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Problems Associated With Small Scale Plasmid DNA Isolation, Restriction Digestion and Agarose Gel Electrophoresis

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Abstract

Two experiments on alkaline lysis method of plasmid DNA isolation was carried out utilizing strains of E.coli JM109, a non- pathogenic bacteria that has been deliberately disabled. Restriction digests and gel electrophoresis carried out. The result of the first gel electrophoresis reveals only traces of RNA, hence no DNA fragments on the samples loaded labeled A to D this could be attributed to possible contamination from the procedures. For the second gel electrophoresis samples, A is plasmid free, B and C contains small fragments approximately 2.3 to 5.6 and D contains the fertility plasmids. Special enzymes termed restriction enzymes have been discovered in many different bacteria and other single-celled organisms. These restriction enzymes are able to scan along a length of DNA looking for a particular sequence of bases that they recognize. This recognition site or sequence is generally from 4 to 6 base pairs in length. Once it is located, the enzyme will attach to the DNA molecule and cut each strand of the double helix. The restriction enzyme will continue to do this along the full length of the DNA molecule which will then break into fragments. The size of these fragments is measured in base pairs or kilobase (1000 bases) pairs.

Since the recognition site or sequence of base pairs is known for each restriction enzyme, we can use this to form a detailed analysis of the sequence of bases in specific regions of the DNA in which we are interested. In the presence of specific DNA repair enzymes, DNA fragments will reanneal or stick themselves to other fragments with cut ends that are complimentary to their own end sequence. It doesn't matter if the fragment that matches the cut end comes from the same organism or from a different one. This ability of DNA to repair itself has been utilized by scientists to introduce foreign DNA into an organism. This DNA may contain genes that allow the organism to exhibit a new function or process. This would include transferring genes that will result in a change in the nutritional quality of a crop or perhaps allow a plant to grow in a region that is colder than its usual preferred area.

In this experiment, we will perform a full restriction digestion. After overnight digestion, the reaction is stopped by addition of a loading buffer. The DNA fragments are separated by Electrophoresis, a process that involves application of an electric field to cause the DNA fragments to migrate into an agarose gel. The gel is then stained with a methylene blue stain to visualize the DNA bands and may be photographed. This laboratory will take approximately 3 days. The restriction digestion takes place overnight and can be kept in the freezer until the next class period when it will be used for gel electrophoresis. The gels may be stained overnight prior to photographing or recording results.

Plasmids have been found to be wide distribution in bacteria. They are autonomously replicating extra chromosomal elements which are not essential for the growth of their host cells. However, they may encode a wide range of genetic products which may permit their host to adapt better to adverse conditions, for example, in the presence of antibiotics. In cloning work, very often the recombinant plasmids have to be isolated from their transformed hosts in order to characterize by restriction analysis and sequencing. The information from these analyses provides a basis for the mode of their presentation to the transformants and the planning of future experiment for the recombinant molecules. Among the various methods available for the preparation of plasmid DNA for rapid screening, a protocol involving the use of an alkaline solution to lyse the cells, salt precipitation to remove cell debris and chromosomal DNA and application to hibind DNA column to eliminate proteins and other contaminants has been widely employed. Gel electrophoresis, which is easily performed, rapid, inexpensive and reproducible, has become the most popular resolution technique in nucleic acid research. Gel electrophoresis using agarose, a highly purified linear polysaccharide derived from agar, has been widely used in the detection and characterization of plasmids, also the linear DNA fragments. Plasmids of sizes ranging from less than one kilo-base (kb) to over a few hundred kb can resolved by conventional agarose gel electrophoresis.

Under the influence of an electric field, the motilities of different DNA species through a gel are inversely related to their respective molecular sizes. Therefore, those with larger sized will move more slowly. If it so happens that two DNA species are of the same size, but different conformation, for example a covalently closed circular (CCC) species versus its open circular (OC) counterpart, agarose gel electrophoresis can still be used to separate them as CCC molecules are more compact, so less retarded than those of OC form.