Ligation

For use if you have compatible sticky ends.

Buffers and Solutions

ATP (10 mM)
Omit ATP from the ligation reaction in Step 5 if the ligation buffer contains ATP.
Sodium acetate (3 M, pH 5.2)
TE (pH 8.0)

1. Digest the vector (10 µg) and insert DNA with the two appropriate restriction enzymes.
2. Purify the digested insert DNA by extraction with phenol:chloroform and ethanol precipitation.
3. Purify the vector DNA by spin-column chromatography followed by ethanol precipitation.
4. Redissolve the precipitated DNAs separately in TE (pH 8.0) at a concentration of approx. 100 ng/µl. Calculate the concentration of the DNA, assuming that 1 bp has a mass of 650 daltons.
5. Transfer appropriate amounts of the DNAs to microfuge tubes as follows:

vector (30 fmoles [approx. 100 ng])
insert (30 fmoles [approx. 10 ng])

The molar ratio of plasmid vector to insert DNA fragment should be approx. 1:1 in the ligation reaction. The final DNA concentration should be approx. 10 ng/µl.

add:
10x Ligation buffer1.0 µl
Bacteriophage T4 DNA ligase 0.1 units
10 mM ATP 1.0 µl
H_2O to 10 µl

The DNA fragments can be added to the tubes together with the H_2O and then warmed to 45°C for 5 minutes to melt any cohesive termini that have reannealed during fragment preparation. Chill the DNA solution to 0°C before the remainder of the ligation reagents are added.

6. Incubate the reaction mixtures overnight at 16°C or for 4 hours at 20°C.
7. Transform competent ***E. coli*** with the ligation reactions. As controls, include known amounts of a standard preparation of superhelical plasmid DNA to check the efficiency of transformation and vector without insert.