

Training in molecular laboratory techniques

(Professional training 2 - REPORT)

Submitted

By

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INTERNSHIP CERTIFICATE

THIS IS TO CERTIFY THAT

GOWSIGA .K

**HAS UNDERGONE TRAINING/ INTERNSHIP IN MOLECULAR BIOLOGY TECHNIQUES AT
OUR LABORATORY AT CHENNAI FROM OCTOBER 1ST TO 15TH OCTOBER 2021**

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Lab Manager

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1. **ANSTRACT**

MOLECULAR BIOLOGY methods used to study the molecular basis of biological activity. Most commonly used methods are protein methods, immunostaining methods, nucleic acid methods. These methods used to explore cells, their characteristics, parts, and chemical processes, and pays special attention to how molecules control a cell's activities and growth. Molecular Biology Techniques include DNA cloning, cut and paste DNA, bacterial transformation , transfection, chromosome integration, cellular screening, cellular culture, extraction of DNA, DNA polymerase DNA dependent, reading and writing DNA, DNA sequencing, DNA synthesis, molecular hybridization, rewriting DNA: mutations, random mutagenesis, point mutation, chromosome mutation. Most important techniques are Polymerase Chain Reaction (PCR), Expression cloning, Gel electrophoresis, Macromolecule blotting and probing, Arrays (DNA array and protein array).

2. **LIST OF ABBREVIATION AND SYMBOLS**

- TAE BUFFER - Tris base, acetic acid and EDTA
- SSC - SALINE SODIUM CITRATE
- MOPS - 3-(N-Morpholino)propane sulfonic acid
- UV - ultra visible
- SDS PAGE - sodium dodecyl sulphate – polyacrylamide gel electrophoresis
- PVDF - Polyvinylidene fluoride
- DNA - Deoxyribonucleic acid
- PCR - Polymerase Chain Reaction
- RT –PCR - reverse transcription-polymerase chain reaction

INTRODUCTION

Molecular biology is the branch of biology that studies the molecular basis of biological activity. A Molecular biology lab is where molecular biologist is to develop an understanding of how biological molecules come together to function as organelles, and ultimately as cells performing a particular function within the body. Molecular biologists conduct research and academic activities.

Aim :

To know the molecular biology laboratory techniques.

Principle :

The basic principle is that DNA, RNA, and proteins can all be separated by means of an electric field and size. In agarose gel electrophoresis, DNA and RNA can be separated on the basis of size by running the DNA through an electrically charged agarose gel. Proteins can be separated on the basis of size by using an SDS-PAGE gel, or on the basis of size and their electric charge by using what is known as a 2D gel electrophoresis.

Types of techniques :

- **Molecular cloning**
- **Polymerase Chain Reaction [PCR]**
- **RT - PCR ,,**
- **Gel electrophoresis**
- **Southern blotting**
- **Northern blotting**
- **Western blotting**

Procedure:

➤ Southern blotting

1. DNA isolation
2. Restriction digestion: digest the DNA with a restriction enzyme, and if necessary, concentrate digested DNA.
3. Gel electrophoresis: prepare an agarose gel and either TAE or TBE buffer (buffer selection will depend on the duration of the run and the size of the DNA fragments). Load samples into wells and include a DNA molecular weight marker. Run the gel.
4. Transfer:
 - Place the gel in a container with denaturing solution, and wash twice for 15 minutes on a shaker.
 - Rinse with water, then wash with neutralization solution.
 - During the previous step, begin to prepare Whatman paper and nylon membrane for the transfer.
 - Assemble the transfer apparatus with the membrane, Whatman paper, and gel and transfer in SSC or SSPE buffer.
 - When transfer is complete, cross-link DNA in a cross-linker, then rinse the membrane.
5. Pre-hybridization (blocking):
 - Blocking reduces non-specific binding to the membrane. Prepare the pre-hybridization solution and add sample DNA. Remove the blot from the cross-linker, add the pre-hybridization solution and incubate.
6. Hybridization:
 - Prepare the probe mixture (a complementary DNA strand) and buffer.
 - Remove the pre-hybridization solution and incubate the blot with the probe (incubation times will vary depending on the application).
 - Following incubation, perform a low-stringency wash followed by a high-stringency wash to refine the DNA
6. Probe detection:
 - Rinse the membrane, transfer to a container with blocking solution and incubate.
 - Discard blocking solution, replace with antibody solution and incubate.
 - Discard antibody solution, wash the membrane.
7. Follow manufacturer directions for chemiluminescent detection

➤ **Northern blotting**

1. RNA isolation

2. Electrophoresis:

- For a formaldehyde agarose gel: prepare the gel and insert the gel tray into the apparatus. Fill with MOPS buffer, load the samples and include a molecular weight marker. Run the gel, then trim the gel prior to blotting.
- For a glyoxal agarose gel: prepare the gel and insert the gel tray into the apparatus. Fill with MOPS buffer, prepare samples and load into wells along with RNA ladder.
- For a denaturing polyacrylamide gel: cast the gel, and mount it in the electrophoresis unit. Prepare samples, load into the gel, and run with TBE running buffer.

3. Transfer:

- For a formaldehyde agarose gel or glyoxal agarose gel: wash the gel in SSC, then assemble the transfer unit with the gel, filter paper, and nylon membrane. When transfer is complete, place the membrane in a UV cross-linker.
- For a denaturing polyacrylamide gel: assemble the transfer unit including gel, filter paper, and nylon membrane ensuring they are flooded with TBE. When transfer is complete, place the membrane in a UV cross-linker to fix the RNA to the membrane.

4. Pre-hybridization (blocking):

- Pre-hybridize the membrane in hybridization solution.

5. Hybridization:

- Add probe to the hybridization solution and incubate.
- Wash the membrane in low-stringency washes to remove hybridization solution and unhybridized probe, and high-stringency washes to remove partially hybridized molecules.
- Follow manufacturer directions for chemiluminescent detection.

➤ **Western Blot**

1. Prepare lysate from cell culture or tissue.
2. Sample preparation:
 - Determine the protein concentration of each sample with a protein quantification assay (ie. Bradford assay).
 - Add an equal volume of 2X Laemmli sample buffer to each sample.
 - Some samples may need to be reduced or denatured, this is achieved by boiling samples in buffer.
3. Electrophoresis:
 - Prepare an SDS-PAGE gel, load samples along with molecular weight marker.
 - Run the gel in running buffer.
4. Transfer:
 - Following electrophoresis, assemble the transfer unit including the gel, PVDF or nitrocellulose membrane, and filter paper.
 - Transfer the proteins to the membrane with transfer buffer.
5. Antibody Staining (indirect detection):
 - Prepare blocking buffer and incubate the membrane to reduce non-specific binding.
 - Incubate the membrane with primary antibody diluted in blocking buffer.
 - Wash the membrane in TBST, and incubate with conjugated secondary antibody diluted in blocking buffer.
 - Wash the membrane in TBST.
 - Follow manufacturer directions for chemiluminescent detection.

PCR

PCR means polymerase chain reaction. It's a test to detect genetic material from a specific organism, such as a virus. The test detects the presence of a virus if you have the virus at the time of the test.

Reagents used in PCR:

- Template DNA,
- PCR primers,
- Nucleotides,
- PCR buffer,
- Sterile water
- Taq polymerase

Taq polymerase

Taq DNA polymerase is a thermostable enzyme derived from the thermophilic bacterium *Thermus aquaticus*. It is commonly used to amplify DNA fragments in PCR. The enzyme is in a recombinant form, expressed in *E. coli*. It is able to withstand repeated heating to 95 °C (as is demanded by the PCR technique) without significant loss of activity. The enzyme has a molecular weight of approximately 94 kDa by SDS-PAGE with no detectable endonuclease or exonuclease activity. It has 5'→3' DNA polymerase activity and 5'→3' exonuclease activity. Each lot of *Taq* DNA Polymerase is tested for PCR amplification and double-stranded sequencing.

STEPS IN PCR

1. **Add the reagents to an appropriately sized tube** in the order provided in the table. (Select appropriate table for reaction setup: standard or ready-mix reagent.) For a large number of reactions, a master mix without the template should be set up and aliquoted into reaction tubes. At the end, template should be added to appropriate tubes.
2. **Mix gently by vortex and briefly centrifuge** to collect all components to the bottom of the tube.

3. **Amplify.** The amplification parameters will vary depending on the primers and the thermal cycler used. It may be necessary to optimize the system for individual primers, template, and thermal cycler.
4. The amplified DNA can be evaluated by agarose gel electrophoresis and subsequent ethidium bromide staining.

RESULT :

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