LIGATION

This method works well for blunt end ligations, as well as sticky end ligations. Procedure from BRL:

1. Generally, 50 ng-100 ng of vector is used per ligation reaction.

2. Calculate from this amount of vector the amount of insert needed to achieve either a 1:1 or 2:1 molar ratio of insert:vector.

3. Add these amounts of vector and insert to Eppendorf tube in a combined volume of $\leq$15 µl. (If volume exceeds this amount, vector and insert DNAs can be concentrated by ethanol precipitation.)

4. Add 4 µl of 5x ligation buffer, made as recommended by BRL:
   250 mM Tris-HCl pH 7.6
   50 mM $MgCl_2$
   25% (w/v) polyethylene glycol 8000
   5 mM ATP
   5 mM DTT

5. Add 1-2 µl T4 DNA ligase (BRL) for a final volume of 20 ul.
   * NOTE: 1 ul is 1 unit of the BRL stock enzyme and this is plenty for 100 ng of vector and insert. Do not use 5 unit of ligase as this is too much enzyme and the concentration of glycerol in the reaction will inhibit ligase activity.

6. Ligate at least 4 hours (to overnight) at room temperature.