Protocol: Ligation Reactions

Author: Akshitha Yarrabothula
Date: April 8, 2011
Trinh Lab

A. Obtain the following materials to set up the ligation reaction:
   1. Vector
   2. Insert
   3. T4 DNA Ligase
   4. T4 DNA Ligase Buffer
   5. Deionized Water

B. Measure the concentrations of the vector and insert using the nanodrop measuring device.

C. Set up the following four reactions, making sure to use 5 ng of vector in each of the reactions for optimization:
   1. Vector to insert ratio of 0:1
   2. Vector to insert ratio of 5:1
   3. Vector to insert ratio of 10:1
   4. Vector to insert ratio of 25:1

   Ex: Ligation of pCT17 (pETite + lucC) with lucD
   Number of base pairs of pCT17 = 3.6 kbp
   Number of base pairs of lucD = 0.9 kbp

   Normalization of length: [I] / [P] = [(0.9) / (3.6)]k

   $n_{lucD} / n_{pCT17} = 0.25k$
   $n_{lucD} = n_{pCT17}(0.25k)$

   Using n_{pCT17} = 5 ng, n_{pCT17} becomes 1.25k

k	n_{lucD}
5	6.25 ng
10	12.5 ng
25	31.25 ng

Table 1

We want to use the following amounts (in ul) of our reactants:

V:I Ratio	V	I	Buffer	Enzyme	Water	Total
1:0	1	0	1	1	7	10
1:5	1	1	1	1	6	10
1:10	1	2	1	1	5	10