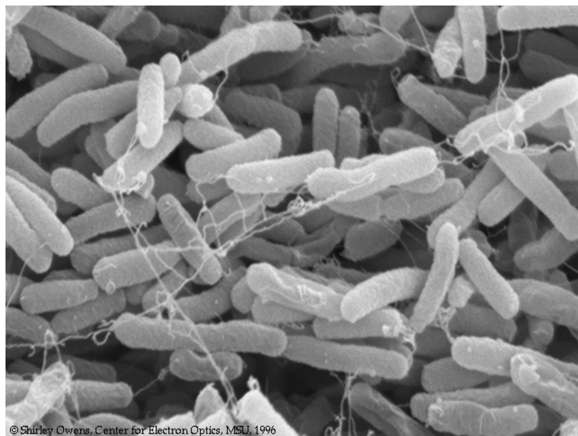
"Real Time"-PCR (RT-PCR)

A Genetic Tool for Bioremediation

Timothy J. Mayotte, Ph.D. P.E.

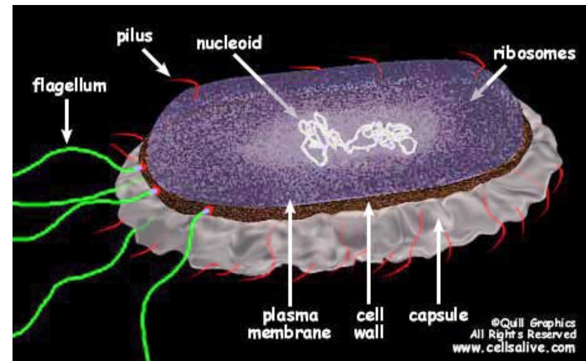
Ribisomal ribonucleic acids (rRNAs) are large molecules that serve as components of the protein synthesizing system within the cytoplasm of bacteria and archea. rRNA molecules evolved early in evolutionary history. They are therefore functionally constant and universally distributed throughout the microbial world.



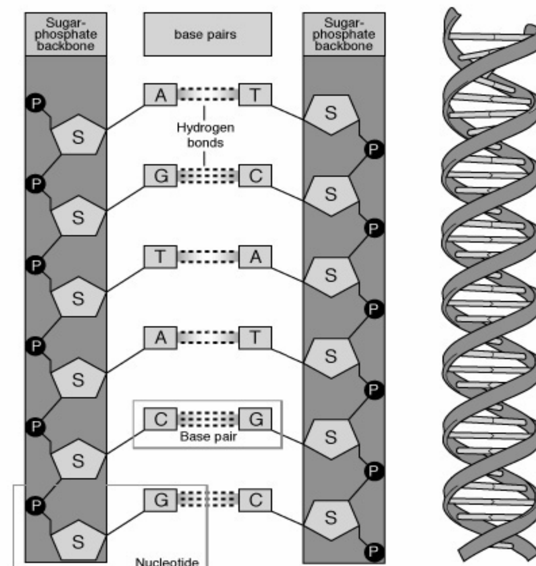
***E. coli* cells**

The genes that encode (e.g., provide the script) for the synthesis of rRNA are moderately well conserved in sequence across a diverse range (or phylogenetic distance) of the microbial world. These genes, or sequences of nucleotides of the DNA in a cell, are also large molecules. The number of different DNA sequences possible in such a large polynucleotide molecule is immense. Therefore, if two DNA sequences are compared and reveal some similarity, a phylogenetic relationship is apparent. The degree of similarity indicates the relative evolutionary relatedness.

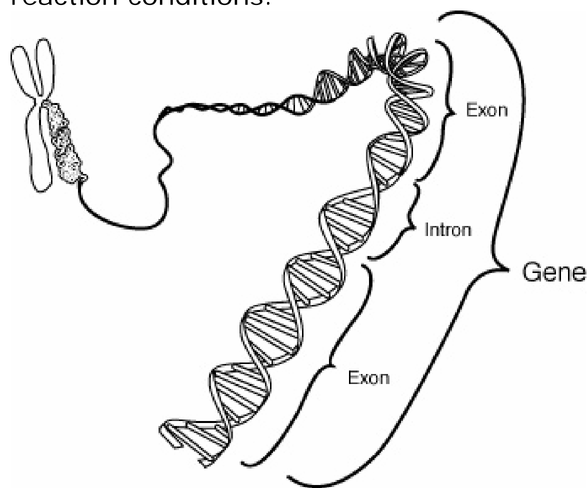
"Small sub-unit (16S) rRNA" genes are specific sequences of DNA approximately 1,500 nucleotides in length found in all microorganisms. These genes have highly conserved regions that serve well for aligning two or more sequences for comparison. The variabilities present in other regions of the DNA sequence are what make these genes excellent evolutionary chronometers.



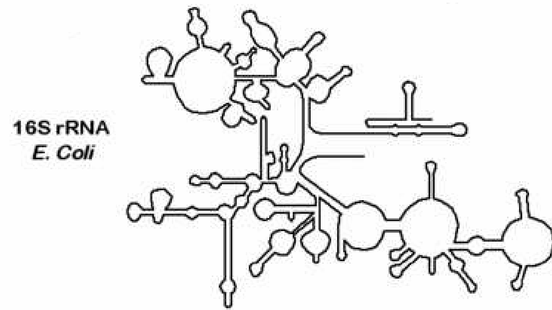
Through many years of comparative analyses and sequencing by researchers, it has become possible to identify 16S rRNA genes that are associated with specific populations and strains of microorganisms. From this information (e.g., knowledge of specific nucleotide sequence associated with the gene), it has been possible to commercially develop primers and probes, which are themselves polynucleotide sequences that facilitate replication and, therefore, amplification of the quantity of the gene from DNA extracted from a sample of source material.



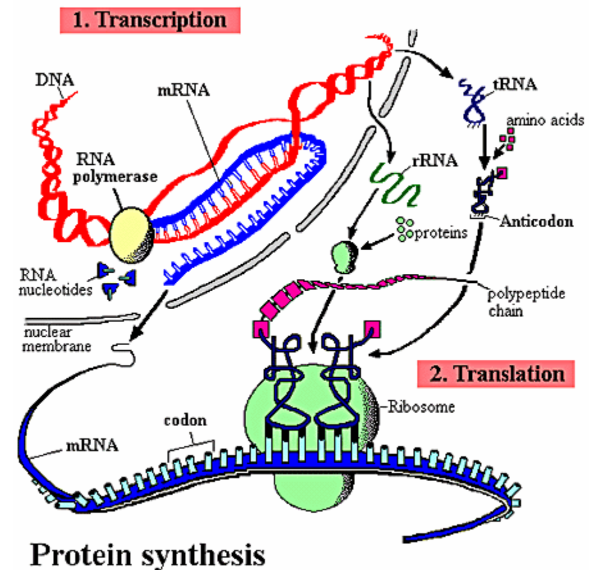
In environmental samples, such as soil and groundwater, it is possible to remove and concentrate biomass associated with microorganisms within these media. DNA is extracted from the concentrated biomass. The "total community" DNA solution can then be treated to amplify specific polynuleotide sequences using probes and primers designed to facilitate the transcription and amplification of genes like 16S rRNA in a process called polymerase chain reaction (PCR). PCR simply entails a series of "thermocycles" during which DNA is denatured (split apart), annealed (bonded) with the primer, and then elongated to form a new pair of genes by a transcription enzyme. Because the amount of DNA doubles with each thermocycle, the amplification of DNA occurs exponentially under optimum reaction conditions.



In recent years, primers for 16S rRNA genes associated with certain microorganisms, such as *Dehalococcoides* sp. and *Desulfuromonas* sp. (degraders of PCE and TCE) have been synthesized with fluorescent dyes and quenchers. When used in a specially designed thermocycling device, the increase in fluorescence detected after each cycle can generally be correlated to the concentration of the gene in the original sample. Therefore, we have a tool for determining in real time the presence and quantity of these and other specific microbial populations in environmental samples. This process has come to be known as Real-Time PCR (RT-PCR).



Mayotte Design & Engineering, PC specializes in biological methods for the treatment of environmental pollutants. To learn more about bioremediation and the use of genetic tools in the application of biotreatment technologies, contact us.



MD&E

Civil & Environmental Engineering

2407 Boston Boulevard
Lansing, Michigan 48910
Phone: 517-367-2674
Fax: 517-374-6228
Cell No: 517-388-2623
E-Mail: mayottet@comcast.net
Web: mayottedesign.net