Blunt-ended Ligation

The maximum number of "correct" clones can generally be obtained from ligation reactions containing equimolar amounts of plasmid and target DNAs, with the total DNA concentration being <100 µg/ml. Blunt-end ligation catalyzed by bacteriophage T4 DNA ligase is suppressed by high concentrations (5 mM) of ATP and polyamines such as spermidine.

Buffers and Solutions

ATP (10 mM)
Omit ATP from the ligation reaction in Step 5 if the ligation buffer contains ATP.
PEG 8000 (30% w/v)
Sodium acetate (3 M, pH 5.2)
TE (pH 8.0)

1. In separate reactions, digest 1-10 µg of the plasmid DNA and insert DNA with the appropriate restriction enzyme(s) that generate blunt ends.
2. Purify the digested insert DNA and vector DNA by extraction with phenol:chloroform and ethanol precipitation.
3. Dissolve the precipitated DNAs separately in TE (pH 8.0) at a concentration of approx. 100 ng/µl. Calculate the concentration of the DNAs assuming that 1 bp has a mass of 650 daltons.
4. Dephosphorylate the plasmid vector DNA
5. Transfer appropriate amounts of the DNAs to microfuge tubes as follows:

vector* (60 fmoles [approx. 100 ng])
insert (60 fmoles [approx. 10 ng])
linearized vector (contains 5'-terminal phosphates) (60 fmoles)
superhelical vector (6 fmoles [approx. 10 ng])
****Vector DNA is dephosphorylated***
The molar ratio of plasmid vector to insert DNA fragment should be approx. 1:1 in the ligation reaction. The total DNA concentration in the ligation reaction should be approx. 10 ng/µl.
add:
10x Ligation buffer1.0 µl
Bacteriophage T4 DNA ligase 0.5 units
5 mM ATP 1.0 µl
H_2O to 8.5 µl
30% PEG 8000 1-1.5 µl

To achieve the maximum efficiency of ligation, set up the reactions in as small a volume as possible (5-10 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 help dissociate any clumps of DNA that have formed 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, vector without insert and vector without dephosphorylation.