This protocol based on a protocol on openwetware somewhere.
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∅∅∅∅∅ T4 Ligase Protocol

T4 allows for the fusion of free DNA ends provided they are complementary or blunt. T4 is the "mainstream" method for fusing DNA backbones.

Total DNA concentration should be below 100 nanograms per reaction. A 6:1 concentration ratio of insert to vector will be required, so ensure you have sufficient amounts of both.

The total volume in the PCR tube should come to 8.5µL. Any volume left below 8.5µL should be filled with ddH2O. Calculate the DNA amounts as well as the insert amounts ahead of time.

Compounds
10x T4 ligase buffer
T4 ligase
ddH2O
Linearized vector (in EB or ddH2O)
Linearized insert (in EB or ddH2O)

Materials
Pipeteman P5, tips
PCR tube

You will also need access to a
Thermocycler
Heat bath

Procedure

1. Add (8.5 - vector - insert)µL **ddH2O** to a **PCR tube** with a **P5**.
2. Add the correct volume of **vector** to the PCR tube with the P5.
3. Add the correct volume of **insert** to the PCR tube with the P5.
4. Add 1µL **T4 Ligase buffer** with a **P5**. Mix the solution by intaking and expelling the liquid repeatedly with your pipette about 5 times.
5. Place the PCR tube in a **thermocycler.** Heat to **42°C** for **2 minutes.** This will break the sticky ends.
6. *Carefully* add 1µL **T4 ligase** to the PCR tube with a P5. Do not dip the tip of the pipette too far into the solution-just inject the contents onto the surface.
7. Mix the solution with a **fresh P5** by intaking and expelling about *half* of the liquid repeatedly with your pipette about 5 times.

8. Place the PCR solution in the thermocycler again, this incubating it at **16°C** for **60 minutes.**

9. Place the PCR tube in a **heat bath** at **65°C** for **10 minutes.**

The resulting solution can be used immediately or stored in at -20°C.