DNA Ligation

Generally, we are using the Quick Ligase protocol from New England Biolabs. The setup below should be similar with other ligases in terms of the calculation of amounts of DNA to be put into the reaction. The buffers and time of ligation will be different. **Remember to set up a vector only control for each ligation. An insert only control may also be required for some ligations.**

Each ligation reaction should contain:
~ 50 ng vector (cut with enzymes and treated with SAP)
X ng insert DNA in a 3:1, 1:1, or 1:3 insert:vector ratio. I typically use a 3:1 I:V ratio. determined by the calculations below
ddH_2O or Qiagen EB (what the DNA is usually eluted in) to 10 µl

add 10 µl 2X Quick Ligase Buffer (warmed to room temperature; try to not freeze-thaw too many times- aliquotting into smaller portions is recommended)
add 1 µl Quick Ligase

Briefly vortex and spin, keep at room temperature for 5 minutes.

Chill on ice, and use 2-3 µl to transform 50 µl of competent *E. coli.*

The way that I determine the amounts of vector and insert to use is as follows:

1. Run 1-3 µl of your DNA to be ligated together (i.e. your gel isolated fragments) on a gel along with various amounts of a DNA mass ladder (such as the DNA mass ladder sold by Invitrogen). Estimate the amount of DNA in your sample by comparing the intensity of your band compared to the known amount of DNA in the band closest in size in the ladder. be sure to account for the volume of your sample that was loaded.
2. Determine how much vector DNA you will need to have 50 ng. Be sure that you will have enough room to add insert DNA to have 10 µl in the end.
3. Determine the concentration of your insert DNA as in step 1.
4. Calculate the moles of ends of your vector using the following formula (assumes that 50 ng vector is being used- be sure to change the calculation accordingly if you are using more/less):

$$\text{M ends vector if 50 ng used in a 20 µl reaction} = \frac{(0.05 \text{ ug DNA}) \times 2}{(\text{vector size in bp}) \times (650 \text{ daltons/bp}) \times 20 \text{ ul}}$$

Amount Vector needed in the reaction = 50 ng / (X ng/µl as determined on gel)

Moles ends of insert needed for 3:1 I:V ratio = (M ends of vector calculated above) x 3

$$\text{ng insert DNA needed for a 3:1 I:V ratio} = \frac{(\text{M ends of insert needed for 3:1}) \times (\text{insert size in bp}) \times (650 \text{ dalton/bp}) \times 20 \text{ µl} \times (1000 \text{ ng/µg})}{2}$$

Amount insert needed in the reaction = amount calc above / (X ng/µl as determined on gel)