## Preparation of animal cell culture media and membrane filtration

**Aim:**

The aim of this procedure is to prepare a suitable growth medium for animal cell culture and to ensure its sterility through membrane filtration.

**Principle:**

Animal cell culture media are formulated to provide all essential nutrients, growth factors, salts, and a suitable pH environment for the growth and proliferation of animal cells in vitro. Membrane filtration is employed to sterilize the media by removing microorganisms and particles larger than the pore size of the filter membrane.

**Materials:**

- Various cell culture media components (amino acids, vitamins, salts, sugars, etc.)
- Sterile water
- pH meter and/or pH indicator strips
- Membrane filter (0.2 µm pore size)
- Filtration unit (e.g., vacuum filtration setup or syringe filters)
- Autoclave or sterilization equipment
- Sterile containers or bottles for media storage

**Methodology:**

1. **Media Preparation:**
    - Calculate the quantities of each component required according to the formulation of the specific cell culture media (e.g., DMEM, RPMI-1640).
    - Prepare a stock solution or dissolve each component in sterile water, adjusting the pH as necessary to achieve the desired final pH of the medium.
    - Combine all components in a sterile container or bottle and mix thoroughly.
2. **Sterilization by Membrane Filtration:**
    - Ensure the media preparation area and all equipment are sterile.
    - Attach a sterile membrane filter (0.2 µm pore size) to a filtration unit (e.g., filtration flask or syringe).
    - Pour the prepared media through the membrane filter using aseptic technique to prevent contamination.
    - Apply a vacuum or pressure to facilitate the filtration process.
    - Collect the sterilized media in a sterile container or bottle

**Procedure:**

1. **Preparation of Media:**
    - Weigh and dissolve each component of the media in sterile water according to the formulation.
    - Adjust the pH of the media solution using a pH meter or indicator strips, typically between 7.2 and 7.4 for most cell lines.
    - Mix the components thoroughly until completely dissolved.
2. **Sterilization:**
    - Set up the membrane filtration unit under sterile conditions.
    - Ensure the membrane filter is securely attached and the filtration system is sealed to prevent air leaks.
    - Slowly pour the prepared media through the membrane filter.
    - Apply gentle vacuum or pressure to facilitate filtration without damaging the filter membrane.
    - Collect the sterilized media in a sterile container or bottle.

**Expected Result:**

Upon completion of the procedure, you should have a clear and sterile cell culture medium ready for use in maintaining animal cell cultures. The media should be free from visible particulates and microbial contamination, as confirmed by the sterilization process through membrane filtration.

This outline provides a structured approach to preparing animal cell culture media and ensuring its sterility using membrane filtration, essential for maintaining healthy and viable cell cultures in a laboratory setting.

**Preparation of single cell suspension from spleen and thymus**.

**Aim:**

To obtain a single-cell suspension from spleen and thymus tissues for downstream cellular analysis or experimentation.

**Principle:**

The principle involves mechanical and enzymatic dissociation of the tissue to release individual cells from the extracellular matrix and cellular aggregates.

**Materials:**

- Spleen and thymus tissues
- Dulbecco's Modified Eagle Medium (DMEM) or RPMI-1640 medium (or other appropriate cell culture medium)
- Fetal bovine serum (FBS)
- Collagenase, DNase, and/or trypsin (enzymes for tissue dissociation)
- PBS (Phosphate-buffered saline)
- Sterile forceps and scissors
- 70 µm cell strainer
- Centrifuge tubes
- Pipettes and pipette tips

**Methodology:**

1. **Preparation of Enzyme Solutions:**
    - Prepare enzyme solutions (e.g., collagenase, DNase) in appropriate concentrations in PBS or cell culture medium.
2. **Tissue Dissection:**
    - Sacrifice the animal and aseptically remove the spleen and thymus.
    - Place each organ in a sterile dish containing cold PBS.
3. **Mechanical Disruption:**
    - Using sterile forceps and scissors, mince the tissue into small pieces (1-2 $mm^3$).
4. **Enzymatic Digestion:**
    - Transfer the minced tissue pieces into a tube containing the prepared enzyme solution.
    - Incubate at 37°C for a specified time (usually 30-60 minutes) with periodic agitation.
5. **Cell Release:**
    - After digestion, triturate the tissue suspension gently with a sterile pipette to further dissociate clumps into single cells.

6. **Filtering:**
    - Pass the cell suspension through a 70 µm cell strainer to remove undigested tissue debris and obtain a uniform single-cell suspension.
7. **Washing and Centrifugation:**
    - Collect the filtered suspension in a new tube.
    - Wash the cells by adding PBS and centrifuge at low speed (e.g., 300-400 × g for 5 minutes) to pellet the cells.
8. **Resuspension:**
    - Discard the supernatant and resuspend the cell pellet in an appropriate volume of complete medium (DMEM/RPMI supplemented with FBS).
9. **Cell Counting:**
    - Perform cell counting using a hemocytometer or automated cell counter to determine cell concentration and viability.

**Expected Result:**

You should obtain a homogeneous suspension of single cells suitable for further analysis or experimental procedures, such as flow cytometry, cell culture, or molecular biology assays.

## MTT assay for cell viability and growth

**Aim:**

The aim of the MTT assay is to assess cell viability and proliferation (growth) by measuring the metabolic activity of cells. This assay is widely used in research and drug development to evaluate the effects of compounds, treatments, or conditions on cell health.

**Principle:**

The principle behind the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay is based on the reduction of MTT by mitochondrial enzymes in viable cells to form insoluble formazan crystals. These crystals are then solubilized and quantified spectrophotometrically, providing a measure of the metabolic activity of the cells. Thus, higher absorbance values indicate higher cell viability and proliferation.

**Materials and Methodology:**

**Materials:**

- MTT reagent
- Cell culture medium
- Experimental treatments or compounds
- 96-well plates
- Spectrophotometer
- Solubilization solution (commonly DMSO)

**Methodology:**

1. **Cell Culture:** Cultivate cells of interest in appropriate culture conditions (e.g., temperature, CO2 levels).
2. **Treatment:** Treat cells with experimental compounds or conditions as per the experimental design.
3. **MTT Addition:** Add MTT solution to each well containing cells. MTT is typically added at a concentration of 0.5 mg/ml.
4. **Incubation:** Incubate cells with MTT solution for a specified period (usually 2-4 hours) to allow the conversion of MTT to formazan crystals by mitochondrial enzymes.
5. **Formazan Solubilization:** After incubation, carefully remove the medium and add a solubilization solution (commonly DMSO) to dissolve the formazan crystals formed by viable cells.
6. **Measurement:** Measure the absorbance of the solubilized formazan solution at a wavelength around 570 nm (with a reference wavelength around 630 nm) using a spectrophotometer.

**Procedure:**

1. Prepare cell culture plates with appropriate cell densities and conditions.
2. Treat cells with experimental compounds or conditions and include appropriate controls (e.g., untreated cells, vehicle controls).
3. Add MTT reagent to each well and incubate under standard cell culture conditions.
4. Remove the medium carefully and add DMSO or another suitable solvent to dissolve the formazan crystals.
5. Measure the absorbance of each well at 570 nm using a spectrophotometer.
6. Analyze the data to determine relative cell viability and growth compared to controls.

**Given Result:**

The results of an MTT assay are typically presented as absorbance values at 570 nm after correcting for background absorbance at 630 nm. These values reflect the metabolic activity of the cells, which correlates with cell viability and proliferation. Higher absorbance indicates higher cell viability and growth, whereas lower absorbance suggests reduced viability or cytotoxic effects.

In summary, the MTT assay provides a quantitative assessment of cell viability and growth based on metabolic activity, making it a valuable tool in biomedical research and drug discovery.

## Demonstration of sections of human ovary, testis and aborted human embryos.

**Aim:**

To study the histological features and structures of human ovary, testis, and aborted human embryos for educational or research purposes.

**Principle:**

Histological examination involves the microscopic study of tissues to understand their structure, organization, and cellular composition. This helps in identifying specific features and functions of organs and developmental stages.

**Materials:**

- **Human ovary and testis sections**: Obtained from medical specimens or research repositories, prepared for microscopic analysis.
- **Aborted human embryo sections**: Obtained from ethical sources with appropriate permissions and consent, prepared similarly.

**Methodology:**

***Preparation of Specimens:***

1. **Fixation**: Specimens are fixed using formalin or other appropriate fixatives to preserve tissue structure.
2. **Embedding**: Specimens are embedded in paraffin wax to facilitate thin sectioning.
3. **Sectioning**: Thin sections (typically 5-10 micrometers thick) are cut using a microtome.
4. **Mounting**: Sections are mounted on glass slides and dried.

***Staining:***

1. **Hematoxylin and Eosin (H&E) Staining**: This is the most common staining method used in histology to visualize cell nuclei (hematoxylin stains nuclei blue) and cytoplasm (eosin stains cytoplasm pink).

**Procedure:**

1. **Microscopic Examination**:
   - Place the prepared slides under a light microscope.
   - Start at low magnification (10x or 20x) to locate general structures.
   - Switch to higher magnifications (40x, 100x) to examine cellular details.
2. **Identification of Structures**:

- **Human Ovary**: Look for ovarian follicles at various stages of development, including primordial, primary, secondary, and Graafian follicles.
- **Human Testis**: Identify seminiferous tubules containing germ cells (spermatogonia, spermatocytes, spermatids) and Sertoli cells.
- **Aborted Human Embryos**: Examine embryonic tissues to observe early developmental stages, including germ layers (ectoderm, mesoderm, endoderm) and primitive organ formation if identifiable.

**Expected Results:**

- **Human Ovary**: Presence of different types of ovarian follicles depending on the stage of development.
- **Human Testis**: Seminiferous tubules with varying populations of germ cells and supporting Sertoli cells.
- **Aborted Human Embryos**: Structures indicative of early developmental stages, possibly showing rudimentary organs or tissues from ectoderm, mesoderm, and endoderm layers.

## Identification of Commercially Important Aquatic Species

### Aim

To identify commercially important aquatic species using morphological and molecular methods.

### Principle

Identification of aquatic species can be achieved through a combination of morphological observations and molecular techniques. Morphological identification involves examining physical characteristics, while molecular identification uses DNA barcoding or other genetic markers to confirm species identity.

### Reagents

1. Sample collection tools (e.g., nets, containers)
2. Ethanol (70%) for sample preservation
3. DNA extraction kit
4. PCR reagents (primers, Taq polymerase, dNTPs, buffer)
5. Agarose gel and electrophoresis apparatus
6. DNA sequencing reagents and equipment
7. Molecular weight marker (DNA ladder)
8. Morphological identification keys or guides

### Apparatus

1. Dissecting microscope
2. Compound microscope
3. Forceps and dissecting tools
4. Thermal cycler (PCR machine)
5. Microcentrifuge tubes
6. Pipettes and tips
7. Gel electrophoresis apparatus
8. UV transilluminator
9. DNA sequencing machine (or access to sequencing services)

### Procedure

#### 1. Sample Collection and Preservation:

- Collect samples of commercially important aquatic species from their natural habitat using appropriate tools.
- Preserve samples in 70% ethanol for molecular analysis.

- Keep some samples alive for morphological observations.

**2. Morphological Identification:**

- Examine the preserved and live samples using a dissecting microscope.
- Identify key morphological features (e.g., body shape, fin structure, coloration) using identification keys or guides.
- Record observations and compare with known species descriptions.

**3. DNA Extraction:**

- Extract DNA from preserved samples using a DNA extraction kit following the manufacturer's protocol.
- Ensure the DNA is of high quality and free of contaminants.

**4. PCR Amplification:**

- Prepare the PCR reaction mix in a microcentrifuge tube. A typical reaction (total volume 25 µL) may include:
    - 1-5 µL extracted DNA template
    - 0.5-1 µL forward primer (e.g., for COI gene)
    - 0.5-1 µL reverse primer
    - 0.5 µL dNTPs mix (10 mM each)
    - 0.5-1 µL DNA polymerase (e.g., Taq polymerase)
    - 2.5 µL 10X PCR buffer
    - Nuclease-free water to make up the final volume to 25 µL
- Mix gently by pipetting up and down.

**5. PCR Cycling Conditions:**

- Place the reaction tube in the thermal cycler and set the cycling conditions. A typical PCR program consists of:
    1. Initial denaturation: 95°C for 2-5 minutes
    2. 30-35 cycles of:
        - Denaturation: 95°C for 30 seconds
        - Annealing: 50-65°C for 30 seconds (depending on the melting temperature of the primers)
        - Extension: 72°C for 1 minute (time depends on the length of the target sequence, typically 1 minute per 1 kb of DNA)
    3. Final extension: 72°C for 5-10 minutes
    4. Hold: 4°C (indefinitely, until samples are removed)

**6. Gel Electrophoresis:**

- Prepare an agarose gel (1-2%) and set up the gel electrophoresis apparatus.
- Mix a portion of the PCR product with a DNA loading dye.
- Load the samples into the wells of the agarose gel along with a DNA ladder (molecular weight marker).
- Run the gel at an appropriate voltage (e.g., 100-120 V) until the dye has migrated sufficiently.
- Stain the gel with ethidium bromide or another DNA stain.
- Visualize the DNA bands under a UV transilluminator.

**7. DNA Sequencing:**

- Purify the PCR products if necessary.
- Send the purified PCR products for DNA sequencing or use an in-house DNA sequencing machine.
- Analyze the sequencing results using bioinformatics tools to compare with known sequences in databases (e.g., GenBank).

**Results**

Record the morphological observations and compare them with known species descriptions. For molecular identification, record the DNA sequences obtained and compare them with reference sequences in databases.

Present the results in a tabular form:

| **Sample ID** | **Morphological Identification** | **PCR Product Size (bp)** | **DNA Sequence Match (Species)** | **Confidence Level (%)** |
|---|---|---|---|---|

Analyze the results to determine the species identity of the samples based on both morphological and molecular data.

## Estimation of Water Quality Parameters: Dissolved Oxygen, Alkalinity, Hardness

**Aim**

To determine the water quality by estimating the levels of dissolved oxygen (DO), alkalinity, and hardness in water samples.

**Principle**

1. **Dissolved Oxygen (DO):** DO is a measure of the amount of oxygen dissolved in water. It is crucial for the survival of aquatic life and is an indicator of water quality. The Winkler method is commonly used to determine DO, involving titration of water samples with reagents that react with the oxygen present.
2. **Alkalinity:** Alkalinity is the capacity of water to neutralize acids, mainly due to the presence of bicarbonates, carbonates, and hydroxides. It is determined by titrating the water sample with a strong acid to a specific pH endpoint.
3. **Hardness:** Hardness is a measure of the concentration of calcium and magnesium ions in water. It is determined by titrating the water sample with a solution of ethylenediaminetetraacetic acid (EDTA), which forms complexes with the calcium and magnesium ions.

**Reagents**

1. **Dissolved Oxygen (DO):**
   - Manganese(II) sulfate solution
   - Alkaline potassium iodide solution
   - Concentrated sulfuric acid
   - Sodium thiosulfate solution (0.025 N)
   - Starch solution (indicator)
2. **Alkalinity:**
   - Hydrochloric acid (0.1 N)
   - Phenolphthalein indicator
   - Methyl orange or bromocresol green indicator
3. **Hardness:**
   - EDTA solution (0.01 M)
   - Ammonia buffer solution (pH 10)
   - Eriochrome Black T indicator

**Apparatus**

1. Burettes
2. Pipettes
3. Conical flasks
4. Graduated cylinders

5. Dissolved oxygen bottles
6. pH meter
7. Magnetic stirrer
8. Analytical balance

**Procedure**

**1. Dissolved Oxygen (DO) by Winkler Method:**

1. **Sample Collection:**
   - Collect water samples in BOD bottles, ensuring no air bubbles are present.
2. **Reagent Addition:**
   - Add 1 mL of manganese(II) sulfate solution and 1 mL of alkaline potassium iodide solution to the sample.
   - Stopper the bottle and mix well. A brown precipitate will form if DO is present.
   - Add 1 mL of concentrated sulfuric acid to dissolve the precipitate. Mix thoroughly.
3. **Titration:**
   - Transfer 50 mL of the treated sample to a conical flask.
   - Titrate with sodium thiosulfate solution until the yellow color fades.
   - Add a few drops of starch solution, which turns the solution blue.
   - Continue titrating until the blue color just disappears.
   - Record the volume of sodium thiosulfate used.

**2. Alkalinity:**

1. **Sample Preparation:**
   - Take 50 mL of the water sample in a conical flask.
2. **Titration with Phenolphthalein:**
   - Add 2-3 drops of phenolphthalein indicator.
   - If the solution turns pink, titrate with 0.1 N HCl until the pink color disappears. Note the volume used (P).
3. **Titration with Methyl Orange or Bromocresol Green:**
   - Add 2-3 drops of methyl orange or bromocresol green indicator to the same

   - Identification and partial characterization of fish and shrimp pathogens. prepare aim,principle reagents procedure result for the given experiment

## Identification and Partial Characterization of Fish and Shrimp Pathogens

### Aim

To identify and partially characterize pathogens affecting fish and shrimp through microbiological, biochemical, and molecular techniques.

### Principle

Identification and characterization of pathogens involve isolating and identifying bacteria, viruses, or fungi from infected fish and shrimp tissues. This can be achieved through culturing techniques, biochemical tests, and molecular methods such as PCR. These methods allow for the determination of the type and nature of the pathogens.

### Reagents

1. **Microbiological Media:**
   - Nutrient agar
   - Tryptic soy agar
   - Selective media (e.g., TCBS agar for Vibrio spp.)
2. **Biochemical Reagents:**
   - Catalase reagent
   - Oxidase reagent
   - Indole reagent
   - Methyl red and Voges-Proskauer reagents
   - Citrate agar
   - Urease broth
3. **Molecular Reagents:**
   - DNA extraction kit
   - PCR reagents (primers specific to pathogen genes, Taq polymerase, dNTPs, buffer)
   - Agarose gel and electrophoresis apparatus
   - Ethidium bromide or other DNA stains
   - DNA ladder (molecular weight marker)

### Apparatus

1. Incubator
2. Sterile petri dishes
3. Inoculating loop
4. Micropipettes and tips
5. Centrifuge

6. PCR machine (thermal cycler)
7. Gel electrophoresis apparatus
8. UV transilluminator
9. Microscope
10. Glass slides and coverslips
11. pH meter
12. Sterile containers for sample collection

**Procedure**

**1. Sample Collection and Initial Processing:**

1. **Collection:**
   - Collect tissue samples from infected fish and shrimp (e.g., gills, skin lesions, hepatopancreas) using sterile tools.
   - Transport samples in sterile containers to the laboratory.
2. **Homogenization:**
   - Homogenize tissue samples in sterile saline or phosphate-buffered saline (PBS).
3. **Inoculation:**
   - Streak homogenized samples onto appropriate microbiological media (e.g., nutrient agar, TCBS agar) and incubate at suitable temperatures (e.g., 25-30°C for 24-48 hours).

**2. Isolation and Identification of Bacteria:**

1. **Colony Morphology:**
   - Examine the colonies for morphological characteristics such as color, shape, and size.
2. **Gram Staining:**
   - Perform Gram staining on isolated colonies and observe under a microscope to determine Gram reaction (positive or negative).
3. **Biochemical Tests:**
   - Conduct a series of biochemical tests to identify bacterial species:
     - Catalase test: Add a drop of hydrogen

## Fish Pituitary Hypophysation

**Aim**

To induce and collect fish pituitary gland extract for use in aquaculture practices.

**Principle**

Pituitary hypophysation involves extracting and processing pituitary glands from fish to obtain gonadotropic hormones. These hormones are used in aquaculture to induce spawning and improve reproductive efficiency.

**Reagents**

1. Mature fish (male and female)
2. Hypophysation equipment (scalpel, forceps, petri dish)
3. Isotonic saline solution
4. Ice
5. Ethanol or acetone (for pituitary extraction)
6. Glassware (beakers, test tubes)

**Apparatus**

1. Dissecting microscope
2. Magnetic stirrer
3. Centrifuge
4. pH meter
5. Autoclave
6. Refrigerator

**Procedure**

**1. Preparation of Fish**

1. **Selection of Fish:** Choose mature male and female fish of the desired species with well-developed gonads.
2. **Anesthesia:** Anesthetize the fish using an appropriate anesthetic (e.g., MS-222) to minimize stress and facilitate handling.

**2. Pituitary Extraction**

1. **Dissection:**

- Place the anesthetized fish on a dissecting board.
- Make an incision behind the operculum to expose the gills and brain.
- Carefully remove the brain and pituitary gland using sterile techniques and tools.

2. **Pituitary Gland Collection:**
   - Transfer the pituitary glands to a petri dish containing isotonic saline solution.
   - Clean and separate the pituitary glands from surrounding tissues.
3. **Storage:**
   - Place the pituitary glands in a storage vial with ethanol or acetone.
   - Store the vial on ice or refrigerate until further processing.

**3. Hormone Extraction and Processing**

1. **Extraction:**
   - Transfer the pituitary glands to a clean glass beaker.
   - Add a known volume of ethanol or acetone to the beaker and homogenize the glands.
2. **Centrifugation:**
   - Centrifuge the homogenate to separate the glandular tissue from the hormone extract.
   - Collect the supernatant containing the pituitary hormones.
3. **Sterilization:**
   - Filter the hormone extract using a sterile filter to remove particulates.
   - Store the filtered extract in sterile containers at low temperatures (4°C).

**4. Hormone Assay**

1. **Determination:**
   - Use specific assays (e.g., ELISA) to quantify the concentration of gonadotropic hormones (FSH, LH) in the extract.

**5. Application in Aquaculture**

1. **Administration:**
   - Administer the pituitary gland extract to fish to induce spawning.
   - Monitor fish behavior and hormone levels to optimize spawning conditions.

**Results**

Document the process yield and hormone concentration in the extract. Present findings in a structured format detailing the effectiveness of pituitary hypophysation in inducing spawning and enhancing reproductive success in fish.

## PCR Diagnosis of White Spot Syndrome Virus (WSSV)

### Aim

To detect the presence of White Spot Syndrome Virus (WSSV) DNA in shrimp samples using Polymerase Chain Reaction (PCR) for diagnostic purposes.

### Principle

PCR is used to amplify specific DNA sequences. In the case of WSSV, specific primers designed for regions of the virus genome are used to amplify and detect WSSV DNA if present in the shrimp samples. This method is sensitive and specific, allowing for rapid diagnosis of WSSV infection in shrimp populations.

### Reagents

1. DNA extraction kit
2. PCR reagents:
   - Forward and reverse primers specific to WSSV DNA
   - Taq DNA polymerase
   - dNTPs (deoxynucleotide triphosphates)
   - PCR buffer
3. Agarose gel
4. Ethidium bromide or other DNA stain
5. DNA ladder (molecular weight marker)
6. Sterile water

### Apparatus

1. Thermal cycler (PCR machine)
2. Micropipettes and tips
3. PCR tubes or plates
4. Gel electrophoresis apparatus
5. UV transilluminator
6. Sterile tubes for sample collection and storage

**Procedure**

**1. Sample Collection and DNA Extraction**

1. **Sample Collection:**
   - Collect tissue samples (e.g., gills, hepatopancreas) from shrimp suspected of WSSV infection using sterile techniques.
   - Transfer samples into sterile tubes and store at -20°C until processing.
2. **DNA Extraction:**
   - Follow the instructions provided with the DNA extraction kit to isolate DNA from shrimp tissue samples.
   - Ensure high-quality DNA extraction to optimize PCR sensitivity.

**2. PCR Amplification of WSSV DNA**

1. **PCR Setup:**
   - Prepare the PCR reaction mix on ice in a sterile environment. A typical reaction (total volume 25 µL) may include:
     - 1-5 µL DNA template
     - 0.5-1 µL forward primer specific to WSSV DNA (10 µM)
     - 0.5-1 µL reverse primer specific to WSSV DNA (10 µM)
     - 0.5 µL dNTPs mix (10 mM each)
     - 0.5-1 µL Taq DNA polymerase
     - 2.5 µL 10X PCR buffer
     - Nuclease-free water to make up the final volume to 25 µL
   - Mix gently by pipetting up and down.
2. **PCR Cycling Conditions:**
   - Place the PCR tubes or plate into the thermal cycler and program the following cycling conditions:
     1. Initial denaturation: 95°C for 5 minutes
     2. 35 cycles of:
        - Denaturation: 95°C for 30 seconds
        - Annealing: 55-60°C (depending on primer Tm) for 30 seconds
        - Extension: 72°C for 1 minute per kb of expected PCR product length
     3. Final extension: 72°C for 10 minutes
     4. Hold: 4°C (indefinitely, until samples are removed)

**3. Gel Electrophoresis**

1. **Preparation of Agarose Gel:**
   - Prepare a 1-2% agarose gel in 1X TAE or TBE buffer with ethidium bromide.
   - Set up the gel in the electrophoresis apparatus.

2. **Loading and Running the Gel:**
   - Mix the PCR products with DNA loading dye.
   - Load the samples into the wells of the agarose gel along with a DNA ladder (molecular weight marker).
   - Run the gel at 100-120 V until the dye has migrated sufficiently.
3. **Visualization of PCR Products:**
   - Place the gel on a UV transilluminator.
   - Visualize the DNA bands under UV light and capture images of the gel using a gel documentation system.

**Results**

Analyze the gel images to determine the presence or absence of PCR products corresponding to the expected size of WSSV DNA. Positive samples will show a band at the expected size on the gel.