6.24

Transform plasmids to Trans5a. pSB1A3, pSB1AC3, pSB1AK3, pSB1AT3, pSB1C3, pSB1K3, pSB1T3, pSB3C5, pSB3T5, pSB4C5, pSB4K5, pMAL-C4X, pET-39b.

6.26

Prepare plasmid DNA. pSB1A3, pSB1AC3, pSB1AK3, pSB1AT3, pSB1C3, pSB1K3, pSB1T3, pSB3C5, pSB3T5, pSB4C5, pSB4K5, pMAL-C4X, pET-39b

Digest the plasmid DNA with EcoRI &PstI.

Retrieve the digested product

6.27

Transform plasmids to Trans5a.1-12O, pSD-MBD, pASK-MBD, NRI.

6.28

Picking colonies and shaking at 37℃ overnight. 1-12O

Prepare plasmid DNA. pSD-MBD, pASK-MBD, NRI.

6.29

PCR Strain NRI with two pairs of primers

6.30

PCR Strain NRI with two pairs of primers

Retrieve the PCR product

Prepare plasmid DNA. 1-12O

7.1

Digest the plasmid DNA with EcoRI &XbaI. 1-2M, MerR, E0840

Retrieve the digested product

7.17

Transform plasmids to Trans5a. merP(mutant)-E0840, 1-18I-MerR, merP-E0840.

7.18

Transform s merP-E0840 and 1,18I-MerR to Mach-1.

7.19

PCR to build merT promoter mutants library

Retrieve the PCR product

Digest the PCR product by EcoRI and PstI

Induce merTP-E0840+1-18I-MerR by 1E-5M Hg(II)

Retrieve the digested product

7.20

Connect digest product with pSB3K3.

7.21

Pfu-library Picking colonies and shaking at 37℃ overnight.

Taq-library Failed. Connect digest product again.

Digest the PCR product by EcoRI and PstI

Retrieve the digested product

Digest the PCR product by EcoRI and PstI overnight.

7.22

Prepare plasmid DNA. The library.

Retrieve the digested product. Rbs-MerR. Failed and digest again.

Retrieve the digested product

Connect the digest product overnight.
7.23
Digest the PCR product by EcoRI and PstI. Rbs-MerR
Connect digest product with I-18C overnight.
7.24
Retrieve the digested product
Connect digest product with I-18C overnight.
7.24
Picking colonies and shaking at 37℃ overnight.1-18C-rbs-MerR
Transform the connect product to Mach-1.
7.25
Prepare plasmid DNA. Failed
PCR to build merT promoter mutants library
7.26
Transform the plasmid of library to E.coli containing 1-18I-MerR.
7.27
Picking colonies and shaking at 37℃ overnight. The library.
Connect MerR with rbs
Transform the connect product to Mach-1.
7.28
Picking colonies and shaking at 37℃ overnight. rbs-MerR.
Activate every mutant in the promoter library until the OD600 was between 0.4 and 0.6.
Then induced them by 10^-5M Hg(II), 2 hours. Centrifuged and resuspensed with PBS.
GFP intensity and OD600 were measured by Tecan Microplate Reader.
7.29
Prepare plasmid DNA. Rbs-MerR
Activate every mutant in the promoter library until the OD600 was between 0.4 and 0.6.
Then induced them by 10^-6M Hg(II), 2 hours. Centrifuged and resuspensed with PBS.
GFP intensity and OD600 were measured by Tecan Microplate Reader.
7.30
Sequenced, rbs-MerR.
Activate every mutant in the promoter library until the OD600 was between 0.4 and 0.6.
Then induced them by 10^-6M Hg(II), 2 hours. Centrifuged and resuspensed with PBS.
GFP intensity and OD600 were measured by Tecan Microplate Reader.
7.31
Activate every mutant in the promoter library until the OD600 was between 0.4 and 0.6.
Then induced them by 10^-7M Hg(II), 2 hours. Centrifuged and resuspensed with PBS.
GFP intensity and OD600 were measured by Tecan Microplate Reader.
8.1
Activate every mutant in the promoter library until the OD600 was between 0.4 and 0.6.
Then induced them by 10^-5M Hg(II), 2 hours. Centrifuged and resuspensed with PBS.
GFP intensity and OD600 were measured by Tecan Microplate Reader.
8.2
Activate every mutant in the promoter library until the OD600 was between 0.4 and 0.6.

Then induced them by 10^-6M Hg(II), 2 hours. Centrifuged and resuspensed with PBS.
GFP intensity and OD600 were measured by Tecan Microplate Reader.
8.3
Activate every mutant in the promoter library until the OD600 was between 0.4 and 0.6.
Then induced them by 10^-7M Hg(II), 2 hours. Centrifuged and resuspensed with PBS.
GFP intensity and OD600 were measured by Tecan Microplate Reader.
8.9
Lab meeting
8.11
Activate mutant3 and mutant81 until the OD600 was between 0.4 and 0.6.
Then induced them by different Hg(II) concentrations, 2 hours. The final concentrations are 0, 1E-9M, 1E-8M, 1E-7M, 1E-6M, 1E-5M. Centrifuged and resuspensed with PBS.
GFP intensity and OD600 were measured by Tecan Microplate Reader.
8.12
Activate mutant88 and mutant94 until the OD600 was between 0.4 and 0.6.
Then induced them by different Hg(II) concentrations, 2 hours. The final concentrations are 0, 1E-9M, 1E-8M, 1E-7M, 1E-6M, 1E-5M. Centrifuged and resuspensed with PBS.
GFP intensity and OD600 were measured by Tecan Microplate Reader.
8.13
Activate mutant1 and mutant25 until the OD600 was between 0.4 and 0.6.
Then induced them by different Hg(II) concentrations, 2 hours. The final concentrations are 0, 1E-9M, 1E-8M, 1E-7M, 1E-6M, 1E-5M. Centrifuged and resuspensed with PBS.
GFP intensity and OD600 were measured by Tecan Microplate Reader.
8.14
Activate mutant88 until the OD600 was between 0.4 and 0.6. Failed.
8.15
Lab meeting
8.16
Activate mutant88 until the OD600 was between 0.4 and 0.6.
Then induced them by different Hg(II) concentrations, 2 hours. The final concentrations are 0, 1E-9M, 5E-9M, 1E-8M, 2E-8M, 4E-8M, 6E-8M, 8E-8M, 1E-7M, 2E-7M, 4E-7M, 6E-7M, 8E-7M, 1E-6M, 2E-6M, 4E-6M, 6E-6M, 8E-6M, 1E-5M, 2E-5M. Centrifuged and resuspensed with PBS.
GFP intensity and OD600 were measured by Tecan Microplate Reader.
8.17
Activate mutant3 until the OD600 was between 0.4 and 0.6.
Then induced them by different Hg(II) concentrations, 2 hours. The final concentrations are 0, 1E-9M, 5E-9M, 1E-8M, 2E-8M, 4E-8M, 6E-8M, 8E-8M, 1E-7M, 2E-7M, 4E-7M, 6E-7M, 8E-7M, 1E-6M, 2E-6M, 4E-6M, 6E-6M, 8E-6M, 1E-5M, 2E-5M. Centrifuged and resuspensed with PBS.
GFP intensity and OD600 were measured by Tecan Microplate Reader.
8.18
Activate mutant44 and mutant85 until the OD600 was between 0.4 and 0.6.
Then induced them by different Hg(II) concentrations, 2 hours. The final concentrations are 0, 1E-9M, 1E-8M, 1E-7M, 1E-6M, 1E-5M. Centrifuged and resuspensed with PBS.
GFP intensity and OD600 were measured by Tecan Microplate Reader.

8.19

8.20

Activate mutant3 until the OD600 was between 0.4 and 0.6.

Then induced them by different Hg(II) concentrations, 2 hours. The final concentrations are 0, 1E-9M, 5E-9M, 1E-8M, 2E-8M, 4E-8M, 6E-8M, 8E-8M, 1E-7M, 2E-7M, 4E-7M, 6E-7M, 8E-7M, 1E-6M, 2E-6M, 4E-6M, 6E-6M, 8E-6M, 1E-5M, 2E-5M. Centrifuged and resuspensed with PBS.

GFP intensity and OD600 were measured by Tecan Microplate Reader.

8.21

Activate mutant81 until the OD600 was between 0.4 and 0.6. Failed

8.22

Lab meeting

8.23

Activate mutant88 until the OD600 was between 0.4 and 0.6.

Then induced them by different Hg(II) concentrations, 2 hours. The final concentrations are 0, 1E-9M, 5E-9M, 1E-8M, 2E-8M, 4E-8M, 6E-8M, 8E-8M, 1E-7M, 2E-7M, 4E-7M, 6E-7M, 8E-7M, 1E-6M, 2E-6M, 4E-6M, 6E-6M, 8E-6M, 1E-5M, 2E-5M. Centrifuged and resuspensed with PBS.

GFP intensity and OD600 were measured by Tecan Microplate Reader.

8.24

Activate mutant81 until the OD600 was between 0.4 and 0.6.

Then induced them by different Hg(II) concentrations, 2 hours. The final concentrations are 0, 1E-9M, 5E-9M, 1E-8M, 2E-8M, 4E-8M, 6E-8M, 8E-8M, 1E-7M, 2E-7M, 4E-7M, 6E-7M, 8E-7M, 1E-6M, 2E-6M, 4E-6M, 6E-6M, 8E-6M, 1E-5M, 2E-5M. Centrifuged and resuspensed with PBS.

GFP intensity and OD600 were measured by Tecan Microplate Reader.

8.25

Activate mutant81 until the OD600 was between 0.4 and 0.6. Failed

8.29

Lab meeting

8.30

Activate mutant94 until the OD600 was between 0.4 and 0.6.

Then induced them by different Hg(II) concentrations, 2 hours. The final concentrations are 0, 1E-9M, 5E-9M, 1E-8M, 2E-8M, 4E-8M, 6E-8M, 8E-8M, 1E-7M, 2E-7M, 4E-7M, 6E-7M, 8E-7M, 1E-6M, 2E-6M, 4E-6M, 6E-6M, 8E-6M, 1E-5M, 2E-5M. Centrifuged and resuspensed with PBS.

GFP intensity and OD600 were measured by Tecan Microplate Reader.

8.31

Activate mutant94 until the OD600 was between 0.4 and 0.6.

Then induced them by different Hg(II) concentrations, 2 hours. The final concentrations are 0, 1E-9M, 5E-9M, 1E-8M, 2E-8M, 4E-8M, 6E-8M, 8E-8M, 1E-7M, 2E-7M, 4E-7M, 6E-7M, 8E-7M, 1E-6M, 2E-6M, 4E-6M, 6E-6M, 8E-6M, 1E-5M, 2E-5M. Centrifuged and resuspensed with PBS.

GFP intensity and OD600 were measured by Tecan Microplate Reader.

9.1

Activate mutant81 until the OD600 was between 0.4 and 0.6.

Then induced them by different Hg(II) concentrations, 2 hours. The final concentrations are 0, 1E-9M, 5E-9M, 1E-8M, 2E-8M, 4E-8M, 6E-8M, 8E-8M, 1E-7M, 2E-7M, 4E-7M, 6E-7M, 8E-7M, 1E-6M, 2E-6M, 4E-6M, 6E-6M, 8E-6M, 1E-5M, 2E-5M. Centrifuged and resuspensed with PBS.

GFP intensity and OD600 were measured by Tecan Microplate Reader.

9.2

Activate mutant1 until the OD600 was between 0.4 and 0.6. Failed.

9.3

Activate mutant3 until the OD600 was between 0.4 and 0.6.

Then induced them by different Hg(II) concentrations, 2 hours. The final concentrations are 0, 1E-9M, 5E-9M, 1E-8M, 2E-8M, 4E-8M, 6E-8M, 8E-8M, 1E-7M, 2E-7M, 4E-7M, 6E-7M, 8E-7M, 1E-6M, 2E-6M, 4E-6M, 6E-6M, 8E-6M, 1E-5M, 2E-5M. Centrifuged and resuspensed with PBS.

GFP intensity and OD600 were measured by Tecan Microplate Reader.

9.4

Activate mutant44 until the OD600 was between 0.4 and 0.6. Failed.

9.5

Lab meeting

9.6

Activate mutant1 and mutant44 until the OD600 was between 0.4 and 0.6.

Then induced them by different Hg(II) concentrations, 2 hours. The final concentrations are 0, 1E-9M, 5E-9M, 1E-8M, 2E-8M, 4E-8M, 6E-8M, 8E-8M, 1E-7M, 2E-7M, 4E-7M, 6E-7M, 8E-7M, 1E-6M, 2E-6M, 4E-6M, 6E-6M, 8E-6M, 1E-5M, 2E-5M. Centrifuged and resuspensed with PBS.

GFP intensity and OD600 were measured by Tecan Microplate Reader.

9.7

9.8

Activate mutant25 and mutant85 until the OD600 was between 0.4 and 0.6.

Then induced them by different Hg(II) concentrations, 2 hours. The final concentrations are 0, 1E-9M, 5E-9M, 1E-8M, 2E-8M, 4E-8M, 6E-8M, 8E-8M, 1E-7M, 2E-7M, 4E-7M, 6E-7M, 8E-7M, 1E-6M, 2E-6M, 4E-6M, 6E-6M, 8E-6M, 1E-5M, 2E-5M. Centrifuged and resuspensed with PBS.

GFP intensity and OD600 were measured by Tecan Microplate Reader.

9.9

Activate mutant1 and mutant44 until the OD600 was between 0.4 and 0.6. Failed

9.11

Prepare plasmid DNA.I-18I-pSB1A2.

Digest the plasmid DNA with EcoRI &PstI.

Connect digest product with pSB3K3.

9.12

Transform connect product to Mach-I

Positive-transform plasmids to Trans5a. mutant1, 3, 25, 44, 85, 88-pSB3K3.

Lab meeting

Picking colonies and shaking at 37℃ overnight

9.13

Prepare plasmid DNA. mutant1, 3, 25, 44, 85, 88-pSB3K3.

Digest the plasmid DNA with EcoRI &PstI.

Connect digest product with pSB1A2.

Prepare plasmid DNA. I-18I-pSB3K3.

9.14

Transform connect product to Tran5a.

Picking colonies and shaking at 37℃ overnight

9.15

Prepare plasmid DNA. mutant1, 3, 25, 44, 85, 88-pSB1A2.

Lab meeting

9.16

Transform mutant1, 3, 25, 44, 85, 88-pSB1A2 and 1-18I-pSB3K3 to Mach-I

9.17

Picking colonies and shaking at 37℃ overnight

9.18

Activate mutant1 and mutant44 until the OD600 was between 0.4 and 0.6.

Then induced them by different Hg(II) concentrations, 2 hours. The final concentrations are 0, 1E-9M, 5E-9M, 1E-8M, 2E-8M, 4E-8M, 6E-8M, 8E-8M, 1E-7M, 2E-7M, 4E-7M, 6E-7M, 8E-7M, 1E-6M, 2E-6M, 4E-6M, 6E-6M, 8E-6M, 1E-5M, 2E-5M. Centrifuged and resuspensed with PBS.

GFP intensity and OD600 were measured by Tecan Microplate Reader.

9.19

Activate mutant25 and mutant85 until the OD600 was between 0.4 and 0.6. Failed.

9.20

Activate mutant25 and mutant85 until the OD600 was between 0.4 and 0.6.

Then induced them by different Hg(II) concentrations, 2 hours. The final concentrations are 0, 1E-9M, 5E-9M, 1E-8M, 2E-8M, 4E-8M, 6E-8M, 8E-8M, 1E-7M, 2E-7M, 4E-7M, 6E-7M, 8E-7M, 1E-6M, 2E-6M, 4E-6M, 6E-6M, 8E-6M, 1E-5M, 2E-5M. Centrifuged and resuspensed with PBS.

GFP intensity and OD600 were measured by Tecan Microplate Reader.

9.21

Activate mutant3 and mutant88 until the OD600 was between 0.4 and 0.6.

Then induced them by different Hg(II) concentrations, 2 hours. The final concentrations are 0, 1E-9M, 5E-9M, 1E-8M, 2E-8M, 4E-8M, 6E-8M, 8E-8M, 1E-7M, 2E-7M, 4E-7M, 6E-7M, 8E-7M, 1E-6M, 2E-6M, 4E-6M, 6E-6M, 8E-6M, 1E-5M, 2E-5M. Centrifuged and resuspensed with PBS.

GFP intensity and OD600 were measured by Tecan Microplate Reader.

9.23

Activate mutant1 and mutant44 until the OD600 was between 0.4 and 0.6.

Then induced them by different Hg(II) concentrations, 2 hours. The final concentrations are 0, 1E-9M, 5E-9M, 1E-8M, 2E-8M, 4E-8M, 6E-8M, 8E-8M, 1E-7M, 2E-7M, 4E-7M, 6E-7M, 8E-7M, 1E-6M, 2E-6M, 4E-6M, 6E-6M, 8E-6M, 1E-5M, 2E-5M. Centrifuged and resuspensed with PBS.

GFP intensity and OD600 were measured by Tecan Microplate Reader.

9.24

Activate mutant25 and mutant85 until the OD600 was between 0.4 and 0.6. Failed.

9.25

Activate mutant25 and mutant85 until the OD600 was between 0.4 and 0.6.

Then induced them by different Hg(II) concentrations, 2 hours. The final concentrations are 0, 1E-9M, 5E-9M, 1E-8M, 2E-8M, 4E-8M, 6E-8M, 8E-8M, 1E-7M, 2E-7M, 4E-7M, 6E-7M, 8E-7M, 1E-6M, 2E-6M, 4E-6M, 6E-6M, 8E-6M, 1E-5M, 2E-5M. Centrifuged and resuspensed with PBS.

GFP intensity and OD600 were measured by Tecan Microplate Reader.

9.26

Lab meeting

9.27

Activate mutant25 and mutant85 until the OD600 was between 0.4 and 0.6. Failed

9.29

Activate mutant3 until the OD600 was between 0.4 and 0.6.

Then induced them by different Hg(II) concentrations, 2 hours. The final concentrations are 0, 1E-9M, 5E-9M, 1E-8M, 2E-8M, 4E-8M, 6E-8M, 8E-8M, 1E-7M, 2E-7M, 4E-7M, 6E-7M, 8E-7M, 1E-6M, 2E-6M, 4E-6M, 6E-6M, 8E-6M, 1E-5M, 2E-5M. Centrifuged and resuspensed with PBS.

GFP intensity and OD600 were measured by Tecan Microplate Reader.

9.30

Activate mutant88 until the OD600 was between 0.4 and 0.6.

Then induced them by different Hg(II) concentrations, 2 hours. The final concentrations are 0, 1E-9M, 5E-9M, 1E-8M, 2E-8M, 4E-8M, 6E-8M, 8E-8M, 1E-7M, 2E-7M, 4E-7M, 6E-7M, 8E-7M, 1E-6M, 2E-6M, 4E-6M, 6E-6M, 8E-6M, 1E-5M, 2E-5M. Centrifuged and resuspensed with PBS.

GFP intensity and OD600 were measured by Tecan Microplate Reader.

10.1-10.3

Wiki

10.3-10.6

Chang Backbone of promoter mutant1-E0840, from pSB3K3 to pSB1C3.

Chang Backbone of promoter mutant3-E0840, from pSB3K3 to pSB1C3.

Chang Backbone of promoter mutant25-E0840, from pSB3K3 to pSB1C3.

Chang Backbone of promoter mutant44-E0840, from pSB3K3 to pSB1C3.

Chang Backbone of promoter mutant85-E0840, from pSB3K3 to pSB1C3.

Chang Backbone of promoter mutant88-E0840, from pSB3K3 to pSB1C3.

Chang Backbone of T7-rbs-DsbA-MBP-Terminater, from pSB3K3 to pSB1C3.

Chang Backbone of T7-rbs-DsbA-MerR-Terminater, from pSB3K3 to pSB1C3.

10.7

Transform connect mutant3, 88-pSB1A2 and 1-18I-pSB3K3 to Mach-I

Picking colonies and shaking at 37℃ overnight

10.8

Activate mutant3E until the OD600 was between 0.4 and 0.6.

Then induced them by different Hg(II) concentrations, 2 hours. The final concentrations are 0, 1E-9M, 5E-9M, 1E-8M, 2E-8M, 4E-8M, 6E-8M, 8E-8M, 1E-7M, 2E-7M, 4E-7M, 6E-7M, 8E-7M, 1E-6M, 2E-6M, 4E-6M, 6E-6M, 8E-6M, 1E-5M, 2E-5M. Centrifuged and resuspensed with PBS.

GFP intensity and OD600 were measured by Tecan Microplate Reader.

10.9

Activate mutant88E until the OD600 was between 0.4 and 0.6. Failed.

10.10

Lab meeting

10.11

Activate mutant88E until the OD600 was between 0.4 and 0.6.

Then induced them by different Hg(II) concentrations, 2 hours. The final concentrations are 0, 1E-9M, 5E-9M, 1E-8M, 2E-8M, 4E-8M, 6E-8M, 8E-8M, 1E-7M, 2E-7M, 4E-7M, 6E-7M, 8E-7M, 1E-6M, 2E-6M, 4E-6M, 6E-6M, 8E-6M, 1E-5M, 2E-5M. Centrifuged and resuspensed with PBS.

GFP intensity and OD600 were measured by Tecan Microplate Reader.

10.12

Activate mutant3E and mutant88E until the OD600 was between 0.4 and 0.6.

Then induced them by different Hg(II) concentrations, 2 hours. The final concentrations are 0, 1E-9M, 5E-9M, 1E-8M, 2E-8M, 4E-8M, 6E-8M, 8E-8M, 1E-7M, 2E-7M, 4E-7M, 6E-7M, 8E-7M, 1E-6M, 2E-6M, 4E-6M, 6E-6M, 8E-6M, 1E-5M, 2E-5M. Centrifuged and resuspensed with PBS.

GFP intensity and OD600 were measured by Tecan Microplate Reader.

10.14

Connect promoter mutant3 and mutant88 with pSB1C3.

Transform connect product to trans5a.

10.15

Prepare plasmid DNA.promoter3-pSB1C3 and promoter88-pSB1C3