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DNA ISOLATION and PURIFICATION

Isolation of high-molecular weight DNA has become very important with the increasing demand for DNA finger-printing, construction of genomic or sequencing libraries and PCR analysis in research laboratories and industry. Also, DNA isolation is the first step in the study of specific DNA sequences within a complex DNA population, and in the analysis of genome structure and gene expression. The quantity, quality and integrity of DNA will directly affect these results.

General requirements of effective DNA isolation are:

1. The method should yield DNA without major contaminants (protein and RNA).
2. The method should be efficient: most of the cellular DNA should be isolated and purified.
3. It should be non-selective: all species of DNA in the cells should be purified with equal efficiency.
4. The method should not physically or chemically alter DNA molecules.
5. The DNA obtained should be of high molecular weight and with few single-stranded breaks.
6. The method should be relatively fast and simple enough that it will not take a long time or much effort to prepare DNA. This is important since preparation of DNA is just the beginning of an experiment and not an end in itself.

Two of the most common problems in obtaining a high yield of high molecular weight DNA are hydrodynamic shearing and DNA degradation by non-specific DNases. To avoid these problems, some general precautions should be taken. All solutions should

contain DNase inhibitors and all glass-ware, plastic pipettes tips, centrifuge tubes, and buffers should be sterilized. The use of molecular biology grade or ultra-pure chemical reagents is strongly recommended.

There are several methods for isolation of DNA that, in general, fulfil most of the requirements listed above. All methods involve four essential steps:

- Cell breakage.
- Removal of protein and RNA.
- Concentration of DNA.
- Determination of the purity and quantity of DNA.

Each DNA isolation task should begin with careful planning of the amount of DNA needed, the purity required and an estimation of the size of DNA molecules needed. Preparation of a genomic library requires 100 to 300 µg of large molecular weight DNA (more than 100 000 bp long), completely free of protein contamination. Southern blot analysis of a single copy gene requires 5 to 10 µg of eukaryotic, genomic DNA per single gel lane. The purity and the size of this DNA is not as critical as it is for genomic library preparation. A single PCR reaction requires only a very small amount of DNA (50 to 500 ng) and can tolerate a considerable amount of contaminating proteins. However, care must be taken to remove excess RNA from the DNA preparation, because a large amount of RNA can severely inhibit the PCR reaction.

BREAKAGE OF CELLS

Cell breakage is one of the most important steps in the isolation of DNA. The primary ways to break cells are chemical, mechanical and enzymatic. The mechanical means of cell breaking, such as sonication, grinding, blending or high pressure cannot be used for DNA preparations. These procedures apply strong forces to open cells that shear the DNA into small fragments. The best procedure to open the cells and obtain intact DNA is through application of chemical (detergents) and/or enzymatic procedures. Detergents can solubilize lipids in cell membranes resulting in gentle cell lysis. In addition, detergents have an inhibitory effect on all cellular DNAases and can denature proteins, aiding the removal of proteins from the solution.

The lysis of animal cells is usually performed using anionic detergents such as SDS or Sarcosyl. These detergents are useful for isolating DNA from cells grown in cell cultures. To apply detergent lysis to tissue-derived cells, tissue is usually frozen in liquid nitrogen and gently crushed into small pieces that are accessible to detergent treatment.

Usually, plant and bacterial cells cannot be broken with detergent alone. To lyse these cells, they are first treated with enzymes that make the cell membrane accessible to detergents. Before the application of detergents, bacterial cells are treated with lysozyme. Plant cell walls can be removed by treatment with enzymes that partially or totally remove the cellulose-based cell wall. Since enzymatic treatment of plant cells is expensive and time consuming, it can be substituted by gentle grinding in liquid nitrogen. Treatment does not mechanically disrupt plant cells but forms small cracks in the cell

wall, permitting detergent access to the cell interior and subsequent lysis of cell membranes.

The method of cell breakage used should avoid strong forces that shear the DNA. DNA, in solution, should always be pipetted slowly with wide-bore pipettes (about 3 to 4 mm orifice diameter). The tip of the pipette should always be immersed in the liquid when pipetting DNA. The DNA solution should never be allowed to run down the side of a tube nor should it be vigorously shaken or vortex.