IDENTIFICATION OF MICROORGANISMS USING POLYMERASE CHAIN REACTION

Objectives

- To get familiar with the common methodology and instrumentation used in molecular biology today.
- To be able to quickly identify a microorganism based on the polymerase chain reaction (PCR) and sequences of nucleotides of a particular gene.

Introduction

In order to determine the relatedness of organisms from all domains of life (bacteria, archaea, and eukaryotes), it is important to find a trait that would be present in all living organisms. In the 1980s Karl Woese suggested the use of DNA sequences of certain common genes. Such a molecular chronometer not only needed to be present in all organisms, but also needed to have retained the same function. Woese proposed using a gene that encoded one of the RNA molecules found in ribosomes, the protein-RNA complexes on which proteins are synthesized in all prokaryotes and eukaryotes. Although there are differences in size between the ribosomes of prokaryotes and eukaryotes, the sequences of the rRNA molecules (and their corresponding rDNA genes) from all sources contain regions that are very similar, allowing the alignment and comparison of these sequences. Further, the gene is small enough to be easily sequenced and large enough to contain enough information for genetic comparisons.

Using extensive computer analyses of rRNA gene sequences, it has been possible to identify the so-called signature sequences, which are short oligonucleotides that are unique to certain groups of organisms. Not only are these signature sequences specific for each of the three domains of life, but they can also be specific within a domain or, a particular genus or even a single species. The specificity of the signature sequences is useful for many purposes. For example, signatures can be used for quickly placing a newly isolated or misclassified microorganism into its correct phylogenetic group. Using the sequence of 16S rRNA genes isolated from DNA of environmental samples allows us to define the composition of a microbial community without relying on the ability to culture all organisms present in these environments.

Before being able to sequence the 16S rRNA gene of an unknown species, we need to have a large number of copies of this gene. As we will soon see, a rather easy method of amplifying a certain gene from just a single copy of that gene is the use of polymerase chain reaction (PCR) techniques. PCR has many uses and is especially an indispensable tool in forensic sciences where millions of copies of a specific piece of DNA can be obtained from a single hair follicle or a single cell found at a crime scene.

In our lab, we will use a few sessions to study the different aspects of using PCR as applied to identification of microorganisms. Before we embark on the actual exercise, some points need to be clarified:

**Labeling:** Choose a 4-6 lettered code for your label and use this code on all your tubes and plates for your PCR-ID unknown. If you are working in a group, make sure all the members of your group know the label code because the sequencing data that we will post on the BlackBoard Website later will bear this code.

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Microfuging Samples: In many cases you may have just a single tube or an odd number of tubes that you want to microfuge. Whenever using the microfuge, make sure that it is balanced by checking that each tube has an opposite tube (with the same weight or volume) in the microfuge rotor. Always place your tubes in the microfuge so that the "hinge" of the Eppendorf tube is pointed toward the outside of the rotor. This is a convenient way to indicate the position of the pellet after centrifugation. Close the lid. You can set a spin time or do a short manual spin at this time. [Your TA will show you how to microfuge your first time samples] The rotor will stop spinning after the allotted time. The bacterial cells will have now settled into a "pellet" at the bottom of the tube. The clear solution on top is called the "supernatant". If the hinge of the Eppendorf were placed towards the outside of the rotor, the pellet would be at the bottom but to the side of the hinge of the Eppendorf.