LIGATION OF DNA

1. Add vector and insert to each other at a molar ratio of 1 : 3 (V : I), keeping volume as small as possible.

2. Add:

   2 μl 10 x ligase buffer (manufacturer's)
   10 units T4 ligase
   up to 20 μl with dH_2O

3. Incubate 15°C overnight if staggered ends, 4°C overnight (or more) if blunt ends.

4. Transform into bacteria.

Controls:
cut phosphatased vector religated
cut non-phosphatased vector religated
uncut vector