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

dd$H_2O$

10X Digest buffer

Digest Enzyme PstI

Digest Enzyme EcoRI

Digest Enzyme DpnI

Digest Enzyme SpeI (BcuI)

Digest Enzyme XbaI

10X T4 DNA Ligase buffer

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 dd$H_2O$

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 dd$H_2O$

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 dd$H_2O$

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 37C/30 min, heat kill 80C/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 16C/30 min, heat kill 80C/20 min

12、Transform with 1-2 ul of product