Dept. Medical Microbiol., College of Medicine
Omar Abd Ulkareem Ali, M.Sc. Gen. Engineering

Methods for extracting genomic DNA from whole blood samples

Human health studies in the field of molecular biology require the use of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein samples. Successful use of available downstream applications will benefit from the use of high-quantity and high-quality DNA. Therefore, nucleic acid extraction is a ***key step*** in laboratory procedures required to perform further molecular research applications. It is essential to choose a suitable extraction method, and there are a few considerations to be made when evaluating the available options. These may include technical requirements, time efficiency, cost-effectiveness, as well as biological specimens to be used and their collection and storage requirements.

Whole blood is one of many different available sources to obtain genomic DNA (gDNA), and it has been widely used in facilities around the world. Therefore, we will focus on DNA extraction protocols using whole blood samples.

Main types of DNA extraction methods from human whole blood samples

Table 1 shows the main categories and subcategories of DNA extraction methods from whole blood samples that are generally used in research facilities worldwide. Laboratory reagents commonly used for each stage of the nucleic acid extraction protocol are included in this table in order to highlight similarities and differences between them.

In recent years, some of these protocols have been adapted to micro-devices that develop miniaturized total chemical analysis systems or micro-fluidic genetic analysis microchips. However, we will limit the scope of our review to those techniques that are available for macroscale and microscale nucleic acid extraction.

Table 1: DNA extraction methods commonly used for extraction from whole blood samples

DNA extraction method (main category)	DNA extraction method (subcategory)	DNA extraction protocol stage			
		Cell lysis	**Denaturation of nucleoproteins/ inactivation of cellular enzymes**	**Removal of contaminants**	**DNA precipitation**
Solution-based DNA extraction methods	Salting out methods	• SDS • SDS/proteinase K • Triton X-100	• Proteinase K • Laundry powder	• Potassium acetate • Sodium acetate • Sodium chloride	• Ethanol • Isopropanol
	Organic solvent/ chaotropes methods	• SDS • SDS/proteinase K	• Guanidine thiocyanate • Phenol	• Phenol • Phenol–chloroform • Phenol–chloroform, isoamyl alcohol	• Sodium acetate/ ethanol • Sodium acetate/ isopropanol
Solid-phase DNA extraction Methods (Adsorption methods)	Glass milk/silica resin methods	• SDS • Triton X-100	• Guanidine thiocyanate	• Glass milk (silica in chaotropic buffer) • Silica matrix • Diatomaceous earth	• Ethanol • Isopropanol
	Anion exchange methods	• Heat	• Chelex • Chelex/proteinase K	• Chelex	• N/A
	Magnetic beads methods	• SDS	• N/A	• Sodium chloride/ polyethylene glycol	• Magnetic beads

Abbreviations: DNA, deoxyribonucleic acid; N/A, not applicable; SDS, sodium dodecyl sulfate.

Solution-based DNA extraction methods

As previously mentioned, solution-based protocols have two main approaches: 1solution-based methods using organic solvents and 2 those based on a salting out technique. Further description of both methods follows.

Solution-based DNA extraction methods using organic solvents

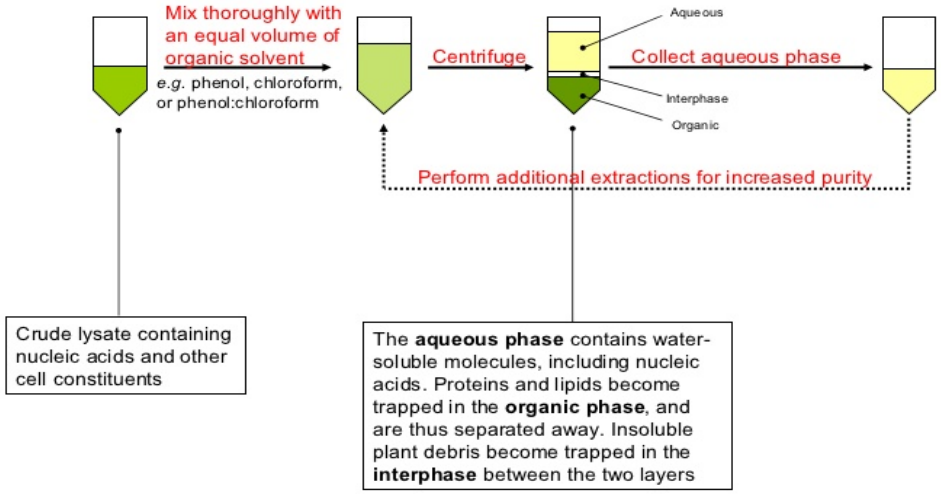
DNA extraction protocols using organic solvents derived originally from a series of related RNA extraction methods.

Some of the main steps used in these methods are:

1. cell lysis: undertaken by adding a detergent/chaotropic-containing solution, including SDS or N-Lauroyl sarcosine
2. inactivation of DNases and RNases, usually through the use of organic solvents
3. purification of DNA and removal of RNA, lipids, and proteins
4. resuspension of extracted nucleic acids.

Phenol is a carbolic acid that denatures proteins quickly, but it is highly corrosive, toxic, and flammable. This organic solvent is usually added to the sample and then, using centrifugal force, a biphasic emulsion is obtained. The top hydrophilic layer contains diluted DNA, and the bottom hydrophobic layer is composed of organic solvents, cellular debris, proteins, and other hydrophobic compounds. DNA is then precipitated after centrifugation by adding high concentrations of salt, such as sodium acetate, and ethanol or isopropanol in 2:1 or 1:1 ratios. Excess salt can be removed by adding 70% ethanol, and the sample is then centrifuged to collect the DNA pellet, which can be resuspended in sterile distilled water or TE buffer (10 mM Tris; 1 mM EDTA pH 8.0).

Phenol–chloroform needs to be equilibrated to an adequate pH, and protocol conditions should be optimized. In an effort to improve the safety and ease of use of these protocols, certain modifications have been introduced in order to avoid physical contact with solvents. These include incorporating a silica gel polymer or replacing solvents with other substances like benzyl alcohol.



Solution-based DNA extraction methods using salting out

Some nucleic acid extraction techniques that avoid the use of organic solvents have also been developed over the years. In 1988, Miller *et al* published a protocol that achieved DNA purification through protein precipitation at high salt concentration. The traditional protocol involves initial cell disruption and digestion with SDS–proteinase K, followed by the addition of high concentrations of salts, usually 6 M sodium chloride. The mixture is then centrifuged to allow proteins to precipitate to the bottom, with the supernatant containing DNA then transferred to a new vial. DNA is then precipitated using ethanol or isopropanol in the same manner as described for organic solvent methods.

Solid-phase DNA extraction methods (Adsorption methods)

Solid-phase extraction methods for DNA extraction from blood samples were initially described in 1989 by McCormick, who published a technique using siliceous based insoluble particles, chemically similar to phenol, which interact with proteins to allow DNA purification.

These techniques will absorb DNA under particular pH and salt content conditions through any of the following principles:

1. hydrogen binding in the presence of a chaotropic agent to a hydrophilic matrix
2. ionic exchange using an anion exchanger under aqueous conditions
3. affinity and size exclusion mechanisms.

Most of these methods follow a series of similar steps to achieve cell disruption, DNA adsorption, nucleic acid washing, and final elution. Most solid-phase techniques use a spin column to bind nucleic acid under centrifugal force.

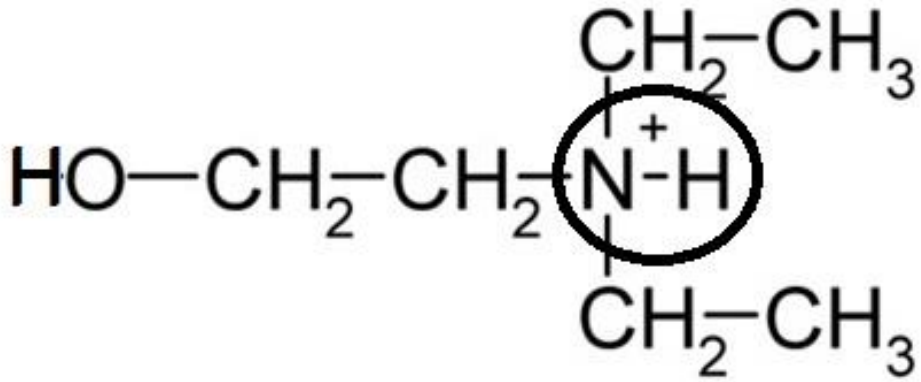
DNA extraction methods using silica and silica matrices

Silica matrices have unique properties for DNA binding, they are positively charged and have high affinity toward the negative charge of the DNA backbone. High salt conditions and pH are achieved using sodium cations, which bind tightly to the negatively charged oxygen in the phosphate backbone of DNA.

Contaminants are removed with a series of washing steps, followed by DNA elution under low ionic strength (pH ≥7) using TE buffer or sterile distilled water.

In these protocols, blood samples are incubated for a few minutes with a lysis buffer. Most protocols take about 40 minutes to 1 hour to complete, producing high yields of DNA with minimum contamination.

A substance that contains high amounts of silica Diethylaminoethanol (DEAE), it binds DNA in the presence of chaotropic agents, followed by washing with a buffer containing alcohol, and finally DNA is eluted in a low salt buffer or sterile distilled water.



DNA extraction using anion exchange resins

Positively charged chemical substances able to bind to negatively charged nucleic acids or contaminants or enzymes, such as nucleases, are called anion exchange resins, and they have also been used as part of DNA extraction protocols from blood samples.

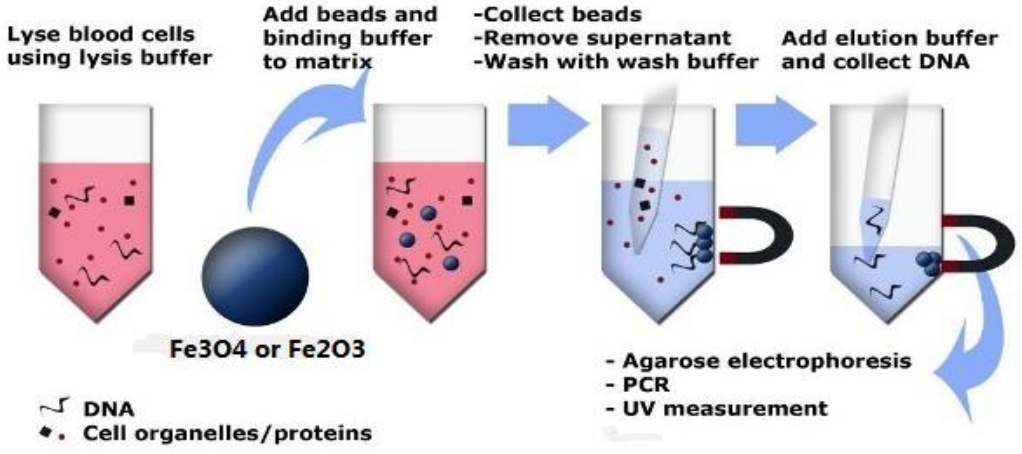
These methods require small sample volumes (under 1 ml of blood) and are usually performed in a single tube reaction with different steps and reagents involved. Blood samples could be lysed using proteinase K and/or incubation at high temperature.

DNA extraction methods using magnetic beads

Nucleic acid extraction techniques using magnetic separation have been emerging since the early 1990s.

Magnetic particles are made of one or several magnetic cores, such as magnetite (Fe3O4) or maghemite (gamma Fe2O3), coated with a matrix of polymers, silica, or hydroxyapatite with terminal functionalized groups. 30 µl of whole blood is mixed with an equal volume of 1% (weight/volume [w/v]) SDS solution. The tube is mixed by inversion two or three times and incubated at room temperature for 1 minute. Ten microliters of magnetic nanoparticles is added to this mixture, followed by the addition of 75 µl of binding buffer (1.25 M sodium chloride and 10% polyethylene glycol 6000). The solution is mixed by inversion and allowed to rest for 3 minutes at room temperature, and the magnetic pellet is immobilized using an external magnet to discard the supernatant. The magnetic pellet is washed with 70% ethanol and dried.

The magnetic pellet is resuspended in 50 µl of TE buffer, and magnetic particles bound to DNA are eluted by incubation at 65°C with continuous agitation.



Storage of DNA

- DNA can be stored at 4oC for extended periods, however for long term storage, - 20oC is usually utilized.
- Avoid repetitive freeze thawing of DNA, since this can cause degradation.
- The storage of DNA at 4ºC is better than -20ºC and storage at room temp dried with stabilizer is even better.

Estimation of quantity and quality of isolated DNA

Nucleic acids absorb light at 260 nm ,the A260 reading should be between 0.1–1.0. However, DNA is not the only molecule that can absorb UV light at 260nm. Since RNA also has a great absorbance at 260nm will contribute to the total measurement at 260nm.

Using TE buffer as the diluent, make an appropriate dilution of your DNA depending on the size of the cuvettes available (e.g. for 1ml cuvettes, dilute 10 microliter DNA solution in 990 micro liters of TE). Determine the absorbance of DNA at 260 nm using TE as the reference solution (i.e. as a blank)

one optical density unit (or absorbance unit) at 260 nm is equivalent to 50 microgram/ml of DNA and 40 microgram/ml of RNA. Multiply the absorbance reading by the conversion factor and the dilution factor to find the concentration of nucleic acid.

- DNA Concentration (microg/ml) = (A260) x dilution factor x 50microg/ml

Total yield is obtained by multiplying the DNA concentration by the final total purified sample volume:

- DNA Yield (microgram) = DNA Concentration x Total Sample Volume (ml)

  - DNA UV absorbance at 260 nm.
  - protein UV absorbance at 280 nm .
  - The ratio of the absorbance at 260 nm/280 nm is a measure of the purity of a DNA sample from protein contamination; it should be between 1.7 and 2.0

- DNA sample purity = 260 nm/280 nm

If the 260 nm/280 nm ratio is less than 1.7, the nucleic acid preparation may be contaminated with unacceptable amounts of protein and not of sufficient purity for use. Such a sample can be improved by reprecipitating the nucleic acid step of the isolation procedure,
A DNA preparation with a ratio higher than 2.0 may be contaminated with RNA. If RNA may interfere or react with DNA detection components, RNase should be used to remove the contaminating RNA.

Example 1. a DNA total volume of 0.5 ml., diluted 1:100, the absorbance reading of 0.200 at 260 nm. and 0.102 at 280nm:

- To obtain the **concentration** in micro gram/ml:

  0.2 x 100 x 50 microg/ml =1000 microg/ml

- The **yield** of the sample is calculated using the volume of the preparation. If in the case illustrated above, the DNA was eluted or resuspended in a volume of 0.5 ml :

  1000 microg/ml x 0.5 ml = 500 microgram

- The **purity** of DNA sample measured by the ratio of the absorbance at 260/280 nm

  0.200nm / 0.102nm = 1.960

Note: If you have a NanoDrop machine, that will be easy. Just use water as blank, and read the OD (260/280nm), the software will estimate the concentration for you right away.