iGEM Tianjin interlab Notebook
Team：iGEM 2018 Tianjin
Dates：2018-7-09 to 2018-8-24

Dates：2018-07-09 to 2018-07-15, the 1st week
Deletion of the three genes-Gal4, Gal80, BarI

Monday 2018-07-09
Synthesis of genes and primers

Wednesday 2018-07-11
Construction of gRNA plasmid and donor DNA
• PCR the genes and purify with 1%Sepharose agarose gel electrophoresis. Gain the pure gene through DNA gel extraction kit.
• Overlap the genes to get donor DNA and purify with the same method. Name as donor DNAI, donor DNAII and donor DNAIII, which are to delete Gal4, Gal80 and BarI, respectively.
• Constructe the three plasmids containing gRNA, which are to delete Gal4, Gal80 and BarI, respectively.
• Purify the plasmid through DNA purification kit.
Culture the wild type yeast-BY4741 in the following condition:
• 30℃, 220rpm, overnight(~ 12h), in YPD liquid medium.

Thursday 2018-07-12
Yeast transformation
• Centrifuge(5000rpm, 2min), wash and centrifuge(5000rpm, 2min) cells cultured last night.
• Incubate cells in SC-Leu-Ura liquid medium on ice.
• Perpare the transformation system consisting of PEG3500, 1M LiOAc, ssDNA and transformation gene(~100ng).
·Mix the transformation system and cells that are centrifuged and resuspended in water(~100μL)
·Heat shock at 42℃ for 18 min.
·Leave on ice for 2min, centrifuge(3600rpm, 1min) and resuspend with 5mM $CaCl_2$ solution(~400μL).
• Centrifuge and resupend the cells in 100μL water.
• Coat on the SC-Leu-Ura solid media and culture in 30℃ incubator for at least 48h.

Saturday 2018-7-14
Streak plate
Select the single colony and culture by the streak method on the SC-Leu-Ura solid media. Culture in 30℃ incubator overnight.

Sunday 2018-7-15
PCR validation
colony PCR
·Boil the cells in 20mM NaOH(50μL) with the protocl(find this on our wiki).

·PCR validation.

·Select the right single colony and culture by the streak method on a new YPD solid media. Culture in 30℃ incubator overnight.

·Store the right cells with glycerinum method at -80℃ and number as **A.**

Dates: 2018-07-16 to 2018-07-22, the 2nd week

Loss of plasmids

Culture the yeast on YPD solid media. And validate the yeast in real time until the plasmids used to delete genes are lost.

Validation of the colony that has lost the plasmid.

Dates：2018-07-23 to 2018-08-05, the 3rd and the 4th weeks

Constructe the plasmids in yeast two-hybrid system

Monday 2018-7-23

Amplification of genes

PCR the genes TEF1P, TEF1T, PGK1P, PGK1T, TDH3P, ADH1T, KaiA, KaiB, KaiC, CikA, RpaA, SasA, AD and BD(You can see what theae genes are on our main page.)

Tuesday 2018-7-24

Purification of the genes PCRed the last day.

The genes in each table make up one yeast two-hybrid system(Hereinafter referred to as Y2H).

Table.1 The first combination of Y2H

Genes	KaiA	KaiB	AD&KaiC	CikA	RpaA	BD&SasA
Promoters	TEF1P	PGK1P	TDH3P	TEF1P	PGK1P	TDH3P
Terminatiors	TEF1T	PGK1T	ADH1T	TEF1T	PGK1T	ADH1T
Genes' order	P-KaiA-T	P-KaiB-T	P-AD-KaiC-T	P-CikA-T	P-RpaA-T	P-BD-SasA-T

Table.2 The second combination of Y2H

Genes	KaiA	KaiB	AD&KaiC	BD&CikA	RpaA	SasA
Promoters	TEF1P	PGK1P	TDH3P	TEF1P	PGK1P	TDH3P
Terminatiors	TEF1T	PGK1T	ADH1T	TEF1T	PGK1T	ADH1T
Genes' order	P-KaiA-T	P-KaiB-T	P-AD-KaiC-T	P-BD-CikA-T	P-RpaA-T	P-SasA-T

Table.3 The third combination of Y2H

Genes	KaiA	AD&KaiB	BD&KaiC	CikA	RpaA	SasA
Promoters	PGK1P	TDH3P	TDH3P	TEF1P	PGK1P	TEF1P
Terminatiors	PGK1T	ADH1T	ADH1T	TEF1T	PGK1T	TEF1T
Genes' order	P-KaiA-T	P-AD-KaiB-T	P-BD-KaiC-T	P-CikA-T	P-RpaA-T	P-SasA-T

• Purify with 1%Sepharose agarose gel electrophoresis. Gain the pure gene through DNA gel extraction kit.

Wednesday 2018-7-25

Enzyme digestion of plasmids pRS413&pRS415

Cut the two plasmids with the restriction endonuclease NotI and EcoRI. Purify the plasmid through DNA purification kit.

Culture A in the following condition:

·30℃ , 220rpm, overnight(~ 12h), in YPD liquid medium.

Thursday 2018-7-26

Yeast transformation

We planned to constructe our plasmids with the principle yeast homologous recombination.

• Centrifuge(5000rpm, 2min), wash and centrifuge(5000rpm, 2min) cells cultured last night.

·Incubate cells in SC liquid medium on ice.

·Perpare the transformation system consisting of PEG3500, 1M LiOAc, ssDNA and transformation gene(~100ng).

·Mix the transformation system and cells that are centrifuged and resuspended in water(~100μL)

·Heat shock at 42℃ for 18 min.

·Leave on ice for 2min, centrifuge(3600rpm, 1min) and resuspend with 5mM $CaCl_2$ solution(~400μL).

·Centrifuge and resupend the cells in 100μL water.

·Coat on the SC-Leu solid media or SC-His solid media and culture in 30℃ incubator for at least 48h.

Notes: When carrying on yeast transformation, performe the two experiment groups simultaneously. One imports pRS413 and the other imports pRS415. And the kind of media used to culture yeast depends on the plasmids imported.

Saturday 2018-7-28

Streak plate

Select the single colony and culture by the streak method on the SC-Leu solid media or SC-His solid media . Culture in 30℃ incubator overnight.

Sunday 2018-7-29

PCR validation

colony PCR

• Boil the cells in 20mM NaOH(50μL) with the protocl(find this on our wiki).

·PCR validation.

·Select the right single colony and culture by the streak method on a new SC-Leu solid media or SC-His solid media. Culture in 30℃ incubator overnight.

·Store the right cells with glycerinum method at -80℃ and number as **B**(pRS413) and **C**(pRS415).

Monday 2018-7-30

Enzyme digestion of plasmids pRS413&pRS415

Cut the two plasmids with the restriction endonuclease NotI and EcoRI. Purify the plasmid through DNA purification kit.

Culture B and C in the following condition:
For B:30℃ , 220rpm, overnight(~ 12h), in SC-His liquid medium.
For C:30℃ , 220rpm, overnight(~ 12h), in SC-Leu liquid medium.

Tuesday 2018-7-31
Extraction of plasmid
Extract the plasmids pRS413 and pRS415 with Yeast plasmid extraction kit
Culture B and C in the following condition:
For B:30℃ , 220rpm, overnight(~ 12h), in SC-His liquid medium.
For C:30℃ , 220rpm, overnight(~ 12h), in SC-Leu liquid medium.

Wednesday 2018-8-1
Yeast transformation
• Centrifuge(5000rpm, 2min), wash and centrifuge(5000rpm, 2min) cells cultured last night.
·Incubate cells in SC liquid medium on ice.
·Perpare the transformation system consisting of PEG3500, 1M LiOAc, ssDNA and transformation gene(~100ng).
·Mix the transformation system and cells that are centrifuged and resuspended in water(~100μL)
·Heat shock at 42℃ for 18min.
·Leave on ice for 2min, centrifuge(3600rpm, 1min) and resuspend with 5mM $CaCl_2$ solution(~400μL).
·Centrifuge and resupend the cells in 100μL water.
·Coat on the SC-Leu-His solid media and culture in 30℃ incubator for at least 48h.
Notes: When carrying on yeast transformation, performe the two experiment groups simultaneously. C imports pRS413 and B imports pRS415.

Saturday 2018-8-4
Streak plate
Select the single colony and culture by the streak method on the SC-Leu-His solid media. Culture in 30℃ incubator overnight.

Sunday 2018-8-5
PCR validation
colony PCR
• Boil the cells in 20mM NaOH(50μL)
• PCR validation.

Dates: 2018-08-06 to 2018-08-12, the 5th week
Reconstruction the cell containing the pRS413 and pRS415 again.

Monday 2018-8-06
Enzyme digestion of plasmids pRS413&pRS415
Cut the two plasmids with the restriction endonuclease NotI and EcoRI. Purify the plasmid through DNA purification kit.

Culture B and C in the following condition:
For B:30℃ , 220rpm, overnight(~ 12h), in SC-His liquid medium.
For C:30℃ , 220rpm, overnight(~ 12h), in SC-Leu liquid medium.

Tuesday 2018-8-07
Extraction of plasmid
Extract the plasmids pRS413 and pRS415 with Yeast plasmid extraction kit
Culture B and C in the following condition:
For B:30℃ , 220rpm, overnight(~ 12h), in SC-His liquid medium.
For C:30℃ , 220rpm, overnight(~ 12h), in SC-Leu liquid medium.

Wednesday 2018-8-08
Yeast transformation
• Centrifuge(5000rpm, 2min), wash and centrifuge(5000rpm, 2min) cells cultured last night.
• Incubate cells in SC liquid medium on ice.
• Perpare the transformation system consisting of PEG3500, 1M LiOAc, ssDNA and transformation gene(~100ng).
• Mix the transformation system and cells that are centrifuged and resuspended in water(~100μL)
• Heat shock at 42℃ for 18 min.
• Leave on ice for 2 min, centrifuge(3600rpm, 1min) and resuspend with 5mM $CaCl_2$ solution(~400μL).
• Centrifuge and resupend the cells in 100μL water.
• Coat on the SC-Leu-His solid media and culture in 30℃ incubator for at least 48h.
Notes: When carrying on yeast transformation, performe the two experiment groups simultaneously. C imports pRS413 and B imports pRS415.

Friday 2018-8-10
Streak plate
Select the single colony and culture by the streak method on the SC-Leu-His solid media. Culture in 30℃ incubator overnight.

Sunday 2018-8-12
PCR validation
colony PCR
• Boil the cells in 20mM NaOH(50μL)
• PCR validation.
• Store the right cells with glycerinum method at -80℃ and number as **D**.

Dates: 2018-08-13 to 2018-08-26, the 6th and 7th weeks
Construct the Biobricks needed to be submitted on the plasmid pSB1C3

Monday 2018-08-13
Synthesis of primers

Wednesday 2018-8-15

Amplification of genes with new primers and purification of the genes

• PCR the genes TEF1P, TEF1T, PGK1P, PGK1T, TDH3P, ADH1T, KaiA, KaiB, KaiC, CikA, RpaA, SasA, AD and BD(You can see what theae genes are on our main page.)

• Purify with 1%Sepharose agarose gel electrophoresis. Gain the pure gene through DNA gel extraction kit.

Thursday 2018-8-16

Preparation of competent E.coli

Friday 2018-8-17

We planned to construct the plasmid with the method **Gibson Assembly**.

• Preparation the reaction system.

• React on the PCR instrument at 37℃ for 30min.

E.coli transformation

• Mix the reaction system with competent E.coli(~50μL)

• Leave on ice for 30min

• Heat shock at 42℃ for 90s

• Leave on ice for 2min and mix with 500μL LB liquid medium

• Culture at 37℃, 180rpm for 45min

• Centrifuge(3600rpm, 1min) and resuspend with 100μL water

• Coat on the LB+CM solid media and culture in 37℃ incubator overnight.

Saturday 2018-8-18

Streak plate

Select the single colony and culture by the streak method on the LB+CM solid media. Culture in 30℃ incubator overnight.

Sunday 2018-8-19

PCR validation

colony PCR, select the right single colony.

Culture the right cell on the following condition:

• 37℃, 220rpm, in LB+CM liquid medium overnight(~8h)

Monday 2018-8-20

Extraction of plasmid

Extract the plasmids pSB1C3 with Plasmid extraction kit.

Tuesday 2018-8-21

Gibson Assembly.

• Preparation the reaction system.

• React on the PCR instrument at 37℃ for 30min.

E.coli transformation

- Mix the reaction system with competent E.coli(~50μL)
- Leave on ice for 30min
- Heat shock at 42℃ for 90s
- Leave on ice for 2min and mix with 500μL LB liquid medium
- Culture at 37℃, 180rpm for 45min
- Centrifuge(3600rpm, 1min) and resuspend with 100μL water
- Coat on the LB+CM solid media and culture in 37℃ incubator overnight.

Wednesday 2018-8-22

Streak plate

Select the single colony and culture by the streak method on the LB+CM solid media. Culture in 30℃ incubator overnight.

Thuseday 2018-8-23

PCR validation

colony PCR, select the right single colony.

Culture the right cell on the following condition:

- 37℃, 220rpm, in LB+CM liquid medium overnight(~8h)

Friday 2018-8-24

Extraction of plasmid

Extract the plasmids pSB1C3 with Plasmid extraction kit.