SEED 2009

Day 6: Ligation

Learning Objective: In this lab you will learn what components go into a ligation reaction and the conditions under which T4 DNA ligase is able to join DNA pieces.

Background: In earlier weeks you learned how DNA can be cleaved in specific locations by restriction endonucleases. The opposite process, ***ligation*** joins DNA strands together. Enzymes called ligases can join two double-stranded pieces of DNA together or a single double-stranded piece of DNA to itself. There are two general varieties of ligation, ***blunt-end*** ligation and ***sticky-end*** ligation, which refer to the absence or presence overhangs on the ends of the DNA being ligated. Blunt-end ligation has the benefit of being very general applicable, while sticky-end has the benefit of high specificity and efficiency.

The exercise: You will be doing a "three-way" ligation to put together all of your cloning parts in a bio-bricks style assembly. This will finalize the work you do directly with DNA.

Materials:
Vector, pSB4A5 (digested with EcoRI and PstI)
Promoters, J231xx (treated with kinase)
RBS-PCR Product, B003x.LacZa-GFP (digested with XbaI & PstI)
T4 DNA Ligase
T4 DNA Ligase Buffer (10X)
1.5 mL Microcentrifuge Tubes
Deionized water

Protocol

1. For each combination of vector (1), promoter (2), and RBS-PCR Product (2), label a 1/5 mL microcentrifuge tube.

2. Add 10 ng of each: vector, promoter, and RBS-PCR (1 uL each)

3. To each tube add 1 uL of the 10X T4 DNA ligase buffer

4. Bring total volume in each tube up to 4.5 uL with sterile deionized water

5. Add 0.5 uL T4 DNA Ligase Enzyme to each reaction mixture

6. Mix by vortexing and spin to collect the liquid drop at the bottom of the tube

7. Incubate the ligations at room temperature for 2 hours