

DNA Extraction Lab: Background Information

Here's a brief handout that may help you answer the lab questions:

The purpose of this experiment is to extract DNA from Cow calf thymus gland. There are three basic steps in extracting or isolating DNA:

1. Disrupting the cell membrane
2. Dissociating proteins that are bound to your DNA or what is called the protein-DNA complex (although, you'll still have a lot of protein that will precipitate out)
3. Separating the DNA from other soluble compounds.

The first step is to place the grounded up calf thymus into a medium that maintains the DNA's chemical stability. The NaCl solution keeps the DNA biologically active and the membrane intact. To disrupt the cell membrane and release the DNA, you need to add the SDS (Sodium Dodecyl Sulfate). The SDS causes the cell membrane to dissociate thus lysing the cells. The SDS is also responsible for denaturing some proteins as well. It is important to avoid vigorous shaking since this will denature the DNA. Cold ethanol (EtOH) is then added to precipitate the DNA out and to get rid of any salts. The EtOH is used to wash/rinse the DNA that you will "spool". Unfortunately, this does not purify your DNA. What else would you expect to find with the DNA? (Consider what you have been studying about in the class.)

In a research-based lab such as those found in a pharmaceutical or biotech companies it is important to purify and isolate the DNA. This important in order to have confidence in the data collected. One way to prevent your DNA from being contaminated is to use sterile techniques and equipment. Consider the procedures outlined in the lab write up. Would it be better to use a sterile technique in the beginning or at the end? In which procedure (s) do you think the DNA is most prone to contamination? Other factors that should be considered to successfully isolate DNA are:

1. Chemical stability (i.e. hydrogen bonding, salt solution, and temperature.
2. Cellular conditions (Enzymes may degrade the DNA)
3. Mechanical stress (don't shake it vigorously!)