7.2 – 7.4

RBS(B0034)- merR construction

Vector Digestion:

B0034 plamid	5 μL
SpeI(NEB)	1.5 μL
PstI(NEB)	1.5 μL
Buffer	2 μL
ddH_2O	10 μL

Gel extraction the digested product

Ligation:

RBS-merR(digested by EcoRI & PstI)	3 μL	(prepared by Ao Liu)
BBa_ B0034 (s.p. digested)	1 μL	
ddH_2O	4 μL	
buffer	1 μL	
ligase	1 μL	

Transformed all the 10μL to trans 5a competent cell.

Colony Picking-up & PCR certification

Taq Mix	5 μL
Univ-For	0.5 μL
Univ-Rev	0.5 μL
ddH_2O	4 μL

miniprep 3 right clones and sequence them.

PmerT – GFP (E0840) construction

The PmerT insert preparation: Two reverse and complementary strains of PmerT were synthesized with artificial sticky ends if they were annealed.

Vector Digestion:

E0840 plamid	5 μL
EcoR I(NEB)	1.5 μL
XbaI(NEB)	1.5 μL
EcoRI Buffer	2 μL
ddH_2O	10 μL

Gel extraction the digested product

Annealing: 95℃ 5min, cooled done in metal bath.

PmerT-For	5μL
PmerT-Rev	5μL

Phosphalation: 37℃ for 30min

T4 PNK	1μL
T4 ligase buffer	1μL
ddH_2O	4μL
Annealing product	3μL

Ligation

PmerT Phosphalation product	9μL
Ligase	1μL
E0840 vector (E. X. digested)	1μL

Transformed all the 11μL to trans 5a competent cell.

Colony Picking-up & PCR certification

Taq Mix	5 μL
PmerT-For	0.5 μL
E0840-Rev	0.5 μL
ddH_2O	4 μL

miniprep 3 right clones and sequence them.

7.5-7.11

Pc(J23100) – RBS- merR construction

Vector Digestion:

J23100 vector digested by S.P.

RBS-merR digested by X.P.

Gel extraction of vector and insert

Ligation

Transformed all the 10μL to trans 5a competent cell.

Colony Picking-up & PCR certification

Primer: Univ-For & Univ-Rev

miniprep 3 right clones and sequence them.

Changed the backbone of PmerT-GFP from pSB1A2 to pSB3K3

Vector Digestion:

pSB3K3 vector digested by S.P.

RBS-merR insert digested by X.P.

Gel extraction of vector and insert

Ligation

6 μL insert + 2 μL vector

Transformed all the 10μL to trans 5a competent cell.

Colony Picking-up & PCR certification
Primer: PmerT-For & Univ-Rev
miniprep 3 right clones

7.12-7.18

Reconstruct Pc(J23100) – RBS- merR construction since the sequence results were wrong.

Made competent bacteria containing the PmerT-GFP(pSB3K3)

Cultured the bacteria in LB overnight
Dilute the bacteria 1:100 with LB and culture it until OD reached 0.4-0.6
Centrifuge 500μL bacteria 5000rpm for 5min
Resuspended the cell with 500μL chilled 0.1M $CaCl_2$, incubated in ice for 30min
Centrifuge 500μL bacteria 5000rpm for 5min
Resuspended the cell with 100μL chilled 0.1M $CaCl_2$

Constructed strains with both PmerT-GFP(pSB3K3) and Pc(1-18I)-RBS-MerR(pSB1A2)

Transformation the competent bacteria of PmerT-GFP(pSB3K3) with 2μL Pc(1-18I)-RBS-MerR(pSB1A2) plasmid.

7.19-7.25

Inducement of the strain with two plasmids which were constructed last week

7.20

With Hg(II)'s concentration = 1E-6 mol/L, 1E-7 mol/L and 1E-8 mol/L to certify that the strain worked.

7.22

With Hg(II)'s concentration = 1E-10, 1E-9, 1E-8, 2E-8, 4E-8, 6E-8, 8E-8, 1E-7, 1E-6, 1E-5(mol/L)
3 duplicates
GFP's intensity was measured by microplate reader.

7.23

With Hg(II)'s concentration = 1E-10, 1E-9, 1E-8, 4E-8, 7E-8, 1E-7, 4E-7, 7E-7, 1E-6, 1E-5(mol/L)
3 duplicates

7.24

With Hg(II)'s concentration = 1E-10, 1E-9, 1E-8, 4E-8, 7E-8, 1E-7, 4E-7, 7E-7, 1E-6, 1E-5(mol/L
The OD was measured by Spectrophotometer to conform Hg(II)'s effect on bacterial growth

7.25

With Hg(II)'s concentration = 1E-10, 1E-9, 4E-9, 1E-8, 1.5E-8, 2E-8, 3E-8, 4E-8, 7E-8, 1E-7, 1.5E-7, 2E-7, 3E-7, 4E-7, 7E-7, 1E-6, 4E-6, 1E-5(mol/L)
OD600 of each sample was also measured by Spectrophotometer.

7.26-8.2

Measurement of the 3 – dimensional figure which represented the time and dose response curve to mercury both for OD600 and GFP intensity.

I first tried to sample 100μL every 20min to the black-96-well plate and stored the plate in 4℃, OD was measured by spectrophotometer right after sampling.

The optimized protocol was to incubate the bacteria with Hg(II) in the microplate (black one for GFP intensity measurement and transparent one for OD600 measurement), then the two plates were measured by the microplate reader every 20min. During the interval of measurement, the two plates were cultured in the 37℃ shaker.

8. 3- 8.11

In Shanghai for the EXPO!

8.12-8.23

Induced the strain containing (PmerT-GFP+ Pc(1-18I) –RBS-merR) with Pb(II) to certify its specificity.

Construction of pPbrA-GFP(E0840) by the same stratagy used in the construction of PmerT-GFP(E0840).

Changed the backbone of pPbrA-GFP from pSB1A2 to pSB3K3

8.24-8.29

Re-changed the backbone of pPbrA-GFP from pSB1A2 to pSB3K3

Since the the concentration of low replicates pSB3K3(E. P. digested) was too low, the transformation was failed last week, I minipreped this plasmid again and by several tricks such as repeat elution for several times, the concentration finally reached the requirement for ligation.

8.30-9.5

Insert the two promoters - PmerT and pPbrA - before CrtEBI.
SDS-PAGE to certify the expression of the T3 polymerase

9.6-9.12

Changed the backbone of PmerT-GFP-merT-merC-merP from pSB1A2 to pSB3K3.

Since the size of PmerT-GFP-merT-merC-merP was similar to pSB1A2 backbone, I found another site for restriction enzyme.

Characterized CrtEBI's expression towards Hg(II)'s concentration.

Double transformation of PmerT-GFP-merT-merC-merP(pSB3k3) & Pc(1-18I) –merR(pSB1A2)

9.13-9.19

Construction of Pc-RBS-pbrR based on the RBS-pbrR constructed by Heng Pan.

9.20-9.26

Transformation the plasmids of Psid and Pll promoter in the parts sent by MIT.

Miniprep the plasmid for further use.

Construction Psid – PmerT-GFP
vector: psid(pSB1A2)
insert: PmerT-GFP(E0840)

Change the backbone of Psid – PmerT-GFP from pSB1A2 to pSB3K3
vector: pSB3K3
insert: Psid – PmerT-GFP

9.27-10.3

Construction of Pc(2-2E) – phiR73 delta

Construction of Psid – PmerT-GFP –PmerT –Ogr
Vector: Psid – PmerT-GFP(pSB3K3)
Insert: PmerT –Ogr(constructed by Mei Chen)

Psid – PmerT-GFP –PmerT –Pag and Psid – PmerT-GFP –PmerT –phiR73 delta in backbone pSB3k3 were constructed in the same way.

Construction of PmerT mutant 3 –lacZ alpha, PmerT mutant 88 –lacZ alpha, PmerT mutant 3 –lacZ, PmerT mutant 88 –lacZ.
Used the same strategy as PmerT's insert.

Change the backbone of PmerT-GFP and pPbra-GFP to pSB1C3 for parts requirement.

10.4- 10.10

Construction of Pc – phiR73 delta with different strength of the promoter by using PCR primer which corresponded the sequence of different primer region.

Transformed Psid – PmerT-GFP –PmerT-Ogr(pag, phiR73 delta) to the competent bacteria containing the plasmid of Pc(1-18I) –RBS-merR.

10.11-10.17

Characterize the response curve of the strain (containing Psid – PmerT-GFP –PmerT-Ogr(pag, phiR73 delta) + Pc(1-18I) –RBS-merR) to Hg(II)