

Standard Assembly

Materials

- Your two Part Samples, A and B: Miniprep DNA (in BioBrick RFC [10] plasmid backbones)
- EcoRI, XbaI, SpeI, DpnI
- NEB Buffer 2
- BSA
- dH₂O

Digest

Enzyme Master Mix for Part A (25ul total/ 5 rxns) ▪ 5 ul NEB Buffer 2 ▪ 0.5 ul BSA ▪ 0.5 ul EcoRI-HF ▪ 0.5 ul SpeI ▪ 18.5 ul dH₂O	Enzyme Master Mix for Part B (25ul total/ 5 rxns) ▪ 5 ul NEB Buffer 2 ▪ 0.5 ul BSA ▪ 0.5 ul XbaI ▪ 0.5 ul PstI ▪ 18.5 ul dH₂O
Digest Part A ▪ Add 4 ul Part A (25ng/ul for 100ng total) ▪ Add 4 ul of Enzyme Master Mix	Digest Part B ▪ Add 4 ul Part B (25ng/ul for 100ng total) ▪ Add 4 ul of Enzyme Master Mix

Ligation

- Add 2ul of digested Plasmid Backbone (25 ng)
- Add 25ng of Part A (EcoRI - HF SpeI digested fragment) (< 3 ul)
- Add equimolar amount of Part B (EcoRI - XbaI digested fragment) (< 3 ul)
- Add 1 ul T4 DNA ligase buffer. *Note: Do **not** use quick ligase!*
- Add 0.5 ul T4 DNA ligase
- Add water to 10 ul
- Ligate 16C/30 min, heat kill 80C/20 min
- Transform with 1-2 ul of product

Adapted from http://parts.igem.org/Assembly:Standard_assembly