3.1 DNA can be seen!!

Hello again. Today we are going to extract DNA and we will see it. Can you believe it? Visualize the DNA!

The DNA is in the interior of cells, well protected by the nuclear membrane, by the cytoplasm and by the plasma membrane. So we have to eliminate sequentially all these components and retain only DNA. We will tell you how it is done in the laboratory of Virology, but in the additional information you'll see that you can extract DNA, for example, from a plant or from any other cell.

In some samples, we will need to isolate first the cells that we are interested in. For example, in a blood sample, we will need to delete the red blood cells (or erythrocytes), which, as you know, have no nucleus and therefore they lack DNA. To do this, it is enough to dilute the blood in distilled water so the red blood cells burst when they fill with water. After centrifuging, we will have a sediment of nucleated cells.

The next step is to break the cell membranes and to disaggregate the components. As the membranes are formed by lipids, it is best to use a detergent or a surfactant. Proteins, lipids and RNA are aggregated by means of a hypersaline solution, or eliminated using proteases and RNAses that destroy them. With any of these options, when we centrifuge, the cellular remains are retained in the pellet while the supernatant contains the DNA.

Good. We have separated the DNA from the other cellular components, but it is accompanied by residual quantities of lipids, proteins, salts, detergents, and other reagents used. To remove them, a mixture of phenol and chloroform/isoamyl alcohol is frequently used. After centrifuging, we can see a lower organic phase that contains proteins that are degraded by phenol, and an upper aqueous phase with the DNA retained by the chloroform and the isoamyl alcohol while lipids stay in the interface. We carefully remove the upper aqueous phase, which contains the DNA.

The last problem that we encounter is how to concentrate the DNA in the chloroform. Well, the DNA precipitates with cold isopropanol, so by adding this reagent and inverting the tube a few times, we can see a kind of cotton-like material. This is precisely the DNA. When we centrifuge, the DNA will be retained in the pellet that we will wash with ethanol to remove all traces of isopropanol and prior reagents. After drying we will have our sample of DNA ready to work with it. Bravoooo!

This procedure is useful when handling samples, but when the volume of samples to handle is high, or there are small amounts of cells in the starting material (and therefore little DNA), it is more practical to use commercial mini-columns that isolate and purify DNA with high performance. There are also columns for isolating and purifying RNA.

When we have purified the nucleic acid, it is usually dissolved in an adequate buffer or in double distilled water. We can estimate its concentration in a spectrophotometer, by evaluating the relationship between the absorbance at 260 nm and at 280 nm. Pure DNA will have an absorbance greater than or equal to 1.8, while if there are residual proteins, the value will be lower.

We already have our DNA. Now let's work with it! Meet me in the following video.

Thank you for your attention.