Ligation Procedure - taken from OpenWetWare

8/7/10

10µL Ligation Mix

3:1 molar ratio of insert to vector (~10ng vector)

Component	Amount (ul)
dH2O	1
FimB (PCR product)	1.91
pBAD	1.68
10x Ligase Buffer	1
T4 DNA Ligase	1

*Run one sample w/out ligase and one sample w/out insert (pBAD) for controls

Calculating Insert Amount

Insert mass in ng = 3 x (insert length in bp)/(vector length in bp) x (vector mass in ng)

Insert mass of FimB = 3 x (FimB length)/ (pBAD length) x pBAD mass

Mass of FimB = 3 x 649/4104 x 100 ng

Mass of FimB = 47.4 ng

100 ng pBAD / 59.7 ng/ul = 1.68 ul

47.4 ng FimB / 24.8 ng/ul = 1.91 ul

(Concentrations from nanodrop)

The insert to vector molar ratio can have a significant effect on the outcome of a ligation and subsequent transformation step. Molar ratios can vary from a 1:1 insert to vector molar ratio to 10:1. It may be necessary to try several ratios in parallel for best results.

Procedure

1. Add 1 ul of deionized H_2O to sterile 0.6 mL tube
2. Add 1 µL ligation buffer to the tube.
   Vortex buffer before pipetting to ensure that it is well-mixed.
   Remember that the buffer contains ATP so repeated freeze, thaw cycles can degrade the ATP thereby decreasing the efficiency of ligation.

3. Add 1.91 ul of FimB to the tube.
4. Add 1.68 ul of pBAD to the tube.
5. Add 1 µL ligase.
   Vortex ligase before pipetting to ensure that it is well-mixed.
6. Let the solution sit at 22.5°C for 30 mins
7. Denature the ligase at 65°C for 10min
8. Dialyze for 20 minutes if electroporating
9. Use disks shiny side up
10. Store at -20°C

a. Digested DNA lengths
   i. pBAD: 4104
   ii. FimB: 649