Cohesive-end Assembly Cloning

This protocol was used to perform all the Biobricks assemblies.

Insert calculation:

$$ng\ of\ insert = \frac{ng\ of\ vector\ \times kb\ size\ of\ insert}{kb\ size\ of\ vector} \times insert{:}\ vector\ molar\ ratio$$

Materials

- Insert 1 previously digested with *Eco*RI and *Spe*I
- Insert 2 previously digested with *Xba*I and *Pst*I
- pSB1C3 previously digested with *Eco*RI and *Pst*I
- 10X T4 DNA Ligase Buffer
- T4 DNA Ligase (5U/µL) (Thermo Scientific)
- Water, nuclease free
- 0.5 mL microtube

Apparatus

- Incubator

Method

1. After thawing and vortexing the solutions (except the enzyme solution), prepare the following mixture in a 0.5 sterile microtube. Keep the microtube on ice.

Insert 1 digest with *Eco*RI and *Spe*I (vector:insert)	1:3 to 1:6
Insert 2 digest with *Xba*I and *Pst*I (vector:insert)	1:3 to 1:6
pSB1C3 digested with *Eco*RI and *Pst*I	50 ng
10X T4 DNA Ligase Buffer	2 µL
T4 DNA Ligase (5 U/µl)	1 µL (5U)
Water, nuclease free	to 20 µL

2. Spin the mixture.
3. Incubate overnight at 4°C.
4. Transform the ligation in *E. coli* DH5α. (**see the Transformation in *E. coli* Protocol**) [http://2014.igem.org/wiki/images/a/a5/Transformation_in_Escherichia_coli_DH5.pdf]
5. Plate out the suspension on a LB agar plate containing chloramphenicol (25 µg/mL) and incubate at 37° for 12 hours.
6. Select the white colonies.