1

Overview of Nucleic Acid Purification and Isolation

Purification or isolation of nucleic acids is the first step in most molecular biology studies and all recombinant DNA techniques.
As a plethora of methods exists for extraction and purification of nucleic acid, researchers usually choose the technique most suited to their:

- Target nucleic acid (ssDNA, dsDNA, total RNA, mRNA, etc.)
- Source organism (mammalian, lower eukaryotes, plants, prokaryotes, viruses, etc.)
- Starting material (whole organ, tissue, cell culture, blood, etc.)
- Desired results (yield, purity, purification time required, etc.)
- Downstream application (PCR, cloning, labeling, blotting, RT-PCR, cDNA synthesis, RNase protection assays, etc.)

Extraction methods

The extraction of nucleic acids from biological material requires cell lysis, inactivation of cellular nucleases, and separation of the desired nucleic acid from cellular debris.

Often, the ideal lysis procedure is a compromise. It must be rigorous enough to fragment the complex starting material (*e.g.*, blood, tissue), yet gentle enough to preserve the target nucleic acid. Common lysis procedures include:

- Mechanical disruption (for example, grinding, hypotonic lysis)
- Chemical treatment (for example, detergent lysis, chaotropic agents, thiol reduction)
- Enzymatic digestion (for example, proteases)

Cell membrane disruption and inactivation of intracellular nucleases may be combined. For instance, a single solution may contain detergents to solubilize cell membranes and strong chaotropic salts to inactivate intracellular enzymes.

After cell lysis and nuclease inactivation, cellular debris may easily be removed by filtration or precipitation.

Purification methods

Methods for purifying nucleic acids from cell extracts are often combinations of extraction/precipitation, chromatography, centrifugation, electrophoresis, and affinity separation.

Extraction/precipitation
Solvent extraction is often used to eliminate contaminants from nucleic acids. For example, a combination of phenol and chloroform are frequently used to remove proteins.

Selective precipitation can also purify nucleic acids. For example, high concentrations of salt (“salting out”) or changes in pH can be used to precipitate proteins.

Precipitation may also be used to concentrate nucleic acids. For example, the target nucleic acids are often precipitated with isopropanol or ethanol. If the amount of target nucleic acid is low, an inert carrier (such as glycogen) can be added to the mixture to increase precipitation efficiency.

Chromatography

Chromatography methods may utilize gel filtration, ion exchange, selective adsorption, or affinity binding.

Gel filtration exploits the molecular sieving properties of porous gel particles. A matrix with defined pore size allows smaller molecules to enter the pores by diffusion whereas bigger molecules are excluded from the pores and eluted at the void volume. Thus, molecules are eluted in order of decreasing molecular size.

Ion exchange chromatography depends on an electrostatic interaction between a target molecule and a functional group on the column matrix. The technique allows concentration and separation of molecules from a large volume in a short time. Nucleic acids – highly negatively charged, linear polyanions – can be eluted from ion exchange columns with simple salt buffers.

In *adsorption chromatography*, nucleic acids adsorb selectively onto silica or glass in the presence of certain salts (such as chaotropic salts), while other biological molecules do not. A low salt buffer or water then elutes the nucleic acids, thereby producing a sample that may be used directly in downstream applications.

Affinity chromatography is a highly specific adaptation of adsorption chromatography. An immobilized ligand recognizes and binds a particular structure on a biomolecule. Washes then remove unbound components (with different structures). Finally, a "competitor molecule" (which also recognizes the immobilized ligand) floods the binding sites on the affinity matrix, releasing the bound biomolecule.

Centrifugation

Selective centrifugation is a powerful purification method. For example, ultracentrifugation in self-forming CsCl gradients at high g-forces has long been used for plasmid purification.

Frequently, centrifugation is combined with another method. For example:

- Spin column chromatography combines gel filtration and centrifugation to purify DNA or RNA from smaller contaminants (salts, nucleotides, etc.), for buffer exchange, or for size selection.
- Some procedures combine selective adsorption on a chromatographic matrix (see above) with centrifugal elution to selectively purify one type of nucleic acid.

Electrophoresis

Nucleic acids may be separated electrophoretically according to their size. This separation is most commonly done on agarose gels. In the presence of ethidium bromide, the separated nucleic acids may be seen under UV light.

Electrophoretic separation is also frequently used to determine size and purity of DNA. For example, after PCR, electrophoresis is used to quickly check product length and purity (absence of byproducts).

Affinity purification

In recent years, more and more purification methods have combined affinity immobilization of nucleic acids with magnetic separation. For instance, poly(A)$^+$ mRNA may be bound to streptavidin-coated magnetic particles by biotin-labeled oligo(dT) and the particle complex removed from the solution (and unbound contaminants) with a magnet. This solid phase technique simplifies nucleic acid purification, since it can replace several centrifugation, organic extraction, and phase separation steps with a single, rapid magnetic separation step.