Yizhe Zeng, Xiunan Huo, Bingbing Liu, Beibei Li, Pan Li, Jiacheng Shi explored an experimental method for testing the activity of pelA.  

8.3–8.15  
Yizhe Zeng, Yanjun Li, Xiunan Huo, Bingbing Liu, Beibei Li, Pan Li, Jiacheng Shi  
Extractionplasmidsof pelA-PHO1, pelA-PHO10pro, pelA-FLO10, pelA-FLO10pro, pelA-SUC2, pelA, Pag1A  
Single enzyme digestion(with Sac I).  

Making competent cells of Pichia pastoris GS115: We need the pelA gene with a signal peptide integrate into GS115 genome. transformation (into Pichia pastoris GS115) Extraction of transformed GS115 Genome to verified whether the target gene has been integrated into. However there is no strip of destination length. We suspect it’s the primers or the gene sequences error.
Yizhe Zeng, Yanjun Li, Xianan Huo, Bingbing Liu, Beibei Li, Pan Li, Jiacheng Shi
Determination of pelA-PHO1 and pelA-FLO10pro enzyme activity by titration

Xianan Huo
Redesign primers
Yizhe Zeng
Design path of pGAP-pgada gfp-ppg-mecherry to verification of promoter activity.

8.24–8.30
Xianan Huo, Jiacheng Shi, Bingbing Liu
Extraction plasmids of pelA-PHO1, pelA-PHO1pro, pelA-FLO10, pelA-FLO10pro, pelA-SUC2, pelA.
Single enzyme digestion (with SacI).

Making competent cells of Pichia pastoris GS115: We need the pelA gene with a signal peptide integrate into GS115 genome. Transformation (into Pichia pastoris GS115)
Extraction of transformed GS115 Genome to verified whether the target gene has been integrated into.
9.2~9.8
Determination of pelA,pelA-FLO10,pelA-SUC2,pelA-PHO1 upregulation enzyme activity by titration

9.30~10.6
Jiacheng Shi, Yizhe Zeng
Extraction plasmids of pGAP,pgada, gfptt,pgas, mcherry in TOP10.

Assembly pGAP,pgada, gfptt,pgas, mcherry, transformation (into E.coli TOP10)
PCR the TOP10 to verify if assembly and transfer successfully.

SLAC Group
7.7-7.13  7.14-7.20  7.21-7.27  7.28-8.3  8.4-8.10  8.11-8.17  8.18-8.24  8.25-8.31

7.10
Spotted culture yeast
7.20
Extraction of yeast genome
7.21
Made competent cells of GS115, electro-transformation
7.23
Plasmid extraction, PCR
7.24
Cultured the GS115-PHO5-SLAC, GS115-FLO10-SLAC, GS115-FLO10-apro-SLAC,
    GS115-FLO10-pelA, GS115-PHO5-pelA, GS115-FLO10 apro-pelA, GS115-PHO5
    apro-pelA, GS115-FLO10 apro-VP
7.25
Electro-transformation of ppic9k-Pgas-GFP, ppic9k-Pgada-GFP into GS115
7.26
Verify electrical transformation

<table>
<thead>
<tr>
<th>SLAC-PHO5</th>
<th>SLAC-FLO10</th>
<th>SLAC-FLO10-apro</th>
<th>pelA-FL010</th>
<th>pelA-PHO5</th>
<th>pelA-FL010-apro</th>
<th>pelA-PHO5-apro</th>
<th>VP-FLO10-apro</th>
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<td>✔️</td>
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</tr>
</tbody>
</table>

Genomic extraction of pichia, Run DNA gel verification (19.7.26)

7.27
Cultured the GS115-Pgas-GFP, GS115-Pgada-GFP in BMGY medium.

7.20-8.3
Measured activities of pelA-FLO10, pelA-PHO5, pelA-FLO10 apro, pelA-PHO5 apro,
VP-FLO10 apro
SDS-PAGE (19.7.29)

Ran the SDS-PAGE of pelA-PHO5, pelA-FLO10pro, VP-FLO10pro, GS115 (negative control) (19.7.30)

Measured the activity of pelA by titration.
Measured the fluorescence of Pgad-GFP, FgadA-GFP
Purified pelA-PHO5 and pelA-FLO10 pro by His-tag.
SDS-PAGE (19.8.3)

8.4
Purified GS115 (negative control), VP-FLO10, VP-SUC2, VP by His-tag.
8.5
Ni-NTA purification (pelA-PHO5, VP, VP-FLO10, VP-SUC2)
SDS-PAGE (VP, VP, VP-FLO010, VP-FLO10, VP-SUC2, VP-SUC2, pelA-PHO5, pelA-PHO5) (19.8.5)
Methanol induce expression.
8.6
Measured the activities of VP, VP-FLO10, VP-SUC2, VP-FLO10 apro, SLAC-FLO10pro, SLAC-FLO10, SLAC-PHO5
8.7
Measuring activities of SLAC-FLO10 apro, SLAC-FLO10, SLAC-PHO5, GS115 (negative control)
Cu2+ needed
SDS-PAGE of SLAC-FLO10 apro, SLAC-FLO10, SLAC-PHO5 (19.8.7)

8.8
Cultured GS115-ppic9k-PHO5-SLAC, GS115-ppic9k-FLO10-SLAC, GS115-ppic9k-FLO10 apro-SLAC in BMGY medium.
8.9
BMGY to BMM
8.10
Plasmid extraction of ppic9k-SLAC, ppic9k-FLO10-SLAC, ppic9k-FLO10 apro-SLAC, ppic9k-PHO5-SLAC, ppic9k-PHO5 apro-SLAC, ppic9k-SUC2-SLAC

Yuanhao Liang
Single enzyme digestion (SacI)  Bohan Wang
DNA gel retraction  Chunxiu Pan & Siyao Gu
1. SLAC  2. SLAC-FLO10  3. SLAC-FLO10 pro  4. SLAC-PHO5  5. SLAC-PHO5 pro
6. SLAC-SUC2
(19.8.10)

Figure. SacI single endonuclease digestion.

Making competent cells of GS115  Chunxiu Pan & Bohan Wang
DNA gel retraction  Siyao Gu
Electro-transformation  Xinran Rao & Bohan Wang & Yuanhao Liang

8.12
Ran the SDS-PAGE of SLAC-FLO10, SLAC-FLO10 pro, SLAC-PHO5
(19.8.12)

8.14
electronic transformation and pick clones, seeding BMGY

8.15
Genomic extraction of pichia: Bohan Wang & Zhi Li
PCR  Chunxiu Pan & Yingying Yang
DNA gel maker: DS5000  Yuanhao Liang & Siyao Gu
1. GS115  2. SLAC  3. SLAC-FLO10  4. SLAC-FLO10 pro  5. SLAC-PHO5
6. SLAC-PHO5 pro  7. SLAC-SUC2
(19.8.15)
8.17
Measured the activities of SLAC-PHO5, SLAC-FLO10, SLAC-FLO10 apro SDS-PAGE
(19.9.17)

8.18
Measured the activities of SLAC
8.19
seeding GS115, SLAC-pho1 pro to BMM from BMGY Siyao Gu & Chunxiu Pan & Bohan Wang
Measured the activities of VP, VP-FLO10, VP SUC2, VP-PHO5 apro Siyao Gu & Chunxiu Pan & Bohan Wang & Yuanhao Liang
8.20
Add heme, measure enzyme activity after 20 min water bath
8.21
Seeding VP, VP-suc2, VP-FLO10, VP-PHO5 from BMGY to BMMY
Add methanol
Measured the activity of SLAC-PHO5 apro
Ran the SDS-PAGE

8.22
Cultured GS115-ppic9k-VP, GS115-ppic9k-FLO10-VP, GS115-ppic9k-PHO5-VP in BMGY medium.
Measured the activity of SLAC-PHO5 apro
Measured the activity of VP

8.23
Seeding GS115, GS115-ppic9k-VP, GS115-ppic9k-FLO10-VP, GS115-ppic9k-FLO10 apro-VP, GS115-ppic9k-SUC2-VP, GS115-ppic9k-PHO5-VP to BMM medium.
Measured the activity of VP

8.24
Seeding GS115, GS115-ppic9k-FLO10-SLAC, GS115-ppic9k-FLO10 apro-SLAC, GS115-ppic9k-PHO5 apro-SLAC, GS115-ppic9k-PHO5-SLAC to BMM medium.
Cultured GS115 in YPD medium.
8.25
Measured the activities of GS115 (negative control), VP, VP-SUC2, VP-FLO10, VP-FLO10 apro, VP-PHO5 apro.
Measured the activities of SLAC-PHO5, SLAC-PHO5 apro, SLAC-FLO10, SLAC-FLO10 apro.
8.26-9.2
Measured the activities of SLAC-PHO5, SLAC-PHO5 apro, SLAC-FLO10, SLAC-FLO10 apro.

**VP Group**

7.7.
Inoculated with 9 tubes.
TOP10-pPIC9K-FLO10-pelA, TOP10-pPIC9K-PHO1-SLAC,
TOP10-pPIC9K-PHO1pro-SLAC,
TOP10-pPIC9K-PHO1pro-pelA, TOP10-pPIC9K-PHO1-pelA

Overnight culture at 37 °C.

7.8.
Sterilize some equipment.
extract nine kinds of plasmid.
pPIC9K-FLO10-VP, pPIC9K-FLO10-SLAC, pPIC9K-FLO10pro-SLAC,
pPIC9K-FLO10-pelA, pPIC9K-PHO1-SLAC, pPIC9K-PHO1pro-SLAC,
pPIC9K-PHO1pro-pelA, pPIC9K-FLO10pro-pelA, pPIC9K-PHO1-pelA.
30 µL per tube.
Sac1 single enzyme cleavage makes plasmid linearization.
System:
Sac1 4.5ul.
DdH2O 65ul.
10 mL buffer 10ul.
Plasmid 20ul.
TdaL 99.5ul.
Running electrophoresis to verify the strip and recovery.
Preparation of yeast (GS115) competent cell.

Yeast electrottransformation.


7.20.

extract plasmid 10 tube.


Sac1 single enzyme digestion.

electrophoresis

![Image of gel electrophoresis](image1)

Figure 1. Ten kinds of plasmid with Sac1 digestion. 1-2 pPIC9K-SUC2-VP 3-4 pPIC9K-SUC2-SLAC 5-6 pPIC9K-PHO1-VP 7. Marker 8-9 pPIC9K-SUC2-pelA 10-11 pPIC9K-PHO1pro-SLAC

![Image of gel electrophoresis](image2)

Figure 1. Ten kinds of plasmid with Sac1 digestion. 1-2 pPIC9K-SLAC 3-4 pPIC9K-PHO1pro-VP 5-6 pPIC9K-FLO10-VP 7. Marker 8-9 pPIC9K-pelA 10-11 pPIC9K-VP

Gel recovery.

Extraction of yeast Genome which from previous Electric transfer

7.21.

electrotransformation.

7.22.

There are eight plates have colony

Pick up colony and do colony PCR electrophoresis.

7.28.

Make ypd, md medium.

Inoculation.

7.29  
extract plasmid 10 tube.  
PpIC9k-VP/ pPIC9k-SUC2-VP/ pPIC9k-SLAC/ pPIC9k-POH1-pro-pelA/pPIC9k-FLO10-VP/pPIC9k-FLO10-pelA/pPIC9k-POH1-pro-SLAC/pPIC9k-SUC2-SLAC/pPIC9k-pelA/pPIC9k-POH1-pro-VP.  
Among them, pPIC9k-SUC2-pelA was turbid after adding solution 2, and pPIC9k-POH1-VP was not pure, so it was abandoned.  
Sac1 single enzyme digestion.  
The system is the same as last time.  
PpIC9k-VP/ pPIC9k-SUC2-VP/ pPIC9k-SLAC/ pPIC9k-POH1-pro-pelA/pPIC9k-FLO10-VP/pPIC9k-FLO10-pelA/pPIC9k-POH1-pro-SLAC/pPIC9k-SUC2-SLAC/pPIC9k-pelA/pPIC9k-POH1-pro-VP.  
electrophoresis and recovery.  
PpIC9k-VP/ pPIC9k-SUC2-VP/ pPIC9k-SLAC/ pPIC9k-POH1-pro-pelA/pPIC9k-FLO10-VP/pPIC9k-FLO10-pelA/pPIC9k-POH1-pro-SLAC/pPIC9k-SUC2-SLAC/pPIC9k-pelA/pPIC9k-POH1-pro-VP.  
Preparation of receptive state, electrorotation.  
7.31  
Expand culture, each with two colony.  
The other four kinds of yeast grew slowly and then expanded and cultured in the future.  

8. 1 Yeast genome extraction (fox ws), PCR (yyy hxy)  

![Verification of the transformation of FLO10-VP, SUC2-VP and VP into GS115](image)

8. 2/8. 3 waiting the growth of the E.coli and GS115  
8. 4 Yeast genome extraction (yyy hxy), PCR (fox ws)
8.5/8.6  search paper about VP

8.7/8.8 Plasmid Extraction, single enzyme digestion (SacI), and we transferred the targeted plasmid into Pichia pastoris GS115 through electroporation (yyy,hxy,wjk,yhby,fcx,ws)

8.9 Plasmid Extraction, single enzyme digestion (SacI), and we transferred the targeted plasmid into Pichia pastoris GS115 through electroporation (wjk,yhby,fcx,ws,yyy,hxy)

8.10/8.11 Plasmid Extraction, single enzyme digestion (SacI), and we transferred the targeted plasmid into Pichia pastoris GS115 through electroporation (fcx,ws,yyy,hxy,wjk,yhby)

8.12 Search paper about basic protein

8.13 Yeast genome extraction, PCR(yyy,hxy),false

GS115 PCR: PeLA (1.2.3) SUC2-PeLA (4.5.6) PHO1-PeLA (7.8.9) PHO1-VP (10.11.12) SUC2-SLAC (13.14.15) 17.30.37
Microscopy
8.16 Plasmid Extraction, single enzyme digestion (SacI), and we transferred the targeted plasmid into Pichia pastoris GS115 through electrotransformation (fcm, ws, yyy, lxy)
pelA PHO1-VP Marker4 DS5000 SUC2-pelA SUC2-SLAC 18.26.38

Verification of the enzyme digestion (SacI) of pelA and SUC2-SLAC

Yeast genome extraction, PCR (wjk, yhby) false
8.17 Plasmid Extraction, single enzyme digestion (SacI), and we wanted to transfer the targeted plasmid into *Pichia pastoris* GS115 through electroporation but GS115 had some problems, so we failed again.

8.18 Yeast genome extraction, PCR(wjckyhb) false

DS5000  FLO10-pelA1  FLO10-P-SLAC1  PHO1-SLAC1  FLO10-SLAC1  FLO10-pelA2  FLO10-SLAC2  PHO1-SLAC2  PHO1-P-SLAC2  FLO10-P-SLAC2  DS5000
8.19-8.21 we wanted to transfer but failed. First time the concentration of E. coli solution was not enough and next we forgot to put nucleic acid fluorescent dye in the gel.
8.22 Plasmid Extraction, single enzyme digestion (SacI), and we wanted to transfer the targeted plasmid into Pichia pastoris GS115 through electroporation (yyj, hxy, fxc, ws, wjk).
PHO1-1-SLAC2 PHO1-P-SLAC PHO5-SLAC PHO1-SLAC FLO10-pelA FLO10-pelA SLAC SLAC (failed)

![Image of gel electrophoresis](image)

Verification of the Enzyme digestion (SacI) of 1.2 PHO1pro-SLAC
3.4 PHO1-SLAC  5.6 FLO10-Pela  7.8 SLAC

8.24 Plasmid Extraction, single enzyme digestion (SacI) and we stopped because we found GS115 infected by other bacteria (ikw, fxc, ws, yyy, hxy, wjk).

![Image of gel electrophoresis](image)

Verification of the Enzyme digestion (SacI) of 1.PHO1pro-Pela  2.Pela 3.FLO10-Pela  4.SUC2-Pela  5. Plasmid positive control  6.Pgad

That day we found most conservation of our GS115 and other yeasts we conserved at the beginning of August had been infected by other bacteria.
8.27 Plasmid Extraction, single enzyme digestion (SacI) (wjk, ikw, yyy, hxy)
VP,FLO10-VP,FLO10-P-VP,SUC2-VP,PHO1-VP

![Image of gel electrophoresis](image)

Verification of the Enzyme digestion(SacI) of 1.VP  2.FLO10-VP  3.FLO10pro-VP  4.SUC2-VP  5.PHO1-VP

8.28 Electroporation the targeted plasmid into Pichia pastoris GS115 (wjk, yyy, ikw, hxy, fxc, ws)
8.29 Group discussion of the experiments.
8.30 Plasmid Extraction, single enzyme digestion (SacI) (hxy, ws, yyy, fcx)

Verification of the Enzyme digestion (SacI) of
1. PHO1-VP  2. FLO10pro-VP  3. FLO10-VP  4. VP

8.31 Electroporation the targeted plasmid into Pichia pastoris GS115 (ws, fcx, wjk, yhb)
Yeast genome extraction, PCR(yyy, hxy)

Verification of the transformation of 1. FLO10pro-VP  2. FLO10-VP  3. PHO1-VP  4. VP

September 1
Yingying Yang, Xinyu Huan, Changxin Fan, Shi Wang, Ziqi Yin, Kaiwen Liu, Jingkai Wen
( Test the activity of the Versatile peroxidase, including PHO1-VP, VP, FLO10-VP, FLO10-P-VP. We don’t have any useful results in the first day.)

September 2
Yingying Yang, Xinyu Huan
( Test the activity of the Versatile peroxidase, including PHO1-VP, VP, FLO10-VP, FLO10-P-VP. We don’t have any useful results in the second day.)

September 3
Ziqi Yin, Kaiwen Liu, Jingkai Wen
( Test the activity of the Versatile peroxidase, including PHO1-VP, VP, FLO10-VP, FLO10-P-VP. We don’t have any useful results in the third day.)

September 4
Changxin Fan, Shi Wang
Test the activity of the Versatile peroxidase, including PHO1-VP, VP, FLO10-VP, FLO10-P-VP, GS115. We don't have any useful results in the forth day.

September 5
Ziqi Yin, Kaiwen Liu

( Test the activity of the Versatile peroxidase, including PHO1-VP, VP, FLO10-VP, FLO10-P-VP, GS115. We don't have any useful results in the fifth day. )

September 7
Changxin Fan, Shi Wang

( Test the activity of the Versatile peroxidase, including PHO1-VP, VP, FLO10-VP, FLO10-P-VP, GS115. We don't have any useful results in the seventh day. )

September 8
Changxin Fan, Yingying Yang

(We break the Pichia pastoris GS115 cells, sodium dodecyl sulfate-polyacrylamide gel electrophoresis)

We didn’t get the any useful results about Versatile peroxidase from the picture.
September 14
Jingkai Wen

( Test the activity of the Versatile peroxidase, including PHO1-VP, VP, FLO10-VP, FLO10-P-VP, GS115. We don’t have any useful results in the fourteenth day. )

10.1 PCR to gain FLO10-SLAC and Pgap(false)
1-4(SLAC), 5-6(VP) Pgap, SLAC-FLO10, pEA-FLO10-P, PgapEA,FLO10-SLAC, Pgap 19.58.52

Verification of (PCR) FLO10-SLAC

10.2 PCR to gain PHO1-pelA and Pgap
1 pgpd 2 pela 3 SLAC 4 pgp 5 PHO1-pela 6 pgap 17.07.29

Verification of (PCR) 1. PHO1-PelA 2. Pgap

10.10 PCR to gain PFTT

Verification of (PCR) 1.2 SLAC-TT

10 12 PCR to verify construction of Pgap-PHO1-pelA-TT-PgapEA-FLO10-SLAC-TT
2019-10-12 20:46:05 assembly: SLAC group 1(1,2), 2(1,2), 3(1,2), 4; VP group 1(1,2), 2(1,2)

Verification of (PCR) 1.2 FLO10-SLAC