

## Review Article

# DNA, RNA, and Protein Extraction: The Past and The Present

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Extraction of DNA, RNA, and protein is the basic method used in molecular biology. These biomolecules can be isolated from any biological material for subsequent downstream processes, analytical, or preparative purposes. In the past, the process of extraction and purification of nucleic acids used to be complicated, time-consuming, labor-intensive, and limited in terms of overall throughput. Currently, there are many specialized methods that can be used to extract pure biomolecules, such as solution-based and column-based protocols. Manual method has certainly come a long way over time with various commercial offerings which included complete kits containing most of the components needed to isolate nucleic acid, but most of them require repeated centrifugation steps, followed by removal of supernatants depending on the type of specimen and additional mechanical treatment. Automated systems designed for medium-to-large laboratories have grown in demand over recent years. It is an alternative to labor-intensive manual methods. The technology should allow a high throughput of samples; the yield, purity, reproducibility, and scalability of the biomolecules as well as the speed, accuracy, and reliability of the assay should be maximal, while minimizing the risk of cross-contamination.

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## 1. Introduction of Biomolecules Extraction

The extraction of biomolecules, DNA, RNA, and protein, is the most crucial method used in molecular biology [1]. It is the starting point for downstream processes and product development including diagnostic kits. DNA, RNA, and protein can be isolated from any biological material such as living or conserved tissues, cells, virus particles, or other samples for analytical or preparative purposes [1].

Two categories that involved in purifying DNA include the isolation of recombinant DNA constructs such as plasmids or bacteriophage and the isolation of chromosomal or genomic DNA from prokaryotic or eukaryotic organisms [2]. Generally, successful nucleic acid purification required four important steps: effective disruption of cells or tissue; denaturation of nucleoprotein complexes; inactivation of nucleases, for example, RNase for RNA extraction and DNase for DNA extraction; away from contamination [2]. The target nucleic acid should be free of contaminants including

protein, carbohydrate, lipids, or other nucleic acid, for example, DNA free of RNA or RNA free of DNA [3]. Quality and also integrity of the isolated nucleic acid will directly affect the results of all succeeding scientific research [4].

On the other hand, RNA is an unstable molecule and has a very short half-life once extracted from the cell or tissues [5]. There are several types of naturally occurring RNA including ribosomal RNA (rRNA) (80%–90%), messenger RNA (mRNA) (2.5%–5%) and transfer RNA (tRNA) [3]. Special care and precautions are required for RNA isolation as it is susceptible to degradation [3, 6]. RNA is especially unstable due to the ubiquitous presence of RNases which are enzymes present in blood, all tissues, as well as most bacteria and fungi in the environment [3, 5]. Strong denaturants has always been used in intact RNA isolation to inhibit endogenous RNases [2]. RNA extraction relies on good laboratory technique and RNase-free technique. RNase is heat-stable and refolds following heat denaturation. They are difficult to inactivate as they do not require cofactors [2].

The most common isolation methods can be divided into two classes: utilization of 4 M guanidinium thiocyanate and utilization of phenol and SDS [2].

Purification of protein is one of the most important parts in protein research to understand their function, as they may partly or completely be involved in any DNA synthesis activity. Protein purification is required to determine its unique characteristics, including size, charge, shape, and function [7]. Cell-based extraction is the starting step for almost all protein purification. Protein can be extracted by a few methods such as detergent lysis, shearing force, treatment with low ionic salt (salting out), and rapid changes in pressure, which aimed to weaken and break the membranes surrounding the cell to allow proteins to escape [7]. Some factors should be considered when handling proteins. Normally, protein extraction is performed at a very low temperature (4°C) as proteins are easily denatured once they are released from the cells. Buffer condition is one of the major factors that need to be considered. Specific buffer conditions are recommended to be maintained because of the sensitivity of proteins toward environmental pH changes [4]. The purity of water will affect the yield of end products as unpurified water contains a lot of microorganisms or proteases that will result in protein degradation [4]. Protein inhibitor, which may exist in solution or buffers, causes the hydrolyzation of proteins. Detergent, another significant factor that cannot be neglected in purification of protein, consists of a hydrophobic portion of a linear or branched hydrocarbon “tail” and a hydrophilic “head” [4]. They solubilize the membrane protein and are amphiphatic molecules which form micelles with the hydrophilic head of proteins [4]. Reducing agents will be added into solution or buffer for protein extraction and purification to avoid the loss of activity of proteins or enzymes which is caused by oxidization. Storage of proteins is important as the half-life of protein is commonly dependent on the storage temperature [4].

The purification of protein requires specific assay. A quick and easy assay method must be known for protein purification so that a known molecular weight, specific affinity, or immunoaffinity of nonenzymatic protein of interest can be detected using appropriate method [7]. There are several methods commonly used in protein purification. They are ion exchange chromatography, gel filtration, affinity chromatography and gel electrophoresis [4].

## 2. History

**2.1. Nucleic Acid Extraction.** The very first DNA isolation was done by a Swiss physician, Friedrich Miescher in 1869 [8]. He hoped to solve the fundamental principles of life, to determine the chemical composition of cells. He tried to isolate cells from lymph nodes for his experiment but the purity of lymphocytes was hard and impossible to be obtained in sufficient quantities. Therefore, he switched to leucocytes, where he obtained them from the pus on collected surgical bandages.

Initially, Miescher focused on the various type of protein that make up the leukocytes and showed that proteins were the main components of the cell's cytoplasm. During his tests, he noticed that a substance precipitated from the solution when acid was added and dissolved again when alkali was added. This was, for the first time he had obtained a crude precipitate of DNA.

To separate DNA from the proteins in his cell extracts, Miescher developed new protocol to separate the cells' nuclei from cytoplasm and then isolated DNA. However, his first protocol failed to yield enough material to continue with further analysis. He had to develop a second protocol to obtain larger quantities of purified nuclein, which had been named as ‘nucleic acid’ later by his student, Richard Altman [8].

**2.2. Protein Extraction.** In the eighteenth century, proteins were known as a distinct class of biological molecules by Antoine Fourcroy and others. They distinguished this molecule by its ability to coagulate under treatment with heat or acid. However, the first description of protein was carried out by Gerhardus Johannes Mulder, a Dutch chemist, in 1893 [9]. His studies on the composition of animal substances, mainly fibrin, albumin, and gelatin, showed the presence of carbon, hydrogen, oxygen, and nitrogen [9]. Furthermore, he recognized that sulfur and phosphorus were present sometimes in animal substances that consisted large number of atoms and he established that these “substances” were macromolecules [9].

Most of the early studies focused on proteins that could be purified in large quantities. For example, blood, egg white and various toxins. Most of the proteins are hard to purify in more than milligram quantities even with today's highly advanced methods. A majority of techniques for protein purification were developed in a project led by Edwin Joseph Cohn, a protein scientist, during World War II. He was responsible for purifying blood and worked out the techniques for isolating the serum albumin fraction of blood plasma, which is important in maintaining the osmotic pressure in the blood vessels, which help keep soldier alive [10].

## 3. Current Tendency

After the fated event where Miescher managed to obtain DNA from cell, many others have followed suit which lead to further advancement in the DNA isolation and purification protocol. The initial routine laboratory procedures for DNA extraction were developed from density gradient centrifugation strategies. Meselson and Stahl used this method in 1958 to demonstrate semiconservative replication of DNA [3]. Later procedures made use of the differences in solubility of large chromosomal DNA, plasmids, and proteins in alkaline buffer [3].

Currently, there are many specialized method of extracting out pure DNA, RNA, or protein. Generally, they are divided into solution-based or column-based protocols. Most of these protocols have been developed into commercial kits that ease the biomolecules extraction processes.

### 3.1. Type of Nucleic Acid Extraction

#### 3.1.1. Conventional Method

(1) *Guanidinium Thiocyanate-Phenol-Chloroform Extraction*. Salt is the common impurity in nucleic acid samples. It has always been required to be removed from nucleic acid samples before any downstream processes and analysis can be done. Therefore, single or multiple separation and/or purification steps are needed to desalt the sample comprising the nucleic acid [11]. The general steps of nucleic acid purification include cell lysis, which disrupts the cellular structure to create a lysate, inactivation of cellular nucleases such as DNase and RNase, and separation of desired nucleic acid from cell debris [2]. Organic solvent—phenol-chloroform extraction is one of the examples, which is widely used in isolating nucleic acid.

Although phenol, a flammable, corrosive, and toxic carboxylic acid can denature proteins rapidly, it does not completely inhibit RNase activity [12]. This problem can be solved by using a mixture of phenol: chloroform: isoamyl alcohol (25:24:1). Proteins, lipids, carbohydrates, and cell debris are removed through extraction of the aqueous phase with the organic mixture of phenol and chloroform [12, 13]. A biphasic emulsion forms when phenol and chloroform are added. The hydrophobic layer of the emulsion will then be settled on the bottom and the hydrophilic layer on top by centrifugation [3]. The upper phase which contained DNA is collected and DNA can be precipitated from the supernatant by adding ethanol or isopropanol in 2 : 1 or 1 : 1 ratios and high concentration of salt [3]. DNA precipitate is collected by centrifugation, and excess salt is rinsed with 70% ethanol and centrifuged to discard the ethanol supernatant. The DNA pellet is then dissolved with TE buffer or sterile distilled water [3].

The use of guanidinium isothiocyanate in RNA extraction was first mentioned by Ulrich et al. (1977). The method was laborious. Therefore, it has been displaced by a single-step technique, which is known as Guanidinium thiocyanate-phenol-chloroform extraction, by Chomczynski and Sacchi (1987) [12], whereby the homogenate is extracted with phenol/chloroform at reduced pH. Guanidinium thiocyanate is a chaotropic agent used in protein degradation. The principle of this single-step technique is that RNA is separated from DNA after extraction with acidic solution consisting guanidinium thiocyanate, sodium acetate, phenol, and chloroform [13]. In the acidic conditions, total RNA will remain in the upper aqueous phase of the whole mixture, while DNA and proteins remain in the interphase or lower organic phase. Recovery of total RNA is then done by precipitation with isopropanol [12].

(2) *Alkaline Extraction Method*. Alkaline lysis has been used to isolate plasmid DNA and *E. coli* [12]. It works well with all strains of *E. coli* and with bacterial cultures ranging in size from 1 mL to more than 500 mL in the presence of Sodium Dodecyl Sulfate (SDS). The principle of the method is based on selective alkaline denaturation of high molecular weight chromosomal DNA while covalently closed circular DNA

remains double stranded [14]. Bacterial proteins, broken cell walls, and denatured chromosomal DNA enmeshed into large complexes that are coated with dodecyl sulfate. Plasmid DNA can be recovered from the supernatant after the denatured material has been removed by centrifugation.

(3) *CTAB Extraction Method*. For plant extraction, the initial step that needs to be done is to grind the sample after freezing it with liquid nitrogen. The purpose of doing this step is to break down cell wall material of sample and allow access to nucleic acid while harmful cellular enzymes and chemicals remain inactivated. After grinding the sample, it can be resuspended in a suitable buffer such as CTAB.

Cetyltrimethylammonium bromide (CTAB) is a non-ionic detergent that can precipitate nucleic acids and acidic polysaccharides from low ionic strength solutions [15]. Meanwhile, proteins and neutral polysaccharides remain in solution under these conditions. In solutions of high ionic strength, CTAB will not precipitate nucleic acids and forms complexes with proteins. CTAB is therefore useful for purification of nucleic acid from organisms which produce large quantities of polysaccharides such as plants and certain Gram-negative bacteria [15].

This method also uses organic solvents and alcohol precipitation in later steps [12]. Insoluble particles are removed through centrifugation to purify nucleic acid. Soluble proteins and other material are separated through mixing with chloroform and centrifugation. Nucleic acid must be precipitated after this from the supernatant and washed thoroughly to remove contaminating salts. The purified nucleic acid is then resuspended and stored in TE buffer or sterile distilled water.

(4) *Ethidium Bromide (EtBr)-Cesium Chloride (CsCl) Gradient Centrifugation*. CsCl gradient centrifugation is a complicated, expensive, and time-consuming method compared to other purification protocols. It requires large scale bacterial culture. Therefore, it is not suitable for the minipreparation of plasmid DNA [4]. Nucleic acids can be concentrated by centrifugation in an EtBr-CsCl gradient after alcohol precipitation and resuspension. Intercalation of EtBr alters the swimming density of the molecule in high molar CsCl. Covalently closed circular molecules will accumulate at lower densities in the CsCl gradient because they incorporate less EtBr per base pair compared to linear molecules. The hydrophobic EtBr is then removed with appropriate hydrophobic solvents after extraction. The purified nucleic acid will be reprecipitated with alcohol [1].

(5) *Purification of Poly (A)<sup>+</sup> RNA by Oligo(dT)-Cellulose Chromatography*. Poly (A)<sup>+</sup> RNA is the template for protein translation and most of the eukaryotic mRNAs carry tracts of it at their 3' termini [4, 15]. It makes up 1 to 2% of total RNA and can be separated by affinity chromatography on oligo (dT)-cellulose. Poly (A) tails form stable RNA-DNA hybrids with short chains of oligo (dT) that attach to various support matrices [4, 15]. High salt must be added to the chromatography buffer to stabilize the nucleic acid duplexes

as only a few dT-A base pairs are formed. A low-salt buffer is used after nonpolyadenylated RNAs have been washed from the matrix. This buffer helps to destabilize the double-stranded structures and elute the poly (A)<sup>+</sup> RNAs from the resin [15].

There are two methods commonly used in the selection of Poly (A)<sup>+</sup> RNA—column chromatography on oligo (dT) columns and batch chromatography. Column chromatography normally used for the purification of large quantities (>25 µg) of nonradioactive poly (A)<sup>+</sup> RNA isolated from mammalian cells. Batch chromatography is the preferred method when working with small amounts (<50 µg) of total mammalian RNA. It can be used when many RNA samples are to be processed, whether radioactive or not. Batch chromatography is carried out with a fine grade of oligo (dT) cellulose at optimal temperatures for binding and elution [15].

**3.1.2. Solid-phase Nucleic Acid Extraction.** Solid-phase nucleic acid purification can be found in most of the commercial extraction kits available in market. It allows quick and efficient purification compared to conventional methods [16]. Many of the problems that are associated with liquid-liquid extraction such as incomplete phase separation can be prevented. Solid phase system will absorb nucleic acid in the extraction process depending on the pH and salt content of the buffer. The absorption process is based on the following principles: hydrogen-binding interaction with a hydrophilic matrix under chaotropic conditions, ionic exchange under aqueous conditions by means of an anion exchanger, and affinity and size exclusion mechanisms.

Solid-phase purification is normally performed by using a spin column, operated under centrifugal force [17]. This method can purify nucleic acid rapidly compared to conventional methods. Silica matrices, glass particles, diatomaceous earth, and anion-exchange carriers are examples that have been utilized in solid-phase extraction method as solid support. Four key steps involved in solid-phase extraction are cell lysis, nucleic acids adsorption, washing, and elution [6].

The initial step in a solid phase extraction process is to condition the column for sample adsorption. Column conditioning can be done by using a buffer at a particular pH to convert the surface or functional groups on the solid into a particular chemical form. [17]. Next, the sample which has been degraded by using lysis buffer is applied to the column. The desired nucleic acid will absorb to the column with the aid of high pH and salt concentration of the binding solution [17]. Other compounds, such as protein may have strong specific bond with the column surface as well. These contaminants can be removed in the washing step by using washing buffer containing a competitive agent [17]. For the elution step, TE buffer or water is introduced to release the desired nucleic acid from the column, so that it can be collected in a purified state [17]. Normally, rapid centrifugation, vacuum filtration, or column separation is required during the washing and elution steps of purification process.

A mixed-bed solid phase nucleic acid extraction and its use in the isolation of nucleic acid have been disclosed [18].

The mixed-bed solid phases of this invention are the mixtures of at least two different solid phases, can be solid or semisolid, porous or non-porous. Each solid phase can bind to the target nucleic acid under different solution conditions and release the nucleic acid under similar elution conditions [18].

(1) *Silica Matrices.* The basis for most of the products related to nucleic acid purification is the unique properties of silica matrices for selective DNA binding. Types of silica materials including glass particles, such as glass powder, silica particles, and glass microfibers prepared by grinding glass fiber filter papers, and including diatomaceous earth [19]. Hydrated silica matrix, which was prepared by refluxing silicon dioxide in sodium hydroxide or potassium hydroxide at a molar ratio of about 2:1 to 10:1 for at least about 48 hours, had been introduced in DNA purification. DNA binds to the inorganic matrix and is released in heated water [20].

The principle of silica matrices purification is based on the high affinity of the negatively charged DNA backbone towards the positively charged silica particles [21]. Sodium plays a role as a cation bridge that attracts the negatively charged oxygen in the phosphate backbone of nucleic acid [22]. Sodium cations break the hydrogen bonds between the hydrogen in water and the negatively charged oxygen ions in silica under high salt conditions (pH ≤ 7) [22]. The DNA is tightly bound, and extensive washing removes all contaminations. The purified DNA molecules can be eluted under low ionic strength (pH ≥ 7) later by using TE buffer or distilled water [21].

Besides silica matrices, nitrocellulose and polyamide membranes such as nylon matrices are also known to bind with nucleic acids, but with less specificity. These materials are often used as solid-phase nucleic acid transfer and hybridization matrices [23]. Polyamide matrices are more durable than nitrocellulose and are known to bind nucleic acids irreversibly. Nucleic acids can be immobilized on polyamide matrices in low ionic strength buffer [23].

(2) *Glass Particle.* Glass particles, powder and beads are useful for nucleic acid purification. For example, DNA isolation from agarose gels involved the use of chaotropic salts to facilitate binding of DNA to common silicate glass, flint glass, and borosilicate glass (glass fiber filter). The adsorption of nucleic acid onto the glass substrate occurs most likely based on the mechanism and principle that similar to adsorption chromatography [24]. Nucleic acid purification can also be done on silica gel and glass mixture [19]. This invention has discovered that a mixture of silica gel and glass particles can be used to separate nucleic acid from other substances in the presence of chaotropic salts solution.

(3) *Diatomaceous Earth.* Diatomaceous earth, which is also known as kieselguhr or diatomite, has silica content as high as 94% [25]. It has been used for filtration and in chromatography and it is useful for the purification of plasmid and other DNA by immobilizing DNA onto its particles in the presence of a chaotropic agent. The resulting diatomaceous earth-bound DNA is then washed with an

alcohol-containing buffer. The alcohol-containing buffer is then discarded and DNA is eluted out in a low salt buffer or in distilled water [25].

(4) *Magnetic Bead Based Nucleic Acid Purification.* Magnetic separation is a simple and efficient way which is used in purification of nucleic acid nowadays. Many magnetic carriers are now commercially available. Particles having a magnetic charge may be removed by using a permanent magnet in the application of a magnetic field. Often, magnetic carriers with immobilized affinity ligands or prepared from biopolymer showing affinity to the target nucleic acid are used for the isolation process. For example, magnetic particles that are produced from different synthetic polymers, biopolymers, porous glass or magnetic particles based on inorganic magnetic materials such as surface-modified iron oxide. Materials with a large surface area are preferred to be used in the binding of nucleic acids. Magnetic particulate materials such as beads are more preferable to be a support in isolation process because of their larger binding capacity. The nucleic acid binding process may be assisted by the nucleic acid “wrapping around” the support. A magnet can be applied to the side of the vessel, which contains the sample mixture for aggregating the particles near the wall of the vessel and pouring away the remainder of the sample [26].

Particles having magnetic or paramagnetic properties are employed in an invention where they are encapsulated in a polymer such as magnetizable cellulose [27]. In the presence of certain concentrations of salt and polyalkylene glycol, magnetizable cellulose can bind to nucleic acids. Small nucleic acid required higher salt concentrations for strong binding to the magnetizable cellulose particles. Therefore, salt concentration can be selectively manipulated to release nucleic acid bound to magnetizable cellulose on the basis of size. The magnetizable cellulose which bound with nucleic acid will be washed with suitable wash buffer before they are contacted with a suitable elution buffer to separate out the desired nucleic acid with cellulose. Separation of magnetizable cellulose from supernatant during all the purification steps can be done by applying a magnetic field to draw down or draw them to the side of the vessel [27]. The magnetizable cellulose used in this invention has an iron oxide content of up to around 90% by weight of the total mass of the cellulose. The magnetic component of cellulose can also be substituted by other magnetic compounds such as ferrous oxide or nickel oxide [27].

An extraction kit based on the principle of magnetic bead based nucleic acid purification is commercially available in the market [28]. The special part of this kit is that the reagents provided are intended for use with magnetic tools. This magnetic tool is recommended if working in microtube format. It is a practical device for performing separations based on magnetic particle technology. The kit does not require any organic solvents and eliminates the need for repeated centrifugation, vacuum filtration or column separation. The protocol is based on a modified alkaline lysis procedure followed by binding of the nucleic

acid to magnetic particles. The magnetic tool is used to capture magnetic particles with the bound nucleic acid and contaminants are removed by washing with wash buffer provided. The nucleic acid is then eluted from the magnetic particles with the elution buffer [28].

Another extraction kit has the same principle as the extraction described above, which used the magnetic-particle technology for nucleic acid purification [29]. It combines the speed and efficiency of silica-based DNA purification with the convenient handling of magnetic particles. A magnetic rod protected by a rod cover is used for the capture of magnetic particles. It enters a vessel containing the samples and attracts the magnetic particles. Then, the magnetic rod cover is positioned above another vessel and the magnetic particles are released [29].

Nucleic acid purification by using zirconia bead is another type of magnetic bead based purification. These microspherical paramagnetic beads have a large available binding surface and can be dispersed in solution. This characteristic allowed thorough nucleic acid binding, washing, and elution. The total nucleic acid isolation kit, which uses this technology for the nucleic acid purification, makes use of the mechanical disruption of samples with zirconia beads in a guanidinium thiocyanate-based solution that not only releases nucleic acid but also inactivate nuclease in the sample matrix [30]. After the lysis step, dilution of samples is done by using isopropanol. Paramagnetic beads are added to the samples for the nucleic acid binding purpose. The mixture of beads and nucleic acid are immobilized on magnets and washed to remove protein and contaminants. Removal of residual binding solution is done with a second wash solution and finally the nucleic acid is eluted in low-salt buffer [30].

Solid-phase reversible immobilization paramagnetic bead-based technology has been utilized for a PCR purification system to deliver quality DNA. It requires simple protocol without centrifugation and filtration. PCR amplicons bind to paramagnetic particles which draw them out of solution, allowing contaminants such as dNTPs, primers, and salts to be rinsed away [31].

Magnetic oligo (dT) bead is an alternative to other oligo (dT) matrices for the purification of poly(A)<sup>+</sup> RNA from total RNA sample [4]. The poly(A)<sup>+</sup> RNA can be extracted by introducing magnetic beads coated with oligo (dT). RNA with a poly-A tail attach to the oligo (dT). The beads will then be drawn to the bottom of a tube removing mRNA directly from total RNA. The magnetic beads which are specially treated minimize the nonspecific binding of other nucleic acids and ensure the purity of mRNA [32].

(5) *Anion-Exchange Material.* Anion exchange resin is one of the popular examples that utilized the anion-exchange principle [33]. It is based on the interaction between positively charged diethylaminoethyl cellulose (DEAE) groups on the resin's surface and negatively charged phosphates of the DNA backbone. The anion-exchange resin consists of defined silica beads with a large pore size, a hydrophilic surface coating and has a high charge density [34]. The

large surface area of resin allows dense coupling of the DEAE groups. The resin works over a wide range of pH conditions (pH 6–9) and/or salt concentration (0.1–1.6 M) which can optimize the separation of DNA from RNA and other impurities [34]. Therefore, salt concentration and pH conditions of the buffers are one of the main factors that determine whether nucleic acid is bound or eluted out from the column. DNA can bind to the DEAE group over a wide range of salt concentration. Impurities such as protein and RNA are washed from the resin by using medium-salt buffers, while DNA remains bound until eluted with a high-salt buffer [34].

The method of utilizing anion exchange materials to isolate nucleic acid has been disclosed in an invention [35], where the commercially available strong or weak positively charged anion exchanger materials were used with selected solutions of known ionic strength for adsorption and elution. Most of the water-soluble components such as protein can be washed through the column by employing a solution with a known ionic strength for the binding of nucleic acids to the anion exchange column materials. The ionic strength for elution is generated by using known salt concentration, which mixed with a buffer to control pH strength, ideally corresponding to the lowest ionic strength at which the nucleic acids will completely elute [35].

**3.2. Type of Protein Extraction Method.** The first step in protein purification is cell lysis. In order to purify and analyze protein efficiently, they must be first released from their host cell in a soluble form. The plasma membrane of mammalian cells, composed of phospholipids and proteins, is easy to be disrupted [36]. In comparison, protein extraction from fungi and bacteria appears more challenging due to their stable cell wall that is stronger than the plasma membrane.

Plant tissues contain a wide range of proteins which vary in their properties. Some specific factors must be taken into account when developing protein extraction protocol for plant [37]. For example, the presence of rigid cellulose cell wall must be sheared in order to release the cell contents. Specific contaminating compounds such as phenolics and a range of proteinases may result in protein degradation or modification. Therefore, specific conditions are required for protein extraction and purification from plant [38].

Mechanical disruption techniques, such as French Press or glass beads are used to remove the cell wall, followed by detergent based extraction of total protein [39].

**3.2.1. Ion Exchange Chromatography.** Ion exchange chromatography separates proteins based on their surface ionic charge using resin that are modified with either positively-charged or negatively-charged chemical groups [4, 7]. Most proteins have an overall negative or positive charge depending on their isoelectric point (pI) at a given pH, which makes them possible to interact with an opposite charged chromatographic matrix [7]. If the net charge of the protein is positive at a pH below pI value, the protein will bind to a cation exchanger; at a pH above the pI value the net charge of

the protein is negative and the protein will bind to an anion exchanger [38].

Proteins that interact weakly with the resins, for example a weak positively charged protein passed over resin modified with a negatively charged group, are eluted out in a low-salt buffer. On the other hand, proteins that interact strongly required more salt to be eluted. Proteins with very similar charge characteristics can be separated into different fractions as they are eluted from the column by increasing the concentration of salt in elution buffer [7].

Ion exchange column is one of the technologies that utilized the principle of ion exchange chromatography [33]. It uses membrane-absorbent technology as a chromatographic matrix to separate proteins. The membrane absorbents in columns are stabilized cellulose-based with highly porous structure that provides proteins access to the charged surface easily. Interactions among molecules and active sites on the membrane happened in convective through-pores. Therefore, the adsorptive membranes have the potential to maintain high efficiencies when purifying large biomolecules with low diffusion [33].

**3.2.2. Gel Filtration Chromatography.** Gel filtration chromatography, also called size-exclusion or gel-permeation chromatography, separates proteins according to molecular sizes and shape and the molecules do not bind to the chromatography medium [39]. It is a process in which large molecules passes through the column faster than small molecules. Small molecules can enter all of the tiny holes of the matrix and access more of the column. Small-sized proteins will pass through those holes and take more time to run out of the column compared with large-sized proteins that cannot get into those holes but run out directly of the column through void space in the column [4, 7].

Gel filtration chromatography kit applies the principle of gel filtration chromatography [40]. The target sample is applied on top of the column which contained porous beads, an example of matrix in the column. The molecules get separated when the molecules pass through the column of porous beads. The separation of molecules can be divided into three main types: total exclusion, selective permeation, and total permeation limit. Total exclusion is the part that large molecules cannot enter the pores and elute fast. For selective permeation region, intermediate molecules may enter the pores and may have an average residence time in the particles depending on their size and shape. As for total permeation limit, small molecules have the longest residence time once they enter the pores on the column [40]. An advantage of gel filtration-based chromatography is that it is suited for biomolecules that may be sensitive to pH changes, concentration of metal ions, and harsh environmental conditions [39].

**3.2.3. Affinity Chromatography.** Affinity chromatography depends on a specific interaction between the protein and the solid phase to affect separation from contaminants. It consists of the same steps as ion exchange chromatography [38]. It enables the purification of a protein on the basis of

TABLE 1: Typical biological interactions used in affinity chromatography [42].

Types of ligand	Target molecules
Enzyme	Substrate analogue, inhibitor, cofactor
Antibody	Antigen, virus, cell
Lectin	Polysaccharide, glycoprotein, cell surface receptor, cell
Nucleic acid	Complementary base sequence, histones, nucleic acid polymerase, nucleic acid binding protein
Hormone, vitamin	Receptor, carrier protein
Glutathione	Glutathione-S-transferase or GST fusion proteins
Metal ions	Poly (his) fusion proteins, native proteins with histidine, cysteine and/or tryptophan residues on their surfaces

its biological function or individual chemical structure [41]. Proteins that have a high affinity towards the specific chemical groups such as ligands will covalently attach and bind to the column matrix while other proteins pass through the column [38]. Electrostatic or hydrophobic interactions, van der Waals' forces and hydrogen bonding are the biological interactions between ligands and the target proteins [41]. The bound proteins will be eluted out from the column by a solution containing high concentration of soluble form of the ligand [36].

A biospecific ligand that can attach to a chromatography matrix covalently is one of the requirements for successful affinity purification. The binding between the ligand and target protein molecules must be reversible to allow the proteins to be removed in an active form [41]. After washing away the contaminants, the coupled ligand must retain its specific binding affinity for the target proteins. Some examples of biological interactions that are usually used in affinity chromatography are listed in Table 1 (see [41]).

Chromatographic separation by differential affinity to ligands immobilized on a beaded porous resin is fundamental to protein research [42]. A complete kit that contains pack beaded affinity resin columns based on principle of affinity chromatography has been introduced to the market [42]. An affinity resin can be used in batch or microcentrifuge spin column format depending on the scale and type of experiment to be carried out. Furthermore, it can be packed into some sort of larger gravity-flow column as well [42].

**3.2.4. Gel Electrophoresis.** Gel electrophoresis is a method to separate protein according to their size and charge properties. The partially purified protein from the chromatography separations can be further purified with nondenaturing polyacrylamide gel electrophoresis (PAGE), or native gel electrophoresis [4]. In PAGE, the proteins are driven by an applied current through a gelled matrix [43]. The movement of protein through this gel depends on the charge density (charge per unit of mass) of the molecules. The molecules with high density charge migrate rapidly. The size and shape of protein are another two important factors that

influence PAGE fractionation [43]. The acrylamide pore size plays a role as a molecular sieve to separate different sizes of proteins [4]. The larger the protein, the slower it migrates as it becomes more entangled in the gel [43]. Shape is also one of the factors because compact globular proteins move faster than elongated fibrous proteins of comparable molecular mass [43].

PAGE is usually carried out in the presence of the sodium dodecyl sulfate (SDS) [44]. A protein treated with SDS will usually eliminate the secondary, tertiary and quaternary structure of protein [4, 7]. Proteins unfold into a similar rod-like shape because of the electrostatic repulsion between the bound SDS molecules. The number of SDS molecules which bind to a protein is approximately proportional to the protein's molecular mass (about 1.4 g SDS/g protein) [43]. Each protein species has an equivalent charge density and is driven through the gel with the same force [43]. In addition, PAGE can minimize the denaturation of proteins. Many proteins still retain their biological activities after running PAGE [7]. However, larger proteins are held up to a greater degree than smaller proteins because the polyacrylamide is highly cross-linked [43]. Consequently, proteins become separated by SDS-PAGE on the basis of their molecular mass. SDS-PAGE can be used to determine the molecular mass of the mixture of proteins by comparing the positions of the bands to those produced by proteins of known size [43]. SDS used in electrophoresis resolve mixture of proteins according to the length of individual polypeptide chains [7].

A technique called two-dimensional gel electrophoresis was developed by Patrick O'Farrell in 1975. It is used to fractionate complex mixtures of proteins by using two different techniques—isoelectric focusing and SDS-PAGE [43]. First, proteins are separated according to their isoelectric point in a tubular gel. After this separation, the gel is removed and placed on top of a slab of SDS-saturated polyacrylamide. The proteins move into the slab gel and separated according to their molecular mass [43]. Two-dimensional gel electrophoresis is suitable to detect changes in proteins present in a cell under different conditions, at different stages in development or the cell cycle, or in different organisms [43].

**3.2.5. Southwestern Blotting (Immunoblotting).** Southwestern blotting is a method that is used to isolate, identify, and characterize DNA-binding proteins by their ability in binding to specific oligonucleotide probes [44, 45]. Many of the DNA-binding proteins in the cell need to be isolated individually and characterized to define the gene function [44]. Three steps are involved in this method. First, nuclear protein extracts are separated by SDS-PAGE electrophoresis. Next, separated proteins are transferred to a nitrocellulose filter, polyvinylidene difluoride (PVDF) or cationic nylon membrane [12]. The filter will then be incubated with oligonucleotide probes to analyze the adsorbed proteins [44, 45]. However, this technique is beset with problems such as large amounts of nuclear proteins are required (typically 50–100 mg), protein degradation during isolation, facing difficulties in achieving efficient electrophoretic separation and transfer of a wide molecular size range of proteins [45].

**3.3. All-in-One Biomolecules Extraction.** Generally, the extraction or purification techniques or kits available in the market can only allow the extraction of one type of nucleic acid, either DNA or RNA, or protein from a targeted organism. When the cellular material is limiting, it is desirable to extract DNA, RNA and protein from the same source.

A variation on the single-step isolation method of Chomczynski and Sacchi (1987), that the guanidinium thiocyanate homogenate is extracted with phenol:chloroform at reduced pH, allows the preparation of DNA, RNA and protein from tissue or cells. This method involves the lysis of cells with guanidine isothiocyanate and phenol in a single-phase solution. A second phase forms after the addition of chloroform where DNA and proteins are extracted, leaving RNA in the aqueous supernatant. The DNA and proteins can be isolated from the organic phase by precipitation with ethanol or isopropanol and the RNA precipitated from aqueous phase with isopropanol [15].

Several all-in-one extraction kits have been introduced in the market nowadays. For example, a column-based extraction kit that designed to purify genomic DNA, total RNA and total protein from a single biological sample simultaneously, without the usage of toxic substances such as phenol or chloroform and alcohol precipitation [46]. It is compatible with small amounts of a wide range of cultured cells and harvested tissue of animal and human origin. The targeted sample does not need to be separated into 3 parts before the purification of DNA, RNA and protein [46].

A solution-based 3-in-1 extraction kit that is available in the market is another example of non-organic solutions kit that can extract and purify DNA, RNA and protein, from different organisms in any types and sizes [47]. Its three simple steps protocol, which takes around 15 to 30 minutes, provides a fast and easy way to do the extraction of different biomolecules. Therefore, higher yield can be expected as fewer steps leads to fewer loss [47].

**3.4. Automated Extraction System.** Automated extraction system, a large, expensive and complex instrumentation designed for high-throughput sample processing, has helped to simplify the isolation of nucleic acids [48]. This system was designed for medium to large laboratories which has grown in presence over recent years [49]. Automating nucleic acid extraction process is potentially beneficial for a number of reasons including to reduce working time, decrease labor costs, increase worker safety and in the midst provides opportunity in increasing reproducibility and quality of results [50]. Besides, it is a key solution to increasing the laboratory efficiency [48].

In clinical laboratories, purification of high-quality biomolecules such as DNA, RNA and protein from a variety of starting material will be used in downstream testing applications. It is crucial to obtain purified samples in sufficient quality and purity [48]. Therefore, automated extractions should be more consistent and reproducible. The speed, accuracy and reliability of the whole extraction process should be maximal and at the same time minimize

the risk of cross-contamination [49]. A solution has to be introduced to increase sample preparation efficiency without sacrificing the quality. The possibility of cross-contamination should be reduced and the systems are amenable to bar-coded sample tracking [51].

An extraction system that is available in the market has met the requirements stated above. It offers forensic laboratories fast and reliable sample processing along with high-quality automated DNA purification [52]. It is a paramagnetic-particle handling system to process sample and provide consistent yield and purity as there is no detectable cross-contamination between samples. The whole extraction process takes about 20 minutes from start to the end because only three simple steps are needed: (1) add liquid samples to reagent cartridge; (2) place reagent cartridges into the machine; (3) press Start button. DNA is eluted into elution buffer at the end of the process [52].

Another example of automated system that is flexible and efficient for extraction of nucleic acids and proteins has been introduced [53]. Various starting materials can be processed by using this system, which is designed for small and medium sample throughput. It utilized surface-functionalized paramagnetic particles to adsorb the isolated nucleic acid [53]. The flexibility of this system allows the extraction of nucleic acid from up to twelve samples simultaneously. The extraction process requires around 20 to 40 minutes depending on the application. The kits that optimized for this system can extract genomic DNA, cellular RNA, viral or bacterial nucleic acids [53].

#### 4. Possible Future Direction

Biomolecules extraction is the first step that needs to be performed for the following analysis or manipulation process. The liquid handling requirement is the most challenging aspect. Therefore, any automatic system must include not only automatic equipment for each extraction step but also equipment for automating the transfer of liquid between machines. Automation has aided in increasing the throughput and improving the reliability of the process, but these systems are still designed for use in a laboratory environment only. Some of the nucleic acid extraction system that are available in the market are large and require manual pre-processing stages by laboratory staff with technical expertise [54]. Therefore, robotic workstations for nucleic acid extraction should fulfill a true “walk-away” automation, which means a fully automated process [49]. A combination of all-in-one biomolecules extraction solution and method with fully automated extraction system can be a prospective invention in the future. The purification of DNA, RNA or protein from various organisms can be performed simultaneously using this type of extraction system with just a single extraction method.

It is often inconvenient that targeted biomolecules sample from an animal, plant or even a clinical sample must be sent to a laboratory for it to be extracted and analyzed [54]. The samples, especially clinical sample such as blood, need to be refrigerated and transferred to the nearest laboratory



for extraction and analyzing. Hence, a portable biomolecules extraction system, which brings several advantages such as reduced labour, reduced waste and increased speed of extracting process, can be a potential development in the future [54]. The combination of portable extraction system with DNA, RNA, or protein analyzer can be build up in the future to help researchers in reducing working time and increasing the work efficiency.

Continued improvement in miniaturization will be the future trend of robotic automation in the laboratory [28]. Many clinical laboratories are performing workflow analysis and finding that smaller systems with lower throughput are more consistent with clinical laboratory workload. Besides, this automation system can be implemented at relatively low cost, improving the turnaround times and also reduce the labor costs [55].

## 5. Conclusion

Since the first DNA isolation was successfully done by Friedrich Miescher in 1869 and the initial DNA extraction developed from density gradient centrifugation strategies by Meselson and Stahl in 1958, many techniques for biomolecules purification has been developed. From guanidinium thiocyanate-phenol-chloroform extraction to the column-technology that is widely used in DNA and RNA extraction, and chromatography purification method to immunoblotting that used to extract proteins, biomolecules extraction has helped researchers and scientists in manipulating subsequent molecular biology analysis in order to have a better understanding in the biological materials of the earth.

The automated nucleic acid extraction system has been developed due to the influence of rapid growth of automation technology nowadays. Automating nucleic acid extraction process is potentially beneficial for a number of reasons including to reduce working time, decrease labor costs, increase worker safety and at the same time provides opportunity in increasing reproducibility and quality of results. However, improvement of the weaknesses for some of the instruments needs to be conducted all the time. In the mean time, an all-in-one biomolecules extraction system, or the invention of a miniature and portable extraction system can become a prospective development in the future.

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