2018.10.01 - 2018.10.07 WEEK 11

2018.10.01

Parts

Ligate KX-Ag to pExoS54F plasmid backbone. Transform the pExoS54F plasmid into DH5α，culture in plates with ampicillin.

------ Ma Xinyue, Zhang Yue

Safty - Light Control

We use the light-off system from 2017 SSTi-SZGD to kill our bacteria after effecting. We test this system in *Pseudomonas aeruginosa* delta 9.

1. Medium preparation: LB+Streptomycin (40 μg/mL)

Bacteria electrotransformation: Transfer the plasmid_BBa_K2244010 to *Pseudomonas aeruginosa* delta 9, which contains ColE promotor, supernova gene and Lev1 gene. Inoculate transformed bacteria into LB with streptomycin, tape the tubes to the platform of a shaker at 37℃ for 2 hours.

2. Medium preparation: LB+agar+Streptomycin (40 μg/mL)

Plate spreading. Divide these plates into two groups, light and dark, and there are 2 plates in each group. Add 100 μL bacteria in each plate. Then culture in 37℃ incubator in dark (covered with silver paper) or light (natural light) for 24 and 48 hours.

------Ma Ningjia, Liang Ruijuan

Collaboration

Electrotransformation of Pseudomonas fluorescens (cooperation project with Shandong University). The pf-5 bacteria were transformed with 13 plasmids containing different promoters separately. They are PK, P2, P3, P4, P5, P6, P8, P9, P10, P11, P12, P13, and P14.

First day, Several LB plates containing kanamycin were prepared.

------Ma Ningjia, Liang Ruijuan

2018.10.02

Parts

Pick single colonies contain KX-pExoS54F plasmid, shake at the speed of 300 r/min for 10 hours.

------ Ma Xinyue, Zhang Yue

Safty - Light Control

Result observation for 24 hours: Each plate grow a film of bacteria.

------Ma Ningjia, Liang Ruijuan

Collaboration

Electroporation: The initial OD600 = 0.085 of the bacterial solution was transferred to 1300 μL of LB medium and cultured at 30°C for 2.5 h.

Competent preparation: operation on ice, centrifuge precooled to 2°C.

After centrifugation, the supernatant was discarded, and the precipitate was washed three times with Buffer (10% sucrose + 2 mM HPEPS). Then resuspend with 30 μL Buffer and add 300 ng of plasmid DNA (the addition volume was determined according to the concentration).Blow and mix, add to the electric rotor (pre-cooled on the electric rotor).

Electric voltage: 1250 V

Resuscitation: LB medium, 30°C, 1 h 40 min.

After that, the cells were collected by centrifugation and uniformly coated on kanamycin-resistant plates, and cultured overnight at 30 ° C incubator.

------Ma Ningjia, Liang Ruijuan

2018.10.03

Parts

Plasmid extraction

Extract KX- pExoS54F plasmid from bacterium solution:

Name	T3A	T3B	T3C	T2	TNA	TNB
Concentration (ng/μL)	518.64	541.57	305.53	354.51	531.19	466.80

------ Ma Xinyue, Zhang Yue

Safty - Light Control

Result observation for 48 hours: Each plate grow a film of bacteria, but the light group has less.

The result is not our expected. We think that maybe the illumination intensity is not strong and we didn't light when shaking before the plate spreading.

------Ma Ningjia, Liang Ruijuan

Collaboration

Pick a single colony and incubate at 30°C overnight.

------Ma Ningjia, Liang Ruijuan

2018.10.04

Parts

Digest KX-T3SS plasmid with EcoRI and XbaI. Gel electrophoresis. Gel extract part of around 1166bp.

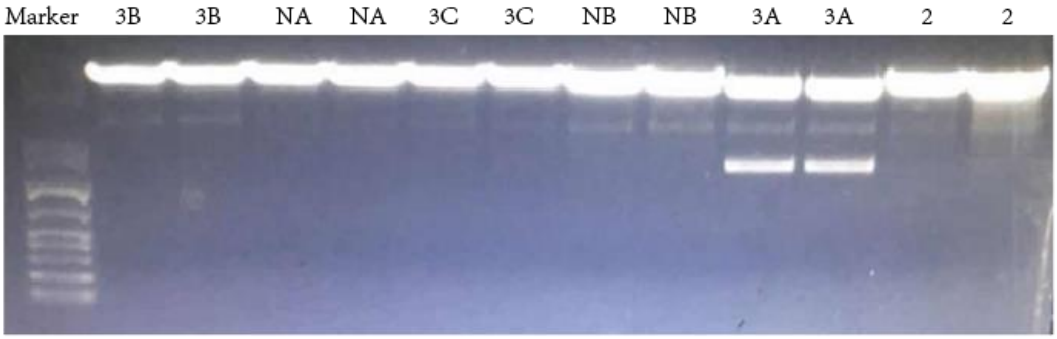

Fig.1 | Restriction Digestion

Result analysis: Only plasmid with 3A part showed expected stripe, which suggested that other plasmids may not ligate successfully or the time of digestion was not long enough.

Transform the pSB1C3 into DH5α, culture in plates with chloramphenicol.

Ligate KX-Ag to pExoS54F plasmid backbone (abbreviation in "KX-pExoS54F plasmid").

Transform the pExoS54F plasmid into DH5α, culture in plates with ampicillin, add negative control (DH5α without plasmid transformed).

Result analysis: There were colonies in the plate of negative control, which showed that only group 3A was digested successfully with expected stripes.

------ Ma Xinyue, Zhang Yue

Safty - Light Control

We repeat the experiment. Take some bacteria transformed last time and shake at 37℃. Sub-cultures are grown overnight.

------Ma Ningjia, Liang Ruijuan

Collaboration

Extract the plasmid.

------Ma Ningjia, Liang Ruijuan

2018.10.05

Parts

Preparation of LB culture solution with chloramphenicol, 1000 mL in total.
Preparation of LB culture plate with chloramphenicol, 30 plates in total.

Anneal pSB1C3 complement part.

Digest pSB1C3 linearized plasmid following the protocol from IGEM part website.

Ligate pSB1C3 complement part with pSB1C3 backbone.

Transform the pSB1C3 into DH5α，culture in plate with chloramphenicol.

------ Ma Xinyue, Zhang Yue

Collaboration

Digest the plasmid with BstB1 enzyme.

Component	Volume
Cut Smart buffer	1 μL
BstB1	0.2 μL
DNA	4 μL
ddH_2O	4.8 μL

Set up the reaction following the instruction in the table above at 65℃ for 1.5 hours.

The digested product was then electrophoresed on a 0.1% agarose gel. Unfortunately, we didn't get the expected electrophoresis bands.

Positive plasmid digestion result:

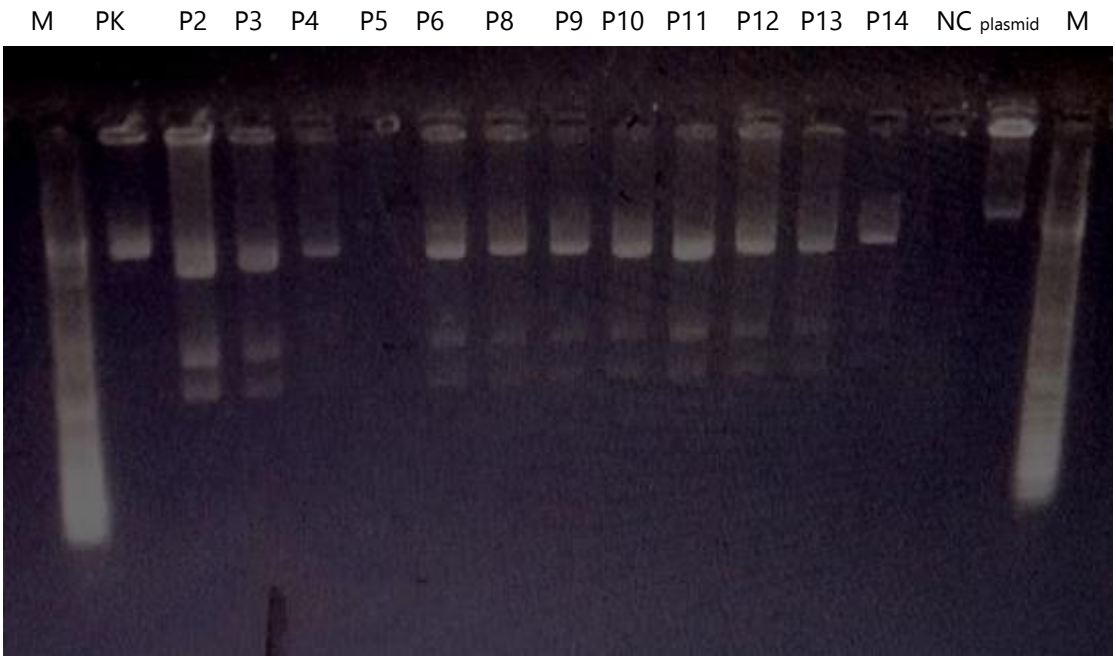

Fig.2 | Restriction Digestion

The results showed that all plasmids except PK and P5 could be digested into three segments.

Plasmid digestion results after transformation:

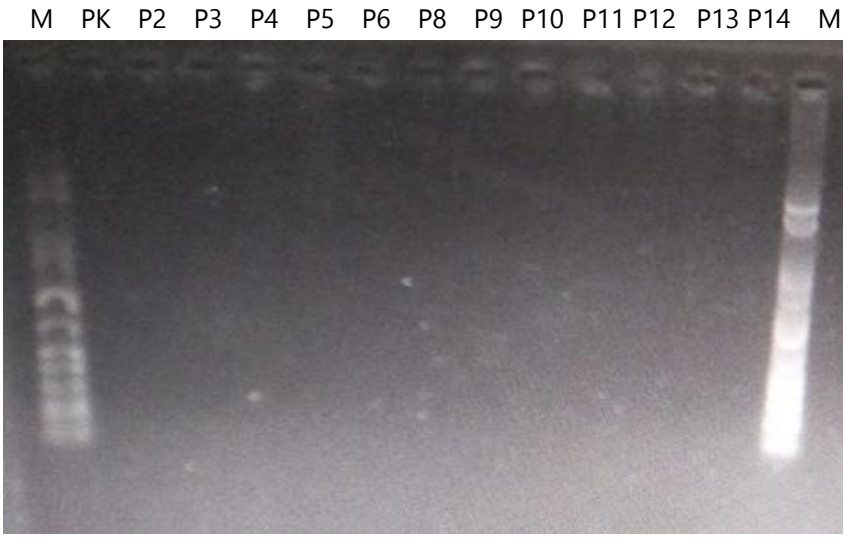

Fig.3 | Restriction Digestion

The results suggest that perhaps our bacterial transformation is a failure.

------Ma Ningjia, Liang Ruijuan

2018.10.06

Parts

Pick monoclonal colony of KX-T3SS plasmid, shake at the speed of 300 r/min for 10 h. Extract KX-pExoS54F plasmid from bacterium solution.

Result analysis: 254.38 ng/μL.

Digest KX-pExoS54F plasmid with enzymes EcoRI and XbaI. Gel electrophoresis. Gel extract part of around 1166bp.Digest pSB1C3 plasmid with enzymes EcoRI and XbaI. Gel electrophoresis. Gel extract part of around 2000bp.

Result analysis: From the result of gel electrophoresis, all samples have expected bright stripes.

Ligate parts of Ag parts (6 in total), T3SS-Ag parts (6 in total) and T3SS part (1 in total) with pSB1C3 backbone, transform into DH5α, culture in plate with chloramphenicol.

------ Ma Xinyue, Zhang Yue

2018.10.07

Parts

Pick single colony of KX-pExoS54F plasmid, shake at the speed of 300 r/min for 10 h.

------ Ma Xinyue, Zhang Yue