University: Basrah
College: Veterinary Medicine
Stage: Postgraduate
Course: Practicle Molecular Biology
Topic: Nucleic Acid Extraction
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Extraction and purification of nucleic acids

The field of biology relies heavily on molecular methods, such as PCR and sequencing, that target nucleic acid. Many methods have been described to extract and purify nucleic acids. There are two basic steps in the extraction and purification of nucleic acid: (1) Extraction: it is release of the nucleic acid from the cell (2) Purification: it is separation of the nucleic acid from other cellular structure components.

Extraction

The most common methods used to release nucleic acids from cells involve the use of heat, osmotic shock, detergents, chaotropic salts and organic solvents, either alone or in combination, all of which lead to denaturation of proteins.

1- **Heat:** The simplest method to release nucleic acids from cells is to heat the sample (typically to 45–100°C). This alone is sufficient for obtaining nucleic acids from cell where nuclease contamination is expected to be minimal. If nucleases are expected to be present, the heating should be carried out in the presence of a chelating agent such as ethylenediamine-tetraacetic acid (EDTA). Ethylene glycol-bis (2-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA) may also be added. EDTA and sometimes EGTA is included in buffers used with DNA, because they chelate divalent and trivalent cations, which are a required cofactor for certain nucleases. The presence of these chelators therefore protects DNA from degradation. DNase is itself irreversibly inactivated by heating to 65°C, but the disintegration of cell can occur faster. Cellular DNA can therefore be lost if cells are heated to 65°C in the presence of DNase and the absence of EDTA. Neither EDTA nor

EGTA inactivates RNase, so other Rnase inhibiting agents may be required if one plans to extract RNA by simple thermal destabilization. Rnase inhibitors include ribonucleoside-vanadyl complex and RNasin, as well as other commercially available proprietary reagents (e.g., RNAsecure, Ambion; RNase Out, Invitrogen).

2- **Osmotic shock:** Osmotic shock can be used to disintegrate the cell membrane, but others are resistant to this treatment. This phenomenon may therefore facilitate some extraction protocols, but is generally not relied on, since exchanges of buffers having very different osmolarities may lead to unintentional release and potential loss of nucleic acids.

3- **Detergent:** Sodium dodecyl sulfate (SDS) is an ionic detergent frequently added to extraction buffers. SDS may be used alone, but is typically used in combination with heating and enzymatic digestion of proteins to effect the release of nucleic acids, alternative to treatment with heat, SDS and proteinase K digestion for extraction of nucleic acid from cells.

4- **Chaotropic salts:** Chaotropic salts such as sodium iodide (NaI) or guanidinium thiocyanate (GTC) can also disrupt cells by denaturing proteins. The guanidinium and thiocyanate ions of GTC are particularly strong denaturants and consequently facilitate disintegration of cell membrane while simultaneously inactivating nucleases. Because GTC so effectively inactivates RNase, it remains a common ingredient in RNA extraction protocols since it is first choice.

5- **Organic solvents**: The nonpolar organic solvent such as phenol also has a long history of use in nucleic acid extraction. Although typically used for its ability to extract proteins from nucleic acid solutions, phenol will simultaneously effect the disruption of cell membrane by denaturing the proteins.

Purification

Once nucleic acids have been released from the cells, it may be necessary to separate the nucleic acids from other macromolecules in the lysate. There are four general approaches to do this task: (1) organic purification (2) differential precipitation (3) solid-phase extraction (4) density gradient fractionation.

1- **Organic purification:** In organic purification, proteins and lipids are purified from a nucleic acid solution using an alkaline buffer–saturated phenol or phenol plus chloroform (1:1). A small amount of isoamyl alcohol (IAA) is also commonly added to the chloroform as an antifoaming agent (phenol:chloroform:IAA; 25:24:1). After emulsification, the aqueous and organic phases are separated by centrifugation. Nucleic acids remain soluble in the upper aqueous phase, which is harvested, whereas lipids and proteins in the lower organic phase. The nucleic acids are removed from the aqueous phase by alcohol precipitation. In ether extractions, the aqueous phase is on the bottom. After removing the bulk of the ether by pipetting, residual amounts can be easily removed by evaporation by warming the sample with the lid open.
2- **Differential precipitation:** When separating proteins and nucleic acids by differential precipitation, the proteins can be “salted out” directly with ammonium sulfate or precipitated as SDS–protein complexes by the addition of salt to SDS-containing lysates. In either case, the proteins are removed by centrifugation followed by recovery of the DNA-containing supernatant. Instead of precipitating protein, DNA can be selectively precipitated from buffers of low ionic strength with the cationic surfactant cetyltrimethylammonium bromide (CTAB). In this case, the proteins are

discarded with the supernatant, and the DNA in the pellet is resuspended in a high-ionic-strength buffer.

3- **Solid-phase purification:** One of the most common purification techniques in use today is a solid-phase purification, which use the selective binding of nucleic acids to silica under conditions of high salt concentration and low pH, and their subsequent elution at low salt concentrations. This phenomenon is the basis for a wide variety of commercial nucleic acid extraction kits, in which the silica is supplied as a fine particle suspension ("glass milk") or a silica-impregnated membrane.
4- **Density gradients:** Nucleic acids can be very effectively and cleanly separated from other macromolecules using density gradient centrifugation. DNA, RNA, proteins, and lipids have sufficiently different buoyant densities that they can be separated in equilibrium buoyant density gradients in an ultracentrifuge. CsCl are commonly used as gradient media for this purpose. DNA can be banded in gradients of salt. RNA, because of its high buoyant density, will pellet in CsCl gradients.

Commercial extraction kits

Commercial extraction or purification kits or reagents are available that rely on the extraction principles outlined above (e.g., MasterPure™, Epicenter; Gentra® Puregene®, Qiagen).

Nucleic acid quantitation

In molecular biology, quantitation of nucleic acids is commonly performed to determine the average concentrations of DNA or RNApresent in a mixture, as well as their purity. Reactions that use nucleic acids often require particular amounts and purity for optimum performance. One of the more commonly used practices to

quantitate DNA or RNA is the use of spectrophotometric analysis using a spectrophotometer (Nanodrop). A spectrophotometer is able to determine the average concentrations of the nucleic acids DNA or RNA present in a mixture, as well as their purity. Spectrophotometric analysis is based on the principles that nucleic acids absorb ultraviolet light in a specific pattern. In the case of DNA and RNA, a sample is exposed to ultraviolet light at a wavelength of 260 nanometres (nm) and a photo-detector measures the light that passes through the sample. Some of the ultraviolet light will pass through and some will be absorbed by the DNA / RNA. The more light absorbed by the sample, the higher the nucleic acid concentration in the sample.

A260 as Quantity Measurement

The "A260 unit" is used as a quantity measure for nucleic acids. One A260 unit is the amount of nucleic acid contained in 1 mL and producing an OD of 1.

1 A260 unit dsDNA = 50 µg/ml

1 A260 unit ssDNA = 33 µg/ml

1 A260 unit ssRNA = 40 µg/ml

Nucleic Acids Purity (260/280 Ratios)

It is common for nucleic acid samples to be contaminated with other molecules (i.e. proteins, organic compounds, other). The secondary benefit of using spectrophotometric analysis for nucleic acid quantitation is the ability to determine sample purity using the 260 nm/280 nm ratio. Nucleic acids have an A260 nm and proteins have A280 nm. The A260/A280 ratio is therefore indicative of the degree of purity of the nucleic acid. A260/A280 ratios of 1.6-1.8 or 1.8-2.0 are usually acceptable for DNA and RNA, respectively.