Ligation of insert and vector DNA

1) The ratio of vector and insert depends on the amount recovered from your restriction digests. If enough is available, it is wise to try two ligations at different ratios/amounts insert:vector. Usually want large excess of insert:vector, but sometimes the [vector] is limiting.

2) Example 1:

i.	Insert DNA	4.5 µl
ii.	Vector DNA	2.5 µl
iii.	10x ligase buffer	1 µl
iv.	DNA ligase	2 µl
	TOTAL	**10 µl**

3) Example 1:

v.	Insert DNA	5.0 µl
vi.	Vector DNA	4.0 µl (low vector concentration)
vii.	10x ligase buffer	1 µl
viii.	DNA ligase	2 µl
	TOTAL	**12 µl**

4) Also ligate the vector alone as a control for intramolecular recircularization. Substitute water for insert DNA.
5) Incubate at room temperature for 2-4 hours.
6) Transformation the entire ligation into ~75-100 µl of competent cells- want a relatively large volume of cells since the ligation volume will dilute the [$CaCl_2$] that makes the cells competent.