3A assembly side project

Project: iGEM uppsala 2019
Authors: JINWENYUAN

THURSDAY, 13/6/2019

MONDAY, 17/6/2019

Prepare solutions for future usage (Johan)

Aim:

prepare solutions as follows:
0.9% NaCl (10ml)
1 M $CaCl_2$ (10ml)
LB media (600ml)
LB Agar Plates (600ml) and Addition of Antibiotics

Materials:

NaCl, $CaCl_2$, Yeast extract , Bacto^{TM}tryptone, agar, double destilled H_2O and a 5M NaOH solution.

Method:

A NaCl solution was prepared according to the protocol:

0.9% NaCl (10ml)

The ammount of NaCl that was used was 0.093g

A $CaCl_2$ solution was prepared according to the protocol but 1.11g of $CaCl_2$ was used

1 M CaCl2 (10ml)

Liquid-Broth media was produced according to the protocol:

LB media (600 mL)

But the ammount of chemicals used can be seen in the table below: NaCl used was 5.9619g and the ammount of tryptone was 6g.

Ammount of chemicals used.

	A	B	C
1	NaCl	5.9619g	
2	BactoTMtryptone 1% (w/v)	6.0078g	
3	yeast extract 0.5% (w/v)	3.0009g	
4	ddH2O	600ml	
5	5 M NaOH	100ul	

A LB agar media was prepered by following the protocol:

LB Agar Plates (600 ml LA) and Addition of Antibiotics

Table1

	A	B	C
1	NaCl	5.9592g	
2	BactoTMtryptone 1% (w/v)	6.0027g	
3	yeast extract 0.5% (w/v)	3.0069g	
4	ddH2O	600ml	
5	5 M NaOH	100ul	
6	Agar	9.0016g	

Transformation of pSB1C3_K608006 (Johan & Manish)

Aim:

Since other groups prepared: pSBIC3_K592009 (amcilCP) for the CDS part, our group only need to transform the plasmid: pSB1C3_K608006 for the Promoter+RBS part, from the well 5E on plate 1 in 2019 IGEM distribution kit into DH5α competent cells for the amplification.

Materials:

DNA Plasmid: pSB1C3_K608006 (conc. 200~300 pg/uL, 1uL)
DH5α Competent cells (50 uL)
LB media
LA+Cm

Procedure:

Following steps were done according to the protocol: Transformation

1. Dilution of DNA sample was done according to the instruction of 2019 IGEM distrubution kit. (10 uL ddH_2O was added into the well 5E on Plate1 for resuspension of the plasmid:pSB1C3_K608006)
2. Competent cells was thawed on ice for more than 25 mins.
3. 1 uL of plasmid resuspension solution and 1uL of ddH_2O (as negative control) were added above to 50μL of competent cells.
4. Then the mixtures were Incubate for 30 minutes on ice
5. 950μL of LB Media (pre-heated to 37°C) were added to the mixtures.
6. Mixtures then were incubated for 80 minutes at 37°C, with occasional gentle mixing by inversion of the tubes.
7. For the mixture containing plasmid: pSB1C3_K608006, it was mixed gently and plated 100μL (=1/10th) on an agar plate containing kanamycin.
8. For the rest mixures, they were spined down at 4000rpm for 5min, at RM (Room Temperature).
9. All but 100μL of the supernatant of the remaining mixtures were discarded and the pellets were resuspended in the remaining 100μL.
10. The remaining suspension were spread on an agar plate.

To-to-list for following days :

- [x] **Day 1:** Tranformation
- [ ] **Day 2**: Calculate transformation efficiency (colonies/μg) of the competent cells. In the evening, re-streak appropriate colonies
- [ ] **Day 3**: Set overnight cultures for preparation of plasmid miniprep.
- [ ] **Day 4**: Make plasmid miniprep and glycerol cell stocks.

TUESDAY, 18/6/2019

Continued from 2019/06/17:

Conclusion and discussion:

We failed the transformation yesterday, no colonies on plates of all groups. It may be due to the bad transformation efficiency of our competent cells since the competent cells we used yesterday were from last year. So, we plan to make our competent cells again.

Preperation of competent E.coli(DH5a) cells (Johan, Manish & Lilli)

Aim:

Due to the failed transformation yesterday new competent cells are neccery to perform a new transformation of the plasmid. The new copetent cells are hoped to bring a succesfull transformation.

Protocol:

The copetent cells are produced by the following protocol:

Preperation of E.coli cells for transformation

WEDNESDAY, 19/6/2019

Prepare solutions for future usage (Johan & Manish)

Aim:

prepare solutions as follows:
2xLB media (600mL)

LB media with low NaCl concentration (600mL)

Materials:

NaCl , Yeast extract , Bacto^{TM}tryptone, agar, double destilled H_2O and a 5M NaOH solution.

Method

Two Liquid-Broth media was produced according to the protocol:

LB media (600 mL)

But the NaOH was added after autoclaving and was then autoclaved again.

A Liquid-Broth media with low NaCl concentration was produced according to the protocol:

LB media (600 mL)

But the ammount of NaCl that was added was 2.98 g.

THURSDAY, 20/6/2019

3A Assembly and gel analysis (Lilli)

Aim: to build a J23110+RBS+amcilCP(CDS)+vector(pSB1K3) construct

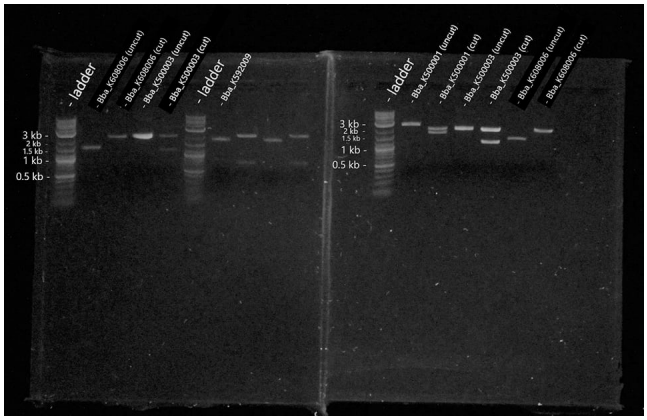
Materials:

ddH_2O, 2 DNA plasmids: psBIC3 and psBIK3/psBIAS, 10x reaction buffer, 3 restriction endonucleases: EcoRI, XbaI, and SpeI.

Method:

Proceeded according to protocol:

image.png



MONDAY, 1/7/2019

3A assembly on Jinwens samples (Lilli)

Digestion

Aim:

To build a Lac+Bba_K50003 construct (we are not gonna do the backbone now, another group)

Materials:

94.5 ng/µl Gene (Bba_K50003), 83,1 ng/µl Promotor (Lac), ddH_20, 5 µl Reaction Buffer, 1 µl EcoR1, Spe1, and Xba1.

Methode:

Proceeded according to protocol 3.

We also did control samples of the gene and promotor with a total of 20 µl.

Gel analysis

Aim:

See if the digestion was succesful.

Materials:

Our digestion samples (Promotor, Control promoter, Gene, Control gene) , Purple Dye, Loading dye, GeneRuler. (The gel was already pre-done)

Methode:

Methode proceeded according to protocol 3.

Result:

image.png

Ladder, Promotor, Control Promotor, Gene, Control Gene

TUESDAY, 2/7/2019

Digestion of backbone pSB1C3 (Lilli)

Aim:

Part of yesterdays 3A assembly since we didnt have a backbone. We want to cut the backbone so its complemetary to our gene and promotor samples.

Materials:

25 ng/µl Backbone (pSB1C3), ddH_20, Solution Buffer, EcoR1 and Pst1

Methode:

Methode proceeded according to protocol 3.

Made a 500 ng sample of backbone with enzymes and a control sample of 200 ng backbone.

Gel analysis of digested backbone

Proceeded like yesterdays gel analysis.

Results:

Not sucesfull. No result.

Ligation

Aim:

SInce our backbone digestion not was sucesful we are gonna use Vanjas groups backbone (PSB1A). This we are gonna ligate with our DIgested gene and promotor from yesterday.
Bba_K50003 + Lac + PSB1A3

Materials:

2 µl of Bba_K50003, Lac and PSB1A3, 11 µl ddH_2O, 2 µl reaction buffer, 1 µl T4 DNA ligase

Methode:

Methode proceeded according to protocol 3.

During the incubaation in romm temperature i accidentally put the sample in ice for about 5 min. So it was incubated in room temperature for about 15 min then put on ice, then incubated at 80 degres.

Transformation

Aim:

Putting our, hopefully ligated, plasmids in to bacteria.

Materials:

Ewerything in protocol 6.

Methode:

Proceeded according to protcol 6.

THURSDAY, 4/7/2019

Transformation (Lilli)

Aim:

We are changing our pSBIK3 backbone because we did not digest it properly according to our gel analysis. We are instead using the ampicilin resistance pSBIA3 bacbone. Transforming a plasmid with the compondments Lac+ GLOX+ pSBIA3. This so we can put our plasmid in to backterias.

Materials/Methode:

See protocol 6.

MONDAY, 8/7/2019

3A assembly(Johan and Manish)

Aim:

The aim of this 3A assembly is to produce a Lac+GLOX+pSBIA3 plasmid.

Digestion (Johan and Manish)

Aim:

To prepare the diffrent genes for ligation.

Materials:

See the following protocols:

3A assembly-Digestion and ligation

The concentratiobn of the samples used and the volume used to reach a amount of 500 ng.

Table2

	Components	Concentrations	Volume
1	GLOX	83.1 ng/mL	6 µL
2	Lac	90.4 ng/mL	5.5 µL
3	pSBIA3	25 ng/mL	20 µL

Methode:

See the following protocols:

3A assembly-Digestion and ligation

Ligation (Johan and Manish)

Aim:

To prepare the construct for transformation.

Materials:

See the following protocols:

3A assembly-Digestion and ligation

Methode:

See the following protocols:

3A assembly-Digestion and ligation

Transformation(Johan and Manish)

Aim:

To introduce the construct into bacteria.

Materials:

See the following protocols:

3A assembly-Digestion and ligation

Methode:

See the following protocols:

3A assembly-Digestion and ligation

With the exception that the heat shock was carried out in 80°C inseted of 42°C

WEDNESDAY, 10/7/2019

No new growth was detected on agar plates with the transformed cells.

THURSDAY, 11/7/2019

3A assembly(Johan and Manish)

Ligation (Johan and Manish)

Aim:

To prepare the construct for transformation.

Materials:

See the following protocols:

3A assembly-Digestion and ligation

Methode:

See the following protocols:

The ammount of GLOX used was 9 µL (90 ng) of GLOX instead of 2 µL (20 ng).

3A assembly-Digestion and ligation

Transformation(Johan and Manish)

Aim:

To introduce the construct into bacteria.

Materials:

See the following protocols:

3A assembly-Digestion and ligation

Methode:

See the following protocols:

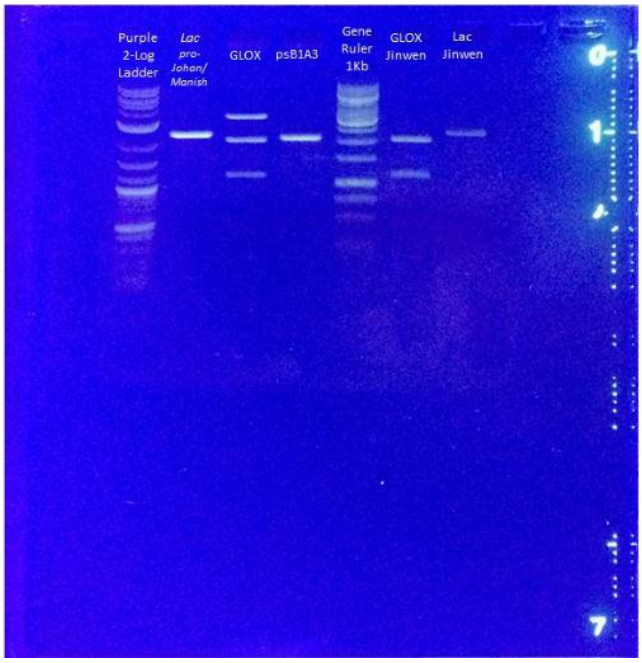
3A assembly-Digestion and ligation

The temprature for heat shock was 80°C insted of 42°C

0.7% Agaros gel analysis (Johan and Manish)

Reuslts:

GelManishJohan.JPG



Conclusion:

Thiere is suspected that thiere is a problem with the enzymes EcoRI

MONDAY, 15/7/2019

3A assembly(Johan and Manish)

Aim:

The aim of this 3A assembly is to produce a Lac+GLOX+pSBIA3 plasmid.

Digestion (Johan and Manish)

Aim:

To prepare the diffrent genes for ligation.

Materials:

- (1) 8-tube strip, or (3) 0.6ml thin-walled tubes
- BioBrick Part in BioBrick plasmid (Purified DNA, > 16ng/ul)
- dH2O
- NEB Buffer 2
- BSA
- Restriction Enzymes: EcoRI, SpeI, XbaI, PstI

Table3

	Components	Concentrations	Volume
1	GLOX	83.1 ng/mL	3 µL
2	Lac	90.4 ng/mL	3 µL
3	pSBIA3	25 ng/mL	3 µL

Method:

1. Add 250ng of DNA to be digested, and adjust with dH20 for a total volume of 16ul.
2. Add 2.5ul of NEBuffer 2.
3. Add 0.5ul of BSA.
4. Add 0.5ul of EcoRI.
5. Add 0.5ul of PstI.
6. There should be a total volume of 20ul. Mix well and spin down briefly.
7. Incubate the restriction digest at 37C for 30min, and then 80C for 20min to heat kill the enzymes. *We incubate in a thermal cycler with a heated lid*
8. Run a portion of the digest on a gel (8ul, 100ng), to check that both plasmid backbone and part length are accurate.

Validety analysis(Johan and Manish)

Aim:

The aim of this was to analyse the validety of the restriction enzymes EcoRI, XbaI,SpaI and PstI

Matrial:

Agaros
1xTBEbuffert

Method:

A 0.7% w/w agaros gel was made and the digest was but in the wells and aelectrophoresis was run at ~90 V for two houres.

Ligation (Johan and Manish)

Aim:

To prepare the construct for transformation.

Materials and Methode:

- Add 2ul of digested plasmid backbone (25 ng)
- Add equimolar amount of EcoRI-HF SpeI digested fragment (< 3 ul)
- Add equimolar amount of XbaI PstI digested fragment (< 3 ul)
- Add 1 ul T4 DNA ligase buffer. **Note:** Do not use quick ligase
- Add 0.5 ul T4 DNA ligase
- Add water to 10 ul
- Ligate 16C/30 min, heat kill 80C/20 min

Transformation(Johan and Manish)

Aim:

To introduce the construct into bacteria.

Materials:

See the following protocols:

3A assembly-Digestion and ligation

Methode:

See the following protocols:

3A assembly-Digestion and ligation

TUESDAY, 16/7/2019

Two cultures had apperd on the plates of the transformed cells one red and one transparent

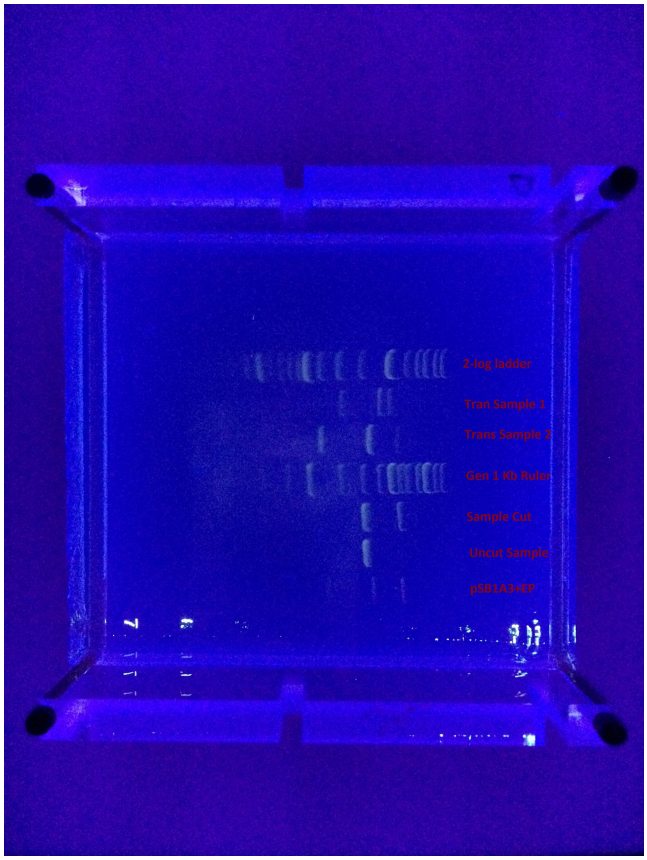
Overnight cultures were run by putting cell colonys in LB and leving over night for 14 hour in 37°C

WEDNESDAY, 17/7/2019

Miniprep was run on the overnight cultures in accordence with the instructions for the miniprep kit.
Analytical digestion and gel electrophoresis was done in accordence with the protocoll:

Analytical Digestion and Agarose Gel Electrophoresis

iGEM_trans_before_gibbs.jpg



THURSDAY, 18/7/2019

Cells from prior this week was digested with the digestion part of the protcoll:

3A assembly-Digestion and ligation

Colony PCR was performed on the digested cell in accordence with the protocoll:

PCR for synthesizing DNA fragments

Gel electrophoresis was performed on the digest and bacteria in accordence with:

3A assembly-Digestion and ligation

TUESDAY, 23/7/2019

3A Assembly (Lilli)

Main project

Project: iGEM uppsala 2019
Authors: JINWENYUAN

THURSDAY, 20/6/2019

FRIDAY, 21/6/2019

Transformation of Shuttle vector (Lilli)

To amplify our shuttle vector pPICZaB through transformation.

Materials:

150 µl Competent cells, 1 ng/µl pPlcz α-B dilution and ddH_2O.

Methode:

The methode was proceeded according to this protocol:

Transformation of Competent E.coli cells

However in step 2 our competent cells were thawed for 30 min on ice instead o 15 min.
In step 3 we added 10 µl of our shuttle to 50 µl of our competent cells and in another tube 10 µl of ddH_2O to 50 µl competent cells.

TUESDAY, 25/6/2019

O/N culture (Lilli)

Aim:

Grow an O/N culture so we can peform a plasmid miniprep.

Materials:

100 ml LB media and 25 µl Zeocin

Methode:

The methode was proceeded according to protocol 2.

6 ml LB + Zeocin mixture was placed in each of the 6 tubes.

(This O/N culture was discarded the following day)

WEDNESDAY, 26/6/2019

O/N culture (Lilli)

Aim:

Grow an O/N culture so we can peform miniprep

Materials:

Low salt LB meda and Zeocin

Methode:

The methode was proceeded according to protocol 2.

50 ml LB media and 2,5 µl Zeocin.
6 ml of mixture was placed in 6 falcon tubes each.

THURSDAY, 27/6/2019

Miniprepkit (Lilli)

Aim:

To purify our pPICzaB shutter vector. So we can do an analytic digestion with our samples.

Materials:

Our O/N culture, 250 µl Respuspenstion Solution, 250 µl Lysis solution, 350 µl Neutralization Solution, 500 µl Wash Solution and 50 µl Elution Buffer

Methode:

Methode was proceeded according to Thermo Scientific GeneJET Plasmid Miniprep Kit (Quick Protocol)

Values for purified DNA was also checked. (Can be found in LIllis lab journal if needed)

Digestion

Aim:

To cut our shuttle vector.

Materials:

200 ng DNA, 20 µl digeston mixture, EcoR1

Methode:

Proceeded according to protocol 3.

6 samples.

MONDAY, 1/7/2019

Gel analysis on Lillis pPICZaB samples (Lilli)

Aim:

See if the digsteion was succesful.

Materials:

The 6 digested samples , ladder (1 µl GeneRuler, 1 µl Purple Dye, 4 µl ddH_20)

Methode:

Methoded proceeded according to protocol 4

Samples: Total volume of 6 µl (5 µl digested samples and 1 µl Purple Dye)

Result:

The gel was empty, no results. Probably something was wrong with the gel or the Purple Dye.

TUESDAY, 2/7/2019

Gel analysis on Lillis pPICZaB samples #2 (Lilli)

Ewerything was proceeded like yesterday exept that the Purlple Dye was exanged for BLue Dye.

Result:

The results this time were pretty OK. We can see the bands and hey are about the right size they should be. The shutter vector samples are saved.

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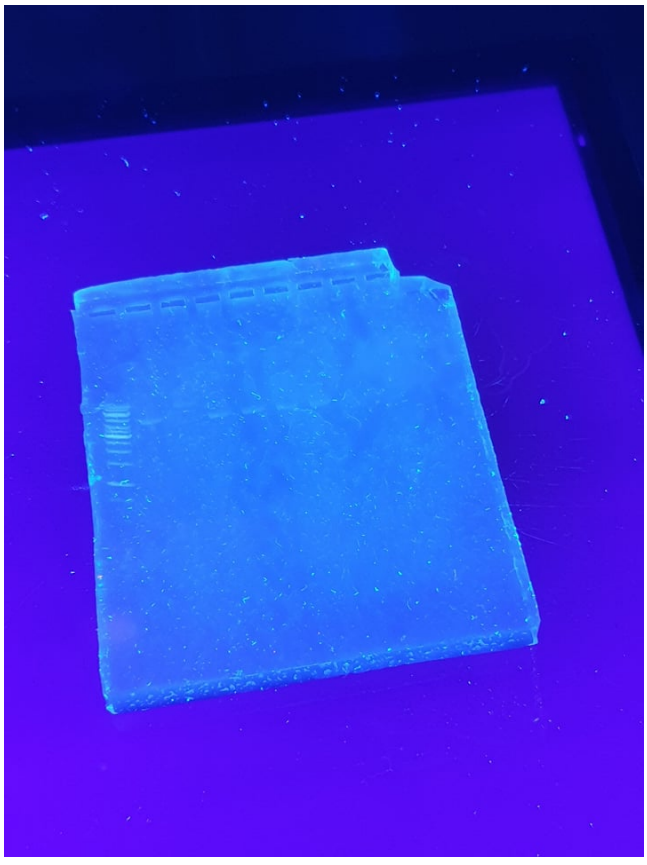


Ladder, sample 1-6

TUESDAY, 23/7/2019

PCR to add overhangs, which have overlaps to pPICZaB, to AAO_P.pastoris, HRP_P.pastoris and MnP, LiP, GLOX genes. (Jinwen & Johan)

refer to PCR protocol (WHICH WAS WRONG ORIGINALLY)
Result: we got nothing, according to gels.

We did this again today, refer to PCR protocol.

HRP reaction mix

Table1

	A	B
1	ddH2O	14.5μL
2	2 mM dNTPs	5 μL
3	Forward primer (5 μM)	5 μL
4	Reverse primer (5 μM)	5 μL
5	5x Phusion® HF buffer	10 μL
6	HRP (10ng/μL)	10μL
7	Phusion® HF DNA polymerase	0.5 μL
8	Total	50 μL

AAO reaction mix

Table2

	A	B
1	ddH2O	14.5μL
2	2 mM dNTPs	5 μL
3	Forward primer (5 μM)	5 μL
4	Reverse primer (5 μM)	5 μL
5	5x Phusion® HF buffer	10 μL
6	AAO (10ng/μL)	10μL
7	Phusion® HF DNA polymerase	0.5 μL
8	Total	50 μL

PCR program setting up for HRP

Table3

	A	B	C	D
1	Description of Step	Temp (°C)	Time: (hh:mm:ss)	Number of cycles
2	Initial denaturation	98	00:00:30	1x
3	Denaturation	98	00:00:10	2x
4	Annealing temp. + 4°C	67	00:00:30	
5	Extension	72	00:00:38	
6	Denaturation	98	00:00:10	6x
7	Annealing temp. + 2°C	63	00:00:30	
8	Extension	72	00:00:38	
9	Denaturation	98	00:00:10	25x
10	Extension	72	00:00:38	
11	Final extension	72	00:07:00	1x
12	Storage	4	∞	1x

PCR program setting up for HRP

Table4

	A	B	C	D
1	Description of Step	Temp (°C)	Time: (hh:mm:ss)	Number of cycles
2	Initial denaturation	98	00:00:30	1x
3	Denaturation	98	00:00:10	2x
4	Annealing temp. + 4°C	63	00:00:30	
5	Extension	72	00:01:00	
6	Denaturation	98	00:00:10	6x
7	Annealing temp. + 2°C	61	00:00:30	
8	Extension	72	00:01:00	
9	Denaturation	98	00:00:10	25x
10	Extension	72	00:01:00	
11	Final extension	72	00:07:00	1x
12	Storage	4	∞	1x

Gel result:

WEDNESDAY, 24/7/2019

Gibson assembly of AAO/HRP/MnP/GLOX/LiP to pPICZaB

prepare reaction mix for AAO

Table5

	A	B	C
1		test group	Positive Control**
2	AAO_PCRpurification	2.4 µl	10 µl
3	pPICZaB_PCR purification	7.2 µl	
4	Gibson Assembly Master Mix (2X)	10 µl	10 µl
5	Deionized H2O	0.4 µl	0
6	Total Volume	20 µl	20 µl

TUESDAY, 30/7/2019

PCR for pPICZaB_HRP minipreps(1,2,3) (Jinwen)

see PCR protocal

miniprep for pPICZaB_AAO_2A_eGFP(E1,E2,E3,E4,E5) & PCR & Gel analysis

see miniprep, PCR, Gel analysis protocal

Colony PCR for pPICZaB_HRP colonies (Jinwen)

see colony PCR protocal

PCR for pPICZaB_HRP minipreps(1,2,3)(Johan)

The pPICZaB_HRP minipreps was removed from the frezer and put on ice. Then the following PCR protocoll was followed:

PCR (Dreamtaq)

The anneling temperature was set to 50°C and the exstension time was set to 1 minute and 30 secondes. A master mix of dNTPs, 2 mM; phosphorylated forward primer, 5 µM, phosphorylated reverse primer, 5 µM and 10X DreamTaq Buffer. A 1% agarose gel was made and the three samples were analised next to a 1kb gene ruler ladder.

WEDNESDAY, 31/7/2019

MONDAY, 5/8/2019

Sequencing of PCR products. (Johan)

Aim:

To investigate if transformation was succsesfull.

Materials:

ddH_2O, Samples that code for diffrent genes and SeqPrimer.

Methode:

Teplates were made of 15 µl of purified DNA with a concentration of about 10 ng/µl of the DNA and then 2 µl of Seqprimer ot a concentration of 10 pmol/µl the tubes were then seal and sent for sequncing. The serialnumber and the content of the test tubes can be found in the table below:

Table6

	A	B
1	EF30500622	HRP-2A-AAO-2
2	EF30500623	HRP-2A-AAO-1
3	EF30500624	PPIC-GLOX-3
4	EF30500625	PPIC-MnP-2
5	EF30500626	PPIC-MnP-3
6	EF30500612	PPIC-MnP-1
7	EF30500611	PPIC-HRP-1
8	EF30500610	PPIC-HRP-2
9	EF30500609	PPIC-HRP-3

WEDNESDAY, 7/8/2019

Gibson assembly (Jinwen)

Aim:

insert HRP_2A_eGFP into pPICZaB & insert AAO_2A_eGFP into pPICZaB

Materials:

HRP_2A_eGFP DNA fragments
AAO_2A_eGFP DNA fragments
ddH2O
Gibson assembly Master Mix

Methods:

refer to the Gibson assembly protocol

Transformation (Jinwen)

Aim:

to tranform the asembled products from 8/7 and also from 26/7 into DH5a for further construc amplification

Materials:

Gibson assembled products (HRP_2A_eGFP+pPIC, AAO_2A_eGFP+pPIC, AAO+pPIC)
DH5a super competent cells from NEB
ddH20 (negative control)
pPICZaB (positive control)
SOB LB media
LSLB(+zeocin) plates

Methods:

refer to the transformation protocol

Ordered DNA fragments and primers for signal peptide mutation side project (Jinwen)

Sequence confirmed for pPICZaB-MnP, HRP, HRP_2A_AAO construct, and for GLOX, we had a point mutation, which may come from the distribution kit, or caused by amplification in DH5A.

THURSDAY, 8/8/2019

We got plenty colonies from transformation yesterday

O/N culture from 6 colonies on each plate (Jinwen, Qian, Erik, Irina)

see O/N culture protocal

Plan for the next day:

1. colony PCR and Gel analysis in the morning, and glycerol stock from O/N of each sample
2. if we got any right size gene in the gel, prepare midiprep O/N for the next day after tomorrow from the relevant glycerol stock

FRIDAY, 9/8/2019

Colony PCR, glycerol stock & Gel analysis (Jinwen, Qian, Johan, Erik, Jenny)

Aim:

verify the right construct among colonies on the transformation plates, through making colony PCR directly on cells in O/N yesterday with pPIC sequencing primers.

Methods:

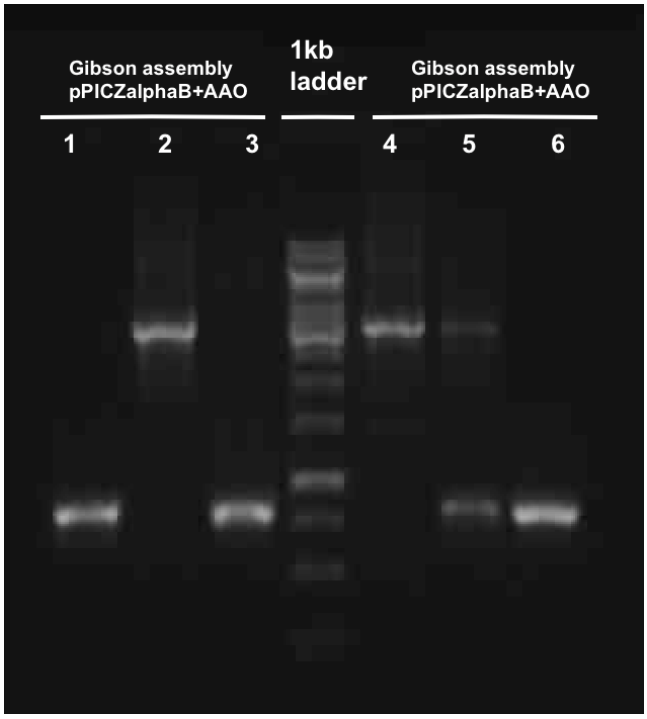
refer to glycerol stock protocol
600 micro liters culture from each sample, mixed with 400 micro liters 50% glycero stock

refer to colony PCR protocol
400 microliters culture from each sample were spun down (6000 rpm, 5 mins), cells were taken by dipping into the pellet with a tip and resuspended into 30 microliters water.

refer to gel analysis protocal

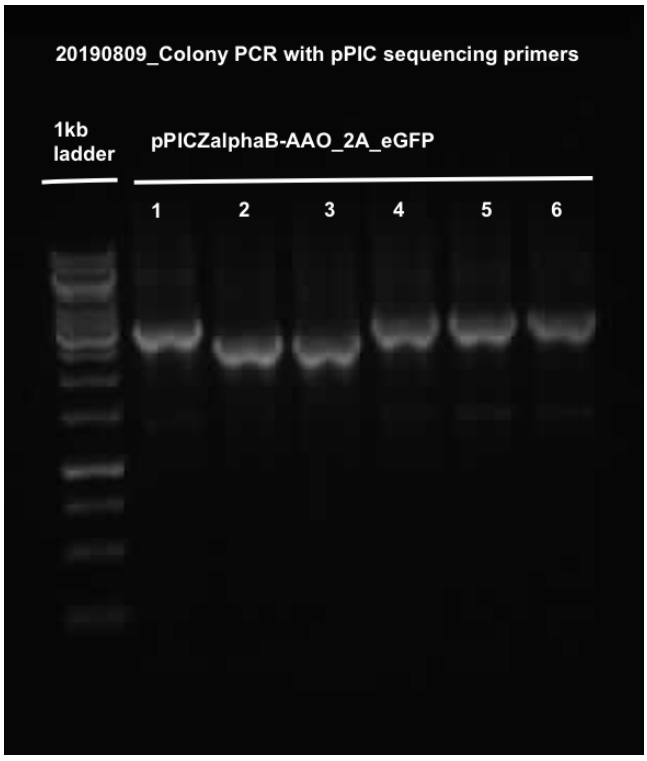
Results:

pPICZaB_AAO (Jinwen)



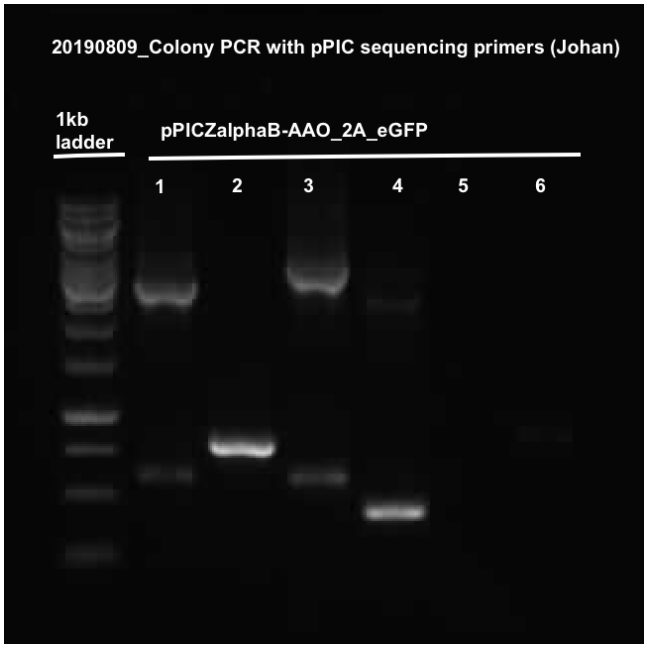
conclusion & discussion: all samples from AAO failed, may due to the wrong Gibsonassembly products was taken, redo AAO's transformation with Qian's groups' AAO+pPIC Gibson assembly product. Samples may be mixed up with AAO-2A-eGFP, this may explain why we got band around 3kb.

pPICZaB_AAO_2A_eGFP (Jenny)



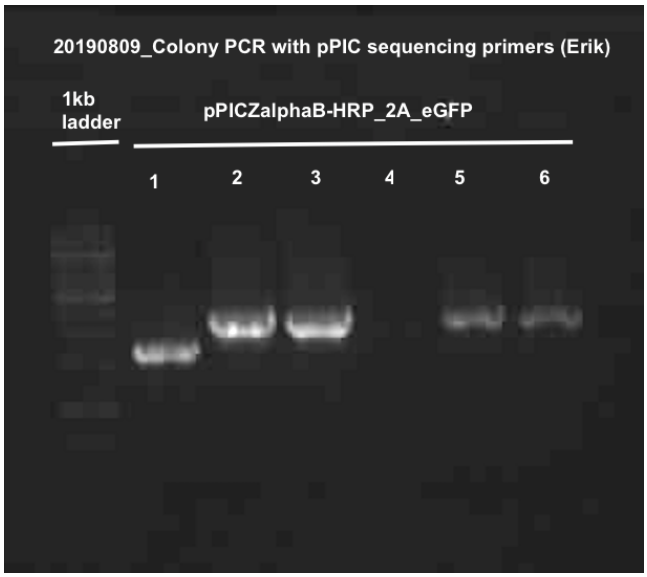
#2& #3 Glycerol stocks were discarded, #6 was taken to make O/N for the nextday's midiprep.

pPICZaB_AAO_2A_eGFP (Johan)



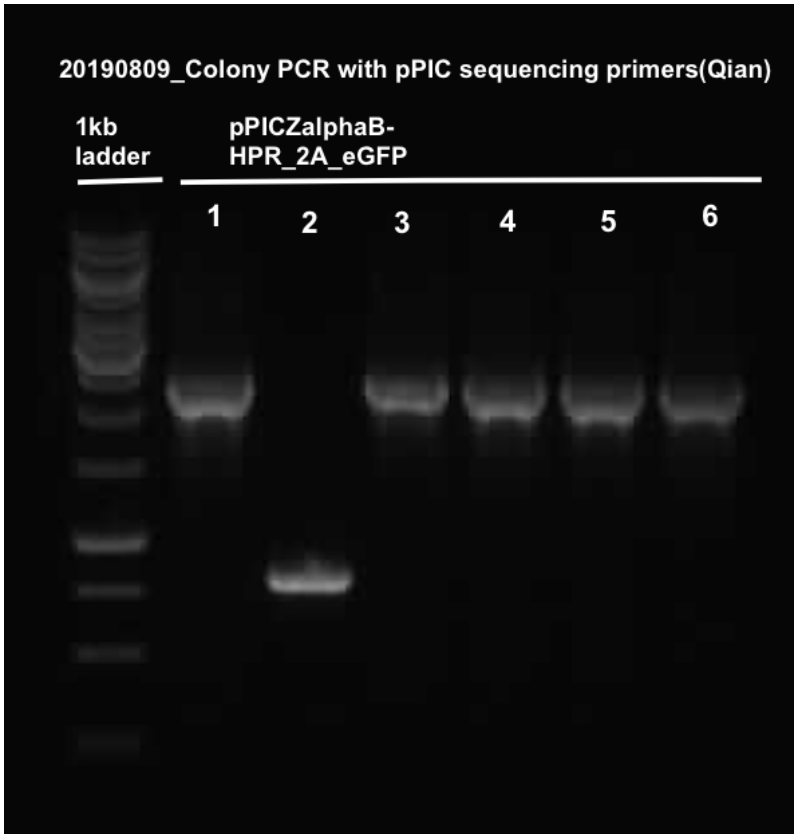
From the gel, the upward band in Lane 1&3 is correct, but Lane 1&3 also contains the band around 300bp, so we supposed that Sample 3 was contaminated with cells carrying the empty plasmid. All samples related with this gel were discarded.

pPICZaB_HRP_2A_eGFP (Erik)



the amplicon we expected here for HRP-2A-eGFP is around 2100bp, so we got the right construct in the #2, #3, #5, #6 samples of Erik's. 1&4 's glycerol stock was discarded.

pPICZaB_HRP_2A_eGFP (Qian)



#2 was discarded, the others are all fine

O/N culture (50ml) for the next day's midiprep (Jinwen & Qian)

Aim:

to prepare DNA for transformation in yeast, which needs at least 1 micro grams DNA.

methods:

refer to O/N protocol

Plan for the next:

1. midi prep for the O/N culture of AAO-2A-eGFP & HRP-2A-eGFP (done by 10/8)
2. DNA preparation for transformation in yeast (DIgestion with SacI) (done by 10/8)
3. Transformation of the Gibson assembly product for AAO & LiP (done by 11/8)

MONDAY, 12/8/2019

Sequencing

Send construct of pPICZaB-HRP_2A_eGFP & pPICZaB_AAO_2A_eGFP & AAO? for seqeuencing with pPIC sequencing fwd primer.

Table7

	A	B
1	EF30500574	pPICZaB-AAO-midiprep
2	EF30500575	pPICZaB-HRP-2A-eGFP midiprep
3	EF30500576	pPICZaB-AAO-2A-eGFP midiprep

We got colonies on transformation plates for pPICZaB-AAO and pPICZaB-Lip GA products, and growed O/N culture on Sunday.

Colony PCR with pPICZ sequenceing primer and gel analysis for pPICZaB-AAO and pPICZaB-Lip constructs.

Aim:
to check if we get the right construct or not.
method:
refer to colony PCR protocol
result:

uv room computer， by qian

WEDNESDAY, 14/8/2019

Table8

	A	B
1	EF30500577	pPICZaB-AAO-midiprep
2	EF30500578	pPICZaB-LiP midiprep
3		

FRIDAY, 16/8/2019

PCR to add overhangs to LiP/MnP in pPICZaB construct, and to mAAO fragment ordered from IDT.

Components in PCR for adding overhangs

	A	B	C	D
1	LiP PCR	1 ng PICZaB-LiP	25uM OvH_ProK_LiP_fwd	25uM pPIC-XXX_Rev
2	MnP PCR	1 ng PICZaB-MnP	25uM OvH_ProK_MnP_fwd	25uM pPIC-XXX_Rev
3	mAAO PCR to add overlap with proa	2 ng mAAO	25uM OvH_Proa_mAAO_fwd	25uM mAAO_Rev
4	mAAO PCR to add overlap with proK	2 ng mAAO	25uM OvH_ProK_mAAO_fwd	25uM mAAO_Rev
5	pPICZaB linerize w/o a signal peptide	1 ng PICZaB	25uM mutLinerize_fwd	25uM mutLinerize_Rev

refer to protocal

Gel analysis of adding overhangs result