 1/10 until a millionth (getting progressively less concentrated)
- ➔ 63 µL LB for rows 2-7



- ➔ 7 agar plates
  - ◆ 0 µL F (Parmis)
  - ◆ 2 µL F (Nichole)
  - ◆ 5 µL F (Camille)
  - ◆ 10 µL F (Sachi)
  - ◆ 20 µL F (Parmis)
  - ◆ 50 µL F (Nichole)
  - ◆ Ampicillin (Camille)

06/28/19

Purpose: To repeat last year's assay and confirm predicted CHOP and FBM results.

NOTES: Nichole

- Serial dilution plating assays of CHOP and FBM
    - 7 dilutions each, decreasing ten-fold in concentration from top to bottom
    - 7 plates of bacteria (6 concentrations of fluoride and 1 ampicillin plate without chloramphenicol to act as a control because CHOP and FBM both have resistance to ampicillin)
        - Ampicillin - Xinyi
        - 0 umol/mL F - Norman
        - 1 umol/mL F - Xinyi
        - 5 umol/mL F - Will
        - 10 umol/mL F - Norman
        - 20 umol/mL F - Will
        - 50 umol/mL F - Rhese
- Results: The serial dilutions of FBM and CHOP plated 06/27 showed no growth on plates with chloramphenicol, but around five dots of growth for plates with only ampicillin.

7/23/19

Calculating agar plate environment concentrations:

Fluoride:

- 50mM F = 0.50 mL
- 20mM F = 0.20 mL
- 10mM F = 0.10 mL
- 5mM F = 0.05 mL
- 2mM F = 0.02 mL
- 1mM F = 0.01 mL
- 0mM F = 0.00 mL

Amount of chloramphenicol:

- 0.294 mL or approximately 300 μL

7/26/19

Purpose: To create the plates for running assays and to determine if FBM stock is available

Agar - 37g (9.25g/well) dissolved in 250mL H_2O

Autoclave (P2)

Cooled before gelation then add antibiotic and pour and dry

Sequenced FBM plasmid

Sequenced 2 23A for AT mutation

07/30/19

Purpose: To test optical density of ΔcrcB and CHOP to determine their growth

- Tested the OD (optical density) of ΔcrcB and CHOP
    - The OD_{600} of ΔcrcB 1-100 and CHOP 1-100 were both 0.8
    - Optimal OD would be around 0.6.
- Plated dilutions in well plates as followed:

	0 uM F stock (0uM)	.04 uM F stock (1uM)	1 uM F stock (25 uM)	2 uM F stock (50 uM)	3uM F stock (75 uM)	4 uM F stock (100uM)	6 uM F stock (150uM)	10 uM F stock (250uM)
E	CHOP (no CAM)	X	X	X	X	X	X	X
C	CHOP	CHOP	CHOP	CHOP	CHOP	CHOP	CHOP	CHOP
D	ΔcrcB	ΔcrcB	ΔcrcB	ΔcrcB	ΔcrcB	ΔcrcB	ΔcrcB	ΔcrcB
F	ΔcrcB (no CAM)	X	X	X	X	X	X	X

E1 (CHOP) and F1 (ΔcrcB) act as controls and don't have chloramphenicol in them.

07/31/19

Purpose: Rhese found an article that detailed a procedure where you grow cells, put them into the environment of interest (in our case, with fluoride), take them out of the environment, and put them into an environment where they'll experience unrestricted growth. The growth correlates with the number of viable bacteria available in the sample.

- Para-filmed outside of 96 well plate
    - C 1-8 = CHOP delta crcB in increasing concentrations of fluoride
    - D 1-8 = delta crcB in increasing concentrations of fluoride
    - E 1 = CHOP delta crcB unhindered (just LB)
    - F 1 = delta crcB unhindered (just LB)
    - Put 2 µL of C 1-8 then E 1 into G 1-9 into 198 µL of LB
    - Put 2 µL of D 1-8 then F 1 into H 1-9 into 198 µL of LB
        - 1/100 dilution of cells from previous round of growth