Lab5: DNA and DNA Extraction

DNA

Deoxyribonucleic acid, more commonly known as DNA, is a complex molecule that contains all of the information necessary to build and maintain an organism. All living things have DNA within their cells. In fact, nearly every cell in a multicellular organism possesses the full set of DNA required for that organism.

However, DNA does more than specify the structure and function of living things; it also serves as the primary unit of heredity in organisms of all types. In other words, whenever organisms reproduce, a portion of their DNA is passed along to their offspring. This transmission of all or part of an organism's DNA helps ensure a certain level of continuity from one generation to the next, while still allowing for slight changes that contribute to the diversity of life.

DNA Extraction

DNA extraction is a process of purification of DNA from sample using a combination of physical and chemical methods. Methods used to extract DNA depends on the source, age, and size of the sample.

In general, the aim to separate DNA present in the nucleus of the cell from other cellular components. Isolation of DNA is needed for genetic analysis, which is used for scientific, medical, or forensic purposes.
Scientists use DNA in a number of applications, such as introduction of DNA into cells and animals or plants, or for diagnostic purposes. On the other hand, forensic science needs to recover DNA for identification of individuals (for example rapists, petty thieves, accident, or war victims), paternity determination, and plant or animal identification.

Purpose of DNA Extraction

To obtain DNA in a relatively purified form that can be used for further investigations, i.e. PCR, sequencing, etc

Sources of DNA

Sources for DNA isolation are very diverse. Basically, it can be isolated from any living or dead organism.

- Common sources for DNA isolation include whole blood, hair, sperm, bones, nails, tissues, blood stains, saliva, buccal (cheek) swabs, epithelial cells, urine, paper cards used for sample collection, bacteria, animal tissues, or plants.
- Stored samples can come from archived tissue samples, frozen blood or tissue, exhumed bones or tissues, and ancient human, animal, or plant samples.

What are the essential components of a DNA extraction procedure?

1. Maximize DNA recovery
2. Remove inhibitors
3. Remove or inhibit nucleases
4. Maximize the quality of DNA

Basic Protocol in DNA Extraction

Most DNA extraction protocols consist of two parts

1. Enzymatic or chemical methods to remove contaminating proteins, RNA, or macromolecules
2. A technique to lyse the cells gently and solubilize the DNA.

In plants, the nucleus is protected within a nuclear membrane which is surrounded by a cell membrane and a cell wall. Four steps are used to remove and purify the DNA from the rest of the cell.

1. Lysis
2. Precipitation
3. Wash
4. Resuspension

LYSIS:

In DNA extraction from plants, this step commonly refers to the breaking of the cell wall and cellular membranes (most importantly, the plasma and nuclear membranes)

- The cell wall (made of cellulose) is disrupted by mechanical force (for example, grinding the leaves)
- Then the addition of a detergent which breaks down the cell membranes. Detergents are able to disrupt membranes due to the amphipathic (having both hydrophilic and hydrophobic regions) nature of both cellular membranes and detergent molecules. The detergent molecules are able to pull apart the membranes.
- The end result of LYSIS is that the contents of the plant cells are distributed in solution.

PRECIPITATION (In a research lab):

This a series of steps where DNA is separated from the rest of the cellular components

In a research lab, the first part of precipitation uses phenol/chloroform to remove the proteins from the DNA (Phenol denatures proteins and dissolves denatured proteins and Chloroform is also a protein denaturant).

- The second part of research lab DNA precipitation is the addition of salts. The salts interrupt the hydrogen bonds between the water and DNA molecules.
- The DNA is then precipitated from the protein in a subsequent step with isopropanol or ethanol. In the presence of cations, ethanol induces a structural change in DNA molecules that causes them to aggregate and precipitate out of solution.
- The DNA is pelleted by spinning with a centrifuge and the supernatant removed.

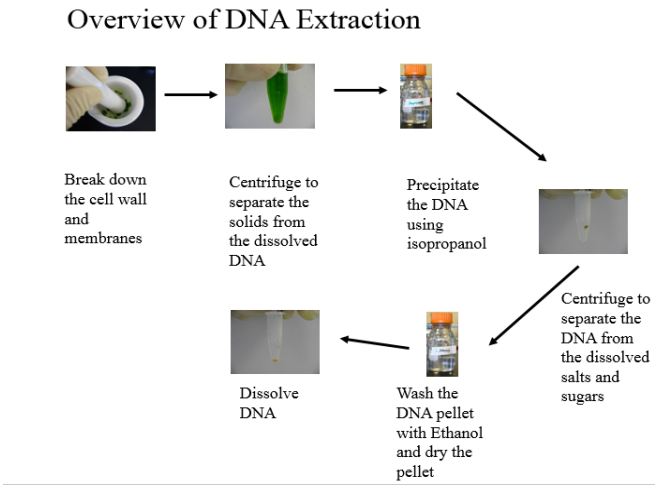
Washing and Resuspension

Washing

- The precipitated DNA is laden with acetate salts. It is "washed" with a 70% ethanol solution to remove salts and other water soluble impurities but not re-suspend the DNA.

Resuspension:

- The clean DNA is now re-suspended in a buffer to ensure stability and long term storage. The most commonly used buffer for resuspension is called 1xTE



Checking the Quality of your DNA

The product of your DNA extraction will be used in subsequent experiments

- Poor quality DNA will not perform well in PCR
- You will want to assess the quality of your DNA extraction using the following simple protocol:
    i. Mix 10 µL of DNA with 10 µL of loading buffer
    ii. Load this mixture into a 1% agarose gel
    iii. Analyze results