

3A Assembly

Introduction

Used to ligate two parts into a backbone

Materials

- › Digested plasmid backbone (different from backbones parts A and B come in)
- › Part A
- › Part B
- › T4 ligase buffer
- › T4 ligase
- › Water
- ›

Procedure

Ligation Reaction:

1. Add 2ul of digested **Plasmid Backbone** (25 ng)
2. Add equimolar amount of **Part A** (EcoRI-HF SpeI digested) fragment (< 3 ul)
3. Add equimolar amount of **Part B** (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