

Index: common abbreviations used

1. Groups ABCD stand for:

A: pET22b

B: endo

C: endo w/ overhang

D: YebF

7/11 (Fri)

Participants: Ariel, Amy, Bokari

1. Cleaning bench, confirm lab materials.

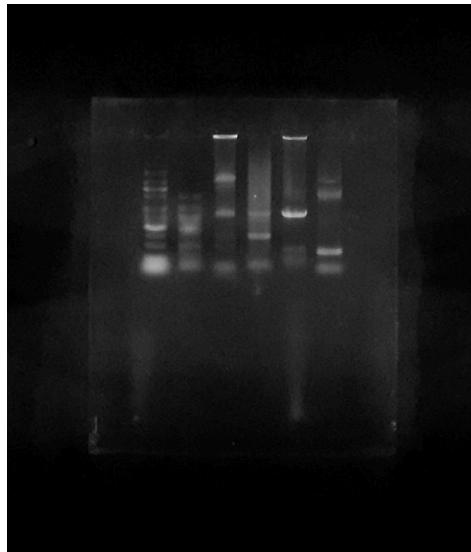
7/14 (Mon)

Participants: Ariel, Amy, Bokari, Gary, Tai

1. Miniprep
2. PCR Endoglucanase and YebF DNA template.
3. Gel electrophoresis pET22b x2 (from miniprep), endo, YebF
4. Test Optical density (OD)

	ng/pmol	260/280	260/230
pET22b	17.7	1.87	1.64
Endoglucanase	20.3	1.81	2.45
YebF	17.6	1.86	2.63

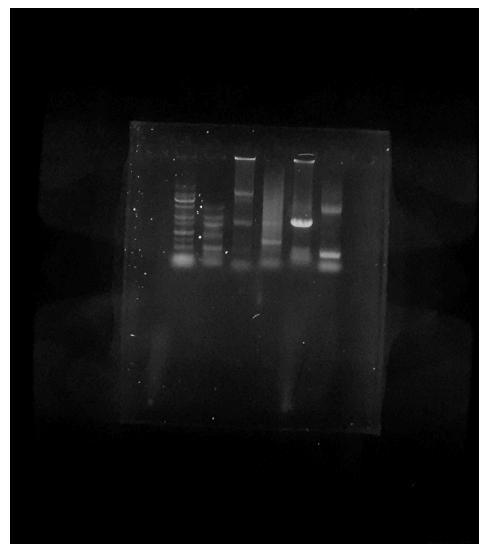
5. PCR Tm 62: (Groups from left to right: 1kb ladder, 100bp, pET22b, endo, endo w/ overhang, YebF)



7/15 (Tue)

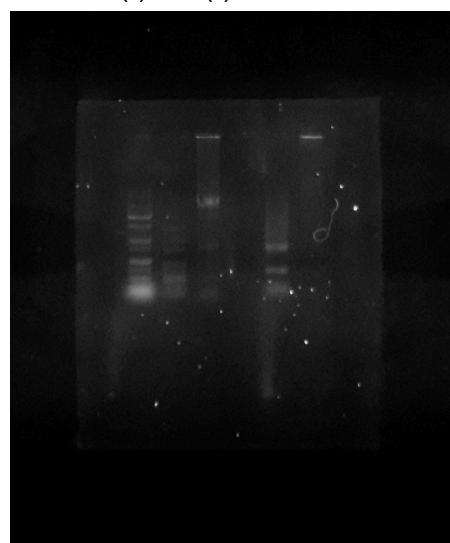
Participants: Ariel, Gary, Bokari

1. Gel electrophoresis 7/14 Tm 62



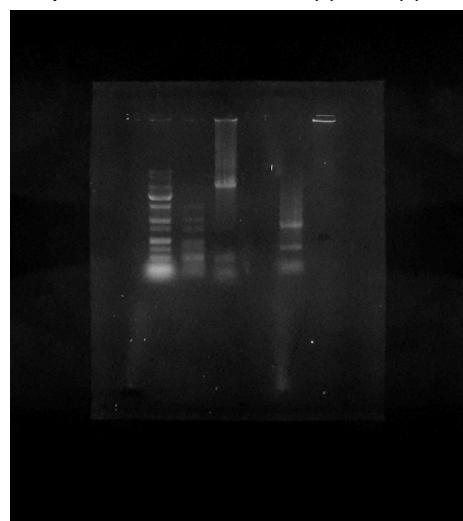
a.

2. PCR Tm 63 A A(-) B B(-)



a.

3. Gel electrophoresis Tm 65 A A(-) B B(-)



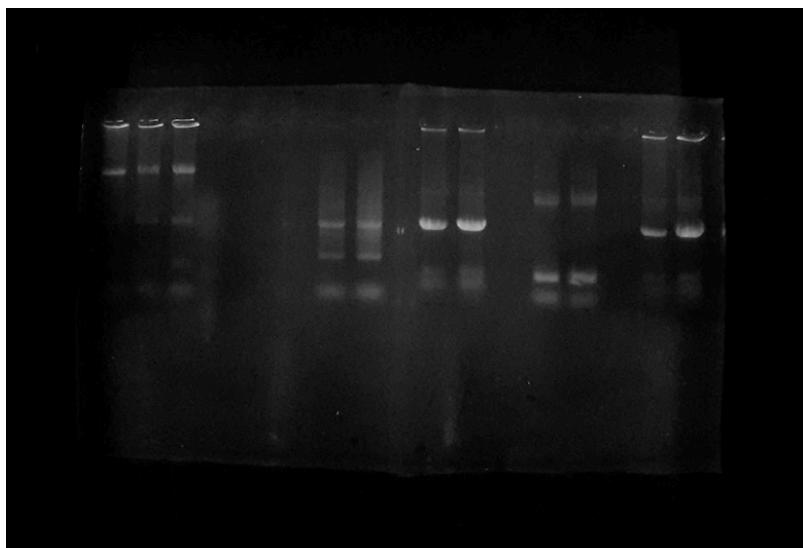
a.

4. PCR Tm63 AB, Tm65 AB

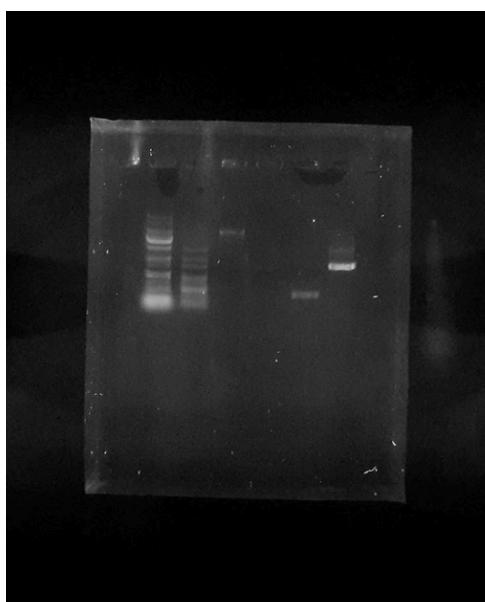
7/16 (Wed)

Participants: Ariel, Amy, Gary, Bokari

1. Gel extraction: using 7/15 Tm63, 65 both groups to fill the wells.



2. After Gel Extraction Gel electrophoresis

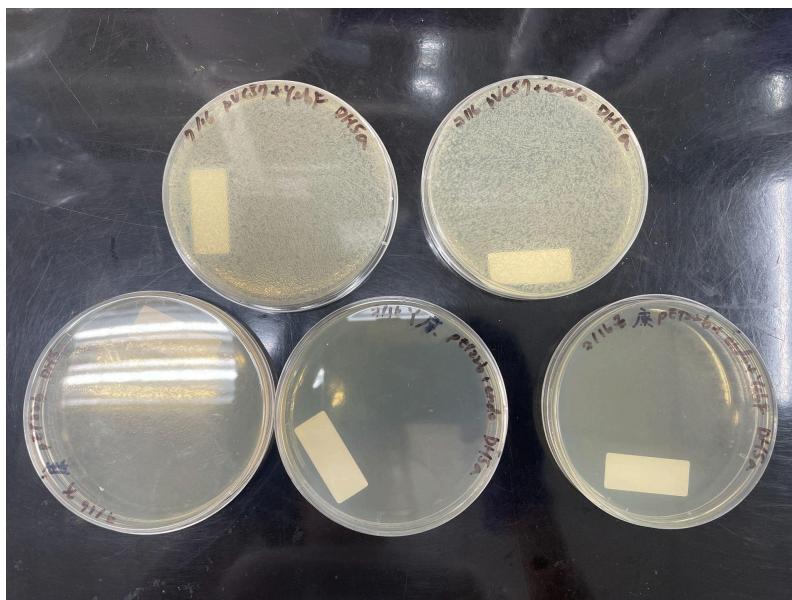


3. Gibson assembly
4. Transformation DH5a, bacterial culture

7/17 (Thu)

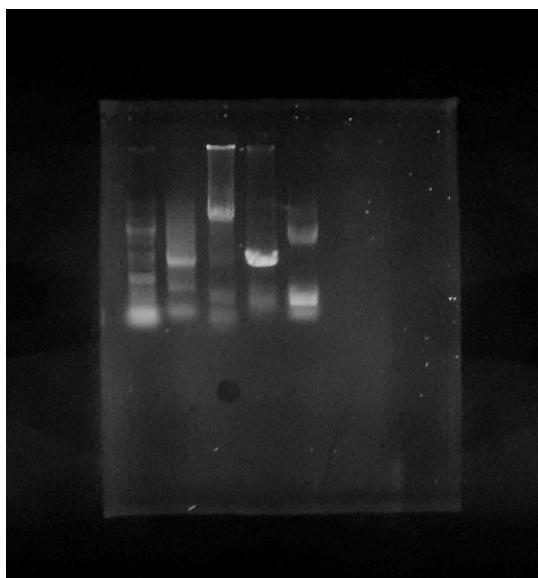
Participants: Ariel

No Colony Shown for experimental groups, positive control groups show clear colonies.



PCR Tm63:

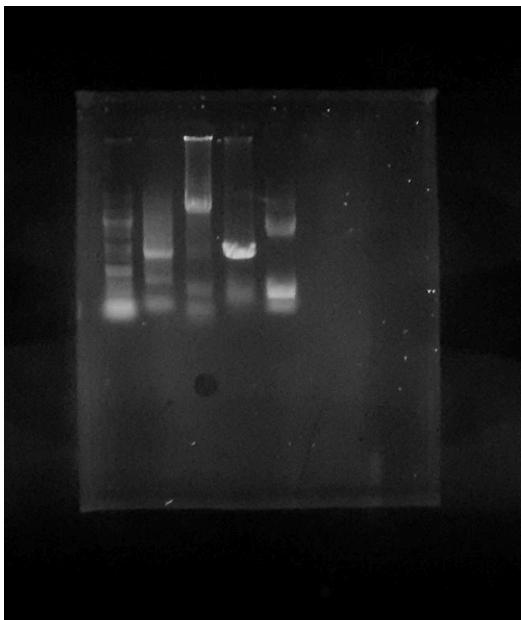
1. pET22b
2. endo
3. endo w/ overhang
4. YebF



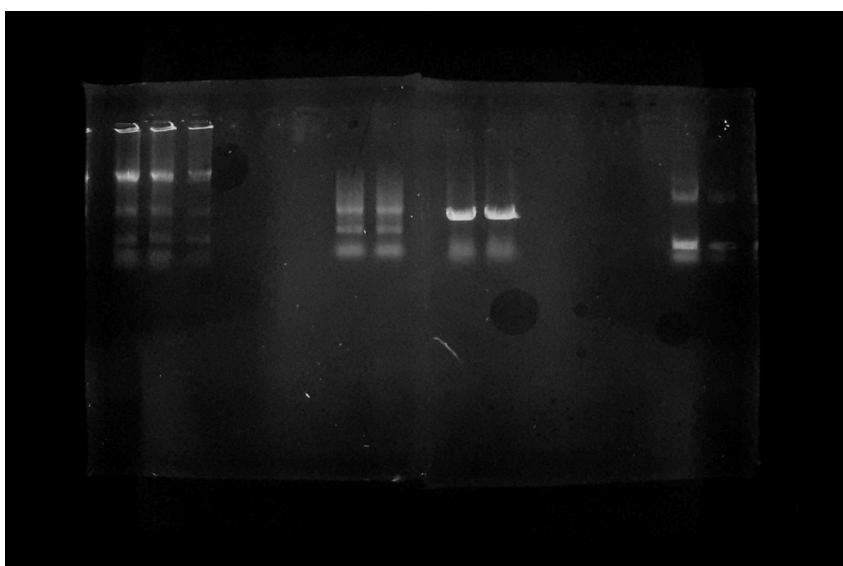
7/21(Mon)

Participants: Ariel, Gary, Bokari

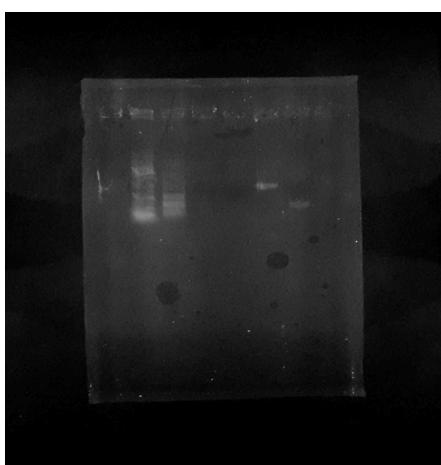
PCR 7/21 Tm65 ABCD



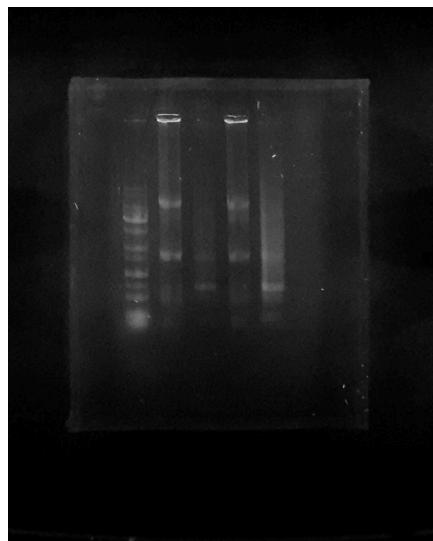
Gel Extraction 7/17 Tm63+65 ABCD



After Gel electrophoresis



Gel electrophoresis: 7/15 Tm63 AB Tm 65 AB



redo PCR, 7/16 Gel extraction products as template

- A: Hifi Kapa
- B: Hifi Kapa
- C: Hifi Kapa
- D: Hifi Kapa

E(pET22b): Q5 polymerase —> though it's also a proofreading enzyme (similar to Kapa Hifi Kit), we tried it and hoped for a better result in Gel extraction due to its large base pair.

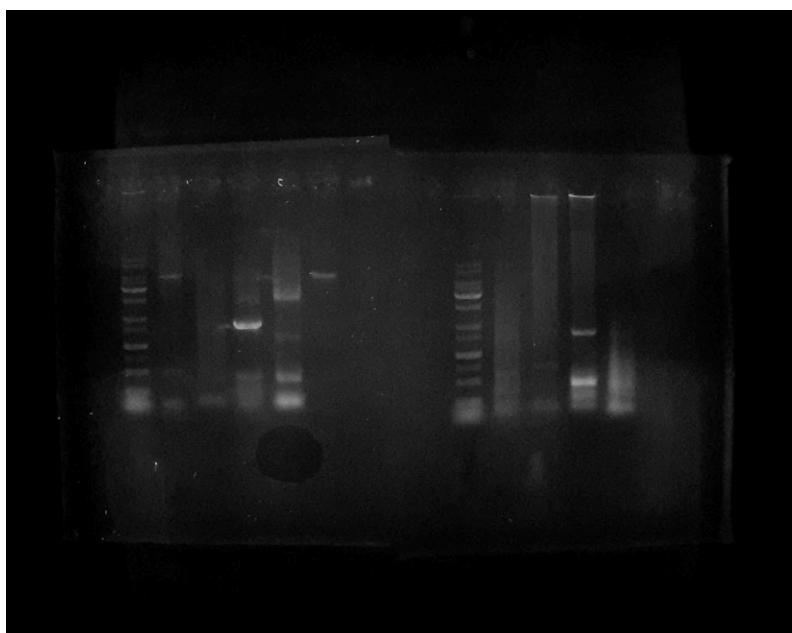
Gel 1 (template whole plasmid): 1kp, A, B, C, D, E(Q5 pET22b)

Gel 2(template gel extraction products) : 1kp, A, B, C, D, E(Q5 pET22b)

7/23(三)

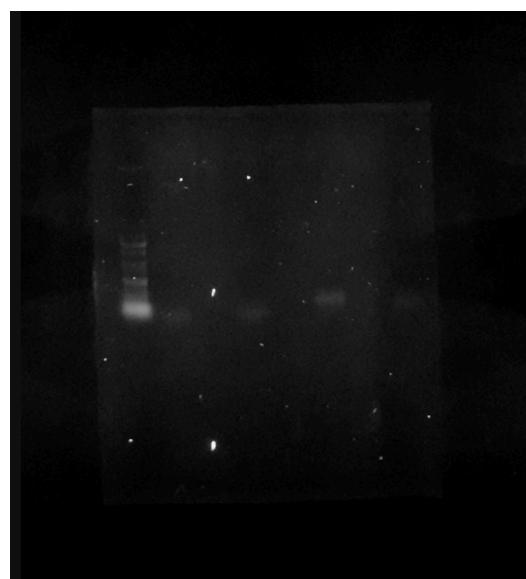
Participants: Ariel, Amy, Vina

1. Gel: 7/21 PCR



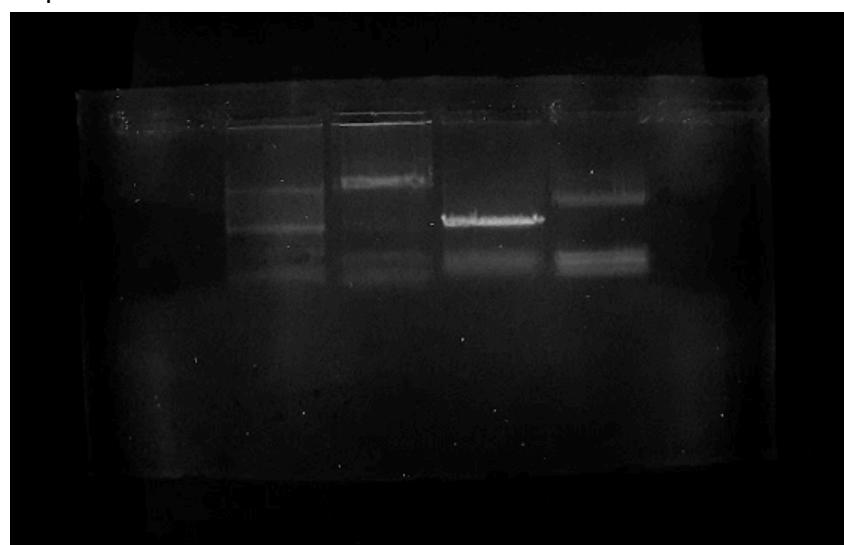
2. PCR: Try changing into the Q5 enzyme

Groups	enzyme	template
pET22b	Q5 DNA polymerase	pET22b
endo	Q5 DNA polymerase	*endo w/ overhang
endo w/ overhang	Q5 DNA polymerase	endo w/ overhang
YebF	Q5 DNA polymerase	YebF



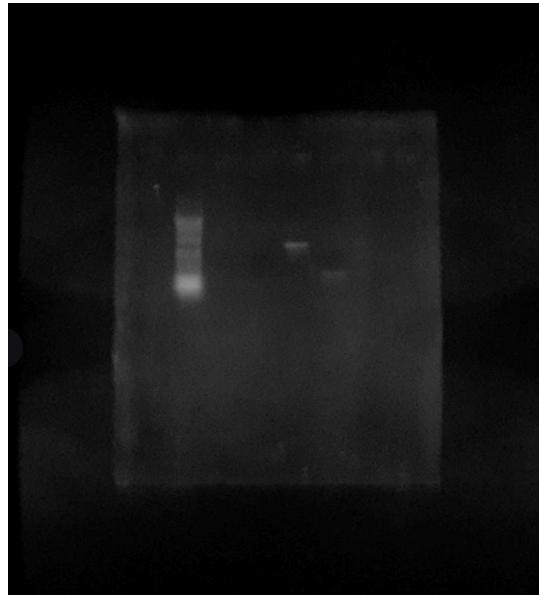
1.

3. 7/21 PCR products Gel extraction



1.

4. After Gel Extraction



5.

7/24 (Thu)

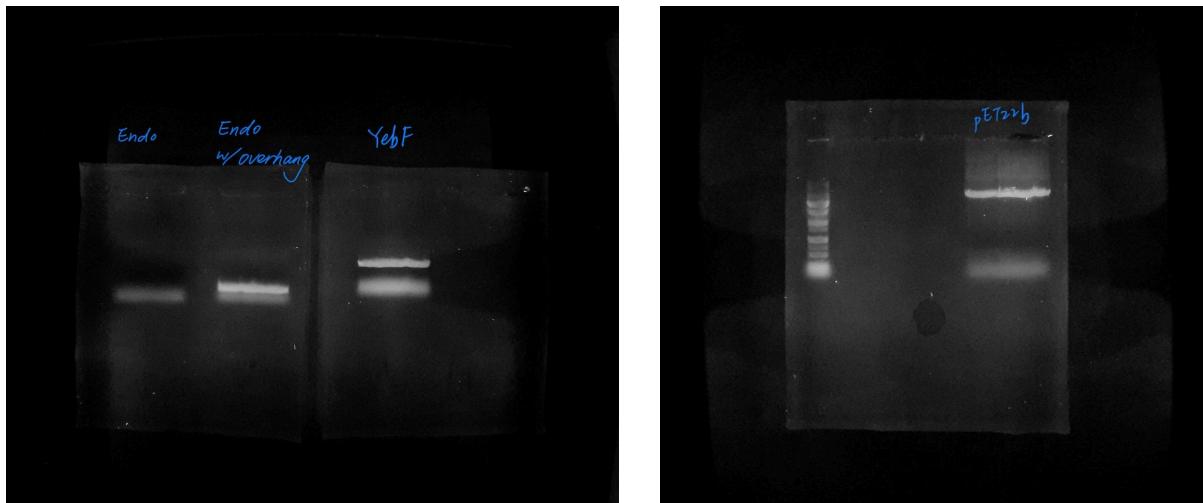
Participants: Ariel

PCR: changing PCR time & sticking to Q5 enzyme (shown on graph below)

PCR ver. 2 (excluding pET22b)		
Temp	Time	Notes
98°C	3 min	Raise the temp for Q5 enzyme
98°C	20 sec	
65°C	15 sec	
72°C	2 min	Lower time, separate groups BCD due to their shorter base pair.
72°C	6 min	
16°C	$\infty$	

Groups	enzyme	template
pET22b	Q5 DNA polymerase	pET22b
endo	Q5 DNA polymerase	endo w/ overhang
endo w/ overhang	Q5 DNA polymerase	endo
YebF	Q5 DNA polymerase	YebF

\*Shown on gel below



\* Products were kept for gel extraction for pET22b, Endo w/ overhang, YebF

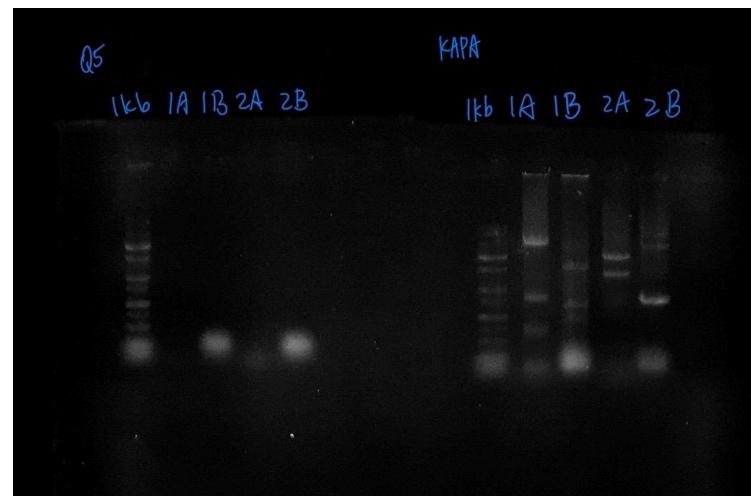
7/25 (Fri)

Participants: Ariel

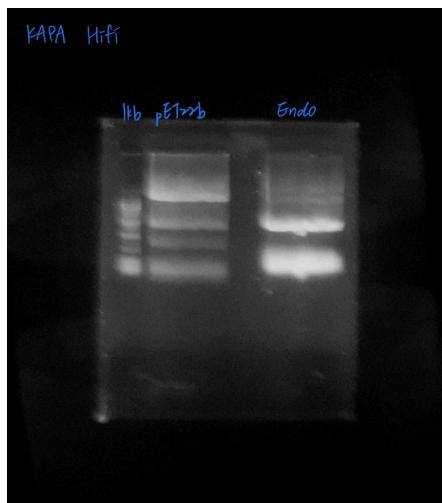
PCR: Change template, use 2 types of enzymes

PCR groups (Shown on graph below)		
Q5 enzyme	(1) pET22b template	(2) endo template
(A) pET22b primer	1A	2A
(B) endo primer	1B	2B

PCR groups (Shown on graph below)		
KAPA Hifi enzyme	(1) pET22b template	(2) endo template
(A) pET22b primer	1A	2A
(B) endo primer	1B	2B



Gel Extraction KAPA 1A, 2B



\* The results showed that endo has two clear bands. We extracted both of them and named them:

1. endo upper band
2. lower endo band

More is shown in the next step.

After gel extraction (Gel electrophoresis)



\* All results clear, move to Gibson Assembly next Monday.

7/28 (Mon)

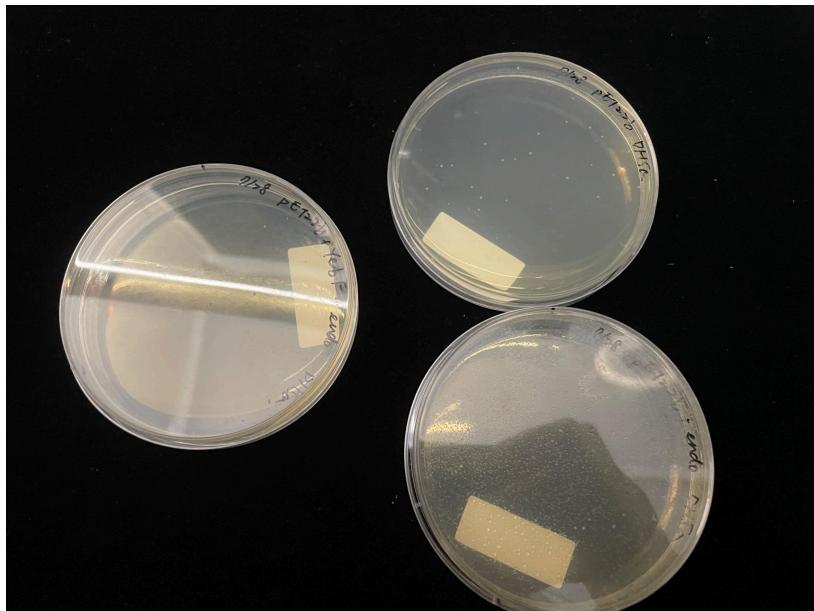
Participants: Ariel

1. Gibson Assembly
  - a. X: pET22b
  - b. Y: pET22b + endo
  - c. Z: pET22b + endo + YebF
2. Transformation DH5a
3. PCR pET22b

7/29 (Tue)

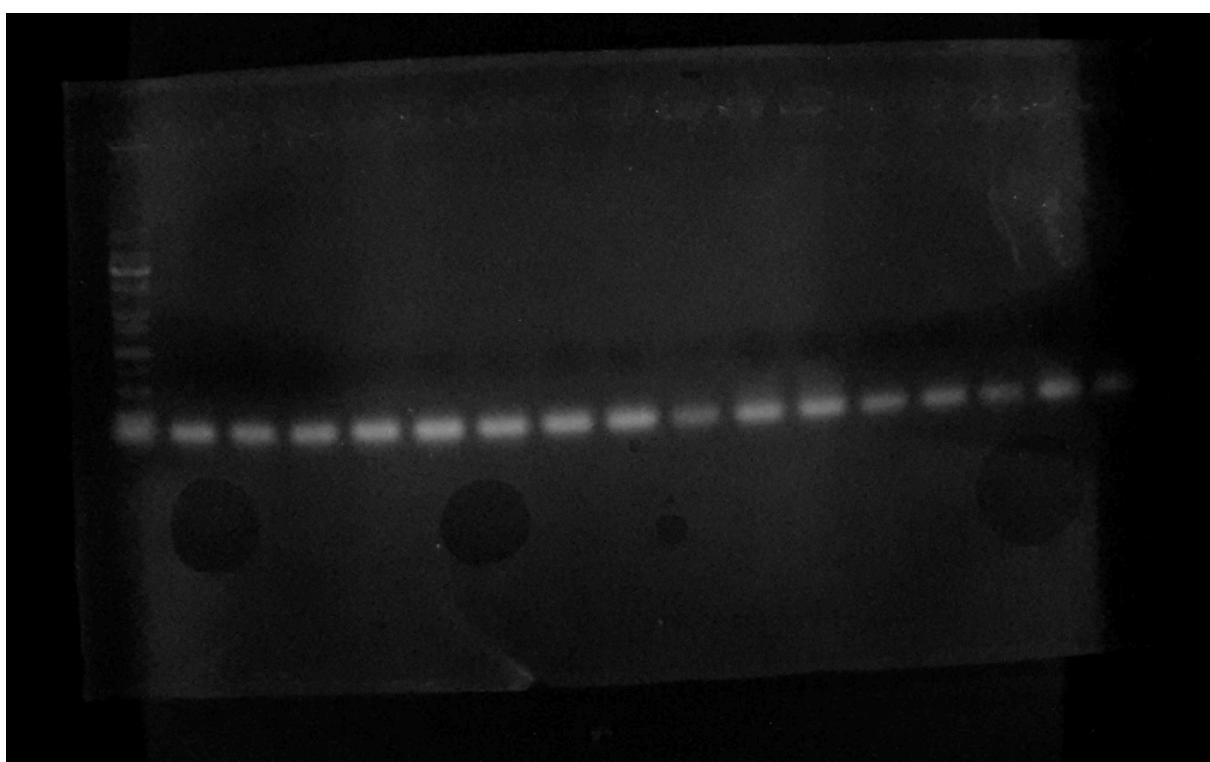
Participants: Ariel

1. Check colony conditions



2. Colony PCR

- a. pET22b + endo
  - i. F: pET22b-F
  - ii. R: endo-R
- b. pET22b+endo+YebF
  - i. F: pET22b-F
  - ii. R: endo-R



3. Gel electrophoresis

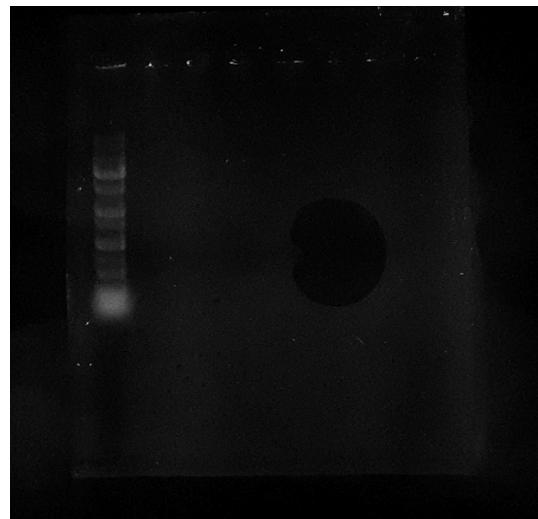
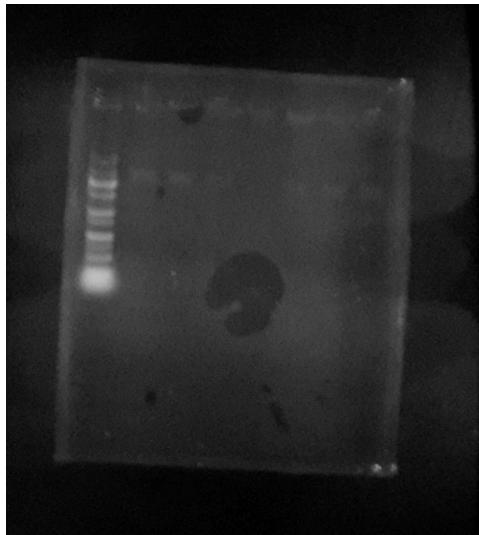
- a. 7/28 PCR pET22b

4. Bacterial Liquid, prepare for miniprep

7/30 (Wed)

Participants: Ariel

1. Miniprep X, Y, Z
2. Gel electrophoresis Y, Z (after miniprep)



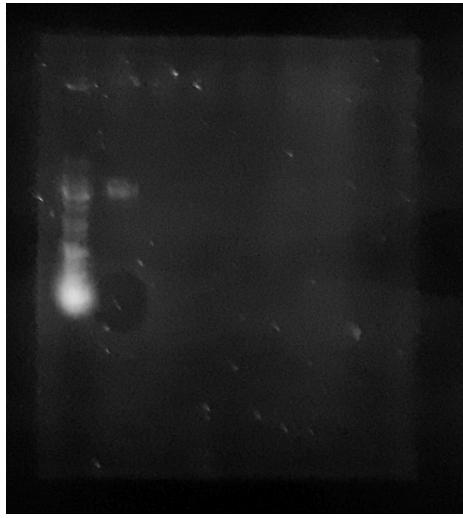
7/31(Thu)

Participants: Ariel

1. Miniprep Z
2. Gel electrophoresis Group Z



3. Gel electrophoresis Group Y

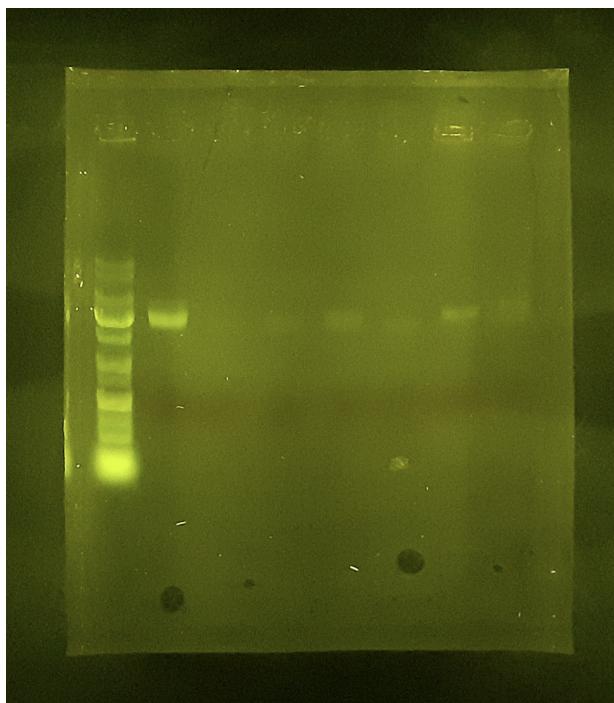


4. Bacterial Culture Group Y, bigger colonies

8/4 (Mon)

Participants: Ariel

1. Miniprep Group Y bigger colonies, Gel electrophoresis

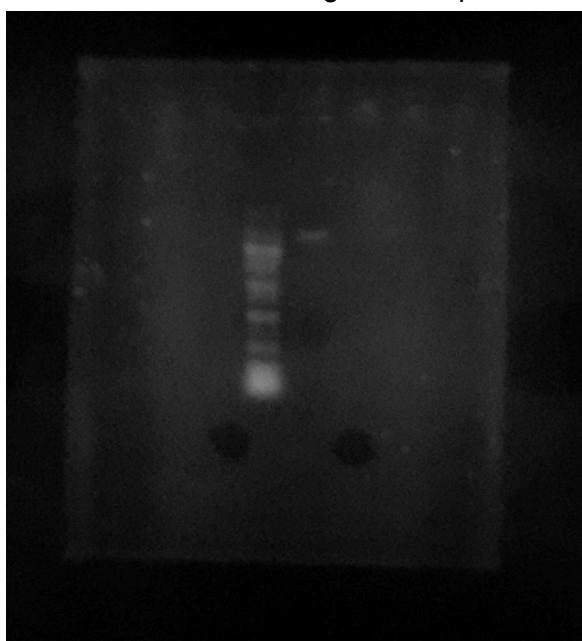


2. Prep Gel for A (Tmr gel extraction)

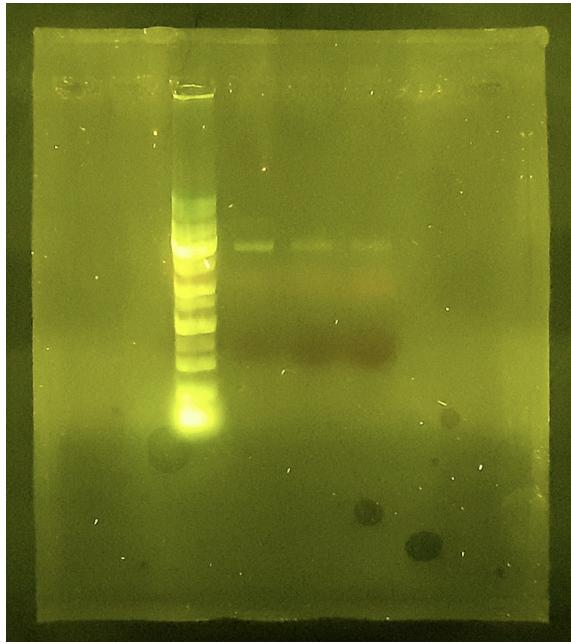
8/5 (Tue)

Participants: Ariel

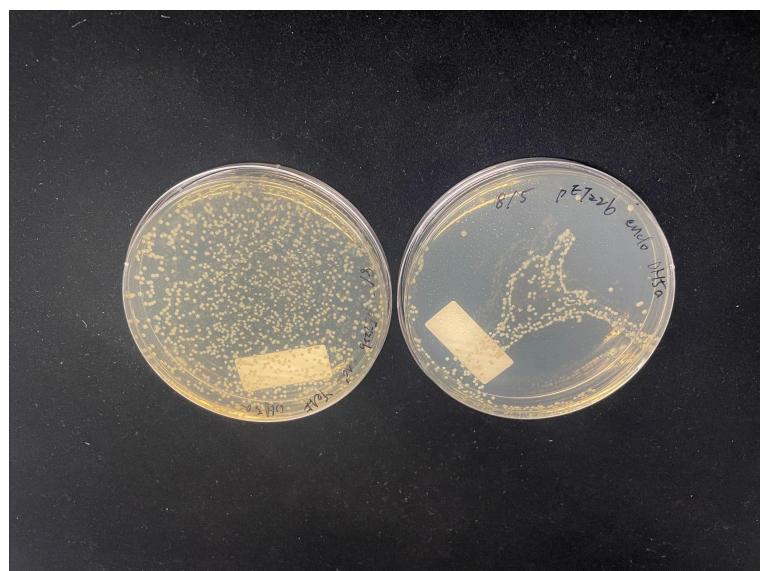
1. PCR A, B (prep for 2nd time gibson)
2. Gel extraction A, gel electrophoresis



3. Gel electrophoresis miniprep Group Y bigger colonies, longer time



4. Gibson assembly
  - a. 1.5 hours, 50 degrees (ok)
  - b. overnight in 37 degrees (ok)
5. Transformation in DH5a

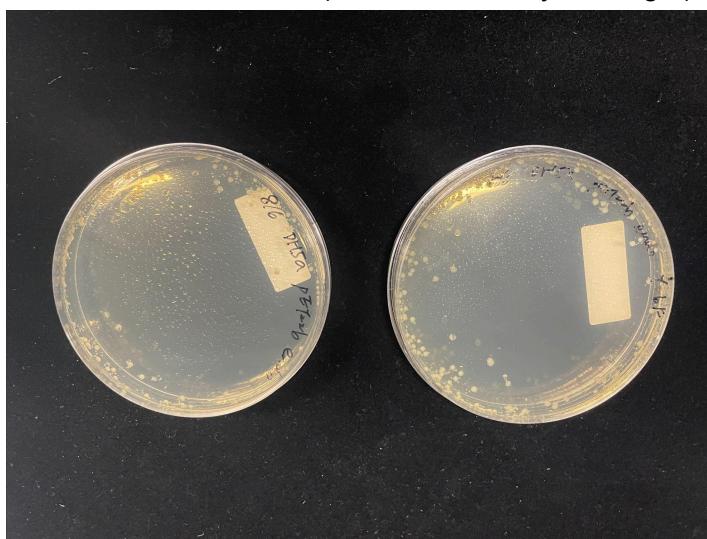


6.

8/6 (Wed)

Participants: Ariel, Bokari

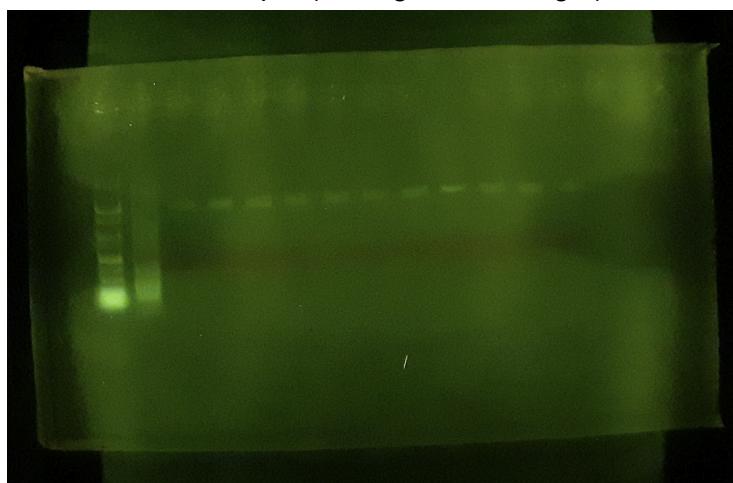
1. Bacterial culture in liquid (prep for miniprep)
2. Transformation in DH5a (Gibson assembly overnight)



8/7 (Thu)

Participants: Ariel

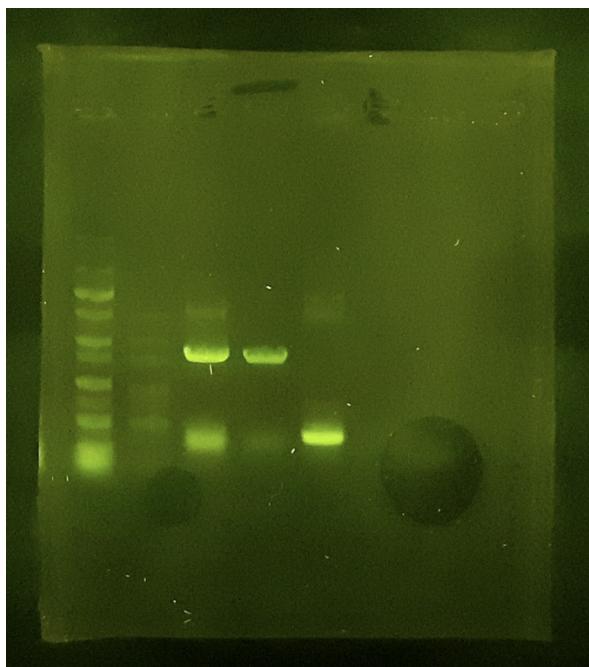
1. miniprep DH5a
  - a. Group 1 (37 degrees overnight)



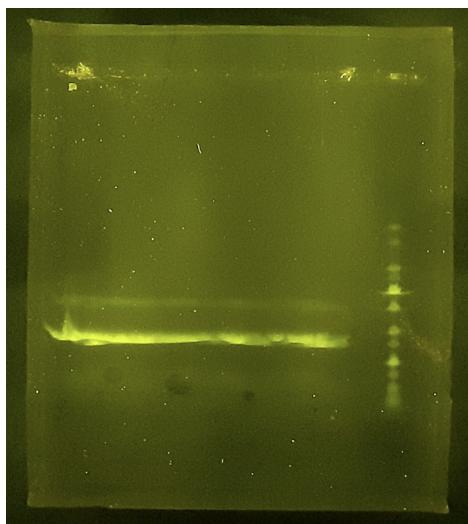
8/11(Mon)

Participants: Ariel, Amy, Bokari

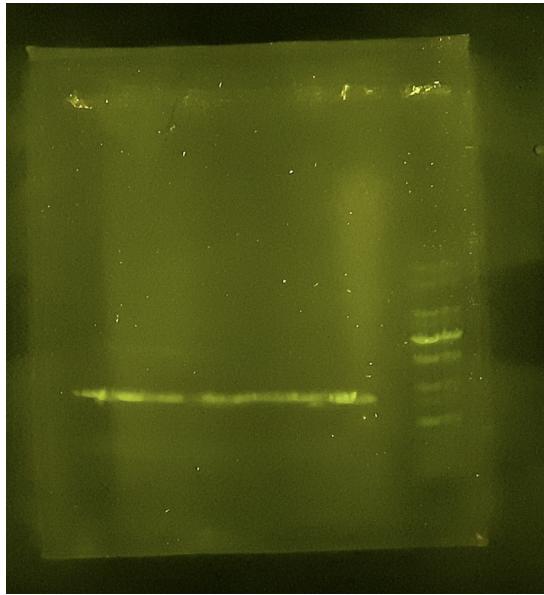
1. PCR
  - a. B, C, D
2. Gel ok (1kb, 100bp, B, C, D)



3. miniprep pET22b(+)
  - a. Gel (x)
4. prep bacterial liquid for tmr miniprep
5. Gel extraction
  - a. Group B



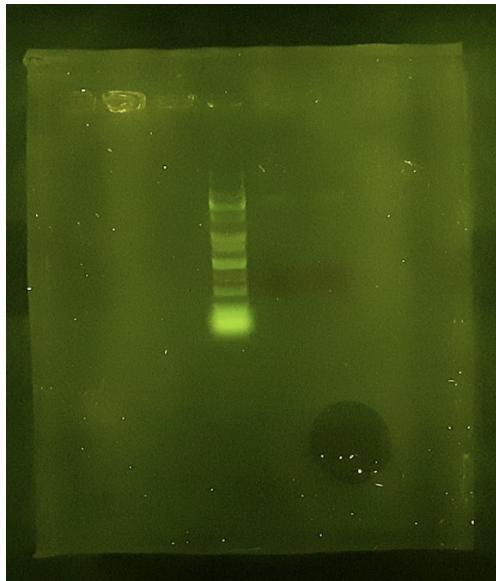
b. Group C



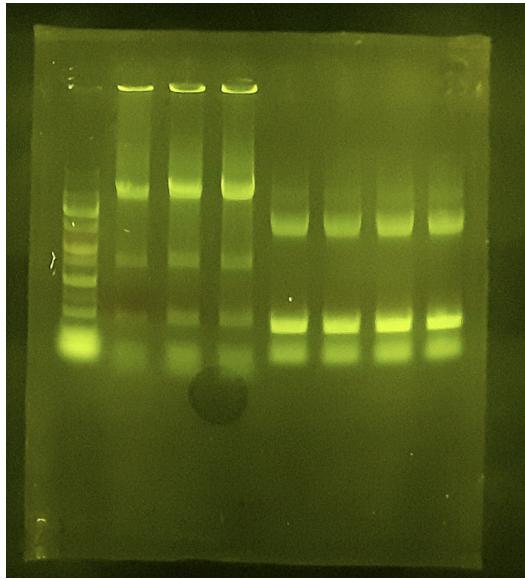
8/12 (Tue)

Participants: Ariel, Amy

1. miniprep A



2. PCR A, D

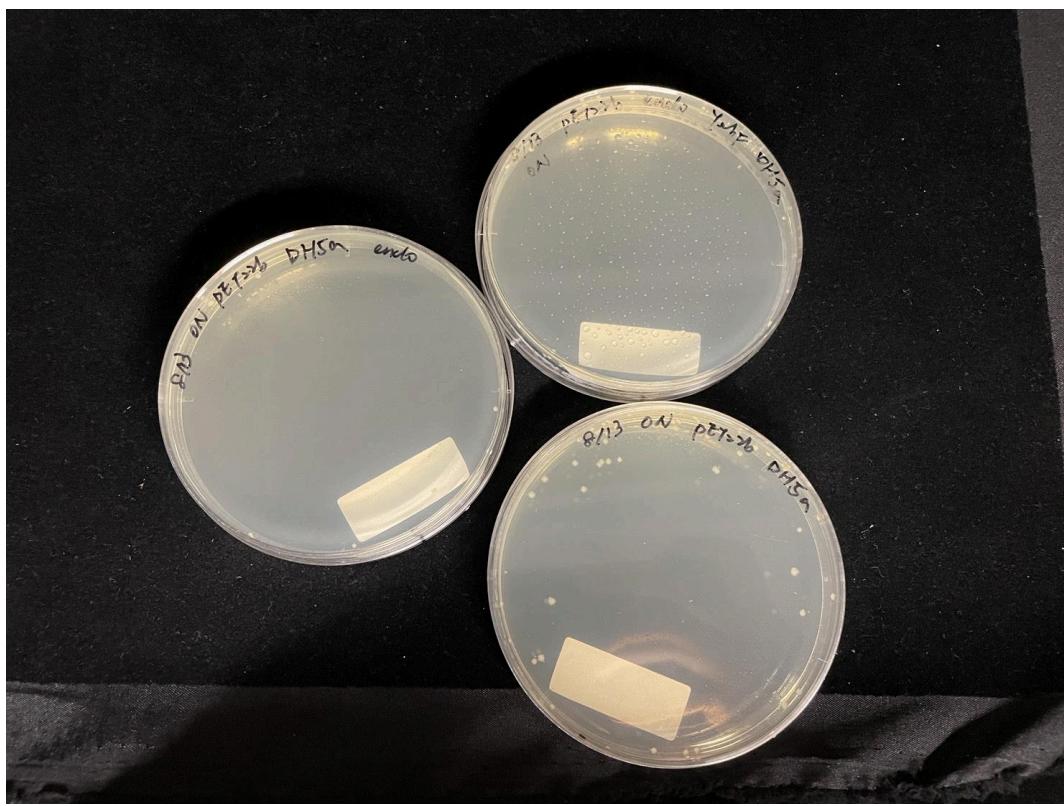
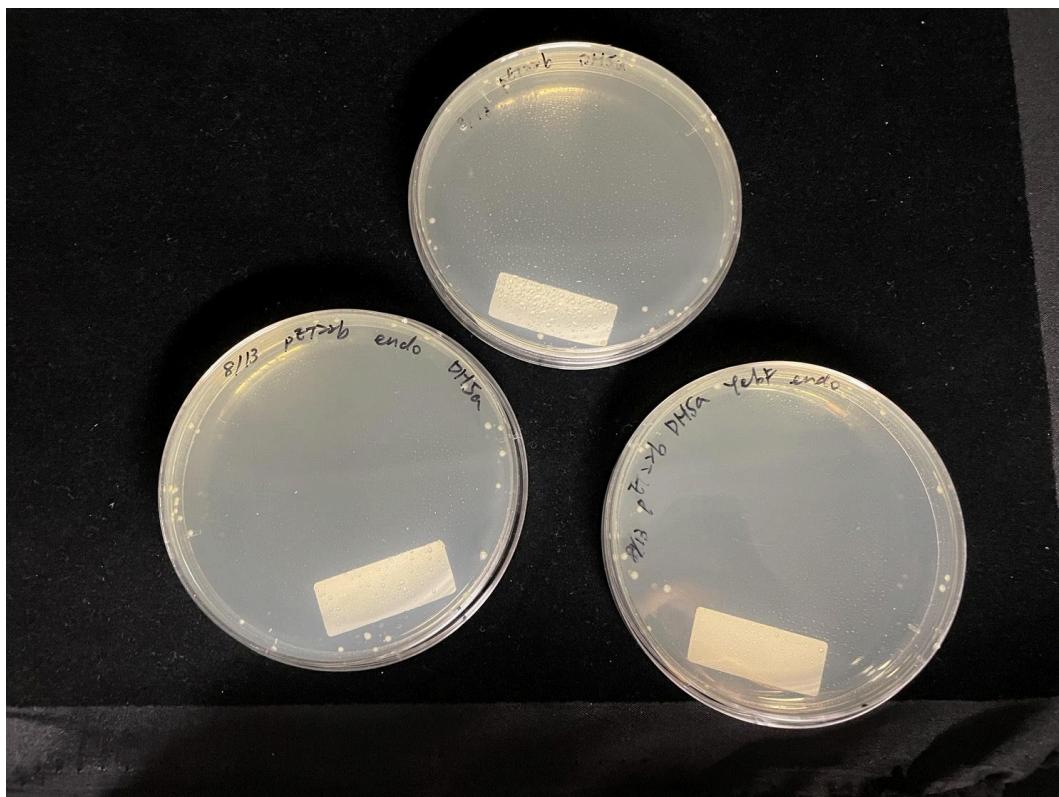


3. gel extraction A, D
4. Gibson assembly
  - a. 1H incubation
  - b. overnight incubation

8/13(Wed)

Participants: Ariel, Amy, Bokari

1. transformation DH5a, plate
  - a. Group 1: pET22b 1H
  - b. Group 2: pET22b endo 1H
  - c. Group 3: pET22b endo YebF 1H
  - d. Group 5: pET22b overnight
  - e. Group 6: pET22b endo overnight
  - f. Group 7: pET22b endo YebF overnight



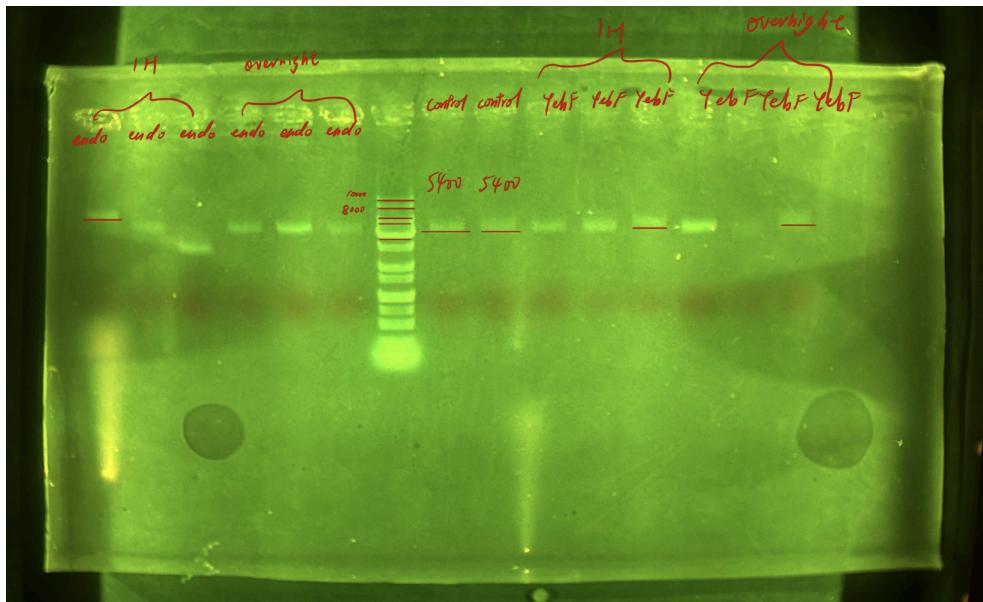
8/14(Thu)

Participants: Ariel, Amy

1. bacterial liquid
  - a. All 3 tubes (only endo, endo YebF → 12 tubes)

8/15(Fri)

Participants: Ariel, Amy



We choose colonies with visibly a larger band and send them to sequencing

\*Low concentration of DNA

8/16(Sat)

The results of sequencing are shown below:



訂單編號: SD250815106

樣品名稱	處理方式
在re-Seq資料夾中的樣品	隨機挑選部分樣品重新調整條件再次定序， 預計需時1-2個工作天

經辦人員: 陳映先 分機: 136

定序分析代碼代表解釋		
定序結果	可能原因	建議事項
(1)沒有訊號	(1-1)DNA濃度不足 (1-2)Primer濃度標示錯誤 (1-3)沒有Primer粘合位點 (1-4)clone有問題	(1-1a) 提高DNA濃度 (1-2a) 檢視primer濃度 (1-3a) 檢視所使用Primer的正確性 (1-4a) 請檢視clone有無選殖成功 (1-4b) 請檢視載體是否修改過
(2)訊號雜亂	(2-1) Primer專一度不佳 (2-2) DNA濃度不足 (2-3) Primer degraded (N-1,N-2..) (2-4) Multiple templates (2-5) Multiple priming sites (2-6) Frameshift mutation (SNP, insertion, deletion) (2-7) 樣品品質不佳(poor quality DNA) (2-8) 樣品smear	(2-1a) 重新設計Primer(最適Tm=50°C~55°C)或更換Primer (2-2a) 提高DNA濃度 (2-3a) 確認primer保存方式或更換使用fresh primer (2-4a) 非單一產物請重新純化分離單一template (2-4b) 請重新純化單一菌落 (2-5a) 更換其他具有單一黏合位點的primer (2-7a) 請完全去除定序反應中的抑制劑(salt,phenol,EDTA..) (2-8a) 請重新製備樣品
(3)訊號提前中斷	(3-1) Homopoly(T),(A),(G),(C) (3-2) Secondary structure (Repeat sequence, hair pin, siRNA..)	(3-1a) 請更換另一端primer (3-1b) 避開重複序列重新設計Primer (3-2a) 請更換另一端primer (3-2b) 使用二級結構試劑(dGTP, DMSO..)

9/4(Wed)

There's still hope(?)

change to TA cloning

## PCR

Enzymes: 0.8 Q5, 0.2 Taq polymerase

5x Q5 buffer 20 microliter

1 dNTP

