Ligation Reaction
December 10, 2010

Description: Ligating an insert to a vector fragment, to form a plasmid construct for eventual transformation into bacteria.

Lab time: ~0.5hr Incubation time: overnight (or 1-2hrs at room temp)

1. Measure concentration of vector and inserts using NanoDrop in 3rd floor common room. Record ng/ul for each fragment.
    a. Open ND1000 software for spectrophotometer on the laptop.
    b. Initialize spectrophotometer by adding a drop of ultrapure H20 to the tip. Click 'ok' to initialize on program. Avoid bubbles (will mess up reading). Kimwipe top and bottom of tip after each step.
    c. Black by adding a drop of ultrapure H20.
    d. Drop 1ul of each sample to measure. Record ng/ul measurements.

2. You will need at least 100ng vector for the ligation reaction. In order to get this, divide 100ng by the concentration of your vector in water (ng/ul). The answer you get is the volume of vector needed for each sample in ul of vector.

3. Use the website below to calculate the amount of insert needed for the reaction.
   http://www.insilico.uni-duesseldorf.de/Lig_Input.html
   Divide the answer by the concentration of the insert and you will get the volume of insert you need to add for the reaction.

4. Prepare ligation reaction
   **use one small epi-tube for each insert
   **keep buffer on ice, keep enzyme in -20C freezer (add last!)
   ***alternative (Zeller) method: 5 ul insert with 1 ul vector

   x ul vector
   y ul insert
   2ul T4 DNA ligase buffer (10x)
   1ul T4 DNA ligase
   H2O fill to volume
   = 20ul reaction solution

5. Incubate ligation tubes in 18C overnight. (mini ascidian fridge)
   *if only incubating for 1-2 hours, leave on top of bench at room temp.