Lab Notebook

7.25

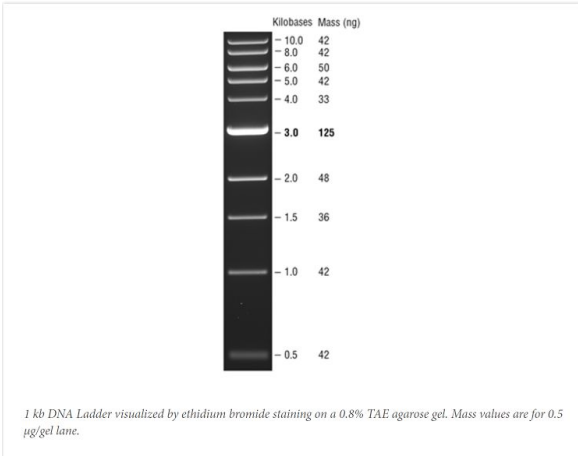
1. Primers arrived
2. Yuya ran the PCR for the iGEM plasmid backbone and the cyanobacteria vector

	25 µL Reaction
5x Q5 buffer	5 µL
10 mM dNTP	0.5 µL
10 µM forward primer	0.75 µL
10 µM reverse primer	0.75 µL
DNA template 1ng/ml	17.75 µL
Q5 polymerase	0.25 µL

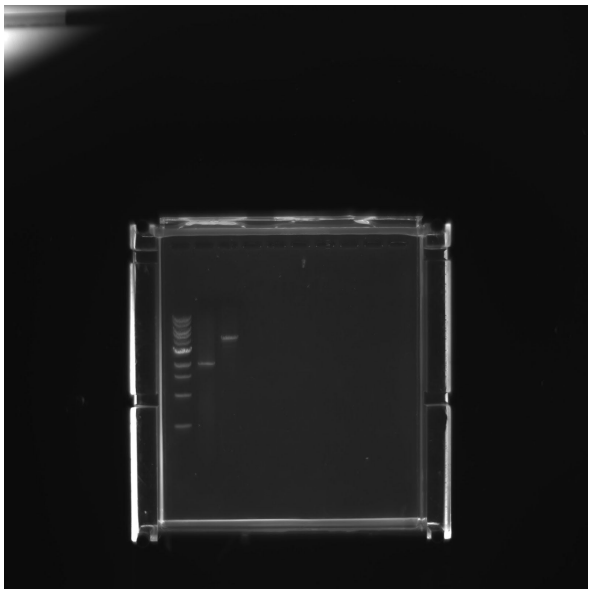
	32 cycles				
98℃	98 ℃	62℃/60℃	72℃	72℃	4℃
30s	10s	30s	2min15s	2min	forever

Yuya running gel from yesterday's PCR

1. Make gel: 40 mL of 0.7% agarose + 4 mL cyber safe
2. Loading: 5 µLof 1kb ladder
3. Get a piece of parafilm. Put a little dye on it
4. Take 5µL of DNA and mix with the dye on parafilm. Then load into the gel
5. Run 100V 30 mins. Then 120V for another 30 mins



1 kb DNA Ladder visualized by ethidium bromide staining on a 0.8% TAE agarose gel. Mass values are for 0.5 µg/gel lane.



Gel looks good, we have the expected result

DNA cleaning:

1. Add equal volume of membrane binding solution and mix with DNA (20 µL)
2. Load DNA+binding solution into column
3. Centrifuge 30k rpm for 1 min
4. Add 700µL membrane wash solution (with ethanol) into column
5. Centrifuge 30k rpm for 1 min
6. Discard flow through and centrifuge 30k for another 1min
7. Transfer column into clean 1.5 mL tube
8. add 30 µL elution buffer
9. Elute for 1 min
10. Centrifuge 1 min 30k rpm

11. Remove column. Store elute at -20 ℃

Concentration:
PSB1C3: 44 ng/µL
AM4941: 33.8 ng/µL

7.31

Seamless reaction

1. Dissolve the DNA
    a. Pipette 100uL water to dried samples
    b. Use water instead of TE buffer because the samples don't need to last long
    c. Prepare a fifth NC group using ARI
    d. Vortex briefly and spin
    e. Incubate at 42oC for 20min

2. Digest DNA
    a. Adjust the DNA amount so no need to add water
    b. Add sample (~3)
    c. Add digested vector (~0.3-0.5)
    d. Add buffer (~1.0) (iced, not for NC)
    e. Add enzyme (~0.5) (iced, not for NC)
    f. Vortex, spin, and incubate at 25oC for 30min in BioRad PCR machine

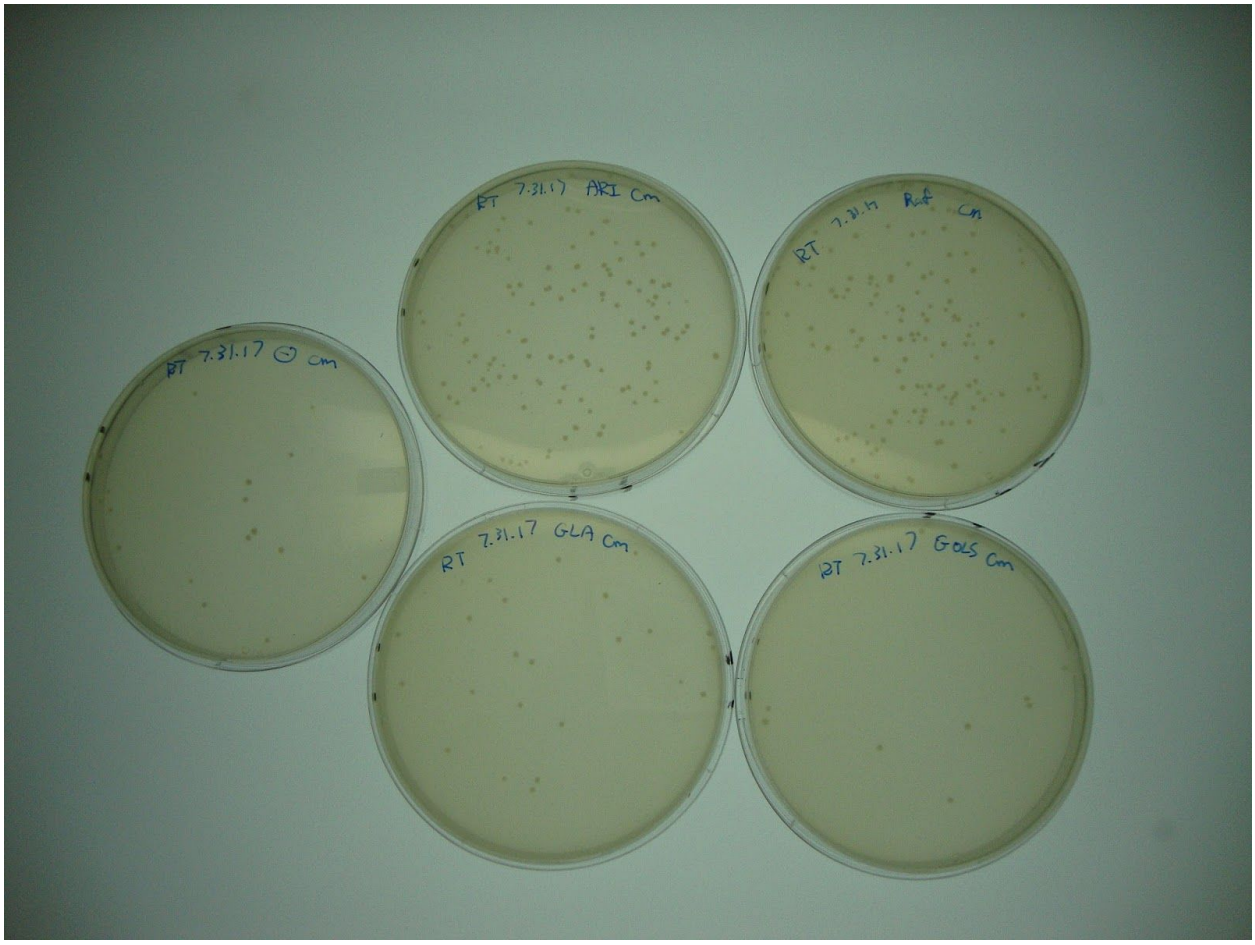
3. Transformation
    a. Thaw the competent cell (OneShot TOP10)
    b. Add the samples to the cells
    c. Transformation at 42oC for 30sec
    d. Place back on ice for 3min
    e. Add 200uL SOC liquid, incubate at 37oC for 1hr in a rotating incubater
    f. Plate the colony

8.1

Performed by: Yuya and Yansen
Notes: Yuya

1. Picking colonies and PCR

2. Examine the plate cultured from yesterday
    a. There are a few colonies on the negative control plate
    b. RafS and ARI plates have a lot of colonies
    c. GolS and GLA plates don't have many colonies (similar number to the negative control)



We have the expected result

3. PCR to check if DNA are integrated into the vector
    a. Make master mix

	25 µL	18x master mix
10x Taq buffer	2.5	45
10 mM dNTP	0.5	9
10 µM forward primer	0.5	9

10 µM reverse primer	0.5	9
Template	N/A	N/A
Taq polymerase	0.125	2.25
water	20.375	366.75
Total	24.5	441

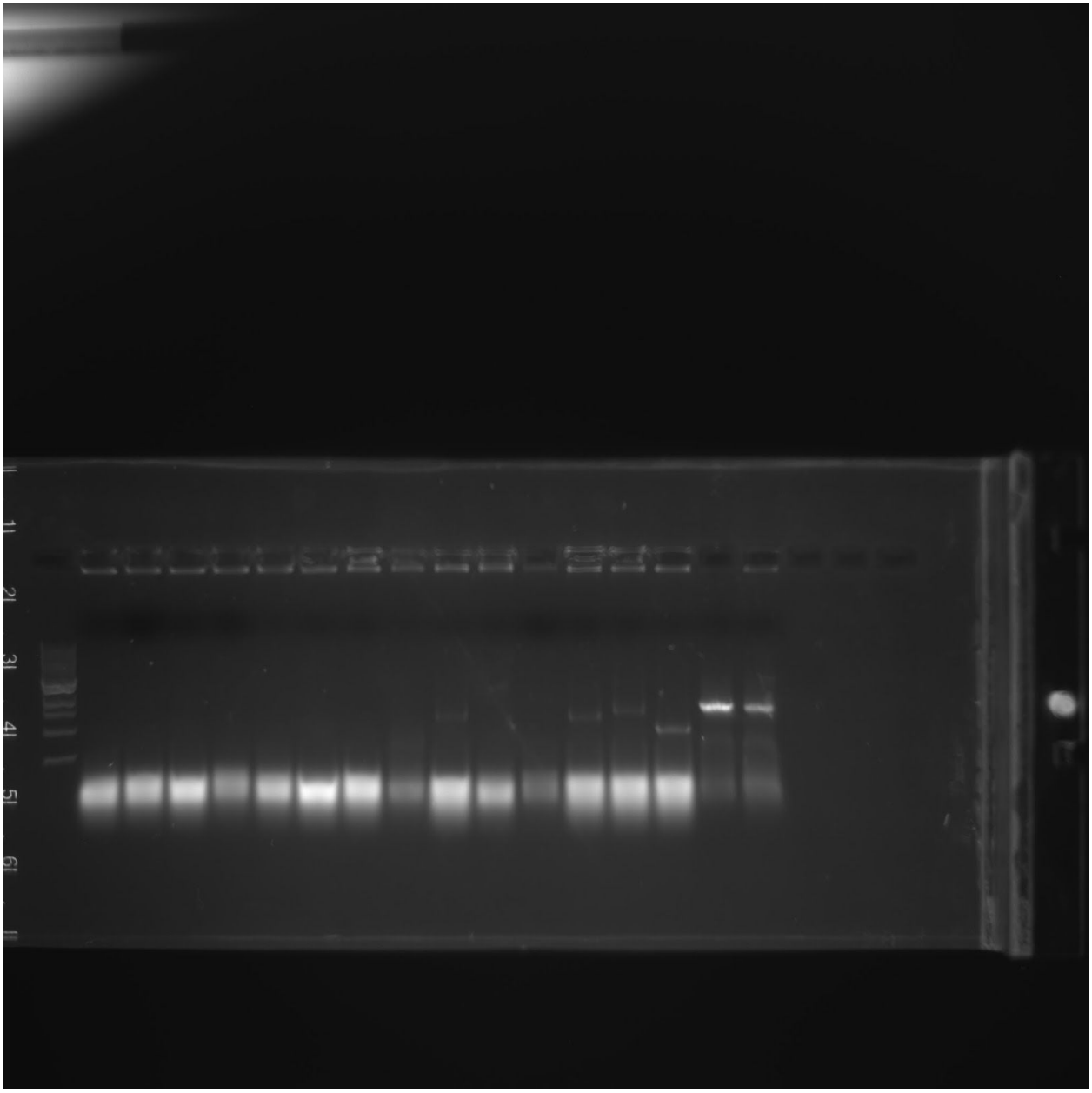
b. Label glass tubes and PCR tubes
c. Add 5 mL LB with chloramphenicol (50µL/100mL) into each glass tube

1	2	3	4	5	6	7	8
RafS1	RafS2	RafS3	RafS4	GolS1	GolS2	GolS3	GolS4
9	10	11	12	13	14	15	16
GLA1	GLA2	GLA3	GLA4	ARI1	ARI2	ARI3	ARI4

d. Add 24.5 µL of master mix into each PCR tube
e. Use 200µL pipette tip to pick one colony. When the tip goes into the tube, but before touching the liquid, push down pipette
f. Mix master mix using the pipette tip. When taking out, only push to the first stop so that there is some liquid remain inside the pipette tip
g. Dispose the pipette tip into corresponding glass tube
h. Run PCR

		30 cycles			
Initial denaturation	denaturation	Annalnation	Extension	Final extension	
95℃	95℃	52℃	68℃	68℃	4℃
30s	15s	30s	3min	5min	forever

4. Run the gel
   125V 25 mins

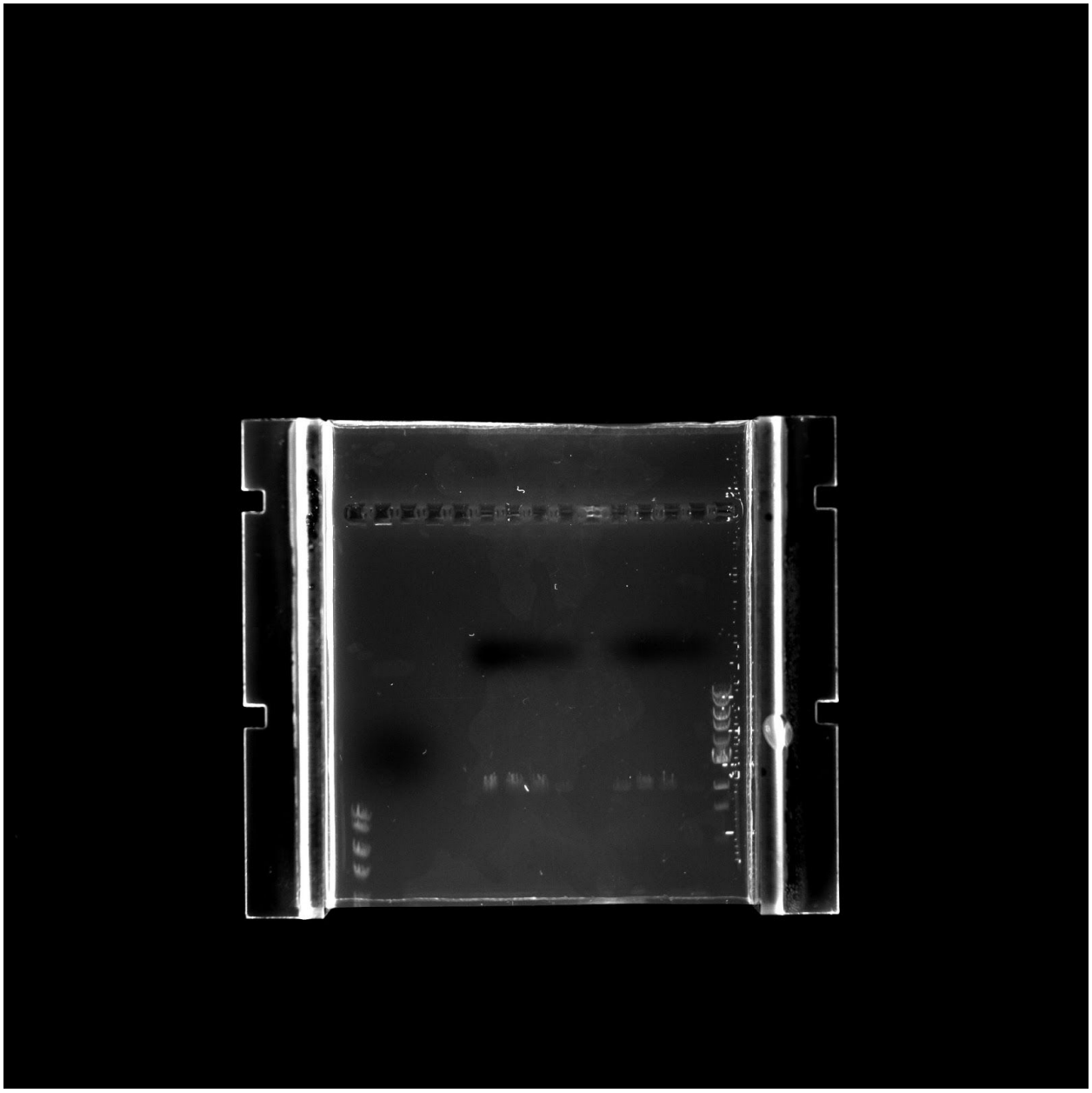


We have the expected result

8.2

1. Performed mini prep following the protocol
2. Performed digestion analysis following the protocal.
   Enzyme: EcoR1, Spel
   Buffer: 2.1

3. Run the gel: 140V 25 mins



We didn’t get the expected result.

8.3

Performed by: Yuya, Victoria
Recorded by: Yuya

1. Add 5 mL LB (with 50 µL chloramphenicol) into each glass tubes
2. Pick a colony the plate with a stick and drop the stick into the glass tube
3. stir the stick a bit and take the stick out

4. Culture the colonies 37 ℃ overnight

8.4

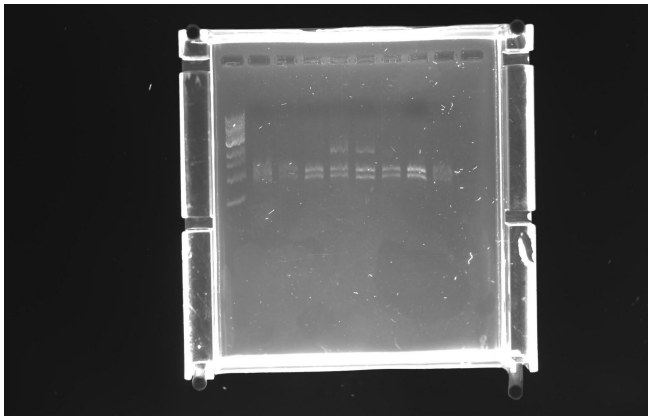
Performed by: Yuya, Victoria
Notes by Yuya

1. Mini Prep for the 8 samples from yesterday
   Result:

RafS1	111.5	ng/µl
RafS2	110.7	ng/µl
RafS3	105	ng/µl
RafS4	125.8	ng/µl
GOLS1	119.2	ng/µl
GOLS2	146.5	ng/µl
GOLS3	179.9	ng/µl
GOLS4	140	ng/µl

2. Digestion analysis
   Enzyme: EcoR1, Sac1
   Buffer: Cutsmart

3. Run a gel



We didn’t get the expected result.

8.7

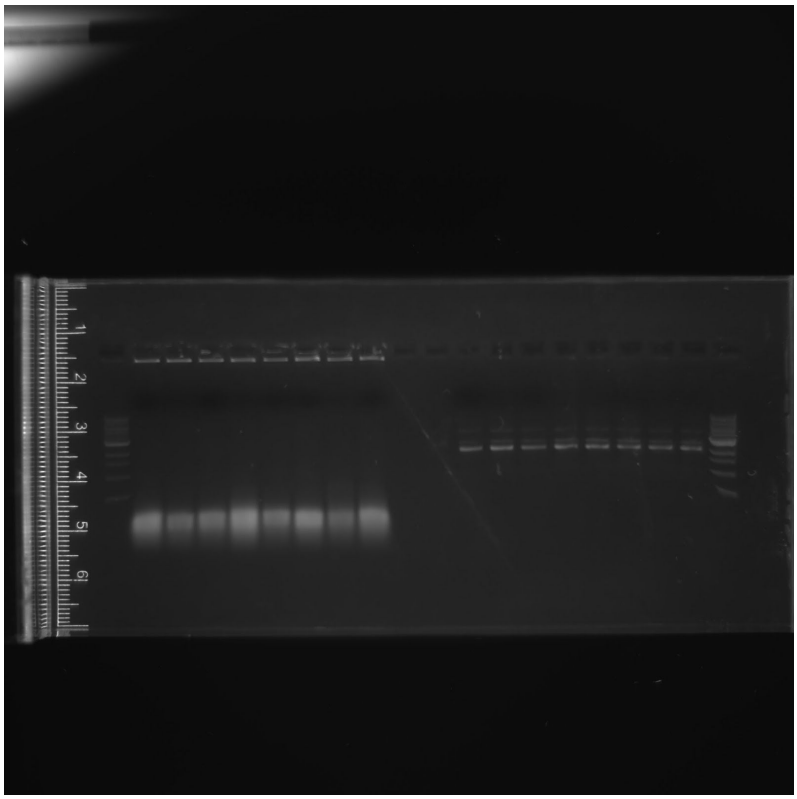
1. Seamless reaction following protocal

	RafS	GOLS
Tempate (DNA from IDT)	3.28µl	3.14µl
Digested vector	0.2µl	0.36µl
Buffer	1.0µl	1.0µl
Enzyme	0.5µl	0.5µl

2. Transformation

8.8

1. Pick four colonies from each plate and do PCR



We didn’t get the expected result

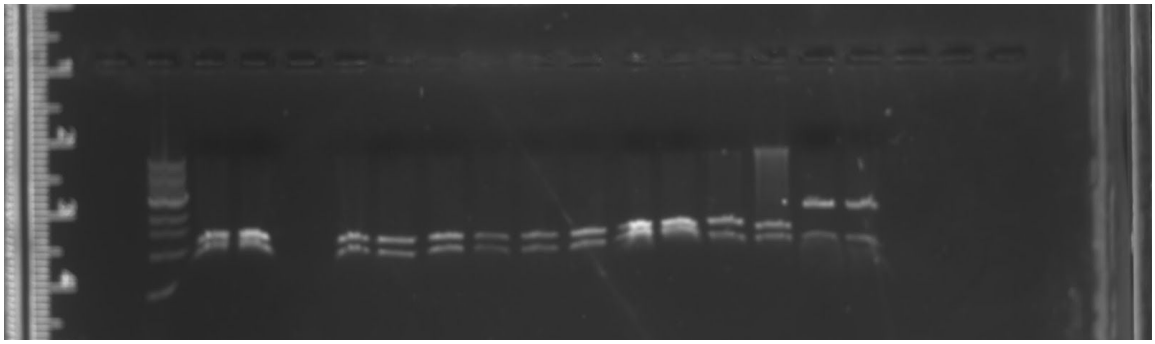
2. Pick four more RafS and the rest of 2 GOLS

8.9

1. Mini prep the 14 samples from yesterday

	Nucleic Acid Conc.	Unit
RafS1	174.9	ng/µl
RafS2	166.2	ng/µl
RafS3	198.6	ng/µl
RafS4	205.9	ng/µl
RafS5	198.5	ng/µl
RafS6	138.4	ng/µl
RafS7	190.3	ng/µl
RafS8	214.6	ng/µl
GolS1	211.3	ng/µl
GolS2	202.7	ng/µl
GolS3	185.4	ng/µl
GolS4	266.9	ng/µl
GolS5	173	ng/µl
GolS6	225.8	ng/µl

2. Digestion analysis
   Enzyme: EcoR1, Sac1
   Buffer: Cutsmart



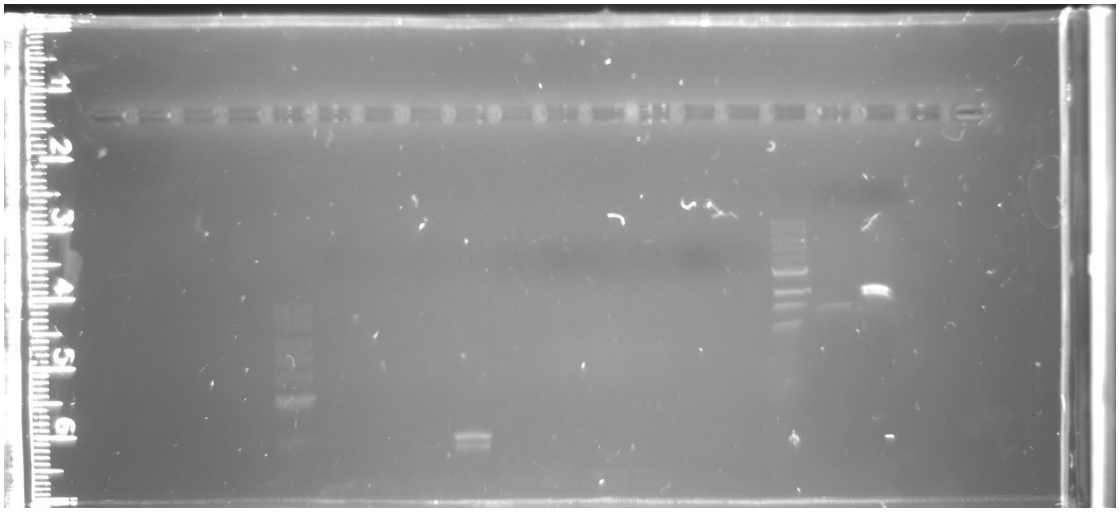
GolS 5, 6 are good. RafS still fail
Pick another 10 RafS colonies (6 from second time plate and 4 from the first time) and culture

8.10

1. Mini prep 10 E coli samples cultured from yesterday
2. 1-6 second plate, 7-10 first plate

1	213.2	ng/µl
2	214.9	ng/µl
3	159.1	ng/µl
4	265.7	ng/µl
5	164.6	ng/µl
6	128.9	ng/µl
7	182.6	ng/µl
8	156.6	ng/µl
9	125.7	ng/µl
10	141.9	ng/µl

3. Digestion analysis with SacI and EcoRI, cutsmart buffer
4. Run a gel



We didn't get the expected result.

8.14

1. Seamless reaction, 2 samples, vector:DNA=1:4, 1:5

Template (DNA)	Digestion vector	Buffer	Enzyme	Total
4.57µl	0.2µl	1µl	0.5µl	4.27µl
5.71µl	0.2µl	1µl	0.5µl	4.27µl

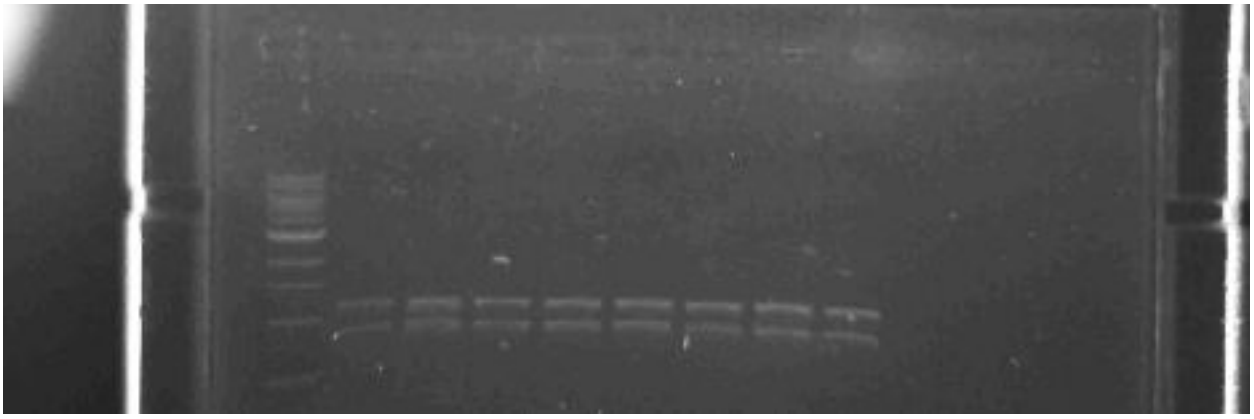
2. Spread on plates

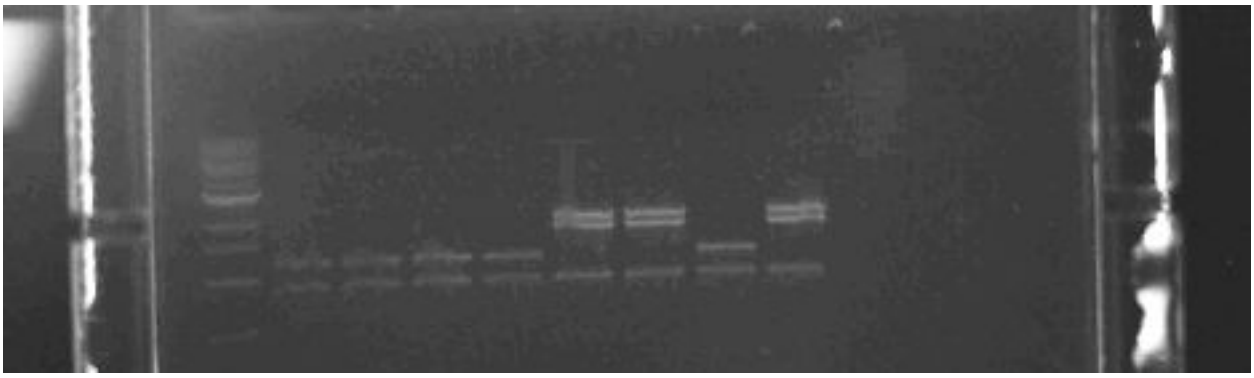
8.15

1. Roughly 15-20 colonies on each plate
2. PCR 4 colonies on each plate (Taq)
3. I did not cap the cap very well and all the PCR evaporate :(
4. Pick 4 more colonies on each plate and culture the total of 16 colonies

8.16

1. Mini prep all 16 samples
2. Digestion analysis with SacI and EcoRI
3. Run the gel





Gel 1 were all empty vectors. Gel 2 has 3 wired bands

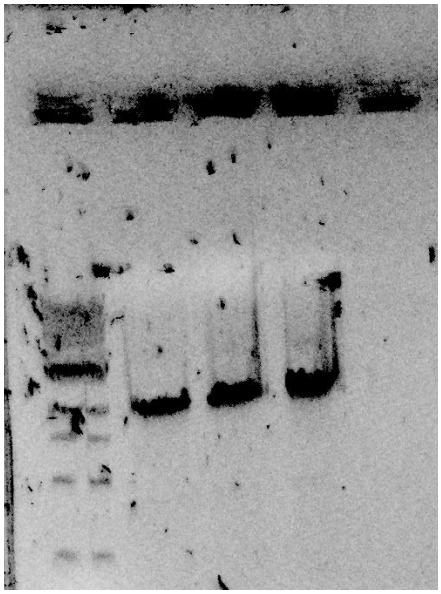
4. Do PCR on the three wired samples

10x Taq Buffer	2.5 µl
10 mM dNTP	0.5 µl
10 µM forward primer	0.5 µl
10 µM reverse primer	0.5 µl
Taq enzyme	0.125 µl
1 ng/µl DNA	20.875 µl

Sample 13: 237.31 ng/µl: 0.2µl DNA into 47.30 µl of water
Sample 14: 278.4 ng/µl: 0.2µl DNA into 55.48 µl of water
Sample 16: 236.2 ng/µl: 0.2 µl DNA into 47.24 µl of water

8.17

1. Run gel of PCR yesterday



Seems like we have the right insert

2. Prepare samples to be sequencing

1	2	3	4	5	6	7	8
RafS 13	Gol 6	GLA 9	ARI 15	RafS 13	Gol 6	GLA 9	ARI 15
5’	5’	5’	5’	3’	3’	3’	3’

a. Primer (pSB1C3 forward or reverse) 2.5µl
b. DNA 3µl (at least 500ng)
c. Water 9.5 µl
d. Total 15µl

8.18

Redo sequencing with the correct primer

RafS 13	RafS 13	Gol 5	Gol 5	Gla 9	Gla 9	ARI 15	ARI 15
5’	3’	5’	3’	5’	3’	5’	3’

1. Primer (enzyme4941 forward or reverse) 2.5µl
2. DNA 3µl (at least 500ng)

3. Water 9.5 µl
4. Total 15µl

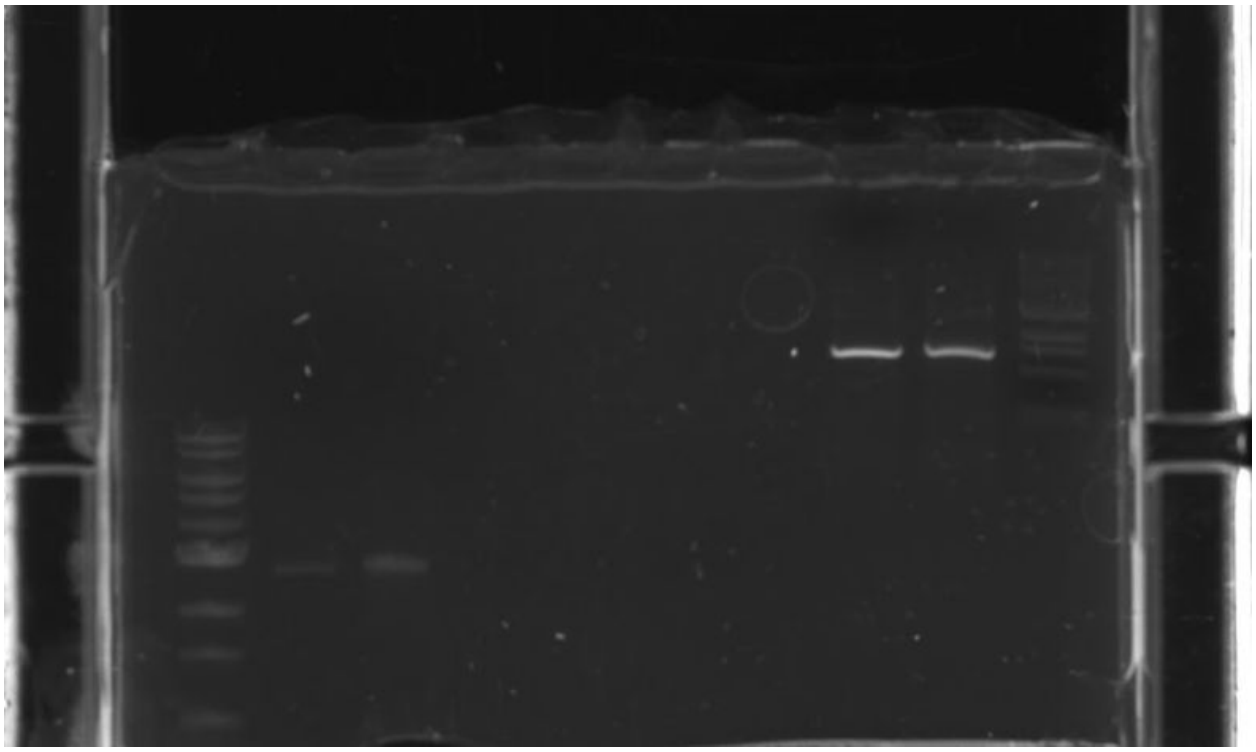
8.21

1. Check sequencing result. Others are fine. But GOLS 3' end fail both time. Need to check
2. PCR with Taq protocol

Gel looks good. Bands have the right size (1.3k)

8.22

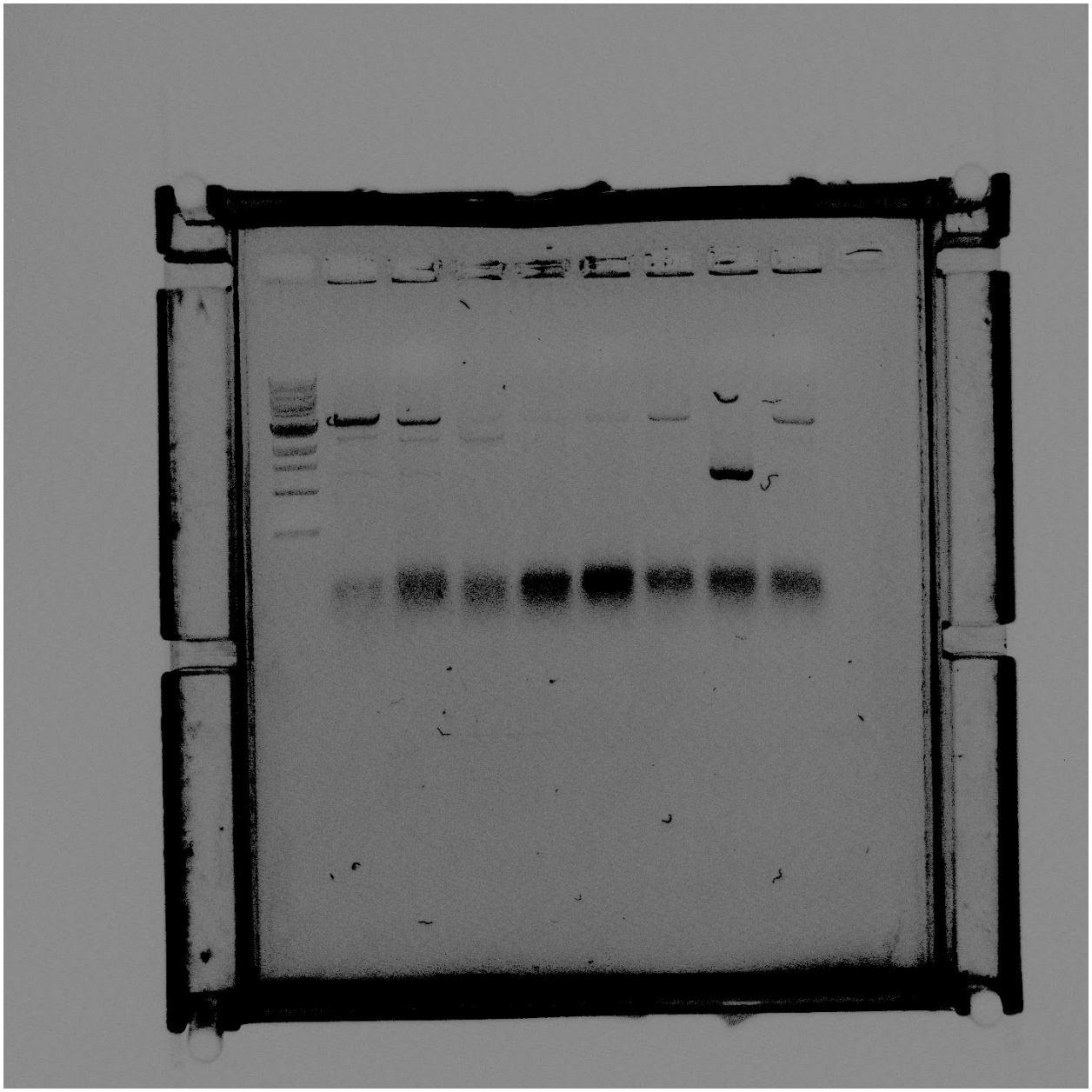
1. 3A assembly GOLS 5 (part A), RafS (part B)
2. For digestion,
    a. 100 ng part A = 0.54 µl
    b. 100 ng part B = 0.364 µl
3. For ligation, use 1:2 of A:B for equimolar amount
    a. 1.3 µl part A
    b. 2.6 µl part B
4. For transformation, use Top 10 Oneshot competent cell, plate on LB+ap
5. Make plasmid stocks with electro shock, plate on LB+cm



We get the expected result.

8.23

1. There are many colonies on the 3A plates, but only 2 on control. There are super many colonies on the stock plates. All the colonies connect to each other and spread on the plate
2. Pick 8 colonies on 3A plate to do Taq PCR. Reculture these 8 colonies in 5 mL LB+ap
3. Pick a stick of colonies on stock plates and reculture in 5 mL LB+cm
4. Gel. 1 and 2 are strong but have impurities. 6 and 8 are weaker but seem purer. Use 6 or 8 to make the next 3A assembly



We get the expected result

8.24

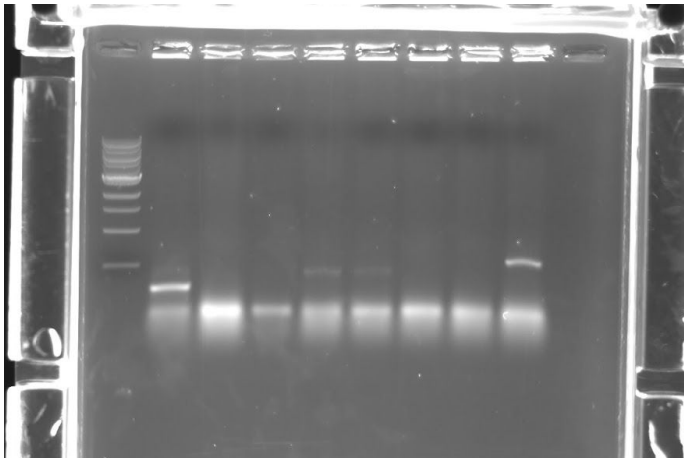
1. Mini prep 6 and 8, and 4 stocks
   6 has 317ng/ul, 8 has ~300ng/ul, all the stocks have ~60ng/ul.
2. 3A assembly for PSB1A3 Gols+Rafs 6 (PartA) and GLA5 (Part B) and PSB1K3 (PB)
   a. For digestion, used 4ul PB, 0.32ul A (317.4ng/ul), and 0.57ul B (175.9ng/ul)
   b. For ligation, use 2.0ul PB, 2.5ul A, and 1.0ul B.

8.25

Check the plate, the positive plate has many colonies while the negative control plate has only two. So the result is reasonable.

8.28

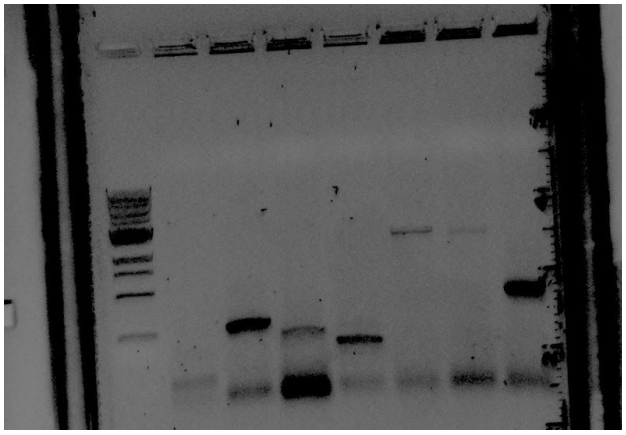
1. PCR 8 colonies on Gols+RafS+GLA km plate
2. Run gel



Gel is not good. Seems like have many empty vectors

8.29

1. Pick another 8 colonies from the G+R+G plate and do PCR
2. Gel



Seems like 5 and 6 has only part A (G+S), and 7 has GLA only, 1 has an empty vector. Not sure what is 2, 3, 4

3. Redo 3A assembly. Plasmid backbone: pSB1K3. Part A: GOLS+RafS 8. Part B: GLA(sequenced)
    - Make master mix for PB, A and B (for 3 reactions)
    - Digest as the protocol but doubling everything
    - 2 ligations. One 1/2, the other 3/4

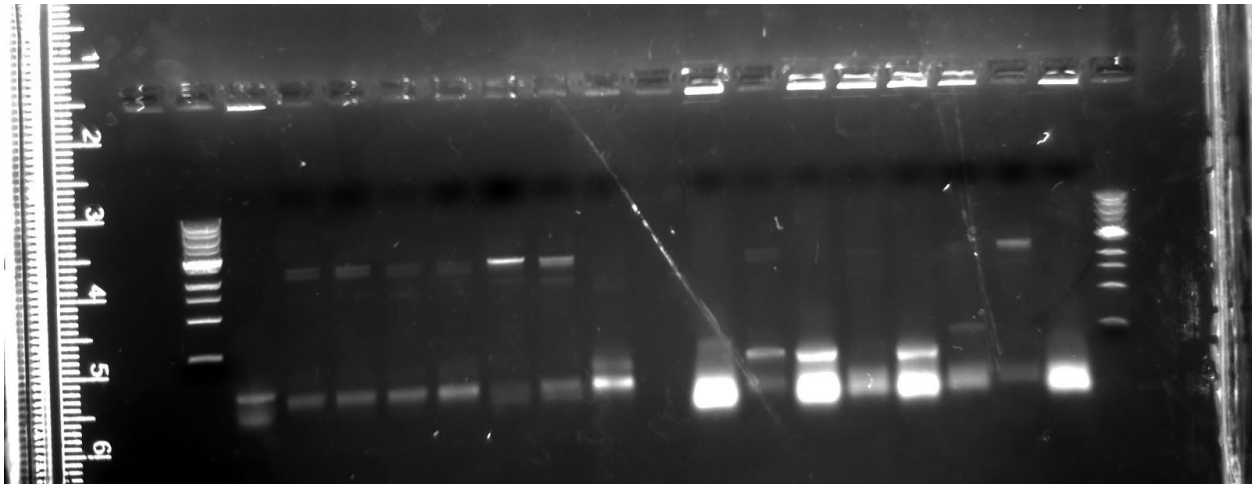
    For 1/2:
    a. 1ul of digested PB
    b. 3.75 ul digested part A
    c. 1.5 ul digested part B
    d. 1 ul T4 DNA ligase buffer
    e. 0.5 ul T4 DNA ligase
    f. 2.25 ul water

    For 3/4
    a. 1.5 ul of digested PB
    b. 3.25 ul of digested part A
    c. 1.3 ul of digested part B
    d. 1 ul of T4 DNA ligase buffer
    e. 0.5 ul of T4 DNA ligase
    f. 2.45 ul water

    - Follow the standard protocol for transformation

8.30

1. PCR (Taq, primer enzyme_4941) 8 colonies from each plate, total 16 samples
2. Gel



Looks like there are many plasmids with only part A inserted

8.31

3A assembly of GOLS+RafS+GLA with excess part B (PB: pSB1K3, Part A: GOLS+RafS on pSB1A3, Part B: GLA on pSB1C3)

1. Digestion following standard protocol
    a. 0.63 µl part A + 7.37 µl water (2 rxn)
    b. 1.14 µl part B + 6.86 µl water (2 rxn)

2. Ligation

    Tube 1:
    a. PB 2.0 µl
    b. Part A 2.0µl
    c. Part B 2.0 µl
    d. T4 ligase 0.5 µl
    e. T4 ligase buffer 1.0 µl
    f. Water 2.5 µl

    Tube 2:
    a. PB 2.0 µl
    b. Part A 3.0 µl
    c. Part B 3.0 µl
    d. T4 ligase 0.5 µl
    e. T4 ligase buffer 1.0 µl
    f. Water 0.5 µl

3. Standard protocol for transformation

9.1

3A assembly of GLA+ARI (PB: pSB1K3, Part A: GLA on pSB1C3, Part B: ARI on pSB1C3)

1. Standard protocol for digestion
    a. 0.57 µl part A + 3.43 µl water + 4 µl master mix
    b. 0.49 µl part + 3.51 µl water + 4 µl master mix

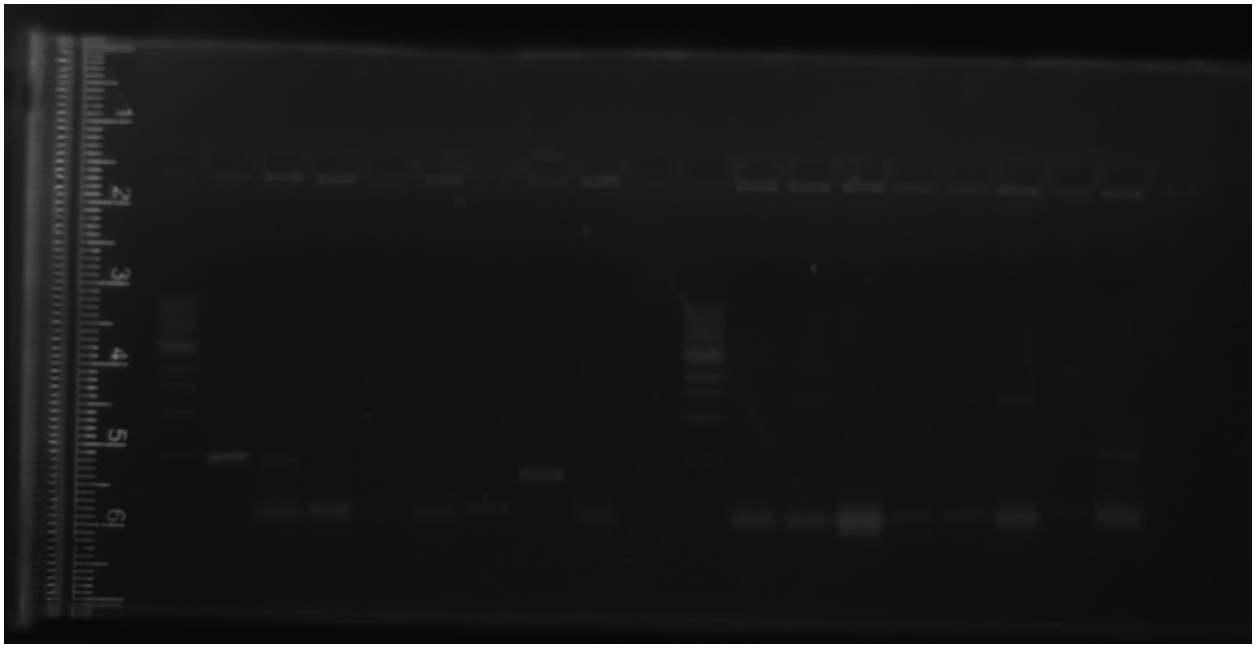
2. Standard protocol for ligation
    a. PB: 2.0 µl
    b. Part A: 2.4 µl
    c. Part B: 2.55 µl
    d. T4 buffer: 1.0 µl
    e. T4 ligase: 0.5 µl
    f. Water: 1.55 µl

3. Standard protocol for transformation

9.5

1. PCR of GLA + ARI 3A (Gols + rafs + gla)
    a. Tap buffer: 45 µl
    b. dNTP : 9µl
    c. FWD primer : 9µl
    d. Rev primer : 9µl
    e. Template : 9µl
    f. Taq : 2.25µl
    g. H2O : 344.75µl

    Tubes 1-4: Gols + Rafs + Gla 1:1:1
    Tubes 5-8: Gols + Rafs + Gla 1:1:1.5
    Tubes 9-16: Gla+ARI



We didn’t get the product we expected.

9.6

1. Mini prep of GLA + ARI / Digestion
   (18 was spilled so less was transfered to column)

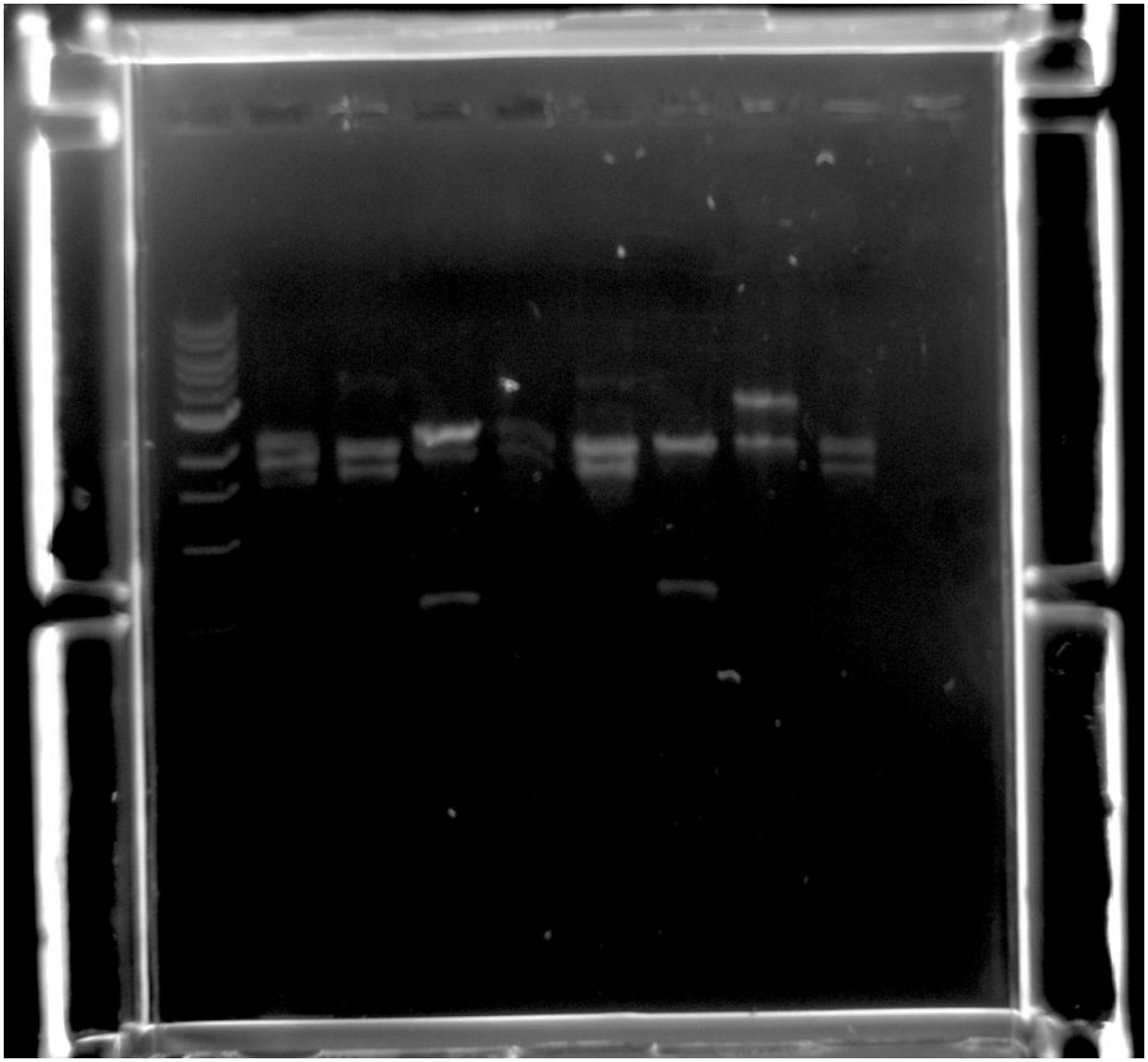
2. Nano drop:

#	Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1		GoldenLab	9/6/2017 10:39 AM	186.4	ng/µl	3.728	2.002	1.86	1.58	DNA	50
2		GoldenLab	9/6/2017 10:40 AM	188.1	ng/µl	3.762	2.016	1.87	1.61	DNA	50
3		GoldenLab	9/6/2017 10:41	195.1	ng/µl	3.903	2.096	1.86	1.61	DNA	50

			AM								
4		GoldenLab	9/6/2017 10:42 AM	71.2	ng/µl	1.425	0.762	1.87	1.57	DNA	50
5		GoldenLab	9/6/2017 10:43 AM	104.1	ng/µl	2.081	1.176	1.77	1.17	DNA	50
6		GoldenLab	9/6/2017 10:43 AM	70	ng/µl	1.4	0.814	1.72	0.99	DNA	50
7		GoldenLab	9/6/2017 10:44 AM	419.4	ng/µl	8.388	4.403	1.9	2.06	DNA	50
8		GoldenLab	9/6/2017 10:45 AM	434.6	ng/µl	8.692	4.579	1.9	2.06	DNA	50
9		GoldenLab	9/6/2017 10:46 AM	54.7	ng/µl	1.093	0.663	1.65	0.87	DNA	50

3. Digestion analysis:
    a. Mini prep 24 µl
    b. Enzyme 10 µl EcoR1 HF Pat 1
    c. Buffer 20µl NEB 2.1
    d. H20 140µl

    Total 160µl



21 is selected because it has the 2 genes GLA + ARI and plasmid.
GLA+ ARI= 2.8 kbp plasmid 2.2 kbp

9.7

222.3A of Gols + Rafs + Gla + Ari

PB: Psblc3
A : Gols + Rafs 317.4 ng/µl
B : Gla + Ari 419.4 ng/µl

1. Digestion
   Duplicate the process because too much mm (12 µl for A B)

Save the digested pieces for next time.

2. Ligation

PB	Saturated	Equimolar	NC
A	2 µl	1.5 µl	2 µl
B	3 µl	2.5 µl	3 µl
Dh20	0.9µl	2.2 µl	2.6 µl
Buffer	1 µl	2.3 µl	2.4 µl

Unfortunately, Soc media was not added. The experiment will restart tomorrow.

9.8

1. 3A of Gols + Rafs + Gla + Ari
    a. PB: Psblc3
    b. A : Gols + Rafs 317.4 ng/µl
    c. B : Gla + Ari 419.4 ng/µl

2. Digestion
   Duplicate the process because too much mm (12 µl for A B)
   Save the digested pieces for next time.

3. Ligation

PB	Saturated	Equimolar	NC
A	2 µl	1.5 µl	2 µl
B	3 µl	2.5 µl	3 µl
Dh20	0.9µl	2.2 µl	2.6 µl
Buffer	1 µl	2.3 µl	2.4 µl

9.11

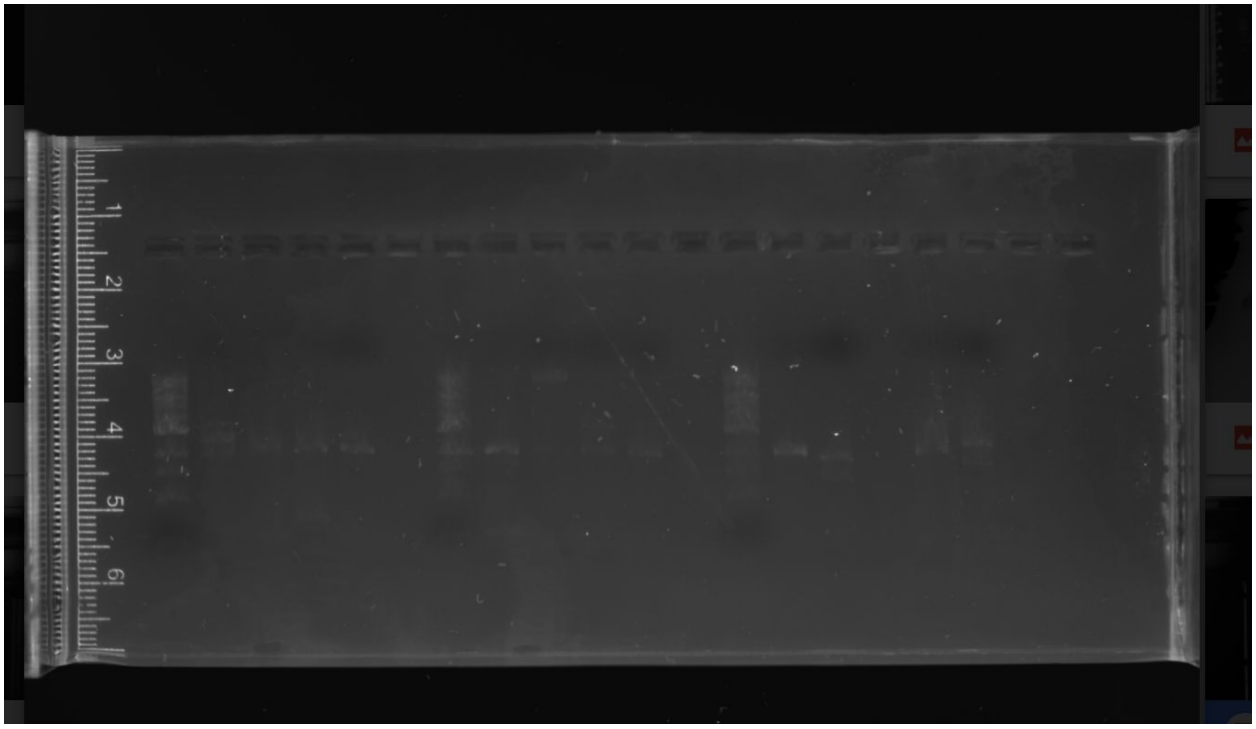
1. PCR/Digestion Analysis
    a. Tubes 1-4 are plate 1
    b. Tubes 5-8 are plate 2
    c. Pick 4 colonies from plate 1(12/9)

1. Miniprep
    a. Saturated 4/8 empty, 2/8 weired
    b. Equimolor ¼ empty, weired

9.12

1. Sequencing
    a. Reaction 1: G+R+G+A
    b. Reaction 2 in reverse.

Sequencing result not good.



2. Analyse the
    a. PB: pSB1C3, E,P,D
    b. A: G+R B: G+A

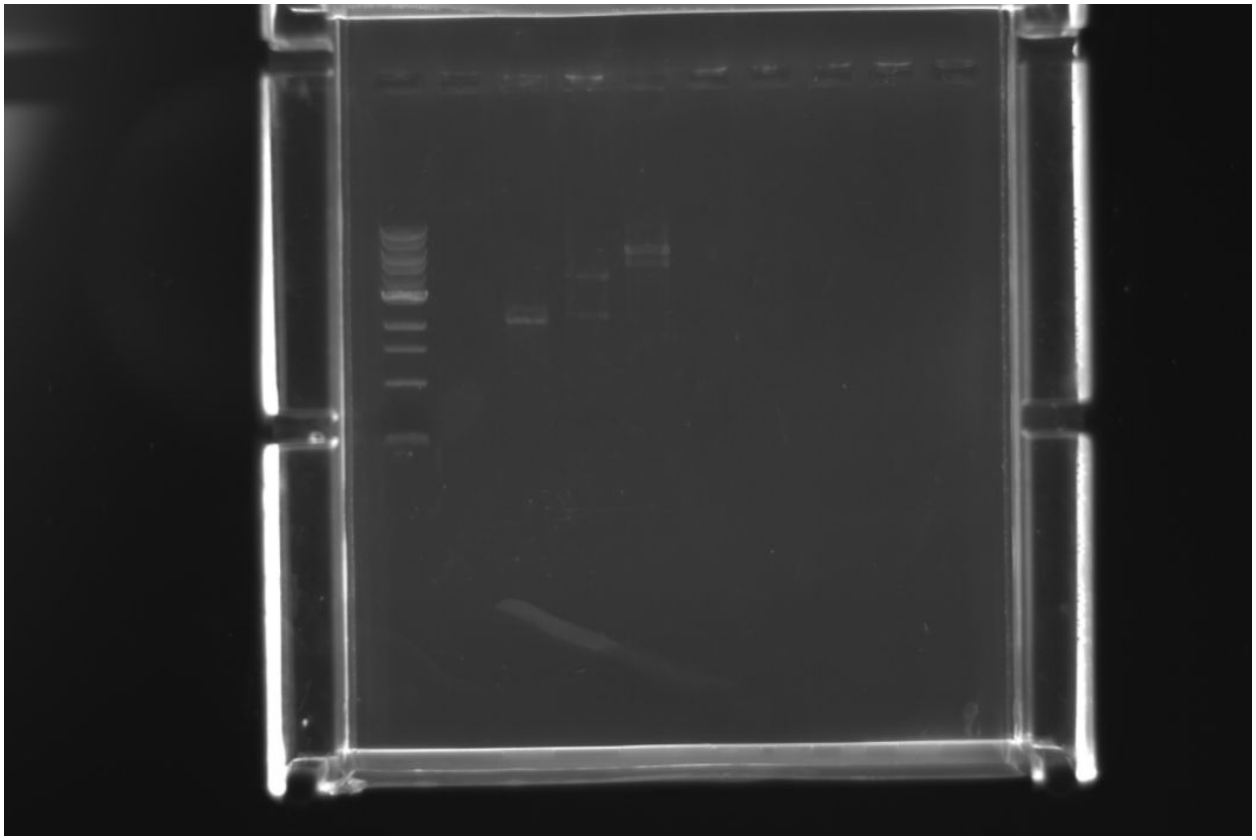
    Add 5 ul of sample to each lane, save the rest for 3A ligating step.

9.14

1. Digestion of G+A21
   Digestion using
   a. EcoR2 + Pst 1
   b. Xbal + Pst 1
   c. Reduce amount by half
   Incubate for an hour.

9.15

3A of G+R+G+A
Same as p28, however, this time, instead of ligation for 30 min. Ligation overnight was used. Because last time it didn't work. Yansen read some paper and realized that we should spend more time.

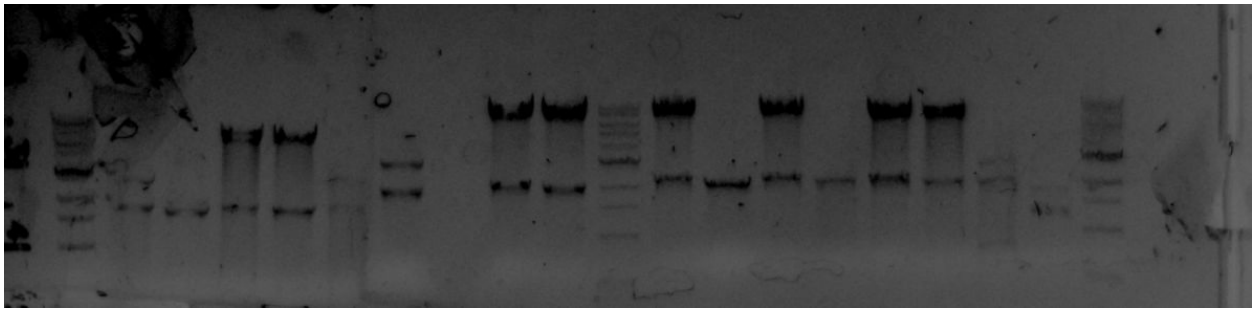


9.17

1. Transformation 2 plates. Incubate overnight.
2. Each plate has a lot of colonies. Pick 8 from each.

9.18

1. Miniprep
2. Digestion analysis



As shown: 3,4,7,8,9,11,13,14 worked.

9.19

1. Nanodrop
   #8 has the highest concentration and is selected to sequence and PCR.
   #8: 441.8 ng/ul

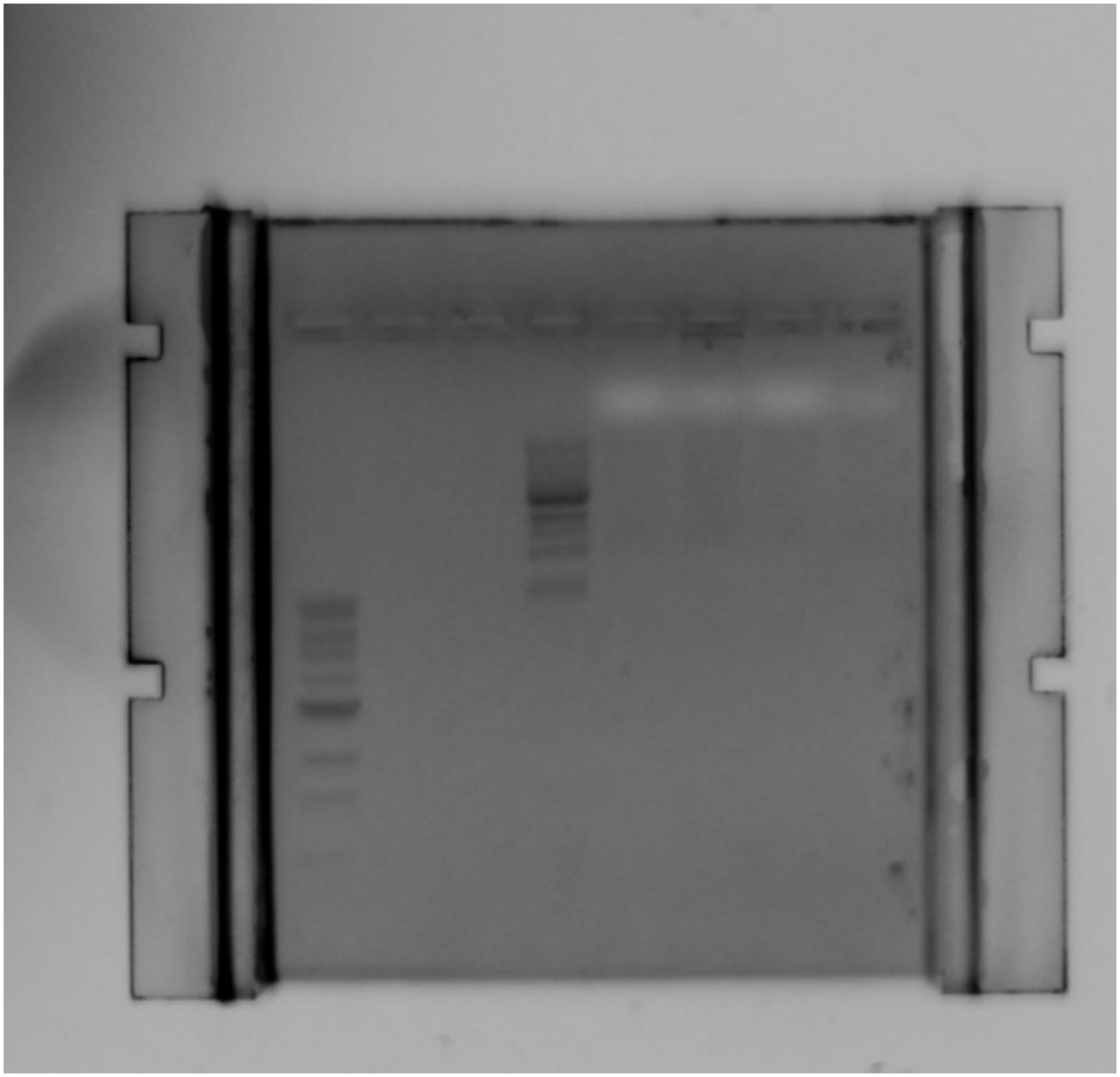
2. Digestion of AM 4941 dPN1 33.8 changed to 22.0 ng/ul
3. PCR

9.20

1. Re-do PCR from yesterday.
   a. Gradient PCR: 60°C, 62.3°C,63.7°C,66°C

b. Run for 4 hours.

2. Run the gel. Didn't work .



9.22

1. Double digestion of 4 genes.
   Digestion with E, S

2. After reaction, run gel, cut

3. Gel purification.
4. Nanodrop: 51.3 ng/ul

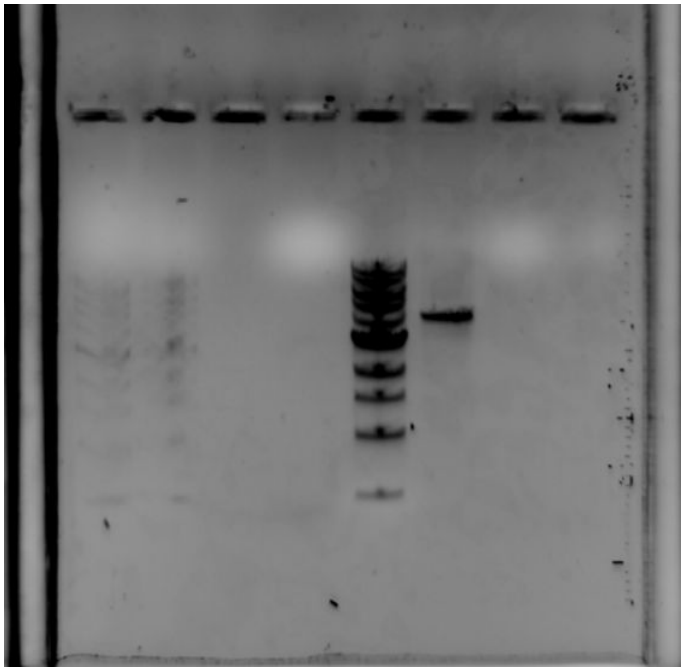
9.25

1. Q5 Reaction: For AM 4941, using new primers, follow the protocal on P46. After PCR load gel with 5 ul sample.

2. a.Digestion of templates: add 0.5 ul dPN1 to the remaining sample.
   b. Incubate at 37 °C for an hour (plate incubator).

3. Purification: using wizard gel/PCR clean up kit. Follow the protocol on P49.

4. Seamless reaction



5. We didn't get the expected result. Plate don't have colonies, redo next time.

9.29

1. Repeat seamless reaction
2. Incubate 25 °C for one hour instead of 30 minutes.

10.1

1. Miniprep 2 colonies
2. Mano drop
    a. 171.2 ng/ul
    b. 95.2 ng/ul

3. Digestion Gel: