iGEM 2017 Lab Notebook

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Exp. iGb1

I. Title: pUC57-mini-6his-aph(3’)-VIa and
Ptac-N6-specR-IMPP2.4-pUC57-mini Assembly

Performed by: Cyrillus Tan
Recorded by: Cyrillus Tan
First Entry: 6/7/2017 1:32 PM

II. Purpose: to subclone kanamycin resistant aph(3') into pUC57 backbone; to subclone spectinomycin resistance specR into Ptac-N6 backbone.

III. Procedure

1a. Plasmid Preparation

6 mL LB-amp
Donor: 1776. pUC57-simple-6his-aph(3’)-VIa
(kan resistance)
Recipient: 573. 6his-gusA-pUC57-mini
(containing ribosome binding site but no promoter)
37 deg rotator 6/7/2017 3:16 PM

6 mL LB-amp
Donor: 1212. pCDF-specR-IMBB/pSL1180
(spec resistance)
Recipient: 2116: Ptac-N6-tagRFP-IMPP2.4-pUC57-mini
(containing weak constitutive promoter)

37 deg rotator 6/7/2017 3:16 PM

QIAprep miniprep on 6/8/2017
elute 40 uL

Take 3 concentration measurements
573. 6his-gusA-pUC57-mini 53.92 ng/uL
1212. pCDF-specR-IMBB/pSL1180 75.645 ng/uL
1776. pUC57-simple-6his-aph(3’)-VIa 16.587 ng/uL
2116: Ptac-N6-tagRFP-IMPP2.4-pUC57-mini 62.593 ng/uL

1b. Restriction Digestion

573. 6his-gusA-pUC57-mini: desire 1882 bp, not 2688 bp
30 uL sample
38 uL ddH2O
8 uL 10x 3.1 NEB buffer
2 uL NcoI
2 uL PstI
----
80 uL

1212. pCDF-specR-IMBB/pSL1180: desire 1242 bp, not 3112 bp
30 uL sample
38 uL ddH2O
8 uL 10x 3.1 NEB buffer
2 uL PstI
2 uL NheI
----
80 uL

Gel Lane:
1/ 2-log DNA ladder
2/
3/ digested 2116: Ptac-N6-tagRFP-IMPP2.4-pUC57-mini
4/ digested 1212. pCDF-specR-IMBB/pSL1180
5/ digested 573. 6his-gusA-pUC57-mini
6/ digested 1776. pUC57-simple-6his-aph(3')-VIa
pic: iGb1b1

Band Isolation, concentration test
573. 6his-gusA-pUC57-mini: 17.257 ng/uL
2116: Ptac-N6-tagRFP-IMPP2.4-pUC57-mini: 12.763 ng/uL
1212. pCDF-specR-IMBB/pSL1180: 12.464 ng/uL
1776. pUC57-simple-6his-aph(3')-VIa: no visible band under UV, abort

pUC57-mini-6his-aph(3')-VIa assembly end hereto

1c. Ligation

20 fmole 2116: Ptac-N6-tagRFP-IMPP2.4-pUC57-mini = 13 ng/kb x 1.94 kb
= .22 ng = 1.935 uL

20 fmole 1212. pCDF-specR-IMBB/pSL1180 = 13 ng/kb x 1.24 kb = 16.12 ng = 1.295 uL

Enz = 1 uL 1/6 dil NEB T4 DNA ligase

#	vec	ins	enz	H2O
1	2	0	1	14
2	2	0	1	13
3	2	1.5	1	11.5
4	0	1.5	1	13.5

+ 4 uL 5x T4 DNA ligase buffer
---
20 uL

17 deg water bath 6/9/2017 7:00 PM

Gel lane
1/ 2-log DNA ladder
2/ 2116: Ptac-N6-tagRFP-IMPP2.4-pUC57-mini
3/ 1212: pCDF-specR-IMBB/pSL1180
4/ #1 ligation VOE
5/ #2 ligation VOE
6/ #3 ligation VIE
7/ #4 ligation OIE
pic: iGb1c1

1d. Bacterial Transformation

Heat shock transformation with Mach 1 E. coli
spread on LB-amp/spec plate, each 100 ug/mL
37 deg room 6/10/2017 4:30 PM

Collected at 6/11/2017 1:30 PM
iGb1d1 VOE: 0 cfu
iGb1d2 VOE: 2 cfu
iGb1d3 VIE: ~700 cfu
iGb1d4 OIE: 3 cfu

1e. Restriction Mapping

Pick 3 colonies from iGb1d3 (LB-amp/spec) plate to be iGb1e1, iGb1e2, iGb1e3, culture in LB-amp 100 ug/mL
37 deg rotator 6/11/2017 3:55 PM

Miniprep
Elute 30 uL

Take 3 concentration test
1e1: 52.19 ng/uL
1e2: 83.702 ng/uL
1e3: 75.43 ng/uL

Exp. iGb2

I. Title: (TBD) Assembly

Performed by: Cyrillus Tan
Recorded by: Cyrillus Tan
First Entry: 6/11/2017 3:56 PM

II. Purpose: to subclone _ resistant _ into pUC57 backbone.

III. Procedure

2a. Plasmid preparation

Set 1
6 mL LB-amp
Recipient: 2113: Ptac-N6-tagRFP-IMBB2.4-pUC57-mini
Donor: 1213: pACYC-chlR-IMBB-pSL1180

Set 2
6 mL LB-amp
Recipient: 2113: Ptac-N6-tagRFP-IMBB2.4-pUC57-mini
Donor: 1843: ampC ADC-33-pUC57-mini

Set 3
6 mL LB-amp
Recipient: 2113: Ptac-N6-tagRFP-IMBB2.4-pUC57-mini
Donor: 1212: pCDF-specR-IMBB-pSC1180

Set 4
6 mL LB-amp
Recipient: 2116: Ptac-N6-tagRFP-IMBB2.4-pUC57-mini
Donor: 1213: pACYC-chlR-IMBB-pSL1180

Set 5
6 mL LB-amp
Recipient: 2116: Ptac-N6-tagRFP-IMBB2.4-pUC57-mini
Donor: 1843: ampC ADC-33-pUC57-mini

Set 6
6 mL LB-amp
Recipient: 573: 6his-gusA-pUC57-mini
Donor: 538: rpoBC v3a-c pUC57

37 deg rotator 6/11/2017 5:31 PM

Miniprep, elute amount
1v: 40 uL 1i: 40 uL
2v: 50 uL 2i: 50 uL
3v: 40 uL 3i: 40 uL
4v: 40 uL 4i: 40 uL
5v: 60 uL 5i: 60 uL
6v: 50 uL 6i: 50 uL

Take 3 concentration test

1v: 93.537 ng/uL, 1i: 84.523 ng/uL
2v: 81.859 ng/uL, 2i: 35.873 ng/uL
3v: 88.933 ng/uL, 3i: 185.915 ng/uL
4v: 67.172 ng/uL, 4i: 53.257 ng/uL
5v: 68.366 ng/uL, 5i: 76.496 ng/uL
6v: 70.134 ng/uL, 6i: 19.683 ng/uL

2b. Restriction Digestion

1v. Recipient: 2113: Ptac-N6-tagRFP-IMBB2.4-pUC57-mini
5 uL 10x buffer 2.1
1 uL SpeI-HF
1 uL PstI-HF
39 uL sample
21.6 mqH2O

67.6 uL

1i. Donor: 1213: pACYC-chlR-IMBB-pSL1180
5 uL 10x buffer 2.1
1 uL NheI
1 uL PstI-HF
39 uL sample
19 uL mqH2O
---
65 uL

2v. Recipient: 2113: Ptac-N6-tagRFP-IMBB2.4-pUC57-mini

8 uL 10x buffer 2.1
1 uL SpeI
1 uL PstI
49 uL sample
46 uL mqH2O
---
105 uL

2i. Donor: 1843: ampC ADC-33-pUC57-mini
8 uL 10x buffer 2.1
1 uL XbaI
1 uL PstI
14 uL mqH2O
49 uL sample
---
63 uL

3v. Recipient: 2113: Ptac-N6-tagRFP-IMBB2.4-pUC57-mini
5 uL 10x NEB buffer 2.1
22.5 uL sample
1 uL SpeI-HF
1 uL PstI
20.5 uL mqH2O
---
50 uL

3i. Donor: 1212: pCDF-specR-IMBB-pSC1180
5 uL 10x NEB buffer 2.1
11 uL sample
1 uL NheI
1 uL PstI
32 uL mqH2O
---
50 uL

4v. Recipient: 2116: Ptac-N6-tagRFP-IMBB2.4-pUC57-mini
Donor: 1213: pACYC-chlR-IMBB-pSL1180

5v. Recipient: 2116: Ptac-N6-tagRFP-IMBB2.4-pUC57-mini
59 uL sample
5 uL 10x NEB buffer 2.1
1 uL SpeI
1 uL PstI

13 uL mqH2O
---
74 uL

5i. Donor: 1843: ampC ADC-33-pUC57-mini
59 uL sample
5 uL 10x NEB buffer 2.1
1 uL XbaI
1 uL PstI
17 uL mqH2O
---
83 uL

6v. Recipient: 573: 6his-gusA-pUC57-mini
29 uL sample
5 uL 10x NEB buffer 3.1
1 uL NcoI
1 uL PstI-HF
13 uL mQH2O
---
50 uL

6i. Donor: 538: rpoBC v3a-c pUC57
50 uL sample
12 uL 10x NEB buffer 3.1
1 uL NcoI
1 uL PstI-HF
56 uL mqH2O
---
120 uL

37 deg rotator 6/12/17 2:45 PM

Gel lane
pic: iGb2b1
1/ 1 kb DNA ladder
2/ 2b1v (2113) digested vector
3/ 2b1i (1213) digested insert
4/ 2b2v (2113) digested vector
5/ 2b2i (1843) digested insert
6/ (empty)
7/ 2a5v (2116) digested vector
8/ 2a5i (1843) digested insert

pic: iGb2b2
1/ 1 kb DNA ladder
2/ 2a3v (2113) undigested vector
3/ 2b3v (2113) digested vector
4/ 2a3i (1212) undigested insert
5/ 2b3i (1212) digested insert
6/ (empty)
7/ 2b4v (2116) digested vector
8/ 2a4v (2116) undigested vector

pic: iGb2b3
1/ 1 kb DNA ladder
2/ 2a4i (1213) undigested vector
3/ 2b4i (1213) digested vector
4/ (empty)
5/ 2a6v (573) undigested vector
6/ 2b6v (573) digested vector
7/ 2a6i (538) undigested vector
8/ 2b6i (538) undigested vector

2c. Gel extraction

gel band weight:
1v (2113), 1942 bp
1i (1213), 1428 bp
2v (2113), 1942 bp
2i (1842), 1217 bp
3v (2113), 1942 bp
3i (1212), 1242 bp
4v (2116), 1942 bp: 428 mg
4i (1213), 1213 bp
5v (2116), 1942 bp
5i (1843), 1217 bp
6v (573), 1882 bp
6i (538), 8532 bp

Elute amount: all 30 uL

Take 3 concentration test
1i: 19.48 ng/uL
2v: 29. 05 ng/uL

2i: 3.81 ng/uL
3v: 21.53 ng/uL
3i: 6.82 ng/uL
4v: -12.38 ng/uL
4i: -2.87 ng/uL
5v: -10.31 ng/uL
5i: -9.15 ng/uL
6v: 9.44 ng/uL
6i: -8.63 ng/uL

2d. Ligation for Ptac-N6-chlR-IMBB2.4-pUC57-mini assembly

2v + 1i.

Enz = 1/6 dil NEB T4 DNA ligase

Rxn	vec	ins	enz	H2O
1	1	0	0	15
2	1	0	1	14
3	1	1	1	13
4	0	1	1	14

+ 4 uL homemade 5x T4 ligase buffer
---
20 uL

3v + 1i.

Enz = 1/6 dil NEB T4 DNA ligase

Rxn	vec	ins	enz	H2O
1	1.2	0	0	14.8
2	1.2	0	1	13.8
3	1.2	1	1	12.8
4	0	1	1	14

+ 4 uL homemade 5x T4 ligase buffer
---
20 uL

17 deg water bath 6/14/17 1:13 PM

Gel lane:
pic: iGb2d1
1/ 11 (3v+1i) VOO
2/ 12 (3v+1i) VOE

3/ 13 (3v+1i) VIE
4/ 14 (3v+1i) OIE
5/ 21 (2v+1i) VOO
6/ 22 (2v+1i) VOE
7/ 23 (2v+1i) VIE
8/ 24 (2v+1i) OIE

pic: iGb2d2
1/ 31 (2v+1i) VOO
2/ 32 (2v+1i) VOE
3/ 33 (2v+1i) VIE
4/ 34 (2v+1i) OIE
5/ 61 (3v+1i) VOO
6/ 62 (3v+1i) VOE
7/ 63 (3v+1i) VIE
8/ 64 (3v+1i) OIE

2e. Transformation Ptac-N6-chlR-IMBB2.4-pUC57-mini plasmid into E.coli

2v+1i: (21,22,23,24; 31,32,33,34; 41,42,43,44)
Rxn 1 (VOO): 1 ng ligation = 0.7 uL
Rxn 2 (VOE): 1 ng ligation = 0.7 uL
Rxn 3 (VIE): 1 ng ligation = 0.5 uL
Rxn 4 (OIE): 1 ng ligation = 1 uL

3v+1i: (11,12,13,14; 61,62,63,64)
Rxn 1 (VOO): 1 ng ligation = 0.77 uL
Rxn 2 (VOE): 1 ng ligation = 0.77 uL
Rxn 3 (VIE): 1 ng ligation = 0.5 uL
Rxn 4 (OIE): 1 ng ligation = 1.1 uL

25 uL E. coli Mach 1
85 uL spread on LB-amp plates
37 deg incubate 6/15/17 12:36PM

Results: 6/16/17 9:30 AM
11 (VOO): 0 cfu/85 uL
12 (VOE): 1 cfu/85 uL
13 (VIE): 26 cfu/85 uL
14 (OIE): 6 cfu/85 uL
21 (VOO): 3 cfu/85 uL

22 (VOE): 41 cfu/85 uL
23 (VIE): ~400 cfu/85 uL
24 (OIE): 3 cfu/85 uL
31 (VOO): 0 cfu/85 uL
32 (VOE): 0 cfu/85 uL
33 (VIE): ~300 cfu/85 uL
34 (OIE): 0 cfu/85 uL
41 (VOO): 0 cfu/85 uL
42 (VOE): 0 cfu/85 uL
43 (VIE): ~1000 cfu/85 uL
44 (OIE): 310 cfu/85 uL
61 (VOO): 0 cfu/85 uL
62 (VOE): 1 cfu/85 uL

63 (VIE): 3 cfu/85 uL
64 (OIE): 0 cfu/85 uL

2f. Re-streak colonies from plates

select 3 colonies each from plate 13, 23, 63
to be 2f11, 2f12, 2f13; 2f21, 2f22, 2f23; 2f61, 2f62, 2f63
cultured in 6 mL LB-amp,chl (amp 100 ug/mL, chl 34 ug/mL)
37 deg rotator 6/16/17 11:30 AM

2g. Restriction mapping of Ptac-N6-chlR-IMBB2.4-pUC57-mini

Plasmid preparation for group 2g11, 2g12, 2g13; 2g21, 2g22, 2g23;
2g61, 2g62, 2g63

Elute amount:
2g11, 2g12, 2g13: 30 uL
2g21, 2g22, 2g23:
2g61, 2g62, 2g63: 40 uL

Take 3 concentration test:
2g11: 53.92 ng/uL
2g12: 51.537 ng/uL
2g13: 52.567 ng/uL
2g21
2g22
2g23

2g61: 63.3 ng/uL
2g62: 47.489 ng/uL
2g63: 93.424 ng/uL

For 2g11, 2g12, 2g13, 2g21, 2g22, 2g23; 2g61, 2g62

Digested with ScaI, expect 2 fragments, 1841 bp and 1529 bp

20 uL plasmid
15 uL mqH2O
1 uL ScaI
4 uL 10x NEB buffer 3.1
---
40 uL

37 deg incubated 6/17/17 4:06 PM

Gel lane:
pic iGb2g1
pic iGb2g2

Select sample 11 and make -80 deg frozen stock

exp. MA3

I. Title: Phosphate Assay

II. Purpose: to develop a working phosphate assay for the WaterHub phosphate removal project

III. Calculations

Malachite Green solution:
-made 7/6/17

10 mL of sulfuric acid (H_2SO_4)
50 mL of DI water
0.075 g of malachite green
*can be kept at room temperature for up to a year

Ammonium Molybdate Solution:
-made 7/6/17

7.5% ammonium molybdate= 10 mL of water + 0.75 g of ammonium molybdate

Tween 20 solution:
-made 7/6/17

11% tween 20= 1.1 mL of tween 20 + 8.9 mL of water

From: Baykov AA, Evtushenko OA, Avaeva SM. 1988. A malachite green procedure
for orthophosphate determination and its use in alkaline phosphatase-based enzyme immunoassay. Anal Biochem. 171: 266-270.

Phosphate Standard Concentrations

compound	MW	g/100 mL for 0.2 M
monobasic sodium phosphate monohydrate	137.99	2.76
dibasic sodium phosphate heptahydrate	268.07	5.364

IV. Procedure

MA3a. Creation of color reagent

1. Slowly add concentrated sulfuric acid to water
2. cool solution to room temperature
3. add malachite green

on the day of use:
4. add 2 mL of color reagent to 500 uL of the ammonium molybdate solution
5. add 40 uL of 11% tween 20

MA3b. Creation of Phosphate Concentration Curve

1. create 0.1 M phosphate buffer at pH 7
    - Add 39 mL of 0.2 M monobasic sodium phosphate to 61 mL of 0.2 M dibasic sodium phosphate
    - Add 100 mL to total 200 mL and end up with 1 M
    - made at 2 pm on 7/3/17 pH of 7.088

2. Add one volume of the color reagent to 4 volumes of sample in a clear 96 well
   plate and let the mixture sit at room temperature for 10 minutes
     -50 uL of color reagent with 200 uL of phosphate sample
     -leave lid on for readings
3. measure absorbance at 630 nm
     -Phosphate Test protocol on gen5
4. record results and graph to create concentration curve

MA3b1.
Concentrations measured at (uM):
100000, 50000, 25000, 12500, 6250, 3125, 1560, 780, 390, 195, 97.5, 48.75, 24.375, 12.2, 6.09, 3.045, 1.5225, 0.76

Concentrations measured on plate reader every minute for 15 minutes six minutes after the color reagent was added
Above 97.5 uM did not produce any usable data, absorbances measured below 97.5 uM showed pretty consistent data points

MA3b2.

Concentrations measured at (uM):
90, 72, 57.6, 46.08, 36.864, 29.4912, 23.59, 18.87, 15.099, 12.08, 9.66, 7.73, 6.18, 4.94, 3.96,
3.17, 2.54, 2.03, 1.62, 1.29, 1.0387

Waterhub samples:
C1-C6:
1. raw sewage= 152.6
2. MBBR2= 136.06
3. MBBR3= 130.90
4. HR4= 112.90
5. HR5= 71.44
6. Final Product= 10.58

Trendline was taken from data with a r-squared of 0.9799
However, trendline does not take into account data from the waterhub range so the numbers are extrapolated

MA3b3.

Concentrations measured at (uM):

300, 270, 243, 200, 180, 162, 145.8, 131.22, 118.098, 106.288, 95.66, 90, 72, 57.6, 46.08, 36.864, 29.4912, 23.59, 18.87, 15.099, 12.08, 9.66, 7.73, 6.18, 4.94, 3.96,
3.17

Test samples:
LB
LB + cells
3.17 uM phosphate buffer + cells

recorded absorbance every minute for 45 minutes
Trendlines were made, but r-squared was lower than last test
Same phosphate concentrations yielded different results
Phosphate readings were very high for both LB, LB with cells, and phosphate buffer with cells

exp. MA4

I. Title: Phosphate Accumulating E coli. from keio and aska collection

II. Purpose: To make keio collection cells competent in order to engineer them to uptake, and accumulate phosphates.

III. Calculations

IV. Procedure

MA4a. Growing Keio and ASKA collection cells

Streak out these cells and put in 37 deg overnight 6/29/17 2:15 pm- 6/30/17 11:20 am
Put in 4 deg 6/30/17 11:35 am for the weekend

Keio Collection
MA4a1- parental strain BW25113- 1540 in frozen stock
MA4a2- delta ppx (plate 47 row 7 column A)
MA4a3- delta ppk (plate 5 row 10 column D)

ASKA Collection
MA4a4- ppk strain (plate 23 row 12 column F)
MA4a5- ppx strain (plate 23 row 12 column G)
MA4a6- Lac Z strain (plate 4 row 4 column B)

MA4b. Making keio collection cells competent (Protocol from general lab protocol)

1. Pick a single colony from a plate and grow to saturation, a 2mL LB only culture,
   grow at 37 deg for about eight hours.

     MA4b1- parental strain
     MA4b2- delta ppx
     MA4b3- delta ppk

     7/3/17 2:45 pm- 7/4/17 10:15 am

2. Then dilute 1:1000 in another batch of the same media it was grown to saturation in.
     - 25 uL in 25 mL of LB put in 18 deg shaker at 200 rpm at 7/4/17 at 11:05 am

3. Grow culture in 18°C shaker for a few hours until it reaches an O.D. of 0.4-0.6 (almost fully saturated, at 600nm).
     *Ask about growth rate of specific strains. Most take overnight. Record in growth rates folder the times it took to grow to mid-log. Make sure to blank machine.
     a. After it reaches OD of 0.2, check every 15 min

     - 10:15 AM 7/5/17 Parent Strain OD 600= 0.38
                Delta ppx OD 600= 0.34
                Delta ppk OD 600= 0.18
     - 10:50 AM 7/5/17 Parent Strain OD 600= 0.42
                Delta ppx OD 600= 0.34
     - 1:27 PM 7/5/17 Delta ppk OD 600= 0.30

4. Transfer culture to sterile plastic bottle. Spin culture at 3000rpm in JA-10 rotor for 10 min at 4°C.
     a. Can use falcon tube b/c of low speed spins
     b. Smaller rotor: 3.5k x 15min

5. Dump supernatant and resuspend in TB ice-cold at 0.4 the volume of culture media very, very, very gently on ice.
     a. Resuspend, put on ice, resuspned, put on ice repeat until pellet is dissolved; little TB at a time.

6. Spin culture at 3000rpm in JA-10 rotor for 10 min at 4°C.
    a. Smaller rotor: 3.5k x 15min

7. Dump supernatant and resuspend in TB at 0.08 the volume of culture media. *resuspend, put on ice, resuspend, put on ice repeat until pellet is dissolved.
    - 1.86 mL of TB buffer and 140 uL of DMSO

8. Then add DMSO (wear gloves) so it equals 7% of the current volume (560uL) and distribute into micro tube in 200uL aliquots on dry ice (use pipette with multi-dispenser).
    -Blue= parent strain
    -Green= delta ppx
    -Pink= delta ppk
    -Shelf 3 box 9

MA4c.Transforming Competent Keio Cells with Aska plasmids

Picked Colonies of Aska cells to grow overnight
    -4 mL culture with 4 uL of Chl
    -put in 37 deg shaker at 4 pm 7/5/17
Taken out at 10:30 am 7/6/17
    -plasmid prep in the QIAcube
    -eluted in 50 uL of EB buffer
Concentrations from take 3

MA4a4 ppk= 204.919 ng/uL
MA4a5 ppx= 291.675 ng/uL
MA416 lac z= 257.196 ng/uL

each diluted to about 1 ng/uL

Transformations

Strains	ppk	ppx	lac Z
parental	ppk + parental (1.4)	ppx + parental (1.5)	lac Z + parental (1.6)
delta ppx	ppk + delta ppx (2.4)	ppx + delta ppx (2.5)	lac z + delta ppx (2.6)

delta ppk ppk + delta ppk (3.4) ppx + delta ppk (3.5) lac z + delta ppk (3.6)

1. 3.4 uL of bme in each tube of competent cells
2. 25 uL of cells per reaction tube
3. 1 uL of plasmids per tube
4. heat shock program
5. 100 uL of SOC at the end of program
6. 37 deg for 1 hour
7. cells plated on LB+chl plates
8. plates put in 37 deg overnight
   -3:11 pm 7/6/17- 10:05 am 7/7/17
   -got a lot of transformants on all plates!
   -put in refrigerator for storage

Exp. iGb3

Title: Identifying the bacteria in the waterhub water sample

7/14/17
iGb3a. Plating water samples and bacteria samples onto LB plates

samples:
1. raw sewage
2. MBBR2 - processing tss and bod anoxic
3. MBBR3 - aerobic, moving beds
4. HR1 (hydroponic reactors) - after tank 4
5. HR5 - before clarifier
6. final product

MB1-2. Moving bed from water sample 3 source

1-6. 300 uL of sewage water
MB1. From a dried moving bed bioreactor
MB2. From a wet moving bed bioreactor

Plated on LB and incubated in 37 deg 7/14/17 3:43 PM

Growth:
1. ~1000+ cfu
2. ~1000+ cfu
3. ~200 cfu
4. ~200 cfu
5. ~200 cfu
6. 0 cfu

MB1. ~100 cfu
MB2. ~50 cfu

Stored in 4 deg room 7/15/17 12:45 PM

Select morphologically different bacteria from each plate and streak on LB individually, as
1i, 1ii, 1iii, 1iv;
2i, 2ii, 2iii, 2iv;
3i, 3ii, 3iii, 3iv, 3v;
4i, 4ii, 4iii, 4iv, 4v, 4vi;
5i, 5ii, 5iii, 5iv;
MB1i, MB1ii, MB1iii, MB1iv, MB1v;
MB2i, MB2ii, MB2iii, MB2iv

Incubated in 37 deg room 7/17/17 2:30 PM
Moved to 4 deg fridge 7/18/17 2:30 PM

Making -80 deg frozen stock of 3iv:
Raise 6 mL LB culture
37 deg rotator at 9/17/17 2:20 PM
retrieved at 9/18/17 1:30 PM
imc stock 2365

iGb3b. Test the phosphate assimilation ability of sample (water hub) bacteria

culture bacteria for control set in 10 mL LB broth
1. E. coli parent strain WB25113
2. delta ppx, E. coli
3. delta ppx + ppk plasmid (positive)
4. parental E. coli + ppk plasmid
5. parental E. coli + lacZ plasmid (negative)

culture sample bacteria in 12 mL LB broth
Bacteria samples from water hub moving bed bioreactor:
MB1i as 11, MB1ii as 12, MB1iii as 13, MB1iv as 14, MB1v as 15,
MB2i as 21, MB2ii as 22, MB2iii as 23, MB2iv as 24

Incubated on 37 deg rocker 7/25/17 5:30 PM

Taken out from 37 deg rocker 7/26/17 10:15 PM

Centrifuge in 4 deg at 2500 rpm for 5 or 10 min, discard the supernatant

Add 30 mL of 1500 uM phosphate buffer to each, resuspend cell pellet by inverting

Centrifuge in 4 deg at 2500 rpm for 10 min, discard the supernatant

Add 32 mL of 1500 uM phosphate buffer to each, resuspend cell pellet by inverting 7/26/17 12:25 PM

Water hub bacteria sample 12, 13, 15 not able to distribute evenly in the solution, stop testing

Reference buffer concentrations:
0 uM, 9.66 uM, 12.07 uM, 15.1 uM, 18.9 uM, 23.6 uM, 29.5 uM, 36.86 uM, 46.08 uM, 57.6 uM, 72 uM, 90 uM, 95.66 uM, 106 uM, 118.1 uM, 131.2 uM, 145.8 uM, 162 uM, 180 uM, 200 uM

Two replications for each test, four time points
Distribute each sample into eight 1.7 mL tubes labeled as .1.0, .1.1, .1.2, .1.3 for the first replicate, and as .2.0, .2.1, .2.2, .2.3 for the second replicate

First time point (.1.0 and .2.0): 7/26/2017 1:20 PM
Centrifuged 13 kpm for 5 min, take 8 uL and diluted with 72 uL mqH2O as sample

Second time point (.1.1 and .2.1): 7/26/2017 2:40 PM
Centrifuged 13 kpm for 5 min, take 8 uL and diluted with 72 uL diH2O as sample

exp. MA6

I. Title: Testing engineered E. coli in phosphate buffer

II. Purpose: To see whether different variations of the engineered E. coli exhibit variations in phosphate accumulation. To test the validity of our experimental protocol.

III. Calculations

IV. Procedure

MA6a. Preparing engineered E. coli in LB or LB + chl

MA6a1: MA4a1= parent strain
MA6a2: MA4a2= delta ppx
MA6a3: MA4c1.4= delta ppx + ppk
MA6a4: MA4c2.4= parental + ppk
MA6a5: MA4c1.6= parental + lac z

Grown in LB or LB+chl 10 mL culture in 50 mL tube
Grown O/N 5:20 pm 7/25/17- 10:20 am 7/26/17

MA6b. Testing the bacteria's effect on phosphate concentration over time

MA6b1. cells

- centrifuged 2500 rpm for 5 or 10 min depending on the sample
- LB supernatant was dumped
- cells resuspended in 30 mL of 1500 uM phosphate buffer
- centrifuged at 2500 rpm for 10 min
- phosphate buffer supernatant dumped
- cells resuspended in 32 mL of 1500 uM phosphate buffer
- 1 mL of resuspended cells put into 1.5 mL tubes labeled with the strain and time point
- tubes centrifuged at 10k rpm for 5 mins at specified time point
    -specified time points
        t0= 30 mins
        t2= 1 hr
        t3= 2.5 hrs
        t4= 24 hrs?
- 8 uL of supernatant put into corresponding 96-well plate position
- 72 uL of DI water added into each well with sample to dilute by a factor of ten
- 20 uL of phosphate assay color reagent added
- absorbances read every minute for 45 minutes

MA6b2. Standard curve concentrations

200-> 180-> 162-> 145.8-> 131.22-> 118.098-> 106.288-> 95.66-> 90-> 72-> 57.6-> 46.08-> 36.864-> 29.4912-> 23.59-> 18.87-> 15.099-> 12.08-> 9.66-> 0

Conclusions:
- Need to use lower range of concentration measurements

MA6c. Testing parental and delta ppx

10 mL culture of each overnight 7/27/17-7/28/17
5 mL used for each concentration tested
MA6c1= parental strain
MA6c2= delta ppx

Concentrations curve:

90-> 72-> 57.6-> 46.08-> 36.864-> 29.4912-> 23.59-> 18.87-> 15.099-> 12.08-> 9.66-> 7.73-> 6.18-> 4.94-> 3.96-> 3.17-> 2.536-> 2.0288-> 1.62-> 0

Preparing the cells:

1500 uM cells
- centrifuged 2500 rpm for 10 min
- LB supernatant was dumped
- cells resuspended in 25 mL of 1500 uM phosphate buffer
- centrifuged at 2500 rpm for 10 min
- phosphate buffer supernatant dumped
- cells resuspended in 15 mL of 1500 uM phosphate buffer
- 1 mL of resuspended cells put into 1.5 mL tubes labeled with the strain and time point
- tubes centrifuged at 13k rpm for 5 mins at specified time point
    -specified time points
        t0= 20 mins
        t2= 2 hr
        t3= 4 hrs
- 2 uL of supernatant put into corresponding 96-well plate position
- 78 uL of DI water added into each well with sample to dilute by a factor of 40
- 20 uL of phosphate assay color reagent added
- absorbances read every minute for 30 minutes

3000 uM cells

- centrifuged 2500 rpm for 10 min
- LB supernatant was dumped
- cells resuspended in 25 mL of 3000 uM phosphate buffer
- centrifuged at 2500 rpm for 10 min

Exp. iGb4

Title: Testing the growth and phosphate assimilation of Keio cell collections

4a. Making low phosphate media based on Davis-Mingioli

500 mL DM-glucose 1000 no phosphate media:
37.9 mL 1 M KCl
0.5 g ammonium sulfate (NH4)2SO4
0.25 g sodium citrate (trisodium, dihydrate)
0.42 mL 1 M MgSO4
10 mL 10% glucose (DM1000)
pH of the media = 6.0

Testing the media
5.5 mL DM-Glc no Pi
5.5 mL x 45.3 mM = 2.49 mL x 0.1 M phosphate buffer
reduce volume to 5.5 mL total
keio cell collection parental+lacZ

37 deg rotator 8/4/17 7:02 PM

retrieved on 8/7/17
cell density 1.12, pH = 5.5

4b. Test the growth rate of delta ppx and its parental bacterial cells

7 mL DM-Glc no Pi
7 mL x 1 mM = 70 uL x 0.1 M phosphate buffer
7 mL x 2 mM = 140 uL x 0.1 M phosphate buffer

Malachite green test works in the range of 0-100 uM = 0-0.1 mM
All sample need to be diluted 1:20

4 uL sample and 76 uL diH2O mixture

on plate iGb4b1:
A1-A12, B1-B7: serial dilution of 120 uM, 108 uM, 97.2 uM, 87.48 uM, 78.7 uM, 70.86 uM, 63.77 uM, 60 uM, 48 uM, 38.4 uM, 30.72 uM, 24.58 uM, 19.66 uM, 15.73 uM, 12 uM, 8.4 uM, 5.88 uM, 4.12 uM, 0 uM
Trendline [Pi] = 70.484 abs + 9.9946, R-sq = 0.9875
C1-C12: reading at 8/08/17 1:00 PM
D1-D12: reading at 8/08/17 4:30 PM
E1-E12: reading at 8/08/17 7:40 PM

on plate iGb4b2:
A1-A12: Pi gradient 100, 90, 80, 70, 60, 50, 40, 30, 20, 10, 5, 0 uM
Trendline [Pi] = 63.33 abs + 8.7663, R-sq = 0.9974
B1-B12: not diluted, ignored
C1-C12: reading at 8/09/17 12:45 PM
E1-E12: reading at 8/09/17 6:25 PM

Cell concentrations:

	0h	4.5h	7.5h	24h	29.5h
parental in 1 mM	0.00	0.00	0.067	0.243	0.1867
parental in 2 mM	0.00	0.003	0.0067	0.3167	0.2833
delta ppx in 1 mM	0.0033	0.0067	0.0133	0.26	0.1967
delta ppx in 2 mM	0.0033	0.01	0.0133	0.31	0.2967

Phosphate levels:

	0h	4.5h	7.5h	24h	29.5h
parental in 1 mM	1134	989	941	802	805
parental in 2 mM	1694	1596	1530	1660	1649
delta ppx in 1 mM	667	1078	1045	896	1032
delta ppx in 2 mM	1536	1538	1485	1677	1798

4c. Test the growth of E. coli with limited phosphate source

6 mL of DM-glc 1000 media with no phosphate

add phosphate buffer

reference: 45 mM x 6 mL, normal DM media level, not experimented

0.1 M x 60 uL = 1000 uM x 6 mL
0.1 M x 18 uL = 300 uM x 6 mL
0.1 M x 6 uL = 100 uM x 6 mL
0.1 M x 1.8 uL = 30 uM x 6 mL
0.1 M x 0.6 uL = 10 uM x 6 mL
1000 uM x 300 uL = 50 uM x 6 mL (mismarked as 5 uM)
1000 uM x 120 uL = 20 uM x 6 mL (mismarked as 2 uM)
1000 uM x 60 uL = 10 uM x 6 mL (mismarked as 1 uM)
1000 uM x 18 uL = 3 uM x 6 mL (mismarked as 0.3 uM)
1000 uM x 6 uL = 1 uM x 6 mL (mismarked as 0.1 uM)
1000 uM x 0 uL = 0 uM x 6 mL, negative control

Bacteria as parental+lacZ from Keio collection

37 deg rotator 8/09/17 9:02 PM

Test on 8/14/17 6:30 PM, 5 days
on plate iGb4c1
A1-A12: (old) Pi gradient 110, 100, 90, 80, 70, 60, 50, 40, 30, 20, 10, 5 uM
B1-B12: Pi gradient 200, 180, 160, 140, 130, 120, 110, 100, 90, 80, 70, 60 uM
C1-C7: Pi gradient 50, 40, 30, 20, 10, 5, 0 uM
D1-D12: samples (double) of 30, 10, 50, 20 uM originally, not centrifuged
E1-F10: samples (double) of 1000, 300, 100, 30, 10, 50, 20, 10, 3, 1, 0 uM originally, centrifuged supernatants
Phosphate levels:

1000 uM set: 786.536 uM &	300 uM set: 172.309 uM &
100 uM set: -1.389 uM	30 uM set: -1.312 uM
10 uM set: -1.719 uM	50 uM set: 0.090 uM
20 uM set: -1.478 uM	10 uM set: -1.739 uM
3 uM set: -1.680 uM	1 uM set: -1.628 uM
0 uM set: -1.569 uM	

4d. Test the phosphate concentration of waterhub sewage sample

reference buffer concentration is set to be (uM)
100, 90, 80, 70, 60, 50, 40, 30, 20, 10, 5, 0

A1-A12: concentration gradient 100 uM, 90 uM, 80 uM, 70 uM, 60 uM, 50 uM, 40 uM, 30 uM, 20 uM, 10 uM, 5 uM, 0 uM
Trendline [Pi] = 63.33 abs + 8.7663, R-sq = 0.9974
D1-D12: serial dilution of waterhub sample,

The concentrations of the dilution of waterhub sample are measured to be:
1/2 dilution x 2 = 231.86 uM
1/4 dilution x 4 = 278.73 uM
1/8 dilution x 8 = 289.86 uM
1/16 dilution x 16 = 278.21 uM
average tested concentration of waterhub sample is 269.67 uM

4e. Test the growth of E. coli on waterhub sewage sample with glucose, MgSO4 and K+/Na+/NH4+

Each well contains:
250 uL waterhub sewage
1 uL keio parental+lacZ LB culture

Options to add to autoclaved sewage sample:
10%(w/v) glucose = 555.06 mM
reference: DM1000 media = 5.55 mM
250 uL x 5.55 mM = 2.5 uL x 555 mM

1 M MgSO4 solution
1:100 diluted to be 10 mM
250 uL x 0.1 mM = 2.5 uL x 10 mM

1:50 ion solution of sodium, potassium and ammonium
1.49 g (0.0255 mol) NaCl, 0.255 M
8.24 g (0.11 mol) KCl, 1.1 M
4.07 g (0.076 mol) NH4Cl, 0.76 M
in 100 mL water
5 uL for each well

#	sewage	culture	glucose	MgSO4	K/Na/NH4
1	250	1	2.5	2.5	5
2	250	1	2.5	2.5	0
3	250	1	2.5	0	5
4	250	1	2.5	0	0
5	250	1	0	2.5	5

6	250	1	0	2.5	0
7	250	1	0	0	5
8	250	1	0	0	0

37 deg shaker 8/12/17 3:00 PM

Cell concentration test at 8/14/17 3:40 PM and 8/16/17 1:11 PM:
Avg abs of 200 uL water 0.0429 and 0.037

1	0.108 ± 0.0077	0.106 ± 0.00846	
2	0.13 ± 0.0041	0.121 ± 0.0106	
3	0.16 ± 0.022	0.162 ± 0.0252	
4	0.28 ± 0.029	0.273 ± 0.0418	&&
5	0.067 ± 0.0031	0.072 ± 0.00438	
6	0.054 ± 0.0055	0.049 ± 0.0015	
7	0.060 ± 0.0079	0.052 ± 0.00123	
8	0.065 ± 0.0078	0.054 ± 0.00123	

4f. Test the growth of E. coli on waterhub sewage sample with various levels of glucose

Each well contains:
250 uL waterhub sewage
1 uL keio parental+lacZ LB culture

Options to add to autoclaved sewage sample:
10%(w/v) glucose = 555.06 mM
reference: DM1000 media = 5.55 mM

diluted 1:10
50 uL x 55.5 mM = 250 uL x 11.1 mM
35 uL x 55.5 mM = 250 uL x 7.77 mM
25 uL x 55.5 mM = 250 uL x 5.55 mM
20 uL x 55.5 mM = 250 uL x 4.44 mM
15 uL x 55.5 mM = 250 uL x 3.33 mM
10 uL x 55.5 mM = 250 uL x 2.22 mM
5 uL x 55.5 mM = 250 uL x 1.11 mM
0 uL x 55.5 mM = 250 uL x 0 mM

37 deg shaker 8/15/17 12:00 PM
retrieved 8/18/17 4:53 PM

OD cell concentration:
11.1 mM: 0.056 ± 0.013
7.77 mM: 0.061 ± 0.007
5.55 mM: 0.085 ± 0.022
4.44 mM: 0.087 ± 0.015
3.33 mM: 0.128 ± 0.049
2.22 mM: 0.149 ± 0.036
1.11 mM: 0.136 ± 0.012
0 mM: 0.062 ± 0.011

4g. Test the growth and phosphate assimilation of ppk and ppx genes in Keio cell collection in controlled phosphate media

Raise cultures of cells in LB
1. parental + lacZ
2. parental + ppk
3. parental + ppx
4. delta ppk + lacZ
5. delta ppx + lacZ
6. delta ppk + ppx
7. delta ppx + ppk
8. delta ppk + ppk
9. delta ppx + ppx
10. no bacteria, negative control

from right to left on the plate

37 deg rotator 8/15/17 5:24 PM
retrieved 8/16/17 12:10 PM

250 uL DM-glc no phosphate media
1 uL culture
0.01 M x 1.5 uL = 60 uM x 250 uL IPTG
0.01 M x 7.5 uL = 300 uM x 250 uL phosphate buffer, diluted 1:10

37 deg shaker 8/16/17 1:18 PM
retrieved 8/18/17 4:53 PM

1:4 diluted for phosphate test (20 uL sample, 60 uL H_2O, 20 uL test reagent)

bacterial cell concentrations: plate iGb4g1 (data omitted)

phosphate level test: plate iGb4g2
A1-A12: -, 100, 90, 80, 70, 60, 50, 40, 30, 20, -, 0 uM buffer
B1-B12: 110, 100, 90, -, 70, 60, 50, 40, 30, 20, 10, 0 uM buffer
(- stands for empty well)
C/D/E/F1-10: Group 10-Group 1, C7 is empty

	phosphate level	
1. parental + lacZ	285.99 ± 3.92	
2. parental + ppk	289.37 ± 3.74	
3. parental + ppx	111.63 ± 7.63	&&
4. delta ppk + lacZ	295.07 ± 25.08	
5. delta ppx + lacZ	233.83 ± 5.50	
6. delta ppk + ppx	226.38 ± 5.63	
7. delta ppx + ppk	198.38 ± 11.41	&
8. delta ppk + ppk	242.33 ± 13.18	
9. delta ppx + ppx	159.71 ± 8.84	&
10. no bacteria	259.54 ± 25.32	

repeating the experiment:

Raise cultures of cells in 400 uL LB
1. parental + lacZ
2. parental + ppk
3. parental + ppx
4. delta ppk + lacZ
5. delta ppk + ppk
6. delta ppk + ppx
7. delta ppx + lacZ
8. delta ppx + ppk
9. delta ppx + ppx
10. no bacteria, negative control

37 deg rotator 8/28/17 5:18 PM
retrieved at 8/29/17 4:40 PM

250 uL DM-glc no phosphate media
1 uL culture
0.01 M x 1.5 uL = 60 uM x 250 uL IPTG
0.01 M x 7.5 uL = 300 uM x 250 uL phosphate buffer, diluted 1:10

Plate planning:
A/B1-12: standard concentration gradient from 110 uM to 0 uM
From left to right, group 1 (C/D/E/F2) to group 10 (C/D/E/F11)
Quadruple set for each group

37 deg shaker 8/29/17 6:16 PM
retrieved at 8/31/17 4:30 PM

bacterial cell concentration: plate iGb4g4
1:4 diluted for phosphate concentration test: plate iGb4g5

	OD cell conc.	Phosphate level	
1. parental + lacZ	0.286 ± 0.028	55.38 ± 3.77	
2. parental + ppk	0.296 ± 0.025	46.92 ± 2.04	
3. parental + ppx	0.233 ± 0.028	54.51 ± 2.61	
4. delta ppk + lacZ	0.317 ± 0.027	52.22 ± 2.62	
5. delta ppk + ppk	0.259 ± 0.037	40.48 ± 15.49	&&
6. delta ppk + ppx	0.322 ± 0.023	43.51 ± 2.57	&
7. delta ppx + lacZ	0.285 ± 0.025	56.47 ± 3.59	
8. delta ppx + ppk	0.323 ± 0.013	44.65 ± 2.78	
9. delta ppx + ppx	0.193 ± 0.026	55.14 ± 2.61	
10. no bacteria	0.229 ± 0.034	54.31 ± 1.95	

4h. Test the growth and phosphate assimilation of ppk and ppx genes in Keio cell collection in waterhub sewage plus glucose

Raise cultures of cells in 400 uL LB
1. parental + lacZ
2. parental + ppk
3. parental + ppx
4. delta ppk + lacZ
5. delta ppk + ppk
6. delta ppk + ppx
7. delta ppx + lacZ
8. delta ppx + ppk
9. delta ppx + ppx
10. no bacteria, negative control

250 uL DM-glc no phosphate media
1 uL culture
0.01 M x 7.5 uL = 300 uM x 250 uL phosphate buffer, diluted 1:10

No IPTG added, failed design

4i. Test the growth and phosphate assimilation of wild waterhub bacteria in controlled phosphate media

Raise culture in 400 uL LB broth
1i-iv, 2i-iv, 3i-v, 4i-vi, 5i-iv, MB1i-v, MB2i-iv

37 deg rotator 8/28/17 5:18 PM
retrieved at 8/29/17 4:40 PM

250 uL DM-glc no phosphate media
1 uL culture
0.01 M x 7.5 uL = 300 uM x 250 uL phosphate buffer, diluted 1:10

Plate planning:
On plate iGb4i5: C/D3-10, E/F3-10
1i 1iii 2i 2iii 3i 3iii 3v 4ii
1ii 1iv 2ii 2iv 3ii 3iv 4i 4iii
On plate iGb4i6: C/D3-10, E/F3-10
4iv 4vi 5ii 5iii MB1ii MB1iv MB2i MB2iii
4v 5i 5iv MB1i MB1iiiMB1v MB2ii MB2iv
double set for each group, blank set on the left with LB added on both plates at B4-5

37 deg shaker 8/29/17 6:16 PM
retrieved at 8/31/17 4:30 PM

Cell concentration test: plate iGb4i5 and iGb4i6
Phosphate level test: plate iGb4i7
Plate planning:
A1-12: 110 uL down to 0 uL phosphate buffer diluted with diH2O
B1-12: 110 uL down to 0 uL phosphate buffer diluted with mqH2O
C/D1-12: 1i, 1iii, 2i, 2iii, 3i, 3iii, 3v, 4ii, 1ii, 1iv, 2ii, 2iv
E/F1-12: 3ii, 3iv, 4i, 4iii, 4iv, 4vi, 5ii, 5iii, MB1ii, MB1iv, MB2i, MB2iii
G/H1-10: 4v, 5i, 5iv, MB1i, MB1iii, MB1v, MB2ii, MB2iv, blank iGb4i5 and iGb4i6

	cell conc.	Pi level(uM)
1i	0.506	76.2
1ii	0.507	50.9
1iii	0.217	171.1
1iv	0.612	93.5

2i	0.154	225.3
2ii	0.255	237.8
2iii	0.378	27.0 &
2iv	0.497	65.9
3i	0.435	33.7 &
3ii	0.248	179.3
3iii	0.246	160.2
3iv	0.804	-1.3 &&
3v	0.219	93.0
4i	0.433	68.5
4ii	0.390	161.0
4iii	0.253	166.0
4iv	0.166	196.6
4v	0.750	4.0 &&
4vi	0.183	177.7
5i	0.411	148.4
5ii	0.166	199.7
5iii	0.165	189.7
5iv	0.192	204.6
MB1i	0.820	-0.4 &&
MB1ii	0.451	37.6 &
MB1iii	0.263	258.8
MB1iv	0.474	24.0 &
MB1v	0.203	119.0
MB2i	0.266	108.9
MB2ii	0.276	166.7
MB2iii	0.195	194.2
MB2iv	0.320	213.3
Bl.1	0.216	206.3
Bl.2	0.175	187.6

By highest OD: MB1i, 3iv, 4v, 1iv, 1ii, 1i, 2iv, MB1iv, MB1ii
By lowest phosphate: 3iv, MB1i, 4v, MB1iv, 2iii, 3i, MB1ii, 1ii

4j. Test the growth and phosphate assimilation rate of selected Keio bacteria and waterhub bacteria in waterhub sewage water plus glucose

Raise bacteria culture in 400 uL LB media
1. parental + ppk
2. delta ppk + ppk
3. delta ppk + ppx
4. delta ppx + ppk

5. waterhub 2iii
6. waterhub 3i
7. waterhub 3iv
8. waterhub 4v
9. moving bed MB1i
10. moving bed MB1ii
11. moving bed MB1iv
12. no bacteria, negative control

37 deg rotator 9/5/17 7:15 PM
retrieved at 9/6/17 3:39 PM

250 uL autoclaved waterhub sewage sample
10 uL x 55.5 mM = 250 uL x 2.22 mM glucose
1.5 uL x 0.01 M = 250 uL x 60 uM IPTG
add 1 uL bacteria LB culture

Plate planning on iGb4j1:
B11-12: blank, just media no LB
C1-4: delta ppx + ppk; C5-8: 4v; C9-12: negative control (with LB)
D1-4: delta ppk + ppx; D5-8: 3iv; D9-12: MB1iv
E1-4: delta ppk + ppk; E5-8: 3i; E9-12: MB1ii
F1-4: parental + ppk; F5-8: 2iii; F9-12: MB1i
Quadruple sets for each sample

37 deg shaker at 9/6/17 6:10 PM
retrieved 9/8/17 4:10 PM
1:4 diluted for phosphate test, on plate iGb4j2

	cell conc.(OD)	phosphate level	
parental + ppk	0.269 ± 0.011	96.02 ± 6.06	
delta ppk + ppk	0.204 ± 0.007	59.24 ± 2.70	
delta ppk + ppx	0.237 ± 0.010	150.10 ± 2.74	
delta ppx + ppk	0.197 ± 0.004	133.98 ± 5.08	
2iii	0.268 ± 0.010	180.09 ± 7.87	
3i	0.180 ± 0.012	197.44 ± 8.97	
3iv	0.324 ± 0.010	103.59 ± 7.20	
4v	0.168 ± 0.039	11.85 ± 7.08	&&
MB1i	0.306 ± 0.043	150.08 ± 8.49	
MB1ii	0.378 ± 0.013	157.06 ± 5.01	
MB1iv	0.354 ± 0.004	140.10 ± 3.45	
negative control	0.137 ± 0.072	207.08 ± 23.23	

Repeating the experiment with no IPTG:

Raise bacteria culture in 400 uL LB media
1. E. coli parental+lacZ
2. waterhub 2iii
3. waterhub 3i
4. waterhub 3iv
5. waterhub 4v
6. moving bed MB1i
7. moving bed MB1ii
8. moving bed MB1iv
9. no bacteria, negative control

250 uL autoclaved waterhub sewage sample
10 uL x 55.5 mM = 250 uL x 2.22 mM glucose
add 1 uL bacteria LB culture

on plate iGb4j3:
2-10C/D/E/F: Group 1-9, quadruple
11C/D: Just media (sewage+glucose)

37 deg shaker 9/20/17 4:28 PM

4k. Test the optimal additional glucose level for bacteria iGb3a4v to grow in waterhub sewage sample

Raise 400 uL culture of iGb3a4v bacteria in LB
37 deg rotator 9/12/17 3:00 PM
retrieved 9/13/17 5:30 PM

Each well contains:
250 uL waterhub sewage
1 uL keio parental+lacZ LB culture
various glucose solutions

Options to add to autoclaved sewage sample:
10%(w/v) glucose = 555.06 mM
reference: DM1000 media = 5.55 mM

diluted 1:10
50 uL x 55.5 mM = 250 uL x 11.1 mM
35 uL x 55.5 mM = 250 uL x 7.77 mM
25 uL x 55.5 mM = 250 uL x 5.55 mM
20 uL x 55.5 mM = 250 uL x 4.44 mM
15 uL x 55.5 mM = 250 uL x 3.33 mM
10 uL x 55.5 mM = 250 uL x 2.22 mM
5 uL x 55.5 mM = 250 uL x 1.11 mM
0 uL x 55.5 mM = 250 uL x 0 mM

37 deg shaker 9/13/17 7:37 PM
retrieved 9/14/17
OD cell concentration test
11.1 mM: 0.0423 ± 0.0004
7.77 mM: 0.0418 ± 0.0004
5.55 mM: 0.0418 ± 0.0008
4.44 mM: 0.0420 ± 0.0007
3.33 mM: 0.0420 ± 0.0000
2.22 mM: 0.0440 ± 0.0025
1.11 mM: 0.1238 ± 0.0097
0 mM: 0.0475 ± 0.0023

Exp. iGb5

Title: Identify and test phosphate assimilating waterhub bacteria samples

5a. Morphological observation of waterhub bacteria growth on LB plate

Raise bacteria culture in 400 uL LB media
1. parental + ppk
5. waterhub 2iii
6. waterhub 3i
7. waterhub 3iv
8. waterhub 4v
9. moving bed MBi1
10. moving bed MB1ii
11. moving bed MB1iv
12. no bacteria, negative control

37 deg rotator 9/5/17 7:15 PM
retrieved at 9/6/17 3:39 PM

Spread 50 uL of the culture onto LB plate
37 deg room 9/6/17 6:26 PM
retrieved 9/7/17 11:07 AM
colonies are too dense to count
respreads on LB plates, 37 deg room 9/7/17 2:11 PM
retrieved 9/8/17 3:00 PM

5b. 16S rRNA PCR on bacteria samples, trial 1

Raise 400 uL LB culture of
2iii, 3i, 3iv, 4v, MB1i, MB1ii, MB1iv, Keio E.coli (parental+lacZ)

37 deg rotator 9/5/17 4:00 PM
retrieved 9/6/17 3:00 PM

PCR recipe:
5 uL 10x Invitrogen buffer
2 uL 5 uM SP/Eubac/27F forward primer, 5'-agagtttgatcctggctcag
2 uL 5 uM ASP/Eubac/1492R reverse primer, 5'-ggttaccttgttacgactt

5 uL 2 mM dNTPs
0.5 uL bacteria LB culture
35 uL mqH2O
0.5 uL homemade Taq polymerase
----
50 uL

in silico predicted 1503 bp product

PCR Groups:
1. 2iii bacteria + Invitrogen buffer B
2. 3i bacteria + Invitrogen buffer B
3. 3iv bacteria + Invitrogen buffer B
4. 4v bacteria + Invitrogen buffer B
5. MB1i bacteria + Invitrogen buffer B
6. MB1ii bacteria + Invitrogen buffer B
7. MB1iv bacteria + Invitrogen buffer B
8. negative control, no bacteria
9. Keio E. coli + Invitrogen buffer A
10. Keio E. coli + Invitrogen buffer B
11. Keio E. coli + Invitrogen buffer C
12. Keio E. coli + Invitrogen buffer D
13. Keio E. coli + Invitrogen buffer F
14. Keio E. coli + Invitrogen buffer J
15. Keio E. coli + Invitrogen buffer N

PCR procedure:
Hot start at 80 deg, keep for 30 sec, then add Taq polymerase
36 cycles of
94 deg 30 sec
46 deg 30 sec
72 deg 40 sec

PCR purification following Ichiro manual PCR purification protocol

Take 3 DNA concentration test:
1. 7.243 ng/uL
2. 4.779 ng/uL
3. 47.425 ng/uL
4. 29.764 ng/uL
5. 12.947 ng/uL
6. 4.822 ng/uL
7. 5.929 ng/uL

8. 6.136 ng/uL
9. 8.692 ng/uL
10. 8.137 ng/uL
11. 7.747 ng/uL
12. 8.624 ng/uL
13. 7.158 ng/uL
14. 4.298 ng/uL
15. 4.265 ng/uL

Alternative trial: streak out bacteria colonies for PCR, following the same recipe (except bacteria), groups, and procedure.

5c. Testing the PCR product on trial 1

Gel lanes on iGb5c1:
1/ 2-Log DNA ladder
2/ 2iii
3/ 3i
4/ 3iv
5/ 4v
6/ MB1i
7/ MB1ii
8/ MB1iv
9/ Negative control

Gel lanes on iGb5c2:
1/ 2-Log DNA ladder
2/ E. coli + buffer A
3/ E. coli + buffer B
4/ E. coli + buffer C
5/ E. coli + buffer D
6/ E. coli + buffer F
7/ E. coli + buffer J
8/ E. coli + buffer N

observed 1.5 kbp band on 3iv, 4v, MB1i, noises on 0.1 kbp in all lanes

GeneRead Size Selection on 3iv, 4v, MB1i
Elute with 30 uL EB

Gel lanes on iGb5c4:
1/ 2-Log DNA ladder

2/ (empty)
3/ 3iv
4/ 4v
5/ MB1i

Take 3 DNA concentration test
3iv. 48.065 ng/uL
4v. 25.789 ng/uL
MB1i. 4.403 ng/uL

Alternative trial:
No desired product. Refer to gel picture iGb5c3

5d. 16S rRNA PCR on bacteria samples, trial 2

Raise 400 uL LB culture of
2iii, 3i, 3iv, 4v, MB1i, MB1ii, MB1iv, Keio E.coli (parental+lacZ)

37 deg rotator 9/12/17 3:00 PM
retrieved 9/13/17 5:30 PM

PCR recipe:
5 uL 10x Invitrogen buffer
2 uL 5 uM SP/Eubac/27F forward primer, 5'-agagtttgatcctggctcag
2 uL 5 uM ASP/Eubac/1492R reverse primer, 5'-ggttaccttgttacgactt
5 uL 2 mM dNTPs
0.25 uL bacteria LB culture
35 uL mqH2O
0.5 uL homemade Taq polymerase
---
50 uL

in silico predicted 1503 bp product

PCR Groups:
1. 2iii bacteria + Invitrogen buffer B
2. 3i bacteria + Invitrogen buffer B
3. 3iv bacteria + Invitrogen buffer B
4. 4v bacteria + Invitrogen buffer B
5. MB1i bacteria + Invitrogen buffer B
6. MB1ii bacteria + Invitrogen buffer B
7. MB1iv bacteria + Invitrogen buffer B

8. negative control, no bacteria
9. Keio E. coli + Invitrogen buffer A
10. Keio E. coli + Invitrogen buffer B
11. Keio E. coli + Invitrogen buffer C
12. Keio E. coli + Invitrogen buffer D
13. Keio E. coli + Invitrogen buffer F
14. Keio E. coli + Invitrogen buffer J
15. Keio E. coli + Invitrogen buffer N

PCR procedure:
Hot start at 80 deg, keep for 30 sec, then add Taq polymerase
36 cycles of
94 deg 30 sec
55 deg 30 sec
72 deg 90 sec

Gel lane on iGb5d1:
1/ 2-Log DNA ladder
2-9/ raw PCR products from Group 1-8, 5 uL
observe 1.5 kbp band on 3iv, 4v, MB1i

Gel lane on iGb5d2:
1/ 2-Log DNA ladder
2-8/ raw PCR products from Group 9-15, 5 uL

5e. 16S rRNA PCR on bacteria samples, trial 3

Raise 400 uL LB culture of
2iii, 3i, 3iv, 4v, MB1i, MB1ii, MB1iv, Keio E.coli (parental+lacZ)

37 deg rotator at 9/17/17 2:20 PM
retrieved at 9/18/17 1:30 PM

PCR recipe:
5 uL 10x Invitrogen buffer
2 uL 5 uM SP/Eubac/27F forward primer, 5'-agagtttgatcctggctcag
2 uL 5 uM ASP/Eubac/1492R reverse primer, 5'-ggttaccttgttacgactt
5 uL 2 mM dNTPs
0.4 uL bacteria LB culture
35 uL mqH2O
0.5 uL homemade Taq polymerase

PCR Groups:
3 x 8 = 24 groups, two digits
1_. +Invitrogen buffer C
2_. +Invitrogen buffer D
3_. +Invitrogen buffer J
_1. +Keio E. coli
_2. +2iii bacteria
_3. +3i bacteria
_4. +3iv bacteria
_5. +4v bacteria
_6. +MB1iv bacteria
_7. +MB1ii bacteria
_8. +MB1i bacteria

PCR procedure:
Hot start at 80 deg, keep for 30 sec, then add Taq polymerase
36 cycles of
94 deg 30 sec
55 deg 30 sec
72 deg 90 sec

Gel lane on iGb5e1:
1-8/ Group 11-18
9/ 2-Log DNA ladder
Gel lane on iGb5e2:
1-8/ Group 21-28
9/ 2-Log DNA ladder
Gel lane on iGb5e3:
1-8/ Group 31-38
9/ 2-Log DNA ladder
No observable 1.5 kbp band

5f. 16S rRNA PCR on bacteria samples, trial 4

Raise 400 uL LB culture of
2iii, 3i, 3iv, 4v, MB1i, MB1ii, MB1iv, Keio E.coli (parental+lacZ)

37 deg rotator at 9/19/17 7:26 PM
retrieved at 9/20/17 1:00 PM

PCR recipe:
5 uL 10x Invitrogen buffer B

2 uL 5 uM SP/Eubac/27F forward primer, 5'-agagtttgatcctggctcag
2 uL 5 uM ASP/Eubac/1492R reverse primer, 5'-ggttaccttgttacgactt
5 uL 2 mM dNTPs
0.35 uL bacteria LB culture
35 uL mqH2O
0.5 uL homemade Taq polymerase

PCR Groups:
6 parallel groups of identical setups
1. Keio E. coli
2. 2iii bacteria
3. 3i bacteria
4. 3iv bacteria
5. 4v bacteria
6. MB1iv bacteria
7. MB1ii bacteria
8. MB1i bacteria

PCR procedure:
Hot start at 80 deg, keep for 30 sec, then add Taq polymerase
36 cycles of
94 deg 30 sec
55 deg 30 sec
72 deg 90 sec

Gel lane on iGb5f1:

5g. Sequencing

5e1 to 5e6
iGb5d3,4,5

label: 380-385
1965FAA3AB___

minimal prime

100 uM 1:4 -> 25 uM, need 3 uL
2 uL conc + 6 uL H2O
1 uL to each reaction
1.7 mL microfuge tube
1 uL of 25 uM primer
9 uL PCR product (250 ng at least)
10 uL total

sticker on each one

PCR with CD or J, 5 paralel 50 uL reaction
Pour all five reaction together and then purify together

Exp. iGb6

Title: Testing and subcloning phoA-tagRFP phosphate biosensor

6a. Testing PphoA-tagRFP-IMBB2.4-pUC57-mini biosensor in low phosphate media

Raise 400 uL culture of PphoA-tagRFP-IMBB2.4-pUC57-mini in LB-amp (frozen stock 2328)
37 deg rotator 9/12/17 3:00 PM
retrieved 9/13/17 5:30 PM

Each well contains:
250 uL waterhub sewage
1 uL PphoA-tagRFP-IMBB2.4-pUC57-mini LB-amp culture
various phosphate buffer

dilute phosphate buffer: 0.1 M x 10 uL = 1000 uM x 1 mL
continue diluting: 1000 uM x 100 uL = 100 uM x 1000 uL
0 uL x 100 uM = 250 uL x 0 uM
0.5 uL x 100 uM = 250 uL x 0.2 uM
1 uL x 100 uM = 250 uL x 0.4 uM
2 uL x 100 uM = 250 uL x 0.8 uM
4 uL x 100 uM = 250 uL x 1.6 uM
8 uL x 100 uM = 250 uL x 3.2 uM

1.6 uL x 1000 uM = 250 uL x 6.4 uM
3.2 uL x 1000 uM = 250 uL x 12.8 uM
6.4 uL x 1000 uM = 250 uL x 25.6 uM
12.8 uL x 1000 uM = 250 uL x 51.2 uM

Plate planning:
From C-F2 to C-F10, 0 uM group to 51.2 uM group
Quadruple each set

37 deg shaker 9/13/17 7:37 PM
retrieved 9/15/17

OD cell concentration test:
0 uM: 0.064 ± 0.010
0.2 uM: 0.069 ± 0.009
0.4 uM: 0.075 ± 0.014
0.8 uM: 0.076 ± 0.019
1.6 uM: 0.082 ± 0.020
3.2 uM: 0.084 ± 0.021
6.4 uM: 0.073 ± 0.008
12.8 uM: 0.080 ± 0.007
25.6 uM: 0.087 ± 0.004
51.2 uM: 0.116 ± 0.003

Exp. iGb7

Title: iGEM interlab measurement studies

7a. Heat shock transform plasmid into E. coli DH5-alpha cells, trial 1

Collect samples from Plate 7 by adding 10 uL mqH2O

Samples 21B (P), 21D (N), 21F (1), 21H (2), 21J (3), 21L (4), 21N (5), 21P (6) in PCR tubes

Take 3 concentration test:
Tube P: 144.777 ng/uL
Tube N: 171.2 ng/uL

Tube 1: 131.713 ng/uL
Tube 2: 138.002 ng/uL
Tube 3: 82.75 ng/uL
Tube 4: 126.847 ng/uL
Tube 5: 124.415 ng/uL
Tube 6: 118.669 ng/uL

1:100 dilute each sample with mqH2O, stored in 17 uL tubes

Heat shock transformation with pre-made E. coli DH5-alpha competent cells
Add 1 uL of diluted plasmid sample into each reaction

Spread 100 uL of transformed culture onto LB-chl plates
37 deg room 9/19/17 7:27 PM
no growth at 9/20/17 2:28 PM

7b. Heat shock transform plasmid into E. coli DH5-alpha cells, trial 2

Samples 21B (P), 21D (N), 21F (1), 21H (2), 21J (3), 21L (4), 21N (5), 21P (6) in PCR tubes, diluted from iGEM kit plate by 10 uL mqH2O

Heat shock transformation with pre-made E. coli Mach1 and E. coli DH5-alpha competent cells
Add 1 uL of plasmid sample into each reaction

Spread on LB-chl plates
37 deg room 9/20/17 3:42 PM

7c. Heat shock transform plasmid into E. coli DH5-alpha cells for device 1

7c1- DH5-alpha
7c2- omnimax 2 (control)

Heat shock transformation with pre-made E. coli Omnimax 2 and E. coli DH5-alpha competent cells
Add 2 uL of plasmid sample into each reaction

Spread on LB-chl plates
37 deg room 9/23/17 5:22 PM

Exp. iGb8

Title: Compare phosphate assimilation ability between Waterhub bacteria sample, lab Bsl strains and E.Coli cells

8a. Compare phosphate assimilation ability between Waterhub bacteria sample and lab Bsl strains SCK16 and REG19

Grow 6 strains of Waterhub bacteria and Bsl SCK16 and REG19 in 2mL LB.
37 deg. Rotator overnight

Each well contains:
250 uL DM-glc
7.5 uL 0.01 M diluted phosphate buffer
1 uL cells

37 deg. Shaker over 2 nights

Plate iGb8 read at 2017/10/7 8:50p.m.
Excel File misnamed as iGb5.9 Bacillus strain20171007