JULY

27/07/21

- Preparation of stock & working solution of primer and combo BC1 & BC2
- PCR of BC1 & BC2
- Gel Run
- PCR Clean up
- Overnight restriction digestion

Stock & working solution of primers and gene fragments BC1 & BC2

Name of Construct	Concentration	
	Primary Vial	Secondary Vial
BC1	10ng/uL	1ng/uL
BC2	10ng/uL	1ng/uL
BC1 FP	100uM	10uM
BC1 RP	100uM	10uM
BC2 FP	100uM	10uM
BC2 RP	100uM	10uM

PCR OF BC1 & BC2

Tm = 50℃

Protocol (for one reaction)

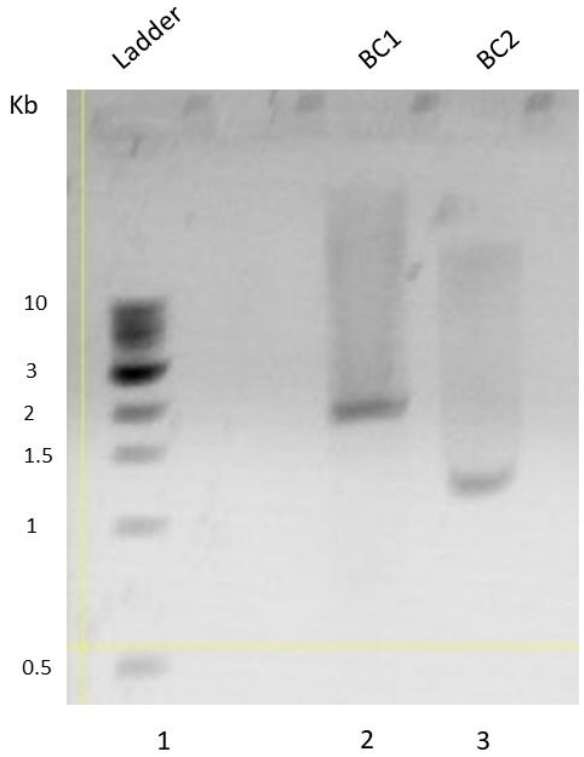
KOD 10X buffer	5 uL
MgSO4 (25mM)	2.5 uL
dNTPs	2.5 uL
KOD Polymerase	0.5 uL
10uM FP	1.5 uL
10uM RP	1.5 uL
Template(10 ng)	1 uL
Water	35.5 uL
Total	50 uL

Reaction Temp:

95℃	3 min	
95℃	20 s	X 35
50℃	20 s	
70℃	**1 min**	
72℃	5 min	
4℃	hold	

50 ul each

Gel run of PCR product



Annotations:
BC1: bacterial Combo 1
BC2: Bacterial Combo 2
Ladder: 1 kb NEB Ladder

Remark: The PCR is successful

PCR CLEAN UP OF BC1 & BC2

Nanodrop result :

S.No.	Concentration (ng/uL)	A260/A280	A260/A230
1	70.3	1.73	0.22
2	61.5	1.94	0.05
1(trial)	47.8	1.66	0.49
2(trial)	30.9	2.2	0.02

Overnight restriction digestion

1ug of :
BC1 = 14.22ul (70.3 ng/ul)
BC2 = 16.26ul (61.5 ng/ul)
Vector = 2.77ul(404.8ng/ul)

Double digestion of pcr purified BC1 & BC2, Vector

BamH1 HF	0.3ul
HindIII HF	0.3ul
Cutsmart	2ul
Template	1 ug
Water	Upto 20ul

Control: Single digestion of vector

BamH1 HF/ HindIII HF	0.3ul
Cutsmart	2ul
Template	1 ug
Water	Upto 20ul

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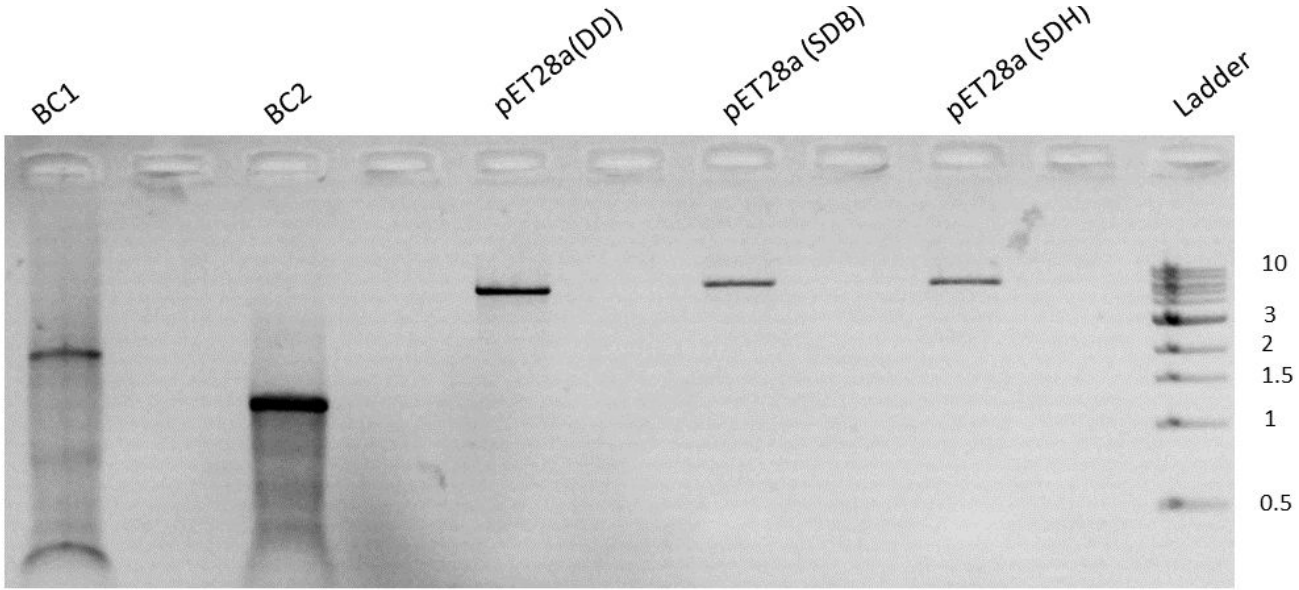
- Gel Run of Digested BC1 & BC2
- PCR of BC1 and BC2
- Restriction Digestion of amplified BC1, BC2 and vector

Gel Run of digested BC1, BC2 & pET28a



Annotations:

DD: Double Digested
SDB: Single Digested with BamH1 HF
SDH: Single Digested with HindIII HF

Conclusion: The size of digested BC1 and BC2 is more. The restriction digestion is not successful. Also, as the conc. of PCR purified product was less its better to do PCR again with Pcr purified product as a template.

PCR using PCR purified template

Protocol (for one reaction)

KOD 10X buffer	5 uL
MgSO4 (25mM)	2.5 uL
dNTPs	2.5 uL
KOD Polymerase	0.5 uL
10uM FP	1.5 uL
10uM RP	1.5 uL
Template(10 ng/uL)	1 uL
Water	35.5 uL
Total	50 uL

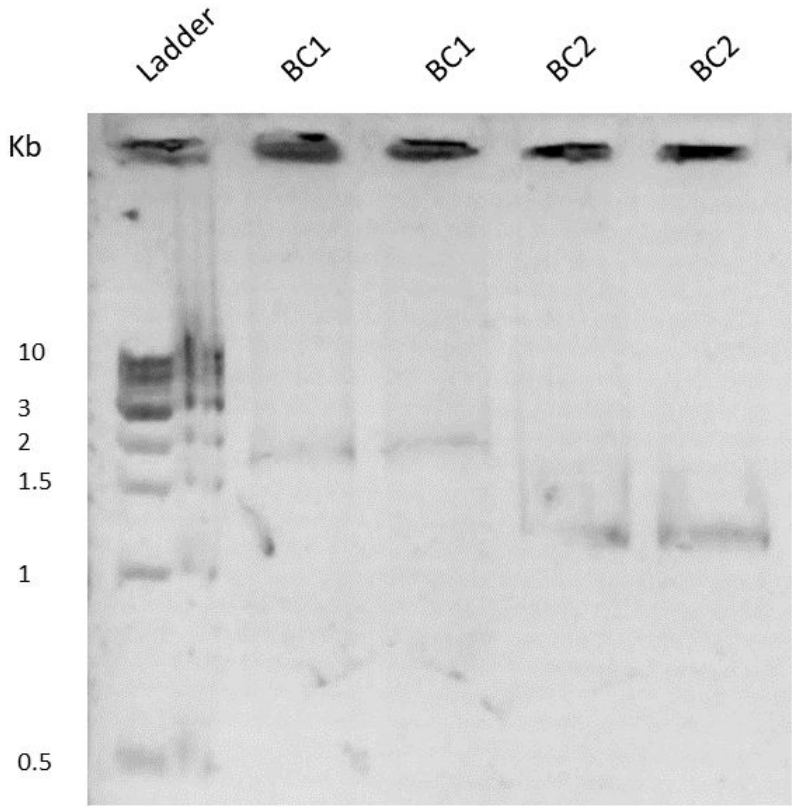
Tm = 50℃

Reaction Temp:

95℃	3 min	
95℃	20 s	X 35
50℃	20 s	
70℃	**1:20 min**	
72℃	5 min	
4℃	hold	

Reaction volume
= 50 ul *2 each

Gel run of PCR product



Annotations:
BC1: bacterial Combo 1
BC2: Bacterial Combo 2
Ladder: 1 kb NEB Ladder

Remark: The PCR is successful

PCR CLEAN UP OF BC1 & BC2

Nanodrop result :

S.No.	Concentration (ng/uL)	A260/A280	A260/A230
BC1	88.1	1.89	0.22
BC2	54.5	1.86	0.22

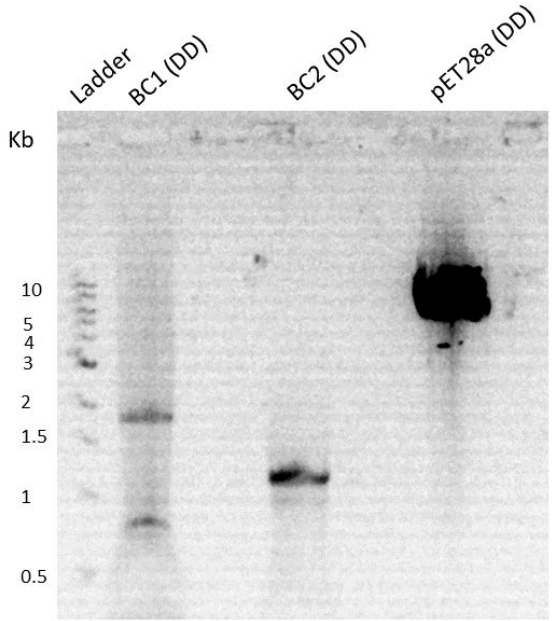
Restriction digestion (2.5hr)

Double digestion of pcr purified BC1 & BC2, Vector

BamH1 HF	0.4ul
HindIII HF	0.4ul
Cutsmart	4ul
Template	35.2 ul (BC17BC2) 4ug vector
Water	Upto 40 ul

4 ug of:
Vector = 21.4ul(404.8ng/ul)

Gel run of digested sample



Annotations:
BC1 (DD): BC1 Double Digested
BC2 (DD): BC2 Double Digested
Ladder: 1 kb NEB Ladder

Conclusion: got the required bands

29/07/21

- Gel elution of digested BC1, BC2 & pet28a
- Ligation of eluted product
- Transformation of ligated BC1 and BC2 into DH5α competent cells

Gel elution of digested BC1, BC2 & pet28a

Nanodrop Result:

S.No.	Concentration (ng/uL)	A260/A280	A260/A230
BC1	2.9	1.79	0.68
BC2	4.9	1.63	0.78
Vector	65.0	1.81	1.88

Ligation of eluted product

Calculation:

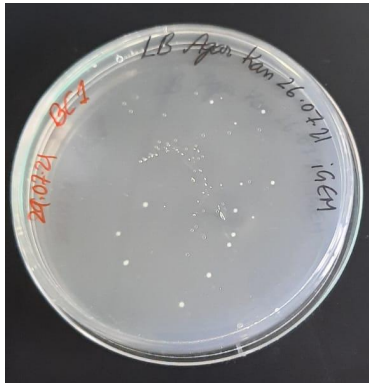
50 ng of Vector
51.03 ng of BC1 (1:3) → 17.59ul
32.59 ng of BC2 (1:3) → 6.65ul

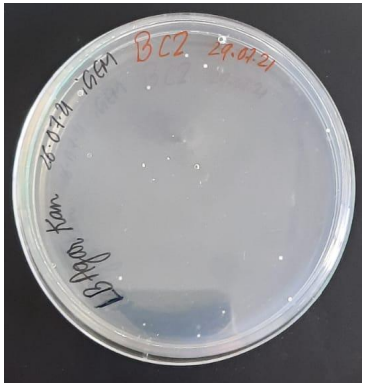
Protocol (for one reaction)

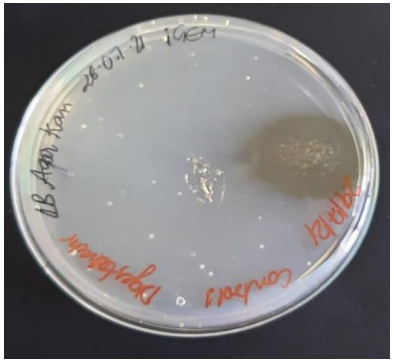
T4 DNA Ligase	0.5ul
10X buffer	2ul
Vector	50ng
Gene	Calculate according to ligation calculator
Water	Upto 20ul

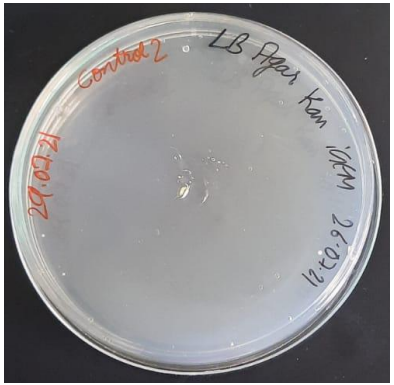
Kept at 23℃ for 1 hr

Transformation of ligated BC1 and BC2 into DH5α competent cells









#	sample	No. of colonies
1	BC1 in competent cell	14-16
2	BC2 in competent cell	12-15
3	Digested vector in competent cells	15-20
4	Competent cell	2-4

Colony PCR of transformed colonies of BC1 &BC2

30.07.21

Sample: use T7 FP and gene specific RP

+ve control: colony with gene specific primers

-ve control: colony from control 1(digested vector in competent cell) with T7 FP and gene specific RP

Tm = 50℃

First keep PCR machine for preheating at 95℃ for 5 mins till it show sample heating, then pause the machine and keep sample.

Extension time: 2 mins

Gel Run of Colony PCR product

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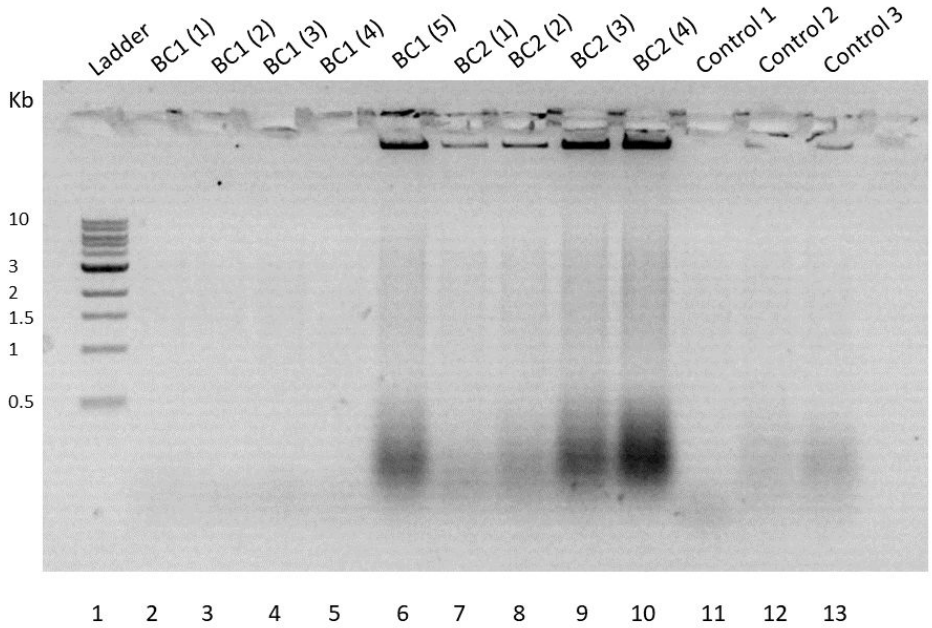

Annotations:
BC1 (1): 1st colony from BC1 plate.
Control 1: +ve control for BC1
Control 2: +ve control for BC2
Control 3: -ve Control
Ladder: 1 KB NEB Ladder

Conclusion: Probably its genomic DNA. Transformation is not successful, most probably vector was not digested properly. Repeat from restriction digestion.