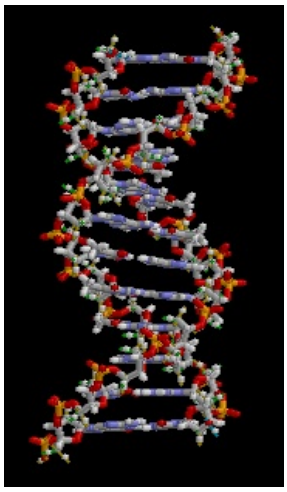
Isolation of DNA

If the molecular-genetic examination starts by the polymerase chain reaction , a very small amount of DNA is sufficient as a sample which could be part of the tissue or several cells lysed with e.g. hydroxide, surfactants or freezing and thawing. However, the quality of the template DNA affects the efficiency of amplification during PCR, and contamination contained in the sample can significantly slow down the polymerase reaction. Contaminations slow the reaction by acting as polymerase inhibitors , or by binding to the template DNA, making it inaccessible to the polymerase reaction. It is therefore often better to **isolate the DNA** from the sample.



DNA

Methods of isolation

If DNA is isolated from cells (eg, from a piece of tissue, buccal mucosal swab cells, peripheral blood leukocytes), the first step is usually to homogenize the tissue and lyse the cells . Cell and nuclear membranes are usually dissolved by the action of surfactants . At the same time, contaminating proteins and histones, to which nuclear DNA is bound, are usually partially hydrolyzed with the help of proteolytic enzymes - most often **proteinase K**. The resulting lysate contains, in addition to DNA, a number of contaminating substances - all low- and high-molecular components of tissue or cells. This is followed by extraction and purification of DNA from the lysate. In practice, various methods of DNA extraction are used, which differ in their labor and demand for chemicals and equipment on the one hand, and the yield and purity of the isolated DNA on the other.

Organic extraction

Contaminating proteins are hydrolyzed by proteases and/or denatured and precipitated. The most frequently used denaturing and precipitating agent is phenol. The precipitated proteins are then separated by shaking in chloroform and centrifugation. At the same time, the hydrophobic components of the mixture, e.g. lipids, are also removed.

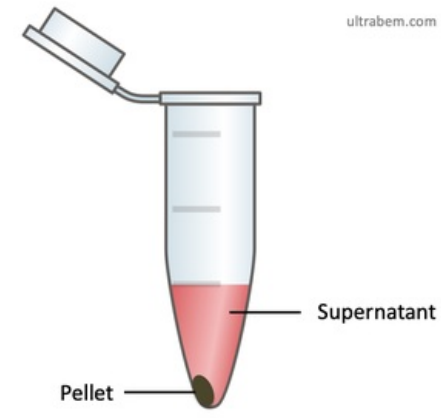
After removal of contaminating proteins and lipids, DNA is still contaminated mainly by low molecular weight substances. This is followed by the precipitation of DNA from the solution, e.g. by salting it out or using simple alcohols (ethanol or isopropanol). The precipitated DNA is then washed and redissolved in an appropriate buffer.

The advantages of organic extraction include high yield and high purity of purified DNA. The phenol-chloroform technique is therefore still the "gold standard" for nucleic acid purification. However, the method is time-consuming and involves working with dangerous chemicals. It is difficult to automate when processing a large number of samples. It is also necessary to ensure that at the end of the extraction, phenol and chloroform residues are reliably removed, which would interfere with subsequent methods (e.g. they would denature the polymerase during PCR).

Extraction using silicates

Silicon beads with a large surface area are used, which, for example, are filled with a short chromatography column. In aqueous solutions, the silicate surface is hydrated. By adding a high concentration of so-called chaotropic salts at a suitable pH, the hydrogen bridges between water molecules and the silicate surface will be broken. DNA binds to the dehydrated silicate with its phosphate groups with high affinity. Contaminating substances can then be washed away. The DNA is released from the silicate beads with a low salt buffer or distilled water.

Extraction using a silicate solid phase is relatively simple and suitable for automation. The yields and purity of the isolated DNA are slightly lower, but still quite sufficient for most applications.



Centrifugation

Magnetic separation

In magnetic separation, DNA is reversibly bound to magnetic beads coated with a suitable surface - antibodies against DNA, silicate, ion exchanger or a surface with other suitable functional groups. After binding the DNA, the beads are separated from the contaminating solution using a magnet and washed repeatedly. Purified DNA is then released from them, most often using ethanol.

Magnetic separation is suitable for automation and is mainly used for processing large numbers of samples. Yields and purity of DNA are comparable to silicate extraction, however it is possible to obtain isolated DNA in a smaller amount of solution (i.e. more concentrated). Usually this technique is more expensive.

Other techniques

Other methods of DNA extraction are based, for example, on salting out DNA from a solution, separation of the mixture by centrifugation on a gradient of caessium chloride, ion exchange chromatography, etc.

Phenol-chloroform method of DNA isolation

The phenol-chloroform method is the most widely used technique for organic DNA extraction. Although it is more time-consuming and involves working with dangerous chemicals, it makes it possible to achieve high yields and obtain virtually pure DNA. In a slight modification, it is also used for RNA isolation.

Tissue homogenization

The first step is to homogenize the tissue in a buffer that contains a surfactant (e.g. sodium dodecyl sulfate , SDS). Surfactant dissolves cell membranes. Cell disintegration is facilitated by the addition of **proteinase K.** This effective bacterial enzyme, which has a temperature optimum of around 60°C, does not require calcium or magnesium ions, and which is not inhibited by concentrated surfactants, partially hydrolyzes contaminating proteins. In addition, it very efficiently cleaves DNases, which could degrade the isolated DNA.

Protein removal

This is followed by denaturation of proteins and their precipitation with a mixture of phenol and chloroform. Phenol-chloroform mixture is immiscible with water. At solution pH above 7.6, DNA is dissociated and remains dissolved in the aqueous phase, while proteins denature and fall into the hydrophobic phase. A small amount of isoamyl alcohol is added to the mixture to facilitate phase separation and prevent foaming when processing protein- rich samples . Since even traces of phenol could inhibit the polymerase reaction, the sample is finally purified by shaking with chloroform alone.

Removal of non-protein contaminants

The DNA is then precipitated from the aqueous solution. First, a suitable salt (sodium chloride, sodium acetate, etc.) is added in high concentration. Its ions create a hydration shell, thereby removing the DNA solvent (so-called salting out). Then a slightly polar substance (e.g. ethanol or isopropanol) is added to the mixture; decreasing solvent polarity leads to DNA precipitation.

The precipitated DNA is washed in 70% ethanol. Alcohol in this concentration dissolves the remains of salts and proteins, but the DNA itself remains undissolved.

The isolated and purified DNA is then usually dissolved in an alkaline buffer containing ethylenediaminetetraacetic acid (EDTA). This complex-forming substance facilitates the dissolution of DNA, and by absorbing Ca 2+ acts as a DNase inhibitor and thus stabilizes the sample.