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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:

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

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?

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

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

	Ligation 1	**Ligation 2**	**Ligation 3**	**Ligation 4**
Vector	√	√	--	√
Insert	√	--	√	√
Buffer	√	√	√	√
T4 ligase	√	√	√	--

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