Ligation
iGEM Protocol

After following our restriction digest protocol (which uses 250ng of DNA) you may follow these steps for ligation.

Ligation

1. Add 2ul of digested plasmid backbone (25 ng)
2. Add equimolar amount of EcoRI-HF SpeI digested fragment (< 3 ul)
3. Add equimolar amount of XbaI PstI digested fragment (< 3 ul)
4. Add 1 ul T4 DNA ligase buffer. Note: Do not use quick ligase
5. Add 0.5 ul T4 DNA ligase
6. Add water to 10 ul
7. Ligate 16C/30 min, heat kill 80C/20 min
8. Transform with 1-2 ul of product

Note: For linearized plasmid backbones provided by iGEM HQ, a plasmid backbone with an insert of BBa_J04450 was used as template. As a result any red colonies that appear during your ligation may be due to the template as a background. Digesting with Dpn1 before use should reduce this occurrence.