Molecular cloning and genetic engineering – 3A Assembly

Aim

3A assembly can combine tow Biobricks together into a new DNA vector.

Materials

Linearized Plasmid Backbone (with a different resistance to the plasmid backbones containing your part samples)

Part 1, in a different backbone than the linearized plasmid backbone

Part 2, in a different backbone than the linearized plasmid backbone

ddH_2O

10X Digest buffer

Digest Enzyme PstI

Digest Enzyme EcoRI

Digest Enzyme DpnI

Digest Enzyme SpeI (BcuI)

Digest Enzyme XbaI

10X T4 DNA Ligase buffer 0.1mol/L $CaCl_2$

T4 DNA Ligase

BSA

Procedure

1. Enzyme Master Mix for Plasmid Backbone (25ul total, for 5 rxns):

5 ul NEB Buffer 2

0.5 ul BSA

0.5 ul EcoRI

0.5 ul PstI

0.5 ul DpnI (Used to digest any template DNA from production)

18 ul ddH_2O

2. Enzyme Master Mix for Part 1 (25ul total, for 5 rxns):

5 ul NEB Buffer 2

0.5 ul BSA

0.5 ul EcoRI

0.5 ul SpeI

18.5 ul ddH_2O

3. Enzyme Master Mix for Part 2 (25ul total, for 5 rxns):

5 ul NEB Buffer 2

0.5 ul BSA

0.5 ul XbaI

0.5 ul PstI

18.5 ul ddH_2O

4、Digest Plasmid Backbone：Add 4 ul linearized plasmid backbone (25ng/ul for 100ng total) Add 4 ul of Enzyme Master Mix；

Digest Part 1: Add 4 ul Part A (25ng/ul for 100ng total); Add 4 ul of Enzyme Master Mix；

Digest Part 2: Add 4 ul Part B (25ng/ul for 100ng total); Add 4 ul of Enzyme Master Mix；

Digest all three reactions at 37℃/30 min, heat kill 80℃/20 min.

5. Add 2ul of digested Plasmid Backbone (25 ng).

6. Add equimolar amount of Part 1 (EcoRI SpeI digested) fragment (< 3 ul).

7. Add equimolar amount of Part B (XbaI PstI digested fragment) (< 3 ul).

8. Add 1 ul T4 DNA ligase buffer.

9. Add 0.5 ul T4 DNA ligase.

10. Add water to 10 ul .

11. Ligate 16℃/30 min, heat kill 80℃/20 min.

12. Transform with 1-2 ul of product.