AUGUST

WEEK 2

08/08/21

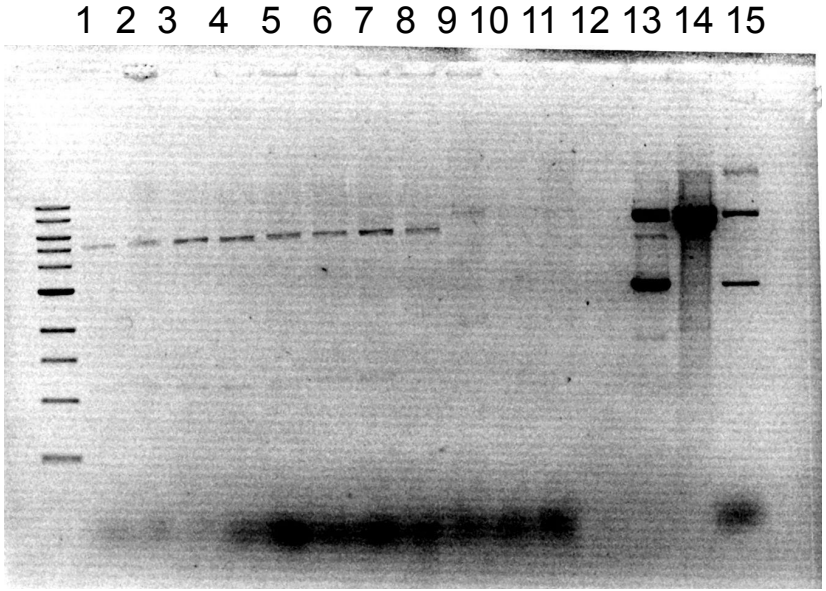
Plasmid Isolation of BC2 transformed colonies

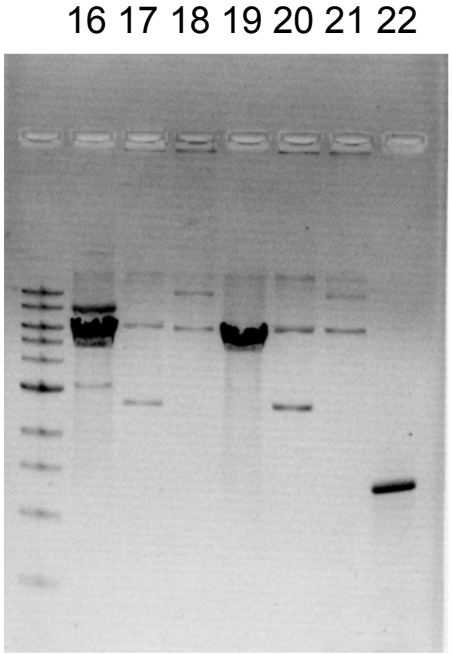
Nanodrop Result

Sample	Concentration (ng/uL)	A260/A280	A260/A230
Cloned BC2 (A)	92.4	1	0.96
Cloned BC2 (B)	15.6	1.83	1.83

Digestion Confirmation of cloned BC1 & BC2

08/08/21





1-8 : BC2 (DH5a) , DD
9-12 : BC1 (BL21DE3),DD
13 : BC1(2,1), MN kit (DD)
14 : pET28a (DD)
15 : BC1(2,1) , Alk. lysis(DD)
16 : pET28a (SD), BamHI
17 : BC1, Alk.lysis(SD),BamHI
18 : BC2, Alk.lysis(SD),BamHI
19 : pET28a(SD), HindIII
20 : BC1, Alk.lysis(SD), HindIII
21 : BC2, Alk.lysis(SD), HindIII
22 : PCR purified product of BC2

Result: Both BC1& BC2 are cloned successfully. Go for sequencing

9/08/21

Sequencing of BC1 & BC2

- Sanger PCR
- Sequencing Clean up
- Sanger sequencing

9/08/21

Sanger PCR

Sequencing buffer	1.75uL
Big Dye	0.5uL
Primer T7 F(10uM)	1uL
Template	200-400 ng
Water	Calculated
Total	10uL

Sequencing Result

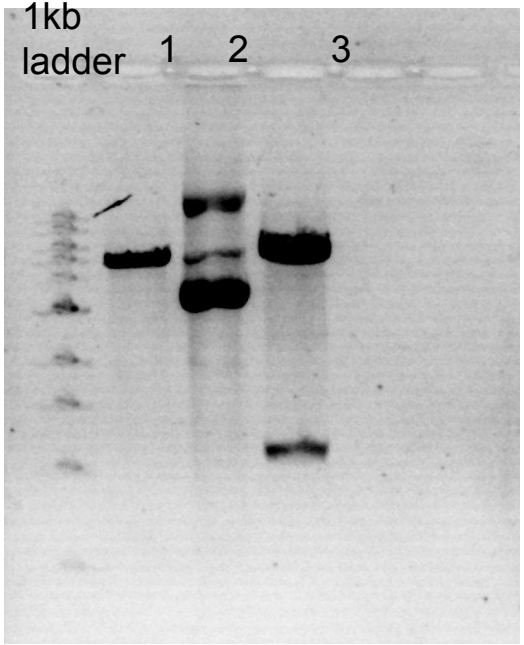
Sample File Name	BC Status	PP Status	Well	Cap #	Peak 1	Base Spacing	# Low QV	# Med QV	# High QV	Sample Score	LOR	'A' S/N	'C' S/N	'G' S/N	'T' S/N	Avg S/N	CR Start	CR Stop
D12_1_04	◆	N/A	D12	4	1132	9.93	121	62	261	24	398	6	7	7	6	7	N/A	N/A
E12_2_05	△	N/A	E12	5	1631	−13.25	5	0	0	0	0	4	4	3	4	4	N/A	N/A
F12_3_06	◆	N/A	F12	6	970	8.59	241	44	799	33	857	16	15	20	13	16	N/A	N/A
G12_4_07	△	N/A	G12	7	1631	−13.25	5	0	0	0	0	4	4	4	4	4	N/A	N/A

Legend: Success ■ Success with warnings ◆ Failed Analysis △
System Error ⬢

Result: BC2 is cloned successfully, BC1 is not cloned

11/08/21

Digestion confirmation of BC2 cloned plasmid

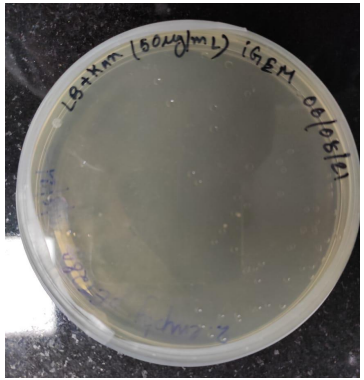


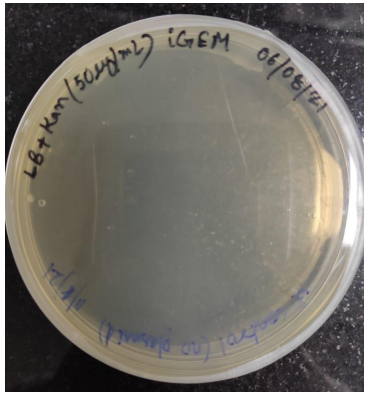
Annotations
1: Double digested vector using BamH1 HF and HindIII HF
2: Undigested vector
3: Double digested cloned BC2 using BamH1 HF and HindIII HF

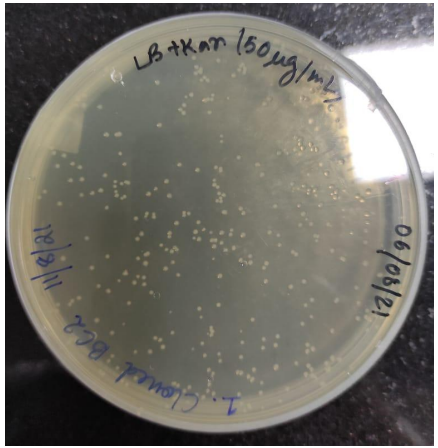
Result: BC2 is successfully cloned

Transformation of cloned BC2 in BL21

11/08/21







#	sample	No. of colonies
1	Cloned BC2 in BL21 cells	35-40
2	Digested vector in BL21 cells	2
4	BL21 cells (negative control)	0