Demonstrate familiarity with databases of information pertaining to genes, markers, maps

Aim

To access and utilize online databases to retrieve information related to genes, genetic markers, and genetic maps for research, analysis, and experimentation.

Principle

Databases containing genetic information provide comprehensive repositories of genetic sequences, markers, maps, and associated metadata. These databases facilitate access to genomic data for understanding gene function, genetic variations, evolutionary relationships, and mapping genetic loci.

Reagents

1. **Internet access:** To connect and browse online databases.
2. **Web browser:** To navigate and interact with database interfaces.
3. **Computational tools:** Such as BLAST (Basic Local Alignment Search Tool) for sequence alignment and analysis.

Procedure

1. Accessing Genetic Databases

1. **Identify Relevant Databases:**
    - Choose appropriate databases based on the specific genetic information needed (e.g., genes, markers, maps, genomes).
2. **Navigate Database Websites:**
    - Visit the websites of major genetic databases such as NCBI (National Center for Biotechnology Information), Ensembl, UCSC Genome Browser, or specific organism-centric databases.

2. Retrieving Genetic Information

1. **Search and Query:**
    - Use database search tools to enter keywords, gene names, genetic markers, or genomic regions of interest.
2. **Explore Data Resources:**
    - Retrieve information such as gene sequences, genomic coordinates, functional annotations, genetic variations (SNPs, indels), and gene expression data.

3. Analyzing and Utilizing Data

1. **Data Interpretation:**

- Analyze retrieved data using bioinformatics tools and algorithms to interpret genetic information.
- Compare sequences, identify conserved domains, predict protein structures, or analyze regulatory elements.

2. **Integration with Experimental Data:**
   - Integrate database findings with experimental results for validation or hypothesis testing.
   - Correlate genetic variations or expression patterns with phenotypic traits or disease outcomes.

Results

Utilize the retrieved genetic information to enhance understanding of gene function, genetic diversity, and evolutionary relationships. Document findings and interpretations derived from database queries and analyses.

Exploration of Disease Information using OMIM and Medline

Aim

To utilize online databases like OMIM and Medline to gather comprehensive information about genetic diseases, their genetic basis, clinical manifestations, and current research.

Principle

OMIM and Medline are comprehensive online resources that provide curated information on genetic disorders (OMIM) and medical literature (Medline). These databases facilitate access to genetic, clinical, and bibliographic information relevant to understanding disease etiology, pathophysiology, and ongoing research.

Reagents

1. **Computer or mobile device:** To access internet and database websites.
2. **Web browser:** For navigation and interaction with database interfaces.

Procedure

1. Accessing OMIM and Medline Databases

1. **Navigate to OMIM:**
   - Visit the OMIM website (https://www.omim.org/) to access genetic disease information.
2. **Explore OMIM Database:**
   - Use search functionalities to look up specific genetic diseases, genes, or phenotypes of interest.
   - Retrieve detailed entries including genetic loci, inheritance patterns, clinical features, and molecular mechanisms.

2. Utilizing Medline (PubMed)

1. **Access Medline (PubMed):**
   - Visit the PubMed website to search medical literature.
2. **Search for Relevant Articles:**
   - Enter disease names, gene names, or keywords related to disease pathogenesis or treatment.
   - Retrieve articles published in peer-reviewed journals covering epidemiology, clinical studies, genetic studies, and therapeutic approaches.

3. Analyzing and Integrating Information

1. **Data Interpretation:**
   - Analyze information from OMIM and Medline to understand disease genetics, clinical manifestations, and current research trends.

- Compare genetic findings with clinical outcomes and treatment strategies reported in the literature.

2. **Integration with Experimental Data:**
   - Integrate database findings with experimental data or clinical observations for comprehensive disease understanding.
   - Correlate genetic mutations or variants with disease phenotypes and outcomes.

Results

Utilize the gathered information from OMIM and Medline to enhance understanding of disease mechanisms, genetic factors, clinical presentations, and therapeutic strategies. Document insights derived from database queries and literature reviews.

Designing Oligonucleotide Primers for PCR and Utilization of Relevant Software

Aim

To understand the principles of designing effective oligonucleotide primers for PCR and utilize bioinformatics software tools for primer design.

Principle

Designing oligonucleotide primers for PCR involves selecting specific sequences flanking the target DNA region, considering factors such as primer length, GC content, melting temperature (Tm), sequence specificity, and potential secondary structures. Bioinformatics tools assist in primer design by predicting these parameters and ensuring primer specificity to amplify the desired DNA fragment efficiently.

Reagents

1. **Computer or laptop:** To access bioinformatics software tools.
2. **Internet access:** Required to connect to online tools or databases.
3. **Primer design software:** Examples include Primer3, NCBI Primer-BLAST, IDT OligoAnalyzer, and others.

Procedure

1. Principles of Primer Design

1. **Sequence Selection:**
    - Identify the target DNA sequence or gene region for PCR amplification.
    - Consider specificity by avoiding regions with repetitive sequences or homology to non-target DNA.
2. **Primer Length and GC Content:**
    - Optimize primer length typically between 18-24 nucleotides.
    - Aim for GC content around 40-60% to balance primer stability and specificity.
3. **Melting Temperature (Tm):**
    - Calculate Tm to ensure primers anneal to the target sequence at the optimal temperature during PCR.
    - Tools predict Tm based on primer sequence, GC content, and ionic conditions.
4. **Avoiding Secondary Structures:**
    - Check for potential hairpins, self-dimers, or heterodimers between primers to prevent non-specific amplification.

2. Utilization of Bioinformatics Software

1. **Access Primer Design Tools:**
    - Choose a suitable primer design software tool based on features and user interface.
    - Examples include Primer3, NCBI Primer-BLAST, and others available online or as downloadable software.

2. **Input Parameters:**
    - Enter target DNA sequence or gene identifier into the software interface.
    - Specify parameters such as desired Tm range, primer length, GC content, and amplicon size.
3. **Evaluate Primer Design:**
    - Analyze output results to review primer sequences, Tm values, GC content, and potential secondary structures.
    - Validate primer specificity using tools that perform sequence alignment against databases to avoid non-specific binding.

3. Primer Validation and Experimental Design

1. **Experimental Setup:**
    - Select validated primers based on bioinformatics predictions and design criteria.
    - Prepare PCR reactions using the designed primers, DNA template, PCR master mix, and appropriate controls.
2. **PCR Amplification:**
    - Perform PCR under optimized conditions (denaturation, annealing, extension cycles).
    - Analyze PCR products using gel electrophoresis or real-time PCR to confirm amplification of the target DNA fragment.

Results

Evaluate the success of primer design by assessing PCR amplification efficiency, specificity, and product yield. Document and interpret results based on gel electrophoresis or real-time PCR analysis.

PCR Applications in Assigning Genotypes to RFLP/VNTR Sequences

Aim

To use PCR to amplify RFLP or VNTR sequences from genomic DNA samples and assign genotypes based on the resulting PCR products.

Principle

PCR amplifies specific DNA sequences (RFLP or VNTR regions) from genomic DNA. RFLP analysis involves digesting PCR products with restriction enzymes to detect variations in DNA fragment lengths. VNTR analysis determines the number of tandem repeats in the amplified DNA region. Genotype assignment is based on the size or presence of PCR products corresponding to different alleles.

Reagents

1. **Genomic DNA samples:** Containing target RFLP or VNTR sequences.
2. **PCR primers:** Designed to flank the RFLP or VNTR region of interest.
3. **DNA polymerase:** Suitable for PCR (e.g., Taq polymerase).
4. **dNTPs:** Deoxynucleotide triphosphates (dATP, dTTP, dCTP, dGTP).
5. **PCR buffer:** Contains salts and stabilizers for enzyme activity.
6. **Restriction enzymes:** Specific to RFLP analysis (if applicable).
7. **Loading dye:** For gel electrophoresis.
8. **DNA size marker:** To estimate fragment sizes.

Apparatus

1. **Thermal cycler:** For PCR amplification.
2. **Electrophoresis unit:** For gel electrophoresis.
3. **UV transilluminator:** For visualizing DNA bands.
4. **Gel documentation system:** For capturing gel images.

Procedure

1. PCR Amplification of RFLP/VNTR Sequences

1. **PCR Setup:**
    - Prepare PCR master mix containing genomic DNA, primers, DNA polymerase, dNTPs, and PCR buffer.
    - Set up PCR reactions in tubes or plates, including positive and negative controls.
2. **PCR Cycling Conditions:**
    - Perform PCR with initial denaturation (94-98°C), followed by cycling denaturation (94-98°C), annealing (50-65°C), and extension (72°C) for multiple cycles (typically 25-35 cycles).
3. **PCR Product Analysis:**
    - Analyze PCR products by agarose gel electrophoresis:
        - Prepare an agarose gel and mix with a DNA intercalating dye.
        - Load PCR products along with DNA size marker onto the gel and run electrophoresis.

- Visualize DNA bands under UV light to confirm amplification of target RFLP or VNTR sequences.

2. RFLP Analysis (if applicable)

1. **Digestion with Restriction Enzymes:**
   - Incubate PCR products with specific restriction enzymes that cleave DNA at recognition sites near the RFLP site.
   - Analyze digested products by gel electrophoresis to detect variation in fragment lengths indicative of different alleles.

3. VNTR Analysis

1. **Fragment Analysis:**
   - Determine the number of tandem repeat units based on the size variation of PCR products.
   - Compare PCR product sizes with a DNA size marker to estimate repeat lengths and assign genotypes.

Results

Interpret gel electrophoresis results to assign genotypes based on the presence and size of PCR products corresponding to different alleles. Document genotypes for each sample based on band patterns observed.

Screening Samples for Identified Mutations

Aim

To detect specific mutations in DNA samples using PCR-based methods for diagnostic or research purposes.

Principle

Screening for identified mutations involves designing PCR primers specific to the mutation site, amplifying the target region from genomic DNA, and analyzing PCR products to identify the presence or absence of mutations. This approach helps in diagnosing genetic disorders, studying genetic variations, or monitoring mutation frequencies.

Reagents

1. **Genomic DNA samples:** Containing the target mutation(s).
2. **PCR primers:** Designed to flank the mutation site(s).
3. **DNA polymerase:** Suitable for PCR (e.g., Taq polymerase).
4. **dNTPs:** Deoxynucleotide triphosphates (dATP, dTTP, dCTP, dGTP).
5. **PCR buffer:** Contains salts and stabilizers for enzyme activity.
6. **Loading dye:** For gel electrophoresis or sequencing.
7. **DNA size marker:** To estimate fragment sizes (for gel electrophoresis).
8. **Sequencing primers:** Optional, for Sanger sequencing confirmation.

Apparatus

1. **Thermal cycler:** For PCR amplification.
2. **Gel electrophoresis unit:** For analyzing PCR products (if applicable).
3. **UV transilluminator:** For visualizing DNA bands (if using gel electrophoresis).
4. **DNA sequencer:** For sequencing PCR products (if performing sequencing).

Procedure

1. PCR Amplification of Mutation Sites

1. **PCR Setup:**
   - Prepare PCR master mix containing genomic DNA, mutation-specific primers, DNA polymerase, dNTPs, and PCR buffer.
   - Include positive (DNA with known mutation) and negative (wild-type DNA) controls.
2. **PCR Cycling Conditions:**
   - Perform PCR with initial denaturation (94-98°C), followed by cycling denaturation (94-98°C), annealing (50-65°C, specific to primer Tm), and extension (72°C) for multiple cycles (typically 25-35 cycles).

2. Analysis of PCR Products

a. Gel Electrophoresis (for PCR-RFLP or fragment analysis)

1. **Prepare Agarose Gel:**
    - Cast and prepare an agarose gel with a suitable concentration (e.g., 1-2% agarose).
    - Mix gel with DNA intercalating dye (e.g., ethidium bromide).
2. **Load and Run Gel Electrophoresis:**
    - Load PCR products along with DNA size marker onto the gel wells.
    - Run electrophoresis at a suitable voltage and duration to separate DNA fragments by size.
3. **Visualize Bands:**
    - After electrophoresis, visualize DNA bands under UV light to detect mutation-specific PCR products (e.g., altered fragment sizes).

b. Sanger Sequencing (for direct mutation confirmation)

1. **Purify PCR Products:**
    - Purify mutation-specific PCR products using a purification kit or enzymatic cleanup.
2. **Sequencing Reaction:**
    - Set up sequencing reactions using purified PCR products, sequencing primers, DNA polymerase, and dNTPs.
    - Perform sequencing reactions according to manufacturer protocols.
3. **Sequence Analysis:**
    - Analyze sequencing data to identify nucleotide changes or mutations compared to a reference sequence.

Results

Interpret results based on gel electrophoresis band patterns or sequencing chromatograms. Determine the presence or absence of mutations in analyzed samples. Document genotypes or mutations identified in each sample.

Immobilization of an Enzyme and Its Assay

Aim

To immobilize the enzyme (amylase or invertase) and evaluate its activity using a spectrophotometric assay.

Principle

Enzyme immobilization involves attaching the enzyme to a solid support or matrix, allowing for reuse and stability. The assay measures the enzyme's ability to catalyze specific reactions, typically monitored by detecting changes in absorbance or color intensity.

Reagents

1. **Enzyme:** Amylase or invertase enzyme solution.
2. **Support Matrix:** Such as agarose beads, activated charcoal, or silica gel.
3. **Buffer Solutions:** Suitable buffers for enzyme stability and activity (e.g., phosphate buffer).
4. **Substrate:** Starch solution (for amylase) or sucrose solution (for invertase).
5. **Assay Reagents:** Iodine solution (for amylase assay) or DNS reagent (for invertase assay).
6. **Spectrophotometer:** For measuring absorbance changes.
7. **Calibration standards:** Known concentrations of enzyme or products for calibration.

Procedure

1. Immobilization of the Enzyme

1. **Preparation of Support Matrix:**
   - Activate the support matrix according to manufacturer instructions (if needed).
   - Prepare a slurry of the matrix in a suitable buffer.
2. **Enzyme Immobilization:**
   - Mix enzyme solution with the support matrix slurry.
   - Incubate under gentle agitation or shaking for sufficient time to allow enzyme binding to the matrix.
3. **Washing and Stabilization:**
   - Wash the immobilized enzyme to remove unbound enzyme or impurities.
   - Stabilize the immobilized enzyme by incubating in buffer to ensure activity and durability.

2. Enzyme Assay

a. Amylase Assay (for immobilized amylase)

1. **Substrate Preparation:**
   - Prepare starch solution in phosphate buffer.
2. **Assay Setup:**
   - Mix immobilized amylase with starch substrate solution in test tubes.
   - Incubate at appropriate temperature and pH for enzyme activity.

3. **Stopping the Reaction:**
    - Add iodine solution to stop the reaction after a defined time interval.
    - The iodine will form a blue complex with the remaining starch.
4. **Measurement:**
    - Measure the absorbance at a specific wavelength (e.g., 540 nm) using a spectrophotometer.
    - Compare with a standard curve of known enzyme concentrations to calculate enzyme activity.

b. Invertase Assay (for immobilized invertase)

1. **Substrate Preparation:**
    - Prepare sucrose solution in phosphate buffer.
2. **Assay Setup:**
    - Mix immobilized invertase with sucrose substrate solution in test tubes.
    - Incubate at appropriate temperature and pH for enzyme activity.
3. **Stopping the Reaction:**
    - Add DNS reagent to stop the reaction after a defined time interval.
    - DNS reagent will react with reducing sugars released by invertase.
4. **Boiling and Measurement:**
    - Boil the mixture to develop color.
    - Measure the absorbance at a specific wavelength (e.g., 540 nm) using a spectrophotometer.
    - Compare with a standard curve of known enzyme concentrations to calculate enzyme activity.

Results

Interpret the assay results by calculating enzyme activity units per milligram of immobilized enzyme. Document the effectiveness of enzyme immobilization in retaining activity and the efficiency of the assay in quantifying enzyme activity.

Expression of an enzyme activity using a western Blotting technique.

Aim:

To detect and quantify the expression levels of [enzyme of interest] in [specify sample or cell line] under [conditions or treatments].

Principle:

Western blotting is a technique used to detect specific proteins in a sample. It involves separating proteins by gel electrophoresis, transferring them to a membrane, and then detecting the target protein using antibodies that bind specifically to it.

Reagents:

1. Protein extraction buffer
2. SDS-PAGE gel and running buffer
3. Transfer buffer
4. Antibodies specific to the enzyme of interest (primary and secondary antibodies)
5. Blocking solution (e.g., BSA or milk)
6. Detection reagents (e.g., ECL substrate)
7. Protein molecular weight markers

Procedure:

1. **Protein Extraction:**
    - Extract proteins from your samples using a suitable protein extraction buffer.
    - Determine protein concentration using a protein assay (e.g., Bradford or BCA assay).
2. **SDS-PAGE Gel Electrophoresis:**
    - Prepare an SDS-PAGE gel according to the desired resolving range.
    - Load equal amounts of protein (based on concentration) into each lane along with molecular weight markers.
    - Run the gel at the appropriate voltage until the dye front reaches the bottom of the gel.
3. **Protein Transfer:**
    - Transfer proteins from the gel to a nitrocellulose or PVDF membrane using a transfer apparatus and transfer buffer.
4. **Blocking and Antibody Incubation:**
    - Block the membrane using blocking solution to prevent nonspecific binding.
    - Incubate the membrane with primary antibody specific to the enzyme of interest overnight at 4°C.
5. **Washing and Secondary Antibody Incubation:**
    - Wash the membrane to remove unbound primary antibody.

- Incubate the membrane with a secondary antibody conjugated to a detection molecule (e.g., HRP) for 1 hour at room temperature.

6. **Detection:**
   - Wash the membrane to remove unbound secondary antibody.
   - Visualize the enzyme bands using an appropriate detection method (e.g., chemiluminescence).
   - Capture and analyze the results using imaging equipment.

Results:

- The Western blot should show bands corresponding to the enzyme of interest at the expected molecular weight.
- Quantify the expression levels of the enzyme by densitometry analysis of the bands.
- Compare expression levels under different conditions or treatments if applicable.

Equation for substrate consumption in an immobilized cell reactor.

Aim:

To investigate the rate of substrate consumption by immobilized cells in a reactor system under specific conditions.

Principle:

Immobilized cell reactors involve entrapping or attaching cells onto a support matrix within a reactor. The cells remain active and catalyze reactions while being physically confined. The rate of substrate consumption can be measured to assess the efficiency of the immobilized cell system.

Reagents:

1. Growth medium suitable for the cells used
2. Substrate of interest (e.g., glucose, ethanol)
3. Buffer solutions (if needed for maintaining pH)
4. Immobilization matrix (e.g., alginate beads, porous carriers)
5. Analytical reagents for measuring substrate concentration (e.g., spectrophotometric assays)

Procedure:

1. **Cell Culture and Immobilization:**
    - Culture the cells under optimal conditions until they reach the desired growth phase.
    - Prepare the immobilization matrix (e.g., alginate beads) according to established protocols.
    - Immobilize the cells onto the matrix by mixing them with the matrix material and forming beads or coating carriers.
2. **Reactor Setup:**
    - Prepare the reactor vessel suitable for immobilized cell culture.
    - Fill the reactor with growth medium containing the substrate at a known concentration.
3. **Monitoring Substrate Consumption:**
    - Start the reactor and allow the cells to catalyze the substrate consumption over a specified time period.
    - Collect samples at regular intervals to measure substrate concentration.
4. **Analytical Measurement:**
    - Use appropriate analytical methods (e.g., spectrophotometry, HPLC) to quantify the remaining substrate concentration in the reactor effluent.
    - Construct a calibration curve if necessary for accurate quantification.
5. **Data Analysis:**

- Calculate the rate of substrate consumption by plotting substrate concentration versus time.
- Determine the specific substrate consumption rate per unit mass or volume of immobilized cells.

Results:

- Plot the substrate concentration versus time to visualize the consumption kinetics.
- Calculate and report the specific substrate consumption rate (e.g., mmol substrate consumed per hour per gram of immobilized cells).
- Discuss the efficiency of substrate utilization by the immobilized cells compared to free cells or other reactor configurations.

Affinity purification of Histidine Tagged proteins.

Aim:

To purify Histidine tagged proteins from a cell lysate using Ni-NTA affinity chromatography.

Principle:

Ni-NTA affinity chromatography utilizes the specific interaction between Histidine tags (His-tags) on proteins and nickel ions immobilized on a solid support (Ni-NTA resin). This allows for selective binding, washing, and elution of the His-tagged proteins while separating them from non-His-tagged proteins.

Reagents:

1. Ni-NTA resin (agarose or magnetic beads)
2. Buffers:
    - Lysis buffer (e.g., containing Tris-HCl, NaCl, imidazole, and protease inhibitors)
    - Binding buffer (e.g., containing Tris-HCl, NaCl, imidazole)
    - Wash buffer (e.g., containing Tris-HCl, NaCl, imidazole)
    - Elution buffer (e.g., containing Tris-HCl, NaCl, imidazole at higher concentration)
3. DTT (Dithiothreitol) or β-mercaptoethanol (for reducing conditions)
4. Protease inhibitors
5. SDS-PAGE sample buffer
6. Protein assay reagent (e.g., Bradford or BCA assay)
7. Molecular weight markers

Procedure:

1. **Cell Lysis and Protein Extraction:**
    - Lyse cells expressing the Histidine Tagged proteins using a suitable lysis buffer containing detergents and protease inhibitors.
    - Centrifuge the lysate to remove cell debris, retaining the supernatant containing soluble proteins.

2. **Ni-NTA Column Preparation:**
    - Equilibrate the Ni-NTA resin with binding buffer to remove storage buffer components.
    - Pack the resin into a chromatography column or use in batch mode (if using magnetic beads).
3. **Binding and Washing:**
    - Apply the cleared cell lysate to the Ni-NTA resin and allow the His-tagged proteins to bind to the resin.
    - Wash the resin with wash buffer to remove nonspecifically bound proteins and contaminants.
4. **Elution:**
    - Elute the His-tagged proteins from the Ni-NTA resin using elution buffer containing a higher concentration of imidazole.
    - Collect eluted fractions into tubes containing SDS-PAGE sample buffer for subsequent analysis.
5. **Analysis:**
    - Analyze the eluted fractions by SDS-PAGE to confirm purity and molecular weight of the His-tagged protein.
    - Optionally, quantify the purified protein using a protein assay if needed.

Results:

- Obtain purified His-tagged protein fractions confirmed by SDS-PAGE.
- Measure the yield and purity of the purified protein.
- Assess the functionality of the protein in downstream assays if applicable.

This experiment allows for efficient purification of Histidine Tagged proteins using Ni-NTA affinity chromatography, demonstrating the specificity and utility of the His-tag for protein

Expression of Eukaryotic protein in a prokaryotic system.

Aim:

To express a eukaryotic protein of interest in a prokaryotic system (Escherichia coli) for subsequent analysis or functional studies.

Principle:

Expression of eukaryotic proteins in prokaryotic systems like E. coli involves cloning the gene encoding the eukaryotic protein into a suitable expression vector compatible with bacterial hosts. The system relies on the prokaryotic machinery to transcribe and translate the eukaryotic gene into protein, despite potential challenges such as codon usage differences or lack of post-translational modifications.

Reagents:

1. E. coli strain suitable for protein expression (e.g., BL21(DE3))
2. Expression vector (e.g., pET series) containing:
    - Promoter (e.g., T7 promoter for inducible expression)
    - Antibiotic resistance marker (e.g., ampicillin resistance gene)
    - Ribosome binding site (RBS)
3. Gene encoding the eukaryotic protein of interest (synthesized or cloned)
4. LB (Luria-Bertani) broth or agar plates with appropriate antibiotics
5. IPTG (Isopropyl β-D-1-thiogalactopyranoside) for induction
6. Lysis buffer (for protein extraction)
7. SDS-PAGE sample buffer

8. Molecular weight markers

Procedure:

1. **Cloning of the Gene:**
    - Clone the gene encoding the eukaryotic protein into the chosen expression vector using standard molecular biology techniques (e.g., restriction digestion, ligation).
    - Verify the correct orientation and sequence of the insert by sequencing.
2. **Transformation and Selection:**
    - Transform the recombinant expression vector into competent E. coli cells (e.g., BL21(DE3)) by heat shock or electroporation.
    - Plate cells on LB agar plates containing appropriate antibiotics (e.g., ampicillin) and incubate overnight at 37°C to select for transformants.
3. **Expression Cultures:**
    - Inoculate a single colony into LB broth supplemented with antibiotics and grow overnight at 37°C with shaking.
    - Subculture into fresh LB broth the next day and grow until reaching an optical density (OD600) of 0.4-0.6.
4. **Induction of Protein Expression:**
    - Add IPTG to a final concentration typically around 0.1-1 mM to induce protein expression.
    - Continue incubation at 37°C for a suitable period (e.g., 4 hours) with shaking to allow protein synthesis.
5. **Protein Extraction and Analysis:**
    - Harvest cells by centrifugation and resuspend in lysis buffer.
    - Lyse cells by sonication or freeze-thaw cycles, then centrifuge to clarify the lysate.
    - Analyze the soluble fraction by SDS-PAGE to confirm expression of the eukaryotic protein at the expected molecular weight.
6. **Purification (if required):**
    - If purification is necessary, use affinity chromatography (e.g., Ni-NTA) or other methods suitable for the His-tagged or otherwise modified protein.
    - Assess purity and yield of the purified protein by SDS-PAGE and protein quantification assays.

Results:

- Confirm expression of the eukaryotic protein in the prokaryotic host by SDS-PAGE.
- Evaluate the yield and solubility of the expressed protein.
- Optionally, perform functional assays or structural studies to validate the activity or conformation of the protein.