ChnR NOTEBOOK

All ligations are T4 ligase and 1:3 ratio

Ladder : 2-Log DNA Ladder (0.1-10.0 kb), NEB

We have synthezied genes (ChnR and pChnB_B0030) from Genewiz. ***

20 August

- Primer design for ChnR and pChnB (BioBrick Prefix and Suffix are added in order to clone pChnB and ChnR into pSB1C3)

primer	**Sequence**
ChnR Fwd(72)	5' gttgttGAATTCGCGGCCGCTTCTAGatgtcgacggacaaggcaaatacg 3'
ChnR Rev(72)	5' gttgttCTGCAGCGGCCGCTACTAGTATTAttaaaaaacgatactcgacacgctg 3'
pChnB Fwd(72)	5' gttgttGAATTCGCGGCCGCTTCTAGgcaactaaaagagattgtttggatc 3'
pChnB Rev(72)	5' gttgttCTGCAGCGGCCGCTACTAGTAtttctcctctttaatcctagggataatc 3'

Table 1: PCR primers

28 August

- PCR is performed for ChnR and pChnB[Q5]

DNA	**Length(bp)**
ChnR	991
pChnB	459

Table 2: Lengths of parts

- *Cut-Ligate*

  Linearized pSB1C3, pChnB and ChnR are digested with EcoRI and PstI
  Ligation of digested pSB1C3 & pChnB and pSB1C3 & ChnR is performed[T4 DNA ligase]

- *Transfromation*

  pSB1C3_ChnR and pSB1C3_pChnB are transformed to PRO competent cell.

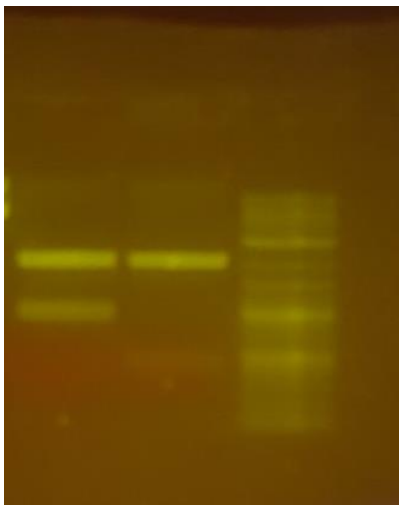
29 August

- One colony is choosen from each plate and they are left overnight culture.

30 August

- Glycerol stock is prepared and Miniprep is performed.

- *Digestion*
  pSB1C3_ChnR and pSB1C3_pChnB are digested with EcoRI and PstI for verification.



pSB1C3_ChnR
pSB1C3_pChnB
Ladder (from left to right)

Figure 1: Agarose Gel Results

20 September

- *Transformation*
  B0032(RBS), K1321337(sfGFP) parts are taken from iGEM 2017 distribution kit and transformed into MG1655 competent cells

25 September

- 1 colony is chosen from each plate (B0032 and K1321337) and left overnight culture.

26 September

- *Miniprep*
  B0032 and K1321337

Sample	**Concentration(ng/ul)**
B0032(RBS)	174.6
K1321337(sfGFP)	75.7

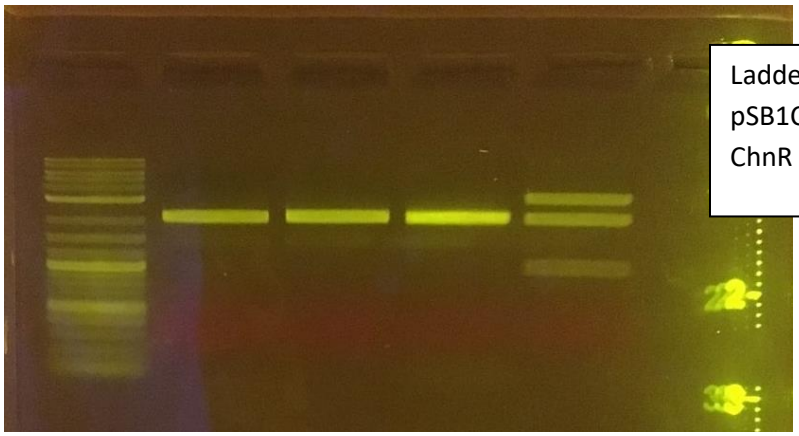
Table 3: Nanodrop results

- *Cut - Ligate*
  Digestion
  B0032 (SpeI/ PstI)
  K1321337(XbaI/PstI)
  ChnR(XbaI/PstI)

DNA	**Length (bp)**
pSB1C3_B0032	2082
K1321337(sfGFP)	711
ChnR	939
pSB1C3	2070

Table 4: Lengths of parts

Gel Electrophpresis



Ladder, pSB1C3_B0032, pSB1C3_B0032, K1321337(sfGFP), ChnR (from left to the right)

Figure2: Agarose Gel Results

- K1321337(sfGFP) digestion is unsucessful therefore 2 more colony is chosen from pSB1C3_K1321337(sfGFP) plate and left overnight culture.

Gel Extraction

Sample	**Concentration(ng/ul)**
pSB1C3_B0032	8.8
pSB1C3_B0032	7.2

ChnR	5.2

Table 5: Nanodrop results

Ligation
pSB1C3_B0032 & ChnR

- *Transformation*
  pSB1C3_B0032 & ChnR (PRO)

27 September

- *Miniprep*
  pSB1C3_K1321337(sfGFP)

Sample	**Concentration(ng/ul)**
Colony 1	75
Colony 2	128

Table 6: Nanodrop results

- *Cut-Ligate*
  Digestion
  pSB1C3_B0032 (SpeI/ PstI)
  pSB1C3_K1321337(XbaI/PstI)

DNA	**Length (bp)**
pSB1C3_B0032	2082
K1321337(sfGFP)	711
pSB1C3	2070

Gel Electrophpresis

Ladder, K1321337 Col 1, K1321337 Col 2, B0032 (from left to the right)

Figure 3: Agarose Gel Results

- K1321337 Col 1 digestion is unsucessful but Col 2 is successful. B0032 is also successful.

Gel Extraction

Sample	**Concentration(ng/ul)**
B0032	17
K1321337 (Col 2)	7.7

Table 7: Nanodrop results

Ligation
pSB1C3_B0032 & K1321337

- *Transformation*
  pSB1C3_B0032 & K1321337 (PRO)
  pSB1C3_J23106(promoter) which is taken from iGEM 2017 distribution kit (PRO)

28 September

- *Overnight Culture*
  pSB1C3_B0032 & K1321337 * 3 Colony is chosen
  pSB1C3_B0032_ChnR
  pSB1C3_J23106 *2 colony is chosen

29 September

- *Miniprep*
  pSB1C3_B0032 & K1321337
  pSB1C3_B0032_ChnR
  pSB1C3_J23106

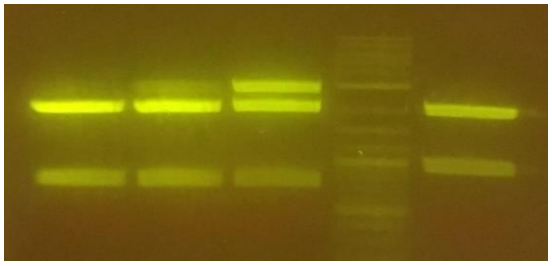
Sample	**Concentration(ng/ul)**
pSB1C3_B0032 & K1321337 Col 1	88.3
pSB1C3_B0032 & K1321337 Col 2	79.3
pSB1C3_B0032 & K1321337 Col 3	53.1
pSB1C3_J23106 Col 1	55.1
pSB1C3_J23106 Col 2	57.4
pSB1C3_B0032_ChnR	67.2

Table 8: Nanodrop results

- *Cut-Ligate*
  Digestion
  pSB1C3_B0032 & K1321337 Col 1 & 2 & 3 (XbaI/PstI)
  pSB1C3_B0032_ChnR (EcoRI/SpeI)
  pSB1C3_B0015(EcoRI/XbaI) *done before

DNA	**Length**
B0032_ K1321337	723
B0032_ChnR	951
pSB1C3_B0015	2199

Gel Electrophpresis



pSB1C3_B0032 & K1321337 Col 1 & 2 & 3, Ladder, pSB1C3_B0032_ChnR (from left to the right)

Figure 4: Agarose Gel Results

Gel Extraction

Sample	**Concentration(ng/ul)**
pSB1C3_B0032 & K1321337 Col 1	10
pSB1C3_B0032 & K1321337 Col 2	17.1
pSB1C3_B0032 & K1321337 Col 3	12.5
pSB1C3_B0032_ChnR	9.6

Table 9: Nanodrop results

Ligation
pSB1C3_B0032_K1321337 & pSB1C3_B0015

- *Transformation*
  pSB1C3_B0032_K1321337 & pSB1C3_B0015(PRO)

1 October

- *Overnight Culture*
  pSB1C3_B0032_ChnR * 2 Colony is chosen
  pSB1C3_B0032_K1321337_B0015 * 2 Colony is chosen

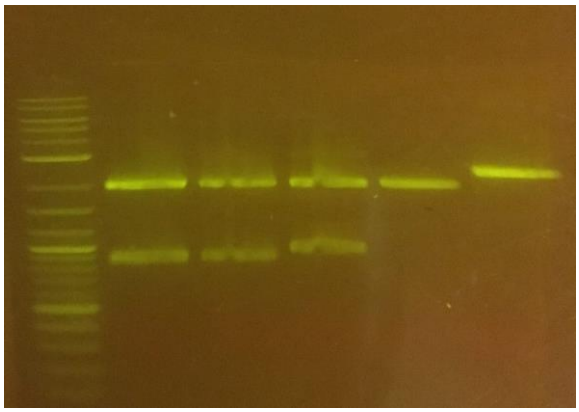
2 October

- *Miniprep*
  pSB1C3_B0032_ChnR Col 1 & 2
  pSB1C3_B0032_K1321337_B0015 Col 1 & 2

- *Digestion*
  pSB1C3_B0032_ChnR Col 1 & 2 (EcoRI/SpeI)
  pSB1C3_B0032_K1321337_B0015 Col 1 & 2 (XbaI/PstI)
  pSB1C3_pChnB (SpeI/ PstI)

DNA	**Length**
B0032_ChnR	951
B0032_K1321337_B0015	852
pSB1C3_pChnB	2529

Gel Electrophpresis



Ladder,
pSB1C3_B0032_ChnR 1 & 2 ,
pSB1C3_B0032_K1321337_B0015 Col 1 & 2,
pSB1C3_pChnB (SpeI/ PstI)
(from left to the right)

Figure 5: Agarose Gel Results

- pSB1C3_B0032_K1321337_B0015 Col 2 digestion is unsuccessful.

Gel Extraction

Sample	**Concentration(ng/ul)**
pSB1C3_B0032_ChnR Col 1	2.7
pSB1C3_B0032_ChnR Col 2	8.1
pSB1C3_B0032-_K1321337_B0015 Col 1	15.5
pSB1C3_pChnB	8.7

Table 10: Nanodrop results

3 October

- *Ligation*
  pSB1C3_B0032_ChnR & pSB1C3_B0015
  pSB1C3_pChnB & pSB1C3_B0032_K1321337_B0015
- *Transformation*
  pSB1C3_B0032_ChnR & pSB1C3_B0015 (PRO)
  pSB1C3_pChnB & pSB1C3_B0032_K1321337_B0015 (PRO)

4 October

- *Overnight Culture*
  pSB1C3_B0032_ChnR & pSB1C3_B0015 *4 colonies are chosen
  pSB1C3_pChnB & pSB1C3_B0032_K1321337_B0015 *4 colonies are chosen

5 October

- *Miniprep*
  pSB1C3_B0032_ChnR & pSB1C3_B0015 Col 1 & 2 & 3 & 4
  pSB1C3_pChnB & pSB1C3_B0032_K1321337_B0015 Col 1 & 2 & 3 & 4

Sample	Concentration(ng/ul)
pSB1C3_B0032_ChnR_B0015 Col 1	89.9
pSB1C3_B0032_ChnR_B0015 Col 2	9.4
pSB1C3_B0032_ChnR_B0015 Col3	10
pSB1C3_B0032_ChnR_B0015 Col 4	5.1
pSB1C3_pChnB_B0032_K1321337_B0015 Col 1	17.3
pSB1C3_pChnB_B0032_K1321337_B0015 Col 2	11.1
pSB1C3_pChnB_B0032_K1321337_B0015 Col 3	87.5
pSB1C3_pChnB_B0032_K1321337_B0015 Col 4	9.7

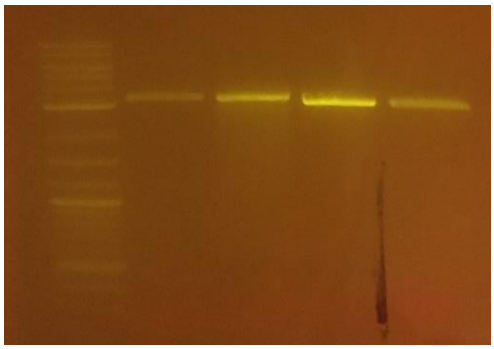
Table 11: Nanodrop results

09 September

- *Digestion*
  pSB1C3_B0032_ChnR_B0015 Col 1 & Col3 (PstI)
  pSB1C3_pChnB_B0032_K1321337_B0015 Col 1 & Col3 (PstI)

DNA	Length
pSB1C3_B0032_ChnR_B0015	3167
pSB1C3_pChnB_B0032_K1321337_B0015	3243

Gel Electrophpresis



Ladder,
pSB1C3_pChnB_B0032_K1321337_B0015 Col 1 & Col3
pSB1C3_B0032_ChnR_B0015 Col 1 & Col3
(from left to the right)

Figure 6: Agarose Gel Results

- Digestion is performed for verification of plasmids, the are verified.

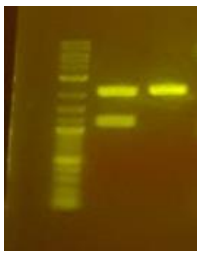
11 September

- *Cut - Ligate*

Digestion
pSB1C3_B0032_ChnR_B0015 Col 1 (XbaI & PstI)
pSB1C3_J23106 Col 2 (SpeI/ PstI)

DNA	**Length**
B0032_ChnR_B0015	1097
pSB1C3_pChnB_B0032_K1321337_B0015	3243

Gel Electrophoresis



Ladder,
pSB1C3_B0032_ChnR_B0015 Col 1
pSB1C3_J23106 Col 2
(from left to the right)

Figure 7: Agarose Gel Results

Ligation
pSB1C3_J23106 & pSB1C3_B0032_ChnR_B0015

- *Transformation*
  pSB1C3_J23106 & pSB1C3_B0032_ChnR_B0015 (MG1655)

13 September

- *Overnight Culture*
  pSB1C3_J23106_B0032_ChnR_B0015
  pSB1C3_pChnB_B0032_K1321337_B0015

16 September

- *Miniprep*
  pSB1C3_J23106_B0032_ChnR_B0015
  pSB1C3_pChnB_B0032_K1321337_B0015

Sample	**Concentration(ng/ul)**
pSB1C3_J23106_B0032_ChnR_B0015	114.8
pSB1C3_pChnB_B0032_K1321337_B0015	124.5

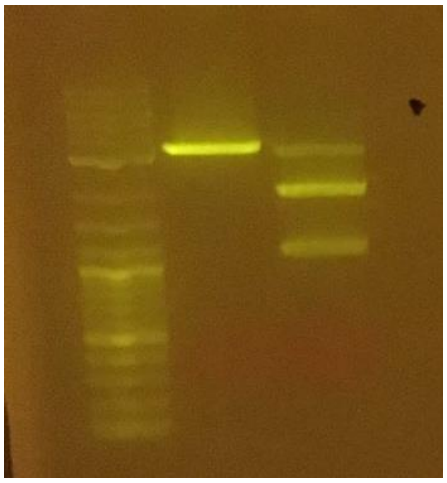
Table 12: Nanodrop results

- *Cut - Ligate*

Digestion
pSB1C3_J23106_B0032_ChnR_B0015 (XbaI/PstI)
pSB1C3_pChnB_B0032_K1321337_B0015(SpeI/PstI)

DNA	**Length**
J23106_B0032_ChnR_B0015	1140
pSB1C3_pChnB_B0032_K1321337_B0015	3243

Gel Electrophoresis



Ladder,
pSB1C3_pChnB_B0032_K1321337_B0015
pSB1C3_J23106_B0032_ChnR_B0015
(from left to the right)

Figure 8: Agarose Gel Results

Gel Extraction

Sample	**Concentration(ng/ul)**
pSB1C3_J23106_B0032_ChnR_B0015	2
pSB1C3_pChnB_B0032_K1321337_B0015	6

Table 13: Nanodrop results

Ligation
pSB1C3_pChnB_B0032_K1321337_B0015 & pSB1C3_J23106_B0032_ChnR_B0015

- *Transformation*
  pSB1C3_pChnB_B0032_K1321337_B0015 &
  pSB1C3_J23106_B0032_ChnR_B0015(MG1655)

***pSB1C3_pChnB_B0032_K1321337_B0015_J23106_B0032_ChnR_B0015 was sent to sequence and its sequence is verified. However, we realized that when we designed a primer for pChnB, RBS (B0030) is also included to target DNA sequence. Therefore all pChnB parts are combined with B0030 unintentionally.