

2017.02-03

Set up the project

2017.05~06

Search for paper data and visit professors who work in the field of protein secreted by yeast and cloning enzyme sequence.

2017.07

Design constructs as following:

3 composite parts---YEplac181-glucyn, YEplac195galSTE12-lipase, YEplac112-lightfusion.

5 biobricks---xylanase, glucanase, lipase, pSTE12, STE12 protein.

2017.08

Send out to IDT for DNA synthesis.

2017.08.23

Practice cloning with iGEM part: Endo-beta-1, 4-glucanase (BBa_K1175006), but failed in ligation and transformation.

2017.09.22

Restriction enzyme analysis of YEplac112 (with suffix): B4, B7, B8 digested with EcoRV+NheI (5108, 4171). Sample B8 was correct.

2017.09.29

Colony PCR of YEplac195 and 181 composite. The latter was correct.

2017.10.03

Restriction enzyme analysis of YEplac181 composite.

2017.10.04

Linearized DNA from IDT synthesis was ligated to pJET, using pJET kit, prefix and suffix primers for biobricks: xylanase, STE12 protein, and pSTE12.

Restriction enzyme analysis of GAL and GPD promoter.

Cloning of YEplac195-galSTE12lipase.

2017.10.05

Light fusion (with prefix) was ligated to YEplac112 (with suffix).

Lipase (with suffix) was ligated to YEplac195 (with prefix).
YEplac195 ligate with pJET-light fusion.
YEplac181 composite was complete. (But mistake after sequencing)

2017.10.06

Cloning of lightfusion to YEplac112.
Colony PCR of YEplac195 composite, pJET-xyn, pJET-STE12 protein.

2017.10.07

YEplac112 was checked by colony PCR. YEplac195 composite was mistakenly thought to be correct due to the band shifted.

2017.10.08

Restriction enzyme analysis of YEplac112 (with suffix): digested with EcoNI+SacI (7554, 1725). Correct. However, fail to successfully ligate into composite parts.
Restriction enzyme analysis of pJET-xyn and STE12 protein. Failed.

2017.10.09

Colony PCR for YEplac112 composite: 8 of 16 colonies showed the band at 1827 bps.
Restriction enzyme analysis of YEplac112 (with suffix): 10/07 mini & original B8.
Restriction enzyme analysis of YEplac112 composite

2017.10.10

Restriction enzyme analysis of YEplac112 composite: failed to see correct bands.
Restriction enzyme analysis of pSB1C3-gluxyn and lipase.

2017.10.11

Got the wrong plate for pSB1C3 cloning when maintaining single colony before staining it into colony PCR.

2017.10.12

Restriction enzyme analysis of pJET1.2-light fusion which digested with NdeI+HindIII (3577, 1224).
pSB1C3-pSTE12lip and pSB1C3-glu were from the wrong plate!!

2017.10.13

Cloning of YEplac112-light fusion. Set up 1:1 and 1:3 of molar ratio of vector and insert in ligation.

2017.10.14

Colony PCR for YEplac112 composite: 24 of 31 colonies showed the band at 2K.

Colony PCR for pSB1C3-GLUXYN (2061 bps) and pSB1C3-GLU (951 bps).

2017.10.15

Restriction enzyme analysis of YEplac112 composite: failed to see correct bands.

Inoculate colony of pSB1C3-GALSTE12 (no. 8, 10, 12) and pSB1C3-GLU (no. 3, 9, 15).

2017.10.16

Restriction enzyme analysis of pSB1C3-GALSTE12 digested with EcoRV+EcoNI (2162, 4952), and pSB1C3-GLU digested with EcoRV+BamHI (1513, 1148, 415).

Trouble shooting for YEplac112 composite by using new primers that overlap in prefix and suffix.

Restriction enzyme analysis of YEplac112 composite: digested with EcoNI+SacI (2678, 3155, 5246)

2017.10.17

1st DNA sequencing: pJET cloning into YEplac181, 195,112 were all correct, and so as pSB1C3-glu.

2017.10.18

Cloning of YEplac112-light fusion. Set up 1:1 and 1:3 of molar ratio of vector and insert in ligation.

Restriction enzyme analysis of pJET-glu.15.

2017.10.20

[iGEM X BioMod meeting]

Restriction enzyme analysis of pJET-XYN, pJET-pSTE12, pJET-LIP, and YEplac112-light fusion. Result: YEplac112-light fusion all ligate to the wrong insert of pJET.

Transformation of YEplac181 half-composite and YEplac195 half-composite that had been sequenced.

Colony PCR for YEplac112 composite. 2 of 24 colonies showed the band.

2017.10.21

Colony PCR for YEplac181 composite and YEplac181 half-composite.

Results: Only 3 of 22 colonies showed the band at 2061 bps and two of them were very dim.

*Colonies grew too tiny and close on the agar plate.

2017.10.22

Restriction enzyme analysis of YEplac195 (with prefix).

2017.10.23

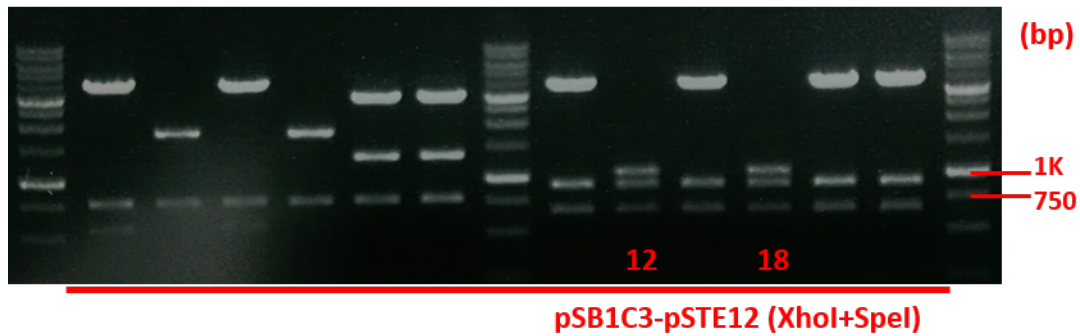
2nd DNA sequencing: pSB1C3-112, 195, 181 backbone with half cloning sequence and corresponding pJET-half sequence.

Colony PCR of YEplac181 and YEplac195 but no bands showed.

2017.10.24

Cloning of YEplac181 and YEplac195 composite again with the previous materials in order to get fewer colonies.

Restriction enzyme analysis of pSB1C3-lipase*6, pSB1C3-pSTE12*13 and result in 4 colonies of pSB1C3-pSTE12 were correct. (no. 1, 5, 12(seq.), 18)

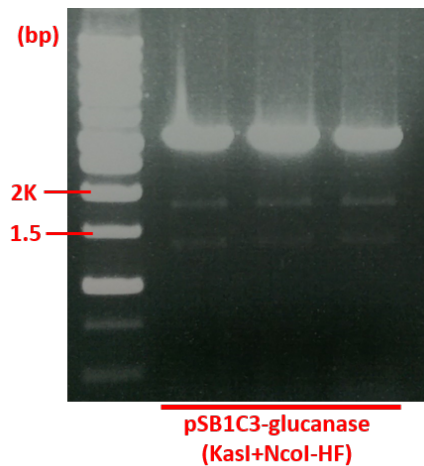


2017.10.25

Use adding primer, gel purify and then ligation to clone xylanase biobricks into pSB1C3.

Half-YEplac181, 195 and 112 composite clone into pSB1C3.

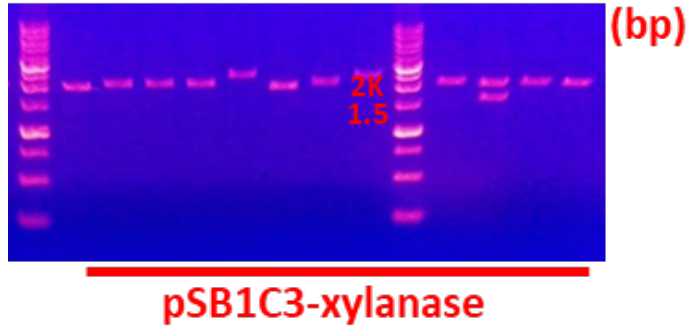
Restriction enzyme analysis of pSB1C3-xyn, lip, STE12 protein, glu; pJET-lip, glu.



2017.10.26

Xyn., lip., and pste12 biobricks clone into pSB1C3.

pJET-xyn is not correct after enzyme analysis.



2017.10.27

Restriction enzyme analysis of YEplac181, 195 and 112 composite. YEplac195 was correct!

Restriction enzyme analysis of pSB1C3-lipase and pJET-lipase.

Clone pste12-lipase which was carried in the YEplac195 into pSB1C3 to get composite parts.

2017.10.28

Restriction enzyme analysis of pSB1C3-pste12-lipase.

Submit our biobricks.

2017.10.31

Again submit our iGEM parts because of the wrong package title. "Plastic plates" is not acceptable.