LIGATIONS

Vector:Insert Ratio

After the vector and insert DNA have been prepared for ligation, determine the concentration either by spectrophotometer or by agarose gel against a known amount of standard marker.
Test various vector:insert DNA ratios in order to find the optimum ratio for a particular vector and insert. In most cases either a 1:1 or a 1:3 molar ratio of vector:insert works well.
The following example illustrates to conversion of molar ratios to mass ratios for a 3.0kb vector and a 500bp DNA insert:

$$\frac{\text{ng of vector X kb size of insert}}{\text{kb size of vector}} \times \text{molar ratio of } \frac{\text{insert}}{\text{vector}}$$

Example:
How much 500bp insert DNA needs to be added to 100ng of 3.0kb vector in a ligation reaction for a desired vector:insert ratio of 1:3?

$$\frac{\text{100ng vector X 0.5kb insert}}{\text{3.0kb}} \times \frac{3}{1} = \text{50ng insert}$$

For Ligation Procedure follow manufacturer's recommendations. Remember to include controls.

	Litigation 1	**Litigation 2**	**Litigation 3**	**Litigation 4**
Vector	X	X	--	X
Insert	X	--	X	X
Buffer	X	X	X	X
T4 ligase	X	X	X	--

Also for transformation include an additional control for your plate solution (bacteria alone).