Session 7

Glycerol Stocks & Sequencing Clones

Learning Objective:

In this lab you will prepare several of your clones for DNA sequencing and make glycerol stock cultures as a stable and uniform starting point for future experiments.

Introduction

In order to interpret the results of biological experiments it is necessary to know precisely with what you are working and have the ability to reproduce the biological aspects of an experiment. Molecular and microbiologists find it extremely useful to keep stocks of microbial strains cryogenically frozen, allowing experiments to be started from a consistent source. DNA sequencing is also an extremely useful tool for identifying the exact genetic composition of a strain and verifying successful constructs. Together, these two methods allow scientists to have confidence in controlling the biological basis for an experiment.

Background:

DNA Sequencing

In addition to being a useful tool for studies of biological evolution, phylogeny, microbial ecology, forensics, and healthcare, DNA sequencing provides a way to verify the identity of cloning products. There are numerous technologies still emerging in the field of DNA sequencing. New methods in DNA sequencing include increasing the throughput of existing methods (such as Emulsion PCR) or using "sequencing by synthesis" methods (such as Pyrosequencing and Reversible Terminator methods). However, chain-termination methods are still the most common and cheapest methods for sequencing.

Sanger sequencing is one of the earliest sequencing technologies and still the most frequently used. This method is a chain-termination method which closely resembles PCR; a template DNA strand binds a known primer and is amplified by a polymerase. However, unlike PCR, only one primer is used for sequencing (no reverse primer is needed) and none of the chains reach full length. Instead, before the polymerase can reach the end of the template sequence, a ***dideoxynucelotide triphosphate (ddNTP)*** is incorporated into the growing chain. ddNTPs are incorporated identically to their corresponding dNTP. Unlike a dNTP, a ddNTP prohibits additional chain elongation. Because the ddNTPs are incorporated randomly along with dNTPS, chains of various lengths are produced.

Only the final base (the ddNTP) is identified for each chain. This is achieved by separating all of the chains of different length using electrophoresis. The different bases (A, T, C, G) can be identified in the same reaction or in separate reactions. In the

classical version of Sanger sequencing, each of the four ddNTP is added to an identical reaction of dNTPs. These products are run on separate lanes and thus each band fixed length and a known base. In a more recent adaptation, fluorescent markers of different colors on the ddNTPs give each band a distinct fluorescent color depending on the terminating base at each position. This allows the sequencing to be done in one reaction and one electrophoresis.

Glycerol Stocks: Preserving the Cloned Strain

An amazing feature of cells is the ability to remain viable after long periods of time at or below -70° C. At these low temperatures, all cellular activity essentially stops. As a result, cells which are defrosted from being cryogenically frozen are the same regardless of when they are defrosted. Biologists use this fact to store stocks of cell lines for long periods of time, growing new cultures from a few frozen cells whenever needed.

Cells cannot simply be cooled to very low temperature and be expected to survive. The water on the inside and outside of the cell expands upon freezing. If nothing is done to protect the cells, they can be crushed or burst by the ice that is formed. To avoid this problem, vitrification solutions are added to the samples to increase the viscosity and decrease the freezing temperature. The vitrified samples remain preserved because they are not damaged as a result of the freezing process. In microbiology, there are two commonly used compounds for vitrification, dimethyl sulfoxide (DMSO) and glycerol. The choice of solution depends on the type of cells being frozen. Glycerol solutions (about 10-20 vol%) are suitable for *E. coli* and are what we will use for making frozen stocks of our strains.

Session 7: Pre-Laboratory Exercises

Name:_____ Date:_____

1) What is a ddNTP and how does it differ from a dNTP?

2) How are chains of different lengths produced in Sanger sequencing?

3) How are chains of different lengths separated in Sanger sequencing?

4) How is the base at each position in the DNA sequence identified in Sanger sequencing? (Hint: think about reporters)

5) Why do biologists use frozen stocks of strains?

6) Why is glycerol used for making frozen stocks of *E. coli*?