Semester VI

17BCU603A **DRUG BIOCHEMISTRY** **4H-4C**

SCOPE

The goal of the paper will ensure the widespread visibility and high impact of Drugs, thereby promoting on emerging research, pointing the way for the establishment of new medicines – from the identification of targets, through to the synthesis and evaluation of putative therapeutic entities.

OBJECTIVES

This paper gives an insight knowledge about the emerging themes, and provides an in depth analysis of specific drug classes, its metabolism and therapeutic approaches.

Unit 1

Introduction to drug Biochemistry: Classification, routes of administration – factors influencing dosage and drug action, Absorption and distribution of drugs, binding of drugs to plasma proteins, Drug Dose relationship (LD_{50}, ED_{50}, therapeutic index), Drug – Receptor interaction, Drug binding forces, Receptor theories, Drug – Receptor interaction. Bioavailability; Pharmaco kinetics.

Unit 2

Drug metabolism: Drug Biotransformation pathways - phase I – oxidation, reduction and hydroxylation. Phase II- Conjugation, Elimination of drugs from body system. Storage of drugs in adipose tissue.

Unit 3

Drug abuse; drug dependence; drug resistance- Biological mechanism, ways to overcome.

Chemotherapy: Antibacterials – Mode of action of sulfonamides, penicillin, streptomycin, tetracycline, chloramphenicol, antiviral drugs, antifungal drugs; Antimetabolites of folate, purines & pyrimidines, Anti tubercular drugs.

Unit 4

Mechanism of action drugs used in the treatment of diabetes mellitus (Acarbose, Biguanides), AIDS (Azidophymidine, Didanosine), cancer(Mechlorethamine, Busulfan), heart (Amrinone, Digoxin) and kidney disorder (Benzophiadiazines, furosemide); antiepileptic drug(Lamictal, Tapclob), drugs for cough (Dextromethorphane Hydrobromide, Noscapine) and bronchial asthma (Salbutamol,Aminophylline), diuretics (Manitol, Xanthine), anti ulcer drugs (Cimetidine, Ranitidine) and drugs for fever (Paracetamol, Ibuprofen).

Unit 5

Toxicology- Introduction, definition and disciplines of toxicology, classification of toxicity and toxicants, Mechanisms of toxic effect, treatment of intoxication, methods in toxicology testing, heavy metal toxicity and chelation therpy. Environmental pollution, mycotoxins, mushroom poisons

TEXTBOOKS

Satoskar, R.S., Bhandarkar, S.P., and Ainapuri, S.S., (2003). Pharmacology and Pharmacotherapeutic, 18th edition,Popular Prakashan, Mumbai.

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Hamilton, D., Philips, R.J., and Scott, D., (2004). Occupational, Industrial and Environmental Toxicology, Moshy Inc Publishers.

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Foye, W., (2012). Principles of Medicinal Chemistry, 7th edition, B.I. Wanerly Pvt. Ltd, New Delhi.

Grahame-Smith, D.G., and Aronson, J.K., (2002). Oxford textbook of Clinical Pharmacology and Drug Therapy: 3rd edition. Oxford University Press.

Tripathy, K.D., (2009). Essentials of Medical Pharmacology, Jaypee brothers medical publishers, New Delhi.

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Practical Planning Report

S.No	Name of the Practical	Plan of Completion
1.	Extraction of total DNA from animal tissue	15.11.2018
2.	Extraction of total RNA from animal tissue	22.11.2018
3.	Isolation of mRNA from yeast by affinity chromatography.	30.11.2018
4.	cDNA synthesis	10.12.2018
5.	Assessment of gene expression using RT-PCR.	18.12.2018
6.	1. Induction of Lac Operon.	25.12.2018

SYLLABUS

1.Extraction of total nucleic acids from plant tissue.
2.Isolation of mRNA from yeast by affinity chromatography.
3.cDNA synthesis
4.Assessment of gene expression using RT-PCR.
5.Induction of Lac Operon.

Ex No.1

ISOLATION OF DNA

Aim

The aim is to isolate the DNA from the given animal tissue sample.

Principle

Extraction of DNA basically consists of four major steps:

- Preparation of a cell extract
- Purification of DNA from cell extract
- Concentration of DNA samples
- Measurement of purity and DNA concentration

Reagents

1. Lysis buffer (50 mM Tris-HCl (pH 8.0), 10 mM EDTA, 0.5% sodium lauryl sarcosiante, 0.5 μg/ml proteinase K).
2. Ribonuclease A (10 ml, 0.5 μg/ml)
3. Phenol (Molecular Biology Grade)
4. Chloroform
5. Isoamyl Alcohol
6. Sodium Acetate (0.5 M)
7. Ethanol

Procedure

1. To extract DNA from cells of interest, cells are lysed with 100 –200 μl of lysis buffer [50 mM Tris-HCl (pH 8.0), containing 10 mM EDTA, 0.5% sodium lauryl sarcosinate and 0.5 μg/ml proteinase K].
2. Incubate for 1 h at 50ºC.

3. Add Ribonuclease A (10 µl, 0.5 mg/ml) and incubate for an additional 1 hr at 50ºC.
4. Add 1ml of phenol, shake well for 5 to 10 min and then centrifuge at 3000 rpm for 5 min at 4ºC.
5. Transfer the supernatant to a new microcentrifuge tube with 500 µl of phenol and 500 µl of chloroform / isoamyl alcohol (24:1), shake it well for 5 to 10 min and centrifuge at 3000 rpm for 5 min at 4ºC.
6. After centrifugation, transfer the supernatant to a new tube and add 25 – 50 µl of 3 M sodium acetate (pH 5.2) and 1 ml of ethanol, shake gently till the DNA to precipitate.
7. Then place it under -8^0C for 20 minutes and centrifuge at 12000 rpm for 20 min to recover the DNA.
8. Rinse the pellet with 1 ml of 70% ethanol and spin for 10min.
9. Discard the supernatant and air dry the pellet at room temperature.
10. Dissolve the DNA in 0.5 – 1.0 ml of Millipore water to determine the concentration and purity of DNA by absorbance at 260/280 nm in a UV-spectrophotometer.

Result

The purity of DNA obtained is _____.

Ex No.2

ISOLATION OF TOTAL RNA

Aim

To isolate RNA from the given tissue sample.

Principle

Single step guanidium acid phenol method emphasizes on the ability of guanidium isothiocyanate (GITC) to lyse cells, denature protein and inactivate intracellular ribonuclease rapidly. The presence of β mercaptoethanol in the mixture increases the solubilization properties of the GITC extraction buffer. Acid phenol extraction (pH< 5.0) selectivity keeps cellular DNA in the organic phase and help in extraction of proteins and lipids. The addition of chloroform further removes lipids and produce two distinct phases containing the DNA, proteins and lipids and an aqueous phase containing the RNA.

Reagents required

1. TRIR kit has the following components:
   Phenol, guanidium isothiocyanate, urea, detergents, buffering agents and stabilizers.
2. Chloroform (molecular biology grade)
3. Isopropanol (molecular biology grade)
4. 75% ethanol (molecular biology grade)

Procedure

1. Add 1 ml of TRIR to the homogenised tissue sample and swirl gently for 15 min and then keep at 4°C for 5 min to permit complete dissociation of nucleoprotein complexes.
2. To this, add 0.2 ml chloroform, shake vigorously for 15 sec and place on ice at 4°C for 5 min.
3. The lysate is to be then centrifuged at 12,000 x g for 15 min at 4°C to yield lower organic phase containing DNA and proteins and upper aqueous phase containing RNA.
4. The volume of the aqueous phase will be approximately 40-50% of the total volume of the lysate.
5. The aqueous phase to be carefully transferred to a fresh eppendorf micro centrifuge tube without disturbing the interphase. Equal volume of isopropanol to be added, mixed and kept at 4°C for 10 min. It is to be again centrifuged at 12,000 x g for 15 min at 4°C to precipitate the RNA.

6. Discard the supernatant and wash the pellet twice with 75% ethanol and air dried. Dissolve the RNA pellet in 50µl of sterile milliQ water and place in a water bath at 60°C for 10 min to ensure maximum solubility of RNA.
7. Vortex gently the RNA sample and quantify before storing at -80°C.

Quantification of RNA

Diluted RNA sample to be quantified spectrophotometrically by measuring the absorbance (A) at 260 nm. An absorbance of 1OD is equivalent to RNA concentration of 40 µg/ml. Therefore, the yield can be calculated by multiplying the absorbance at 260 nm with dilution factor and 40 µg. The purity of RNA preparations were assessed by determining the ratio of absorbance of sample at 260 nm and 280 nm.

Result

The purity of RNA obtained is _____.

Ex No.3

ISOLATION OF mRNA FROM YEAST BY AFFINITY CHROMATOGRAPHY

Aim

To isolate mRNA from the yeast using affinity chromatography technique.

Principle

Messenger RNA (mRNA) comprises approximately 1–5% of total cellular RNA. Although the actual amount depends on the type of cell and its physiological state, at any one time approximately 12,000 genes are being transcribed with ~500,000 mRNA molecules present in each mammalian cell. Eukaryotic mRNAs are heterogeneous in size (ranging from 0.5 kb to over 20 kb) and abundance (from fewer than 15 copies to over 20,000 copies per cell). The presence of a terminal stretch of approximately 200 adenosine residues (the polyA tail) on most eukaryotic mRNAs and its absence in ribosomal and transfer RNAs has important practical consequences, as it allows polyadenylated species (messenger RNAs) to be separated from their nonpolyadenylated counterparts (ribosomal and transfer RNAs, which account for over 90% of total cellular RNA). High-quality mRNA is needed for a number of molecular biology techniques, including cDNA library construction. Not surprisingly, numerous mRNA extraction kits are now commercially available. All use the same basic principle, described in which involves the affinity selection of polyadenylated mRNA using oligodeoxythymidylate (oligo (dT)).

Materials required

All materials used in this procedure should be sterile and of molecular biology grade. All Tris-containing solutions are prepared using RNase-free water and autoclaved. All other solutions, unless otherwise stated, should be treated directly with diethyl pyrocarbonate (DEPC) and autoclaved. DEPC is an efficient, nonspecific inhibitor of RNase activity. It is, however, a carcinogen and should be handled in a fume hood with extreme care. Hands are a major source of RNase activity. Because of this, gloves should be worn for all procedures.

1. RNase-free water: Add 0.1% DEPC to water. Allow to stand overnight at 37°C and autoclave to destroy residual DEPC activity. All solutions except Tris, which inactivates DEPC, can be treated in the same way.
2. SDS (sodium dodecyl sulphate): SDS is dangerous if inhaled and should be weighed in a fume hood. A 10% stock solution is normally prepared. This

solution is unstable if 10 Bryant and Manning autoclaved, however any residual RNase activity can be destroyed by heating the solution at 65°C for 2 h.

3. Oligodeoxthymidylate-cellulose (oligo(dT)): Oligo (dT) cellulose is available commercially. Although the binding capacity of oligo(dT) cellulose varies between different suppliers, a general rule is to use 25 mg of oligo(dT) for each 1 mg of total RNA. Suspend oligo (dT) cellulose in loading buffer at a concentration of 5 mg per 1 mL loading buffer.
4. Oligo (dT) is insoluble and should be resuspended by gentle tapping or inversion. Do not put it in a vortex. It can be stored either dry at 4°C or in suspended in loading buffer at –20°C.
5. RNase-free glass wool and Pasteur pipets: Wrap both the glass wool and pipets in aluminium foil and bake at 200°C for 2–4 h to remove any RNase activity.
6. 5 *M* NaCl: Store at room temperature.
7. 3 *M* Sodium acetate pH6: Store at room temperature.
8. Absolute alcohol: Store at –20°C.
9. 70% ethanol: Prepare this solution using DEPC-treated water. Store at 4°C.
10. Loading buffer: 0.5 *M* NaCl in 0.5% SDS, 1 m*M* EDTA, 10 m*M* Tris-HCl, pH 7.5. Store at room temperature.
11. Elution buffer: 1 m*M* EDTA, 10 m*M* Tris-HCl, pH 7.5. The buffer can be stored at room temperature but should be preheated to 65°C prior to use.
12. Recycling buffer: 0.1 *M* NaOH, which should be prepared immediately before use and used fresh.

Preparing an Oligo (dT) Column

Oligo (dT) columns are available commercially or can be prepared by using a 1–3 mL syringe. Preparing your own columns is both easy and cheap.

1. Remove the plunger from the syringe and plug the base with glass wool.
2. Add oligo (dT) cellulose to the syringe using a sterile RNase-free Pasteur pipet. The oligo (dT) cellulose will collect, as a column, above the glass wool. The loading buffer will escape through the glass wool and can be discarded. To ensure that the oligo (dT) cellulose is packed and free from air locks, add 3 vol of loading buffer using a pipette and allow the solution to run through the column. The column is now ready for immediate use and should not be allowed to run dry.

Isolation of Poly(A+) RNA

1. Resuspend the RNA pellet in loading buffer or, if the buffer is in solution, add 1/10th vol of 5 *M* NaCl.
2. Heat denature RNA and immediately load it onto the column and apply 3 vol of loading buffer.
3. Reapply the eluate to the column.
4. Wash with 3 vol of loading buffer. Discard eluate.
5. Recover the bound poly(A+) mRNA by adding 3 vol elution buffer. Collect the mRNA in a sterile tube on ice.
6. The mRNA is precipitated by adding 1/10th vol of 3 *M* sodium acetate and 2 vol of ice-cold absolute ethanol. An overnight precipitation at –20°C maximizes the precipitation of RNA.
7. Centrifuge at 15,000*g* for 15 min to pellet the RNA. Discard the supernatant.
8. Wash the RNA pellet in ice-cold 70% ethanol. Centrifuge at 15,000*g* for 5 min to repellet the RNA which may have been disturbed by washing. Discard the supernatant.
9. Dry the RNA pellet. Once it is dry, resuspend it in DEPC-treated water.
10. Assess the purity and integrity of mRNA
11. The integrity of the mRNA can be assessed by formaldehyde Gel Electrophoresis of Total RNA.
12. The mRNA should appear on ethidium bromide-stained gels as a smear ranging from 200 bp to greater than 10 kb with no detectable ribosomal RNA. If small amounts of mRNA are added to the gel, visualization by ethidium bromide may be impossible. To circumvent this problem, set up a Northern blot and hybridize using a labeled oligo(dT) primer.

Ex No.4

REVERSE TRANSCRIPTASE-POLYMERASE CHAIN REACTION

Aim

The aim is to reverse transcriptase of the resultant mRNA into cDNA using RT-PCR technique

Principle

The reverse transcriptase-polymerase chain reaction (RT-PCR) involves the conversion of mRNA of gene of interest present in the total RNA into cDNA and then amplifies a specific region of the cDNA. This enzyme reverse transcriptase catalyzes the conversion of mRNA into cDNA. This enzyme is isolated from retroviruses such as murine moloney leukemia virus (MMLV) and avian myeloblastosis virus (AMV). Reverse transcriptase polymerase chain reaction were done using a single step kit in which the reverse transcription reaction and the amplification can be carried out in a single vial.

Materials required

- **One step RT-PCR kit Components**

1. **Enzyme mix** containing 20 mM TrisHCl, 100 mM KCl,1 mM dithiothreitol (DTT), 0.1mM EDTA, 0.5%(V/V) Stabilizer; pH 9.0.
2. **5x RT-PCR master mix** containing Tris HCl, KCl, $(NH_4)_2SO4$, 12.5 mM $MgCl_2$, DTT, pH 8.7.
3. **dNTP mix** containing 10 mM each of dATP, dCTP, dGTP and dTTP.
4. PCR-grade RNase free water.
5. **Oligonucleotide primers**

For amplification of the individual gene, a 18-21 base primer needs to be identified and synthesized (available commercially)

Procedure

To 1.5 µg of total RNA, 2 µl of dNTP mix, 10 µl of 5X buffer, 2 µl of enzyme mix, 2 µl of ß actin (6 µM) and 5 µl of ALP or collagen (6 µM) of each primers were added and made up to final volume of 50 µl using RNAse free water.

- Reverse transcription (RT): 50°C for 30 min
- Termination of RT: 94°C for 2 min

- Denaturation: 94°C for 90 sec
- Annealing: 55°C for 90 sec
- Extension: 72°C for 90 sec

} (30 cycles)

- Final extension: 72°C for 10 min
- Hold at 4°C

Result

cDNA is synthesized from mRNA by reverse transcription.

Ex No.5

AGAROSE GEL ELECTROPHORESIS

Aim

To identify the purified DNA molecules from the given sample using Agarose Gel Electrophoresis

Principle

The generated cDNA fragments were resolved in 2% agarose gel under an applied electric field. DNA molecules migrate towards the anode due to negatively charged phosphate along the backbone of DNA. The rate of migration of linear DNA is inversely proportional to its molecular weight. Thus, the larger molecules travel at a much lower speed when compared to smaller one.

Reagents required

1. **TBE buffer 1X: (Tris, Boric acid. EDTA) (pH 8.0):** 3.78 g tris, 1.925 g boric acid and 0.260 g EDTA were dissolved in 350 ml of autoclaved RNAse and DNAse free water and the pH was adjusted to 8.0.
2. **1% Ethidium bromide in RNAse and DNAse free water**
3. **2% Agarose in 1x TBE buffer**
4. **Gel loading dye**
   The gel loading dye (6X) was procured commercially in ready to use form.

Procedure

1. Add 1 g of agarose to 50 ml 1X TBE buffer (2%). Then melt it on a microwave oven, make up the volume to 50 ml with TBE buffer and add 10 µl of 1% EtBr, evenly mix and cool to 40°C.
2. Then pour the mixed solution into a sealed gel-casting platform and insert the comb after ensuring the absence of air bubbles.
3. Then allow the gel to get harden. Remove the comb after 15 min by taking care of not disturbing the sample wells. Immerse the platform in the electrophoresis buffer tank.
4. Mix the 5 µl of PCR products from each reaction tube with 1µl of 6X gel loading dye and load to each well. Load simultaneously of 100 bp molecular weight marker DNA in the first lane to identify the cDNA products.

5. Turn on the power supply and adjust the electricity to 60 mA. Run the gel for 2 h. And then, visualize the resolved cDNA fragments gel for gel documentation.
6. Normalize the band intensity of cDNA fragments of ALP and Collagen with the internal control ß actin and express in OD units relative to GAPDH.

Ex No.6

INDUCTION OF LAC OPERON

Aim

To prepare competent cell, transform plasmid DNA and to assess induction of Lac Operon.

Introduction

Bacterial transformation is a process which involves genetic alteration of bacteria by incorporation and stable expression of a foreign genetic material from the environment or surrounding medium. Since DNA is a very hydrophobic molecule, it will not normally cross the bacterial cell membrane and hence bacterial cells needs to be made competent to ensure DNA uptake. Competence is the propensity of a bacterial cell to take up extracellular DNA from its environment. There are different methods of carrying out transformation, e.g. chemical transformation, electroporation, gene gun, liposome mediated transfer and microinjection. Chemical transformation includes the usage of Calcium chloride (CaCl2). This mode of transformation is easy to perform and requires minimum number of equipments.

Principle:

For the incorporation of plasmid into a cell, bacteria must first be made "competent". This process includes the treatment of cells with bivalent calcium ions in ice-cold condition. As a result small pores are formed on the cell membrane, which makes it permeable.

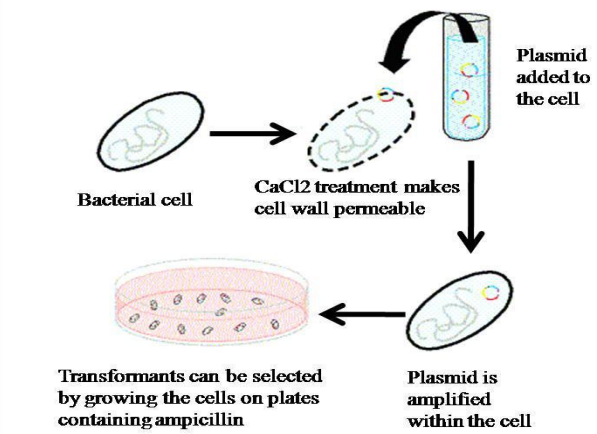

Fig 1: The process of bacterial transformation includes treatment of cells with CaCl2, which makes cells permeable, and plasmid DNA can enter the cell.

The plasmid DNA may adhere to the surface of the cell and uptake is mediated by a pulsed heat shock at 42^{O}C. A rapid chilling step on ice ensures the closure of the pores. These cells are allowed to propagate and selection of transformants can be done by growing the cells on a selective media which will allow only the plasmid containing cells to grow.

Plasmids are extrachromosomal DNA element capable of independent replication inside a suitable host. Plasmids encode a wide variety of genes, including those required for antimicrobial resistance. These genes act as selective markers when a transformation experiment is carried out.

The E. coli plasmid pUC19 encodes a gene that can be utilized as a selectable marker during a transformation experiment. pUC19 has ampicillin resistance marker that facilitate only transformed cells to grow on LB – Ampicillin plates. Transformants, thus having the ability to grow on ampicillin plates can be selected. This process of direct selection of recombinants is called insertional-inactivation. pUC19 also carries the N-terminal coding sequence for β-galactosidase of the lac operon. The E. coli host strain has a deletion at the amino terminal end of the LacZ gene, which codes for β-galactosidase. When pUC19 is transformed into the competent host cells, the truncated products from both complement each other leading to the production of enzymatically active β-galactosidase. This is referred as α-complementation. The tranformants turn blue on X-gal and IPTG containing plates due to the synthesis of β-galactosidase. X-gal is the chromogenic substrate of β-galactosidase and IPTG induce the expression of this β-galactosidase.

Step wise procedure:

1. The entire process needs to be carried out under sterile conditions.
2. **Preparation of 0.1M Calcium chloride (sterile):**
   To prepare 1000 ml of 11.1 gram of Calcium chloride was weighed and dissolved in 700 ml of sterile distilled water and made upto 1000 ml. This solution can be stored at 2-8^{O}C.
3. Pre-chill the tubes before competent cell preparation, 0.1M Calcium chloride solution and centrifuge tubes. Set the centrifuge at 4^{O}C and water bath at 42 ^{O}C.
4. 50 ng/µl of plasmid is used for transformation.
5. **Preparation of LB (Luria Bertani) broth (55 ml):** Dissolve 1.38 g of LB media in 55 ml of distilled water. The solution is to be made sterile by autoclaving.
6. **Preparation of LB (Luria Bertani) agar plates (20 ml):** 0.5 g of LB media and 0.3 g of agar are dissolved in 20 ml of sterile distilled water. The solution is to be made sterile by autoclaving.
7. **Preparation of Ampicillin:** Dissolve 30 mg of ampicillin powder in 600 µl of sterile double distilled water to prepare 50 mg/ml ampicillin solution.
8. **Preparation of LB (Luria Bertani) Agar plates containing Ampicillin, X-Gal and IPTG (100 ml):**
   Dissolve 2.5 g of LB media and 1.5 g of agar in 100 ml of distilled water. Sterilize by autoclaving and allow the media to cool down to 40-45 ^{O}C. Add 100 µl of ampicillin, 200 µl of X-Gal and of 100 µl IPTG to 100 ml of autoclaved LB agar media, mix well and pour on sterile petriplates.

9. Transformation to be carried out as soon as possible after the competent cells are prepared. Storage of competent cells decreases the transformation efficiency.
10. **Preparation of LB (Luria Bertani) agar plates (20 ml):** Dissolve 0.5 g of LB media and 0.3 g of agar in 20 ml of sterile distilled water. Sterilize by autoclaving and pour on sterile petriplate.
11. **Preparation of LB (Luria Bertani) Agar plates containing Ampicillin, X-Gal and IPTG (100 ml):**
    Dissolve 2.5 g of LB media and 1.5 g of agar in 100 ml of distilled water. Sterilize by autoclaving and allow the media to cool down to 40-45^{O}C. Add 100 µl of ampicillin, 200 µl of X-Gal and of 100 µl IPTG to 100 ml of autoclaved LB agar media, mix well and pour on sterile petriplates.

Procedure:

Day 1:

1. Open the bottle containing culture and reconstitute the pellet with 0.25 ml of LB broth.
2. Pick up a loopful of culture and streak onto LB agar plate and incubate overnight at 37^{0}C .

Day 2:

1. Inoculate a single colony from the revived plate in 1 ml LB broth and incubate overnight at 37^{0}C.

Day 3:

1. Take 50 ml of LB broth in a sterile flask. Transfer 1 ml of overnight grown culture into the flask containing 50 ml of LB broth and incubate at 37^{0}C in a shaker set at 3000 rpm for four hours.

A) Preparation of Competent Cells:

Note: Competent cells are to be prepared within 3 days of reviving the strain.

1. Transfer the above culture into a pre-cooled 50 ml polypropylene tube.
2. Incubate the culture at 4^{O}C for 10 minutes on ice.
3. Centrifuge at 5000 rpm for 10 minutes at 4^{O}C in a cooling centrifuge.
4. Decant the medium completely leaving no traces of medium.
5. The cell pellet is resuspended in 30 ml pre-chilled sterile 0.1 M Calcium chloride solution and incubate for 30 minutes on ice.
6. Centrifuge at 5000 rpm for 10 minutes at 4^{O}C.
7. Discard the calcium chloride solution.
8. The pellet is resuspended in 2 ml pre-chilled sterile 0.1M Calcium chloride solution.
9. This cell suspension contains competent cells and can be used for transformation.

B) Transformation of cells:

1. Take 200 µl of the above cell suspension in two 2.0 ml tubes and label control and transformed. Add 2 µl of plasmid DNA to the tube labeled as transformed and mix well.
2. Incubate the tubes at 4^{O}C for 30 minutes.
3. Transfer them to a pre-warmed water bath set at a temperature of 42^{O}C for 2 minutes to produce heat shock.

4. Rapidly transfer the tubes on ice-bath. Allow the cells to cool for 5 minutes.
5. Add 800 µl of LB Broth to both the tubes. Incubate the tubes for 1 hour at $37^{o}C$ to ensure the bacteria to recover and express the antibiotic resistance marker encoded by the plasmid.
6. Take four LB agar plates containing ampicillin, X-Gal, IPTG and label them as control, X, Y and Z. Plate 200 µl of culture from the „control" tube and plate it on the corresponding plate with a sterile spreader. Plate 50 µl, 100 µl and 200 µl of cell cultures from the transformed tube on the plates labeled as X, Y and Z.
7. Store at room temperature till the plates are dry.
8. Incubate the plates overnight at $37^{o}C$.

Observation and Result

After incubation observe the plates for the bacterial growth and count the number of visible colonies. Calculate the efficiency of transformation.

Record your observations as follows:

Sr. No.	Plate	Growth	Number of colonies	Transformation Efficiency
1.	Control plate			
2.	Transformed plate (X)			
3.	Transformed plate (Y)			
4.	Transformed plate (Z)			

Denote +ve when you observe bacterial growth, -ve when there is no growth

Calculation of transformation efficiency

Transformation efficiency is defined as the number of cells transformed per microgram of supercoiled plasmid DNA in a transformation reaction**.**

Transformation Efficiency = Number of colonies x 1000 ng/ Amount of DNA plated (ng) = /g.

Interpretation

On transformation of cells with pUC19 plasmid, antibiotic resistance is conferred on the host as this plasmid carries gene for ampicillin resistance. As a result, those cells that grow in presence of ampicillin are transformed cells. The transformed colonies are blue on X-Gal, IPTG plates due to α-complementation.

Possible viva questions

1. How might you optimize the purity of DNA?
2. Why must you handle DNA gently in the extraction procedure?
3. What are the importance of DNA isolation?
4. What are DNA? Mention its types?
5. What are the principles of DNA isolation?
6. Mention the uses of alcohol in isolation of DNA.
7. What is the need of cell lysis in DNA isolation?
8. What are the action of lysozyme in DNA isolation?
9. How acetate purify DNA during DNA isolation?
10. What are chemicals need for DNA isolation?
11. What is the end use of isolated DNA?
12. What is the importance of RNA?
13. How do you detect RNA in solution?
14. What are the difference between plasmid and chromosomal DNA?
15. What is single digestion and double digestion?
16. Mention properties of agarose.
17. What is electrophoresis?
18. What are the different types of electrophoresis?
19. What are the uses of ethidium bromide DNA seperation?
20. What are the factors associated with nucleic acid mobility in electrophoresis?
21. What are the purposes of using buffer in electrophoresis unit?
22. What are the principles of DNA amplification?
23. Who invented PCR?
24. What is primer?
25. Explain the principle beyond lac operon induction.
26. Mention the principle of affinity chromatography in isolation of mRNA.