This protocol based on a protocol on openwetware somewhere.
This document is version 1.0. Last updated 6.7.11.

∅∅∅∅∅ Quick DNA Ligase Protocol

Quick DNA ligase allows for the fusion of free DNA ends provided they are complementary or blunt. While more expensive than the standard T4 ligase, it is also far faster, ligating ends in 5 minutes instead of 30 for T4.

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 4.5µL. Add together the volumes of vector and insert required for the reaction; any volume left over to reach 4.5µL should be filled with ddH2O. Calculate the DNA amounts as well as the insert amounts ahead of time.

Compounds
2x Quick ligase buffer
Quick ligase (NEB)
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 (4.5-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. Place the PCR tube in a **thermocycler.** Heat to **42°C** for **2 minutes.** This will break the sticky ends.
5. Add 5µL **2X Quick ligase buffer** to the tube with a P5. Mix the solution by intaking and expelling about *half* of the liquid repeatedly with your pipette about 5 times.
6. *Carefully* add 0.5µL **Quick 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 the liquid repeatedly with your pipette about 5 times.

8. Let the solution stand at **room temperature** for **7 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.