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Techniques of Nucleic Acids Purification: A Review

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INTRODUCTION :

Every gene manipulation procedure requires genetic material like DNA and RNA. Nucleic acids occur naturally in association with proteins and lipoprotein organelles. The dissociation of a nucleoprotein into nucleic acid and protein moieties and their subsequent separation, are the essential steps in the isolation of all species of nucleic acids. Isolation of nucleic acids is followed by quantitation of nucleic acids generally done by either spectrophotometric or by using fluorescent dyes to determine the average concentrations and purity of DNA or RNA present in a mixture. Isolating the genetic material (DNA) from cells (bacterial, viral, plant or animal) involves three basic steps-

• Rupturing of cell membrane to release the cellular components and DNA
• Separation of the nucleic acids from other cellular components
• Purification of nucleic acid

WHAT CRITERIA COULD YOU CONSIDER WHEN SELECTING A PURIFICATION STRATEGY

How much purity does your application Require what contaminants will affect your immediate and downstream Application some PCR applications might not require extensively purified DNA. Cells can be lysed, diluted, and amplified without any further steps. Another reason to accurately determine purity requirements is that yields tend to decrease as purity requirements

HOW MUCH NUCLEIC ACID CAN BE PRODUCED FROM A GIVEN A MOUNT OF STARTING MATERIAL

While it is feasible to mathematically calculate the total amount of nucleic acid in a given sample, and values are provided in there search literature the yields from commercial purification products and noncommercial purification strategies are usually significantly less than these maxima, sometimes less than 50%. Since recoveries will vary with sample origin, consider making your plans based on yields published for samples similar if not identical to your own.

REQUIRE HIGH MOLECULAR WEIGHT MATERIAL

The average size of genomic DNA prepared will vary between commercial products and between published procedures. How Important Is Speed to Your Situation? Some purification protocols are very fast and allow isolation of nucleic acids within 30 minutes, but speed usually comes at the price of reduced yield and/or purity, especially when working with complex samples.

HOW IMPORTANT IS COST

Reagents obviously figure into the cost of a procedure, but the labor required to produce and apply the reagents of purification should also be considered. how important is reproducibility.

(Robustness) of the Procedure

Some methods will not give consistent quality and quantity. When planning long-term or high-throughput extractions, validate your methods for consistency and robustness. complex samples.

What Interferes with Nucleic Acid Purification Nuclease

One of the major concerns of nucleic acid purification is the ubiquity of nucleases. The minute a cell dies, the isolation of DNA urns into a race against internal degradation. Samples must be lysed fast and completely and lysis buffers must inactivate nucleasesto prevent nuclease degradation. Most lysis buffers contain protein-denaturing and enzyme inhibiting components. DNases are much easier to inactivate than RNases, but care should be taken not to reintroduce them during or after purification. All materials should be autoclaved or baked four hours at 300°F to inactivate DNases and RNases, or you should use disposable materials. Use only enzymes and materials guaranteed to be free of contaminating nucleases. Where appropriate, work on ice or in the cold to slow down potential nuclease activity. Smears and lack of signal, or smeared signal alone, and fail ureto amplify by PCR are indicative of nuclease contamination. The presence of nuclease can be verified by incubating a small aliquot of your sample at 37°C for a few hours or overnight, followed by valuation by electrophoresis or

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hybridization. If nuclease contaminations minor, consider re-purifying the sample with a procedure that removes protein.

Shearing:

Large DNA molecules (genomic DNA, bacterial artificial chromomoses, yeast artificial chromosomes) can be easily sheared during purification. Avoid vortexing, repeated pipetting (especially through low-volume pipette tips), and any other form of mechanical stress when the isolate is destined for applications that require high molecuar weight DNA.

Chemical Contaminants:

Materials that interfere with nucleic acid isolation or down stream applications involving the purified DNA can originate from the sample. Plants, molds, and fungi can present a challenge because of their rigid cell wall and the presence of polyphenolic components, which can react irreversibly with nucleic acids to create an unusable final product.The reagents of a DNA purification method can also contribute contaminants to the isolated DNA. Reagents that lyse and solubilize samples, such as guanidiniumi sothiocyanate, can inhibit some enzymes when present in trace amounts. Ethanol precipitation of the DNA and subsequent ethanol washes eliminate such a contaminant. Phenol can also be problematic. If you experience problems with DNA purified by a phenol-based strategy, apply chloroform to extract away the phenol. Phenol oxidation products may also damage nucleic acids; hence re-distilled phenol is recommended for purification procedures. A mixture of chloroform and phenol is often employed to maximize the yield of isolated DNA; the chloroform reduces the amount of the DNA-containing aqueous layer at the phenol interphase. Similar to phenol, residual chloroform can be problematic and should be removed by thorough drying. Drying is also employed to remove residual ethanol. Over dried DNA can be difficult to dissolve, so drying should be stopped shortly after the liquid can no longer be observed.

WHAT PRACTICES WILL MAXIMIZE THE QUALITY OF DNA PURIFICATION

The success of DNA purification is dependent on the initial quality of the sample and its preparation. It would be nice to have a simple, straightforward formula that applies to all samples, but some specimens have inherent limitations. The list below will help guide your selection and provide remedies to non ideal situations:

1. Ideally start with fresh sample. Old and necrotic samples complicate purification. In the case of plasmid preparations, cell death sets in after active growth has ceased, which can produce an increase in unwanted by-products such as endo-toxins that interfere with purification or own stream application. The best growth phase of bacterial cultures for plasmid preparation may be strain dependent. During the log phase of bacterial culture, actively replicating plasmids are present that are "nicked" during replication rather than being super coiled. Still some researchers prefer mid to late log phase due to the high ratio of DNA to protein and low numbers of dead cells. Others only work with plasmids that have grown just out of log phase to avoid co-purification of nicked plasmid. If old samples can't be avoided, scaling up the purification can compensate for losses due to degradation. PCR or dot blottingis strongly recommended to document the integrity of the DNA.

2. Process your sample as quickly as possible. There are few exceptions to this rule, one being virus purification. When samples can't be immediately purified, snap freeze the intact sample in liquid nitrogen or hexane on dry ice or store the lysed extract at -80°C. Commercial products, such as those from Ambion, Inc., can also protect samples from degradation prior to nucleic acid purification. Samples can also be freeze-dried

3. Thorough, rapid homogenization is crucial. Review the literature to determine if your sample requires any special physical or mechanical means to generate the lysate.

4. Load the appropriate amount of sample. Nothing will impair the quality and yield of a purification strategy more than overloading the system. Too much sample can cause an increase in the viscosity of the DNA preparation and lead to shearing of genomic DNA. If you do not know the exact amount of starting material, use 60 to 70% of your estimate.

HOW CAN YOU MAXIMIZE THE STORAGE LIFE OF PURIFIED DNA

The integrity of purified DNA in solution could be compromised by nuclease, pH below 6.0 and above 9.0, heavy metals, UV light, and oxidation by free radicals. EDTA is often added to chelate divalent cations required for nuclease activity and to prevent heavy metal oxidative damage. Tris-based buffers will provide a safe pH of 7 to 8 and will not generate free radicals, as can occur with PBS. Free-radical oxidation seems to be a key player in breakdown and ethanol is the best means to control this process. Low temperatures are also important for long-term stability. Storage

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at 4°C is only recommended for short periods (days). Even though some studies have shown that storage under ethanol is safe even at elevated temperatures, better stability is obtained at -80°C. Storage at-20°C can lead to degradation, but this breakdown is prevented by the addition of carrier DNA. RNA stored in serum has also been shown to degrade at -20°C .Another approach for intermediate storage is freeze drying DNA-containing samples intact .The DNA within freeze-dried tissue was stable for 6 months, but RNA be gande rading after 10 weeks of storage. The control of moisture and temperature had a significant effect on shelf life of samples. The long term stability of DNA-containing samples is still being investigated, but some companies offer specialized solutions llowing storage at room temperature.

ISOLATING DNA FROM CELLS AND TISSUES:

What are the fundamental Steps of DNA purification The fundamental processes of DNA purification from cells and tissues are sample lysis and the segregation of the nucleic acid away from contaminants. While DNA is more or less universal to all species, the contaminants and their relative amounts will differ considerably. The composition of fat cells differs significantly from muscle cells. Plants have to sustain high pressure, contain chloroplasts packed with chromophores, and often have a very rigid outer cell wall. Bacteria contain lipo poly saccharides that can interfere with purification and cause toxicity problems when present in downstream applications. Fibrous tissues such as heart and skeletal muscle are tough to homogenize. These variations have to be taken into consideration when developing or selecting a lysis method.

Lysis:

Detergents are used to solubilize the cell membranes. Popular choices are SDS, Triton X-100, and CTAB (hexadecyltrimethyl ammonium bromide). CTAB can precipitate genomic DNA, and
it is also popular because of its ability to remove polysaccharides from bacterial and plant preparations Enzymes attacking cell surface components and/or components of the cytosol are often added to detergent-based lysis buffers. Lysozyme digests cell wall components of gram-positive bacteria. Zymolase, and murienase aid in protoplast production from yeast cells. Proteinase K cleaves glycoproteins and inactivates (to some extent) RNase/DNase in 0.5 to 1% SDS solutions. Heat is also applied to enhance lysis. Denaturants such as urea, guanidinium salts, and other chaotropes are applied to lyse cells and inactivate enzymes, but extended use beyond what is recommended in a procedure can lead to a reduction in quality and yield. Sonication, grinding in liquid nitrogen, shredding devices such as rigid spheres or beads, and mechanical stress such as filtration have been used to lyse difficult samples prior to or in conjunction with lysis solutions. Disruption methods are discussed

Segregation of DNA from Contaminants:

The separation of nucleic acid from contaminants are discussed below within the question, What are the strengths and limitations of contemporary Purification Methods

DNA Precipitation:

To concentrate nucleic acids for re suspension in a more suitable buffer, solvents such as ethanol (75–80%) or isopropanol (final concentration of 40–50%) are commonly used in the presence of salt to precipitate nucleic acids. If volume is not an issue, ethanol is preferred because less salt will co precipitate and the pellet is more easily dried. Polyethylene glycol (PEG) selectively precipitates high molecular weight DNA, but it is also more difficult to dry and can interfere with downstream applications. Tri chloro acetic acid (TCA) precipitate seven low MW polymers down to (5kDa) , but nucleic acids cannot be recovered in a functional form after precipitation. Salt is essential for DNA precipitation because its cations counteract the repulsion caused by the negative charges of the phosphate backbone. Ammonium acetate is useful because it is volatile and easily removed, and at high concentration it selectively precipitates high molecular weight molecules. Lithium chlorideis often used for RNA because Li+ does not precipitate double-stranded DNA, proteins, or carbohydrates, although the single-stranded nucleic acids must be above 300 nucleotides. To efficiently precipitate nucleic acids, incubation at low temperatures (preferably -20°C) for at least 10 minutes is required, followed by centrifugation at 12,000 g for at least five minutes. Temperature and time are crucial for nucleic acids at low concentrations, but above 0.25 mg/ml, precipitation may be carried out at room temperature. Additional washing steps with 70% ethanol will remove residual salt from pelleted DNA. Pellets are dried in a speed vac or on the bench and are re suspended in water or TE (10mM Tris, 1mM EDTA). Do not attempt to precipitate nucleic acids below a concentration of 20 ng/ml

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unless carrier such as RNA, DNA, or a high molecular weight co-precipitant like glycogen is added. In the range from 20 ng/ml to 10mg/ml, either add carrier or extend precipitation time, and add more ethanol. Polyethylene glycol (PEG) precipitation is even more concentration dependent and will only work at DNA concentrations above 10mg/ml. Pellets will dissolve better in low salt buffers (water or TE) and at concentrations below 1 mg/ml.
Gentle heating can also help to re dissolve nucleic acids.

Isolation and Purification of Genomic DNA :

Genomic DNA is found in the nucleus of all living cells with the structure of double stranded DNA remaining unchanged (helical ribbon). The isolation of genomic DNA differs in animals and plant cells. DNA isolation from plant cells is difficult due to the presence of cell wall, as compared to animal cells. The amount and purity of extracted DNA depends on the nature of the cell. The method of isolation of genomic DNA from a bacterium comprises following steps –

1. Bacterial culture growth and harvest.
2. Cell wall rupture and cell extract preparation.
3. DNA Purification from the cell extract.
4. Concentration of DNA solution.

Growth and harvest of bacterial culture:

Bacterial cell culture is more convenient than any other microbe, as it requires only liquid medium (broth) containing essential nutrients at optimal concentrations, for the growth and division of bacterial cells. The bacterial cells are usually grown on a complex medium like Luria-Bertani (LB), in which the medium composition is difficult to decipher. Later, the cells are separated by centrifugation and re suspended in 1% or less of the initial culture volume.

Preparation of cell extract:

Bacterial cell is surrounded by an additional layer called cell wall, apart from plasma membrane with some species of E. coli comprising multilayered cell wall. The lysis of cell wall to release the genetic material i.e. DNA can be achieved by following ways-

• Physical method by mechanical forces.
• Chemical method by metal chelating agents i.e. EDTA and surfactant i.e. SDS or
enzyme (e.g. lysozyme).

Lysozyme :

• present in egg-white, salivary secretion and tears.
• catalyzes the breakdown of cell wall i.e. the peptidoglycan layer.

EDTA (Ethylene di amine tetra-acetic acid) :

• a chelating agent necessary for destabilizing the integrity of cell wall.
• inhibits the cellular enzymes that degrade DNA.

SDS (Sodium dodecyl sulphate) :

• helps in removal of lipid molecules and denaturation of membrane proteins.
Generally, a mixture of EDTA and lysozyme is used. Cell lysis is followed by centrifugation to pellet down the cell wall fractions leaving a clear supernatant containing cell extract.

Purification of DNA:

In addition to DNA, a cell extract contains significant quantities of protein and RNA which can be further purified by following methods-

Organic extraction and enzymatic digestion for the removal of contaminants

It involves the addition of a mixture of phenol and chloroform (1:1) to the cell lysate for protein separation. The proteins aggregate as a white mass in between the aqueous phase containing DNA and RNA, and the organic layer. Treatment of lysate with pronase or protease, in addition to phenol/chloroform, ensures complete removal of proteins from the extract. The RNA can be effectively removed by using Ribo nuclease, an enzyme which rapidly degrades RNA into its ribo nucleotide subunits. Repeated phenol extraction is not desirable, as it damages the DNA.

Using ion-exchange chromatography :

This involves the separation of ions and polar molecules (proteins, small nucleotides and amino acids) based on their charge. DNA carrying negative charge binds to the cationic resin or matrix which can be eluted from the column by salt gradient. Gradual increase in salt concentration detaches molecules from the resin one after another.

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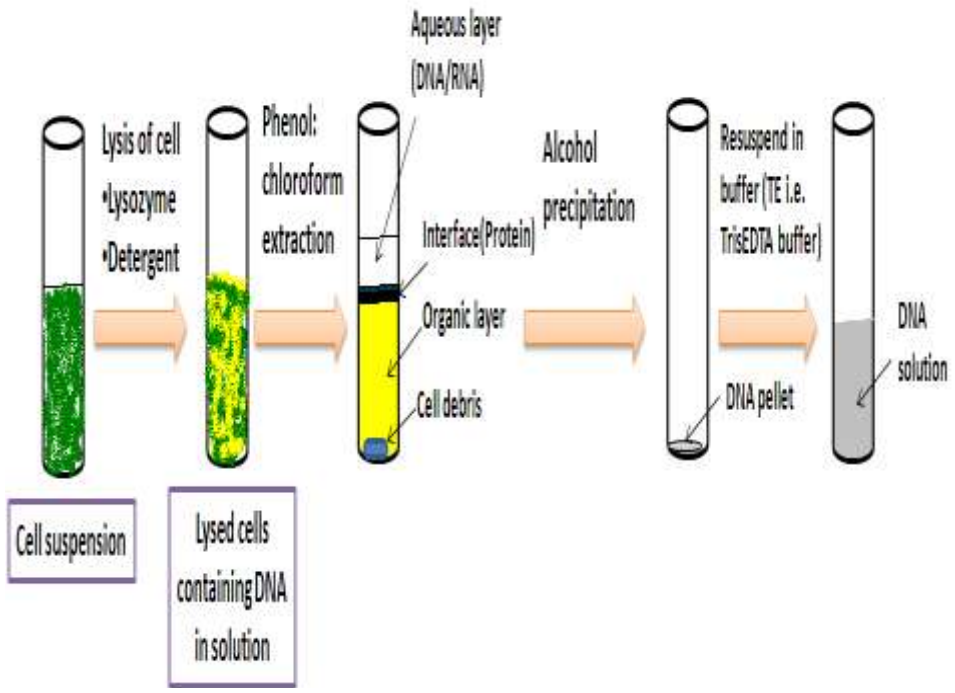

Preparation of genomic DNA

Concentration of DNA samples :

Concentration of DNA can be done using ethanol along with salts such as sodium acetate, potassium acetate etc. These salts provide metal ions like sodium ions (Na+), potassium ions (K+) which help in aggregation and hence precipitation of DNA molecules.

Advantage :

It leaves short-chain and monomeric nucleic acid components in solution. Ribonucleotides produced by the ribonuclease treatment are separated from DNA.

Isolation and Purification of Plasmid DNA :

Plasmids are circular, double stranded extra cellular DNA molecules of bacterium and most commonly used in recombinant DNA technology. The isolation of plasmid DNA involves three major steps-

1. Growth of the bacterial cell.
2. Harvesting and lysis of the bacteria.
3. Purification of the plasmid DNA.

Growth of the bacterial cell :

It involves growth of the bacterial cells in a media containing essential nutrients.

Harvest and lysis of bacteria:

Lysis of bacteria results in the precipitation of DNA and cellular proteins. Addition of acetate-containing neutralization buffer results in the precipitation of large and less super coiled chromosomal DNA and proteins leaving the small bacterial DNA plasmids in solution.

Purification of Plasmid DNA:

This step is same for both plasmid and genomic but former involves an additional step i.e. the separation of plasmid DNA from the large bacterial chromosomal

Methods for separation of plasmid DNA :

Separation of plasmid DNA is based on the several features like size and conformation of plasmid DNA and bacterial DNA. Plasmids are much smaller than the bacterial main chromosomes, the largest plasmids being only 8% of the size of the E. coli chromosome. The separation of small molecules (i.e. plasmids) from larger ones (i.e. bacterial chromosome) is based on the fact that plasmids and the bacterial chromosomes are circular but bacterial chromosomes break into linear fragments during the preparation of the cell extract resulting in separation of pure plasmids. The methods of separation of plasmid DNA are described as below-

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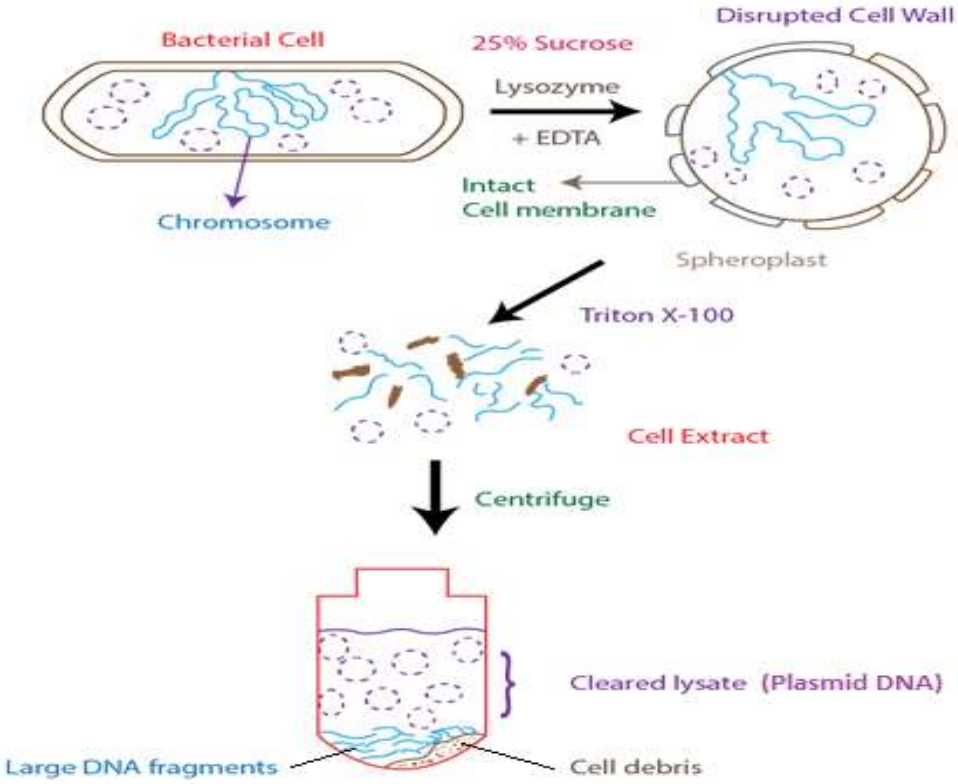
Separation based on size difference :

• It involves lysis of cells with lysozyme and
• Cells with partially degraded cell walls are formed that retain anintact cytoplasmic membrane called as sphaeroplasts.
• Cell lysis is then induced by the addition of a non-ionic detergent (e.g. Triton X-100) or ionic detergents (e.g. SDS) causing chromosomal breakage.
• Bacterial chromosome attached to cell membrane, upon lysis gets removed with the cell debris.
• A cleared lysate consisting almost entirely of plasmid DNA is formed with very little breakage of the bacterial DNA.



Separation of plasmid DNA on the basis of size.

Separation based on conformation:

Plasmids are supercoiled molecules formed by partial unwinding of double helix of the plasmid DNA during the plasmid replication process by enzymes called topo isomerases. The supercoiled conformation can be maintained when both polynucleotide strands are intact, hence called covalently closed-circular (ccc) DNA. If one of the polynucleotide strands is broken, the double helix reverts to its normal relaxed state taking an alternative conformation, called open-circular (oc). Super coiling is important in plasmid preparation due to the easy separation of supercoiled molecules from non-supercoiled ones.

The commonly used methods of separation based on conformation are as follows-

(a). Alkaline denaturation method

• This method is based on maintaining a very narrow pH range for the denaturation of non-supercoiled DNA but not the supercoiled plasmid
• Addition of sodium hydroxide to cell extract or cleared lysate (pH12.0-12.5) results in disruption of the hydrogen bonds of non-supercoiled DNA molecules.
• As a result, the double helix unwinds and two polynucleotide chains separate.

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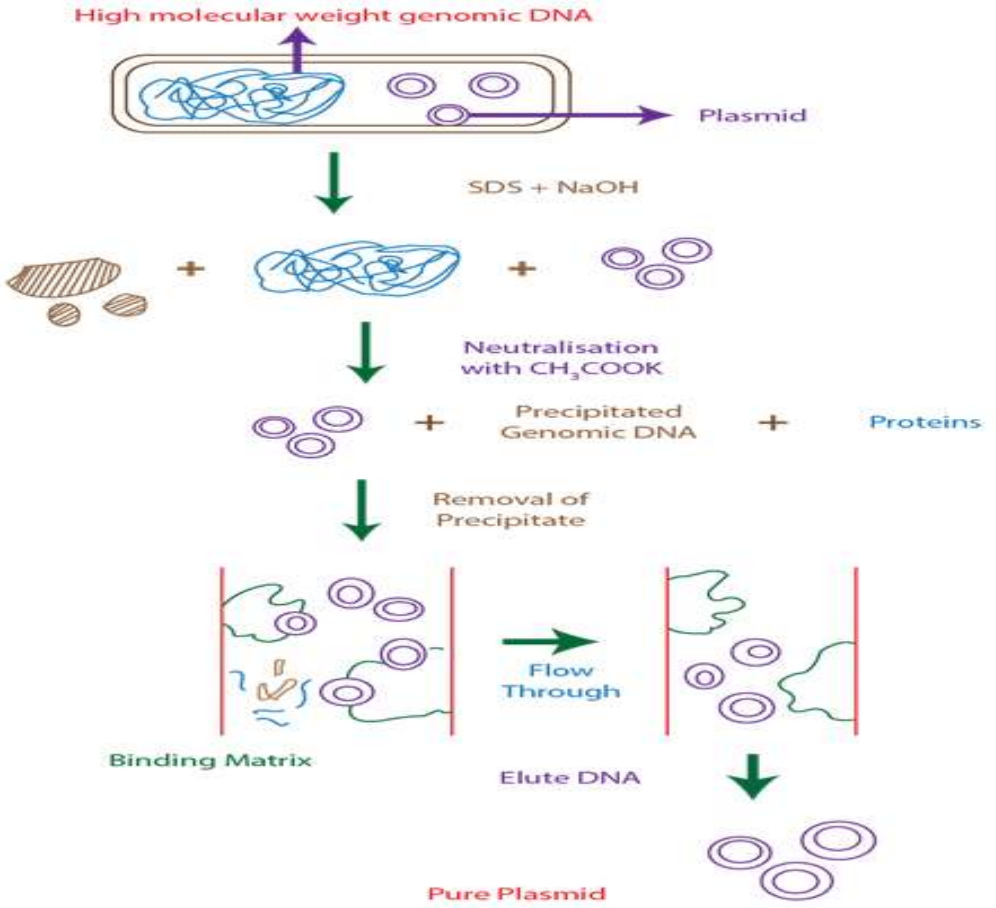
• Further addition of acid causes the aggregation of these denatured bacterial DNA strands into a tangled mass which can be pelleted by centrifugation, leaving plasmid DNA in the supernatant.

Advantage

• Most of the RNA and protein under defined conditions (specifically cell lysis by SDS and neutralization with sodium acetate) can be removed by the centrifugation.
• No requirement of organic extraction.



(a). Separation of plasmid DNA by Alkaline denaturation method
(b). Ethidium bromide-cesium chloride density gradient centrifugation

• Density gradient centrifugation can separate DNA, RNA and protein. It is a very efficient method for obtaining pure plasmid DNA.
• A density gradient is produced by centrifuging a solution of cesium chloride at a very high speed which pulls the CsCl ions towards the bottom. This process is referred as isopycnic centrifugation.
• The DNA migrates to the point at which it has density similar to that of CsCl i.e.1.7 g/cm3 in the gradient.
• In contrast, protein molecules having lower buoyant densities float at the top of the tube whereas RNA gets pelleted at the bottom.

Density gradient centrifugation in the presence of ethidium bromide (EtBr) can be used to separate supercoiled DNA from non-super coiled molecules. Ethidium bromide is an intercalating dye that binds to DNA molecules causing partial unwinding of the double helix. Supercoiled DNA have very little freedom to unwind due to absence of free ends and bind to a limited amount of EtBr resulting in very less decrease in buoyant density (0.085 g/cm3) than that of linear DNA (0.125 g/cm3). As a result, they form a distinct band separated from the linear bacterial DNA. The EtBr bound to DNA is then extracted by n-butanol and the CsCl is removed by dialysis.

Isolation and Purification of RNA :

RNA (Ribonucleic acid) is a polymeric substance consisting of a long single-stranded

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chain of phosphate and ribose units with the nitrogen bases adenine, guanine, cytosine and uracil bonded to the ribose sugar present in living cells and many viruses. The steps for preparation of RNA involve homogenization, phase separation, RNA precipitation, washing and re-dissolving RNA.

The method for isolation and purification of RNA are as follows-

1) Organic extraction method
2) Filter-based, spin basket formats
3) Magnetic particle methods
4) Direct lysis method.

Organic extraction method :

This method involves phase separation by addition and centrifugation of a mixture of a solution containing phenol, chloroform and a chaotropic agent (guanidiniumthiocyanate) and aqueous sample. Guanidiumthiocyanate results in the denaturation of proteins and RNases, separating rRNA from ribosomes. Addition of chloroform forms a colorless upper aqueous phase containing RNA, an interphase containing DNA and a lower phenol-chloroform phase containing protein. RNA is collected from the upper aqueous phase by alcohol (2-propanol or ethanol) precipitation followed by rehydration.

One of the advantages of this method is the stabilization of RNA and rapid denaturation of nucleases. Besides advantages, it has several drawbacks such as it is difficult to automate, needs labor and manual intensive processing, and use of chlorinated organic reagents

Direct lysis methods :

This method involves use of lysis buffer under specified conditions for the disruption of sample and stabilization of nucleic acids. If desired, samples can also be purified from stabilized lysates. This method eliminates the need of binding and elution from solid surfaces and thus avoids bias and recovery efficiency effects.

Advantages :

• Extremely fast and easy.
• Highest ability for precise RNA representation.
• Easy to work on very small samples.
• Amenable to simple automation.

Drawbacks :

• Unable to perform traditional analytical methods (e.g. spectrophotometric method).
• Dilution-based (most useful with concentrated samples).
• Potential for suboptimal performance unless developed/optimized with downstream analysis.
• Potential for residual RNase activity if lysates are not handled properly

POURPOSE OF NUCLEIC ACID PURIFICATION :

- It usually determines the success or failure of all your immediate and dowm stream experiments
- Extract sample amounts of your genomic and / plasmid DNA sample from a limited source to satisfy the requirements of your research
- Purify it to reduce the amount of contaminant thath can comprise te result of your research and shorten the shelf life of your precious samples

Phenol: chloroform Extraction:

It is a liquid-liquid extraction technique in biochemistry and molecular biology for purifying nucleic acids and eliminating proteins and lipids. In brief, aqueous samples lysed cells or homogenised tissues are mixed with equal volumes of a phenol: chloroform mixture. After mixing, the mixture is centrifuged and two distinct phases are formed, because the phenol: chloroform mixture is immiscible with water. The aqueous phase is on top because it is less dense than the organic phase (phenol: chloroform). The proteins and hydrophobic lipids will partition into the lower organic phase while the nucleic acids (as well as other contaminants such as salts, sugars, etc.) remain in the upper aqueous phase. The upper aqueous phase is pipetted off and care is taken to avoid pipetting any of the organic phase or material at the interface. This procedure is often performed multiple times to increase the purity of the DNA.If the mixture is acidic, DNA will precipitate into the organic phase while RNA remains in the aqueous phase due to DNA being more readily neutralised than RNA.

Spin column-based nucleic acid purification :

It is a solid phase extraction method to quickly purify nucleic acids. This method relies on the fact that nucleic acid will bind to the solid phase of silica under certain conditions.

The stages of the method are

- **Lyse** – The cells of a sample are broken open with a lysis procedure.
- **Bind** – A buffer solution is then added to the sample along with ethanol or isopropanol. This forms the binding solution. The binding

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solution is transferred to a spin column and the column is put in a centrifuge. The centrifuge forces the binding solution through a silica gel membrane that is inside the spin column. If the pH and salt concentration of the binding solution are optimal, the nucleic acid will bind to the silica gel membrane as the solution passes though.

- **Wash** – The flow-through is removed and a wash buffer is added to the column. The column is put in a centrifuge again, forcing the wash buffer through the membrane. This removes any remaining impurities from the membrane, leaving only the nucleic acid bound to the silica gel.
- **Elute** – The wash buffer is removed and an elution buffer (or simply water) is added to the column. The column is put in a centrifuge again, forcing the elution buffer through the membrane. The elution buffer removes the nucleic acid from the membrane and the nucleic acid is collected from the bottom of the column.

Boom method:

Boom method (Boom nucleic acid extraction method) is a solid phase extraction method for "isolating nucleic acid (NA) from Biological samples. Essential of this method is the use of Silica beads, capable of binding the NA in the presence of a chaotropic substance according to the effect. This method is one of the most widespread methods for isolating nucleic acids from biological samples and is known as a simple, rapid, and reliable method for the small-scale purification of NA from Biological sample.

The process for isolating nucleic acid from Starting material of Boom method are essentially consist of following 4 steps

(a)**Lysingand/orHomogenizing** the Starting material.Lysate of Starting material is obtained by for example a detergent in the presence of protein degrading enzymes.

(b)**Mixing** chaotropic substance and Silica beads into the Starting material.

Mixing the Starting material', a chaotropic substance to bind the NA to Silica beads, Lysate of Starting material of (a) is mixed with sufficiently large amounts of chaotropic substance. According to the chaotropic effect, releasing-NA will be bound to the silica beads almost instantaneously. In this way, silica-nucleic acid complexes are formed. The reasons "why NA and Silica form bond" are to be described in following section (Basic Principals).

(c)**Washing Silica beads**

In this step, Silica beads of (b) are washed several times to remove contaminants. Process of Washing of the silica-nucleic acid complexes (silica beads) typically consists of following steps,

Collecting Silica beads from the liquid by for example Tajima pipette or by for example Pellet-down (by rapid sedimentation (centrifugation) and disposal of the supernatant (e.g., by suction))

- Re dispersioning Silica beads into the chaotropic salt-containing washing buffer using, e. g., a vortex mixer.
- Collecting re dispersed Silica beads from above mentioned washing buffer again.
- Further washed successively with an alcohol-water solution and with acetone.
- Drying beads are preferably be done.

(d)**Separating the bonded nucleic acids:**

Separating the bonded nucleic acids from the Silica beads. Pure NA are eluted into buffer by decreasing the concentration of chaotropic substance. NA presented in the washed (and preferably dried) silica-nucleic acid

complexes is eluted into elution buffer such as TE buffer, aqua bidest, ... , and so on. The selection of the elution buffer is co-determined by the contemplated use of the isolated NA.

Applications

2-D Electrophoresis and Analysis 2-DE is an essential step in biomarker discovery workflow, whether your goal is protein characterization, purification and profiling, or posttranslational modification studies.

Automated Electrophoresis and Analysis

How can automation in electrophoresis enhance reliability in experiments assessing the purity and yield of proteins or evaluating the integrity of RNA?

Biomarker Analysis

Details on how biomarkers such as hormones, cytokines, chemokines, growth factors, and phosphor proteins are used to investigate physiological or pharmacological mechanisms.

Genomics

Explore different aspects of genomics — from functional genomics and biomarker discovery to mutation analysis, gene expression, transfection, and epigenetics.

Label-Free Biomolecular Interactions

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Various aspects of how to develop, optimize, and validate biomolecular interaction assays using surface plasmon resonance (SPR) technology.

Separation, Transfer, and Analysis of Proteins

Bio-Rad's V3 Western Workflow™ facilitates speed and validation at each step of a western blotting experiment — from running gels to quantifying proteins.

Stem Cell Research

Tips for isolation, maintenance, differentiation, transfection and analysis of stem cells.

Protein Purification and Isolation

Learn how protein purification by chromatography is used in monoclonal antibody discovery, viral contaminant removal, and preparative protein isolation

Technologies

Introduction to Protein Electrophoresis

Protocols, video tutorials, and selection guides to help you at every step of your electrophoresis experiments.

PCR

Learn how PCR works, how to choose the right PCR instrument, how to design, optimize, analyze, and troubleshoot PCR assays.

Introduction to Western Blotting

Learn more about western blotting techniques. Find step-by-step protocols and helpful tips on equipment, membranes, transfer conditions, and detection methods.

What is Real-Time PCR?

Discover an array of solutions to optimize, execute, and troubleshoot MIQE-compliant real-time PCR experiments.

Imaging and Analysis

Comparison of parameters, sensitivity, and data analysis using Bio-Rad imaging systems and software to determine which imaging system is ideal for your application.

Introduction to PCR

Digital PCR is a breakthrough technology that provides ultrasensitive and absolute nucleic acid quantification.

2-D Electrophoresis

Useful information from sample preparation to imaging of 2-D gels, along with protocols, video tutorials, demonstrations, and troubleshooting tips.

Introduction to Transfection

Information and tips on electroporation, viral transfection vector, lipofection, and biolistics transformation for planning and selecting the appropriate transfection method.

Horizontal Electrophoresis

Gel boxes, running buffers, available agarose types, as well as factors affecting resolution of DNA fragments, to help with agarose gel electrophoresis of DNA.

Cell Counting Methods

Manual and automated cell counting methods and their application to gene expression studies are presented and discussed here.

Pulsed Field Gel Electrophoresis

How can large DNA fragments be separated using PFGE? How to prepare plugs, optimize sample resolution, analyze images, and troubleshoot PFGE gels? Explore.

Multiplex Immunoassays

Details on the experimental design, data collection, analysis, and validation of multiplex protein assays using the Bio-Plex® suspension array system.

Experion™ Automated Electrophoresis System

The microfluidic technology used in Bio-Rad's Experion™ system helps to automate protein and nucleic acid separation, detection, and analysis.

ProteOn™ XPR36 Surface Plasmon Resonance (SPR) System

How does this label-free system perform analysis of biomolecular interactions? How do you choose the right sensor chip for your analysis? Find out.

Introduction to Chromatography

Description of the different types of chromatography methods available and how they can be applied to guide you in your protein purification experiments.

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