Quiz 3: Fundamentals of Biology II by Dr. Gaurav Ahuja Date: 17/10/2025

Multiple Choice Questions (2 x 8 = 16 marks, 0.5 marks negative)

Answers for all questions is b

Subjective Questions: Attempt any 4 Questions (4 marks each)

1. Explain the major steps involved in the creation of recombinant DNA, highlighting the role of restriction enzymes and vectors.

Ans: The process of creating recombinant DNA involves six major steps that together allow scientists to insert a desired gene into a suitable vector and express it within a host cell. First, the target gene containing the desired DNA sequence is isolated from the donor organism using molecular techniques such as restriction digestion or PCR. The next step is cutting (cleavage), where both the isolated gene and the vector DNA—commonly a plasmid—are cut with the same restriction enzyme. This ensures that both DNA molecules have compatible ends that can be joined together. Following this, the joining (ligation) step involves inserting the target gene into the plasmid vector, and the enzyme DNA ligase seals the sugar-phosphate backbone to form a stable recombinant DNA molecule.

The recombinant plasmid is then introduced into a host cell, such as *E. coli*, through a process called transformation, enabling the foreign DNA to enter and function within the cell. Once inside, the recombinant DNA replicates as the host cell divides, leading to amplification of the inserted gene. Finally, the transformed cells are subjected to selection (screening) to identify those that have successfully taken up the recombinant plasmid. This is usually achieved using selectable markers, such as antibiotic resistance genes or color-based reporter systems. Together, these six steps—isolation, cutting, joining, transforming, amplifying, and selection—form the foundation of recombinant DNA technology, enabling the creation and propagation of genetically modified organisms for research and biotechnology applications.

2. What are the sources of evidence of phylogeny and explain homologous characteristics with examples?

Ans: Scientists use different types of data to construct evolutionary or phylogenetic trees that depict relationships among organisms. One important source of information is the fossil record, which provides direct evidence of ancestral life forms and allows scientists to trace evolutionary changes over time. However, the fossil record is often incomplete or poorly preserved, leaving significant gaps that make it difficult to form a continuous evolutionary sequence. Another approach is based on phenotypic characteristics, often referred to as the “classic method,” which relies on observable physical traits such as anatomy and morphology. For example, comparing the bone structures of different animals can reveal shared evolutionary origins. In contrast, the modern approach uses molecular data, such as DNA or protein sequences, to determine evolutionary relationships. The greater the similarity in these molecular sequences

between two species, the more closely related they are considered to be. This molecular method has become the most widely used and reliable approach in modern evolutionary biology.

3. Explain the principle and procedure of the Ames test. How does it help in identifying potential chemical mutagens and carcinogens?

Ans: The Ames test is a simple biological assay used to identify whether a chemical compound is a mutagen and therefore potentially a carcinogen. It is based on the principle that mutagenic substances can cause reverse mutations in specific mutant strains of *Salmonella typhimurium* that cannot synthesize histidine (his^-). When these bacteria are exposed to a mutagen, some undergo mutations that restore their ability to produce histidine (his^+), allowing them to grow on a histidine-free medium.

In the procedure, the mutant bacteria are mixed with the test chemical, sometimes along with a liver extract (S9 mix) to mimic metabolic activation. The mixture is plated on a medium lacking histidine and incubated. The appearance of more bacterial colonies compared to the control indicates that the compound is mutagenic.

Thus, the Ames test helps in screening chemicals for their mutagenic and carcinogenic potential, providing a quick and cost-effective method for early detection of DNA-damaging agents.

4. Describe the mechanism of the CRISPR-Cas9 system in gene editing. How do the guide RNA (gRNA) and PAM sequence contribute to the specificity of DNA cleavage?

Ans: The CRISPR-Cas9 system is a powerful tool for gene editing that allows precise modification of DNA sequences in living cells. It is adapted from a natural bacterial defense mechanism used to recognize and cut foreign viral DNA. The system mainly consists of two components: the Cas9 nuclease and a guide RNA (gRNA).

The gRNA is designed to be complementary to the target DNA sequence, guiding the Cas9 enzyme to the exact location in the genome that needs to be edited. Once the gRNA binds to its matching DNA sequence, Cas9 introduces a double-stranded break at that site. The cell then repairs this break through one of two natural repair mechanisms: non-homologous end joining (NHEJ), which may cause small insertions or deletions leading to gene disruption, or homology-directed repair (HDR), which can insert a new DNA sequence if a repair template is provided.

The PAM (Protospacer Adjacent Motif) sequence, which is typically a short sequence like NGG, is essential for Cas9 recognition and cleavage. Cas9 will only cut DNA that is immediately followed by a PAM, ensuring that it targets the correct site. Together, the gRNA provides sequence specificity by base-pairing with the target DNA, while the PAM sequence ensures accurate Cas9 binding, making CRISPR-Cas9 a highly specific and efficient gene-editing tool.

5. What is insertional inactivation in recombinant DNA technology? Explain how it is used to identify recombinant colonies using the example of the pBR322 plasmid.

Ans: Insertional inactivation is a technique used in recombinant DNA technology to identify whether a foreign DNA fragment has been successfully inserted into a plasmid vector. It is based on the principle that when a gene within the plasmid (usually a selectable marker gene) is disrupted by the insertion of foreign DNA, the normal function of that gene is inactivated.

In the pBR322 plasmid, there are two antibiotic resistance genes — ampicillin resistance (amp^R) and tetracycline resistance (tet^R). When a foreign DNA fragment is inserted into the tet^R gene at its unique restriction site (for example, at the *BamHI* site), the insertion disrupts the gene's coding sequence, thereby inactivating the tetracycline resistance. As a result, the recombinant plasmid confers resistance to ampicillin but not to tetracycline.

To identify recombinant colonies, bacterial cells transformed with pBR322 are first grown on plates containing ampicillin. Only those cells that have taken up the plasmid (recombinant or non-recombinant) survive. These colonies are then replica-plated onto tetracycline-containing plates. Colonies that fail to grow on tetracycline medium but grew on ampicillin plates are identified as recombinant colonies, since their tetracycline resistance gene has been inactivated by the insertion of foreign DNA.

Thus, insertional inactivation provides a simple and effective method to screen and distinguish recombinant clones from non-recombinants.