AVIT AARUPADAI VEEDU INSTITUTE OF TECHNOLOGY | VINAYAKA MISSION'S RESEARCH FOUNDATION (Deemed to be University under section 3 of the UGC Act 1956) | Accredited by NAAC | Approved by AICTE

DEPARTMENT BIOTECHNOLOGY

481172L1-GENETIC ENGINEERING LAB

STANDARD OPERATING PROCEDURE

Name of the Lab./facility	**Molecular biology /Genetic Engineering lab**
Purpose	To provide training to the students on - Isolation of DNA from Plant, humans and Bacteria. -To cut the DNA molecule in to specific place using molecular enzymes(Hind –III, Bam HI) -To ligate the fragmented DNA by ligation Process.
Scope	Experimental training on isolation of DNA from Plant, humans and Bacteria and students able to cut the DNA molecule in to small pieces by using restriction enzymes, able to ligate the fragments DNA using ligase enzyme. This will be use full for their project work and their forthcoming research.
Responsibility	Faculty i/c of the facility, HOD/BIOTECH

STANDARD OPERATING PROCEDURE FOR POLYMERASECHAIN REACTION (PCR)

- Always put on a fresh pair of gloves before going anywhere near the PCR bench.
- All users should have their own aliquoted reagents (dNTP's, primers, etc.)—that way if you contaminate your own reagents, you have not contaminated the concentrated stock.
- All regular users should have their own stocks of MgCl, 10X PCR buffer and Taq as well.
- HPLC water has been autoclaved and aliquoted already. Take one and use it, do not put it back for general usage.
- (*OPTIONAL)* No DNA near the PCR preparation bench. Make pooled master mix of all the reagents without the DNA using DNA-free PCR pipettes, then dispense to individual tubes and finally add DNA to individual reactions, using different pipettes and in a different location.
- After PCR has been performed, none of the reaction products should go near the PCR bench

and never use PCR designated pipettors for post-PCR pipetting.

- Observe proper operation of the pipettes on the PCR bench. If solution is sucked up into the pipette tip too fast, the pipette itself can become contaminated (it is for this reason that aerosol tips are incorporated).

- Never remove or add anything to the PCR bench.

- **ALWAYS INCLUDE A NEGATIVE CONTROL AND A VERY DILUTE POSITIVE CONTROL IN EVERY EXPERIMENT**.
    - The negative controls should be an indication of no contamination (use sterile water instead of DNA).
    - The positive control should only use 1-5 ng of DNA.

- The *Taq* polymerase should never be left out at room temperature. It should either stay in the freezer or in a freezer box.

10mM dNTP working stock
5µl dATP
5 µldCTP
5 µldTTP
5 µl dGTP
30 µl sterile HPLC water

In a conventional 25 µl PCR, use 0.25 µl of this 10mM dNTP working stock.
Do not freeze-thaw the individual dNTPs each time, as degradation will eventually occur.

Primers :
When ordered, primers should be reconstituted to 20 µM. Remove 50-100 µl aliquots for individual use. Do not freeze-thaw the stock each time as degradation will eventually occur.
A conventional 25 µL PCR generally uses 0.5 µL of each primer.

Standard Master Mix for a 25µl PCR reaction

COMPONENT	**VOLUME**	**FINAL CONCENTRATION**
$MgCl_2$ (25 mM)	3 µL	3 mM
10x PCR Buffer	2.5 µL	1x
dNTPs (10 mM each nucleotide)	0.25 µL	100 µM (each nucleotide)
forward primer (20□M)	0.5 µL	0.4 µM
reverse primer (20□M)	0.5 µL	0.4 µM
Taq DNA polymerase	0.1 µL	0.75 U/25 µL
DNA/sample	X µL	(1-5 ng)
HPLC water	make up to 25 µl final volume	

This mastermix is only a guideline and when developing new PCR protocols with new primers the concentrations of $MgCl_2$, dNTPs and primers will have to be optimized.

PRECAUTIONS TO BE FOLLOWED

- Wear clean gloves

- Work carefully using aseptic technique
- Don't use too much template DNA
- Don't use PCR products in PCR preparation areas
- *Always, always* include water and very dilute positive controls in every experiment
- Use aerosol pipette tips

RECORD TO BE MAINTAINED

- Laboratory Manual containing the experiments that can be performed with the equipment
- Maintenance Record

HOD

AVIT AARUPADAI VEEDU INSTITUTE OF TECHNOLOGY | VINAYAKA MISSION'S RESEARCH FOUNDATION (Deemed to be University under section 3 of the UGC Act 1956) | Accredited by NAAC | Approved by AICTE

DEPARTMENT BIOTECHNOLOGY

481172L1-GENETIC ENGINEERING LAB

STANDARD OPERATING PROCEDURE

Name of the Lab./facility	**Molecular biology /Genetic Engineering lab**
Purpose	To provide training for students on - Isolation of DNA from Plant, humans and Bacteria. -To cut the DNA molecule in to specific place using molecular enzymes(Hind –III, Bam HI) -To ligate the fragmented DNA by ligation Process.
Scope	Experimental training on isolation of DNA from Plant, humans and Bacteria and students able to cut the DNA molecule in to small pieces by using restriction enzymes, able to ligate the fragment DNA using ligase enzyme. This will be use full for their project work and their forthcoming research.
Responsibility	Faculty i/c of the facility, HOD/BIOTECH
STANDARD OPERATING PROCEDURE FOR **REFRIGERATED CENTRIFUGE**	

- When working in the laboratory the investigator or delegated person must wear protective clothing.
- Handling person should wash their hands before handling blood samples.
- Delegated person must ensure that the centrifuge is switched on at the wall plug socket.
- If refrigerated centrifuge samples are required the investigator or students must set the temperature at least 10 minutes before use and the lid must be closed.
- A person must set the time speed using the “set” key on the time/rotor field on the front panel of the centrifuge and scroll up or down using the “+” and “–“ keys until the desired time is reached. Press “set” a second time on the time/rotator field and this will programme the time.
- Sealed buckets or rotators must be used.

· Check that bucket seals are intact so that they provide adequate protection against liquid dispersion in the event of an accident during use.

· The containers being centrifuged must be strong enough to withstand the centrifugal forces to which they will be exposed.

· The bucket sealing rings must be inspected regularly and changed as necessary.

· The fluid being centrifuged should be introduced into the container carefully, the threads or outside of the container must not be contaminated.

· Containers must be filled according to the marker's instructions. At least 2cm of headspace must be left between the liquid level and the container rim. Excessive pressures can be generated in overfilled containers which may also lead to leakage.

· On a weekly basis or after 20 runs the inside of the centrifuge and its buckets should be cleaned decontaminated and greased. Cleaning products can be found in the laboratory in the Clinical Research Facility.

- Rotator and bowls should not be soiled and can be wiped down with Trigene and alcohol they should then be thoroughly dried with a dry cloth.

- Buckets and inserts that are not obviously soiled can be treated in the same way but any soiledcomponents should be decontaminated appropriately.

- Greasing the bucketsensures smooth movement and less vibration during acceleration anddeceleration of the motor.

- Working person must ensure that the rotator buckets are firmly closed with the covers in place for each centrifugal run.

- The lid of the centrifuge will be open when it is not in use.

PRECAUTIONS TO BE FOLLOWED

- Must never leave the centrifuge in refrigerated mode with the machine switched on and the lid open. This will cause the refrigeration mechanism to break.
- Delegated person does have a blood spillage whilst using the centrifuge; they should inform a senior member of the Clinical Research Facility team.

RECORD TO BE MAINTAINED

- Laboratory Manual containing the experiments that can be performed with the equipment
- Maintenance Record

HOD

AVIT AARUPADAI VEEDU INSTITUTE OF TECHNOLOGY | VINAYAKA MISSION'S RESEARCH FOUNDATION (Deemed to be University under section 3 of the UGC Act 1956) | Accredited by NAAC | Approved by AICTE

DEPARTMENT BIOTECHNOLOGY

481172L1-GENETIC ENGINEERING LAB

STANDARD OPERATING PROCEDURE

Name of the Lab./facility	**Molecular biology /Genetic Engineering lab**
Purpose	To provide training for students on - Isolation of DNA from Plant, humans and Bacteria. -To cut the DNA molecule in to specific place using molecular enzymes(Hind –III, Bam HI) -To ligate the fragmented DNA by ligation Process.
Scope	Experimental training on isolation of DNA from Plant, humans and Bacteria and students able to cut the DNA molecule in to small pieces by using restriction enzymes, able to ligate the fragment DNA using ligase enzyme. This will be use full for their project work and their forthcoming research.
Responsibility	Faculty i/c of the facility, HOD/BIOTECH

STANDARD OPERATING PROCEDURE FOR GEL DOCUMENTATION SYSTEM

- Do not use gloves to open and close the doors of common instrument room as well as any other rooms .
- Designate a separate plastic tray for carrying gel containing EtBr, gloves, tissue paper and 70% ethanol / distilled water.
- Before using gel doc system, clean the surface of trans-illuminator with 70 % ethanol ordistilled water.
- Wear gloves to handle the gel containing EtBr and then place the gel on to the surface oftrans-illuminator.
- Remove the gloves and close the door of gel doc system.
- Document the gel picture on the computer without wearing the gloves.
- At any moment of time, computer, keyboard, mouse and gel doc system should not beused with gloves.

- Wear gloves to remove the gel containing EtBr and clean the surface of trans-illuminator.
- Remove the gloves and close the door of gel doc system.
- Make an appropriate entry in the log book of gel doc system.
- Carry all the material in the designated plastic tray.
- Report immediately to concerned in-charge for problem regarding system, log book etc.
- If you find anyone using gloves for the computer / gel doc system then report to Lab in-charge

PRECAUTIONS TO BE FOLLOWED

• Exposure to ultraviolet radiation can cause serious burns to the skin.

• Exterior part of the equipment may be contaminated with traces amount of ethidium bromide solution from stained agarose gels or gel electrophoresis buffers. Ethidium bromide is a powerful mutagen and toxic.

RECORD TO BE MAINTAINED

- Laboratory Manual containing the experiments that can be performed with the equipment
- Maintenance Record

HOD

AVIT AARUPADAI VEEDU INSTITUTE OF TECHNOLOGY | VINAYAKA MISSION'S RESEARCH FOUNDATION (Deemed to be University under section 3 of the UGC Act 1956) | Accredited by NAAC | Approved by AICTE

DEPARTMENT BIOTECHNOLOGY

481172L1-GENETIC ENGINEERING LAB

STANDARD OPERATING PROCEDURE

Name of the Lab./facility	**Molecular biology /Genetic Engineering lab**
Purpose	To provide training for students on - Isolation of DNA from Plant, humans and Bacteria. -To cut the DNA molecule in to specific place using molecular enzymes(Hind –III, Bam HI) -To ligate the fragmented DNA by ligation Process.
Scope	Experimental training on isolation of DNA from Plant, humans and Bacteria and students able to cut the DNA molecule in to small pieces by using restriction enzymes, able to ligate the fragment DNA using ligase enzyme. This will be use full for their project work and their forthcoming research.
Responsibility	Faculty i/c of the facility, HOD/BIOTECH

STANDARD OPERATING PROCEDURE FOR ELECTORPHORESIS UNIT

- Make sure you have been trained by an experienced worker in the safe use of electrophoresis equipment.
- Familiarize yourself with chemicals you intend to use.
- Check equipment and wiring before use. Look for signs of damage. Do not use worn or frayed leads.
- Use only electrophoresis tanks which have a secure design preventing contact with buffer when connected to a power supply.
- Always disconnect from the power supply before opening.
- Switch off power before moving a tank.
- Clean up spills of electrophoresis buffer or gel mixes immediately – these may contain toxic chemicals e.g. ethidium bromide or acrylamide.
- Latex gloves often contain small holes – use nitrile (or other suitable) gloves when immersing hands in electrophoresis buffers or handling gels.
- When using vertical electrophoresis equipment, take care that leakage

from the upper buffer chamber does not cause arcing.

PRECAUTIONS TO BE FOLLOWED

• Electrophoresis involves the use of high voltages and carries the risk of electric shock.

RECORD TO BE MAINTAINED

- Laboratory Manual containing the experiments that can be performed with the equipment
- Maintenance Record

HOD

AVIT
AARUPADAI VEEDU INSTITUTE OF TECHNOLOGY
VINAYAKA MISSION'S RESEARCH FOUNDATION
(Deemed to be University under section 3 of the UGC Act 1956)
Accredited by NAAC
Approved by AICTE

DEPARTMENT BIOTECHNOLOGY

481172L1-GENETIC ENGINEERING LAB

STANDARD OPERATING PROCEDURE

LABORATORY RULES AND REGULATIONS

1. Always wear a lab coat before entering the laboratory for protecting the clothes and your body from burning, contamination or accidental discoloration by staining solution.
2. Always wear gloves and face mask when handling and weighing potentially hazardous chemicals like phenol, ethidium bromide stained gels.
3. Wash your hands with soap and water upon entering and prior to leaving the laboratory. Before and after each laboratory session wipe your workbench with disinfectant like ethanol.
4. All microbial cultures should be handled as potential pathogens
5. Never pipette any broth cultures or any chemical reagents by mouth
6. Always keep the test tubes containing cultures in an upright position in the rack. When moving around the laboratory, carry the test tubes containing the cultures in a rack
7. If a live culture is spilled over the area, leave the area with disinfectant solution for 15 minutes and then clean it.
8. In the case of accidental splash of hazardous chemical, immediately rinse the area thoroughly with water and inform to the instructor.
9. Materials such as stains, reagents, bottles, test tubes, petri plates pipettes etc., must be returned to the original location after use.
10. Do not place contaminated instruments such as inoculated loops, needles,pippetes tips on bench tops.
11. All cultures, stocks, and other regulated wastes are decontaminated by autoclaving before disposal.
12. Store the chemicals, reagents and kits at the appropriate temperature as prescribed.
13. Always use new micropipette tips to pipette out DNA polymerase and other modifying enzymes. Pipetting should be done according to the instructions given in the protocols.
14. Always clean microscope stage, eye piece and objective lens before and after use.
15. The voltage used for electrophoresis is sufficient to cause electric shock, therefore keep the buffer reservoirs covered during electrophoresis.
16. Always turn off the power supply and unplug the leads before removing the gel from the tank. Avoid exposure to UV radiation while visualizing the gel under gel documentation system.

HOD

AVIT AARUPADAI VEEDU INSTITUTE OF TECHNOLOGY | VINAYAKA MISSION'S RESEARCH FOUNDATION (Deemed to be University under section 3 of the UGC Act 1956) | Accredited by NAAC | Approved by AICTE

DEPARTMENT BIOTECHNOLOGY

481172L1-GENETIC ENGINEERING LAB

STANDARD OPERATING PROCEDURE

Standard Operating Procedure for Operating Laminar Airflow

Name of the Lab./facility	**Molecular biology /Genetic Engineering lab**
Purpose	To describe the procedure for the operation and maintenance of the Laminar Airflow
Scope	This Standard Operating Procedure (SOP) applies to the staff and students using the Laminar Airflow in molecular biology & Genetic Engineering laboratory, Vinayaka missions' Research foundation.
Responsibility	Faculty i/c of the facility, HoD/Biotechnology

STANDARD OPERATING PROCEDURE FOR LAMINAR AIRFLOW

- Switch "ON" the mains
- Switch "OFF" U.V light
- Switch "ON" laminar air flow and light
- Check and ensure manometer reading "0" mm of water gauge before switching "ON". Check and ensure the manometer reading between 10 to 15 mm water gauge after switching "ON" the LAF and keep the record of reading
- In case the manometer reading is found out of limit, inform maintenance department for corrective action
- Clean the LAF bench with 70% IPA before use and after completion of work

PRECAUTIONS TO BE FOLLOWED

- Validate the LAF twice a year by the third party for DOP test/smoke Test for air velocity and for nonviable particle count
- Maintain U.V light burning record

HOD

DEPARTMENT BIOTECHNOLOGY

481172L1-GENETIC ENGINEERING LAB

STANDARD OPERATING PROCEDURE

Name of the Lab./facility	**Molecular biology /Genetic Engineering lab**
Purpose	To describe the procedure for the operation and maintenance of the Microscope
Scope	This Standard Operating Procedure (SOP) applies to the staff and students using the Microscope in molecular biology & Genetic Engineering laboratory, Vinayaka missions' Research foundation.
Responsibility	Faculty i/c of the facility, HoD/Biotechnology

STANDARD OPERATING PROCEDURE FOR LAMINAR AIRFLOW

1. Ensure that the microscope and its surrounding area is clean.

2. Plug the microscope power cord in to electrical out let.

3. Turn on the microscope by rotating the illumination control knob on the bottom left side of the instrument.

4. Set the intensity of light to the lowest setting using illumination control knob.

5. Fully open the aperture diaphragm of the condenser by rotating the ring to the extreme right.

6. Using the sub stage condenser focusing knob, raise the condenser to the top of its excursion. Critical illumination only: If the condenser travel is excessive, limit the travel with the thumbscrew under the sub stage until the top lens of it is just below the stage surface (0.35mm)

7. Place the specimen slide on the stage.

8. Rotate the nosepiece to move the objective (40 X for dry mount and 10 X for wet mount) into working position.

9. Raise the stage by rotating the coarse adjustment knob to its positive stop. Using the fine adjustment knob, bring the specimen into sharp focus.

10. Adjust the eye tubes for inter pupillary distance and eye difference. The left eyepiece tube is focusable to compensate for refractive differences of the eyes.

11. To correctly set the eye tubes, focus on the specimen through the right eyepiece tube only. Use the fine adjustment knob while covering the left eyepiece or closing the left eye.

12. Next, focus the specimen through the left eyepiece by turning the eye tube. Cover the right eyepiece while doing this and be sure to focus with the left eye tube only, without using the focusing knob.

13. Remove an eyepiece and view the back aperture of the objective. Close the condenser aperture diaphragm and then, to obtain the full resolving power of the microscope, reopen until the diaphragm leaves just disappear from view. Replace the eyepiece. The aperture diaphragm can be adjusted to enhance contrast and/or increase the depth of focus.

14. When changing to higher power objectives, the positions of the aperture diaphragm must be reset. As magnification increases, the aperture diaphragm must be opened as required.

Cleaning and Maintenance

1. Whenever lack of contrast, cloudiness or poor definition is encountered, Clean the lower magnification objectives and optical surfaces with a lint free cloth or lens tissue moistened (not wet) with methanol.

2. Clean the front lens with a toothpick covered with a cotton tip wetted with methanol.

3.	Avoid excessive use of solvent for cleaning.
4.	Cover the microscope always with dust cover, whenever the microscope is not in use.
5.	Wipe the bottom of Oil immersion lens of a fast absorbing tissue paper before and after using the lens.
6.	Use Xylene to clean the lens surfaces

HOD