DEPARTMENT OF BIOTECHNOLOGY

17BTCC88-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 (20MM)	0.5 µL	0.4 µM
reverse primer (20µM)	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 master mix 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
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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.
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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.

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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.

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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.

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Name of the Lab./facility	**Molecular biology /Genetic Engineering lab**
Purpose	To provide training for students to visualize DNA band in agarose gel .
Scope	Visualize the colorless DNA band in agarose gel
Responsibility	Faculty i/c of the facility, HOD/BIOTECH

STANDARD OPERATING PROCEDURE FOR UV-TRANSILLUMINATOR

- The power of the unit should be turned off.
- Open the UV-blocking cover to desired angle.
- Place the sample on the filter surface.
- Close the UV-blocking cover or make sure people surrounded are under proper protection.
- Press the switch "power ON/OFF" to turn on the unit. The tubes should become energized and emit a steady glow of light. The light may initially flicker, especially if the lamp is cold, but should stabilize after a few seconds.
- Press "high/low switch" for preferred intensity to visualize. If you need high intensity of UV radiation, then press the switch to "high" for high intensity. (Note: Do not press the switch subito up and down.)
- Visualize or capture image by imaging system or any camera available. (Note: Do not keep the UV transilluminator on for a long period of time, it may reduce filter and UV tube's lifetime. Long time UV exposure may also cause damaged to sample.)
- Turn off the unit before remove the sample.
- Clean the sample touched surface with soft tissue.

PRECAUTIONS TO BE FOLLOWED

• UV transilluminator 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

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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

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STANDARD OPERATING PROCEDURE

Name of the Lab./facility	**Molecular biology /Genetic Engineering lab**
Purpose	To provide training for students to handle microwave oven for preparing agarose gel. -
Scope	Experimental training on preparation of agarose gel using microwave oven.
Responsibility	Faculty i/c of the facility, HOD/BIOTECH

STANDARD OPERATING PROCEDURE FOR MICROWAVE OVEN

- Never put metal into a microwave.
- Containers placed in microwaves must have their tops/caps/closures fully loosened. Agarose in particular is likely to expand when heated, leak into cap threads, dry out, and reseal the cap. When this happens the results can be catastrophic, and if not controlled will sooner or later cause serious injury or worse.
- A label should be visible close to all microwave ovens, close to the control panel, warning of the dangers and giving instructions for safe operation.
- Check that the containers used will withstand heating. Some plastic or wax based containers melt at high temperature. Duran type borosilicate glass containers are suitable for microwaving, but medical flats are not.
- Check that the item does not give off fumes or vapours when heated.
- Do not heat or dry any radioactive materials in the microwave.
- Check door seals and latches regularly for signs of wear and damage.
- Do not slam the door of the oven; this causes damage to latches and seals.
- Clean up any spillages; microwaves will go to the point of any dried matter.
- Microwaves used for laboratory work should never be used for heating food or drinks for human consumption.
- Inform safety personnel if any sparking occurs during heating.
- Safety checks should be made regularly.

PRECAUTIONS TO BE FOLLOWED

• 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.

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