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 pDG1661

Lab plan

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

1. **Double Digestion of Pveg +RBS+YbdN+BMP-2+BBa_0015 in pSB1AK3**
   Double digest construct Pveg+RBS+YbdN+BMP-2 in pSB1C3 with EcoRI and PstI
2. **Gel Electrophoresis of digestion product**
   a. Prepare 1.5% agarose gel
   b. Expected band size: 3189 b.p. and 677 b.p.
3. **Gel Purification of Pveg +RBS+YbdN+BMP-2+BBa_0015**
   a. Cut the gel that shows 677 b.p. band.
   b. Purify gel using Favorgen gel purification kit.
4. **Double Digestion of pBluescript (pBS)**
   Double digest construct Pveg+RBS in pSB1C3 with EcoRi and PstI
5. **Dephosphorylation of digested pBS**
   Into the digestion product of pBS, add 2 μ l of Antarctic phosphatase buffer and 1 μ l Antarctic phosphatase.
6. **DNA purification of digested pBS**
   Purify DNA using Favorgen PCR cleanup kit.
7. **Ligation of Pveg +RBS+YbdN+BMP-2+BBa_0015 and pBS**
   a. Mix the insert: Pveg +RBS+YbdN+BMP-2+BBa_0015 and backbone: pBS 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+BBa_0015 in pBS 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 onto Ampicilin (150 ng/ μ l) plate. Incubate in 37℃ overnight.
g. Observe the colonies formed on next day.

9. Inoculation of of Pveg +RBS+YbdN+BMP-2+BBa_0015 in pBS in *E. coli*

a. Prepare Ampicilin (150 ng/ μ l) 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+BMP2+BBa_0015 in pDG1661

1. Plasmid extraction of Pveg +RBS+YbdN+BMP-2+BBa_0015 in pBS

Extract the plasmid using Favorgen plasmid extraction miniprep kit.

2. Double Digestion of Pveg +RBS+YbdN+BMP-2+BBa_0015 in pBS

Double Digest construct Pveg+RBS+YbdN+BMP2 in pSB1C3 with EcoRI and BamHI

3. Gel electrophoresis of digested Pveg+RBS+YbdN+BMP2 in pBS

a. Prepare 1.5% agarose gel
b. Expected band size: 2961 b.p. and 677 b.p

4. Gel Purification of digested Pveg+RBS+YbdN+BMP2

a. Cut the gel that shows 677 b.p. bands
b. Purify the DNA from the gel using Favorgen gel purification kit.

5. Double Digestion of pDG1661

Double digest pDG1661 with EcoRI and BamHI

6. DNA purification of digested pDg1661

Purify DNA using Favorgen PCR cleanup kit.

7. Ligation of Pveg +RBS+YbdN+BMP-2+BBa_0015 with pDG1661

a. Mix the insert: Pveg +RBS+YbdN+BMP-2+BBa_0015 and backbone: pDg1661 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+BBa_0015 in pDG1661 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/ μ l) plate. Incubate in 37℃ overnight.
g. Observe the colonies formed on next day.

Construct Pveg+RBS+YbdN+BMP2+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.