3A Assembly

Materials

- Your two Part Samples, A and B: Miniprepped DNA (in BioBrick RFC [10] plasmid backbones)
- Linearized Plasmid Backbone (with a different resistance to the plasmid backbones containing your part samples)
- EcoRI, XbaI, SpeI, PstI, DpnI
- NEB Buffer 2
- BSA
- dH_2O

Digest

Enzyme Master Mix for Plasmid Backbone (25ul total/ 5 rxns)	**Enzyme Master Mix for Part A** (25ul total/ 5 rxns)	**Enzyme Master Mix for Part B** (25ul total/ 5 rxns)
▪ 5 ul NEB Buffer 2 ▪ 0.5 ul BSA ▪ 0.5 ul EcoRI-HF ▪ 0.5 ul PstI ▪ 0.5 ul DpnI (Used to digest any template DNA from production) ▪ 18 ul dH_2O	▪ 5 ul NEB Buffer 2 ▪ 0.5 ul BSA ▪ 0.5 ul EcoRI-HF ▪ 0.5 ul SpeI ▪ 18.5 ul dH_2O	▪ 5 ul NEB Buffer 2 ▪ 0.5 ul BSA ▪ 0.5 ul XbaI ▪ 0.5 ul PstI ▪ 18.5 ul dH_2O

Digest Plasmid Backbone	Digest Part A	Digest Part B
▪ Add 4 ul linearized plasmid backbone (25ng/ul for 100ng total) ▪ Add 4 ul of Enzymed Master Mix	▪ Add 4 ul Part A (25ng/ul for 100ng total) ▪ Add 4 ul of Enzyme Master Mix	▪ 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

Ligation

- Add 2ul of digested Plasmid Backbone (25 ng)
- Add equimolar amount of Part A (EcoRI-HF SpeI digested) fragment (< 3 ul)
- Add equimolar amount of Part B (XbaI PstI 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/Help:Protocols/3A_Assembly