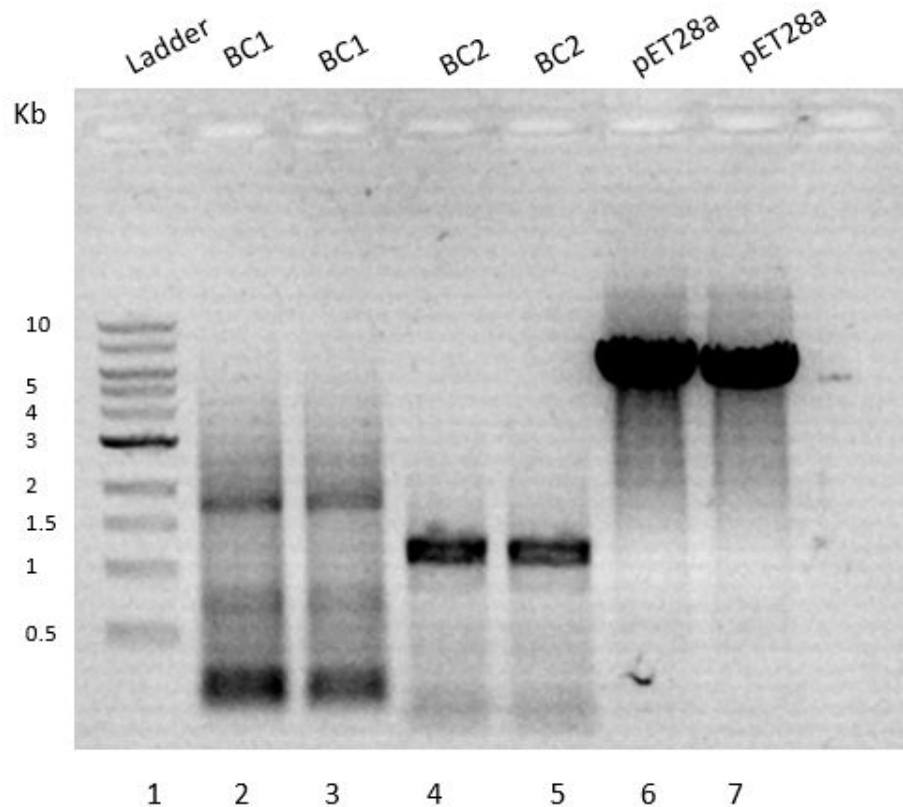


AUGUST

WEEK 1

Restriction digestion of BC1, BC2 & pet28a

02/08/21



Annotations:

BC1 (DD): BC1 Double Digested

BC2 (DD): BC2 Double Digested

Ladder: 1 kb NEB Ladder

02/08/21

Gel Elution of digested BC1, BC2 & pet28a

Sample	Concentration (ng/uL)	A260/A280	A260/A230
BC1 (DD)	6.3	1.59	0.96
BC2 (DD)	15.6	1.83	1.83
pET28a (DD)	79.7	1.85	1.83

Nanodrop Result

DD: Double digested

Ligation and Transformation of BC1 and BC2 in DH5 α

Calculation:

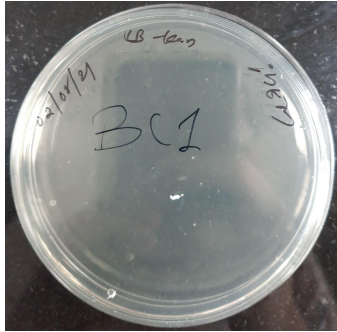
50 ng of Vector

51.03 ng of BC1 (1:3) \rightarrow 8.1 ul

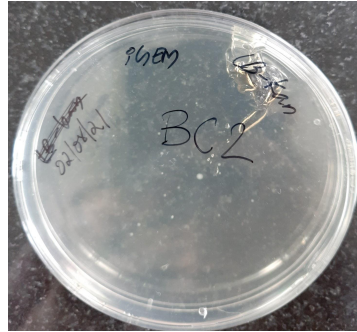
32.59 ng of BC2 (1:3) \rightarrow 2.09 ul

Per Reaction	
T4 DNA Ligase	1 ul
10X buffer	2 ul
Vector	50ng (0.63uL)
Gene	Calculate according to ligation calculator
Water	Upto 20ul

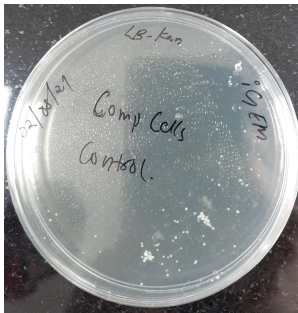
Transformation Result



BC1



BC2



CONTROL

#	sample	No. of colonies
1	BC1 in competent cell	6-10
2	BC2 in competent cell	4-6
3	Digested vector in competent cells	6-10

4-08-2021

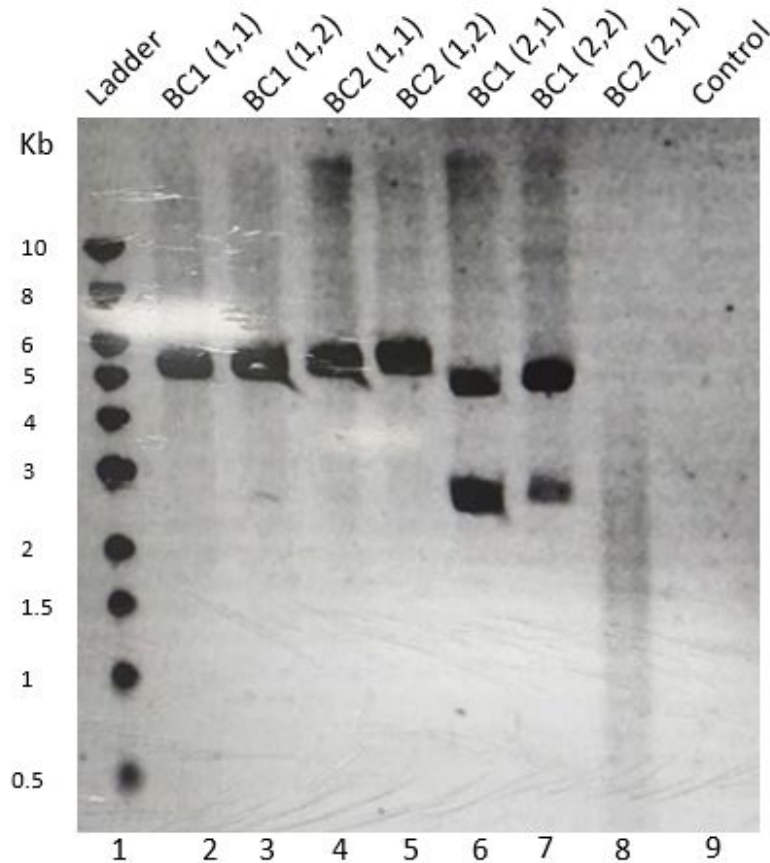
Plasmid Isolation

Sample	LB	Antibiotic (Stock conc.)	Antibiotic (Working conc.)	Observation after 13 hrs
BC1(1,1)	6mL	100 mg/mL Kan	50 ug/mL	turbid
BC1(1,2)	6mL	100 mg/mL Kan	50 ug/mL	turbid
BC1(2,1)	6mL	100 mg/mL Kan	50 ug/mL	turbid
BC1(2,2)	6mL	100mg/mL Kan	50 ug/mL	turbid
BC2(1,1)	6mL	100mg/mL Kan	50 ug/mL	turbid
BC2(1,2)	6mL	100mg/mL Kan	50 ug/mL	turbid
BC2(2,1)	6mL	100mg/mL Kan	50 ug/mL	turbid
BC2(2,2)	6mL	100mg/mL Kan	50 ug/mL	No turbidity
Control	6mL	100mg/mL Kan	50 ug/mL	turbid

Nanodrop Result

	ng/uL	A(260/280)	A(260/230)
BC1 (1,1)	2178.0	2.07	2.32
BC1 (1,2)	1502	2.04	2.22
BC1 (2,1)	3064.3	2.10	2.60
BC1(2,2)	2721.2	2.08	2.34
BC2 (1,1)	2526	2.09	2.46
BC2 (1,2)	1736.2	2.07	2.44
BC2(2,1)	2933.9	2.08	2.34
Control	27.6	1.94	1.27

Restriction Digestion Confirmation & Gel Run



BamH1 HF	0.15ul
HindIII HF	0.15ul
Cutsmart	1ul
Template	2uL
Water	6.7uL
Total	10uL

Annotations:

(1,1): 1st sample from transformation done on 29/07/21

(2,1): 1st sample from transformation done on 02/08/21

Control: pET28a double digested with BamH1 and HindIII HF

Overnight Ligation of digested BC2 & vector at 16°C and Transformation

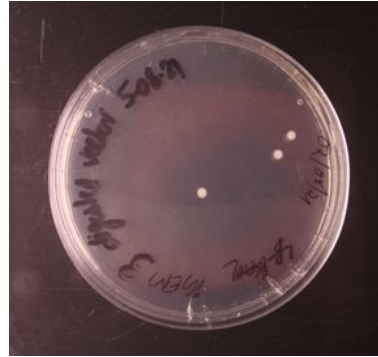
The digested products were kept for overnight ligation at 16°C after which they were transformed into DH5 Alpha and BL21 cells.

Per Reaction	
T4 DNA Ligase	0.5 ul
10X buffer	1 ul
Vector	25ng
Gene	16.29 ng [Calculated according to ligation calculator]
Water	Upto 10ul

Transformation Results



Dh5α + BC2



Dh5α + Digested vector

#	sample	No. of colonies
1	Digested vector in competent cell	2 (+3 contamination)
2	BC1 in competent cell	10-14

Plasmid isolation of BC1 transformed colonies

Nanodrop result :

S.No.	Concentration (ng/uL)	A260/A280	A260/A230
BC1 (A)	150	1.86	2.06
BC1 (B)	96	1.81	1.77