Peosonal Notes

Donghai Liang

7.1
Dissolve primers
Design PCR programme
7.4
MerR PCR, MBP PCR
Retrieve the PCR product
7.5
Digest the plasmid pET-39(B)+ with SacII and EcoRI
Digest the PCR product with SacII and EcoRI
Retrieve the digested product
Ligated the digested plasmid pET-39(b)+ and the PCR product
Transform the ligation product into Trans5αstrain.
7.6
Part I handle the job for YHu
Digest pET-21a with NdeI and XhoI
Retrieve the digested product
Ligated the digested pET-21a with MBP digested fragments
Transform the ligation product
Part II　Periplasmic Construction
Pick the six single clones for the plate transformed last night
PCR the clones to identify the successfully ligated clone
Clones 1\3\5 shows positive result and go on shaking at 37℃ overnight
7.7
Part I handle the job for YHu
Pick clones from the plate transformed yesterday and shake at 37℃ for ten hours
Miniprep the plasmid from the clones
Part II　Periplasmic Construction
Miniprep clone 1\3\5 and send them for sequencing
7.8
Part I handle the job for YHu
Digest the plasmid minipreped yesterday and identify by Electrophoresis
No positive result showed
Redo the experiment starting with PCR the MBP
Part II　Periplasmic Construction
Positive Transformation of DsbA-MerR
Start the construction of DsbA-MBP
PCR MBP overnight
7.9
Part I handle the job for YHu
Redo the ligation of MBP into pET-21a

Transformation
Part II Periplasmic Construction
The sequence of Clone 1 from DsbA-MerR is correct
Retrieve the MBP PCR product
7.10
YHu's job is officially handled by XTeng.I begin to conduct the experiment of the periplasmic module of the bioabsorbent display alone
Digest pET-39(b)+ and the retrieved MBP PCR product
Ligate the product overnight
Start the standardization of DsbA-MerR
PCR DsbA-MerR with standadized primers
7.11
Transformation of the ligated DsbA-MBP
Transformation of standardization of DsbA-MerR
7.12
Plasmid Miniprep from the transformation product
7.13
Digest the product of DsbA-MerR with EcoRI and SacII and do the identificate by Electrophoresis
Digest the product of standardization of DsbA-MerR with EcoRI and PstI and do the identificate by Electrophoresis
No postive result showed
7.14
re-Conduct the experiment
PCR MBP and retrieve the product
PCR DsbA-MerR and retrieve the product
Digest the product with EcoRI and SacII
Digest the the standardization product with EcoRI and PstI
Retrieve the product and ligate with digested pET-39(b)+
Transform the ligated product
7.15
Pick 24 clones from the plates and shake at 37℃ for ten hours
Start the construction of MerR into pET-21a
PCR MerR and retrieve
Digest it with XhoI and NdeI
Retrieve the digested product and ligate with digested pET-21a
7.16
Plasmid Miniprep from the 24 clones
Pick 21 clones from the plates of the standardization of DsbA-MerR and shake at 37℃ for ten hours
Digest the Plasmid Miniprep product and identificate by Electrophoresis
7.17
Positive result showed among the clones,they are A21 A22 A23 A27 C15 C21 C22
Positive transform of A22 A27 C15 C22
7.18-7.24

Go to ShangHai EXPO on a vocation.
The sequence result(done by Xteng) showed the construction of DsbA-MBP failed again.
Meanwhile since we later discovered there is a PstI restriction site right in the middle of DsbA ,unfortuanately we uesd to digest the Standardized PCR product of DsbA-MerR with PstI,so this part failed, too.
7.25
Digest pET-39(b)+ with XbaI and XhoI
PCR MBP and retrieve the product
Digest it with XbaI and XhoI
Ligate the digested vector and the PCR product
7.26
Transform the ligated product
Pick clones from the plate and shake at 37℃ ovenight
7.27
Plasmid Miniprep and send for sequencing
7.28
PCR DsbA-MerR
Positive transform DsbA-MerR into Omni Strain
7.29
The Sequence of DsbA-MBP result failed again, but reveal that the original plasmid containing MBP from Summers is incorrect.
We design to do the three fragment Ligation strategy to construct the MBP
PCR Strain NRI/PASK-MBD with two pairs of primers
Retrieve the product
Start the nested PCR of DsbA-MerR to finish its Standradization.
7.30
Digest the pEt-39(b)+ with XbaI and XhoI
Digest the MBD-Part I with XbaI and BamHi
Digest the MBD-Part II with XhoI and BamHi
Rerieve the three products and ligate them together for three hours
Transform the ligated product
Identificate the Standradization of DsbA-MerR but failed
7.31
Yesterday's transformation failed
Re-ligate the three fragments for three hours
Transform the product
redo the first round nested PCR of DsbA-MerR
Retrieve the product
the Transformation seems to be successful and pick 12 clones from the plate
Do the second round nested PCR of DsbA-MerR
8.1
Retrieve the second round nested PCR product of DsbA-MerR
Identificate the No 442 444 332 (DsbA-MBP) by Electrophoresis
No Positive resluit in the Electrophoresis

Miniprep of the new redo construction of DsbA-MBP
8.2
Digest 442 444 332 product and check the reslut by Electrophoresis
No positive result again
Redo the PCR of MBP
8.3-8.6
Redo the construction of DsbA-MBP (just as the steps on 7.30-8.1)
Standardization of DsbA-MerR was successfully constructed,confirmed by the sequencing result.
Start the western-blot of DsbA-MerR
8.7
Miniprep of 12 clones of DsbA-MBP
Identificate by Electrophoresis but no positive result
Send it for sequencing
8.8
Begin the construction of standardization of DsbA-MBP while it's waiting for sequencing
8.9
Redo the PCR of MBP into two fragments
Digest pET-39(b)+ again with Spe I and Xho I
Ligate the three fragments again
8.10
Transform the ligated product
Retrieve the PCR product of the three fragments
Digest with XbaI and BamHI for N and XhoI and BamHI for C
Retrieve the digested product and Ligate them with digested pET-39(b)+ together
Transform the ligated product
8.11
Pick clones from the plate and shake at 37℃ ovenight
8.12-8.14
Postive results showed some clones is correct
Send for sequencing
8.15-8.17
Sequencing result show that NIC1 6 clone is correct
DsbA-MBP construction is complete
Positive Transform DsbA-MBP
8.18
Transform the DsbA-MBP into Omni and BL21a
8.19-8.21
Begin the Standardization of DsbA-MBP
First Round of Nested PCR of the DsbA-MBP
Second Round of Nested PCR of the DsbA-MBP
Construction Finished and waited for sequence
8.22-8.25
Go home on vocation
8.26

Sequence result show that the standardization of DsbA-MBP is failed

8.27-8.28

Redo the Standardization of DsbA-MBP

Construction Finished and waited for sequence

8.29

Sequencing result correct

Start coworking with Ao Liu on the promoter characterization

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.
9.8
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
9.10
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 connect 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

9.28

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.
===10.1-10.6===
Booking Hotel
Paying reservation fees
===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
===10.16-10.27===

Preparing the team's material for Visa
Sending DNA Submission(parts)
Uploading WIKI as well as personal notes
Conducting the travel plan and Purchasing tickets