Donian Chyong DC 7/17/18-8/27/18

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exp. DC1

I. Title: fly growth

II. Purpose: produce a lot of flies for malathion killing and rescue experiments

III. Calculations

any calculations used to make buffers, media, etc.

IV. Procedure

1a. tap flies for propagation

1 vial into a flask
(don't tap too hard from donor vial or the flies will get stuck in the food at the bottom and die)

Generation Count:
S refers to stock (non-experimental flies, kept for breeding). Number after S refers to #vial created
E refers to experimental (used for experiments). Experiment identification after refer to experiment the vial was used for (ex. DC1A.E.DC2a)

DC1A.S1 -- 7/17/18 -- pupated 7/24/18
DC1A(2a) -- Made from experimental vial 2 of DC2A(7/17/18) -- 7/24/18
25 Adult flies used for DC5
25 Adult flies used for DC5b
Pupates after 7 days
Pupa emerge after 3-4 days
DC1A.S2 -- 7/18/18
DC1A.S3 -- 7/19/18 -- Sacrificed 7/20/18
DC1A.S4 -- 7/20/18

DC1A.S5 -- 7/23/18
DC1A.S6 -- 7/23/18
DC1A.S7 -- 7/24/18
DC1A.S8 -- 7/24/18 -- Sacrificed 7/25/18
            DC5b
DC1A.S9 -- 7/26/18
DC1A.S10 -- 7/26/18
DC1A.S11 -- 7/27/18
DC1A.S12 -- 7/27/18
DC1A.S13 -- 7/30/18
DC1A.S14 -- 7/30/18
DC1A.S15 -- 7/30/18
DC1A.S16 -- 7/30/18
DC1A.S17 -- 7/30/18
DC1A.S18 -- 7/30/18
DC1A.S19 -- 7/30/18
DC1A.S20 -- 7/30/18
DC1A.S21 -- 7/31/18
DC1A.S22 -- 7/31/18
DC1A.S23 -- 7/31/18
DC1A.S24 -- 8/1/18
DC1A.S25 -- 8/1/18
DC1A.S26 -- 8/1/18
DC1A.S27 -- 8/1/18
DC1A.S28 -- 8/2/18 (Sacrificed 8/2 for DC6C, remade with S1)
DC1A.S29 -- 8/1/18
DC1A.S30 -- 8/2/18 (Sacrificed 8/2 for DC6C, remade with S1)
DC1A.S31 -- 8/6/18
DC1A.S32 -- 8/6/18
DC1A.S33 -- 8/6/18
DC1A.S34 -- 8/6/18
DC1A.S35 -- 8/6/18
DC1A.S36 -- 8/6/18
DC1A.S37 -- 8/9/18
DC1A.S38 -- 8/9/18
DC1A.S39 -- 8/9/18
DC1A.S40 -- 8/9/18
DC1A.S41 -- 8/9/18
DC1A.S42 -- 8/9/18
DC1A.S43 -- 8/9/18
DC1A.S44 -- 8/9/18
DC1A.S45 -- 8/9/18
DC1A.S46 -- 8/13/18
DC1A.S47 -- 8/13/18
DC1A.S48 -- 8/13/18
DC1A.S49 -- 8/13/18
DC1A.S50 -- 8/13/18
DC1A.S51 -- 8/13/18
DC1A.S52 -- 8/13/18
DC1A.S53 -- 8/13/18
DC1A.S54 -- 8/20/18
DC1A.S55 -- 8/20/18
DC1A.S56 -- 8/20/18
DC1A.S57 -- 8/20/18

DC1A.S58 -- 8/20/18
DC1A.S59 -- 8/20/18
DC1A.S60 -- 8/20/18
DC1A.S61 -- 8/20/18
DC1A.S62 -- 8/20/18
DC1A.S63 -- 8/20/18
DC1A.S64 -- 8/20/18
DC1A.S65 -- 8/20/18
DC1A.S66 -- 8/24/18
DC1A.S67 -- 8/27/18
DC1A.S68 -- 8/27/18
DC1A.S69 -- 8/27/18
DC1A.S70 -- 8/27/18
DC1A.S71 -- 8/27/18

I recommend watching videos about how to transfer flies, etc. to learn how

YEAST IS IMPORTANT FOR FLIES NUTRITION --
3-5 grains ONLY

According to literature,
the best temperature to incubate flies is at:
22C (basically room temperatuer)
CO2 dosage time recommended is <10 min
CO2 decreases breeding viability but I don't see a way around knocking them out to move them around

Lots of larvae within a day or so of vial transfer
Noticible pupae in a week
Then pupae emerge within 3-4 days (a weekend basically)

exp. DC2

I. Title: measuring malathion MIC (minimum inhibitory concentration)

II. Purpose: produce a lot of flies for malathion killing and rescue experiments

III. Calculations

volume of food in fly vial
2.5 cm diameter x 1.8 cm height
pi 1.25 cm^2 x 1.8 cm = 8.8 cm^3

malathion
2 tsp per gallon = 10 mL per 4000 mL

or 22 uL in 8.8 mL

IV. Procedure

2a. test malathion

4 vials
0. no malathion (see 1a)
1. manufacturer recommended dose of malathion (22 uL + 22 uL ddH2O)
2. E. coli only: imc270s3.1 PliaG-lacO-lacI-Pveg-lacO-gusA-pNW33N (44 uL E. coli culture) -- flies survived and reproduced, 7/24/18 pupated -- see DC1A
3. malathion + E. coli imc270s4.1 PliaG-lacO-lacI-Pveg-lacO-native ArOpdA s308L/Y309A v4-pNW33N (22 uL malathion + 22 uL E. coli culture)
4. malathion + sodium hydroxide (22 uL malathion + 11 uL 1 M NaOH + 11 uL 1 M HCl)
---
44 uL 7/17/2018 11:32 AM-7/17/2018 1:14 PM

Results
all flies in vials 1, 3 and 4 die within 15 minutes
flies in vial 2 (E. coli expressing gusA reporter) remain healthy
When ovserved 7/18/18, there seems to be growth of E. coli on the walls of the tube

exp. DC3

I. Title: Testing for malthion MIC using serial dilutions of pure malthion (no solvent)

II. Purpose: to measure the minimum concentration of malthion applied at the top of the food at the bottom of the vial in order to kill all the flies in the vial

III. Calcuations

Procedure

serial dilutions

Based off of literature:

Malathion is not that toxic to humans, but if ingested it becomes malaoxon, which is substanially more toxic.
I recommend fume hood because researchers will be using relatively larger concentrations than commercial use as well as indoors.
Malathion is flammable , also is a probable carcinogen

Xylene (solvent in commercial Malthion) is toxic.
Thus, use the pure malthion to test for its minimum toxic dose

For dilutions use 200 proof ethanol

Greatest nonlethal dose was:

Source did -- 10 mL in 4 g medium, 15 flies in each jar using 50% malathion solution with xylene solvent using water to dilute

0.0008% Malathion approx LC90
0.0005% Malathion approx LC65
0.0002% Malathion approx LC50

Source: Development of Resistance in Drosophila Melanogaster by Selective Pressure with Malathion (Organophosphate Insecticide) [1972]

Method:

10mL Agarose - 10g agar

exp. DC4

I. Title: Testing whether flies can survive in experimental vials with LB + Agarose + 0.2% sucrose medium

II. Purpose: To determine whether we can conduce experiments in the experimental vials using the agarose medium. If flies can survive in this medium and eat it for food, we can easily, repeatibly, and evenly introduce malathion to the flies

III. Calculations

Methods

Agarose medium formula:

Agarose concentration: 15 g/L
LD concentration: 25 g/L
0.2% sucrose
(in later experiments add varying concentrations of malathion)

2 vials with just agarose medium
2 vials with agarose medium and 34 ug/mL Chl

Made 7/19/18

1. Make agarose medium
2. Autoclave
3. Apply 10mL to bottom of each vial
4. Wait to harden
5. Apply bacteria to one tube with Chl
6. Add 3-5 flies to each vial

Experiment started 7/20/18 10:40:40 AM

Results:
Flies don't get stuck in the gel even though they are tapped down forcibly

Because the breeding vial and the experimental vial are different sizes, I recommend sedating the flies before transfering

(Observed 7/23/18)
Flies are dying, likely of starvation. They are unable to chew on the agar.
I will decrease concentration of agar to .7%

exp. DC5a

I. Title: Testing whether adding malathion in agarose medium with sucrose is an acceptable method of introducing pesticide to flies

II. Purpose: Earlier, we just sprayed malathion at the top of the food. Through this experiment I hope to show malathion can be mixed in the process of creating the agarose medium to create an easy method of administering controllable doses of malathion. The end goal is to discover the minimum dosage necessary to kill all flies

III. Calculations

Total: 10 mL in each experiemental tube
making 300 mL of agarose solution total

w/
25g/L LB ----------> 7.5g LB
7g/L Agar ----------> 2.1g Agar
.7% Sucrose --------> 2.1g Sucrose

Malathion dissolved in 100uL Eth

Malathion concentrations
#1 0 (Negative Control)
#2 0.1 uL/10mL serial dilution (1 uL of 1uL/10mL dilution) (1uL/10mL Eth dilution)
#3 0.3 uL/10mL serial dilution (3 uL of 1uL/10mL dilution) (1uL/10mL Eth dilution)
#4 1 uL/10mL
#5 3 uL/10mL

Methods

Make Agarose solution
Autoclave
Cool
Add sucrose
Make vials
Pour solution
Wait to solidify

Add flies (add 5 flies in each)(knocked out flies)
Observe

Results:
2 hours later, Flies are all dead in #s 5 and 4. 1 is alive in #2, and #3. All are alive in #1

exp. DC5b
I. Title: same as DC5a but with smaller concentrations
II. Purpose: Narrow down how much malathion is necessary to kill all the flies
III. Calculations:

Total: 10 mL in each experiemental tube
making 300 mL of agarose solution total

w/
25g/L LB ----------> 7.5g LB
7g/L Agar ----------> 2.1g Agar
.7% Sucrose ----------> 2.1g Sucrose

Malathion dissolved and serial diluted Eth

Malathion concentrations
#1 0
#2 0.001 uL/10mL
#3 0.003 uL/10mL
#4 0.01 uL/10mL
#5 0.03 uL/10mL
#6 0.1 uL/10mL

Results:

(Checked 7/25/18 10:25am)

Tube	Survival
#1	100%
#2	100%
#3	100%
#4	60%
#5	0%
#6	0%

Will use 0.003 uL/mL as minimun malathion (minimum inhibitory concentration) necessary to kill all flies

exp. DC6a
I. Title: Growing bacteria expressing oph or opdh in experimental fly vials with agar medium with malathion
II. Purpose: To see if these transformed bacteria can break down the malathion in the vials and save the flies
III. Calculations:

Methods

Total: 10 mL in each experiemental tube

w/
25g/L LB ----------> 7.5g LB
7g/L Agar ----------> 2.1g Agar
.7% Sucrose ----------> 2.1g Sucrose

Malathion dissolved and serial diluted Eth
Will use 0.003 uL/mL

means 0.03 uL total in 10 mL medium (3 uL of 0.1% malathion)

300uL of each culture

#1 -- 284R8 - RFP -- (no protective enzyme)
#2 -- RFP but with no malathion
#3 -- 270Z3 - OPDA
#4 -- 270Z4 - OPH
#5 -- Malathion + Medium + NO bacteria

Results
On 7/30/18 2:34pm
#1 - Pink layer at top
#2 - Pink layer at top
#3 - Cloudy medium
#4 - Cloudy medium
#5 - Clear

Will transfer 10 flies into each vial

Results
On 7/31/18 3:14pm
#1 - 5/11 still alive
#2 - 8/10 still alive
#3 - 8/10 still alive
#4 - 8/11 still alive
#5 - 9/10 still alive

exp. DC6b

I. Title: Rethinking how to administer bacterial + lb + malathion solution to experimental fly vial
II. Purpose: To determine how to best evenly administer the solution to the bottom of the vial to expose flies to solution
in an even and repeatable way and minimizing extraneous variables
such as:
gel
eth

food
III. Calculations:

1000 uL -- Covers entire bottom
800 uL -- Covers entire bottom
600 uL -- Covers entire bottom
500 uL -- Covers entire bottom
400 uL -- too little

Using 500 uL of 10% eth in LB

Started drying at 12:00pm 8/1/18
Still not dry at 12:36pm, need to pursue different option
Only rim wet at 2:21pm for vaccum dry; basically no change in vial in fume hood
at 3:57pm Fully Dry in vaccum

4 HOURS NECESSARY, NOT GOOD

experimental vial diameter is 2.5 cm
area : 4.91 cm^2

Filter paper
Using razor blade to cut around lip of each vial is not consistent
1.5cm x 1.5cm squares seem to work
Use paper cutter and stacks of filter paper

Test whether flies drown
250 uL -- flies don't drown but drops off of paper
200 uL -- flies don't drown but drops off of paper

100 uL -- (a few drops off of paper) -- but 90% LB + 10%Eth kills some flies
75 uL --   1-2 drown each time
60 uL --   Also good
50 uL --   Good -- All flies survive
25 uL -- (barely covers square)

Will use 50 uL

exp. DC6c

I. Title: Growing bacteria expressing oph or opdh in experimental fly vials with filter paper
INSTEAD of agar
Growing bacteria in liquid culture with malathion inside each experimental vial
II. Purpose: To see if these transformed bacteria can break down the malathion while in liquid culture and save the flies
III. Calculations:

Methods:

5mL of LB
Cultures made 5:05pm 7/31/18
1# IMC 284R8 - RFP
2# IMC270Z3 - OPDH
3# IMC270Z4 - OPH

How much malathion and E. coli?
malathion MW = 330.358 g/mol
330.358 ug/L = 1 micromolar
10 micromolar = 3.3 mg/L
Km OPH/malathion = 91.2 micromolar = 0.092 mM = 30.128 mg/L

OPH
>PoOPH

ATGCGCCTGTTCTCCCTGTCCACCGCACTTTCCAGCGCTATGATCGCCCTGGTGTCCCTG
CCCCTGCAAGCCATGGCCGCTCCCGCGCAGCAGAAGACGCAGGTGCCGGGCTATTATCGT
ATGGCCCTGGGCGATTTTGAAGTGACCGCCCTGTATGATGGCTATATTGATGGTCCGGCG
TCTCTTCTGAAAGGCATTGATGATAAAGACCTTCAGAGCCTTCTTGCCCGTATGTTCGTG
GCCAGCGAGGGTGGCGTCCAGACGGCAGTGAACGCCTACCTGATTAATACCGGCGATAAC
CTGGTCCTGATTGACACGGGCGCAGCGCAATGCTTCGGTCCCGCCCTTGGCGTCGTCCAA
ACCAACCTGAAGGCCAGCGGCTATCAGCCGGAACAGGTGGATACCGTGCTGATCACCCAT
CTGCATCCCGATCACGCTTGTGGTCTTGTTAACGCGGATGGCTCCCCGGCTTATCCGAAC
GCCACGGTGGAAGTGCCGCAGGCGGAAGCCGAGTTCTGGCTGGATGAAGCCACGATGGCA
AAAGCCCCGGAAGGTATGCAGGGTTTCTTCAAGATGGCCCGTCAAGCCGTCGCGCCGTAT
GCCAAGATGAACAAGCTGAAGCCGTATAAGACGGAAGGCGAACTGCTGCCCGGTGTCTCT
CTTGTTGCCTCCTCCGGTCATACCCCCGGTCACACGTCCTATCTGTTCAAGTCTGGCGGC
CAATCCCTGCTGGTGTGGGGCGACATCCTGATCAATCATGCCGTGCAATTCGCCAAACCG
GAAGTGGCCTGGGAGTTCGACGTGGATTCCGATCAAGCTCGCCAGTCCCGTCAGCGCATC
CTGGCCGAGGCCGCAACCGATAAGCTGTGGGTGGCCGGTGCACATCTGCCGTTCCCGGGT
CTGGGTCACGTGCGCGAAGAAGCTCAGGGCTATGCGTGGGTCCCGGTGGAGTTCTCTCCG
ATTCGCAGCGACCGCAAACTTGCTGCCGCACTGGAAAAGCTTTGA

Translation of DNA sequence:

```
1 atgcgcctgttctccctgtccaccgcactttccagcgctatgatcgccctggtgtccctg
1  M  R  L  F  S  L  S  T  A  L  S  S  A  M  I  A  L  V  S  L

61 cccctgcaagccatggccgctcccgcgcagcagaagacgcaggtgccgggctattatcgt
21  P  L  Q  A  M  A  A  P  A  Q  Q  K  T  Q  V  P  G  Y  Y  R

121 atggccctgggcgattttgaagtgaccgccctgtatgatggctatattgatggtccggcg
41   M  A  L  G  D  F  E  V  T  A  L  Y  D  G  Y  I  D  G  P  A

181 tctcttctgaaaggcattgatgataaagaccttcagagccttcttgcccgtatgttcgtg
61   S  L  L  K  G  I  D  D  K  D  L  Q  S  L  L  A  R  M  F  V

241 gccagcgagggtggcgtccagacggcagtgaacgcctacctgattaataccggcgataac
81   A  S  E  G  G  V  Q  T  A  V  N  A  Y  L  I  N  T  G  D  N
```

```
301 ctggtcctgattgacacgggcgcagcgcaatgcttcggtcccgcccttggcgtcgtccaa
101  L  V  L  I  D  T  G  A  A  Q  C  F  G  P  A  L  G  V  V  Q

361 accaacctgaaggccagcggctatcagccggaacaggtggataccgtgctgatcacccat
121  T  N  L  K  A  S  G  Y  Q  P  E  Q  V  D  T  V  L  I  T  H

421 ctgcatcccgatcacgcttgtggtcttgttaacgcggatggctccccggcttatccgaac
141  L  H  P  D  H  A  C  G  L  V  N  A  D  G  S  P  A  Y  P  N

481 gccacggtggaagtgccgcaggcggaagccgagttctggctggatgaagccacgatggca
161  A  T  V  E  V  P  Q  A  E  A  E  F  W  L  D  E  A  T  M  A

541 aaagccccggaaggtatgcagggtttcttcaagatggcccgtcaagccgtcgcgccgtat
181  K  A  P  E  G  M  Q  G  F  F  K  M  A  R  Q  A  V  A  P  Y

601 gccaagatgaacaagctgaagccgtataagacggaaggcgaactgctgcccggtgtctct
201  A  K  M  N  K  L  K  P  Y  K  T  E  G  E  L  L  P  G  V  S

661 cttgttgcctcctccggtcatacccccggtcacacgtcctatctgttcaagtctggcggc
221  L  V  A  S  S  G  H  T  P  G  H  T  S  Y  L  F  K  S  G  G

721 caatccctgctggtgtggggcgacatcctgatcaatcatgccgtgcaattcgccaaaccg
241  Q  S  L  L  V  W  G  D  I  L  I  N  H  A  V  Q  F  A  K  P

781 gaagtggcctgggagttcgacgtggattccgatcaagctcgccagtcccgtcagcgcatc
261  E  V  A  W  E  F  D  V  D  S  D  Q  A  R  Q  S  R  Q  R  I

841 ctggccgaggccgcaaccgataagctgtgggtggccggtgcacatctgccgttcccgggt
281  L  A  E  A  A  T  D  K  L  W  V  A  G  A  H  L  P  F  P  G

901 ctgggtcacgtgcgcgaagaagctcagggctatgcgtgggtcccggtggagttctctccg
301  L  G  H  V  R  E  E  A  Q  G  Y  A  W  V  P  V  E  F  S  P

961 attcgcagcgaccgcaaacttgctgccgcactggaaaagctttga
321  I  R  S  D  R  K  L  A  A  A  L  E  K  L  *

1021
341
```

Translated protein sequence: 35.9 kDa

```
1 MRLFSLSTAL SSAMIALVSL PLQAMAAPAQ QKTQVPGYYR MALGDFEVTA LYDGYIDGPA
61 SLLKGIDDKD LQSLLARMFV ASEGGVQTAV NAYLINTGDN LVLIDTGAAQ CFGPALGVVQ
121 TNLKASGYQP EQVDTVLITH LHPDHACGLV NADGSPAYPN ATVEVPQAEA EFWLDEATMA
181 KAPEGMQGFF KMARQAVAPY AKMNKLKPYK TEGELLPGVS LVASSGHTPG HTSYLFKSGG
241 QSLLVWGDIL INHAVQFAKP EVAWEFDVDS DQARQSRQRI LAEAATDKLW VAGAHLPFPG
301 LGHVREEAQG YAWVPVEFSP IRSDRKLAAA LEKL*
```

yield of PoOPH = 100 mg/L = 100 mg/35,900,000 mg /L = 2.8 x 10^-6 M
PoOPH MW = 35,900 g/mol

ideally, a 91.2 uM malathion = 30.128 mg/L (33,192-fold) should be incubated in a saturated culture of OPH-expressing E. coli (2.8 uM).

Dilute pure malathion in ethanol as follows:

final volume = 1 mL culture
in 2 mL tubes

dil 4 uL into 196 uL uL ethanol = 1/50 (stock)
6.04 uL malathion stock into 1 mL E. coli

-- (12.08 uL of 1/100 stock) --

The initial velocity should be 1/2 of max = 24.2/sec divided by 2 = 12.1/sec. Assuming perfect membrane permeability, the entire reaction should
take 2.7 seconds at 30 deg (but let it incubate as long as possible).

Incubate over night
8/1/18 5:00 pm

Taken from 30C room @ 1:26pm 8/2/18

Spot 50 uL onto each postage stamp sized Whatman 1 filter.

Insert 10 flies for each vial
record how many flies are alive at different times

Tubes:

with malathion
- #1 tagRFP (no enzyme): death?
- #2 PoOPHM9 (experimental): survive?
- #3 no E. coli (LB90% + 10%Eth): death?

without malathion
- #4 tagRFP (no enzyme): survive?
- #5 PoOPHM9 (experimental): survive?
- #6 no E. coli (LB90% + 10% Eth): survive?

(number after decimal indicates dilution)

Results (#flies still alive)

Vial	5min	10min	30min	45min	60min	75 min	90min	105 min	120 min
#1.1	10	10	9	3	2	1	1	0	0
#2.1	10	10	8	5	5	4	2	2	2
#3.1	10	10	6	4	2	1	0	0	0

#4.1 10 10 10 10 10 10 10 10
10
#5.1 10 10 10 10 10 10 10 10
10
#6.1 10 10 10 10 10 10 10 10
10

at 30min, 1.1: 1 dead, 4 struggling to move
at 45 min, 3.1: 6 dead, 2 struggling to move
at 45 min 2.1: 5 dead, 1 struggling to move
at 60min 2.1: 5 dead, 1 struggling to move
at 75 min 2.1: 6 dead, 2 struggling to move
at 75 min 3.1: 9 dead, 1 struggling to move
at 90 min 1.1: 9 dead, 1 struggling to move, basically dead, twitching

#1.10 10 10 10 10 10 10 10
#2.10 10 10 10 10 10 10 10
#3.10 10 10 10 10 10 10 10
#4.10
#5.10
#6.10

#1.100 10 10 10 10 10 10
#2.100 10 10 10 10 10 10
#3.100 10 10 10 10 10 10
#4.100
#5.100
#6.100

___
Never did 1000x
#1.1000
#2.1000
#3.1000
#4.1000
#5.1000
#6.1000

6d. replicate malathion survival experiments

15 x 1 mL LB + ampicillin (1-2) or no amp (3-6) cultures

1a-c. imc270z1 Ptac-lacO-syn rplW-ArOpdA s308L/Y309A v4-IMBB2.4-pUC57-mini/GC10
(not previously tested, also called ArPTE S308L/Y309A)

2a-c. imc270z2 Ptac-lacO-syn rplW-PoOPHM9 v4-IMBB2.4-pUC57-mini/GC10 (not previously tested, oph in exp. DC6c)

3a-c. imc270z3 Ptac-lacO-syn rplW-ArOpdA s308L/Y309A v4-IMBB2.4-pUC57-mini/delta rplW DH10B
(not previously tested, also called ArPTE S308L/Y309A)

4a-c. imc270z4 Ptac-lacO-syn rplW-PoOPHM9 v4-IMBB2.4-pUC57-mini/delta rplW DH10B
(note, this was #2 oph in exp. DC6c)

5-6a-c. imc284r8 Ptac-lacO-tagRFP-rplW-IMBB2.4-pUC57-mini/ delta rplW DH10B
(note, these were #1 and #4 tagRFP, with and without malathion, in exp. DC6c)

37 deg rotator x 24 hours 8/4/2018 12:46 PM

add to 1-5 (a-c), 12 uL 1/100 dil malathion
30 deg rotator x 20 hours 8/5/2018 2:30 PM

Removed @ 1:14pm 8/6/18

add 50 uL of each culture to 1.5 cm x 1.5 cm Whatman 1 filter in fly vial
add 10 adult flies to each vial, count survivors every 15 min for 2 hours

see DC6d fly survival data excel spreadsheet document for data

Inoculuated food vials with C vials
C1-6 ---Took picture

Picture saved
1, 3, 6 -- Lots of new flies 8/21/18

6e. agar assay

1 mL LB + ampicillin (1) or no amp (3 and 5) cultures

Cultures put in 37C room @ 3:00pm 8/7/18
Removed @ 12:53pm 8/8/18

1. imc270z1 Ptac-lacO-syn rplW-ArOpdA s308L/Y309A v4-IMBB2.4-pUC57-mini/GC10 (also called ArPTE S308L/Y309A)
(1 uL ampicillin in #1)

3. imc270z3 Ptac-lacO-syn rplW-ArOpdA s308L/Y309A v4-IMBB2.4-pUC57-mini/delta rplW DH10B (also called ArPTE S308L/Y309A)

5. imc284r8 Ptac-lacO-tagRFP-rplW-IMBB2.4-pUC57-mini/ delta rplW DH10B with malathion

6 = 5 without malathion control

Total: 10 mL in each experiemental tube
making 250 mL of agarose solution total

w/
25g/L LB ----------> 6.25g LB
7g/L Agar ----------> 1.75g Agar
.7% Sucrose ----------> 1.75g Sucrose

Malathion dissolved and serial diluted Eth

300uL of each culture for 10mL of agar

When mixed in 10 mL of agar, #1 receives 10uL additional ampicillin.

Will use:
a) 0.1 uL/10mL serial dilution (1 uL of 1uL/10mL dilution) (1uL/10mL Eth dilution)
b) 0.3 uL/10mL serial dilution (3 uL of 1uL/10mL dilution) (1uL/10mL Eth dilution)

1 and 3 uL of 1% malathion

Total 7 vials
(a = with 0.1 uL/10mL malathion)
(b = with 0.3 uL/10mL malathion)

Vials made 1:36pm 8/8/18, put into 30C incubator
vials taken out 12:09pm 8/9/18, 20 flies per vial introduced @ 1:51pm 8/9/18

Larvae?
Time checked:

	# flies alive
Vial	8/13/18
1	
a	1
b	0
3	
a	2
b	1
5	
a	0
b	0
6	
	0

8/13/18: Larvae present in 1A, 3A, 3B, 6
8/21/18: New adult fly (1) in 3A, 6

exp DC6f
Purpose: Use flies that survived from 6d reexpose to new malathion

Procedure:
Knock out flies in 1 ,3 ,6
Remove flies, put in different food vial
Add malathion to food again
Wait overnight
Put flies back into old food vial with malathion in it

Calculations

12.08 uL malathion stock into 1 mL E. coli in 6c, so will do

12.08 uL malathion 1/100 into 1mL LB
50 uL into each emptied food vial 3C, 6C
4:33pm 8/21/18
Flies reintroduced
1:30pm 8/22/18

50 uL into 1C @
1:11pm 8/22/18

Observations:

exp. DC6g

Title: Add malathion to food vial, add bacteria to food, add flies
Procedure:
Make E.coli cultures
1mL culture in 2 mL tubes, 1 mL LB + 2uL 50x ampicillin

(from DC6d)
1. imc270z1 Ptac-lacO-syn rplW-ArOpdA s308L/Y309A v4-IMBB2.4-pUC57-mini/GC10
(also called ArPTE S308L/Y309A)

3. imc270z3 Ptac-lacO-syn rplW-ArOpdA s308L/Y309A v4-IMBB2.4-pUC57-mini/delta rplW DH10B
(also called ArPTE S308L/Y309A)(rplW+ ?)

5. imc284r8 Ptac-lacO-tagRFP-rplW-IMBB2.4-pUC57-mini/ delta rplW DH10B

37 deg rotator x 48 hours 5:01pm 8/22/18

removed 3:43pm 8/24/18

Malathione + LB mixture
200 uL LB + 12 uL malathione

In each food vial, put 50 uL of bacteria and 10 uL of malathione mixture
Add 20 flies in each

1a. imc270z1 -- flies put in 8/24 (Friday)
1b. imc270z1 -- flies put in 8/27 (Monday)

3a. imc270z3 -- flies put in 8/24 (Friday)
3b. imc270z3 -- flies put in 8/27 (Monday)

5a. imc284r8 -- flies put in 8/24 (Friday)
5b. imc284r8 -- flies put in 8/27 (Monday)

1a, 3a, 5a -- All dead when checked on Monday
1b, 3b, 5b -- Checked 8/28/18 -- All mostly alive

Inconclusive result

exp. DC7

I. Title: Bacillus subtilis

II. Purpose: To transform Bacillus subtilis with PTE and OPH expression vectors

III. Calculations:

IV. Procedure

7a. rolling circle amplification of expression vectors

0) no plasmid
1) 1653 BGSC pNW33N
2) 2631 imc270s3.1 PliaG-lacO-lacI-Pveg-lacO-gusA-pNW33N
3) 2632 imc270s4.1 PliaG-lacO-lacI-Pveg-lacO-native ArOpdA s308L/Y309A v4-pNW33N
4) 2633 imc270s5.1 PliaG-lacO-lacI-Pveg-lacO-native PoOPHM9 v4-pNW33N
5) 2634 imc270s7.1 PliaG-lacO-lacI-Pveg-lacO-Lp3117-PoOPHM9-6his-pNW33N
6) 2635 imc270v6.1 PliaG-lacO-lacI-Pveg-lacO-LP3117-ArOpdA s308l/y309a-6his-pNW33N

TempliPhi RCA protocol (10 uL total for up to 60 sequencing reactions)
don't use thin pipet tips (p2 or gel loading) for enzyme or RCA product

0.4 uL template
5 uL TempliPhi sample buffer

95 deg x 3 min (no necessary for purified plasmid)

master mix (per reaction)
5 uL TempliPhi reaction buffer
0.2 uL TempliPhi enzyme mix
keep on ice

add 5 uL of master mix to each sample
30 deg x 4-18 hours 8/17/2018 3:41 PM

65 deg x 10 min (important)

if the RCA worked, the sample should be viscous (use regular p200 tip)

Results
Optical density of B.subtilis = 0.47 absorbance units

1:22pm 8/20/18 - added 1% xylose, put in 37 degree room
removed 1:18pm 8/21/18

exp. DC7b

Title : Liquid culture to test expression of reporter enzyme

1-2. w/ and w/o IPDG - 1653 BGSC pNW33N
3-4. w/ and w/o IPDG - 2631 imc270s3.1 PliaG-lacO-lacI-Pveg-lacO-gusA-pNW33N

Calculations:

5 ug ml^-1 chloramphenicol + 25 g/L LB

--
25 mL LB w/ 5 ug/ml Chloramphenicol means
3.67 uL stock 34 mg/ml Chloramphenicol + 25mL LB
5uL of IPDG per 5 mL culture

5 mL into each 10 mL tube, put into shaker
4:00pm 8/21/18
removed 1:36pm 8/22/18

Measuring growth of bacteria (1 mL into cuvette)
OD - @600nm

7B1.	0.68
7B2.	0.66
7B3.	0.46
7B4.	0.51

--
7B1 and 7B3 untouched, 4mL remaining in tube

7B2 divided into (2 mL removed from original 7B2 and mixed in new tube with 2mL LB + 5 ug/mL Chloramphenicol each)
	7B2.1 - PNW33N w/ IPDG (IPDG added 1 day after 7B1 and 7B3)
	7B2.2 - PNW33N w/o IPDG

7B4 divided into (2 mL removed from original 7B4 and mixed in new tubes with 2mL LB + 5 ug/mL Chloramphenicol each)
	7B4.1 - GusA w/ IPDG (IPDG added 1 day after 7B1 and 7B3)
	7B4.2 - GusA w/o IPDG

Placed back in shaker
4:20 pm 8/22/18

Removed 1:30 pm 8/23/18

GusA enzyme assay
	100uL out of 300 uL total

7B1) PNW33N w/ IPDG

7B2.1) PNW33N w/ IPDG	0
7B2.2) PNW33N w/o IPDG

7B3) GUSA w/ IPDG	22

7B4.1) GusA w/ IPDG	20
7B4.2) GusA w/o IPDG	17

exp. DC7c

Title: liquid cultures of

1) 1653 BGSC pNW33N
2) 2631 imc270s3.1 PliaG-lacO-lacI-Pveg-lacO-gusA-pNW33N
3) 2632 imc270s4.1 PliaG-lacO-lacI-Pveg-lacO-native ArOpdA s308L/Y309A v4-pNW33N
4) 2633 imc270s5.1 PliaG-lacO-lacI-Pveg-lacO-native PoOPHM9 v4-pNW33N
5) 2634 imc270s7.1 PliaG-lacO-lacI-Pveg-lacO-Lp3117-PoOPHM9-6his-pNW33N
6) 2635 imc270v6.1 PliaG-lacO-lacI-Pveg-lacO-LP3117-ArOpdA s308l/y309a-6his-pNW33N

made
1mL into 2 mL tube with 5 ug/ml Chloramphenicol

Put in 37C room 4:34 pm 8/24/18
Removed 2pm 8/28/18 -- Not grown at all

Inconclusive
So, made 5mL cultures of each (1-6) for future experiments
5mL of 25 g/L LB + 5 ug/mL chloramphenicol
put in shaker -- 3:22pm 8/28/18