Lab plan for construct of Pveg+RBS+YbdN+BMP-2+BBa_0015

Final construct:

Pveg promoter + RBS + Signal peptide YbdN + Mature mouse BMP-2+ BBa+0015 double terminator in pSB1AK3

Lab plan

Construction of Pveg +RBS+YbdN+BMP-2 in pSB1C3

1. **Double Digestion of YbdN+BMP-2 in pSB1C3**
   Double digest construct YbdN+BMP-2 in pSB1C3 with XbaI and PstI
2. **Gel Electrophoresis of digestion product**
   a. Prepare 2% agarose gel
   b. Expected band size: 2070 b.p. and 439 b.p.
3. **Gel Purification of YbdN+BMP-2**
   a. Cut the gel that shows 439 b.p. band.
   b. Purify gel using Favorgen gel purification kit.
4. **Double Digestion of Pveg+RBS in pSB1C3**
   Double digest construct Pveg+RBS in pSB1C3 with SpeI and PstI
5. **Dephosphorylation of digested Pveg+RBS in pSB1C3**
   Into the digestion product of Pveg+RBS in pSB1C3, add 2 μl of Antarctic phosphatase buffer and 1 μl Antarctic phosphatase.
6. **DNA purification of digested Pveg+RBS in pSB1C3**
   DNA purification using Favorgen PCR cleanup kit.
7. **Ligation of Pveg+RBS in pSB1C3 and YbdN+BMP-2**
   a. Mix the insert: YbdN+BMP-2 and backbone: Pveg+RBS in pSB1C3 with ratio of backbone:insert equals to 1:4.
   b. Into the mixture also add T4 ligase buffer and T4 ligase
   c. In another tube, prepare the mixture using backbone only without insert as negative control.

8. Transformation of Pveg+RBS+YbdN+BMP-2 in pSB1C3 into *E. coli*

a. Take out the competent cell from -80 freezer.
b. Cool down the cell in ice for 10 minutes.
c. Add all ligation products into the cells. Leave it in ice for 10 minutes.
d. Put the tubes into 42℃ water bath for exactly 90 seconds.
e. Put them back into ice for 2 minutes.
f. Add 1ml LB in all cells. Incubate the tubes in 37℃ for 1 hour.
g. Spin down the cells. Transfer 100 μl of each tube of cells into separate Chloramphenicol (25ng/ μl) plates. Spread the plate. Incubate in 37℃ overnight.
h. Observe the colonies formed on next day.

9. Inoculation of of Pveg+RBS+YbdN+BMP-2 in pSB1C3 in *E. coli*

a. Prepare Chloramphenicol (25ng/ μl) LB solution.
b. Add 5 ml of LB solution into sterile falcon tubes.
c. Pick colony from transformed plate and dip into LB solution in falcon tube.
d. Incubate at 37^0C overnight

Construction of Pveg +RBS+YbdN+BMP-2+BBa_0015 in pSB1AK3

1. Plasmid extraction of Pveg+RBS+YbdN+BMP-2 in pSB1C3

Extract the plasmid using Favorgen plasmid extraction miniprep kit.

2. Double Digestion of Pveg+RBS+YbdN+BMP-2 in pSB1C3

Double Digest construct Pveg+RBS+YbdN+BMP-2 in pSB1C3 with EcoRI and SpeI

3. Gel electrophoresis of digested Pveg+RBS+YbdN+BMP-2 in pSB1C3

a. Prepare 2% agarose gel
b. Expected band size: 2070 b.p. and 570 b.p

4. Gel Purification of digested Pveg+RBS+YbdN+BMP-2

a. Cut the gel that shows 570 b.p. bands
b. Purify the DNA from the gel using Favorgen gel purification kit.

5. Double Digestion of BBa_0015 in PSB1AK3

Double digest biobrick BBA_0015 in PSB1AK3 with EcoRI and XbaI

6. Dephosphorylation of Bba_0015 in pSB1AK3

Into the digestion product of BBa_0015 in pSB1AK3, add 2 μl of Antarctic phosphatase buffer and 1 μl Antarctic phosphatase.

7. DNA purification of digested BBa_0015 in PSB1AK3

DNA purification using Favorgen PCR cleanup kit.

8. Ligation of Pveg+RBS YbdN+BMP-2 with BBa_0015 in PSB1AK3

a. Mix the insert: Pveg+RBS+YbdN+BMP-2 and backbone: BBa_0015 in pSB1AK3 with ratio of backbone:insert equals to 1:4.
b. Into the mixture also add T4 ligase buffer and T4 ligase
c. In another tube, prepare the mixture using backbone only without insert as negative control.

9. Transformation of Pveg+RBS+YbdN+BMP-2+BBa_0015 in pSB1AK3 into E.coli

a. Take out the competent cell from -80 freezer.
b. Cool down the cell in ice for 10 minutes.
c. Add all ligation products into the cells. Leave it in ice for 10 minutes.
d. Put the tubes into 42℃ water bath for exactly 90 seconds.
e. Put them back into ice for 2 minutes.
f. Spread the onto Ampicilin (150 ng/ μl) plate. Incubate in 37℃ overnight.
g. Observe the colonies formed on next day.

Construct Pveg+RBS+YbdN+BMP-2+BBa_0015 screening

1. Colony PCR of Colonies from plate Pveg+RBS+YbdN+BMP-2+BBa_0015 in pSB1AK3 in E.coli

Prepare the following mixture:

Sample	Each PCR Tube
ddH_2O	16
10X thermopol PCR buffer	2
Forward Primer (10μM)	0.5
Reverse Primer (10μM)	0.5
dNTP (10mM)	0.5
Taq polymerase	0.5
Total Volume (μl)	20

Forward Primer sequences:

5’- GATCATTCTAGAGAAAGGAGGTTGTTTGCATGGTG-3’

Reverse Primer sequences:

5’-ATGATCACTAGTATTATTAACGACACCCGCAGCCC-3’

Thermocycle temperature setup

Cycle number	Denaturation	Annealing	Polymerization
Initial denaturation 1 cycle	10 min at 95 °C	-	-
30 cycles	30 sec at 95 °C	30 sec at 64°C	1 min at 68 °C
Final extension 1 cycle	-	-	5 min at 68 °C

2. Gel electrophoresis of colony PCR product

a. Prepare 2% agarose gel
b. Expected band size: 439 b.p.