

**Day 1: March 31, 2005**

**DNA ligation- creation of recombinant DNA construct**

You will be given a cDNA (cDNA #2 or #4) insert that has been digested with EcoRI and purified by agarose gel electrophoresis. You will also receive the pGEM<sup>®</sup>-7Zf(+) plasmid digested with EcoRI and gel purified. You will get an aliquot of the plasmid that has been treated with shrimp alkaline phosphatase (SAP; Promega, see product literature for details) to dephosphorylate the 5' ends of the plasmid and an aliquot of plasmid that has not been treated with SAP.

For ligation reactions, the ideal ratio of insert to vector DNA is variable; however a starting point for ligations into pGEM<sup>®</sup>-7Zf(+) using T4 DNA ligase is 1:3 (vector:insert). A suggested starting point is to use 50 ng of vector.

See T4 DNA ligase (Promega) product information sheet on the course website for equation to calculate the amount of starting insert for various ratios.

1. Set up the following ligation reactions: (for both cDNA #2 and #4)

Component	1	2	3	4
pGem7 vector (100ng) (not dephosphorylated)	4.5	---	4.5	----
Dephosphorylated pGem7 vector (100ng)	---	4.5	-----	4.5
Insert #2 or #4	4	4	-----	-----
10x ligase buffer	1	1	1	1
T4 DNA ligase	0.5	0.5	0.5	0.5
Sterile H2O	----	-----	4	4

2. Incubate your ligation reactions at 4 ° C overnight (the TA will take them out of the refrigerator on the 1<sup>st</sup> and put them in the freezer)

Ligations can also be incubated at: Room temperature for 3 hours or 15 or 16° C for 4-18 hours.