C. Optimizing Insert:Vector Molar Ratios

The pGEM®-T and pGEM®-T Easy Vector Systems have been optimized using a 1:1 molar ratio of the Control Insert DNA to the Vectors. However, ratios of 8:1 to 1:8 have been successfully used. If initial experiments with your PCR product are suboptimal, ratio optimization may be necessary. Ratios from 3:1 to 1:3 provide good initial parameters. The concentration of PCR product should be estimated by comparison to DNA mass standards on a gel or by using a fluorescent assay (5). The pGEM®-T and pGEM®-T Easy Vectors are approximately 3kb and are supplied at 50ng/ml. To calculate the appropriate amount of PCR product (insert) to include in the ligation reaction, use the following equation.

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

Sufficient pGEM®-T or pGEM®-T Easy Vector is provided to vary insert:vector ratios as recommended and to perform control reactions.

Example of insert:vector ratio calculation:

How much 0.5kb PCR product should be added to a ligation in which 50ng of 3.0kb vector will be used if a 3:1 insert:vector molar ratio is desired?

$$\frac{50\text{ng vector} \times 0.5\text{kb insert}}{3.0\text{kb vector}} \times \frac{3}{1} = 25\text{ng insert}$$

Note: Using the same parameters for a 1:1 insert:vector molar ratio, 8.3ng of a 0.5kb insert would be required.

Example:

I'd to ligate SmGPCR like **Smp 043340** insert (**1.6Kb, 20ng/µl**) with pGEM-T vector (**3Kb, 50ng/µl**) in 3:1 molar ratio (insert:vector).
Thus, I need **80ng** insert and since the gel purified insert is **20**ng/µl, then I should use **4µl**

Prtocol:
(1) Pulse-spin pGEM-T vector to collect it in the bottom of the tube.
(2) Vortex 2X Rapid ligation buffer vigorously to dissolve its components prior to use.
Keep the T4-ligase and its buffer on ice always.
(3) **In 10ul ligation rxn tube, addthe following (respect the order)**
2X ligation buffer ----------5µl
Insert* ---------- **4µl**
pGEM-T (50ng/µl) ---------- 1µl
T4 Ligase (3U/µl) ---------- 1µl

Pipette up and down to mix the stuff and either incubate for 1hour at room temperature or O/N at 4°C.