Session 6

Ligation and Transformation

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. Understand transformation of a plasmid into a host strain to complete the cloning process. Gain practical experience with the importance of selection and antibiotic resistance.

Introduction

In previous sessions we have seen how we can get pieces of DNA by isolating it from cells, cutting it with restriction digestion, and amplifying with PCR. Today we will put the DNA pieces together with a process called ligation. Ligation permanently attaches strands of DNA so that they can be put into a cell and maintained. The process of putting DNA into a cell is called transformation. After transformation, the cells containing the plasmid are selected with antibiotic completing the cloning process.

Background:

Ligation: Joining DNA Fragments

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 generally applicable, while sticky-end has the benefit of high specificity and efficiency.

In this lab session you will be doing a sticky-end ligation. The parts that we are going to put together are BBa_J45200, a protein generator for alcohol acetyltransferase I, and pSB4A5. Two sessions ago, using EcoRI and PstI, you digested the backbone and cut out the "purple"-producing enzymes. You will now insert ATF1, the "banana smell"-producing enzyme. These pieces represent everything required to express the gene in *E. coli* and produce a functional protein. The EcoRI overhang from the vector matches the EcoRI overhang in the gene, the PstI overhang in the gene matches the PstI overhang in the vector.

Transformation: Putting Foreign DNA into a Cell

Transformation is a process by which cells pickup foreign DNA from their surroundings and incorporate it into the cytoplasm. Transformation a common method for introducing plasmids into a host cell. For a transformation to be successful, the cells must be ***competent***. There are two ways in which cells can be competent, ***electrocompetent*** and

chemically competent. To make the cells competent, they are treated using cold salt solutions and glycerol. They are cryogenically frozen in the very delicate competent state and defrosted just prior to use. Cells are transformed in different ways depending on the type of competency. For electrocompetent cells, a high voltage is passed through the media in a process called electroporation. For chemically competent cells, the cells are rapidly heated for a short time and then quickly cooled. Each of these processes allows DNA to enter the cell.

Following transformation, the cells are still very fragile. They are grown in rich media to allow them to recover from damaged state. If they contain the target DNA, they begin to build up selective resistance against an antibiotic. Following this period, the cells are plated on selective media so that only successfully transformed cells can grow and survive. After incubation, single monoclonal colonies can be selected which contain and check to verify the presence of the target DNA.

Some microorganisms can be made competent easily, while other cannot be made competent with our current understanding of their physiology. *E. coli* is made competent relatively easily be the methods described above. Many different strains of *E. coli* can be used depending on the desired application or experiment. The selection of a host strain is a complicated topic that requires a significant amount of background in molecular biology and genetics. For the purpose of this course, you only need to know that the strain we are using was derived from a non-pathogenic strain of *E. coli*; it was made stable for cloning applications and does <u>not</u> contain the LacZα.

Session 6: Pre-Laboratory Exercises

Name:_____ Date:_____

1) What are the two parts we will be ligating in lab in this session?

2) Explain how the DNA we have constructed leads to the production of an enzyme. Refer to the specific parts you will be using in this lab.

3) What are the two types of transformation described in this section and how are they performed?

4) Why are cells grown in rich liquid media after transformation before plating?