Chapter 4
Application of Recombinant DNA Technology

EXERCISES

1. What do you mean by DNA fingerprinting? Explain it through RFLP.

Ans: DNA Fingerprinting:

DNA fingerprinting, also known as DNA profiling or DNA typing, is a forensic technique used to identify individuals based on their unique DNA sequences. It relies on the fact that the DNA sequence varies between individuals, except in identical twins. The method involves the analysis of specific regions of the DNA, producing a unique pattern or "fingerprint" for each individual.

RFLP (Restriction Fragment Length Polymorphism):

RFLP is one of the early methods used for DNA fingerprinting. It exploits variations in the lengths of DNA fragments that result from the action of restriction enzymes. Here's a step-by-step explanation of the RFLP process in DNA fingerprinting:

1. **DNA Sample Collection:**
   - DNA is extracted from the biological sample (blood, saliva, hair, etc.) collected from the crime scene or individuals involved.
2. **Restriction Enzyme Digestion:**
   - The extracted DNA is then treated with specific restriction enzymes. These enzymes recognize specific DNA sequences and cleave the DNA at those sites, producing fragments with different lengths.
3. **Gel Electrophoresis:**
   - The digested DNA fragments are separated based on their sizes using gel electrophoresis. In this process, an electric field is applied to a gel matrix, causing the DNA fragments to migrate through the gel. Smaller fragments move faster, and larger fragments move more slowly.
4. **Southern Blotting:**
   - After electrophoresis, the DNA fragments are transferred from the gel to a solid support membrane (usually nylon or nitrocellulose) in a process known as Southern blotting. This transfers the DNA fragments' spatial arrangement on the gel to the membrane.

5. **Probe Hybridization:**
    - The membrane is then incubated with a labeled DNA probe. The probe is a single-stranded DNA molecule that is complementary to a specific sequence of interest. It hybridizes with the target DNA fragments on the membrane.
6. **Autoradiography:**
    - The membrane is exposed to X-ray film, and the labeled DNA fragments bound to the probe create a visible pattern on the film through autoradiography.
7. **Analysis and Interpretation:**
    - The autoradiograph reveals a series of bands corresponding to the DNA fragments. The banding pattern is unique to each individual because it reflects the variations in the lengths of the DNA fragments at specific loci.
8. **Comparison:**
    - The DNA fingerprints obtained from different individuals can be compared. If two individuals share similar banding patterns, they are more likely to be closely related or have a common biological origin.

RFLP-based DNA fingerprinting was widely used in forensics, paternity testing, and genetic identification. However, it has been largely replaced by more advanced techniques like Short Tandem Repeat (STR) analysis and Polymerase Chain Reaction (PCR)-based methods due to their higher sensitivity, speed, and ability to work with degraded DNA samples

2. What are GMOs? Describe the method of development of transgenic plants.

Ans: GMOs (Genetically Modified Organisms):

Genetically Modified Organisms (GMOs) are living organisms whose genetic material has been artificially manipulated in a way that does not occur naturally through mating or natural recombination. Genetic modification involves the introduction of specific DNA sequences into an organism's genome, resulting in desired traits or characteristics.

Method of Development of Transgenic Plants:

Developing transgenic plants involves the introduction of foreign genes into plant cells, leading to the expression of new traits. The following steps outline the general process of creating transgenic plants:

1. **Identification of Target Gene:**
    - The first step involves identifying the specific gene or genes that encode the desired trait. This could be a gene responsible for resistance to pests, tolerance to herbicides, improved nutritional content, or other traits.
2. **Isolation of the Target Gene:**
    - The target gene is isolated from the source organism. This gene is typically selected for its known function and compatibility with the target plant species.
3. **Construction of a Plant Transformation Vector:**
    - The isolated gene is inserted into a plant transformation vector, often a plasmid. The vector contains additional elements such as a promoter (to drive gene expression), terminator (to signal the end of transcription), and selectable marker genes (to aid in the identification of transformed cells).
4. **Transformation of Plant Cells:**
    - Plant cells are transformed by introducing the vector into them. Several methods can be used for plant cell transformation:
        - Agrobacterium-mediated transformation: Agrobacterium tumefaciens, a bacterium, is used as a vector to transfer genes into plant cells.
        - Biolistics (Particle Bombardment): DNA-coated particles are shot into plant cells using a gene gun.
        - Electroporation: An electric field is used to introduce DNA into plant cells.
5. **Selection of Transgenic Cells:**
    - Transformed cells are selected using a selectable marker present in the transformation vector. Common markers include antibiotic resistance genes or herbicide resistance genes. Only cells that successfully incorporate the foreign gene will survive the selection process.
6. **Regeneration of Transgenic Plants:**
    - Transgenic cells are induced to form a whole plant through a process called regeneration. This often involves the use of plant tissue culture techniques.

7. **Verification of Transgene Integration:**
    - PCR and other molecular techniques are used to confirm the presence and integration of the transgene in the regenerated plants.
8. **Field Testing and Commercialization:**
    - Transgenic plants that pass safety and environmental assessments undergo field testing to evaluate their performance under real-world conditions. If deemed safe and beneficial, these plants may be commercialized for agricultural use.

Common examples of genetically modified crops include insect-resistant Bt cotton, herbicide-tolerant soybeans, and virus-resistant papaya. The development of transgenic plants has applications in agriculture, where it can improve crop yields, reduce the use of chemical pesticides, and enhance nutritional content. However, it also raises concerns about environmental impact and potential risks, leading to ongoing discussions about the regulation and ethical considerations surrounding GMOs

3. Differentiate between direct and indirect method of gene transfer. Name one indirect method suitable for gene transfer in dicot plants.

Ans: Direct vs. Indirect Gene Transfer:

1. Direct Gene Transfer:

- **Description:**
    - In direct gene transfer, the foreign DNA is introduced directly into the target organism's cells, bypassing the need for an intermediary vector.
    - Direct gene transfer methods often involve physical methods or direct manipulation of cells to facilitate the uptake of foreign DNA.
- **Example Methods:**
    - Electroporation: Application of an electric field to create temporary pores in cell membranes, allowing DNA uptake.
    - Microinjection: Direct injection of DNA into the nucleus or cytoplasm of a cell using a fine glass needle.

- Particle Bombardment (Biolistics): DNA-coated particles are propelled into target cells using a gene gun.

2. Indirect Gene Transfer:

- **Description:**
  - In indirect gene transfer, an intermediary vector, such as a plasmid or a viral vector, is used to carry and deliver the foreign DNA into the target cells.
  - The vector acts as a vehicle for transporting the genetic material and facilitating its integration into the host organism's genome.
- **Example Methods:**
  - Agrobacterium-Mediated Transformation: The use of the soil bacterium Agrobacterium tumefaciens as a vector to transfer genes into plant cells.

Agrobacterium-Mediated Transformation:

- **Description:**
  - Agrobacterium tumefaciens is a soil bacterium naturally capable of transferring DNA (T-DNA) into plant cells.
  - The Ti plasmid of Agrobacterium contains the T-DNA region, which can be modified to carry the desired genes.
  - The modified Agrobacterium is used to infect plant tissues, leading to the transfer of the T-DNA into the plant genome.
- **Steps:**
  1. **T-DNA Modification:**
     - The gene of interest is inserted into the T-DNA region of the Ti plasmid.
  2. **Infection:**
     - The modified Agrobacterium is introduced into plant tissues, often through a process called co-cultivation.
     - The T-DNA is transferred to the plant cells during infection.
  3. **Integration into Plant Genome:**
     - The T-DNA integrates into the plant genome, leading to the expression of the foreign gene in the transformed plant cells.
  4. **Selection and Regeneration:**

- Transformed cells are selected using antibiotic resistance genes present on the Ti plasmid.
- The selected cells are induced to regenerate into whole plants through tissue culture.

Key Difference:

- In direct gene transfer, foreign DNA is introduced directly into the target cells without the use of an intermediary vector, whereas, in indirect gene transfer, an intermediary vector is used to deliver the foreign DNA into the target cells.

Suitability for Dicot Plants:

- Agrobacterium-mediated transformation is a suitable indirect method for gene transfer in dicot plants. It has been widely used for introducing foreign genes into dicotyledonous plants, leading to the development of genetically modified crops with desired traits

4. What is molecular pharming? Give applications of transgenic animals in molecular pharming.

Ans: Molecular Pharming:

Molecular pharming, also known as biopharming or molecular farming, is the use of genetic engineering techniques to produce pharmaceuticals, therapeutic proteins, and other valuable compounds in plants or animals. The organisms engineered for molecular pharming act as bioreactors, producing complex proteins that are then harvested and used for medical or industrial purposes.

Applications of Transgenic Animals in Molecular Pharming:

1. **Production of Therapeutic Proteins:**
   - Transgenic animals can be engineered to produce therapeutic proteins in their milk, blood, or tissues. For example, cows can be modified to express human proteins in their milk, which can then be collected and used for medical purposes.

2. **Antibody Production:**
    - Transgenic animals can be designed to produce specific antibodies against diseases or toxins. The antibodies can be harvested from the animals' blood or other bodily fluids and used for diagnostic or therapeutic purposes.
3. **Blood Clotting Factors:**
    - Animals can be genetically modified to produce human blood clotting factors. This is particularly valuable for individuals with hemophilia who require regular infusions of clotting factors.
4. **Enzyme Replacement Therapy:**
    - Transgenic animals can be engineered to produce enzymes that are deficient or missing in individuals with certain genetic disorders. This allows for enzyme replacement therapy by harvesting the enzymes from the animals.
5. **Vaccine Production:**
    - Animals can be genetically modified to produce proteins or antigens related to specific pathogens. The harvested proteins can be used in the development of vaccines for human or veterinary applications.
6. **Humanized Organs for Xenotransplantation:**
    - While not yet widely implemented, there is ongoing research in developing transgenic pigs that express human genes to produce organs suitable for xenotransplantation. This could address the shortage of human donor organs for transplantation.
7. **Production of Growth Hormones:**
    - Transgenic animals can be engineered to produce growth hormones, which can be used for therapeutic purposes in individuals with growth disorders.
8. **Spider Silk Production:**
    - Some transgenic animals, such as goats, have been engineered to produce spider silk proteins in their milk. Spider silk is known for its strength and elasticity, and it has potential applications in the development of lightweight and strong materials.
9. **Human Serum Albumin Production:**
    - Transgenic animals can be designed to produce human serum albumin, a protein that is used in medical applications such as blood volume expansion and as a component of certain therapeutic formulations.

Molecular pharming in transgenic animals offers a scalable and cost-effective method for producing complex proteins and compounds with pharmaceutical relevance. While there are ethical and regulatory considerations, advancements in this field hold promise for addressing medical needs and improving the production of therapeutic agents.

5. Differentiate between gene gun and gene therapy.

Ans: Gene Gun:

1. **Definition:**
   - The gene gun, also known as biolistics or particle bombardment, is a method used in genetic engineering to introduce foreign DNA into the cells of living organisms.
2. **Delivery Method:**
   - In the gene gun method, DNA-coated particles (such as gold or tungsten particles) are accelerated and shot into the target cells or tissues.
3. **Mechanism:**
   - The accelerated particles penetrate the cell walls and cell membranes, delivering the foreign DNA into the nucleus of the target cells.
4. **Applications:**
   - The gene gun is widely used in plant transformation and has been employed in the transformation of animal cells as well. It has applications in genetic research and the development of genetically modified organisms.

Gene Therapy:

1. **Definition:**
   - Gene therapy is a medical approach that involves the introduction, removal, or modification of genetic material within an individual's cells to treat or prevent a disease.
2. **Objective:**
   - The primary goal of gene therapy is to correct or replace faulty genes responsible for diseases or to introduce therapeutic genes to enhance the individual's health.

3. **Delivery Method:**
    - Gene therapy can be delivered using various methods, including viral vectors, liposomes, electroporation, and other techniques, depending on the target cells and the nature of the genetic material to be introduced.
4. **Applications:**
    - Gene therapy has potential applications in the treatment of genetic disorders, cancer, and various other diseases. It can involve the addition of a functional copy of a gene, the correction of a mutated gene, or the introduction of therapeutic genes to address specific medical conditions.

Key Differences:

1. **Focus:**
    - The gene gun is a method for physically delivering DNA into cells, often used in genetic engineering for research purposes.
    - Gene therapy is a medical intervention focused on treating or preventing diseases by modifying the genetic material within an individual's cells.
2. **Application Area:**
    - The gene gun is commonly used in plant transformation and has been adapted for some applications in animal cells.
    - Gene therapy is a medical approach applied to the treatment of various diseases in humans and, in some cases, animals.
3. **Delivery Mechanism:**
    - The gene gun uses physical force to deliver DNA-coated particles into cells.
    - Gene therapy can employ various delivery methods, including viral vectors, liposomes, and others, depending on the specific requirements of the therapeutic intervention.
4. **Objective:**
    - The primary objective of the gene gun is to introduce foreign DNA into cells for research or genetic modification purposes.
    - The primary objective of gene therapy is to treat or prevent diseases by modifying the genetic material within the patient's cells.

In summary, while the gene gun is a tool for delivering genetic material into cells, gene therapy is a broader medical approach focused on using genetic interventions to treat or prevent diseases in living organisms

6. Give the procedure of development of recombinant subunit vaccines.

Ans: Recombinant subunit vaccines are a type of vaccine that is developed by expressing and purifying specific protein subunits of a pathogen. These protein subunits serve as antigens to stimulate an immune response, providing protection against the targeted pathogen. Here is a general procedure for the development of recombinant subunit vaccines:

1. **Identification of Target Antigen:**
    - Determine the specific antigenic protein or protein subunit from the pathogen that is capable of inducing a protective immune response. This selection is crucial for the vaccine's effectiveness.
2. **Isolation of the Gene Encoding the Antigen:**
    - Isolate and clone the gene that encodes the selected antigen. This is often done using molecular biology techniques such as PCR (Polymerase Chain Reaction) or other gene cloning methods.
3. **Expression Vector Construction:**
    - Insert the isolated gene into an expression vector. The expression vector is a DNA molecule that can replicate in a host organism (e.g., bacteria, yeast, or mammalian cells) and express the target gene. The vector should include regulatory elements such as a promoter to drive gene expression.
4. **Transformation of Host Cells:**
    - Introduce the constructed expression vector into a suitable host organism, such as bacteria (e.g., Escherichia coli), yeast cells, or mammalian cells. The host organism will then produce the antigenic protein.
5. **Expression and Production of Recombinant Protein:**
    - Allow the host cells to express the recombinant protein. The protein is synthesized using the host cell's machinery, and the antigenic protein is subsequently produced in significant quantities.
6. **Protein Purification:**

- Harvest the host cells and extract the recombinant protein. Purify the protein to remove contaminants and obtain a highly purified form of the antigen.

7. **Formulation of the Vaccine:**
   - Formulate the purified recombinant protein into a vaccine. This may involve combining the protein with adjuvants or other components to enhance the immune response and improve vaccine efficacy.
8. **Preclinical Testing:**
   - Conduct preclinical testing of the recombinant subunit vaccine in laboratory animals to assess safety, immunogenicity, and efficacy. This step helps in identifying any potential issues before proceeding to clinical trials.
9. **Clinical Trials:**
   - If successful in preclinical testing, move on to clinical trials involving human subjects. Clinical trials typically consist of three phases to evaluate safety, immunogenicity, and efficacy in larger populations.
10. **Regulatory Approval:**
    - Submit the data from clinical trials to regulatory authorities for approval. The regulatory approval process involves rigorous evaluation of the vaccine's safety, efficacy, and manufacturing processes.
11. **Production and Distribution:**
    - Upon regulatory approval, scale up the production of the recombinant subunit vaccine. Distribute the vaccine for mass vaccination campaigns or routine immunization programs.

Recombinant subunit vaccines have several advantages, including safety, as they do not contain live pathogens, and the ability to target specific antigens of interest. These vaccines have been successful in preventing diseases such as hepatitis B, human papillomavirus (HPV), and others.

7. Write a short note on DNA vaccines.

Ans: DNA Vaccines:

DNA vaccines represent a type of genetic vaccine that uses a small, circular piece of DNA (plasmid) to induce an immune response against specific pathogens. Unlike traditional vaccines, which use inactivated or attenuated pathogens or their components, DNA vaccines deliver the genetic information encoding antigens directly into the cells of the vaccinated individual. This approach stimulates both the humoral and cellular arms of the immune system, potentially providing robust and long-lasting immune protection.

Key Features of DNA Vaccines:

1. **Plasmid Design:**
   - The DNA used in vaccines is typically a circular, double-stranded plasmid. It carries the genetic information for the production of one or more antigens from the targeted pathogen.
2. **Antigen Expression:**
   - Once the DNA vaccine is delivered into the host cells, the cells use their own machinery to transcribe and translate the inserted genes. This leads to the expression of the antigens encoded by the vaccine.
3. **Immune Response Stimulation:**
   - The expressed antigens are presented on the surface of the host cells, triggering both humoral (antibody-mediated) and cellular (T-cell-mediated) immune responses. This dual immune response is advantageous for combating intracellular pathogens.
4. **Adjuvant Effect:**
   - DNA vaccines inherently possess adjuvant-like properties. The DNA itself can stimulate innate immune responses, enhancing the overall immune reaction to the expressed antigens.
5. **Flexibility and Rapid Development:**
   - DNA vaccines are relatively easy and quick to design and produce. This flexibility is particularly advantageous during the development of vaccines for emerging infectious diseases.
6. **Safety Profile:**
   - DNA vaccines are considered safe because they do not contain live pathogens, reducing the risk of causing disease in the vaccinated individual. Additionally, there is no integration of the DNA into the host genome.

7. **Potential for Therapeutic Vaccination:**
    - DNA vaccines show promise for therapeutic vaccination against chronic infections, cancers, and other diseases. They can stimulate immune responses against pre-existing infections or conditions.

Challenges and Considerations:

1. **Delivery Methods:**
    - Efficient delivery of DNA vaccines into cells can be a challenge. Various methods, including electroporation, gene guns, and viral vectors, have been explored to enhance delivery.
2. **Immune Response Levels:**
    - Achieving optimal immune response levels in humans may require further research and optimization. Strategies to enhance immunogenicity are under investigation.
3. **Human Trials and Commercialization:**
    - While DNA vaccines have shown promise in preclinical studies and some clinical trials, the process of obtaining regulatory approvals and commercialization is ongoing.
4. **Perception and Public Acceptance:**
    - Public perception and acceptance of DNA vaccines, like any new technology, play a crucial role in their adoption. Addressing concerns and educating the public about the safety and efficacy of DNA vaccines is essential.

Prominent examples of DNA vaccines in development include those for diseases like Zika virus, HIV, influenza, and certain types of cancer. Continued research and advancements in the field of DNA vaccines hold the potential to revolutionize vaccine development and disease prevention strategies.

8. Describe the advantages of monoclonal antibodies developed by rDNA technology over that developed by Hybridoma technology.

Ans: Monoclonal antibodies (mAbs) are proteins designed to target specific antigens and have applications in various fields, including medicine, diagnostics,

and research. They can be developed using different technologies, with two primary methods being Hybridoma technology and recombinant DNA (rDNA) technology. Here are some advantages of monoclonal antibodies developed by rDNA technology over those developed by Hybridoma technology:

1. Consistent Quality:

- **rDNA Technology:**
  - Monoclonal antibodies produced using rDNA technology ensure consistent and reproducible quality. The process involves the use of genetically engineered cells that are designed to produce specific antibodies, leading to standardized products.
- **Hybridoma Technology:**
  - In Hybridoma technology, variations in antibody production may occur due to differences in the fusion of cells and the characteristics of individual hybridomas. This can result in variability in antibody quality and performance.

2. Scalability:

- **rDNA Technology:**
  - The production of monoclonal antibodies using rDNA technology is generally more scalable than Hybridoma technology. Recombinant systems allow for the efficient and large-scale production of antibodies, making it easier to meet high demand.
- **Hybridoma Technology:**
  - Hybridoma cell lines may have limitations in terms of scalability. The production of antibodies from hybridomas can be time-consuming and labor-intensive, making large-scale production challenging.

3. Flexibility in Modification:

- **rDNA Technology:**
  - Recombinant DNA technology provides greater flexibility for modifying antibody structures. This includes the ability to engineer specific

functionalities, such as adding tags, altering glycosylation patterns, or creating bispecific antibodies with different specificities.

- **Hybridoma Technology:**
  - Hybridomas produce antibodies with native structures, and modifications are limited. Genetic manipulation for modifying antibody properties is not as straightforward as in rDNA technology.

4. Reduced Batch-to-Batch Variation:

- **rDNA Technology:**
  - Monoclonal antibodies produced through rDNA technology exhibit reduced batch-to-batch variation. The use of standardized production methods and engineered cell lines contributes to the consistency of the final product.
- **Hybridoma Technology:**
  - Hybridomas can exhibit variability between batches, leading to potential differences in antibody characteristics. This variation may necessitate additional quality control measures.

5. Animal-Free Production:

- **rDNA Technology:**
  - Recombinant systems allow for animal-free production of monoclonal antibodies. This reduces concerns related to animal welfare, contamination, and the potential transmission of infectious agents.
- **Hybridoma Technology:**
  - Hybridoma technology initially involves the immunization of animals, raising ethical concerns and requiring subsequent steps to purify antibodies from animal-derived materials.

6. Reduced Cross-Reactivity:

- **rDNA Technology:**
  - Antibodies produced through rDNA technology can be engineered to reduce cross-reactivity with unintended targets. This can enhance specificity and reduce off-target effects.

- **Hybridoma Technology:**
  - Hybridoma-derived antibodies may exhibit cross-reactivity due to the use of whole organisms for immunization, leading to potential challenges in certain applications.

In summary, while both rDNA technology and Hybridoma technology have contributed significantly to the production of monoclonal antibodies, rDNA technology offers advantages in terms of consistent quality, scalability, flexibility in modification, reduced batch-to-batch variation, animal-free production, and reduced cross-reactivity. These factors make rDNA-derived monoclonal antibodies preferable in many research, diagnostic, and therapeutic applications.

9. Briefly describe the development of Humulin through rDNA technology.

Ans: Development of Humulin through rDNA Technology:

Background:

- Before the advent of recombinant DNA (rDNA) technology, insulin used for the treatment of diabetes was extracted from the pancreases of animals, primarily pigs and cows. However, this method posed challenges, including the risk of allergic reactions and limited availability of insulin.

Introduction of rDNA Technology:

- In the early 1980s, the development of rDNA technology revolutionized the production of therapeutic proteins, including insulin. This technology allowed for the creation of genetically engineered microorganisms capable of producing human insulin.

Steps in the Development of Humulin (Recombinant Human Insulin):

1. **Gene Cloning:**
   - The genes encoding the A and B chains of human insulin were identified and isolated.

- The DNA sequences of these chains were then cloned into separate plasmids, creating recombinant DNA constructs.

2. **Expression Vectors:**
   - The recombinant DNA constructs were inserted into expression vectors, typically plasmids or viral vectors, which would serve as vehicles for introducing the genes into host cells.
3. **Host Cells and Transformation:**
   - The expression vectors containing the insulin genes were introduced into host cells, often strains of Escherichia coli (E. coli), through a process known as transformation.
   - These host cells were selected for their ability to efficiently express the human insulin genes and produce the corresponding proteins.
4. **Fermentation:**
   - The transformed host cells were cultured in large fermentation tanks, providing an environment conducive to the growth and replication of the cells.
   - As the cells multiplied, they also expressed the human insulin protein.
5. **Insulin Synthesis and Harvesting:**
   - Within the host cells, the individual A and B chains of insulin were synthesized, and they spontaneously combined to form functional human insulin.
   - The insulin-producing cells were harvested from the fermentation tanks.
6. **Purification:**
   - The harvested cells were lysed (broken open), and the insulin was extracted.
   - The crude extract underwent a series of purification steps to isolate and purify the human insulin protein from other cellular components.
7. **Formulation:**
   - The purified insulin was formulated into pharmaceutical preparations suitable for therapeutic use.
   - This included the creation of insulin formulations with different onset and duration of action to mimic the natural release of insulin in the body.
8. **Quality Control:**
   - Rigorous quality control measures were implemented to ensure the safety, purity, and consistency of the final product.

9. **Commercialization:**
    - The product, named "Humulin," was developed by Eli Lilly and Company and was the first commercial recombinant human insulin approved by regulatory authorities.
    - Humulin became available for clinical use, providing a safer and more abundant source of insulin for individuals with diabetes.

Significance:

- The development of Humulin marked a significant milestone in biotechnology and medicine. It showcased the potential of rDNA technology in producing therapeutic proteins on a large scale, providing a safer and more sustainable source of insulin for diabetes treatment. The success of Humulin paved the way for the development of other recombinant therapeutic proteins, transforming the landscape of pharmaceutical manufacturing.

10. Write a short note on humatrope and Protropin.

Ans: Humatrope and Protropin: Recombinant Human Growth Hormones

1. Humatrope:

- **Description:**
    - Humatrope is a brand name for a recombinant human growth hormone (rhGH) known as somatropin. It is produced using recombinant DNA technology, making it a synthetic version of the human growth hormone.
- **Manufacturer:**
    - Humatrope is manufactured by Eli Lilly and Company.
- **Indications:**
    - Humatrope is used for the treatment of growth hormone deficiency in children and adults. It promotes growth and development, particularly in individuals with insufficient endogenous growth hormone production.

2. Protropin:

- **Description:**
  - Protropin is another brand name for recombinant human growth hormone (rhGH), and its generic name is somatrem. Like Humatrope, Protropin is produced using recombinant DNA technology and is a synthetic form of human growth hormone.
- **Manufacturer:**
  - Protropin was originally manufactured by Genentech, and later, the rights and manufacturing were acquired by Eli Lilly and Company.
- **Indications:**
  - Protropin is also used for the treatment of growth hormone deficiency in children and adults. It serves the same purpose as Humatrope in promoting growth and development.

Key Points:

- **Recombinant DNA Technology:**
  - Both Humatrope and Protropin are examples of therapeutic proteins developed through recombinant DNA technology. This involves the insertion of the human growth hormone gene into host cells, typically Escherichia coli (E. coli) bacteria, to produce the synthetic growth hormone.
- **Indications for Use:**
  - The primary indication for both Humatrope and Protropin is the treatment of growth hormone deficiency. This deficiency can result from various medical conditions and may affect children or adults.
- **Mode of Administration:**
  - Both medications are administered via subcutaneous injection. The frequency and dosage of administration depend on the specific patient and the medical condition being treated.
- **Similarities and Differences:**
  - While both Humatrope and Protropin are recombinant human growth hormones with similar therapeutic indications, they may have differences in formulation, dosing, and patient-specific factors. Medical professionals consider individual patient needs and response to treatment when choosing between these medications.

- **Advancements and Availability:**
  - Over time, advancements in recombinant DNA technology and biopharmaceutical manufacturing have led to the development of newer growth hormone products. While Humatrope remains commercially available, Protropin has been discontinued, and patients are often transitioned to alternative recombinant growth hormone products.

It's important for individuals using or considering the use of recombinant human growth hormones to consult with healthcare professionals for personalized guidance and monitoring. Additionally, newer growth hormone formulations may offer improved convenience and therapeutic options for patients with growth hormone deficiencies.

11. Briefly describe the applications of rDNA technology in crop improvement.

Ans: Applications of rDNA Technology in Crop Improvement:

Recombinant DNA (rDNA) technology, also known as genetic engineering or biotechnology, has been extensively utilized in crop improvement to enhance various agronomic traits. These applications aim to develop crops with improved yield, resistance to pests and diseases, tolerance to environmental stresses, and nutritional quality. Here are some key applications of rDNA technology in crop improvement:

1. **Herbicide Resistance:**
   - Introduction of genes conferring resistance to specific herbicides into crops allows for the selective control of weeds without affecting the crop plants. This technology enables more efficient weed management in agricultural fields.
2. **Insect Resistance (Bt Crops):**
   - Incorporation of genes from the bacterium Bacillus thuringiensis (Bt) into crops produces proteins toxic to certain insect pests. Bt crops, such as Bt cotton and Bt corn, exhibit resistance to specific insects, reducing the need for chemical insecticides and minimizing crop damage.

3. **Disease Resistance:**
   - Transfer of genes encoding resistance to fungal, bacterial, or viral pathogens helps crops withstand various diseases. This approach enhances crop health and reduces yield losses due to diseases.
4. **Abiotic Stress Tolerance:**
   - Introduction of genes associated with tolerance to abiotic stresses, such as drought, salinity, and extreme temperatures, helps crops thrive in challenging environmental conditions. This is particularly crucial for ensuring stable yields in the face of climate change.
5. **Improved Nutritional Content:**
   - Manipulation of metabolic pathways through genetic engineering can enhance the nutritional content of crops. For example, biofortification efforts have focused on increasing levels of essential nutrients, such as vitamins and minerals, in staple crops.
6. **Delayed Ripening:**
   - Incorporation of genes that regulate ethylene production can extend the shelf life of fruits and vegetables. This delayed ripening trait enhances post-harvest storage and transportation, reducing food wastage.
7. **Improved Crop Quality:**
   - rDNA technology has been used to modify the composition of crops, leading to improvements in qualities such as flavor, texture, and appearance. This contributes to consumer acceptance and marketability.
8. **Crop Yield Enhancement:**
   - Genetic modification can target pathways involved in plant growth and development, leading to increased yields. This includes the manipulation of genes associated with photosynthesis, hormone signaling, and nutrient uptake.
9. **Environmental Sustainability:**
   - Genetic engineering contributes to sustainable agriculture by reducing the reliance on chemical inputs. Pest-resistant and disease-resistant crops may require fewer pesticides, leading to environmental benefits and decreased ecological impact.
10. **Precision Breeding:**

- rDNA technology allows for precision breeding by directly introducing specific genes into crops, bypassing the lengthy traditional breeding processes. This accelerates the development of desired traits and shortens the time required for crop improvement.

11.**Development of Transgenic Varieties:**

- The creation of transgenic crops involves introducing foreign genes into plants to confer specific traits. These genetically modified organisms (GMOs) have played a significant role in modern agriculture.

12.**Gene Editing (CRISPR/Cas9):**

- Advanced gene editing techniques, such as CRISPR/Cas9, offer precise modification of specific genes without introducing foreign DNA. This tool allows for targeted genetic modifications to achieve desired traits in crops.

While rDNA technology has demonstrated immense potential in crop improvement, it is essential to consider ethical, environmental, and regulatory aspects associated with genetically modified crops. Ongoing research and advancements in gene editing technologies continue to shape the future of crop biotechnology.

12. List the ethical issues related to the use of transgenic animals?

Ans: The use of transgenic animals, animals that have been genetically modified to carry foreign genes, raises various ethical considerations. These ethical issues encompass concerns about animal welfare, environmental impact, and the broader implications of altering the genetic makeup of living organisms. Here are some key ethical issues related to the use of transgenic animals:

1. **Animal Welfare:**
   - **Suffering and Well-being:** Concerns arise about the well-being and potential suffering of transgenic animals. The genetic modifications may lead to unintended health issues, and researchers must ensure that animals do not experience unnecessary pain or distress.
2. **Intrinsic Value of Animals:**

- **Respect for Animal Life:** Questions are raised about the inherent value of animals and whether genetic modification compromises the natural essence or dignity of the animals. Ethical considerations involve the respect for the intrinsic value of living beings.

3. **Environmental Impact:**
   - **Escape and Interbreeding:** There is a risk of transgenic animals escaping into the wild and interbreeding with wild populations, potentially leading to ecological consequences. This concern is particularly relevant for genetically modified organisms (GMOs) with altered traits that may impact ecosystems.
4. **Human Health and Safety:**
   - **Food Safety:** If transgenic animals are intended for human consumption or produce substances used in food production, ethical concerns relate to the safety of the resulting products. The potential for unintended health effects in humans must be thoroughly assessed.
5. **Intellectual Property and Ownership:**
   - **Ownership of Genetic Information:** The ownership and control of genetic information raise ethical questions. Patents on transgenic animals and their genetic modifications may lead to issues of intellectual property and commercial exploitation.
6. **Informed Consent:**
   - **Lack of Consent:** Animals cannot provide informed consent for genetic modifications. Ethical considerations arise regarding the right to alter an animal's genetic makeup without its explicit consent, as animals are not capable of understanding or communicating such choices.
7. **Moral Status of Transgenic Animals:**
   - **Rights and Consideration:** The ethical status of transgenic animals raises questions about whether they should be granted specific rights or considerations. Philosophical discussions about the moral status of genetically modified organisms touch on issues of sentience, consciousness, and the ability to experience well-being.
8. **Unintended Consequences:**
   - **Unanticipated Outcomes:** The long-term consequences of introducing transgenic animals into ecosystems or human environments may be

unpredictable. Ethical concerns surround the potential for unintended outcomes that could harm other organisms or disrupt natural balances.

9. **Conservation and Biodiversity:**
    - **Impact on Wild Populations:** The release of transgenic animals into the environment may impact wild populations and biodiversity. Questions arise about the ethical implications of altering natural ecosystems and the potential harm to non-modified species.
10. **Social and Cultural Concerns:**
    - **Acceptance and Cultural Values:** The societal acceptance of transgenic animals may vary based on cultural, religious, and ethical values. Ethical considerations include respecting diverse perspectives on the acceptability of genetic modification.
11. **Regulatory Oversight:**
    - **Regulation and Oversight:** Ethical considerations extend to the need for robust regulatory frameworks and oversight to ensure responsible and ethical use of transgenic animals. Lack of adequate regulations may lead to risks and unintended consequences.

Addressing these ethical concerns involves a multidisciplinary approach, engaging scientists, ethicists, policymakers, and the public in discussions about the responsible use of genetic modification technologies in animals. Striking a balance between scientific advancements and ethical considerations is crucial for fostering ethical and sustainable practices in the field of transgenic animals.

13. What is the role of vaccinia virus in the development of recombinant vaccine?

Ans: Vaccinia virus plays a crucial role in the development of recombinant vaccines as a vector for expressing foreign antigens. The vaccinia virus is a member of the poxvirus family and was historically used as the vaccine in the smallpox eradication campaign. Due to its large genome, ease of manipulation, and ability to infect a broad range of host cells, vaccinia virus serves as an excellent platform for the construction of recombinant vaccines. Here is an overview of the key roles of vaccinia virus in the development of recombinant vaccines:

1. **Vector for Gene Insertion:**
   - Vaccinia virus serves as a vector for the insertion of foreign genes. Researchers can engineer the vaccinia virus genome to include genes that code for antigens from other pathogens, such as viruses or bacteria. These inserted genes become part of the viral genome.
2. **Expression of Foreign Antigens:**
   - Once the foreign genes are integrated into the vaccinia virus genome, the virus can replicate and express the encoded antigens during its life cycle. This leads to the production of foreign proteins within infected host cells.
3. **Induction of Immune Response:**
   - The expression of foreign antigens by the recombinant vaccinia virus stimulates an immune response in the host. The host's immune system recognizes the foreign antigens as non-self and mounts an immune response, including the production of antibodies and activation of T cells.
4. **Prime-Boost Strategies:**
   - Vaccinia virus is often used in prime-boost vaccination strategies. In these strategies, an initial immunization with a vaccinia virus-based vector primes the immune system, followed by a booster dose with the same or a different vector expressing the same antigens. This approach enhances and prolongs the immune response.
5. **Safety Features:**
   - While vaccinia virus is capable of efficient gene expression and immune stimulation, it has been modified to be replication-deficient or attenuated to enhance safety. Replication-deficient or attenuated vaccinia viruses can still express foreign antigens but have a reduced capacity to cause disease.
6. **Multivalent Vaccines:**
   - Vaccinia virus can be engineered to express multiple antigens from different pathogens. This capability allows the development of multivalent vaccines that provide protection against multiple diseases with a single vaccination.
7. **Ease of Manipulation:**
   - The large genome of vaccinia virus and its well-established molecular biology techniques make it relatively easy to manipulate for the insertion, expression, and deletion of genes. This facilitates the development and customization of recombinant vaccines.

8. **Broad Host Range:**
    - Vaccinia virus has a broad host range and can infect various mammalian cells, making it suitable for the development of vaccines for different animal species, including humans.
9. **Versatility in Application:**
    - Recombinant vaccinia viruses have been used in the development of vaccines against a variety of infectious diseases, including viral infections, bacterial infections, and parasitic diseases.

Despite its historical significance in smallpox vaccination and its utility as a vector for recombinant vaccines, the use of vaccinia virus has raised safety concerns, particularly in individuals with weakened immune systems. As a result, alternative vector systems and platforms, such as adenoviruses and vesicular stomatitis viruses, are also being explored for the development of recombinant vaccines

14. Write a short note on recombinant therapeutic agents.

Ans: Recombinant Therapeutic Agents:

Recombinant therapeutic agents are medical products derived from genetic engineering techniques, specifically the manipulation of DNA to produce proteins with therapeutic properties. These products have revolutionized medicine by providing safer and more effective treatments for various diseases. The process involves inserting specific genes into host cells, such as bacteria or mammalian cells, to produce therapeutic proteins. Here's a brief overview of some notable recombinant therapeutic agents:

1. **Insulin (Humulin, Novolin):**
    - One of the earliest and most well-known recombinant therapeutic agents is recombinant human insulin. Before the advent of recombinant technology, insulin was extracted from animal pancreases. Recombinant insulin, produced in bacteria or yeast, has replaced animal-derived insulin for the treatment of diabetes.
2. **Human Growth Hormone (Humatrope, Genotropin):**

- Recombinant human growth hormone (rhGH) is used to treat growth hormone deficiency in children and adults. It is produced using recombinant DNA technology, allowing for the mass production of pure and identical growth hormone.

3. **Erythropoietin (EPO):**
    - EPO is a hormone that stimulates the production of red blood cells. Recombinant EPO, produced in mammalian cells, is used to treat anemia associated with chronic kidney disease, cancer chemotherapy, and certain other conditions.
4. **Interferons (e.g., Interferon-alpha, Interferon-beta):**
    - Recombinant interferons are used in the treatment of various viral infections, autoimmune diseases, and certain cancers. They are produced in bacteria or mammalian cells and mimic the body's natural defense mechanisms against viruses.
5. **Monoclonal Antibodies (e.g., Rituximab, Trastuzumab):**
    - Monoclonal antibodies (mAbs) are engineered antibodies designed to target specific proteins in the body. They have revolutionized the treatment of various diseases, including cancer and autoimmune disorders. Recombinant DNA technology allows for the production of therapeutic mAbs with high specificity and reduced immunogenicity.
6. **Clotting Factors (e.g., Factor VIII, Factor IX):**
    - Recombinant clotting factors are essential for the treatment of hemophilia, a genetic disorder affecting blood clotting. These factors are produced in mammalian cells and provide a safer alternative to clotting factors derived from human blood.
7. **Vaccines (e.g., Hepatitis B Vaccine, Human Papillomavirus Vaccine):**
    - Recombinant DNA technology has been employed to produce vaccines against various infectious diseases. For example, the hepatitis B vaccine is produced using yeast cells engineered to express the viral surface antigen.
8. **Enzyme Replacement Therapies (e.g., Imiglucerase, Laronidase):**
    - Recombinant enzymes are used in enzyme replacement therapies for certain genetic disorders where patients lack specific enzymes. For example, imiglucerase is used in the treatment of Gaucher's disease.
9. **TNF-alpha Inhibitors (e.g., Etanercept, Adalimumab):**

- Tumor necrosis factor-alpha (TNF-alpha) inhibitors are used to treat autoimmune diseases such as rheumatoid arthritis. These therapeutic agents are recombinant proteins that block the action of TNF-alpha, reducing inflammation.

10. **Blood Clotting Modulators (e.g., Tissue Plasminogen Activator - tPA):**
    - Recombinant tissue plasminogen activator (tPA) is used to dissolve blood clots in conditions such as acute ischemic stroke or myocardial infarction.

Recombinant therapeutic agents have transformed the treatment landscape by providing precise, targeted, and often safer interventions for various medical conditions. These products continue to be a focus of research and development, with ongoing efforts to expand the range of therapeutic options available through genetic engineering technologies.

15. Write a short note on humanised antibodies.

Ans:
Humanized Antibodies:

Humanized antibodies are a class of monoclonal antibodies (mAbs) that have been engineered to minimize their non-human components while retaining their antigen-binding specificity. Monoclonal antibodies are naturally produced by immune cells and can be developed as therapeutic agents to target specific proteins involved in diseases, such as cancer and autoimmune disorders. Humanization of antibodies involves modifying their structure to make them more similar to human antibodies, thereby reducing potential immunogenicity and improving their therapeutic effectiveness.

Key Steps in Humanizing Antibodies:

1. **Identification of Target Antibody:**
    - The process begins by identifying a non-human monoclonal antibody with the desired specificity for the target antigen. This initial antibody, often derived from mice or other non-human species, is known as the "parent" or "murine" antibody.

2. **Isolation of Antibody Sequences:**
    - The genetic sequences that encode the variable regions of the antibody, responsible for antigen binding, are isolated from the parent antibody. These variable regions are crucial for the specificity of the antibody.
3. **Construction of Chimeric Antibody:**
    - To reduce immunogenicity, a chimeric antibody is initially created by replacing the constant regions (Fc regions) of the parent antibody with human Fc regions. This chimeric antibody retains the antigen-binding specificity of the parent antibody but has a humanized Fc portion.
4. **Framework Shuffling:**
    - Framework shuffling involves further modifying the variable regions of the chimeric antibody to make them more similar to human antibodies. This process helps eliminate potential immunogenic epitopes while maintaining the antigen-binding properties.
5. **Humanization Techniques:**
    - Several techniques, such as CDR grafting and complementarity-determining region (CDR) manipulation, are employed to humanize the antibody. These techniques focus on preserving the antigen-binding regions while minimizing non-human components.
6. **Affinity Maturation:**
    - Affinity maturation involves additional modifications to optimize the binding affinity of the humanized antibody for its target antigen. This step enhances the therapeutic efficacy of the antibody.

Advantages of Humanized Antibodies:

1. **Reduced Immunogenicity:**
    - By minimizing non-human components, humanized antibodies are less likely to elicit an immune response when administered to humans. This is crucial for improving their safety and reducing the risk of adverse reactions.
2. **Extended Half-Life:**
    - Humanized antibodies often exhibit an extended half-life in the bloodstream, allowing for less frequent dosing and improved patient convenience.
3. **Enhanced Therapeutic Efficacy:**

- Humanization techniques aim to maintain or improve the antigen-binding affinity and specificity of the antibody, ensuring its effectiveness in targeting the desired disease-related proteins.

4. **Broad Applicability:**
    - Humanized antibodies can be used in a wide range of therapeutic applications, including cancer treatment, autoimmune disorders, and infectious diseases.
5. **Improved Pharmacokinetics:**
    - Humanization may lead to improved pharmacokinetic properties, such as enhanced tissue penetration and reduced clearance rates, contributing to better therapeutic outcomes.

Examples of Humanized Antibodies:

- Rituximab, trastuzumab, adalimumab, and bevacizumab are examples of humanized antibodies that have been successfully developed and used in the treatment of various diseases.

Humanized antibodies represent a significant advancement in the field of therapeutic monoclonal antibodies, striking a balance between therapeutic efficacy and reduced immunogenicity. These antibodies have become integral components of modern medicine, offering targeted and personalized treatment options for a diverse range of medical conditions.

16. Assertion: In hybridoma technology, B cells are fused with myeloma cells.
Reason: Myeloma cells are immortal.

(a) Both assertion and reason are true and the reason is the correct explanation of the assertion.

(b) Both assertion and reason are true but the reason is not the correct explanation of the assertion.

(c) Assertion is true but reason is false.

(d) Both assertion and reason are false.

Ans: The correct answer is:

(a) Both assertion and reason are true and the reason is the correct explanation of the assertion.

Explanation:

- The assertion that in hybridoma technology, B cells are fused with myeloma cells is true. Hybridoma technology involves the fusion of antibody-producing B cells (from the immune system) with myeloma cells (cancerous B cells). This fusion results in hybrid cells, called hybridomas, which have the ability to produce a specific antibody indefinitely.
- The reason provided, stating that myeloma cells are immortal, is also true and is the correct explanation of the assertion. Myeloma cells, being cancerous, have the property of continuous proliferation and are capable of indefinite growth and division. This immortal nature of myeloma cells is essential for the sustained production of antibodies by the hybridomas, making them a valuable tool in the generation of monoclonal antibodies in large quantities for various applications, including research and medical treatments.

17. Assertion: In Humulin, polypeptide A and polypeptide B are linked with disulfide bridges. Reason: C peptide is removed from proinsulin to

biological active insulin.

(a) Both assertion and reason are true and the reason is the correct explanation of the assertion.

(b) Both assertion and reason are true but the reason is not the correct explanation of the assertion.

(c) Assertion is true but reason is false.

(d) Both assertion and reason are false.

Ans: The correct answer is:

(a) Both assertion and reason are true and the reason is the correct explanation of the assertion.

Explanation:

- The assertion that in Humulin, polypeptide A and polypeptide B are linked with disulfide bridges is true. Humulin is a form of human insulin produced through genetic engineering, and it consists of two polypeptide chains, A and B, which are linked by disulfide bonds.
- The reason provided, stating that C peptide is removed from proinsulin to produce biologically active insulin, is also true and is the correct explanation of the assertion. In the synthesis of insulin, proinsulin is initially formed, which includes an additional peptide called C peptide. In the final step, during the conversion of proinsulin to active insulin, the C peptide is cleaved, and the remaining A and B chains are connected by disulfide bridges to form the mature, biologically active insulin.

Therefore, both the assertion and the reason are true, and the reason provides a correct explanation for the arrangement of polypeptide A and polypeptide B in Humulin.

18. DNA fingerprinting depends on identifying specific:

(a) Coding sequences

(b) Non-coding sequences

(c) mRNA

(d) Promoter

Ans: (b) Non-coding sequences

Explanation: DNA fingerprinting, also known as DNA profiling or DNA typing, relies on identifying specific non-coding sequences in the DNA. These non-coding regions, also called variable number tandem repeats (VNTRs) or short tandem

repeats (STRs), exhibit variations in the number of repeated DNA sequences among individuals. These variations are unique to each individual, except for identical twins.

The analysis of these non-coding regions is the basis for creating a DNA fingerprint, which can be used for various purposes, including forensic identification, paternity testing, and genetic mapping. Coding sequences (option a), mRNA (option c), and promoter regions (option d) are typically not used for DNA fingerprinting purposes.

19. Short stretch of DNA used to identify complementary sequences in a sample is called:

(a) Probe

(b) Marker

(c) VNTR

(d) Minisatellite

Ans: The correct answer is:

(a) Probe

Explanation: A short stretch of DNA used to identify complementary sequences in a sample is called a "probe." Probes are single-stranded DNA sequences that are complementary to a specific target sequence of interest. They are labeled with a detectable marker, such as a radioactive or fluorescent tag, allowing researchers to identify and visualize the presence of the complementary sequence in a sample.

Markers (option b) can refer to various types of molecules used to label or detect specific DNA sequences, but they are not the specific short stretches of DNA used for identification. VNTR (Variable Number Tandem Repeat, option c) and minisatellite (option d) are terms that describe specific types of repetitive DNA sequences used in DNA fingerprinting and genetic profiling techniques.

20. Variable number tandem repeat (VNTR) are:

(a) Repetitive coding short DNA sequences

(b) Non-repetitive non-coding short DNA sequences

(c) Repetitive non-coding short DNA sequences

(d) Non-repetitive coding short DNA sequences

Ans: The correct answer is:

(c) Repetitive non-coding short DNA sequences

Explanation: Variable Number Tandem Repeats (VNTRs) are short DNA sequences characterized by repetitions of specific nucleotide motifs. These repeats are found in non-coding regions of the DNA and exhibit variations in the number of repeated units among individuals. VNTRs are commonly used in DNA fingerprinting and genetic profiling due to their polymorphic nature, allowing for unique identification of individuals based on the pattern of repeats in these non-coding regions.

Option (c) "Repetitive non-coding short DNA sequences" accurately describes VNTRs.

21. Cry genes or Bt genes are obtained from:

(a) Cotton pest

(b) Tobacco plant

(c) Bacillus thuringiensis

(d) E. coli

Ans: The correct answer is:

(c) Bacillus thuringiensis

Explanation: Cry genes, also known as Bt genes, are obtained from the bacterium *Bacillus thuringiensis* (Bt). *Bacillus thuringiensis* is a soil-dwelling bacterium that produces crystal proteins (Cry proteins) toxic to certain insect pests. The Cry genes have been used in genetic engineering to create crops, such as Bt crops, that express these insecticidal proteins.

The introduction of Bt genes into crops, such as cotton and corn, has been employed to confer resistance against specific insect pests. The expression of Cry proteins in these genetically modified crops provides a built-in insecticide, reducing the need for external chemical insecticides and contributing to pest resistance management in agriculture.

22. When gene therapy is done in somatic cells, it is _____.

(a) not-heritable

(b) heritable

(c) rarely heritable

(d) not related to heritability

Ans: (a) not-heritable

23. In gene augmentation therapy, genetic material is _____.

(a) modified

(b) replaced

(c) suppressed

(d) removed

Ans: (b) replaced

24. Germ cell therapy if used for _____.

(a) RBC

(b) Stomach cells

(c) Egg cells

(d) Bone marrow cells

Ans: (c) Egg cells

25. For the first time, from which animal material was isolated for vaccination?

(a) Cat

(b) Cow

(c) Goat

(d) Horse

Ans: (b) Cow

26. Vaccination was invented by:

(a) Jenner

(b) Pasteur

(c) Watson

(d) Crick

Ans: (a) Jenner

7. For the production of insulin by rDNA technology, which bacterium was used?

(a) Saccharomyces

(b) Rhizobium

(c) Escherichia

(d) Mycobacterium

Ans: (c) Escherichia

28. Genetically engineered insulin is called _____.

(a) Humulin

(b) Promulin

(c) Bovulin

(d) Proculin

Ans: (a) Humulin

29. Monoclonal antibodies are produced by_____.

(a) Mutations

(b) Transfection

(c) Hybridoma technology

(d) RNA interference

Ans: (c) Hybridoma technology

SUMMARY

• To study and compare the inherited variations in human DNA without sequencing, a new technique, known as 'DNA fingerprinting' was developed by Sir Alec Jeffreys in 1984 at the University of Leicester.
• In DNA fingerprinting, a stretch of mini-satellite DNA known as Variable Number Tandem Repeats (VNTR) tandemly arranged are exploited using the technique, referred to as Restriction Fragment Length Polymorphism (RFLP).
• The process of insertion of a foreign gene (transgene) into the genome of an organism and its transmission and expression in the organism's progeny is termed

as transgenesis. The organisms carrying the transgene are known as transgenic organisms.

• Transgenic plants are also called genetically modified plants, whose genome is modified, like introduction of one or more genes from another species through genetic engineering techniques.

• Basic requirement for genetic transformation is construction of genetic vehicle, which carries the genes of interest flanked by necessary regulating sequences, like promoter or terminator. The most commonly used techniques for gene transfer are of two types: vector-mediated or indirect gene transfer and vector-less or direct gene transfer.

• Vector-mediated or indirect gene transfer includes transformation using Agrobacterium tumefaciens, in planta transformation, plant virus-mediated transfer while vectorless or direct gene transfer includes particle bombardment, protoplast transformation and microinjection.

• Transgenic animals are animals whose genetic makeup has been transformed by the use of various genetic engineering techniques, such as DNA pronuclear microinjection, embryonic stem cell-mediated gene transfer and retrovirusmediated gene transfer.

• Transgenic plants have been developed with improved agronomic traits in crop plants and products, for example, resistance to biotic and abiotic stresses nutrient quality and delayed fruit ripening, etc.

• Transgenic plants are used in Molecular Farming for large scale production of industrial and therapeutic products.

• Transgenic animals have also been used in molecular pharming for large scale production of proteins, such as α1-antitrypsin, human α-lactalbumin, etc. Transgenic animals have been developed for use in environment benefits and for research purposes.

• There are a number of concerns related to use of GMOs on human health and environment. The Genetic Engineering Approval Committe (GEAC) established by Ministry of Environment, Forest and Climate Change regulates the manufature, use, import, export of hazardous microorganisums.

• Gene therapy is a technique designed to repair faulty genes in humans by introducing correct genetic material inside cells. There are three main approaches for gene therapy, they are: (i) Gene replacement / Gene addition, (ii) Gene

inhibition and (iii) Gene repair/ Gene editing.

• Since, gene therapy includes making changes to the body's basic set of genes, it raises unique ethical considerations.

• A preparation of killed or weakened pathogen or their components given to elicit an immune response that subsequently recognises the infectious agent and confers protection against disease is known as 'Vaccine'.

• To avoid several potential concerns raised by conventional vaccines like reversal of the toxoids to their toxigenic forms, or co-purification of undesirable components and to overcome the complexity involved in obtaining sufficient quantities of purified antigenic components, recombinant vaccines were developed using the various tools of rDNA technologies.

• There are three main types of recombinant vaccines: Live genetically modified vaccines, recombinant subunit vaccines and genetic/DNA vaccines

• rDNA technology enables health care by facilitating large scale biological production of a variety of safe, pure and efficient therapeutic agents, such as a Drugs: Monoclonal antibodies, human proteins e.g. Insulin, HGH.

• With the advances in rDNA technology, it is possible to develop mouse antibodies carrying a few human segments known as chimeric or humanised antibodies possessing higher efficacy and activity.

• In late 1970s, biochemists exploited various tools of rDNA technology for the production of insulin. They isolated the insulin gene from a gene library and then inserted this gene in a bacterial plasmid of E. coli.

• The first, genetically engineered human insulin marketed as Humulin was manufactured in 1982. Successfulproduction of human insulin proved without any ambiguity, the possibility of genetically engineering diverse biological organisms to produce human proteins for medicinal and therapeutic use.

• DNA technologists can now produce Human Growth Hormone entirely using rDNA technology. In 1985, genetically engineered human growth hormone was produced and marketed as Humatrope and Protropin.