Mead

SCHEDULE

	1	2	3	4	5	6	7	8
TIME								
MONDAY								
TUESDAY								
WEDNESDAY								
THURSDAY								
FRIDAY								
SATURDAY								
SUNDAY								

May. 31st

Using the platereader (GH216)

Open "PerkinElmer Workstation"

pulldown protocol

- new protocol
- create new protocol (user2)

press green arrow ▷

Making Plates

1. ~~Hand~~ 40g of LB agar & 1L water (ultra clean) in 2L flask
2. ~~autoct~~ stir until LB disolve
3. cover with foil, leave stir bar in, autoclave tape
4. auto clave (121°C for 20min)
5. stir (agar at bottom)
6. 50°C waterbath for at least 30min
7. add antibiotic (1mL)
8. stir briefly
9. Set up plates : mark with color — CAM - red, AMP - black
10. pour plate in stacks of 5
    - flame flask between each pour
11. rinse flask right away.
12. ~~56~~ plate stays on bench overnight before going into fridge.

Touch tomorrow

team wikis from past years (human practices)
extracting DNAs from strawberrys
bacterias with different smells
DNA bacterial art
William & Mary iGEM team (human practices PDF)

Inside Lab:

- Strawberry DNA
- Bacteria Smell
- Bacteria Art.

Outside Lab:

- Genetics Activity
- Sensory tests: jellybeans, hearing & different frequencies, inverse image

June. 1st

~~0.487~~ ~~0.495~~ 0.514 Colony 1
0.520 Colony 2

~~0.52x~~ ~~0.02~~ / ~~0.52~~ $\frac{0.02}{0.52} = \frac{x}{12}$

$12 \times \frac{0.02}{0.52} = \frac{12}{26} = \frac{6}{13} = 0.46$ mL (2) 11.54

$12 \times \frac{0.02}{0.514} = \frac{12}{25.7} = 0.47$ mL (1) 11.53

dilute to $OD_{600} = 0.1$ ~~in 1mL of LB + Cam media~~.

June. 4th

Project Planning.

Bacteria : E.coli, Salmonella
Location of bacteria

Today: strain that make good biofilms
(model organism)

Integrated practices: risk & regulations on food

Bacteria ~~of~~ on crop surface: more space for bad bacteria?
how does getting rid of biofilm affect?

measurment of biofilm

AFPs
(other proteins)
- tick (pos control) → order from Adgene? → otherwise clone? or
- plant AFPs → - express - purify
- other plant-based proteins

Biofilms
① bacterial strains: E.coli, Salmonella, Liskeria > safe model organism
② making biofilms
- minimal - M9 (MAP paper)
- write a ★ protocol
- what do we already have? - transform or streak (on lab surface)
- test existing AFPs in E.coli inducible biofilm assay
- cloning of plant AFPs
  - GMO plants expressing AFP
  - treating surface or plant in purified AFP
③ measuring biofilm
- crystal violet assay (protocol)
- other? ⇓ hardware/software interface
④ biofilm on plants ?

Plant based small molecules (curcumin, others?)
After biofilms
- apply curcumin to look at biofilm disruption
biosynthetic pathway of curcumin
- can bacteria make it → Eugenol
- transgenic plant make it?

AFP
- order tick gene
- transform/streak AFP that we have

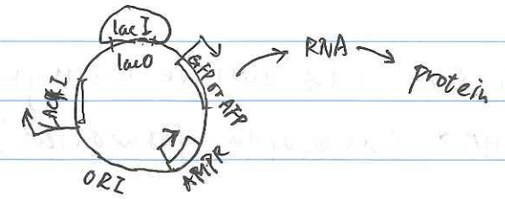
Biofilms
- look into bacteria
- protocol making biofilm
- - - measuring - -
- find/grow E.coli & Staph strains

Touch Tomorrow.

Plant based
- research biosynthetic of curcumin

June. 5th

pET21 -



if lactose present, bind to lacI, comes off, GFP/AFP can be expressed (similar in nature)
inducible gene system
in lab, use IPTG (cell break lactose down)
can't be broken down.

? PET21a - ZEAFP (fish) clone 1, 4
(+) PET21a - IAFGP (tick) clone 4, 5
(-) PET21b - GFP

BL21 (protein strain)
DH5α (strain for growing DNA, DNA purification, cloning)

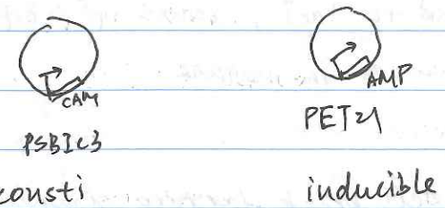
grow biofilm → turn on → see result.

Streak (frozen cells)
be quick: once thawns, the cells are useless
scrape then streak later
label: plasmid name + date

biofilm

E.coli EMG 2kλ in LB culture overnight

- control: DH5α (no biofilm formation)



Gene gun:

Cambridge 2016 biolistics (iGEM)

for plants

June. 6th

Banana Odor Bacteria

- transformation
- 10mL LB, 10μl CAM, 1 colony
- shake for 8 hours
- add arsenic
- shake overnight.

Biofilm Assay.

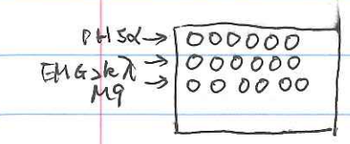
overnight culture - dilute 1:20 (1mL culture + 19mL M9)

check OD_{600} (~~#~~ blank - 1mL LB + 19mL M9)

Adjust OD_{600} to 0.1 using M9 (mate 10mL)

check OD_{600} again to make sure it's 0.1

take OD_{600} 0.1 dilution, 100μl per well



3 ~~plants~~ times, 2 temperature (RT, 37°C) (6 plates)

(24h, 48h, Mon)

OD_{600} (600 nm)

EMG-2kλ 0.33

DH5α 0.198

10mL, 0.1 OD_{600}.

EMG-2kλ

$\frac{0.33}{0.1}$ $10 \times \frac{0.1}{0.33} = 3.03mL$ 6.97

DH5α

$10 \times \frac{0.1}{0.198} = 5.05mL$

0.1 dilution

EMG-2kλ 0.103

DH5α 0.099

5mL LB
5μL CAM
1 colony.

June. 7th Thur
Banana Odor Redo
- 10 mL LB
- 20μL culture
- 10μL CAM
- 20μL Ars (or Ø)

① biofilm crystal violet assay - set up
② set up E.coli & Staph cultures to grow overnight
③ banana odor cultures

Friday
- set up biofilm plates - E.coli & Staph
- run crystal violet assay
- start cultures for TT (smelly)

Crystal Violet Assay.
0.1%, dilute 1:100 (0.001%)
serial dilution
#15 μL water, 5μL crystal violet.

- dry bacteria
- 1:10 dilute crystal violet
- redo with 5 bacteria
- banana odor
- bacteria art out of incubator & in fridge.

	OD_{600}	adjusted		mL	mL
					M9
NCTC9001	0.053	0.099	$10\times\frac{0.1}{0.053}\times\frac{0.1}{0.105}$	9.52	0.48
DH5α	0.159	0.096	$10\times\frac{0.1}{0.159}$	6.29 mL	3.71 mL
EMG2kλ	0.179	0.099	$10\times\frac{0.1}{0.179}$	5.59 mL	4.41 mL
S. epidermis	0.205	0.102	$10\times\frac{0.1}{0.205}$	4.88 mL	5.12 mL
S. aureus.	0.281	0.100	$10\times\frac{0.1}{0.281}$	2.56 mL	6.44 mL

10mL, 0.1

Plates
A E.coli NCTC9001
B E.coli DH5α
C E.coli EMG2kλ
D S.epidermis
E S.aureus
F M9

June. 11th

(original)
150 µL of Crystal Violet in each well

Find similar parts
- part list
- estimated cost

AFPs (current)

IAFGP GFP

zeAFP → - express
- induce
↑
transforming into biofilm stain
- EMG
- NCTC
} make competent cells

BL21 - purify
- coat surface (plate, leaf)

Grow overnight + IPTG

Lyse cells
external protein (column, weak)

check on gels

M - overnight culture
- buffer

T - competent cell protocol
- test + tfm (empty PET21a)

W - check test
- tfm AFP plasmids

Th - AFP colonies in AFP & NCTC
- overnight culture

F - set up biofilm plates

T - set up for sonicator

F - sonicate measure ODs

Worcester Tech Highschool. (~~tued~~) Thur

10:15 - 11:30 AM
- miniprep
- microscopy

New Anti-microbial Protein ?

cloning
- List of plant AFP, look for good candidates
- find current plant AFPs, look for data

curcumin biosynthetic pathway.

Biofilm Assays
- optimizing biofilm growth & staining
- try sonicator method for biofilm.
- coat plates with curcumin

10mL LB (no Abs!)

pick colony

write protocol for - staining
- sonication

DH5α
EMG-2kλ
NCTC
S. aureus
S. epidermis.

make 0.1% CV (0.2g in 200mL water)

M9 media (100mL)

70mL water

20mL M9 salts

0.2mL 1M $MgSO_4$

2mL 20% glucose

5μL 2M $CaCl_2$

adjust to 100mL.

↳ filter

June. 12th, 2018

dilute culture 1:20 — 0.5mL culture + 9.5 mL M9.

	OD_{600} (1:20)	1:20	M9	adjusted value
DH5α	0.153	~~0.1~~ $10 \times \frac{0.1}{0.153} = 6.536$	3.46	0.100
EMG-2kλ	0.178	$10 \times \frac{0.1}{0.178} = 5.62$	4.38	~~0.091~~ 0.097
NCTC 9001	0.065 ~~0.085~~ 0.096	~~$10 \times \frac{0.1}{0.104} = 9.62$~~	~~0.38~~	~~0.096~~ 0.099 0.103
S. epidermidis	0.242	$10 \times \frac{0.1}{0.242} = 4.13$	5.87	~~0.108~~
S. aureus.	0.213	$10 \times \frac{0.1}{0.213} = 4.69$	5.31	~~0.119~~ 0.105.

NCTC $8 \times \frac{0.1}{0.104} = 7.69$ 0.31

M9 additives:

Thiamine

2.37g in 10mL water

1000x L-Arginine

1.26g in 10mL water

1000x Glutamine

June. 13th 2018

Safety Training

Emergency Procedures

Chemical Spill

- alert all personnel, evacuate
- close door behind
- call campus police (508-831-5555)
- tend to injured/contaminated personnel
- stay in general area, safe distance, wait responders
- introduce yourself to emergency responder.

Fire Safety

- Fire extinguisher : PASS system
- get out, pull fire alarm

Safety Equipment

- eye wash
- shower

Injuries and Reporting.

- health service
- report to HR

Chimicals

- know hazards : what, why dangerous, how to deal with
- don't spread contamination when washing
- reactive chemicals:
- look at labels on containers (pictograms ...)
- safety data sheet
- "CAMEO chemicals"
- compatibility ~~tpro~~ incompatabilities

Routes of Exposure

1. injection
2. inhalation: eliminate hazard, fume hood, lab ventilation
3. absorption: eye protection, faceshields, lab coat, glove
4. ingestion

Hazardous Waste

1. appropriate container 2. seperate solid & liquid
3. keep container closed 4. label container as hazardous waste
5. secondary containment 6. keep at point-of-generation.
7. date container when full 8. request container pick-up when FULL.

www.wpi.edu/+ehs

Miniprep

1. overnight culture in centrefuge. 10min
2. dump liquid
3. add 2 buffer
4. incubate at RT for 5 min.

June. 15th 2018

Purple Violet for 72hr plates

+- L-Arginine. 5 bacteria + 1 M9 C-control

bacterial in M9

move growth in additive

June. 16th 2018

- Take overnight culture (5 bacterial)
- dilute 1:20 ~~0.5~~ 500μL + 9.5mL M9
- check OD_{600}
- dilute to OD_{600} 0.1 in 10mL M9.
- put in additive
- 4 plates 48, 72 hr; 37°C. RT.

		OD_{600} (initial)			
1	DH5α	0.036		0.109.	$V_2 = \frac{(0.1-0.05)\cdot 9}{19\times 0.05} = 0.47$
2	EMG2kλ	0.050	1.0	~~0.99~~ 0.099	$V_1 = 8.53$.
3	NCTC 9001	0.017		~~0.98~~. 0.098.	$V_2 = \frac{(0.1-n)\cdot w}{19n}$
4	S. epidermidis	0.045		0.107	$V_1 = w - V_2$.
5	S. aureus	0.033		0.100	$n\cdot V_1 + 20n\cdot V_2 = 0.1\cdot w$

$V_1 + V_2 = w$.

$n(w-V_2) + 20n\cdot V_2 = 0.1w$.

$n\cdot w - n\cdot V_2 + 20n\cdot V_2 = 0.1w$.

$19n\cdot V_2 = 0.1w - n\cdot w$.

$= (0.1-n)w$.

~~$0.05V_1 + 1.0V_2 = 0.1$~~

$0.05V_1 + 1V_2 = 1$.

$V_1 + V_2 = 10$.

$0.05(10-V_2) + V_2 = 1$.

$0.5 - 0.05V_2 + 0.95V_2 = 1$

$0.9V_2 = 0.5$ $V_2 = 0.53$

$V_1 = 9.47$

$V_2 = \frac{(0.1-n)w}{19n}$ ~~= tot~~

3. $V_2 = \frac{(0.1-0.017)\cdot 9}{19 \times 0.017} = 2.31$ ~~per 2.57~~

7.43.

$V_1 = 6.69$.

8.5 mL $\frac{0.111}{0.10}$ 0.085 mL.

0.85.

10 11.

June. 18th
2018

Biofilm Strains → Mon (2)
→ Wed (2)

- rinse, stain, dry, resuspend, plate reader.

New Stuff

- Sonication Protocol
- Curcumin Assay.
- replicates ↔ stains
  ↘ AFP biofilms
- Cloning stuff - 2 plants AFP, DCAFP, LpAFP
  - new proteins
  - peptides (10-30 Amino Acids) [research]
    1-5 weeks

June. 19th 2018

- set up culture plates (set 2)
- check set 1 - 96 hr
- check set 1 - 48 hr dye, read plate
- prepare M9 for set (3)

Final OD_{600}		OD_{600}		dilution	M9	OD_{600} adjusted	
0.096	1 PHSX	0.152	~~$8\times\frac{0.1}{0.152}$~~	$= 5.26$	2.74	0.089	0.092
0.097	2 EMG2k1	0.160	$8\times\frac{0.1}{0.160}$	$= 5$	3	0.110	0.111
0.099	3 NCTC	0.046	~~8x~~			0.106	0.105
0.099	4 S. epi	0.188	$8\times\frac{0.1}{0.188}$	$= 4.26$	3.74	0.112	0.117
0.0925	S. aureus	0.312	$8\times\frac{0.1}{0.312}$	$= 2.56$	5.44	0.108	0.107

Crystal Violet Protocol

1. Rinse plate wells with water 4-5 times (do not rinse directly to bottoms)
2. dye with 125μl CV for 10 min
3. rinse with water 4-5 times
4. dry overnight
5. 200μl of 30% acetic acid in each well
6. take 100μl of CV + acetic acid solution from each well and transform into 96-well flat bottom plate
7. 100μl of 30% acetic in plate for blank
8. read OD_{595} of plate from plate reader.

June. 20th 2018

- set up culture plates (set 3)
- check set 1; 72hr (originally 96hrs), read plate

	Set 1	Set 2	Set 3
Mon	48hr [stained dried]	overnight culture	
Tue	48hr [checked], 72hr [stained dried]	set up	~~set~~ overnight culture
Wed	72hr [check]	—	set up
Thur		48hr [st. dry]	—
Fri		48hr [check], 72 [stain dry]	48hr [st. dry]
Sat		72hr [check]	48hr [check], 72hr [stain dry]
Sun			72hr [check],

June. 22nd 2018

6/22/18

- add acetic acid to set 2 - 48hr plates ; transform into flat bottomed plates ; read plate
- rinse & dye set 2 - 72hr plates ; let dry overnight.

plant AFPs in PET21a (DcAFP LpAFP)
MsrA2 in PET21a
ApBD1 in PET21a

benchling.com
iGEM2018 folder

New cloning method.
Enzyme

① ~~ATT~~ A=T pairing
~~GTC~~ G≡C

② always antiparallel.

③ 5' —— 3' → open 3' end only.
← 3' —— 5'

ordering from company: start with 5'

DcAFP
primer design
codon optimization
different codon preferences

June. 25th

6/25/18

- Sonicator
- finish set 3 replicate

Biofilm Assays
- add AFPs to crystal violet assay.
  - pre-coat with protein
  - add protein to bacteria
  - no protein
  5 strains (E.coli & S. epi & S. aureus).
  48hr, 37°C
- ☆ - sonication protocol.
  37°C, 48hr, 72hr
  5 strains.

Cloning
- LpAFP } plant
- DcAFP }
- CfAFP - insert.

Tue/Wed
- prepare vector (digest, purify)

Thursday
- PCR amplify
- purify on gel
- Gibson assembly

Fri
- check plates

★ AFP purification

- run 16% gel to see ZeAFP

~~run~~ pour gels

run gels

PAGE gel confirmation

of AFP, GFP expression in EMG-2kλ

Curcumin

+/- curcumin to 5 strains (4-5 concentrations)

5 strains

Labs for GeneGun, connections.

type up final protocols.

Today:

- add acetic acid to set2 -72hr plates & check OD
- start overnight culture for ~~set~~ ev-set3 & soni-set1
- check sonication.

6/26/18

Ultra Sterile M9 media

- ~~to~~ add additives & to 100 mL M9 and filter under sterile hood.

Thiamine, L-Arginine (100 µL)

Glutamin (1mL)

6/27/18

- Set up biofilm crystal violet set 3. Lin lab 206).
- set up biofilm sonication set 1

		OD_{600}	culture	M9	final OD_{600}
1	DH5α	0.156	$10 \times \frac{0.1}{0.156} = 6.41$	3.59	0.099
2	EMG5kλ	0.172	$10 \times \frac{0.1}{0.172} = 5.81$	4.19	0.101
3	NCTC9001	0.216	$10 \times \frac{0.1}{0.216} = 4.63$	5.37	0.100
4	S. epi	0.234	$10 \times \frac{0.1}{0.234} = 4.27$	5.73	0.102
5	S. aureus	0.328	$10 \times \frac{0.1}{0.328} = 3.05$	6.95	0.102

Sonication Protocol.

6/28/18 Pouring Gels
- clean

Primers
cf, Cp ~500 pairs spin for 10 min
Dc ~1200 pairs.

µmol.
want concentration to be 100~~mMol~~.
28.3 283 µL of elution buffer.
29.3 293 µL

PCR reaction:
1µL template DNA
1.25µL each primer (10µM)
21.5 µL water
25µL 2x per master mix (green)

6/29/18 - Rinse and dye 48hr plates for crystal violet set 3.
- check sonication set 1, read plate.

7/2/18 PCR clean up
45µL PCR rxn (Lp, Cf, Dc)
+90µL NT1 buffer → protocol elute 40µL
↓ nanodrop

Sonication set 1
- dye cover slips from 6-2, 3-1, 4-1 with CV for 10 min
- rinse & submerge in 30% acidic acid (1mL)
- read with OD600 (blank: 30% acidic acid).

	OD600
M9	0.128
S. epi	0.098
NCTC	0.095

Sonication - optimal conditions
- use only NCTCs, metal rack, sink to bottom
6 samples (1hr, 2hr, 4hr, 8hr)
take PBS and cover slip stain with CV.
(OD)

7/3/2018 - Set up crystal violet set 4 (96well)
- set up sonication ~~set 2~~ : only for NCTC9001 for testing optimal conditions

	OD_{600} (initial)	culture	M9	OD_{600} (adjusted)
1 DH5α	0.146	$10 \times \frac{0.1}{0.146} = 6.85$	3.15	~~0.097~~ 0.096
2 EMG2k1	0.156	$10 \times \frac{0.1}{0.156} = 6.41$	3.59	0.099
3 NCTC9001	0.110	$10 \times \frac{0.1}{0.110} = 9.09$	0.91	0.097
4. S. epi	0.232	$10 \times \frac{0.1}{0.232} = 4.31$	5.69	0.101
5 S. aures	0.334	$10 \times \frac{0.1}{0.334} = 2.99$	7.01	0.097

7/5/2018 - take out cover slip (rinse) put in 3ml of PBS
- put on sonicator for (DH5α, M9, NCTC)
30 mins, 60 mins, 90 mins, 2hrs, 3hrs.
- dye cover slip with crystal violet
- check OD_{600} of PBS in tube

	M9	DH5α	NCTC	(blank of PBS)
30min	0.004	0.004	0.236	
60min	-	0.003	0.003	
90min	-	-	0.002	
2hrs	-	0.006	0.003	0.003
3hrs	0.003	0.005	0.002	~~0.029~~

7/9/18 - Search proper material for sonication (coverslip)
- ~~transform~~ EMG GFP cells (need antibiotic)
- competent S. epidermidis? with E.coli plasmids?

Gene gun
- order parts
- build parts
- water proof test.
- electric building lab.

Set up plates for CV set #5

		OD_{600} (initial)	culture	M9		
1	DH5α	0.160	$10 \times \frac{0.1}{0.16} = 6.25$	3.75	0.080	0.090 0.100
2	EMG2k1	0.171	$10 \times \frac{0.1}{0.171} = 5.85$	4.15	0.096 ✓	
3	NCTC9001	0.097 ✓			0.097 ✓	
4	S. epi	0.271	$10 \times \frac{0.1}{0.271} = 3.69$	6.31	~~0.095~~	~~0.084~~ 0.101
5	S. aureus	0.381	$10 \times \frac{0.1}{0.381} = 2.62$	7.38	~~0.084~~	~~0.089~~ 0.096

~~0.08~~ ~~tox~~

7/10/18 Set up CV culture set 6

		OD600 (initial)	culture	MQ	adjusted
1	DH5α	0.152	6.58	3.42	0.096
2	EMG2KN	0.187	5.35	4.65	0.099
3	NCTC9001	0.099 ✓ ☺			0.099
4	S.epi	0.237	4.22	5.78	~~0.098~~ 0.099
5	S.aureus	0.340	2.94	7.06	0.094

7/11/18 Set up CV culture set 7

		OD600 (initial)	culture (1:20)	MQ	OD600
1	DH5α	0.097 ✓	–	–	
2	EMG2KN	0.161	6.21	3.79	0.1
3	NCTC9001	0.295	3.39	6.61	0.099
4	S.epi	0.139	7.19	2.81	~~0.089~~ 0.095
6	S.aureus	0.141	7.09	2.91	0.1

7/16/18 – stain lettuce, lettuce biofilm formation.
– test lettuce in biofilm assay.

7/17/18 – analyze data
– order parts for gene gun

crystal violet standard curve

~~225μL~~

crystal violet (0.2g in 200mL water) 0.1% CV
diluted 1:10 with ~~acidic~~ acetic acid (= CV : acid = 1:9)

$$\frac{\cancel{0.2\,g}}{\cancel{2}} \quad \frac{0.1\,g}{100\,mL} \times \frac{1\,mol}{407.986\,g} \times \frac{1000\,mL}{L} = 2.451 \times 10^{-3}\,\frac{mol}{L}$$

diluted (first well): $2.4511 \times 10^{-4}\,\frac{mol}{L}$

7/19/18 – check lettuce plate. with (NCTC, S.epi, MQ, water)
1. take lettuce out of plate, rince lettuce with water.
2. rinse 24-well plate with water.
3. put 250μL (?) CV into each well then put lettuce back in to dye lettuce with CV; then put 100μL CV on top.
4. take out lettuce, rinse ~~w~~ with water again
5. rinse plate with water.
6. let dry of lettuce in new 24-well plate.
7. dry original 24-well plate overnight.

7/20/2018

1. resuspend original 24-well plate with 1mL ~~acid~~ acetic acid (30%v)
2. resuspend lettuce with 500μL acetic acid.
3. take 100μl from each original 24-well plate and move into 96-well plate
4. take 50μl from each lettuce well, put extra 50μl acetic acid into 96-well
5. read plate

7/24/18 Lettuce Protocol.

1. Make OD_{600} 0.1 culture for 5 strains.

		OD_{600}	culture	M9	OD_{600} (adjusted)
1	DH5α	0.183	5.46	4.54	0.103
2	EMG2k1	0.099	–	–	0.099
3	NCTC9001	0.150	6.67	3.33	0.104
4	S.epi	0.154	6.49	3.51	~~0.113~~ (+1mL M9.) 0.102
5	S.aureus.	0.204	4.90	5.10	0.095

2. dilute bleach (NaClO) to dip leaf in to 300ppm (0.03%) (Originally 8.25%) with water

$100\,mL \times \frac{0.03\%}{8.25\%} = 0.364\,mL$ bleach

99.636 mL water.

3. cut lettuce with 50mL conical tube
4. dip cut lettuce circles into 300ppm bleach for 3 minutes
5. take leaves out and rinse with deionized water
6. plate leaves onto petri(??) dishes, 4 pieces of leaves each dish, remove excess water with kim whipe.
7. put 50μl of each kind of culture onto ~~leave~~ leaves; spread culture on leaves with bent pipette tip.

7/26/18 weigh lettuce pieces

PHSα 0.0101g.

NcTc9001 0.008g

M9 0.0098g.

put leaves in U-bottomed tubes

put in 400μL of PBS (1X)

7/27/18 Count colonies (picture)

use $1:10^6$ diluted lettuce blend next time for plating.

Set up lettuce set 1

		OD_{600}	culture	M9	adjusted OD_{600}
1	PHSα	0.203	4.93	5.07	0.102 +1mL M9
2	EMG2kλ	0.212	4.72	5.28	~~0.111~~ 0.099
3	NcTc9001	0.149	6.71	3.29	0.100
4	S.epi	0.164	6.10	3.90	0.104
5	S.aureus	0.273	3.66	6.34	0.101

7/29/18 - take lettuce set 1 out and plate

- set up lettuce set 2

7/30/18 - Set up lettuce set 2.

- Check lettuce set 1 colonies.

7/31/18 - Pour plain plates

- Set up lettuce set 4

- Blend and plate lettuce set 2

Blend: rinse with water then

1. take leaf out, put into tubes, 3 leaves from each strain
   (Fisher brand, culture test tube 17×100 mm, polystyrene, with cap)
2. put 800μL of 1×PBS into each tube
3. let sit for
4. blend with blender in eppi tubes
5. dilute 10^5 times 10 times + 100 times + 100 times
6. plate 3 plates for each tube. 100μL on each plate.
7. incubate overnight.

8/1/18 - check plates for set 2

set 2 (diluted 10^5)

	DH5α	EMG:2kJ1	NCTC9001	S.epi	S.aureus	M9
	238	1 cluster 37	132	300+	15	2
Leaf 1	212	44	324	300+	12	9
	193	43	348	300+	17	(1) 54
	172	(3) 33	(6) 144	328	(3) 109	(1 big) 128
Leaf 2	(2) 148	(3) 27	(7) 44	(2) 94	(2) 137	(1 slump) 208
	264	25	~~(2) 152~~ 257	(1) 154	(1) 51	(1) 150
	344	244	(4) 168	300+	(1) 300+	7
Leaf 3	(a few) 167	(8) 152	(2) 136	278	349	7
	(lots) 164	(8) 40	(2) 152	(1) 196	336	7

- set up lettuce set 4

try dipping lettuce in DH5α, Sepi for 2 min

lettuce

- plate set 3

dilute 2×10^5 with epi tubes

50μL in 950μL

10μL in 990μL

10μL in 990μL

8/2/18 count colonies for set 2. (diluted 2×10^5)

	DH5α	EMG:2kJ1	NCTC9001	S.epi	S.aureus	M9
	0	0	4	16	3	0
Leaf 1	0	0	3	11	8	1
	0	0	2	12	3	0
	0	0	5	5	0	0
Leaf 2	0	0	5	4	1	0
	1	0	5	3	4	0
	0	0	2	4	4	0
Leaf 3	0	0	0	6	1	0
	0	0	1	4	2	0

pour plain LB plates

clean up fridge & lab.

8/3/18 – Take out and plate set 4 for lettuce
for serial dilution, dilute 1:2×10^6
use 24-well plates, put 900μL of 1xPBS in each well (6 wells for each strain)
– set up lettuce set 5.

8/7/18 plate set 4 (vortex 55 after adding PBS) (r) rinsed with water)
– rinse lettuce leaves with 800μL of PBS in the tube
– transport PBS with planktonic bacteria into ependorf tubes
– dilute 2×10^5
– plate

dilution using 48 well plates
1:2 100μL : 100μL
1:~~10~~ 100μL : ~~900~~ 900μL
1:10 100μL : 900μL
1:10 100μL : 900μL
1:10 100μL : 900μL
1:10 100μL : 900μL.
culture : PBS

set 4 (diluted 2×10^5)

	DH5α	EMG2(k)	NCTC9001	S. epi	S. au	MG
	4	(r) 11	98	1000+	618	2
Leaf 1	0	0	92	(r) 1000+	618	0
	2	0	90	1000+	(1big) 282	1
	51	0	19	52	(1big) 84	(afew) 420
Leaf 2	56 (r) 32	0	(r) 48	10	(1big) 146	(afew) 350
	36	0	29	13	(1big) 464	(1 big) 406
	0	3	14	(r) 105	(r) 10	456
Leaf 3	2	5	8	(r) 125	(r) 11	765.
	0	0	25	(4) 107	15	(1big) 1131
	(2) 484			680		
dipped	(1 edge) 676			748		
	(1big) 312			980.		

8/9/18 - plate planktonic & biofilm for set 5.

operate under flames.

use 10^5 dilution for both planktonic & biofilm.

	culture	PBS
1:10	100 µl	900 µl
1:10	"	"
1:10	"	"
1:10	"	"
1:10	"	"

- count colonies for set5 ($1:2\times10^5$)

Biofilm	DH5α	EMG2k7	NCTC9001	S.epi	S.au	M9
Leaf1	cont	cont	~~#~~ 29	~~18~~ 6	3	0
	cont (300)	cont	120	9	5	33
	cont	Cont	41	12	2	3
Leaf2	cont	cont	18	3	2	3
	cont	cont	10	10	0	1
	cont	cont	7	1	1	1
Leaf3	300+	51	17	68	14	67
	300+	32	48	40	18	cont + 32
	300+	cont	28	44	23	42

cont: contamination

$1:2\times10^5$

planktonic		DH5α	EMG2k7	NCTC9001	S.epi	S.au	M9.
		0	0	~~#1~~ 52	64	9	0
	Leaf1	0	1	~~#20~~ 70	56	10	0
		0	0	~~29~~ 54	52	3	0
		0	0	cont+21 ~~7~~	356	15	0
	Leaf2	0	0	~~60~~ 34	332	18	0
		0	0	~~78~~ 23	112	15	1
		0	0	~~28~~ 27	128	24	0
	Leaf3	2	0	~~#8~~ 38	236	31	0
		0	0	~~#7~~ 23	260	18	1

~~biofilm~~ ~~DH5α~~ ~~EMG2k7~~

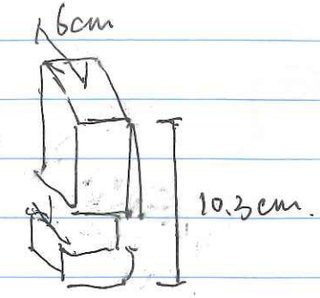
8/14/18 Assemble electronic part of gene gun.
- timer relay & socket
- switch
- solenoid valve
- power cord. (black - phases
green - ground
white - neutural)

9/17 overnight. for set # 7

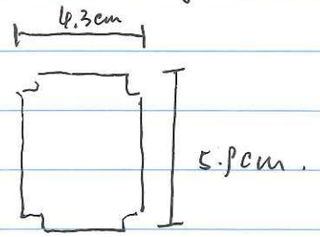
9/18 OD for set 7

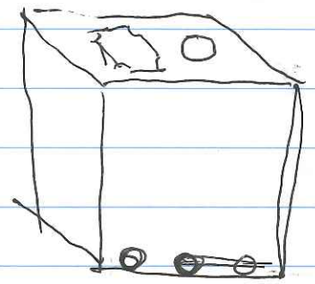
9/20 plating set 7
dilute 1: ~~5~~ 2×10^5
1:2
1:10 x 5 with 48 well plates

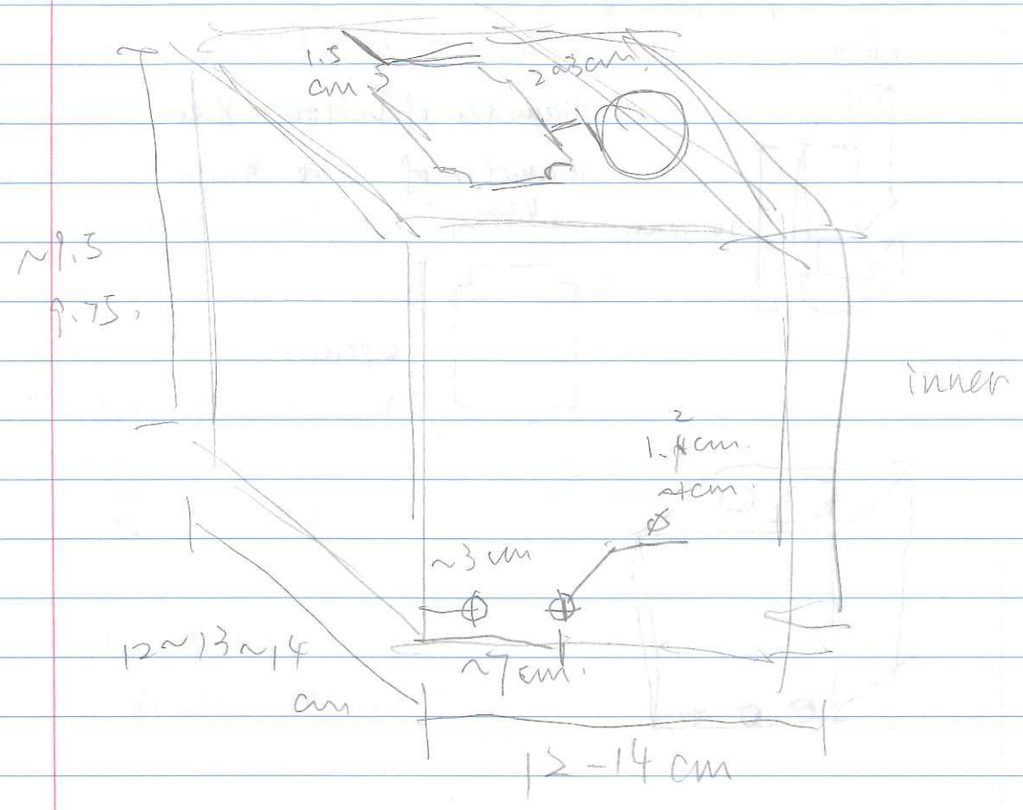
9/21 dimensions for gene gun.



diameter of button 2.8 cm
diameter of wire 8mm



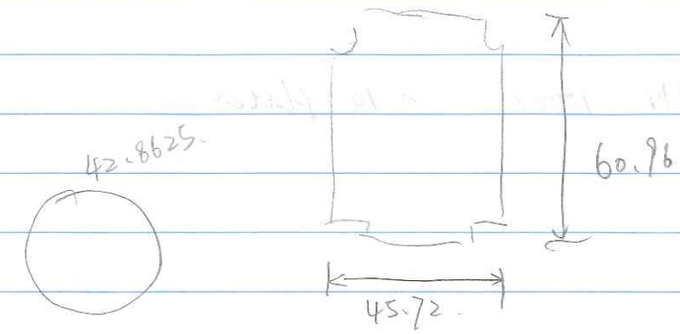


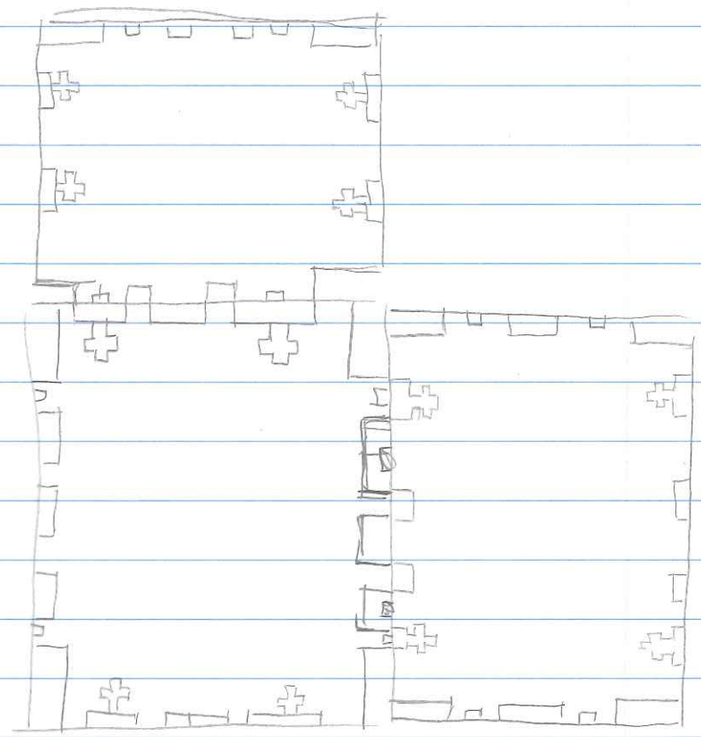


timer

height	2.4"	60.96 mm
width	1.8"	45.72 mm
depth		

sep. 24





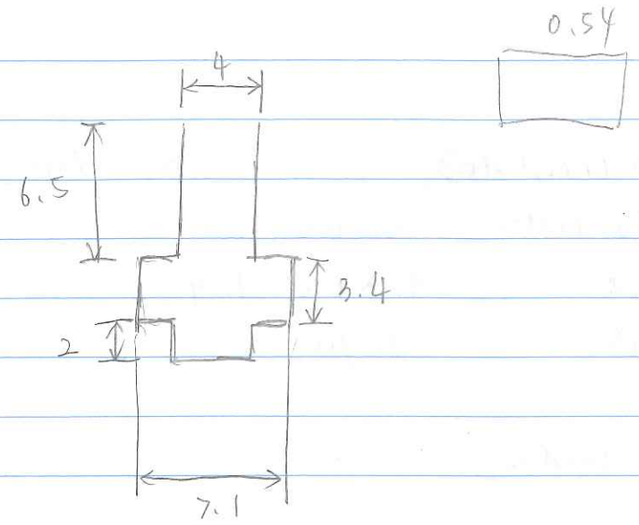
KAN 1000X ~10 plates.

10/5

make KAN plates (need 10).

1000 ml	DI water	300 ml
40 g	agar	40 X 0.3 = 12 g.
1 ml	KAN	300 µl

make plain LB media.



⌀ 4.1 mm.

2.15

2.7.

W3

10/5/18

streak GFP bacteria onto KAN plates

MH - Mei Hao

AR - Alex Robello.

10/6/18

pick colonies and make overnight.

MH - 5 (4 (50mL tube + 10mL) 1 (10mL tube + 5mL).

AR - 5 (3 10mL + 2 5mL).

biobrick - 4 5mL

10/7/18

miniprep for all 14 tubes.