2024/7/24 (Wed)

13:00-20:30 (JST)

Experiment Supervisor:
Koichi Yano

Participants:
・Shiori Kajikawa (all)
・Shoya Inoue (all)
・Kei Hato (-15:00)

Experiment:
・Lab safety guide
・gDNA purification of B. subtilis natto BEST195
・planting 8 different strains of B. subtilis, B. subtilis subsp. Natto, E. Coli

Results:
・Participants learned the safety rules in Sue'tsugu lab.
・gDNA precipitation was visually confirmed

Additional Notes:
・Participants must make sure to take note of, and share cautions within the lab with other members

2024/07/25 (Thu)

10:00-18:30 (JST)

Experiment Supervisor:
Koichi Yano

Participants:
・Mizuho Sakai (11:30-)
・Rikuto Fukushima (-16:30)
・Shiori Kajikawa (-13:00)
・Kei Hato (11:30-15:00)

Experiment
・PCR of aprE fragment
・TAE-EtBr agarose gel creation (2% and 0.8%)

・PCR of extracted gDNA
・Moving bacteria to freezing glycerol stock

Results
None

2024/07/26 (Fri)

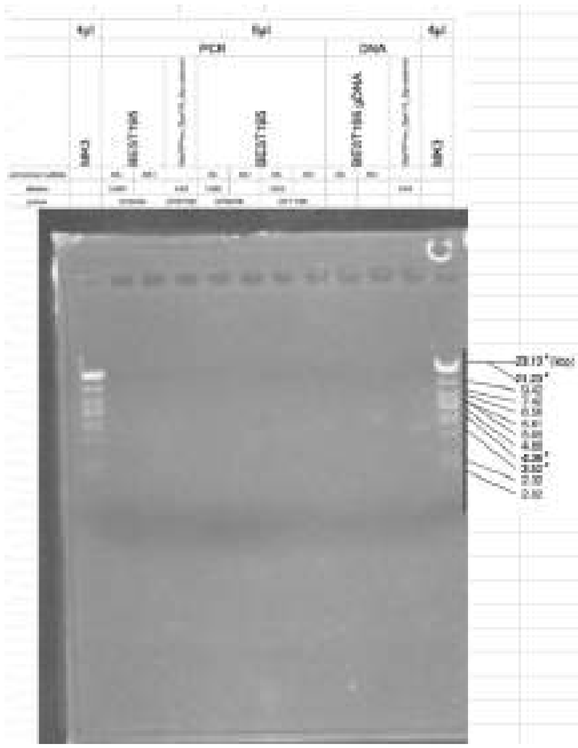
12:30-18:30 (JST)

Experiment Supervisor:
Koichi Yano

Participants:
・Mizuho Sakai (all)
・Rikuto Fukushima(13:00-)
・Shiori Kajikawa (15:50-)

Experiment:
・Autoclaving effluent
・Electrophoresis yesterday's PCR product
・PCR of DNA extracted yesterday through different cycle setting
・Researching about BioBrick RFC10

Results:
・No bands except lane 10 (plasmid) or lane 1/12(ladder)showed.
・Upon troubleshooting, we found that the PCR setup, conducted yesterday, was done in 2-steps instead of 3



Additional Notes:

2024/07/27 (Sat)

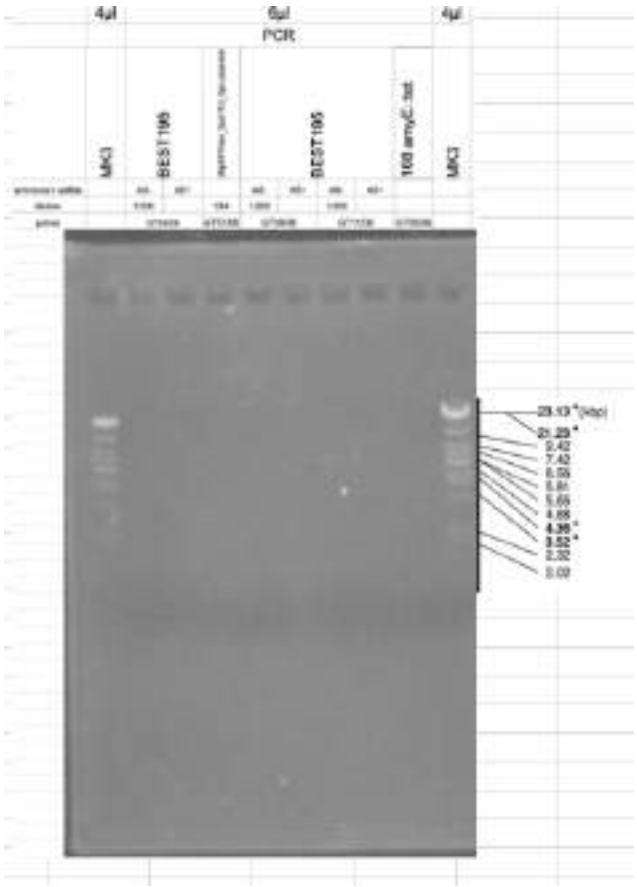
10:30-18:00 (JST)

Experiment Supervisor:
Kazuyuki Fujimitsu

Participants:
・Rikuto Fukushima(-15:30)
・Shiori Kajikawa (11:30-)
・Tatsuhiko Akiyama (17:00-)
・Yukiya Horiba (17:00-)

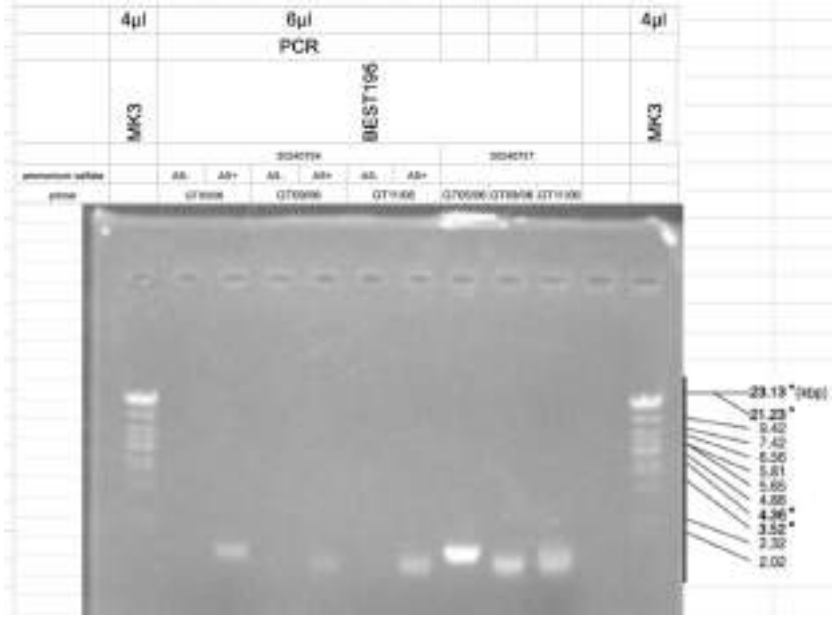
Experiment:
・Electrophoresis of yesterday’s PCR product
・Troubleshooting
・Extraction of gDNA from B. subtilis natto BEST195 (This time, use Wizard kit)
・PCR and electrophoresis (3rd time) of PCR product
・Pipette training session for Tatsuhiko and Yukiya

Results:



・No bands except ladders were seen in electrophoresis.

・Made alterations to PCR= increase extension time, lower annealing temperature, increase the addition of primers and DNA sample, reconducted DNA extraction which may have failed first time



2024/07/29 (Mon)

10:30- 19:30 (JST)

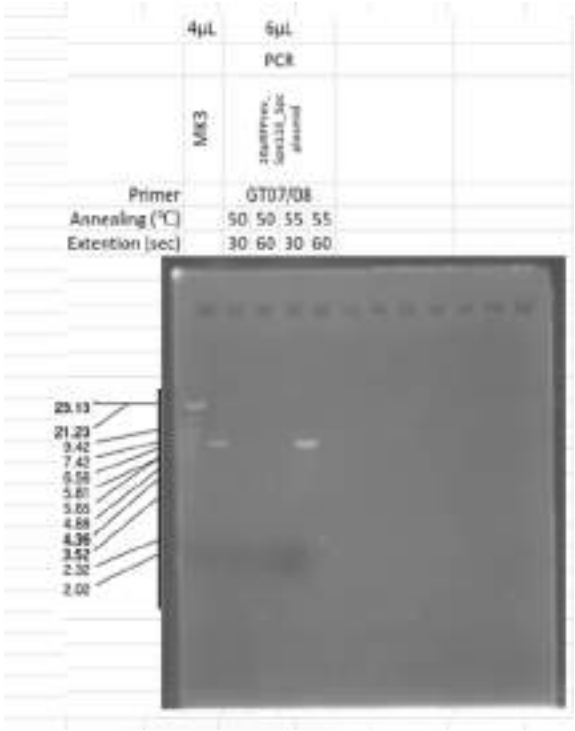
Experiment Supervisor:
Koichi Yano

Participants:
・Rikuto Fukushima (all)
・Shiori Kajikawa (-14:30)
・Kei Hato (-17:00)
・Saki Tsuchiya (13:30-18:00)
・Mizuho Sakai (14:30-15:30)

Experiment:
<Wet lab>
・Clarification of plans for this week (increased production of NK and fibrin plate assay)
・Ordering necessary material for mainly this week's experiments (fibrin, thrombin)
・PCR and electrophoresis of vector plasmid

<Education>
・PCR trial for lab session on Aug 4th

Results:
<Wet lab>
・Experiment was planned for this week
・PCR confirmed via electrophoresis



<Education>
・PCR & electrophoresis committed 3 times, all negative results. Resume trial tomorrow, but adding water and heating in microwave when breaking down the soybeans

2024/07/30 (Tue)

12:30-17:00 (JST)

Experiment Supervisor:
Koichi Yano

Participants:
・Shiori Kajikawa (all)
・Saki Tsuchiya (13:00-17:00)
・Mizuho Sakai (13:00-)
・Kei Hato (13:30-)
・Shoya Inoue (14:00-)

Experiment:
<Wet lab>
・Creation of culture medium for B. subtilis, B. subtilis Natto, and preculture of E. Coli C600
・Ordering primers for colony PCR fragments

<Education>
PCR trial for experiment session on Aug. 4

Results:
<Wet lab>
None

<Education>
・PCR failed. No bands except the ladder.

2024/07/31 (Wed)

11:00-18:00 (JST)

Experiment Supervisor:
Koichi Yano

Participants:
・Shiori Kajikawa (all)
・Mizuho Sakai (11:30-)
・Rikuto Fukushima (14:00-)

Experiment:
・Preparation of E. coli C600 competent cell
・Creation of LB medium for transformation

Results:
・At the end, we realized that the “E. coli” cells we were using was actually B. subtilis NEST115.
・Thus, we made a glycerol stock of competent B. subtilis NEST115 cells.
・We will re-conduct the same experiment using E. coli C600

Additional Notes:

2024/08/01 (Thu)

10:00-17:00 (JST)

Experiment Supervisor:
Koichi Yano

Participants:
・Shiori Kajikawa (all)
・Kei Hato (all)

・Rikuto Fukushima (13:00-)
・Ryuzo Kijima (16:00-)

Experiment:
・Preparation of E. Coli C600 competent cell glycerol stock

Results:
・Made a glycerol stock of competent E. Coli C600 competent cell

Additional Notes:
・Creation of phosphate buffer

2024/08/02 (Fri)

11:00-15:00 (JST)

Experiment Supervisor:
Koichi Yano

Participants:
・Shiori Kajikawa (all)
・Shoya Inoue (-13:00)
・Rikuto Fukushima (13:30-)
・Ryuzo Kijima (14:00-)

Experiment:
・DNA Column purification of aprE and plasmid fragment, DNA quantity determination using Nanodrop and Quantus

Results:
・NanoDrop DNA concentration measurement was 168.8ng/μl, 141.8ng/μl, 137.8 ng/μl, and 103.5 ng/μl for tubes A, B, C, D respectively, for the PCR samples from 7/27 and 7/29
・Quantus DNA concentration measurement was 139.7nM, 140.5nM, 134.8nM, 22.6nM for tubes A, B, C, D respectively

2024/08/05 (Mon)

10:00-21:00 (JST)

Experiment Supervisor:
Koichi Yano

Participants:

・Kei Hato (-13:00)
・Misaki Ozawa (11:00-18:30)
・Ryuzo Kijima (13:30-18:30)
・Shiori Kajikawa (13:30-)

Experiment:
・infusion cloning of aprE fragments with pNK1
・transformation into E. coli DH5α
・Competence measurement of E. coli C600 with transformation

Results:
None

2024/08/06 (Tue)

11:00-19:30 (JST)

Experiment Supervisor:
Koichi Yano

Participants:
・Shiori Kajikawa (-14:00)
・Yukiya Horiba (13:30-)

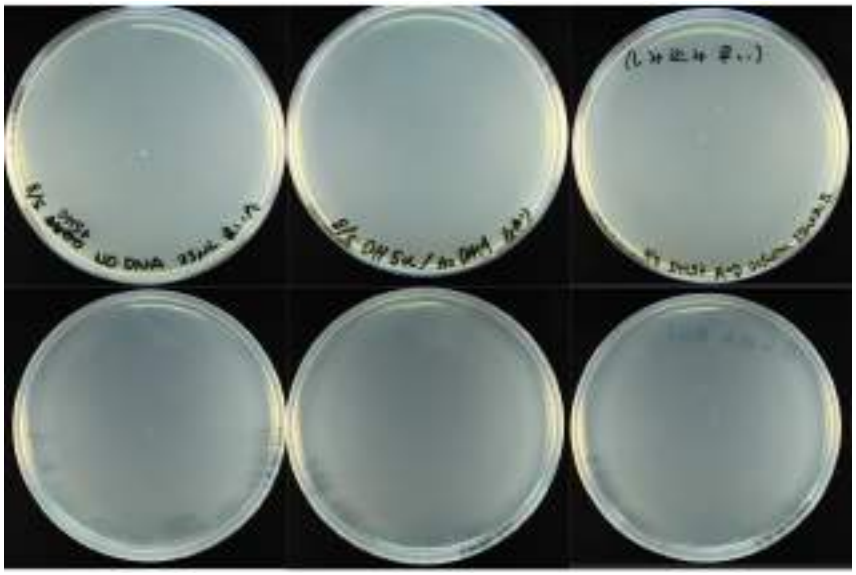
Experiment:
・Colony PCR of transformed E. Coli DH5α/pNK1 and electrophoresis

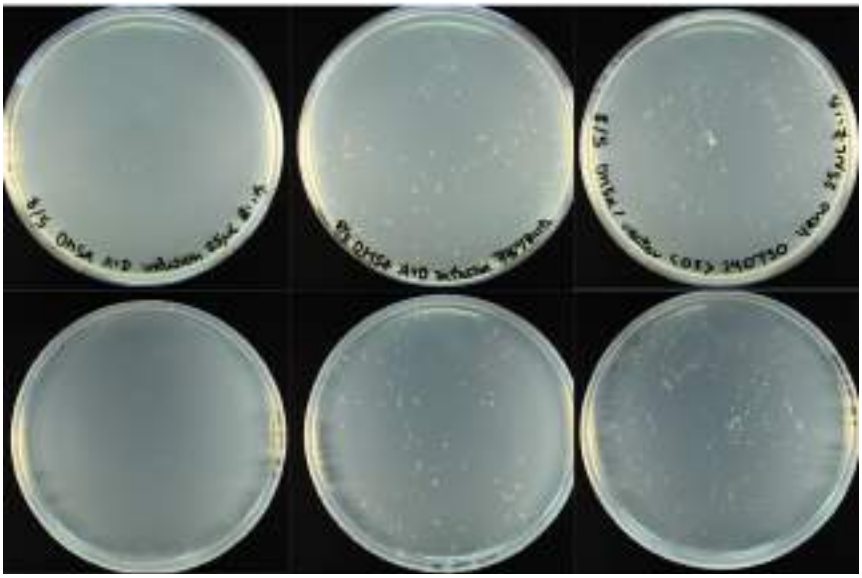
Tube No.	Template
1-23	DH5α infusion colonies
24	Plasmid <05> Yano
25	DH5α/<05> colony

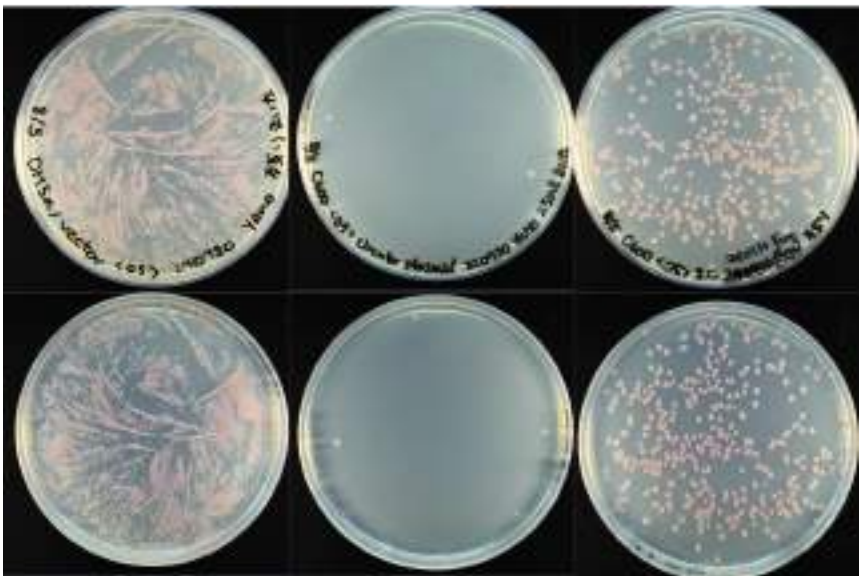
PCR program		
predenaturation	98°c, 3 min	
denaturation	98°c, 10 sec	*130 cycles
annealing	55°c, 5 sec	
extension	68°c, 60sec	
final extension	68°c, 7 min	
hold	12°c	

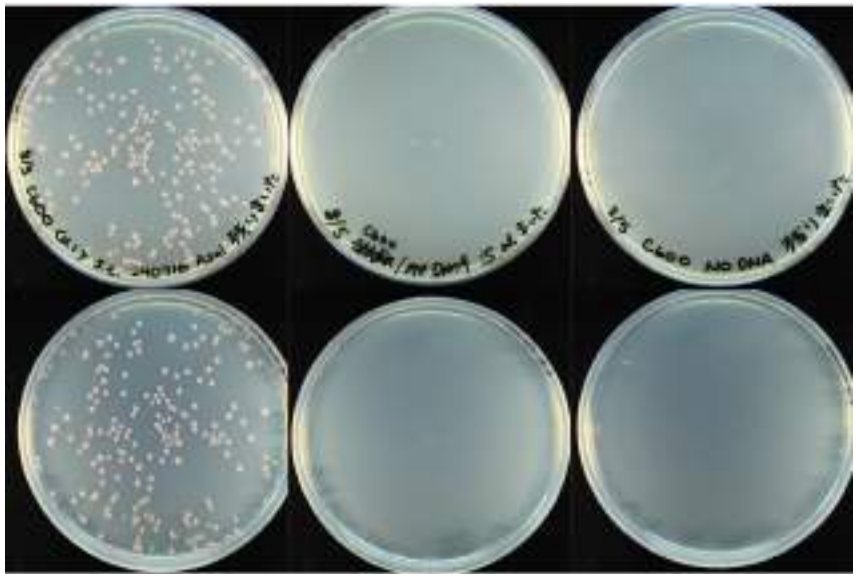
・Inoculation of successful colonies to liquid medium

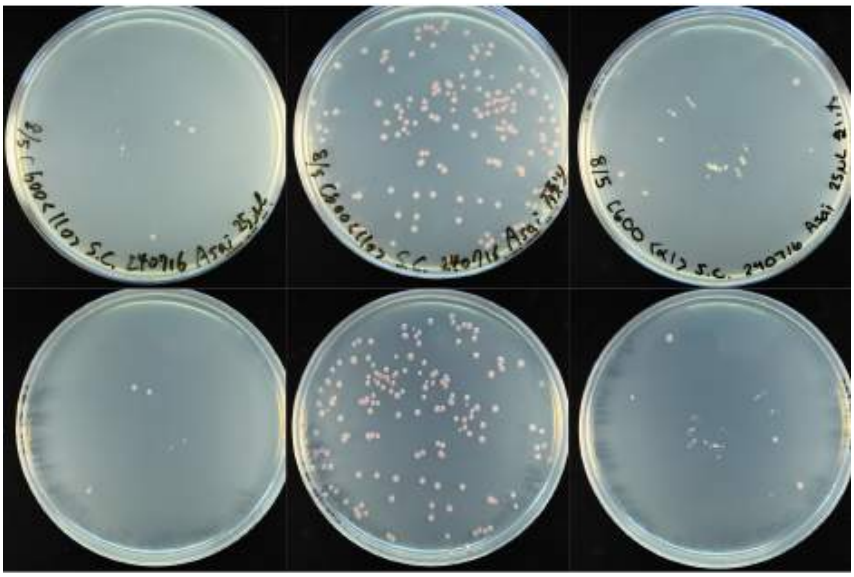
Results:
・Colonies were observed on the transformation plate
・All colonies were estimated to be about the targeted length (7.5kbp)

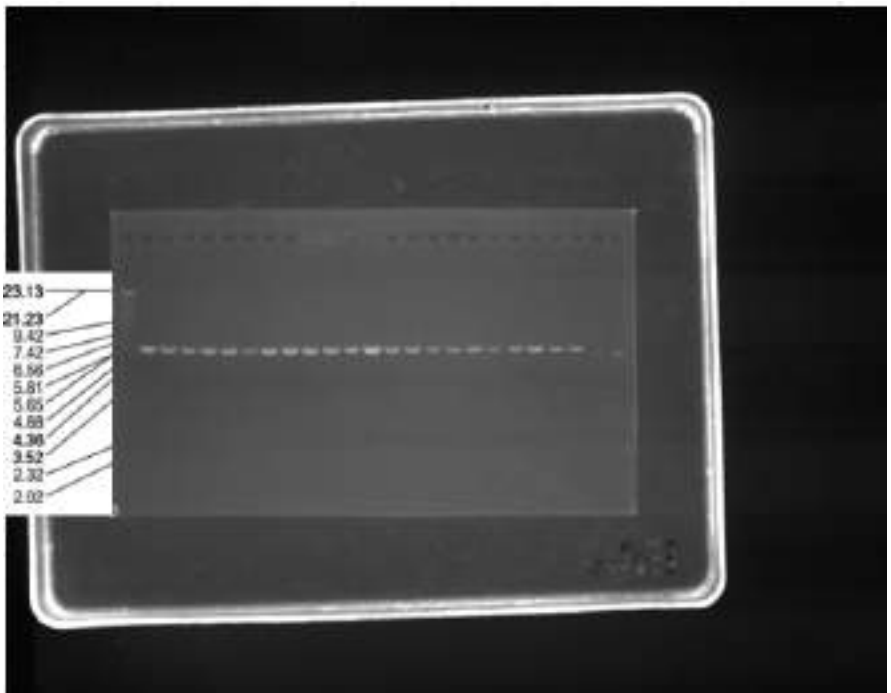











23.13
21.23
9.42
7.42
6.56
5.81
5.65
4.88
4.36
3.52
2.32
2.02

2024/08/07 (Wed)

10:00-17:30 (JST)

Experiment Supervisor:
Koichi Yano

Participants:
・Kei Hato (all)
・Misaki Ozawa (11:00-17:00)
・Rikuto Fukushima (13:00-15:00)

Experiment:
・Trial of creating milk plate/fibrin plate assay

Results:
None

2024/08/08 (Thu)

10:00-20:00 (JST)

Experiment Supervisor:
Koichi Yano

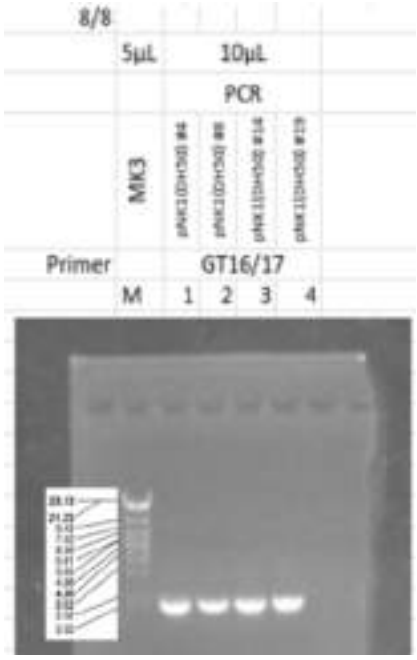
Participants:
・Kei Hato (10:00-17:30)
・Rikuto Fukushima
・Misaki Ozawa (14:00-20:00)

Experiment:
・Creation of milk plate/fibrin plate assay
・PCR and electrophoresis for miniprep pNK1(DH5α)

Tube No.	Template DNA	NanoDrop (ng/μL)
1	pNK1(DH5α) #4 240807	
2	pNK1(DH5α) #8 240807	
3	pNK1(DH5α) #14 240807	
4	pNK1(DH5α) #19 240807	

PCR program		
predenaturatio	98°c, 3 min	
denaturation	98°c, 10 sec	30 cycles
annealing	55°c, 5 sec	
extension	68°c, 30sec	
final extension	68°c, 7 min	
hold	12°c	

Results:



Additional Notes:

2024/08/09 (Fri)

10:00-18:00 (JST)

Experiment Supervisor:
Koichi Yano

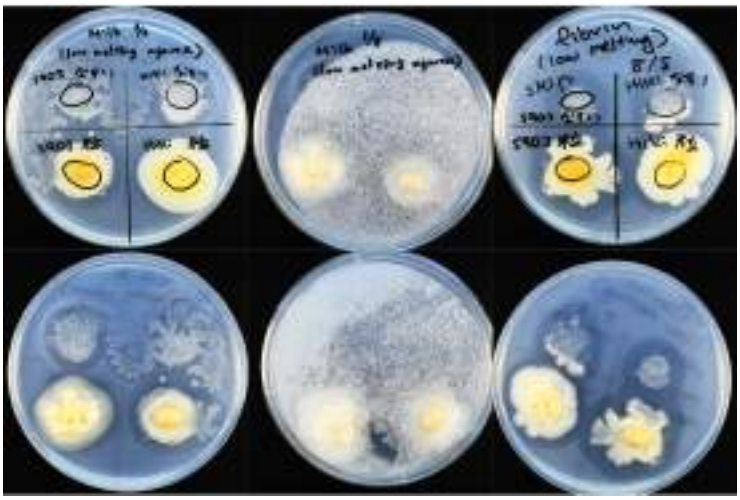
Participants:
・Shiori Kajikawa (all)

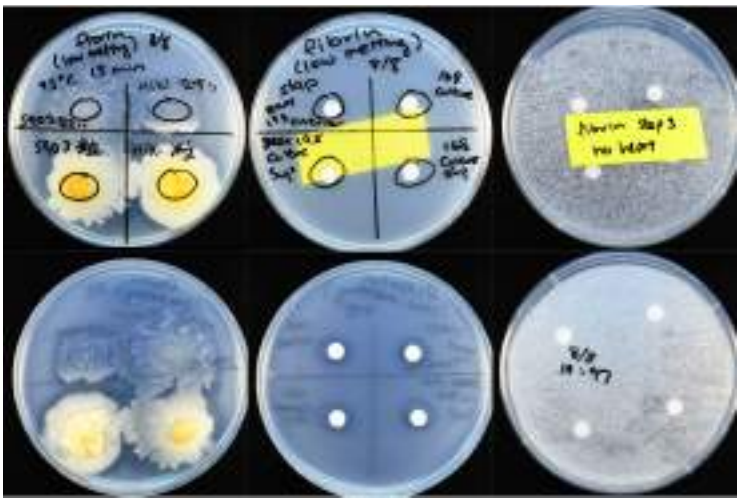
Experiment:

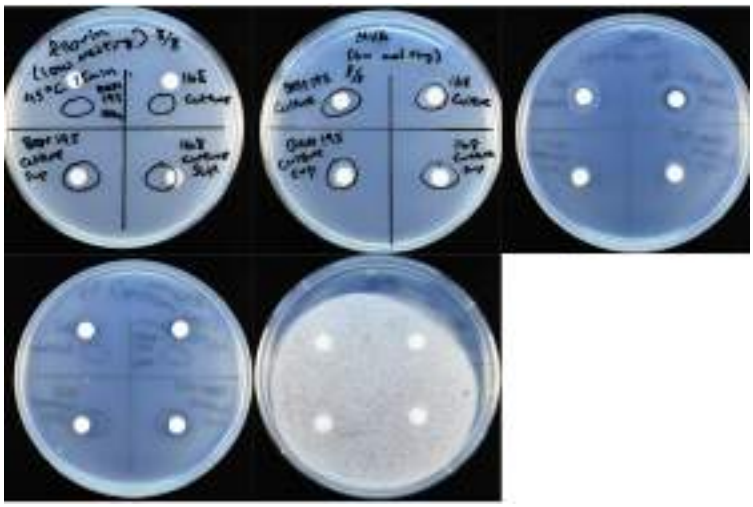
・Creation of competent cells with B. subtilis 168 and B. subtilis subsp. Natto BEST195
・Column purification and NanoDrop
・Observing results of yesterday's fibrin/milk plate assay

Results:

・NanoDrop DNA concentration measurement for the purified PCR column was 141.8ng/μL, 97.7ng/μL, 135.8ng/μL, 133.1ng/μL for tubes 1, 2, 3, and 4 respectively
・We have determined an optimal recipe/protocol for plate creation, as well as how the strains would look depending on whether it is grown on LB medium or soybeans.







Additional Notes:
・Sent column samples for sequencing analysis

2024/08/10 (Sat)

13:00-18:00 (JST)

Experiment Supervisor:
Kazuyuki Fujimitsu

Participants:
・Shiori Kajikawa (all)

Experiment:
・LB agar plate creation
・NanoDrop of miniprep plasmid
・Transformation of pNK1 #4, 8, 14, 19 to E. coli C600

Results:
・NanoDrop DNA concentration measurement of the miniprep plasmid was 31.6ng/μL, 31,3ng/μL, 32.9ng/μL, 45ng/μL for GTstr13, 14, 15, 16 respectively.

2024/08/12 (Mon)

13:30-17:30 (JST)

Experiment Supervisor:
Fujimitsu Kazuyuki

Participants:
・Rikuto Fukushima (all)
・Misaki Ozawa (-16:00)
・Lee Doria (-17:00)

Experiment:
・Inoculation of E. coli C600/<05><110><α1>
・PCR of pNK1 plasmid to create pNK2 and pNK3

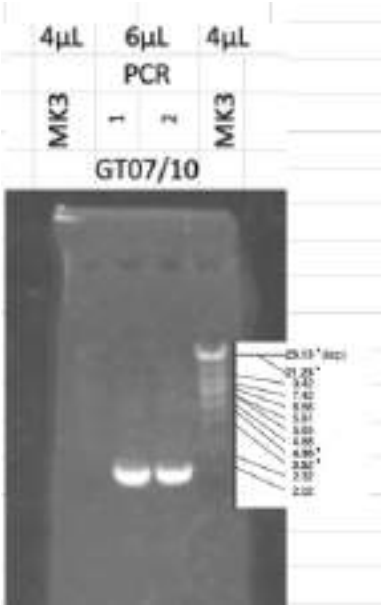
	Tube	
Template DNA	1	240807_miniprep pNK1#4
	2	240807_miniprep pNK1#4

PCR program		
predenaturatio	98°c, 3 min	
denaturation	98°c, 10 sec	
		*130 cycles
annealing	55°c, 5 sec	
extension	68°c, 30sec	
final extension	68°c, 7 min	
hold	12°c	

Results:

・Bands were found near 1.6kbp, when it is supposed to be near 6 kbp….

・After troubleshooting, we found that the primer GT10 had another binding site, other than the one that we anticipated



Additional Notes:

・Reconstructed primers and ordered them (GT34). Will redo this experiment on 8/21

2024/08/19 (Mon)

10:30-17:00 (JST)

Experiment Supervisor:

Koichi Yano

Participants:
・Shiori Kajikawa (all)
・Mizuho Sakai (15:00-)

Experiment:
・Picture record of inoculation plates from 08/10
・LB plate creation (spectinomycin, chloramphenicol included)

Colonies		
	240805	C600/<05>
		C600/<110>
		C600/<α1>
	240810	C600/pNK1 #4
		C600/pNK1 #8
		C600/pNK1 #14
		C600/pNK1 #19
		YAN17632

・Inoculation of E. coli C600 with various plasmids (<05>, <110>, <α1>, pNK #4, 8, 14, 19)

Results:
None

Additional Notes:

2024/08/20 (Tue)

10:00-19:30 (JST)

Experiment Supervisor:
Koichi Yano

Participants:
・Doria Lee (-12:30)
・Shiori Kajikawa (11:30-)

Experiment:
・Miniprep of E. coli strains that were inoculated yesterday
・NanoDrop of strains
・gDNA purification of B. subtilis (stock YAN17632)
・Creation of minimal medium for conjugational transfer
・Checking Sequence analysis of E. coli C600/pNK1 #4

Results:

・NanoDrop DNA concentration was 58.3ng/μL, 55.4ng/μL, 54.8ng/μL, 70.6ng/μL, 75.6ng/μL, 70.8ng/μL, 93.6ng/μL for strains GTstr17, GTstr18, GTstr19, GTstr20, GTstr21, GTstr22, and GTstr23, respectively.
・NanoDrop DNA concentration for the gDNA purification was 116.1ng/μL

Additional Notes:

2024/08/21 (Wed)

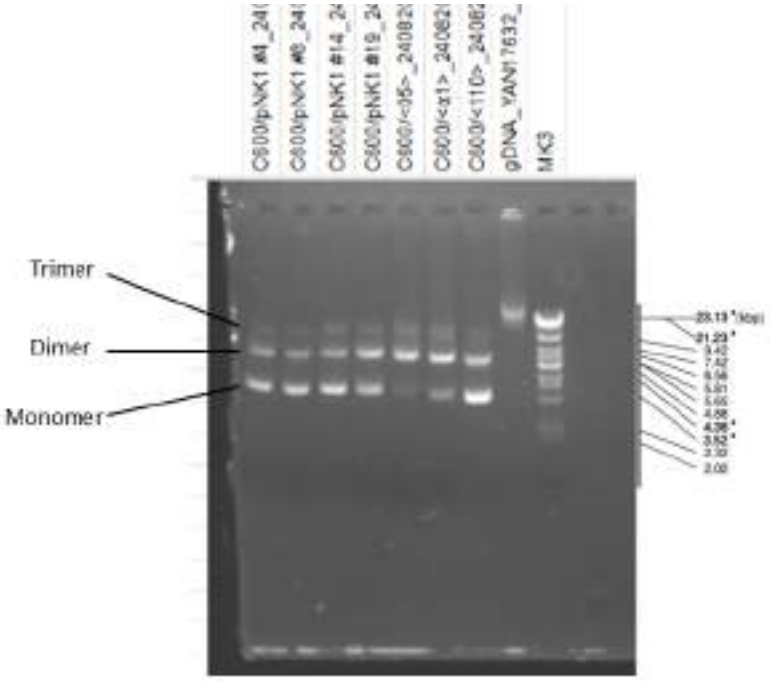
12:00-19:30 (JST)

Experiment Supervisor:
Koichi Yano

Participants:
・Shiori Kajikawa (all)
・Saki Tsuchiya (all)
・Mizuho Sakai (14:00-16:00)

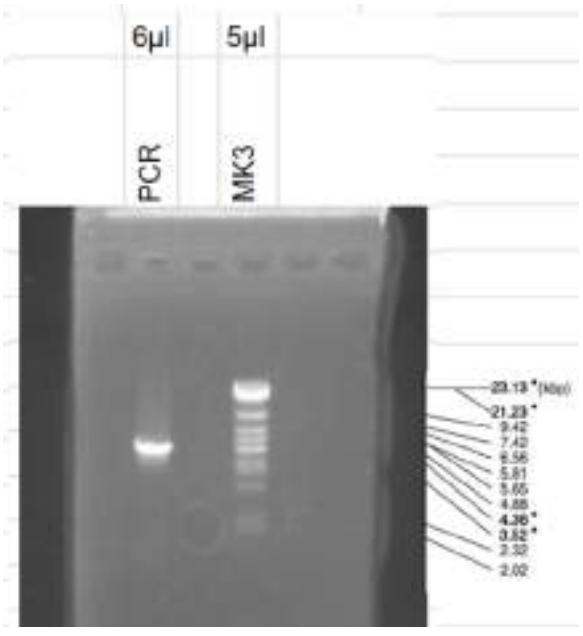
Experiment:
・Electrophoresis of miniprep plasmids/B. subtilis_YAN17632 gDNA
・Nanodrop of gDNA_YAN17632_240820
・PCR of pNK1 to create pNK2 and pNK3, electrophoresis
・Transformation of dimer plasmid into B. subtilis 168/pLS20cat and B. subtilis natto BEST195 (continued from 240809)

Results:



Each miniprep plasmid is the same length. It was divided into a trimer(9.42kbp), a dimer(5.81kbp), and a monomer(3.52kbp). The length of gDNA was 23.13kbp.
Nanodrop DNA concentration measurement gDNA_YAN17632_240820 was 75.3ng/μL

The length of the PCR product was 5.65kbp.



Additional Notes:
When measuring DNA concentration with NanoDrop, we used TE buffer near NanoDrop, and it decreased a lot from yesterdy. Therefore, we redid with TE buffer of our lot.

2024/08/22 (Thu)

11:00-17:30 (JST)

Experiment Supervisor:
Koichi Yano

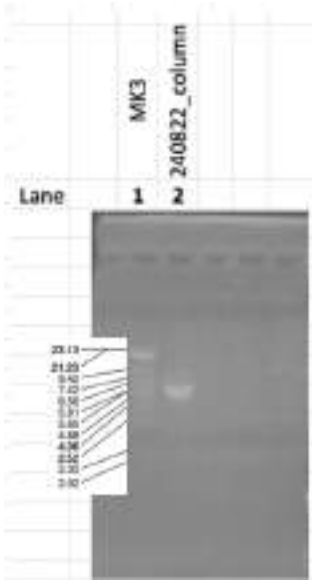
Participants:
・Shoya Inoue (all)

Experiment:
・Column purification of PCR samples from 08/21
・Electrophoresis of column purification sample
・Recording of plasmid transformation into B. subtilis/B. subtilis natto (photo&cell count)
・Colony PCR

Tube No.	Template
1-4	168/pL520cat /pNK1 #4(C600) colonies
5-8	168/pL520cat /<05>(C600) colonies
9-12	168/pL520cat /noDNA colonies
13-16	168/pL520cat /YAN17632 colonies
17-20	168/pL520cat /pNK1 #4(DH5α) colonies
21-24	BEST195/pNK1 #4(C600) colonies
25-28	BEST195/<05>(C600) colonies
29-32	BEST195/noDNA colonies
33-36	BEST195/YAN17632 colonies
37	Plasmid pNK1 #4(C600)_240820 dil. 1/58 (1ng/μl)
38	Plasmid <05>(C600)_240820 dil. 1/75 (1ng/μl)

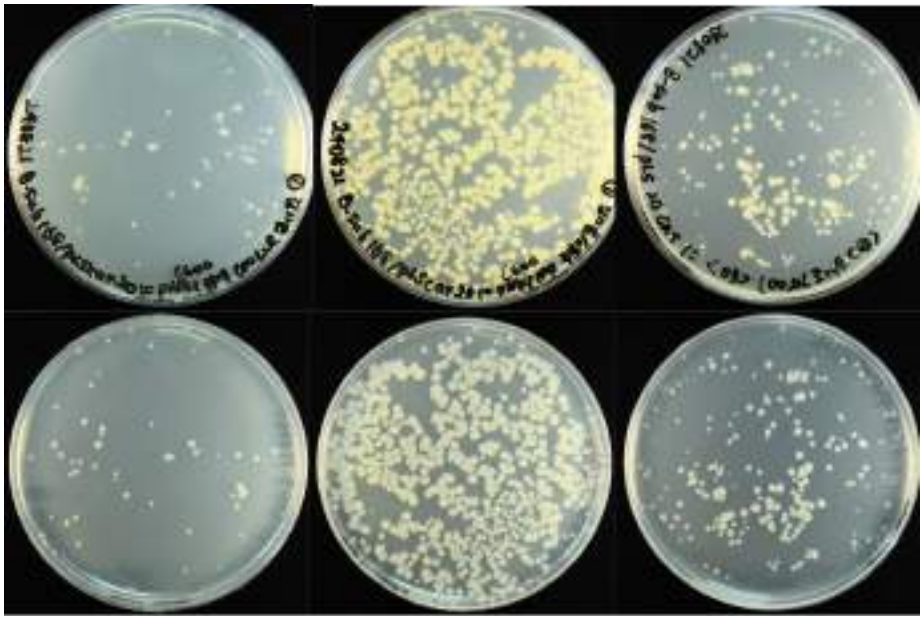
PCR program		
predenaturatio	95°c, 5 min	
denaturation	98°c, 10 sec	↑30 cycles
annealing	55°c, 5 sec	
extension	68°c, 60sec	
final extension	68°c, 7 min	
hold	12°c	

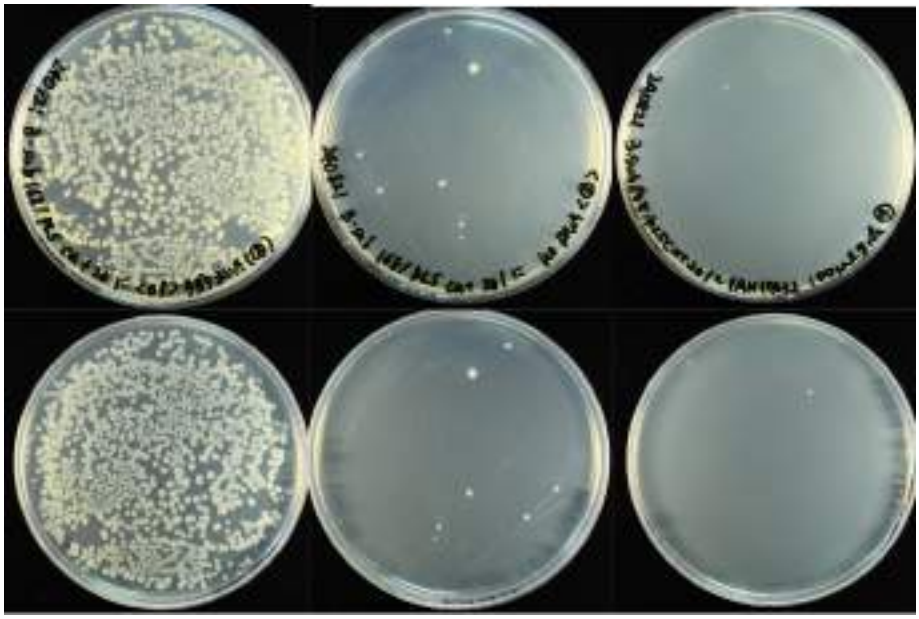
Results:

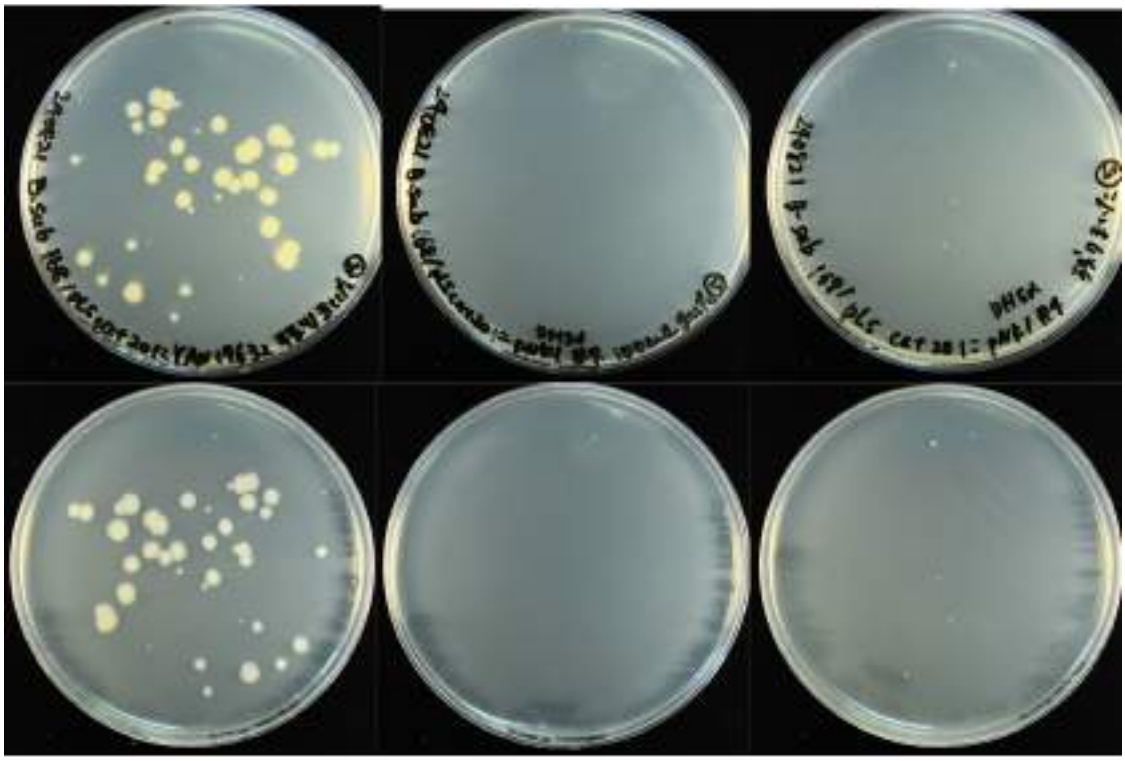


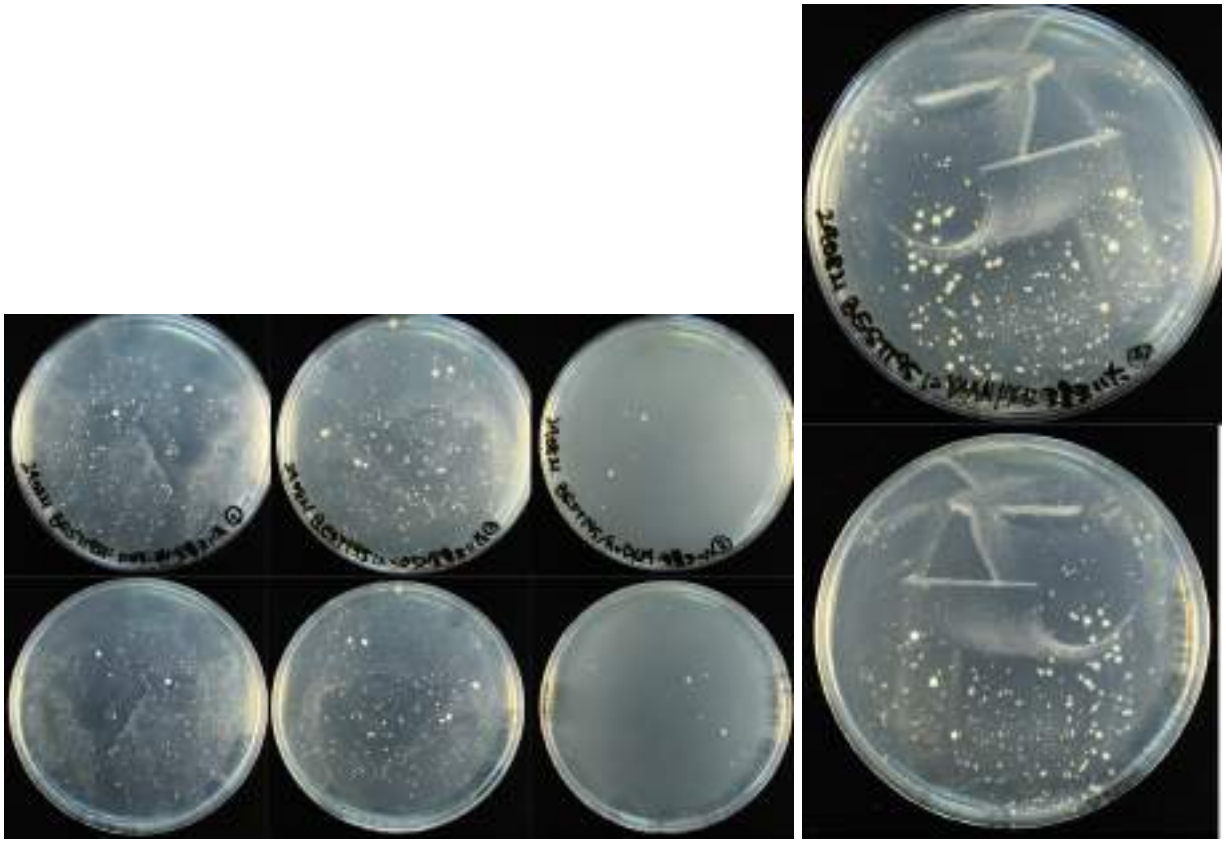
What to add	Volume plated (mL)	Colonies counted	c.f.u. (mL-1 competence cells * µg-1 DNA)
pNK1 #4 (C600)_miniprep240820	0.1	51	5.1E+03
	1.1	564	5.1E+03
<05> (C600) miniprep240820	0.1	198	2.0E+04
	1.1	904	8.2E+03
noDNA	0.1	-	-
	1.2	10	8.3E+00
YAN17632_gDNA_240820 (1/32 dil.)	0.1	2	2.0E+01
	1.1	36	3.3E+01
pNK1 #4 (DH5α) miniprep240807	0.1	0	<1E+01
	1.1	5	4.5E+01

What to add	Volume plated (mL)	Colonies count c.f.u. (mL-1 competence cells * µg-1 DNA)	
pNK1 #4 (C600)_miniprep240820	1.2	286	2.4E+03
<05> (C600) miniprep240820	1.2	330	2.8E+03
noDNA	1.2	18	1.5E+01
YAN17632_gDNA_240820 (1/32 dil.)	1.2	373	3.1E+04









Additional Notes:

2024/08/23 (Fri)

12:00-21:30 (JST)

Experiment Supervisor:
Koichi Yano

Participants:
・Shiori Kajikawa (all)

Experiment:
・0.8% TAE-EtBr agarose gel creation
・Electrophoresis of yesterday's colony PCR
・Quantus Fluoromerter_240822 column

・Infusion cloning to create pNK2 and pNK3

Reagent	Lot
Cloning Enhancer	1812781A
5 x In-Fusion Assembly Master Mix	2211176A

Protocol
Vector=3 µL + Cloning enhancer 1.2 µL
Insert: Make it the same number of moles as the vector

This time
Undiluted vector (240822_column)=56.4 nM
After cloning enhancer: 56.4 * 3/4.2 = 17.0 nMになった。
Thus, use insert as listed below.

Sample	Quantus measurement (nM)	After 1/10 dil. (nM)	Amount to use (µL)
Frag. <B>, PCR→Column purification: 240802 lot	140.5	14.05	1.95
Frag. <C>, PCR→Column purification: 240802 lot	134.8	13.48	2.03

STEPS
1. Vector only, treat with cloning enhancer
2. React the vector with cloning enhancer by incubating at 37 degrees for 15 min, then 80 degrees for 15 min, then on ice. 18:39 - 18:55
3. Mix reagents as listed below
4. Leave at RT for 5 min (Opti reaction), 50 degrees for 15 min, then on ice

Mix_1_pMK2	Concentration		Cloning pMK2	(Weight used)	
Vector (240822_column)	27.4	nM	1 µL	197.64 ng	
Fragment B_240802	14.05	nM	1.95 µL	24.58 ng	
Opti (2 U/µL)	2	U/µL	0.5 µL		
5 x In-Fusion MMP	5	x	0.9 µL		
MQ			0.05 µL		Unstable, so ignore
Total			4.5 µL		

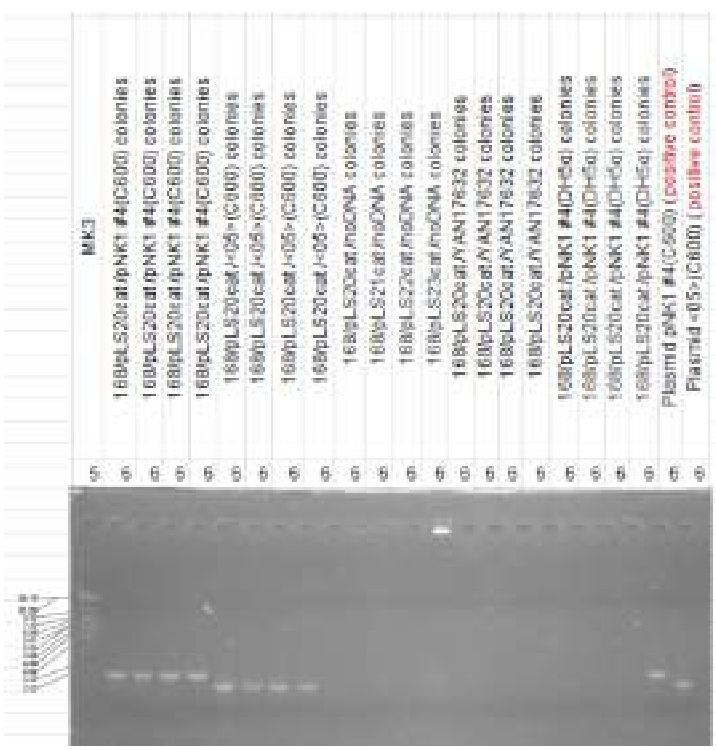
Mix_2_pMK2	Concentration		Cloning pMK2	(Weight used)	
Vector (240822_column)	27.4	nM	1 µL	197.64 ng	
Fragment C_240802	13.48	nM	2.03 µL	25.37 ng	
Opti (2 U/µL)	2	U/µL	0.5 µL		
5 x In-Fusion MMP	5	x	0.9 µL		
MQ			0.07 µL		Unstable, so ignore
Total			4.5 µL		

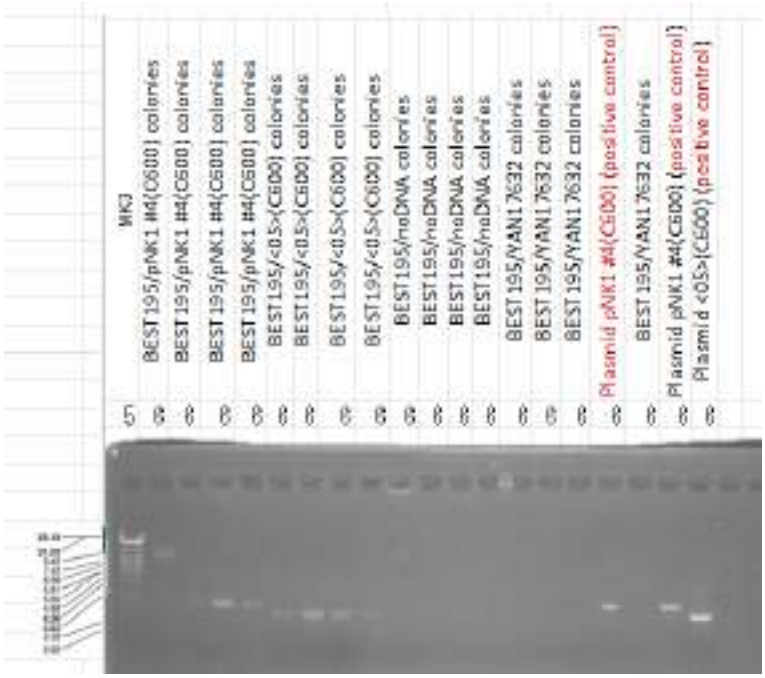
・Inoculation for glycerol stock creation (for tomorrow)

	Antibiotics (final conc. µg/mL)
1. B. subtilis 168/pLS20cat pNK1_240822 colony PCR_#1	Cm 5, Sp 100
2. B. subtilis 168/pLS20cat <05>_240822 colony PCR_#5	Cm 5, Sp 100
3. B. subtilis 168 aprE::spc lacI/pLS20cat_240822 colony PCR_#13	Cm 5, Sp 100
4. B. subtilis natto BEST195/pNK1_240822 colony PCR_#23	Sp 100
5. B. subtilis natto BEST195/<05>_240822 colony PCR_#28	Sp 100
6. B. subtilis natto BEST195 aprE::spc lacI_240822 colony PCR_#33	Sp 100

	Stock conc.	Amount added (to 5ml LB)	Final conc.
Spectinomycin	100mg/ml	5µL	100µg/ml
Chloramphenicol	20mg/ml	1.25µL	5µg/ml

Results:





Additional Notes:

2024/08/24 (Sat)

11:00-16:00 (JST)

Experiment Supervisor:
Kazuyuki Fujimitsu

Participants:
・Kei Hato (11:00-14:00)
・Misaki Ozawa (12:30-)

Experiment:
・glycerol stock preparation
・transformation (pNK2, pNK3 to DH5α)

Results:

Baterial strains culture OD value		
Sample	GTstr No.	OD(600nm)
B. subtilis 168/pLS20cat pNK1 #1	25	0.42
B. subtilis 168/pLS20cat <05> #5	26	0.64
B. subtilis 168 aprE::spc lacI/pLS20cat #	27	0.94
BEST195/pNK1 #23	28	-(error)
BEST195/<05> #26	29	1.09
BEST195 aprE::spc lacI #33	30	-(error)

Additional Notes:

2024/08/26 (Mon)

10:00- 19:00 (JST)

Experiment Supervisor:
Koichi Yano

Participants:
・Shiori Kajikawa (all)
・Doria Lee (-12:30)
・Misaki Ozawa (15:30-)

Experiment:
・LB agar plate creation
・transformation (pNK2, pNK3 to DH5α)

Tube	DNA(1μl)	DH5α
1	infusion product for pNK2 (Mix 1_240823)	25
2	infusion product for pNK3 (Mix 2_240823)	25
3	pNK1 plasmid (C600)	25
4	no DNA	25

Sp100_LB plates	Tube	Amount
240826 DH5α/pNK2-infusion 25μlまいた	1	25 ml
240826 DH5α/pNK2-infusion 残りまいた	1	500 ml
240826 DH5α/pNK3-infusion 25μlまいた	2	25 ml
240826 DH5α/pNK3-infusion 残りまいた	2	500 ml
240826 DH5α/pNK1(C600) 25μlまいた	3	25 ml
240826 DH5α no DNA 全部まいた	4	525 ml

Results:
・No colonies were found from the transformation from yesterday

Additional Notes:

・As wrong tubes were used previously for the transformation, the experiment was redone (have to use 1.5mL tubes instead of 0.6mL tubes to fit into the block) can not be heated properly when transforming

2024/08/27 (Tue)

10:00- 12:30 (JST)

Experiment Supervisor:
Koichi Yano

Participants:
・Doria Lee (all)

Experiment:
・counting colonies from Transformation_pNK2 into E. coli DH5α 240824
・PCR of colonies from transformation with 2 types of primer to create pNK4

Tube No.	Template
1-4	pNK3 clone 240824 TF
5-9	pNK3 clone 240826 TF
10	pNK1 colony 240826 TF
11	pNK1 (DH5α) 240807 miniprep (1ng/μl) 1 μl

PCR program		
predenaturatio	98°c, 3 min	
denaturation	98°c, 10 sec	30 cycles
annealing	55°c, 5 sec	
extension	68°c, 30sec	
final extension	68°c, 7 min	
hold	12°c	

Tube No.	Template
21-22	pNK3 clone 240824 TF #1-2
23-24	pNK3 clone 240826 TF #5-6

PCR program		
predenaturatio	98°c, 3 min	
denaturation	98°c, 10 sec	30 cycles
annealing	55°c, 5 sec	
extension	68°c, 1min10sec	
final extension	68°c, 7 min	
hold	12°c	

Results:

	Transformation_pNK2 into E. coli DH5α 240824 (Plate:SP 100) Plates 1-6	Colonies counted
1	240824 DH5α/pNK2-infusion 25μlまいた	0
2	240824 DH5α/pNK2-infusion 残りまいた	0
3	240824 DH5α/pNK3-infusion 25μlまいた	0
4	240824 DH5α/pNK3-infusion 残りまいた	4
5	240824 DH5α/pNK1(C600) 25μlまいた	60
6	240824 DH5α no DNA 全部まいた	0

Transformation_pNK2 into E. coli DH5α 240826 *USING 1.5mL Tubes
(Plate:SP 100)

	Plates 1-6	Colonies counted
1	240826 DH5α/pNK2-infusion 25μlまいた	0
2	240826 DH5α/pNK2-infusion 残りまいた	0
3	240826 DH5α/pNK3-infusion 25μlまいた	0
4	240826 DH5α/pNK3-infusion 残りまいた	5
5	240826 DH5α/pNK1(C600) 25μlまいた	1
6	240826 DH5α no DNA 全部まいた	0

Additional Notes:

2024/08/28 (Wed)

10:30- 16:00 (JST)

Experiment Supervisor:
Kazuyuki Fujimitsu

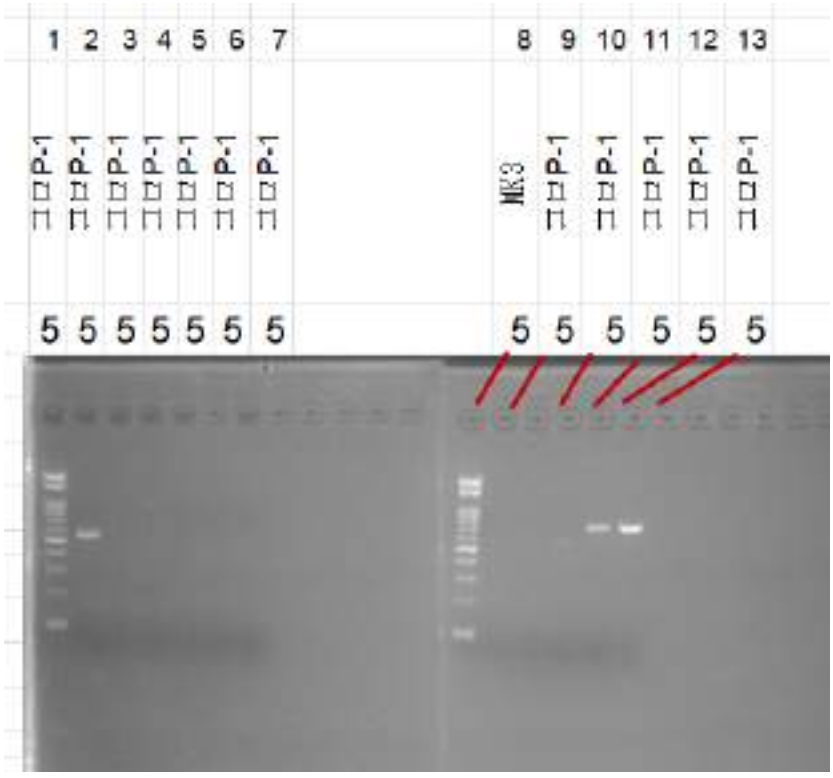
Participants:
・Kei Hato (-14:00)
・Misaki Ozawa (11:00 -)

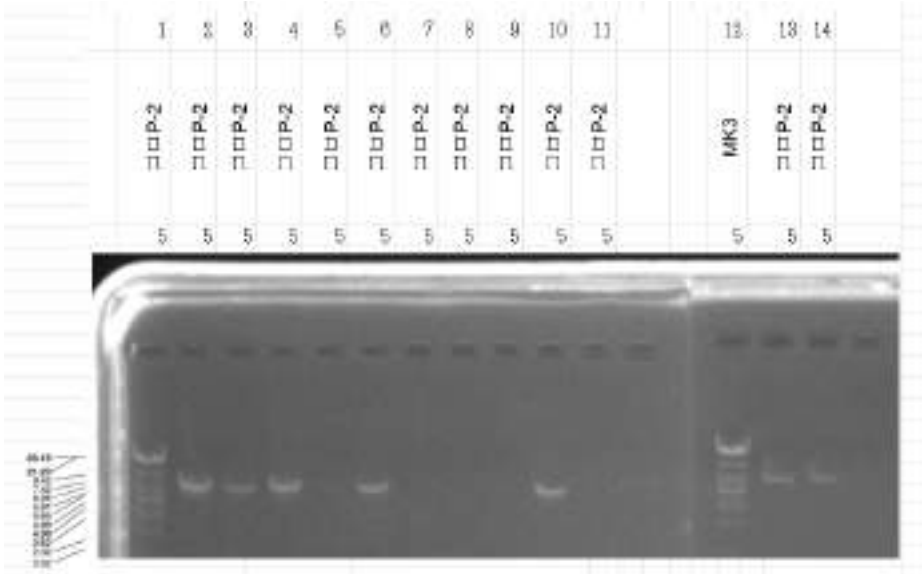
Experiment:
・Electrophoresis of yesterday's colony PCR

Tube No.	Template
1-4	pNK3 clone 240824 TF
5-9	pNK3 clone 240826 TF
10	pNK1 colony 240826 TF
11	pNK1 (DH5α) 240807 miniprep (1ng/μl) 1 μL

・Inoculation of pNK3 from electrophoresis
・Planting colonies (BEST 195 and B. subtilis 168) to difco and star agar plates

Results:





Additional Notes:

2024/09/02 (Mon)

10:00- 13:00 (JST)

Experiment Supervisor:
Kazuyuki Fujimitsu

Participants:
・Kei Hato (all)

Experiment:
・Glycerol freeze stock of bacterial strain DH5a/pNK3 clone, miniprep
・Column purification of PCR sample from 8/27

DNA sample	Sample data:
240827_PCR_#21	**240828**
240827_PCR_#24	**240828**

Results:
None

Additional Notes:

2024/09/03 (Tue)

16:30-19:00 (JST)

Experiment Supervisor:
Koichi Yano

Participants:
・Wingdor Doria Lee (all)

Experiment:
・Quantus of Fluoromerter_240822 column
・In-Fusion of Fluoromerter_240822 column
・Miniprep of E. coli DH5α/pNK3 #6, inoculation into LB+Sp for glycerol stock creation

Results:
None

Additional Notes:

2024/09/04 (Wed)

16:30-19:00 (JST)

Experiment Supervisor:
Koichi Yano

Participants:
・Wingdor Doria Lee (all)

Experiment:
・LB agar plate creation
・Glycerol Freeze Stock of bacterial strains

Results:
None

Additional Notes:

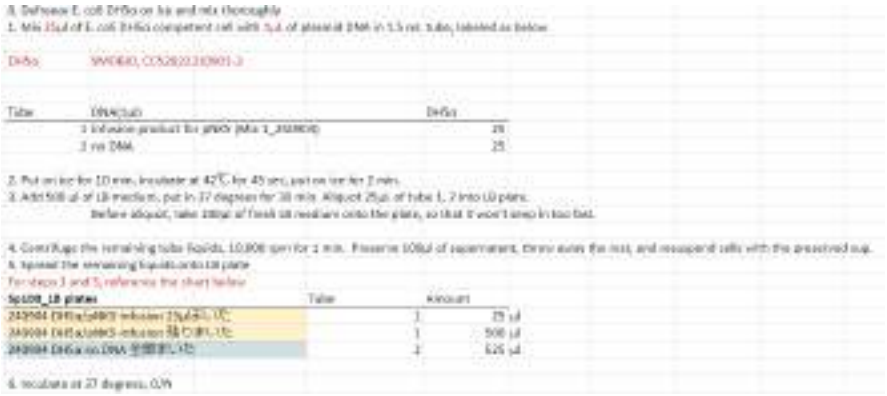
2024/09/05 (Thu)

14:00-19:30 (JST)

Experiment Supervisor:
Koichi Yano

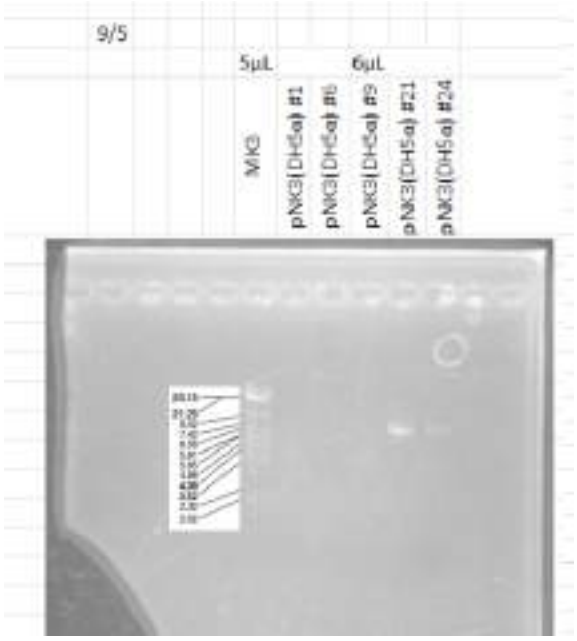
Participants:
・Mizuho Sakai (all)
・Wingdor Doria Lee (16:00-19:00)

Experiment:
・Transformation_pNK5 into E. coli DH5α 240904 *USING 1.5mL Tubes



・QIAprep Spin Miniprep Kit (pNK3 #6)
・Electrophoresis (pNK3 #1, 6, 9, 21, 24)

Results:



Additional Notes:

2024/09/08 (Sun)

11:00-18:30 (JST)

Experiment Supervisor:
Kazuyuki Fujimittsu

Participants:
・Misaki Ozawa (all)

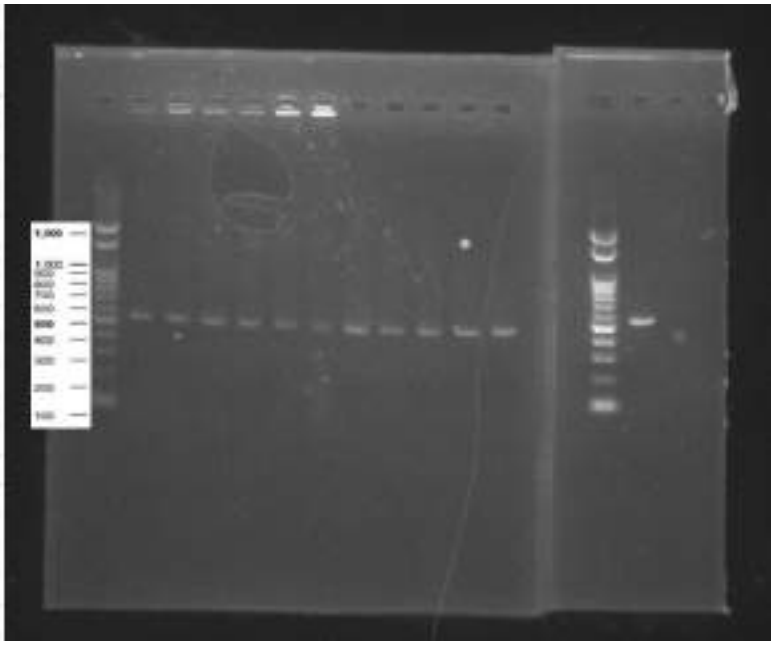
Experiment:
・PCR and electrophoresis of colonies from DH5a/pNK5-infusion

Tube No.	Template
1-3	DH5a/pNK5-infusion 240904 #1
4-6	DH5a/pNK5-infusion 240904 #2
7-9	DH5a/pNK5-infusion 240904 #3
10-12	DH5a/pNK5-infusion 240904 #4

PCR program		
predenaturation	98°c, 3 min	
denaturation	98°c, 10 sec	30 cycles
annealing	55°c, 5 sec	
extension	68°c, 1min10sec	
final extension	68°c, 7 min	
hold	12°c	

Results:

Lane No.	Tube #	[illegible]	6x Loading Dye	TE	[illegible]	[illegible]	
1		-	-	-	5	5	μL
2	1	1	1	4	-	5	μL
3	2	1	1	4	-	5	μL
4	3	1	1	4	-	5	μL
5	4	1	1	4	-	5	μL
6	5	1	1	4	-	5	μL
7	6	1	1	4	-	5	μL
8	7	1	1	4	-	5	μL
9	8	1	1	4	-	5	μL
10	9	1	1	4	-	5	μL
11	10	1	1	4	-	5	μL
12	11	1	1	4	-	5	μL
13	-	-	-	5	5	5	μL
14	12	1	1	4	-	5	μL



Additional Notes:

・Used the wrong ladder, so redid electrophoresis a second time.

2024/09/10 (Mon)

16:00-18:00 (JST)

Experiment Supervisor:
Koichi Yano

Participants:
・Shoya Inoue (all)

Experiment:
・Combine DNA samples from 9/8 colony PCR

	DNA sample	Sample data:
A	240908_colonyPCR #1~3 mix	**240908**
B	240908_colonyPCR #4~6 mix	**240908**
C	240908_colonyPCR #7~9 mix	**240908**
D	240908_colonyPCR #10~12 mix	**240908**

Results:
None

Additional Notes:

2024/09/11 (Wed)

17:00-20:00 (JST)

Experiment Supervisor:
Koichi Yano

Participants:
・Misaki Ozawa (all)

Experiment:
・Glycerol Freeze Stock of DH5a/pNK5-infusion
・Miniprep of DH5a/pNK5-infusion

Results:
None

Additional Notes:

2024/09/16 (Mon)

11:00-18:30 (JST)

Experiment Supervisor:
Kazuyuki Fujimitsu

Participants:
・Shoya Inoue (all)
・Mizuho Sakai (13:00-)

Experiment:
・Transformation_pNK2 into E. coli DH5α 240826
・NanoDrop DNA conc. measurement(240910_column)

Results:
・NanoDrop DNA concentration values for the four samples from 9/10 was 27.7ng/μL, 34.5ng/μL, 90.3ng/μL, and 79.5ng/μL
・When spreading on LB medium, mistakenly used an item that contained Cm.

Additional Notes:

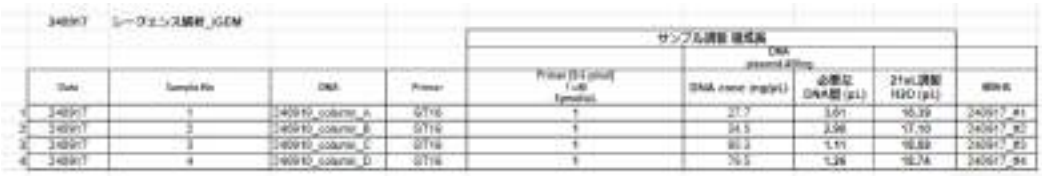
2024/09/17 (Tue)

12:00-19:30 (JST)

Experiment Supervisor:
Koichi Yano

Participants:
・Saki Tsuchiya (all)
・Mizuho Sakai (16:30-）

Experiment:
・Sequence analysis



・Electrophoresis (100V, 25min, 2%agarose gel)
・Transformation_pNK2 into E. coli DH5α 240826

Tube	DNA(1μl)		C600	
	1 C600-1	pNK5 #1_240911	100	
	2 C600-2	pNK5 #2_240911	100	
	3 C600-3	pNK5 #3_240911	100	
	4 C600-4	pNK5 #4_240911	100	
	5 C600-ND	-	100	つまりこのグリセロールには何もいれない、ネガコン。

Sp100_LB plates	Tube	Amount
240917 C600/pNK5 #1_240911 全部まいた	1	525 μl
240917 C600/pNK5 #2_240911 全部まいた	2	525 μl
240917 C600/pNK5 #3_240911 全部まいた	3	525 μl
240917 C600/pNK5 #4_240911 全部まいた	4	525 μl
240917 C600 no DNA 全部まいた	5	525 μl

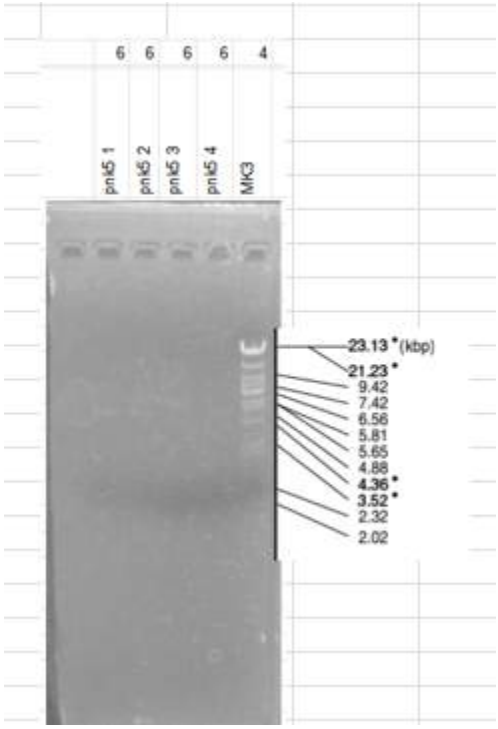
・Transformation of dimer plasmid into B. subtilis 168/pLS20cat (continued from 240809)

Tube	What to add	How much (μl)
1	pNK5 (DH5α) #1_240911	20
2	pNK5 (DH5α) #2_240911	20
3	pNK5 (DH5α) #3_240911	20
4	pNK5 (DH5α) #4_240911	20
5	no DNA	0

・Creating Natto with soybean and NK Day1

Results:

・In electrophoresis, all bands were seen. Only pnk5 3 was seen in different lengths.



Additional Notes:

We redid the ”Transformation_pNK2 into E. coli DH5α 240826” process that I made a mistake in yesterday.

2024/09/18 (Wed)

17:00-21:00 (JST)

Experiment Supervisor:
・Koichi Yano

Participants:
・Arisa Tani (all)

Experiment:
・Creating Natto with soybean and NK Day2
・Colony PCR (168/pLS20cat pNK5)

Tube No.	Template		
1 - 4	168/pLS20cat /pNK5 #1 colonies		
5 - 9	168/pLS20cat /pNK5 #2 colonies		
10 - 14	168/pLS20cat /pNK5 #3 colonies		
15 - 19	168/pLS20cat /pNK5 #4 colonies		
20	Positive control (plasmid pNK5 #1)		
21	Positive control (plasmid pNK5 #3)		
PCR program			
predenaturation	98°C	3 mins	
denaturation	98°C	10 secs	
annealing	55°C	5 secs	x 30
extension	68°C	30 secs	
final extension	68°C	7 mins	
hold	12°C	-	

・Culture of transformed 168, (not transformed) BEST195 and S903 in preparation for conjugal Transmission

Donor	GTstr No.	Antibiotics
168/pLS20cat pNK1	25	Sp100 Cm5
168/pLS20cat <05>	26	Sp100 Cm5
168/pLS20cat pNK5 #1 - 1		Sp100 Cm5
168/pLS20cat pNK5 #1 - 2		Sp100 Cm5
168/pLS20cat pNK5 #2 - 5		Sp100 Cm5
168/pLS20cat pNK5 #2 - 6		Sp100 Cm5
168/pLS20cat pNK5 #3 - 10		Sp100 Cm5
168/pLS20cat pNK5 #3 - 11		Sp100 Cm5
168/pLS20cat pNK5 #4 - 15		Sp100 Cm5
168/pLS20cat pNK5 #4 - 16		Sp100 Cm5

Recipient	GTstr No.	Antibiotics
BEST195	1	None
S903	35	None

Results:
None

Additional Notes:

2024/09/19 (Thu)

16:00-20:30 (JST)

Experiment Supervisor:
・Koichi Yano

Participants:
・Rikuto Fukushima (-18:00)
・Arisa Tani (17:00-)

Experiment:
・Milk plate assay
・Fibrin plate assay
・Creating Natto with soybean and NK Day3
・Conjugation DAY 1
・Preparation of Glycerol Freeze Stock

Results:
Natto creation (sample) is completed.

2024/09/20 (Fri)

17:00-19:00 (JST)

Experiment Supervisor:
・Koichi Yano

Participants:
・Misaki Ozawa (all)

Experiment:
・Glycerol Freeze Stock of bacterial strains
・Conjugation Day2

Results:
None

Additional Notes:

2024/09/22 (Sun)

11:00-17:30 (JST)

Experiment Supervisor:
Kazuyuki Fujimitsu

Participants:
・Arisa Tani (all)

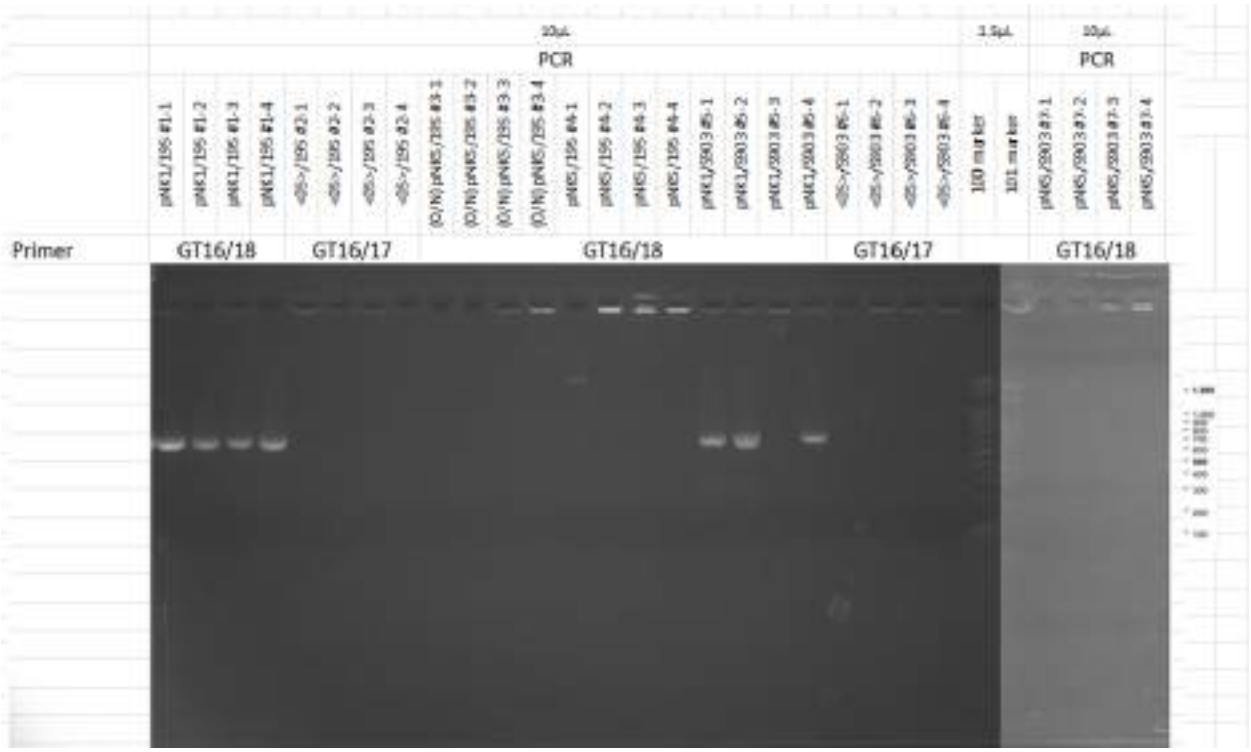
Experiment:
・S903, BEST195 fibrin plate assay preliminary experiment
・Colony PCR

Number	Plasmid / Strain	Samples	Primer_F	Primer_R
1	pNK1 / 195	4	GT16	GT18
2	<05> / 195	4	GT16	GT17
3	pNK5 (O/N) / 195 (O/N)	4	GT16	GT18
4	pNK5 / 195	4	GT16	GT18
5	pNK1 / S903	4	GT16	GT18
6	<05> / S903	4	GT16	GT17
7	pNK5 / S903	4	GT16	GT18
8	Positive control (pNK5-#1)	1	GT16	GT18

PCR program			
predenaturation	98°C	3 mins	
denaturation	98°C	10 secs	x 30
annealing	55°C	5 secs	
extension	68°C	30 secs	
final extension	68°C	7 mins	
hold	12°C	-	

・Electrophoresis of colony PCR product

Results:



Yesterday's Conjugation

Results of conjugation

MM + Ca	Donor / Recipient	BEST195	S903
	168/pLS20cat pNK1	>2,000	>2,000
	168/pLS20cat <05>	>2,000	>2,000
	168/pLS20cat pNK5 #1 - 2	6	35

MM + Ca (overnight)	Donor / Recipient	BEST195
	168/pLS20cat pNK5 #1 - 2	16

MM - Ca	Donor / Recipient	BEST195	S903
	168/pLS20cat pNK1	>2,000	>2,000
	168/pLS20cat <05>	>2,000	>2,000
	168/pLS20cat pNK5 #1 - 2	1	19

MM - Ca (overnight)	Donor / Recipient	BEST195
	168/pLS20cat pNK5 #1 - 2	5

・The colony is clearly visible only on the plate that transmitted NK5. This is unknown because we did not take a negative control, but it may appear spontaneously. If it is spontaneous, the transmission of NK5 failed, while the other >2,000 colonies, pNK1 and <05, were successfully transmitted. The successful plate has the potential to have produced >2,000 colonies because too many were spread on the plate.

Additional Notes:

2024/09/23 (Mon)

13:00-20:00 (JST)

Experiment Supervisor:
Kazuyuki Fujimitsu

Participants:

・Arisa Tani (all)

Experiment:
・Overnight culture creation
・Culture creation for PCR
・LB agar plates for filter membrane method
・Minimal medium agar plates

Results:
None

Additional Notes:

2024/09/24 (Tue)

13:30-22:00 (JST)

Experiment Supervisor:
Koichi Yano
Kazuyuki Fujimitsu

Participants:
・Mizuho Sakai (all)
・Saki Tsuchiya (17:00-20:00)

Experiment:
・Preparation of LB medium for Colony PCR
・Colony PCR

Number	Plasmid / Strain	Colony	Samples	Primer_F	Primer_R
4	pNK1 / 195	1-1	4	GT16	GT18
2	pNK1 / 195	1-3	4	GT16	GT18
3	<05> / 195	2-3	4	GT16	GT17
~~4~~	~~pNK5 (O/N) / 195 (O/N)~~	~~3-4~~	~~4~~	~~GT16~~	~~GT18~~
~~5~~	~~pNK5 / S003~~	~~7-3~~	~~4~~	~~GT16~~	~~GT18~~
6	pNK5 / S003	7-4	4	GT16	GT17
7	Positive control (pNK5 #1)		1	GT16	GT18
8	Positive control (<05>)		1	GT16	GT17

predenaturation	98°C	3 mins	
denaturation	98°C	10 secs	x 30
annealing	55°C	5 secs	
extension	68°C	30 secs	
final extension	68°C	7 mins	
hold	12°C	-	

・Confirmation of the bacteria (Bacillus subtilis or Bacillus subtilis natto)
・Preparation of NB medium for fibrin assay

Results:
None

Additional Notes:

2024/09/25 (Wed)

17:00-22:00 (JST)

Experiment Supervisor:
Koichi Yano
Kazuyuki Fujimitsu

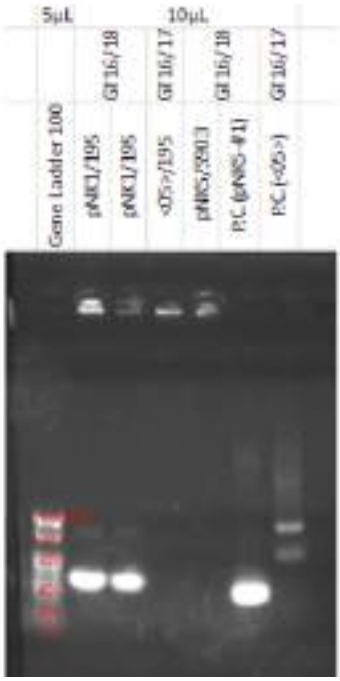
Participants:
・Arisa Tani (all)

Experiment:
・Conjugation Day2
　　・Inoculated on LB medium
・Electrophoresis (yesterday's Colony PCR)

Results:
OD value measured before inoculating LB medium

Strain	OD	amount for 10^9
BEST195	0.91	1.373626374
S903	1.35	0.9259259259
pNK1	1.7	0.7352941176
<05>	0.95	1.315789474
N.C.	1.41	0.8865248227
pNK5	2.19	0.5707762557

・Electrophoresis (yesterday's Colony PCR)



Additional Notes:

2024/09/26 (Thu)

17:00-22:00 (JST)

Experiment Supervisor:
Koichi Yano
Kazuyuki Fujimitsu

Participants:
・Arisa Tani (all)
・Mizuho Sakai (all)

Experiment:
・Preparation of 100mL of NB medium, fibrin assay

Nutrient Broth		
powder	0.8g	
DW	100mL	x 2 bottles

・Colony PCR, Colony count

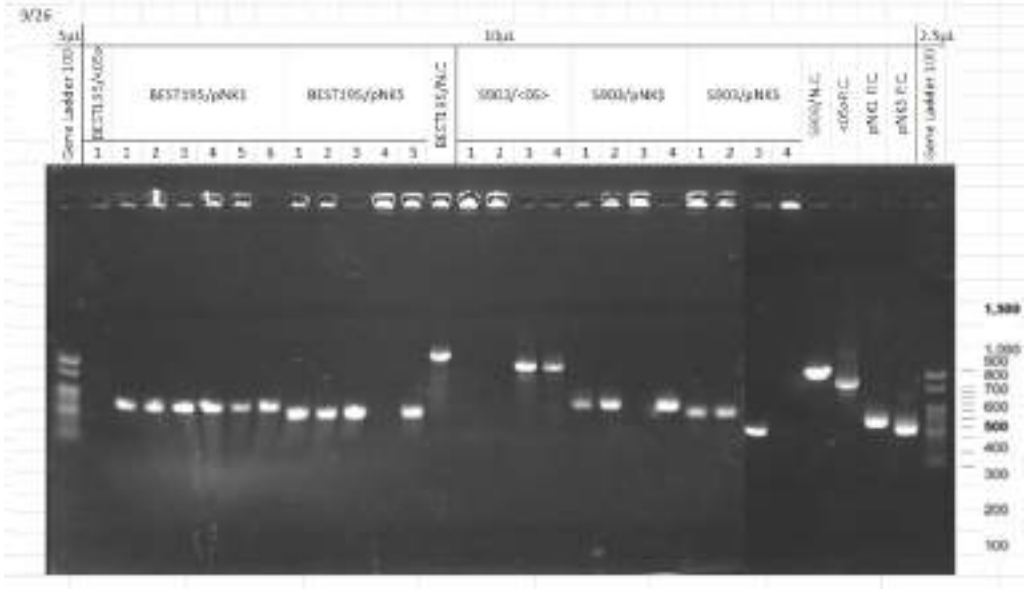
Number	Plasmid / Strain	Colony No.	Primer_F	Primer_R
1	BEST195/<GT>	1		GT17
2		2		
3		3		
4	BEST195/pNK1	1		GT18
5		2		
6		3		
7		4		
8		1		
9		2		
10	BEST195/pNK3	3		GT18
11		4		
12		1		
13	BEST195/N.C	2		GT17
14		3		
15	S903/<GT>	1	GT18	GT17
16		2		
17		3		
18		4		
19	S903/pNK1	1		GT18
20		2		
21		3		
22		4		
23	S903/pNK3	1		GT18
24		2		
25		3		
26	S903/N.C	4		GT17
27	<GT> P.C	1		GT17
28	pNK1 P.C	2		GT18
29	pNK3 P.C	3		GT18

PCR Cycle			
predenaturation	98°C	3 mins	
denaturation	98°C	10 secs	x 30
annealing	55°C	5 secs	
extension	68°C	30 secs	
final extension	68°C	7 mins	
hold	12°C	-	

・Electrophoresis of Colony PCR products
・fibrin plate assay

Results:

・Electrophoresis of Colony PCR products



Colony count

5µL

Donor / Recipient	BEST195	1903
168/pLS20cat <05>	7	0
168/pLS20cat pNK1	3	0
168/pLS20cat pNK5 #3-15	11	0
168/pLS20cat (N.C.)	>2,000*	1

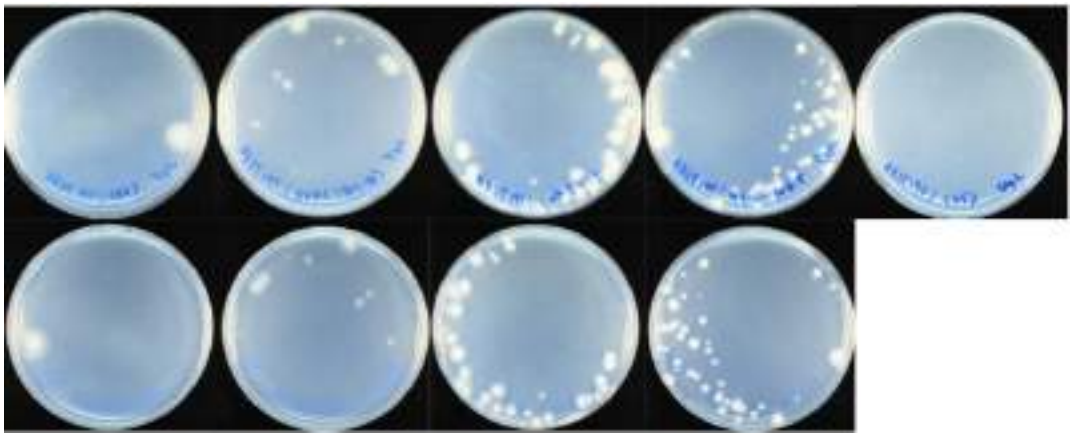
50µL

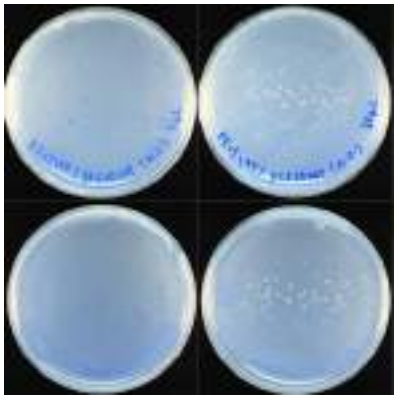
Donor / Recipient	BEST195	9001
168/pLS20cat <06>	6	7
168/pLS20cat pNK3	36	8
168/pLS20cat pNK5 #3-15	41	48
168/pLS20cat (N.C.)	62	>2,000*

*The colonies' sizes are too small to count.

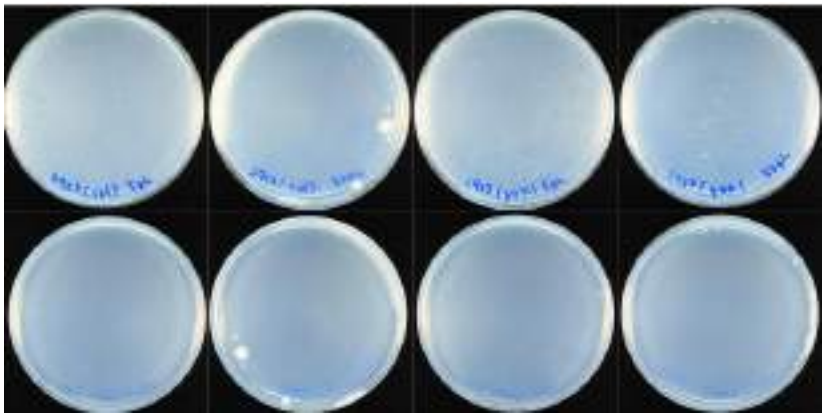
Conjugation results

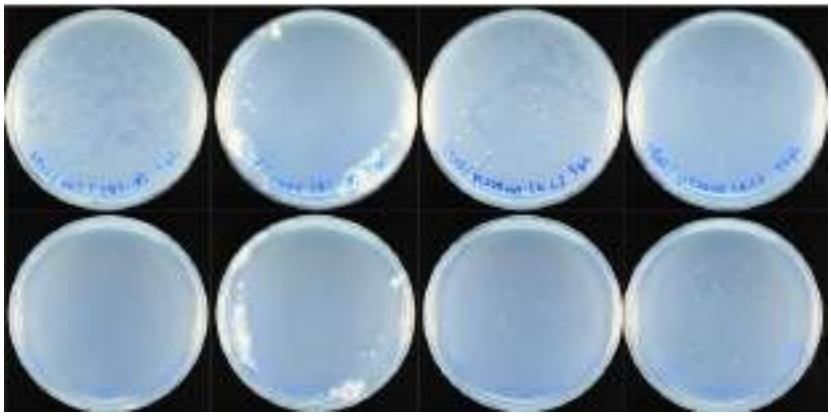
BEST195





S903





Additional Notes:

2024/09/27 (Fri)

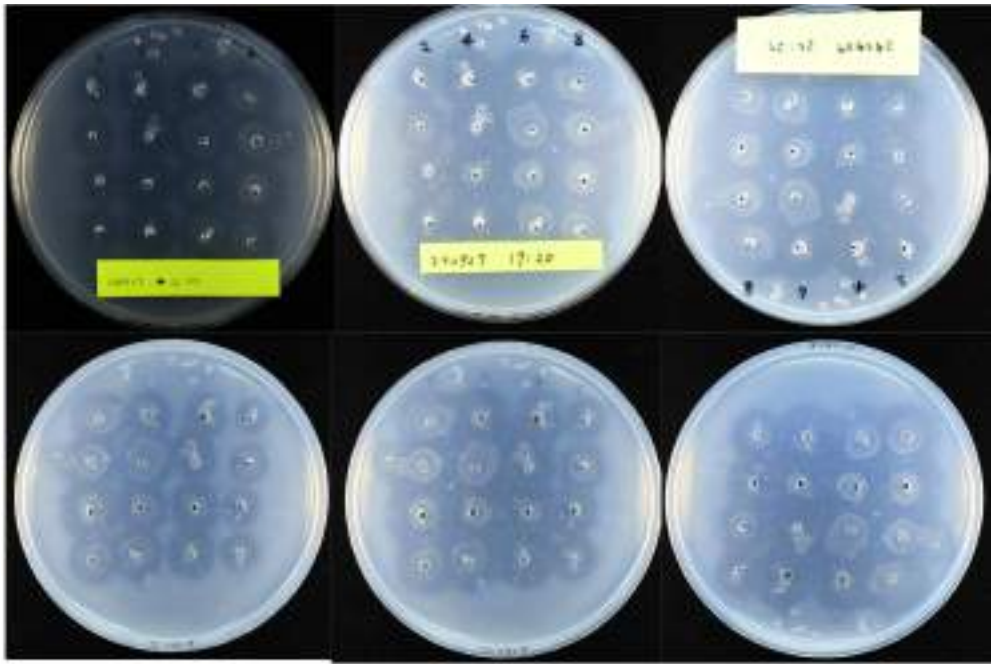
12:30-16:30, 17:30-22:00 (JST)

Experiment Supervisor:
Koichi Yano
Kazuyuki Fujimitsu

Participants:
・Arisa Tani (17:30-22:00)
・Mizuho Sakai (12:30-16:30)

Experiment:
・Confirmation of results of fibrin assay
・Preparation of NB medium
・Preparation of Conjugation

Results:
Results of yesterday's fibrin plate assay



Additional Notes:
・The bacteria did not increase in the NB medium made in yesterday's “Preparation of NB medium” process. The use of thin tubes may have resulted in a lack of oxygen due to the small surface area.

2024/09/28 (Sat)

13:00-21:30 (JST)

Experiment Supervisor:
Kazuyuki Fujimitsu

Participants:
・Mizuho Sakai (all)
・Arisa Tani (14:00-)

Experiment:
・Conjugation
・fibrin plates creation
・Preparation NB medium for fibrin assay, fibrin assay
・Fibrin plate assay
・Made some glycerol stocks

Results:
OD value of inoculated NB medium for fibrin assay

Number	plasmid/strain	OD	dillution for OD=0.5
3	BEST195 (GTstr.1)	1.17	0.234
4	BEST195/pNK1 (3)	1.17	0.234
5	BEST195/pNK5 (3)	2.16	0.432
6	S903/<05> (3)	0.94	0.188
7	S903/pNK1 (4)	1.53	0.306
8	S903/pNK5 (1)	1.83	0.366
9	BEST195/<05>	1.41	0.282
10	S903 (GTstr.35)	1.2	0.24

・Made some glycerol stocks

Glycerol stock		
4	BEST195/pNK1 (3)	47
5	BEST195/pNK5 (3)	48
9	BEST195/<05>	49
6	S903/<05> (3)	50
7	S903/pNK1 (4)	51
8	S903/pNK5 (1)	52

Additional Notes:

2024/09/29 (Sun)

11:00-22:00 (JST)

Experiment Supervisor:
Kazuyuki Fujimitsu

Participants:
・Arisa Tani (all)

Experiment:
・Creation of sodium phosphate buffer

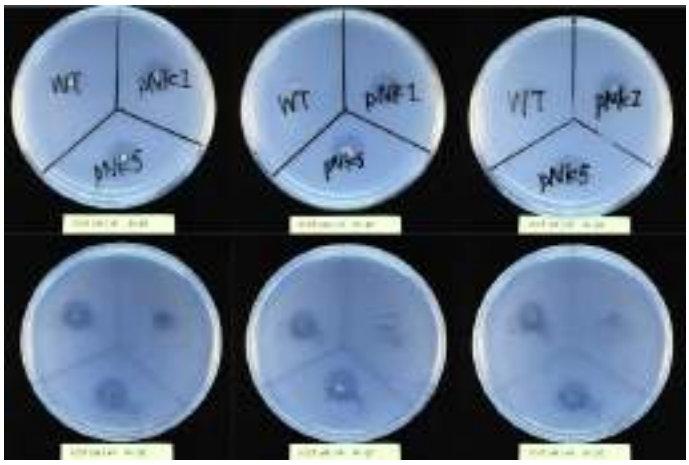
Sodium phosphate buffer		
NaH2PO4 2H2O	1.919	g
Na2HPO4 12H2O	13.503	g
Water	400+x	mL
Total	500	mL

・Fibrin plate creation
・Halo assay with natto suspension liquid and LB medium

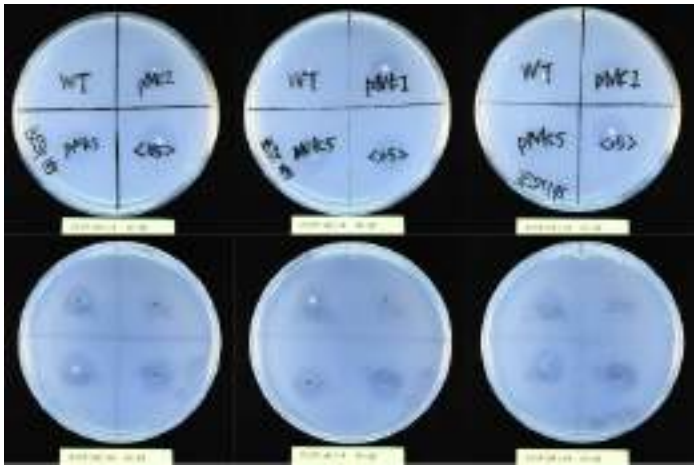
Results:
・Yesterday's results of fibrin plate assay

16h

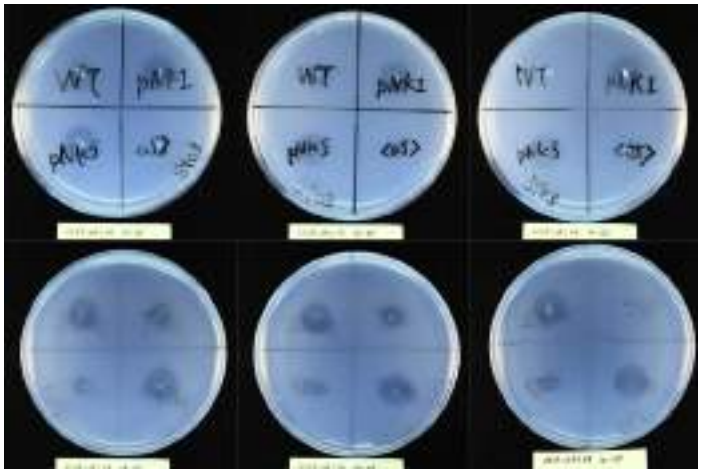
BEST195×3



BEST195×4

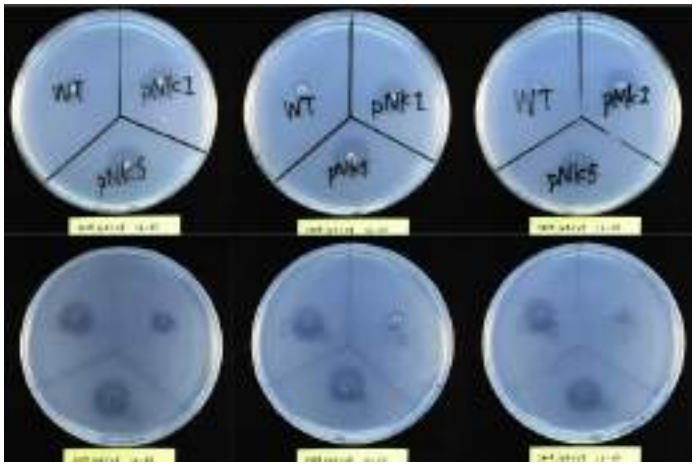


S903×3



20h

BEST195×3



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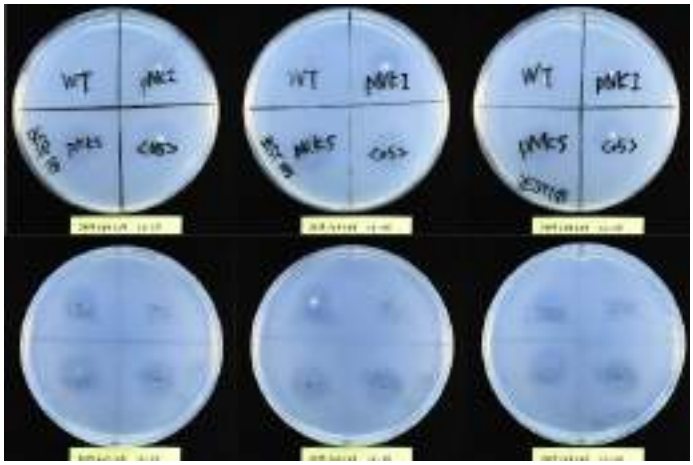
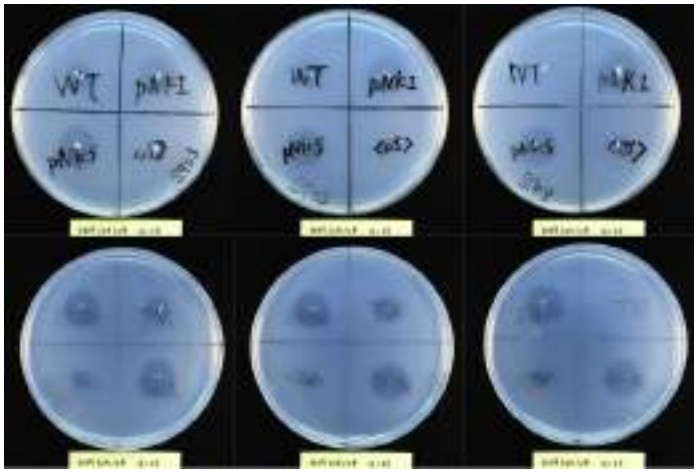
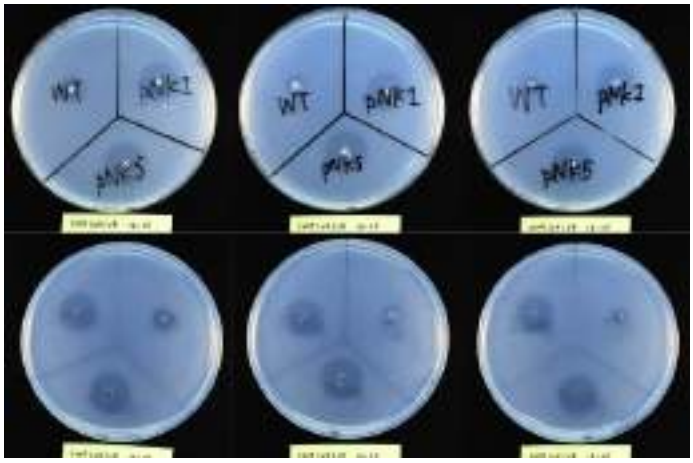


S903×3

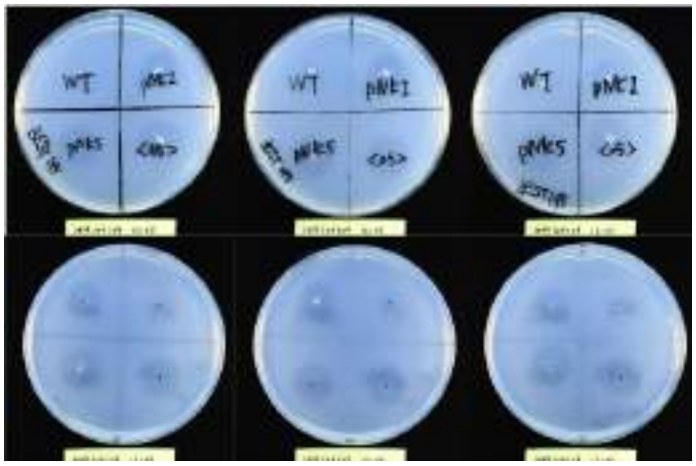


24h

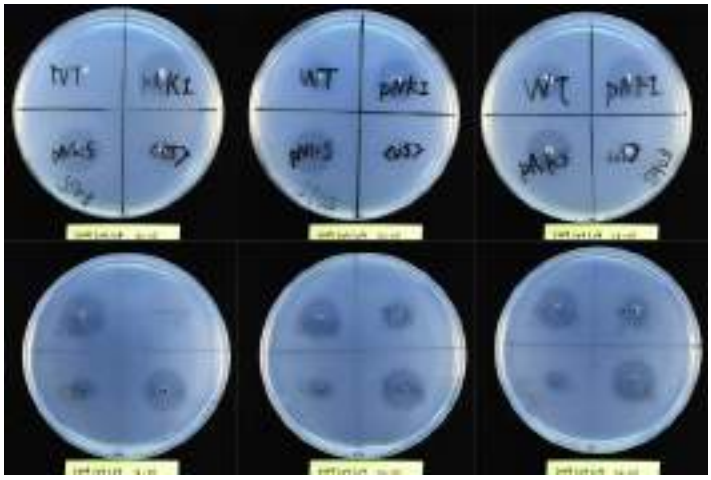
BEST195×3



BEST195×4



S903×3



・OD value of natto solution in the process of “Halo assay with natto suspension liquid.

	Strain	OD	dillution for OD=0.5
1	BEST195	2.92	0.0584
2	BEST195/pNK1	4.64	0.0928
3	BEST195/pNK5	6.08	0.1216
4	BEST195/<05>	6	0.12
5	S903	2.78	0.0556
6	S903/pNK1	4.16	0.0832
7	S903/pNK5	3.92	0.0784
8	S903/<05>	1.74	0.0348

Additional Notes:

2024/09/30 (Mon)

18:00-22:00 (JST)

Experiment Supervisor:
Koichi Yano
Kazuyuki Fujimitsu

Participants:
・Mizuho Sakai (all)
・Arisa Tani (19:00-)

Experiment:
・Fibrin plate assay
    ・Liquid inoculated on LB medium
    ・Liquid suspended natto

Results:
None

2024/10/01 (Tue)

13:30-22:00 (JST)

Experiment Supervisor:
Koichi Yano
Kazuyuki Fujimitsu

Participants:
・Mizuho Sakai (all)

・Arisa Tani (19:00-)

Experiment:

・Confirmation of yesterday's results of the fibrin assay

・Colony PCR

Number	Plasmid / Strain	Primer_F	Primer_R	Amplified length (bp)
1	P.C. 168/<05> LB culture (centrifuge culture, pellet direct into PCR)	GT16	GT17	1,588
2	<05>P.C. (240820 GTor21 C600/<05> 1/75 dil. 3μL)			1,588
3	BEST195/<05> (240927 NB culture No.2, centrifuge pellet, suspend directly into PCR rxn)			1,588
4	BEST195/<05> (240927 NB culture No.3, centrifuge pellet, suspend directly into PCR rxn)			1,588

PCR Cycle			
predenaturation	98°C	3 mins	
denaturation	98°C	10 secs	x 30
annealing	55°C	5 secs	
extension	68°C	30 secs	
final extension	68°C	7 mins	
hold	12°C	-	

・Electrophoresis of Colony PCR products

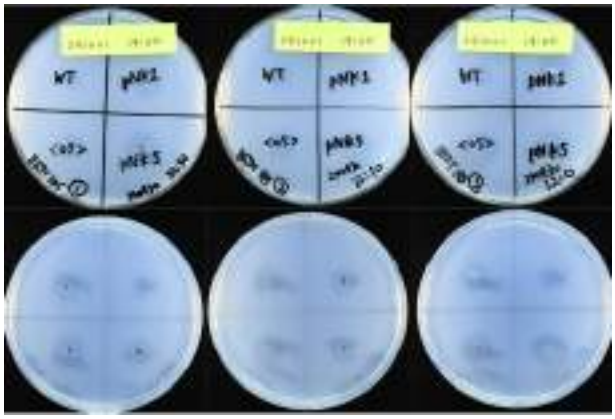
Results:

・Yesterday's results of fibrin assay

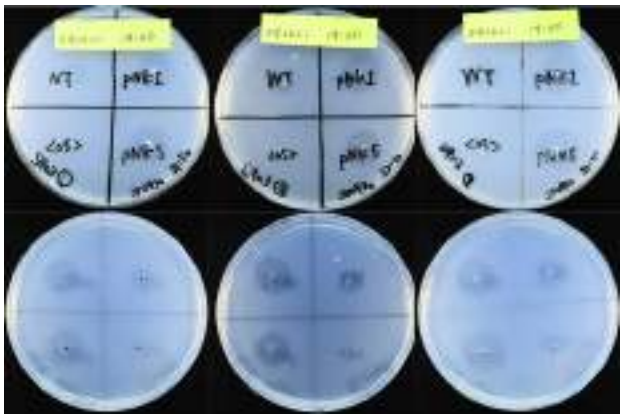
Liquid inoculated on LB medium

16h

BEST195

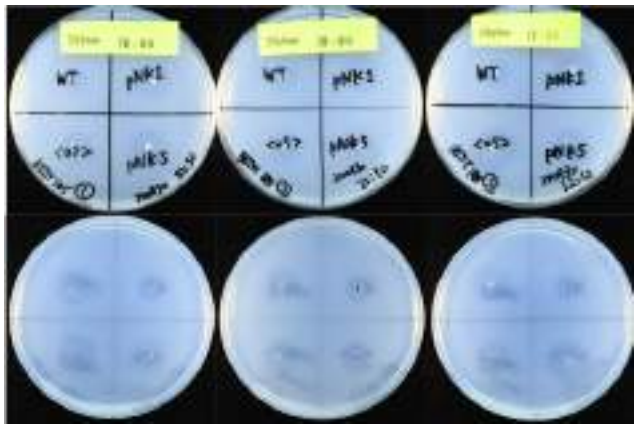


S903



20h

BEST195



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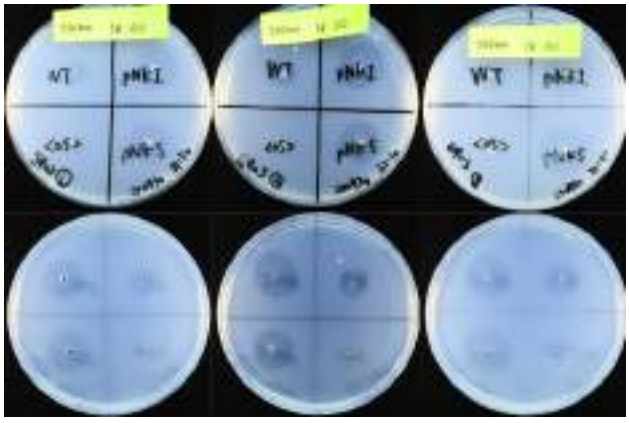
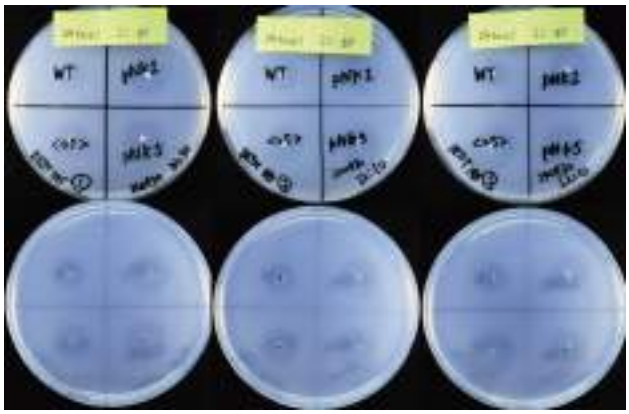


24h

BEST195



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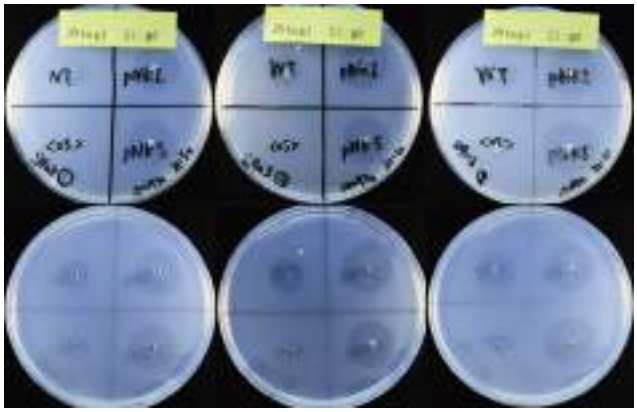
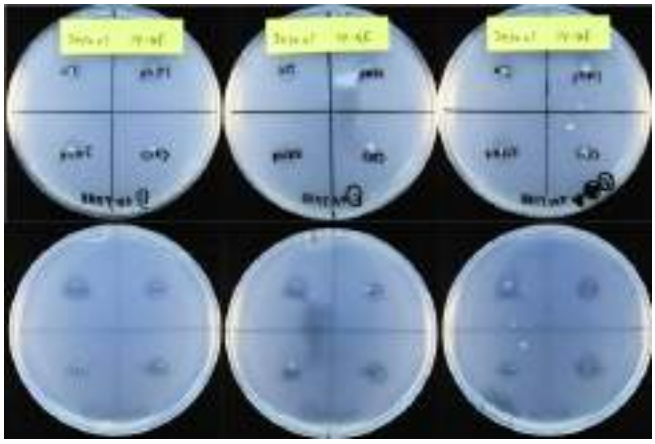


Natto suspension liquid
16h
BEST195



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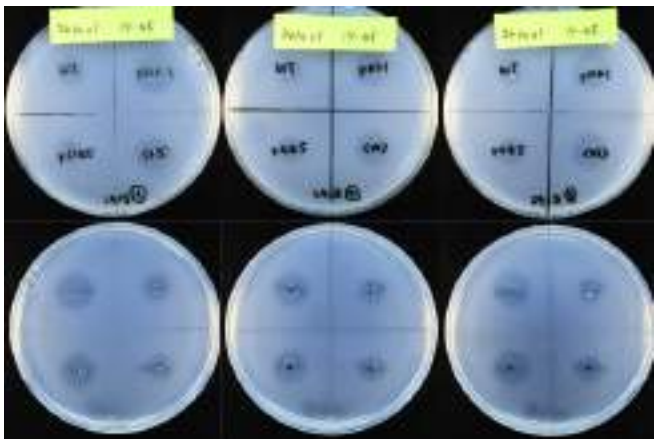
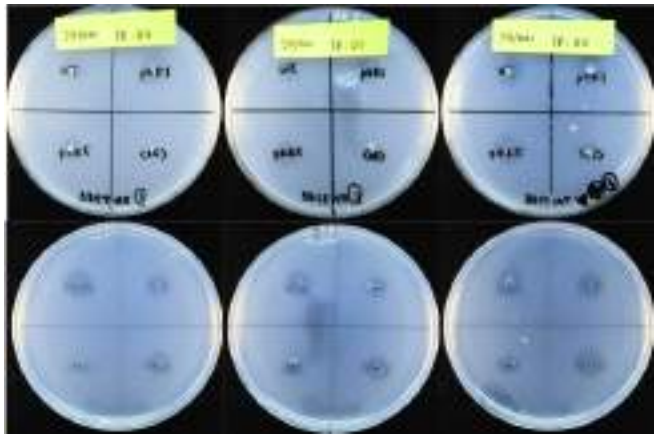


20h
BEST195



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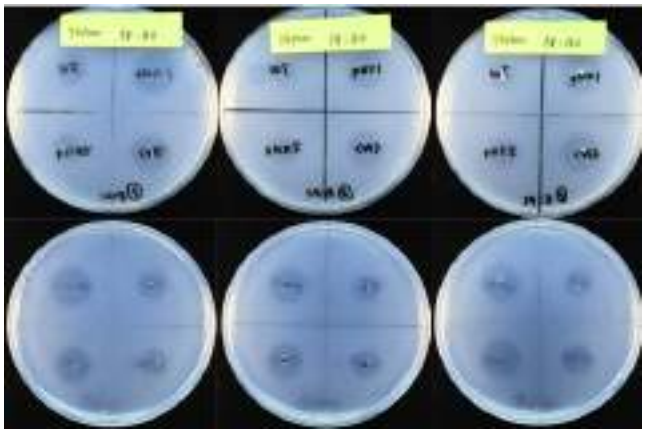
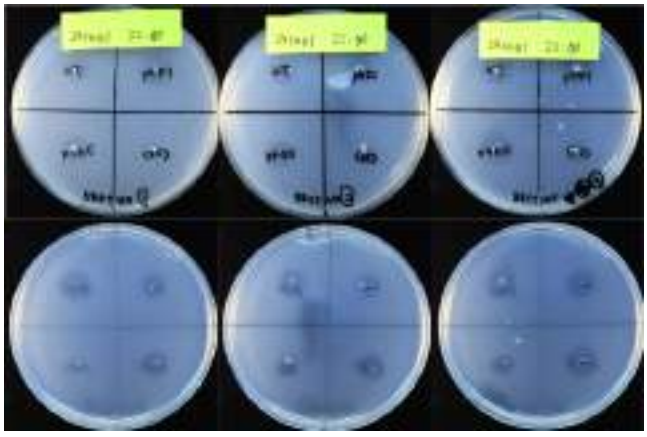


24h
BEST195



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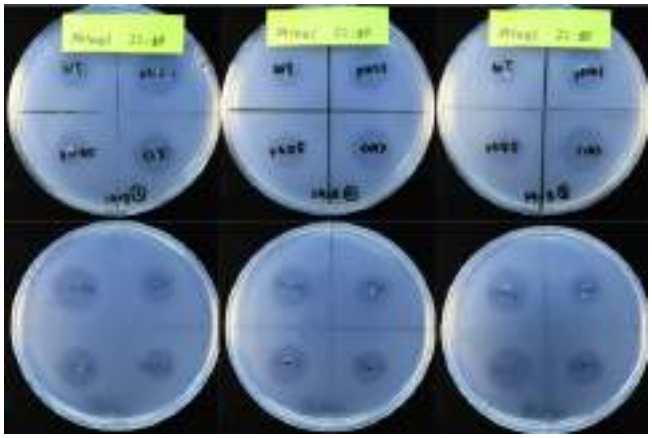

・OD values measured before Colony PCR

	OD
BEST195/<05> (240927 NB culture No.2, centrifuge pellet, suspend directly into PCR rxn)	0.92
BEST195/<05> (240927 NB culture No.9, centrifuge pellet, suspend directly into PCR rxn)	1.31

・Electrophoresis

Additional Notes:

There was a little water on the surface of the fibrin plate. We were able to confirm the halo, but need to keep a close eye on it.