Ligation Reactions (20 µL)

1. Gel purify all digested inserts and plasmid backbones
   - Refer to gel purification of DNA protocol
2. Add the following reaction components to a 0.2 mL PCR tube
   - 11 µL - ddH_2O
   - 2 µL - upstream insert
   - 2 µL - downstream insert
   - 2 µL - destination plasmid (backbone)
   - 2 µL - T4 DNA Ligase Buffer (10x)
   - 1 µL - T4 DNA Ligase
3. Incubate in thermocycler w/ heated lid
   - Overnight @ 16° C
   - Heat inactivate for 20 min. @ 80° C (optional)

Alternative protocol

1. Gel purify all digested inserts and plasmid backbones
   - Refer to gel purification of DNA protocol
2. Add the following reaction components to a 0.2 mL PCR tube
   - 5 µL - ddH_2O
   - 4 µL - upstream insert
   - 4 µL - downstream insert
   - 4 µL - destination plasmid (backbone)
   - 2 µL - T4 DNA Ligase Buffer (10x)
   - 1 µL - T4 DNA Ligase
3. Incubate for ~10 min. @ room temperature
4. Heat inactivate for 20 min. @ 80° C (optional)