Course Code				**23ZOMAP233**			
Title of the Course				**Animal Biotechnology**			
Offered to:				**B.Sc. Hons Zoology**			
L	0	**T**	**0**	**P**	**2**	**C**	**1**
Year of Introduction:		**2024-25**		**Semester:**			**3**
Course Category:		**MAJOR**		**Course Relates to:**		**GLOBAL**	
Year of Revision :		**NA**		**Percentage:**		**NA**	
Type of the Course:				**SKILL DEVELOMENT Employability**			
Crosscutting Issues of the Course :				**GENDER**			
Pre-requisites, if any				**Introduction to recombinant DNA technology**			

Course Description:

Animal biotechnology is a branch of biotechnology in which molecular biology techniques are used to genetically engineer animals in order to improve their suitability for agriculture, industrial and pharmaceutical applications.

Advances in animal biotechnology have been facilitated by recent progress in sequencing animal genomes, gene expression and metabolic profiling of animal cells. Genome editing technologies (Zinc Finger Nucleases, and CRISPR-Cas systems) have opened up new opportunities to easily create genetic variations in animals that can improve their health and well-being, agricultural production, and protection against diseases.

The course create awareness on advanced streams like Stem Cell Biology, Animal Cell Culture, Genomics and Proteomics, Drug Design, Genetic Engineering and Bioinformatics.

Course Objectives:

S. No	COURSE OBJECTIVES
1	To provide knowledge on animal cell and tissue culture and their preservation
2	To understand principles of animal culture, media preparation.
3	To empower students with latest biotechnology techniques like stem cell technology, genetic
4	To explain in vitro fertilization, embryo transfer technology and other reproduction manipulation methodologies.
5	To get insight in applications or recombinant DNA technology in agriculture, production of therapeutic proteins

Course Outcomes

At the end of the course, the student will be able

NO	COURSE OUTCOME	BTL	PO	PSO
CO1	Get knowledge of the Vectors and Restriction enzymes used in biotechnology	**K1**	**2**	**1**
CO2	Describe the gene delivery mechanism and PCR technique	**K2**	**2**	**1**
CO3	Acquire basic knowledge on media preparation and cell culture techniques	**K2**	**2**	**1**
CO4	Understand the manipulation of reproduction with the application of biotechnology	**K3**	**2**	**1**
CO5	Understand the applications of Biotechnology in the fields ofindustry and agriculture including animal cell/tissue culture, stem cell technology and genetic engineering.	**K4**	**2**	**1**

For BTL: K1: Remember; K2: Understand; K3: Apply; K4: Analyze; K5: Evaluate;K6: Create

CO-PO-PSO MATRIX									
CO NO	**PO1**	**PO2**	**PO3**	**PO4**	**PO5**	**PO6**	**PO7**	**PSO1**	**PSO2**
CO1		2						**2**	
CO2		2						**2**	
CO3		2						**2**	
CO4		2						**2**	
CO5		2						**2**	

Use the codes 3, 2, 1 for High, Moderate and Low correlation Between CO-PO-PSOrespectively

Course Structure:

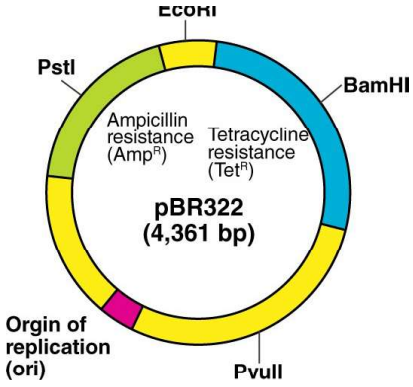
Unit – I: (6 Hrs)

Practical 1

1. Cloning vectors: plasmid vectors: PBR322 and PUC series, Bacteriophage lambda and M13: based vectors, cosmids ,BAC, YAC Demonstration by visual (images,charts)

Plasmid vectors: Plasmid is a small covalently coiled circular extra chromosomal self-replicating double standard DNA molecule .

A) pBR 322:- p: stands for plasmid, B for Bolivar and R for Rodriguez.
The number 322 in pBR322 denotes the order of synthesis that distinguishes it from the other plasmids synthesized in the same laboratory. pBR322 is used in genetic engineering.

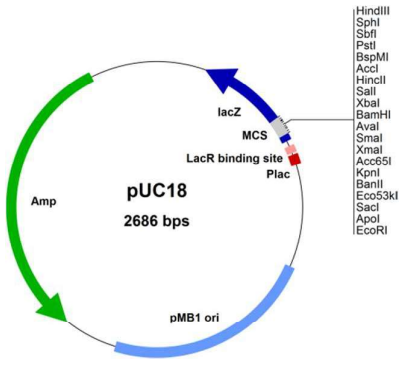


B) pUC 18

pUC18 is a commonly used plasmid cloning vector in E.coli. The molecule is a double-stranded circular DNA (2686 base pairs in length). Due to a small size pUC18 enables successful cloning of large DNA fragments. The pUC18 plasmid confers ampicillin resistance and complement defects in β-galactosidase in appropriate host strains. The multiplecloning site (MCS) is within the β-galactosidase gene and contains unique sites for 13 different restriction enzymes (Acc I, BamH I, EcoR I, Hinc II, Hind III, Kpn I, Pst I, Sac I, Sal I, Sma I, Sph I, Xba I, and Xma I).

Foreign DNA inserted at the MCS interrupts the β-galactosidase gene and abolishes the ability to catabolize lactose. The recombinant plasmids can be verified via blue/white colony screening. Lactose-positive, ampicillin-resistant colonies (host strain containing plasmid) form blue colonies on plates containing ampicillin and X-Gal; lactose-negative, ampicillin- resistant colonies (host strain containing plasmid with foreign DNA inserted at the MCS) form white colonies on this medium.

pUC18 is similar to pUC19, but the MCS region is reversed. pUC18 and pUC19 provide the option to clone DNA fragment generated by restriction enzymes orientation towards the lac promoter.

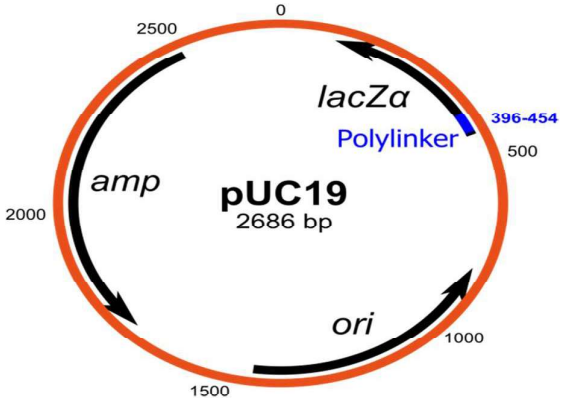


PUC 19

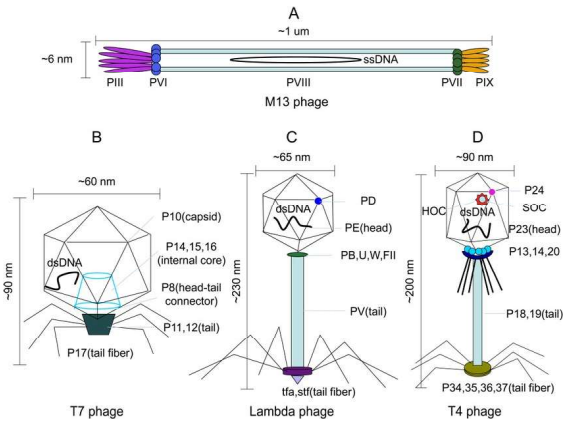
pUC19 is a commonly used cloning vector that conveys the Amp resistance.

The molecule is a small double-stranded circle, 2686 base pairs in length, and has a high copy number.

pUC19 carries a 54 base-pair multiple cloning site polylinker that contains unique sites for 13 different hexanucleotide-specific restriction endonucleases.



C) Bacteriophage lambda and M13

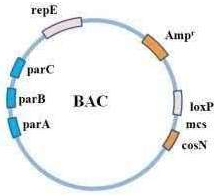


lambda phage and M13 phage is that lambda phage is a head to tail bacteriophage that has a linear double-stranded genome while M13 phage is a filamentous bacteriophage that has a circular single-stranded genome.

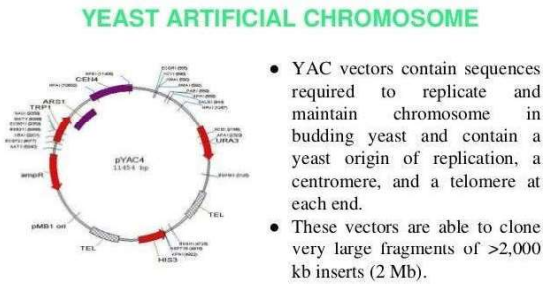
Viruses are infectious agents. They infect plants, animals, fungi, and bacteria. Bacteriophages are bacterial viruses. They specifically infect bacterial cells. In molecular biology, bacteriophages are used as gene delivery vehicles for eukaryotes. Lambda phage and M13 phage are the most studied and exploited phages among different bacteriophages.

Cosmids A cosmid is a type of plasmid vector that contains sequences from bacteriophage lambda, specifically the cos sites.

BACs Bacterial artificial chromosomes



YACs Yeast artificial chromosomes



Practical 2

2.DNA quantification using DPA methods.

Aim

Quantification of DNA in unknown sample by diphenylamine (DPA) reagent.

Apparatus

Chemicals

Standard DNA solution (0.25mg/ml),

Diphenylamine reagent

DNA sample in saline citrate buffer

Saline citrate buffer (0.15M NaCl, 0.015M sodium citrate, pH 7.0)

Glacial acetic Acid

Concentrated H2SO4

Glassware/Plastic ware

All glassware and plastic ware used should be sterilized:

Test tubes

Micro pipettes

Graduated Cylinder

Instruments/ equipment

Spectrophotometer

Water bath

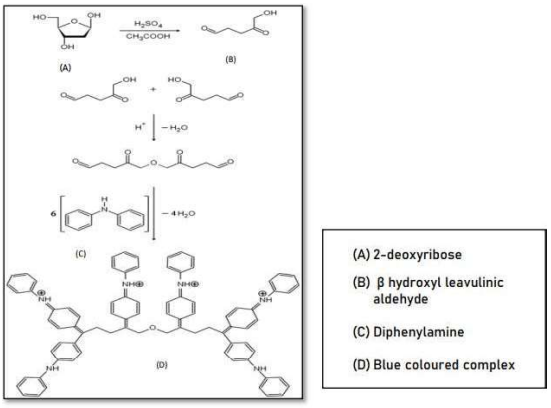
Preparation of diphenylamine reagent

1. Dissolve 1 g of diphenylamine in 100ml of glacial acetic acid.
2. In this, now add 2.5 ml sulphuric acid.

Principle

When DNA is treated with diphenylamine under the acidic condition a blue colored complex is formed which has an absorption peak at 595nm.This reaction is given by 2- deoxypentose in general. In acidic solution deoxypentose are converted into a highly reactive β hydroxyl leavulinic aldehyde which reacts with diphenylamine gives blue complex. In DNA, only the deoxyribose of purine nucleotide reacts so that the value obtained represents one half of the total deoxyribose produced.

Reactions-



Procedure

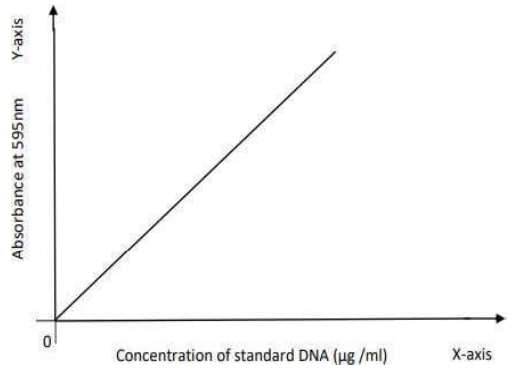
1. Pipette out 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard in to the series of labelled test tubes.
2. The final volume in all the test tubes was made to 1 ml with distilled water.
3. Pipette out 1 ml of the given sample in another test tube which serves as a blank.
4. Pipette out 0.5ml of unknown DNA sample in another test tube labelled as 'unknown' and volume was made upto 1ml with distilled water.
5. Now add 5 ml of DPA reagent to all the test tubes including the test tubes labelled 'blank' and 'unknown'.

6. Place all the test tubes in a boiling water bath for 15 minutes.
7. Now, cool the test tubes and note down the absorbance of the blue colored solution at 595 nm against blank.
8. Plot the standard curve by taking concentration of DNA in βg /ml along X-axis and absorbance at 595 nm along Y-axis.
9. From this standard curve calculate the concentration of DNA in the unknown sample.

Observation and calculation

Sr. no.	Volume of standard DNA (ml)	Concentration of DNA (µg)	Volume of distilled water (ml)	Volume of Diphenylamine reagent (ml)	Incubation	Absorbance at 595nm
1.	0	0	1	5	Place the test tubes in boiling water bath for 15 minutes.	
2.	o.2	100	0.8	5		
3.	0.4	200	0.6	5		
4.	0.6	300	0.4	5		
5.	0.8	400	0.2	5		
6.	1	500	0	5		
7.	0.5			5		

straight line graph is formed by taking concentration of standard DNA in ug/ml along Xaxis and absorbance at 595nm along the Y-axis.



Calculation

Determine the amount of DNA in the unknown sample by plotting a standard curve of A595 on Y-axis and Âµg of DNA on X-axis.

By using the

formulaSlope = y2-

y1/x2-x1 **Result &**

Discussion

The amount of the DNA present in the given unknown solution =____µg/ml.

Deoxyribose in presence of acid converts to a compound that binds with diphenylamine to form a bluecoloured complex. In standard DNA solution the gradient of blue colour represents the corresponding DNA concentration.

Optical density of lowest concentrated DNA (100 βg/ml) is__and optical density of highestconcentrated DNA (1000 Âµg/ml) is_____. The concentration of unknown DNA is_____βg/ml.

Precaution

It is recommended to use freshly prepared diphenylamine reagent. The solution however, can bestored in advance in dark at 2-8 0C

Wear eye protection and use a fume cupboard when preparing this reagent. Diphenylamine is harmfulif ingested or inhaled and may irritate skin or eyes if it comes into contact with them.

Clean all the test tubes properly before use.

Spectrophotometer should be switched on 15-30 minutes before use to allow it to warm up.

Unit – II: (6 Hrs)

Practical 3. Techniques:DNA fingerprinting

Practical 4: separation and purification of biological components by paper chromatography

Aim

To distinguish and study the various pigments present in plants through the process of paper chromatography.

Theory

Plants carry out the process of photosynthesis, during which light energy from the sun is converted into chemical energy (food). The capturing of light energy is carried out by molecules known as pigments, which are present within the plant cells.

What are Pigments?

Pigments are chemical compounds, which are able to reflect only a particular range of wavelengths of visible light. Leaves of plants primarily contain different types of pigments within their tissues. The four different types of pigments are listed below in a tabular column along with their colours.

Pigment	Colour
Chlorophyll A	Dark green
Chlorophyll B	Yellowish-green

Xanthophylls yellow

Carotenoids Orange

What is Chromatography?

It is a technique that is used to distinguish between different molecules. This differentiation is based on these attributes-shape, size, charge, mass, adsorption and solubility.

Types of chromatography:

- Column chromatography
- Paper chromatography
- Partition chromatography
- Thin-layer chromatography

Mechanism of Paper Chromatography

- In this technique, the interaction between three components is involved – solid phase, separation of a mixture and a solvent.
- At first, the mixture is spotted onto the paper and is dried.
- The solvent is made to flow through the capillary attraction.
- While the solvent moves through the paper, the various components of the mixture differentiate into varied coloured spots.
- Later the paper is allowed to dry and the position of various compounds is viewed.
- The substance, which is the most soluble moves further on the paper as compared to the other substances that are less soluble.

Material Required

- ★ Chromatography chamber
- ★ Spinach leaves
- ★ Mortar and pestle
- ★ Scissors
- ★ Ether acetone solvent
- ★ Acetone
- ★ Capillary tube
- ★ Pencil
- ★ Spatula
- ★ Scale
- ★ Filter paper strips
- ★ Stapler
- ★ Thread
- ★ Watch glass

Procedure

- In this experiment, spinach leaves are used to separate different pigments.
- Pick a few fresh and green leaves of spinach and wash it.
- Cut out small pieces of spinach using scissors. Add them to the mortar.
- Accurately measure 5ml acetone using a measuring cylinder and add it into the mortar.
- With the help of mortar and pestle, grind the spinach leaves into a smooth paste.
- Shift the prepared paste of spinach into the watch glass with the help of a spatula.
- Place a filter paper strip with a tapering notch towards one ending of the strip.

- Horizontally trace a line with a scale and a pencil that is 2 to 3 cm apart from the notch's tip.
- Using a capillary tube, add 1 drop of the extract of the pigment in the midsection of the line.
- Let the drop dry. Repeat the same process of adding a drop and allowing it to dry for 4-5 times.
- In the chromatographic chamber, pour the ether acetone solvent.
- Make sure to folded and stapled an end side of the paper.
- Suspend the strip in the chamber.
- The loading spot remains about 1 cm above the level of the solvent.
- Let the chamber remain uninterrupted for a while.
- We can notice that the solvent passes along the paper scattering various pigments of the blend to different distances.
- Once the solvent reaches 3/4th of the strip, carefully take the strip off.
- Allow the strip to dry.

Observation

The dried paper strip displays four different bands. Discrete pigments can be distinguished with the help of colours.

Conclusion

1. The Carotene pigment is observed at the topmost as an orange-yellow band of pigments distinctively.
2. Just below this band, a yellowish band appears which indicates the pigment xanthophyll.
3. The third band appearing dark green indicates chlorophyll-a pigment.
4. The yellowish-green band present at the bottom is the chlorophyll b pigment.

Precautions

- The leaves that are selected should be green and fresh spinach leaves
- From the tip of the notch, the loading spot needs to be 2 to 3 cm apart
- While suspending the filter paper strips in the chamber, one need to ensure that the loading spot needs to be set up above 1 cm from the level of the solvent.

Unit – III (6 Hrs.).

Practical 5. Cleaning and Sterilization of glass wares for cell cultures

UNIT- IV: **(6Hrs)**

Practical 6 Preparation of culture media

Unit – V: (6 Hrs.)

Practical 7 Amplification of PCR

Virtual labs/demos

1. Techniques DNA fingerprinting by demonstration method

Specific Web link:

2. https://www.slideshare.net/slideshow/dna-quantification/238354753

SEMESTER END LAB EXAMINATION

23ZOMAP233: Animal biotechnology **B.Sc. Hons Zoology**

Semester: III Max.Marks: 50M

(A) SEE Evaluation Procedure **35M**

Answer all the questions

1. DNA quantification using DPA method. 'A' or 10M
   Separation and Purification of biological components
2. Identify & write a detail note on vectors.' B or C ' (roll number wise) 1x5=5M
3. Preparation of culture media. $2x2^{1/2}$=5M

d.

e.

4.Identify and write comments upon the given spotters $2x2^{1/2}$=5M

f.

g.

5. Identify and write comments upon the given spotters $2x2^{1/2}$=5M

h.

i.

4. Practical Record 2M
5. Viva voce 3M

(B) CONTINUOUS ASSESMENT(Internal) **15 MARKS**

15 marks for the continuous assessment (Day to day work in the laboratory shall be evaluated for 15 marks by the concerned laboratory teacher based on the regularity/ record/viva). Laboratory teachers are mandated to ensure that every student completes 80%-90% of the lab assessments.

TOTAL: (A)+(B) = **50 MARKS**