

Working Principal of DNA Extraction

NaCl

NaCl provides Na⁺ ions that will block negative charge from phosphates on DNA. Negatively charged phosphates on DNA cause molecules to repel each other. The Na⁺ ions will form an ionic bond with the negatively charged phosphates on the DNA, neutralizing the negative charges and allowing the DNA molecules to come together

CTAB

C-TAB is a detergent that helps lyse the cell membrane; however it is pretty poor with denaturing proteins so something with a longer tail is usually used for extraction.

Note: CTAB was established some time ago as the best detergent to use during the extraction/isolation of highly polymerized DNA from plant material. This detergent simultaneously solubilizes the plant cell wall and lipid membranes of internal organelles and denatures proteins (enzymes). Thus, the DNA is not hydrolyzed during the isolation process and as long as vortexing or vigorous shaking are avoided highly polymerized (i.e., very high intact) genomic DNA is obtained.

EDTA

EDTA is a chelating agent and has great affinity with metal ions and Mg-ion present in DNase as a cofactor and responsible for DNase action that degrades the DNA. EDTA binds with Mg-ion and nullify the action of DNase.

Tris

Tris, or tris(hydroxymethyl) aminomethane, is a common biological buffer, used throughout the DNA extraction process. During extraction from any number of sources, DNA is pH sensitive. During cell lysis, removal of unwanted cellular components and precipitation, tris is used to maintain a stable pH. Additionally, it plays a particularly important role in cell lysis.

Acetic acid

As you know it, TAE buffer contains Tris, EDTA and acetic acid, the acid reacts with water tremendously because it is concentrated. The reactivity with water causes the solution to change the pH, as we know that solutions use is based on their pH in most cases. So, acetic acid purpose is to allow dissolving of salts/EDTA by pulling down acidity in solution and thus tris functions to reduce the state brought by acid.

2-Mercaptoethanol

2-Mercaptoethanol is often included in extraction buffers designed for plant DNA extraction, because it is a strong reducing agent which can remove tannins and other polyphenols often present in the crude plant extract.

PVP

PVP (polyvinylpyrrolidone) is added to remove phenolic compounds from plant DNA extracts.

3M Sodium acetate

Cellular and histone proteins bound to the DNA can be removed either by adding a protease or by having precipitated the proteins with sodium or ammonium acetate, or extracted them with a phenol-chloroform mixture prior to the DNA-precipitation.

Chloroform isoamyl alcohol (24:1)

Chloroform isoamyl alcohol is a type of detergent. It binds to protein and lipids of cell membrane and dissolves them. By this it will disrupt the bonds that hold the cell membrane together and cause it to breakdown. It then forms complexes with these lipids and proteins, causing them to precipitate out of solution. Note that lipids and proteins are non-aqueous compound and DNA/RNA are aqueous compound. The detergent binds to non-aqueous compound.

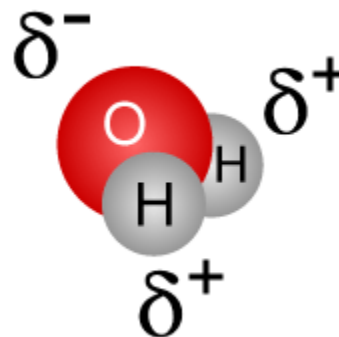
Ethanol Precipitation of DNA and RNA

Ethanol precipitation is a commonly used technique for concentrating and de-salting nucleic acids (DNA or RNA) preparations in aqueous solution.

The basic procedure is that salt and ethanol are added to the aqueous solution, which forces the precipitation of nucleic acids out of solution. After precipitation the nucleic acids can then be separated from the rest of the solution by centrifugation. The pellet is washed in cold 70% ethanol then after a further centrifugation step the ethanol is removed, and the nucleic acid pellet is allowed to dry before being resuspended in clean aqueous buffer.

Ethanol precipitation of nucleic acids is all about solubility...

Water is a polar molecule – it has a partial negative charge near the oxygen atom due the unshared pairs of electrons, and partial



positive charges near the hydrogen atoms (see the diagram on the right).

Because of these charges, polar molecules, like DNA or RNA, can interact electrostatically with the water molecules, allowing them to easily dissolve in water. Polar molecules can therefore be described as hydrophilic and non-polar molecules, which can't easily interact with water molecules, are hydrophobic. Nucleic acids are hydrophilic due to the negatively charged phosphate (PO_3^-) groups along the sugar phosphate backbone.

The role of the salt in ethanol precipitation...

The role of the salt in the protocol is to neutralize the charges on the sugar phosphate backbone. A commonly used salt is sodium acetate. In solution, sodium acetate breaks up into Na^+ and $[\text{CH}_3\text{COO}]^-$. The positively charged sodium ions neutralize the negative charge on the PO_3^- groups on the nucleic acids, making the molecule far less hydrophilic, and therefore much less soluble in water.

The role of the ethanol...

Water has a high dielectric constant, which makes it fairly difficult for the Na^+ and PO_3^- to come together. Ethanol on the other hand has a much lower dielectric constant, making it much easier for Na^+ to interact with the PO_3^- , shield its charge and make the nucleic acid less hydrophilic, causing it to drop out of solution.

The role of temperature in ethanol precipitation...

Incubation of the nucleic acid/salt/ethanol mixture at low temperatures (e.g. -20 or -80°C) is commonly cited in protocols as necessary in protocols. However, according to Maniatis *et al* (Molecular Cloning, A Laboratory Manual 2nd Edition... 2nd edition?? this is not required, as nucleic acids at concentrations as low as 20ng/mL will precipitate at $0-4^\circ\text{C}$ so incubation for 15-30 minutes on ice is sufficient.

Procedure of DNA Extraction

Plant DNA extraction

1. DNA was extracted from the leaf material of the ten days old seedling as shown in using the CTAB method with slight modifications (Suman et al., 1999).
2. 0.1-0.3g fresh weight of plant leaf material was ground with 600 μl extraction buffer to make a fine paste using pestle and mortar.
3. The ground plant tissues were transferred in labeled eppendorf tubes.
4. Microcentrifuge tubes were incubated in a water bath at 65°C for 20 min. Samples were centrifuged at 10,000 rpm for 10 min.

5. After centrifugation, samples were taken out and 600µl chloroform: isoamyl alcohol (24:1) was added. Tubes were inverted several times manually and then centrifuged at 10,000 rpm for five min.
6. By using wide-bore pipette the upper aqueous phase was removed and transferred to new 1.5 ml microfuge tube and 96% (v/v) ethanol was added. Mixed gently to precipitate the DNA and stored at -20°C for one hour to achieve maximum precipitation.
7. Samples were centrifuged at 10,000 rpm for ten min to obtain the DNA pellets and washed with 70% (v/v) chilled ethanol.
8. Pellets were air-dried and re suspended in 50µl 1X TE buffer. The Purified DNA samples were stored at -20°C for further analysis.

Microbial DNA extraction

Following protocol was used to isolate genomic DNA from the harvested microbial biomass:

1. 1 ml culture of isolate was centrifuged at 14,000 rpm in 1.5 ml eppendorf tubes. Supernatant was discarded and cell pellet was mixed with 500 µl TE buffer and 100 µl of lysozyme. Eppendorf tubes were incubated at 37°C for 1 hours.
2. Added 30 µl of 10% SDS , 5 µl of 20 mg / ml proteinase K, and 3 µl RNase mixed and incubated for 1 hour at 37°C.
3. Tubes were centrifuged at 10,000 rpm for 10 minutes to remove cell debris and the supernatant was collected in fresh tubes.
4. 690 µl chloroform: isoamyl alcohol (24:1) was added in supernatant and mixed gently by inverting the tubes to form an emulsion.
5. The tubes were centrifuged at 13,000 rpm for 15 minutes.
6. Aqueous layer was taken in separate fresh tube and discarded the remaining chloroform phase was discarded.
7. Aqueous layer was taken in fresh tube and 70 µl of 3M Na-acetate and 400 µl of isopropanol was added in it.
8. Eppendorf tubes were allowed to stay at -20°C for 1 hour.
9. Centrifuged at 14,000 rpm for 10 minutes and discarded the supernatant. Washed the DNA pellet with 70% ethanol to remove the bases. Then added absolute alcohol to dehydrate the pellet.
10. Suspended the DNA pellet in appropriate volume of deionized H₂O.
11. Quality of DNA was checked by 1% agarose gel.