**Isolation, detection and qualitative analysis of nucleic acid DNA**

There are three basic and two optional steps in a **DNA isolation**:

* Cells which are to be studied need to be collected.
* Breaking the [cell membranes](https://en.wikipedia.org/wiki/Cell_membrane) open to expose the DNA along with the cytoplasm within ([cell lysis](https://en.wikipedia.org/wiki/Lysis)).
  + Lipids from the cell membrane and the nucleus are broken down with [detergents](https://en.wikipedia.org/wiki/Detergent) and [surfactants](https://en.wikipedia.org/wiki/Surfactant).
  + Breaking [proteins](https://en.wikipedia.org/wiki/Protein) by adding a [protease](https://en.wikipedia.org/wiki/Protease) (optional).
  + Breaking [RNA](https://en.wikipedia.org/wiki/RNA) by adding an [RNase](https://en.wikipedia.org/wiki/RNase) (optional).
* The solution is treated with concentrated salt solution to make debris such as broken proteins, lipids and RNA to clump together.
* [Centrifugation](https://en.wikipedia.org/wiki/Centrifugation) of the solution, which separates the clumped cellular debris from the DNA.
* DNA purification from detergents, proteins, salts and reagents used during cell lysis step. The most commonly used procedures are:
  + [Ethanol precipitation](https://en.wikipedia.org/wiki/Ethanol_precipitation) usually by ice-cold [ethanol](https://en.wikipedia.org/wiki/Ethanol) or [isopropanol](https://en.wikipedia.org/wiki/Isopropanol). Since DNA is insoluble in these alcohols, it will aggregate together, giving a *pellet* upon [centrifugation](https://en.wikipedia.org/wiki/Centrifugation). Precipitation of DNA is improved by increasing of ionic strength, usually by adding [sodium acetate](https://en.wikipedia.org/wiki/Sodium_acetate).
  + [Phenol–chloroform extraction](https://en.wikipedia.org/wiki/Phenol%E2%80%93chloroform_extraction) in which [phenol](https://en.wikipedia.org/wiki/Phenol) [denatures](https://en.wikipedia.org/wiki/Denaturation_(biochemistry)) proteins in the sample. After centrifugation of the sample, denaturated proteins stay in the organic phase while aqueous phase containing [nucleic acid](https://en.wikipedia.org/wiki/Nucleic_acid) is mixed with the [chloroform](https://en.wikipedia.org/wiki/Chloroform) that removes phenol residues from solution.
  + [Minicolumn purification](https://en.wikipedia.org/wiki/Minicolumn_purification) that relies on the fact that the [nucleic acids](https://en.wikipedia.org/wiki/Nucleic_acid) may bind ([adsorption](https://en.wikipedia.org/wiki/Adsorption)) to the solid phase (silica or other) depending on the [pH](https://en.wikipedia.org/wiki/PH) and the salt concentration of the buffer.

Cellular and [histone](https://en.wikipedia.org/wiki/Histone) proteins bound to the DNA can be removed either by adding a [protease](https://en.wikipedia.org/wiki/Protease) or by having precipitated the proteins with [sodium](https://en.wikipedia.org/wiki/Sodium_acetate) or [ammonium acetate](https://en.wikipedia.org/wiki/Ammonium_acetate), or [extracted them with a phenol-chloroform](https://en.wikipedia.org/wiki/Phenol-chloroform_extraction) mixture prior to the DNA-precipitation.

After isolation, the DNA is dissolved in slightly alkaline buffer, usually in the [TE buffer](https://en.wikipedia.org/wiki/TE_buffer), or in [ultra-pure water](https://en.wikipedia.org/wiki/Ultrapure_water).

**DNA detection**

A diphenylamine (DPA) indicator will confirm the presence of DNA. This procedure involves chemical hydrolysis of DNA: when heated (e.g. ≥95 °C) in acid, the reaction requires a deoxyribose sugar and therefore is specific for DNA. Under these conditions, the 2-deoxyribose is converted to w-hydroxylevulinyl aldehyde, which reacts with the compound, diphenylamine, to produce a blue-colored compound. DNA concentration can be determined measuring the intensity of absorbance of the solution at the 600 nm with a [spectrophotometer](https://en.wikipedia.org/wiki/Spectrophotometer) and comparing to a [standard curve](https://en.wikipedia.org/wiki/Standard_curve) of known DNA concentrations.

**Determination of the quality of DNA (Purity)**

Measuring the intensity of absorbance of the DNA solution at wavelengths [260 nm and 280 nm](https://en.wikipedia.org/wiki/Quantification_of_nucleic_acids) is used as a measure of DNA purity. DNA absorbs [UV](https://en.wikipedia.org/wiki/UV) light at 260 and 280 nanometres, and aromatic proteins absorb UV light at 280 nm; a pure sample of DNA has a ratio of 1.8 at 260/280 and is relatively free from protein contamination. A DNA preparation that is contaminated with protein will have a 260/280 ratio lower than 1.8.