Isolation of DNA from Blood

Aim

To isolate genomic DNA from blood samples for downstream applications such as PCR, sequencing, or genetic analysis.

Principle

Isolating DNA from blood involves breaking open the blood cells to release the DNA, removing proteins and other contaminants, and precipitating the DNA for purification. This process typically utilizes detergents to lyse cells, proteinase K to degrade proteins, salts for DNA precipitation, and alcohol for DNA precipitation.

Reagents

1. Blood samples (whole blood or buffy coat)
2. Cell lysis buffer (containing detergents and salts)
3. Proteinase K
4. Phenol:chloroform alcohol (25:24:1)
5. Chloroform alcohol (24:1)
6. Ethanol (100% and 70%)
7. RNase A (optional)
8. TE buffer (Tris-EDTA buffer, pH 8.0 or 8.5)

Apparatus

1. Microcentrifuge tubes
2. Centrifuge
3. Vortex mixer
4. Heat block or water bath
5. Microcentrifuge
6. Spectrophotometer or nanodrop for DNA quantification

Procedure

1. Blood Cell Lysis

1. **Preparation:** Collect blood samples in EDTA-coated tubes to prevent clotting.

2. **Cell Lysis:**
    - Transfer the blood sample to a microcentrifuge tube and add cell lysis buffer.
    - Add proteinase K to digest proteins that may degrade DNA.
    - Mix thoroughly and incubate at a specific temperature (e.g., 56°C) for a defined period to lyse cells and release DNA.

2. DNA Extraction

1. **Organic Extraction:**
    - Add an equal volume of phenol:chloroform

      alcohol to the lysed sample.

    - Mix thoroughly by vortexing and centrifuge at high speed to separate the aqueous (upper) phase containing DNA from the organic (lower) phase.
2. **DNA Precipitation:**
    - Transfer the aqueous phase to a new tube and add cold ethanol (100%).
    - Mix gently and incubate at -20°C or -80°C for DNA precipitation.
    - Centrifuge at high speed to pellet DNA.
3. **Washing and Resuspension:**
    - Carefully remove the supernatant and wash the DNA pellet with 70% ethanol to remove residual salts and contaminants.
    - Air-dry the pellet or centrifuge briefly to remove ethanol.
    - Resuspend the DNA pellet in TE buffer or distilled water.

3. DNA Quantification and Storage

1. **Quantification:**
    - Measure the concentration and purity of the isolated DNA using a spectrophotometer or nanodrop.
    - Typical ratios for pure DNA (OD260/OD280) are around 1.8-2.0, indicating minimal protein contamination.
2. **Storage:**
    - Store the isolated DNA at -20°C or -80°C for long-term storage or proceed with downstream applications immediately.

Results : Quantify and report the yield and purity of the isolated DNA. Ensure the DNA is suitable for subsequent applications such as PCR, sequencing, or genetic analysis based on its concentration and purity.

Isolation of RNA from Yeast

Aim

To isolate RNA from yeast cells for downstream applications such as gene expression analysis, RT-PCR, or RNA sequencing.

Principle

Isolating RNA from yeast involves breaking open the yeast cells to release RNA, removing DNA, proteins, and other contaminants, and precipitating the RNA for purification. This process typically utilizes phenol-chloroform extraction to lyse cells, RNase inhibitors to prevent RNA degradation, and alcohol for RNA precipitation.

Reagents

1. Yeast culture
2. Trizol reagent or similar RNA extraction kit
3. Chloroform
4. Isopropanol
5. Ethanol (70% and 100%)
6. RNase-free water
7. RNase inhibitor
8. DNase (optional)
9. TE buffer (Tris-EDTA buffer, pH 8.0 or 8.5)

Apparatus

1. Microcentrifuge tubes
2. Centrifuge
3. Vortex mixer
4. Heat block or water bath
5. RNase-free pipettes and tips

6. RNase-free microcentrifuge tubes
7. Spectrophotometer or nanodrop for RNA quantification

Procedure

1. Cell Lysis and RNA Extraction

1. **Yeast Harvesting:**
   - Harvest yeast cells in mid-log phase by centrifugation at low speed (e.g., 3000 rpm for 5 minutes).
2. **Cell Lysis:**
   - Resuspend yeast pellet in Trizol reagent or similar RNA extraction reagent.
   - Add chloroform and mix thoroughly by vortexing to separate phases.
   - Centrifuge to separate the aqueous phase containing RNA from the organic phase.
3. **RNA Precipitation:**
   - Transfer the aqueous phase to a new tube and add isopropanol to precipitate RNA.
   - Incubate at -20°C or -80°C for RNA precipitation.
   - Centrifuge at high speed to pellet RNA.
4. **Washing and Resuspension:**
   - Carefully remove the supernatant and wash the RNA pellet with 70% ethanol to remove residual salts and contaminants.
   - Air-dry the RNA pellet or centrifuge briefly to remove ethanol.
   - Resuspend the RNA pellet in RNase-free water or TE buffer containing RNase inhibitor.

2. DNase Treatment (Optional)

1. **DNase Treatment:**
   - If DNA contamination is a concern, treat RNA with DNase to degrade any residual DNA.
   - Incubate RNA with DNase according to manufacturer's instructions.
   - Inactivate DNase by adding EDTA and heat inactivation.

3. RNA Quantification and Quality Check

1. **Quantification:**
   - Measure the concentration and purity of the isolated RNA using a spectrophotometer or nanodrop.
   - Typical ratios for pure RNA (OD260/OD280) are around 1.8-2.0, indicating minimal protein and DNA contamination.
2. **Quality Check:**

- Assess RNA integrity using gel electrophoresis or a bioanalyzer to confirm the presence of intact 18S and 28S ribosomal RNA bands.

Results

Quantify and report the yield and purity of the isolated RNA. Ensure the RNA is suitable for subsequent applications such as RT-PCR, gene expression analysis, or RNA sequencing based on its concentration and integrity.

Blotting Techniques

Aim

To transfer and detect specific nucleic acid or protein molecules from a gel matrix to a solid support for further analysis.

Principle

Blotting techniques involve the transfer of biomolecules (DNA, RNA, or proteins) separated by gel electrophoresis onto a solid membrane, where they can be detected using specific probes (DNA probes for nucleic acids or antibodies for proteins). The transfer process typically utilizes capillary action or electric field to move the biomolecules from the gel matrix to the membrane, followed by detection through hybridization with complementary probes.

Reagents

1. Gel electrophoresis apparatus and supplies (agarose or polyacrylamide gel)
2. Nitrocellulose or PVDF (Polyvinylidene fluoride) membrane
3. Transfer buffer (e.g., Tris-glycine buffer for proteins, SSC buffer for nucleic acids)
4. Blocking solution (e.g., non-fat milk, BSA)
5. Primary antibody or probe (specific to target molecule)
6. Secondary antibody or probe (conjugated with enzyme or fluorophore)
7. Substrate for enzyme-linked detection (e.g., chemiluminescent substrate)

Apparatus

1. Semi-dry or wet blotting apparatus
2. Power supply for electrophoresis and blotting
3. Incubation trays or containers
4. Shaker or rocker
5. Imaging system (chemiluminescence imager or UV transilluminator)

Procedure

1. Gel Electrophoresis

1. **Sample Preparation:**
    - Prepare samples containing nucleic acids or proteins and load them onto an agarose or polyacrylamide gel.
    - Run electrophoresis under appropriate conditions to separate molecules based on size or charge.

2. Blotting Procedure (Western Blotting for Proteins)

a. Protein Transfer (Western Blot)

1. **Preparation of Gel and Membrane:**
    - Cut a piece of nitrocellulose or PVDF membrane to the size of the gel and activate the membrane (e.g., by soaking in methanol).
2. **Transfer Process:**
    - Assemble the gel, membrane, and filter papers soaked in transfer buffer in a blotting apparatus.
    - Apply constant voltage or current for semi-dry or wet transfer to facilitate transfer of proteins from the gel to the membrane.
3. **Blocking and Detection:**
    - Block non-specific binding sites on the membrane by incubating in blocking solution (e.g., non-fat milk or BSA).
    - Incubate with primary antibody specific to the target protein.
    - Wash the membrane to remove unbound primary antibody.
    - Incubate with secondary antibody conjugated to an enzyme (e.g., HRP) or fluorophore.
    - Wash to remove unbound secondary antibody.
    - Visualize protein bands using a chemiluminescence substrate for enzymatic detection or fluorescence for fluorophore detection.

3. Nucleic Acid Transfer (Southern or Northern Blot)

1. **Preparation of Gel and Membrane:**
    - Prepare and run agarose gel electrophoresis for DNA (Southern blot) or RNA (Northern blot).
    - Soak the membrane in alkaline solution (for DNA) or ethanol (for RNA) to denature and immobilize nucleic acids.
2. **Transfer Process:**
    - Transfer nucleic acids to nitrocellulose or nylon membrane using capillary or vacuum-assisted transfer methods.
    - Crosslink the nucleic acids to the membrane using UV radiation or baking.
3. **Hybridization and Detection:**
    - Pre-hybridize the membrane in hybridization buffer.
    - Hybridize with labeled DNA or RNA probes specific to the target sequence.
    - Wash away unbound probes and detect bound probes using autoradiography (radioactive probes) or chemiluminescence (non-radioactive probes).

Results

Visualize and analyze the results by detecting specific bands or signals corresponding to the target molecules on the membrane. Quantify the intensity or size of bands to determine the presence and abundance of specific nucleic acids or proteins.

Gene Transfer Techniques

Aim

To introduce foreign genes into target cells or organisms for genetic manipulation, gene therapy, or biotechnological applications.

Principle

Gene transfer techniques involve delivering exogenous DNA (or RNA) into cells to alter their genetic makeup or express desired traits. Various methods are employed, including viral vectors, non-viral vectors (e.g., liposomes), electroporation, and microinjection. These techniques aim to achieve stable integration or transient expression of the introduced genetic material into the host genome or cytoplasm.

Reagents

1. Plasmid DNA or viral vectors containing the gene of interest
2. Cell culture medium

3. Transfection reagents (e.g., lipids for liposome-mediated transfection, calcium phosphate for calcium phosphate transfection)
4. Electroporation buffer (for electroporation)
5. Antibiotics (for selection of transfected cells, if applicable)
6. Reporter genes (optional, for assessing transfection efficiency)
7. PCR primers or antibodies (for verifying gene expression or protein production)

Apparatus

1. Tissue culture plates or flasks
2. Incubator (with appropriate temperature and CO2 levels for cell culture)
3. Microscope
4. Centrifuge
5. Electroporator (for electroporation)
6. Flow cytometer (for flow cytometry-based analysis, if applicable)

Procedure

1. Preparation of Host Cells

1. **Cell Culture:**
    - Grow host cells (e.g., mammalian cells, bacterial cells) to appropriate confluency or density in cell culture medium.

2. Gene Transfer Methods

a. Viral Vector-Mediated Gene Transfer

1. **Preparation of Viral Vector:**
    - Prepare recombinant viral vectors (e.g., retrovirus, lentivirus, adenovirus) containing the gene of interest.
    - Amplify and purify viral vectors to high titer for efficient transduction.
2. **Transduction:**
    - Add viral vectors to cultured cells and allow for infection.
    - Incubate cells to allow viral entry and integration of the exogenous DNA into the host genome.
3. **Selection (if applicable):**
    - Culture cells in the presence of antibiotics or selectivity markers carried by the viral vector.
    - Select for cells that have stably integrated the transgene.

b. Non-Viral Vector-Mediated Gene Transfer (e.g., Lipofection)

1. **Complex Formation:**
   - Mix plasmid DNA or RNA with liposomes or cationic polymers to form complexes.
   - Incubate complexes with cultured cells to facilitate uptake via endocytosis or direct fusion with cell membranes.
2. **Transfection:**
   - Allow cells to incubate with DNA-lipid complexes for a period to achieve transfection.
   - Optimize conditions such as DNA concentration, lipid-to-DNA ratio, and transfection duration for efficient gene delivery.

c. Electroporation

1. **Preparation:**
   - Resuspend cells in electroporation buffer containing the gene of interest and adjust cell density.
2. **Electroporation:**
   - Apply electric pulses of controlled voltage and duration to induce temporary pores in cell membranes.
   - Allow DNA molecules to enter cells through these pores.
3. **Recovery:**
   - Transfer electroporated cells to fresh medium and culture under optimal conditions.
   - Incubate cells to allow for gene expression or integration of transfected DNA.

4. **Analysis of Gene Transfer**

a. Assessing Transfection Efficiency

1. **Reporter Genes:**
   - Use reporter genes (e.g., GFP, luciferase) co-transfected with the gene of interest to assess transfection efficiency.
   - Monitor reporter gene expression using a microscope, flow cytometer, or other detection methods.

Results

Evaluate the success of gene transfer by assessing transfection efficiency, gene expression levels, or the presence of desired traits in transfected cells. Document and analyze results to determine the effectiveness of each gene transfer method used.

PCR Applications

Aim

To amplify specific DNA sequences from a complex mixture, allowing for various applications such as gene cloning, genetic testing, mutation detection, and gene expression analysis.

Principle

Polymerase Chain Reaction (PCR) is a technique used to amplify DNA sequences exponentially. It involves repeated cycles of denaturation, annealing, and extension, facilitated by a DNA polymerase enzyme. PCR requires specific primers that flank the target DNA region, nucleotides (dNTPs) for DNA synthesis, buffer for enzyme activity, and a thermal cycler to control temperature cycles.

Reagents

1. DNA template (genomic DNA or plasmid DNA)
2. PCR primers (specific to target DNA sequence)
3. DNA polymerase (e.g., Taq polymerase, Pfu polymerase)
4. dNTPs (deoxynucleotide triphosphates: dATP, dTTP, dCTP, dGTP)
5. PCR buffer (containing MgCl2 and stabilizers)
6. MgCl2 (magnesium chloride, for enzyme activity)
7. Template DNA (genomic DNA, cDNA, plasmid DNA)
8. Thermal cycler

Apparatus

1. Thermal cycler (PCR machine)
2. Microcentrifuge tubes
3. PCR tubes or plates
4. PCR-grade water
5. Pipettes and tips (PCR-certified, RNase/DNase-free)
6. Agarose gel electrophoresis equipment (for gel analysis, if applicable)

Procedure

1. PCR Setup

1. **Primer Design:**
    - Design specific primers complementary to the target DNA sequence flanking the region of interest.
2. **PCR Master Mix Preparation:**
    - Prepare a PCR master mix containing PCR buffer, dNTPs, MgCl2, primers, DNA polymerase, and template DNA (if not added separately).

2. PCR Cycling Conditions

1. **Denaturation:**
    - Heat the PCR mixture to 94-98°C to denature the double-stranded DNA into single strands.
2. **Annealing:**
    - Cool the reaction mixture to 50-65°C to allow specific binding of the primers to complementary sequences on the DNA template.
3. **Extension:**
    - Increase the temperature to 72°C for DNA polymerase to extend the primers by synthesizing complementary DNA strands from dNTPs.
4. **Cycle Repetition:**
    - Repeat denaturation, annealing, and extension cycles typically 25-35 times (depending on target DNA length and desired amplification).

3. PCR Product Analysis

1. **Agarose Gel Electrophoresis:**
    - Analyze PCR products by loading a small aliquot onto an agarose gel containing DNA stain (e.g., ethidium bromide).
    - Apply an electric field to separate DNA fragments based on size.
    - Visualize DNA bands under UV light to confirm amplification and estimate fragment size.
2. **Quantification (Optional):**
    - Quantify PCR product concentration using a spectrophotometer or fluorometer for further downstream applications.

Results

Evaluate the success of PCR amplification by observing the presence and size of DNA bands on the agarose gel. A specific band at the expected size indicates successful amplification of the target DNA sequence. Document the results based on gel analysis or quantitative measurements of PCR product concentration.