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+2 ZOOLOGY

IMPORTANT QUESTIONS WITH ANSWERS

UNIT IV

APPLICATIONS OF BIOTECHNOLOGY

ONE WORD QUESTIONS:

1. Mention the number of primers required in each cycle of PCR.
Two
2. Write the role of primers and DNA polymerase in PCR.
The primers serve as binding site for the DNA sequences and thus helps in DNA synthesis.
3. Name the source organism of the DNA polymerase used in PCR.
Taq bacterium (*Thermus aquatica*) is the source of DNA polymerase used in PCR.
4. Who gave the first clinical gene therapy to the patient?
The first clinical gene therapy was given in 1990 by French Anderson to a four-year old girl with adenosine deaminase (ADA) deficiency.
5. Who isolated insulin from the pancreatic cells of dog?
Best and Banting in the year 1921.
6. Who developed PCR technique?
Kary Mullis (Nobel laureate, 1993) in the year 1983
7. Name the first GI tagged product in India.
Darjeeling tea was the first GI tagged product in India in 2004-05.

TWO MARK QUESTIONS:

8. If a person thinks he is infected with HIV, due to unprotected sex, and goes for a blood test. Do you think a test such as ELISA will help? If so, why? If not, why?
Yes, ELISA test will help, because ELISA is a tool for determining serum antibody concentration produced in the body infected by pathogens like HIV.
9. What does "gene therapy" mean?
It is a corrective therapy given to a person born with a hereditary disease. This process involves the transfer of a normal gene into a person's cells that carry one or more mutant alleles.
10. What is somatic cell nucleus transfer?
Somatic cell nuclear transfer is a technique for creating a viable embryo from a body cell and an egg cell.
11. What are interferons?
Interferons are proteinaceous, antiviral, species specific substances produced by mammalian cells when infected with viruses.
12. What are the two main types of stem cells?

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In mammals there are two main types of stem cells - embryonic stem cells (ES cells) and adult stem cells.

13. What do you mean by stem cell banking?

Stem cell banking is the extraction, processing and storage of stem cells, so that they may be used for treatment in the future, when required.

14. What are the four kinds of ELISA?

There are four kinds of ELISA namely, Direct ELISA, Indirect ELISA, sandwich ELISA and competitive ELISA.

15. What is the significance of ELISA?

It is a highly sensitive and specific method used for diagnosis. ELISA possesses the added advantages of not requiring radioisotopes or a radiation counting apparatus.

16. What is transgenesis?

Transgenesis is the process of introduction of extra (foreign/exogenous) DNA into the genome of the animals to create and maintain stable heritable characters.

17. What do you mean by totipotency?

Totipotency refers to the potential of a cell to become different cells, a tissue, organs and finally an organism.

18. What is GI?

Geographical indication is a name or sign used on products which correspond to a specific geographical origin and possess qualities or a reputation that are due to that origin.

19. Name the products which are given GI tags in Tamil Nadu.

In Tamil Nādu, Kancheepuram silk, Coimbatore wet grinder, Thanjavur paintings, Madurai Malli, Tirunelveli Halwa and Temple jewellery of Nagercoil are GI tagged.

20. Name the first living organism that was patented and the scientist who genetically engineered it.

The first living organism that was patented was a genetically engineered species of bacteria - *Pseudomonas putida* in 1980.

Ananda Mohan Chakrabarty in 1971.

3 MARK QUESTIONS:

21. Define transgene and transgenic animals.

The foreign DNA that is introduced is called the transgene. The animals that are produced by DNA manipulations are called transgenic animals or the genetically engineered or genetically modified organisms.

22. What are edible vaccines?

Edible vaccines are prepared by molecular pharming using the science of

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genetic engineering. Edible vaccines are mucosal targeted vaccines which cause stimulation of both systemic and mucosal immune response. At present edible vaccines are produced for human and animal diseases like measles, cholera, foot and mouth disease and hepatitis.

23. Say about lactalbumin of human milk.

In human milk, α lactalbumin is the most abundant protein comprising 25% of total protein found in human milk.

It is synthesized by the mammary glands. α lactalbumin binds calcium and zinc ions and possesses bactericidal and anti-tumour activities.

24. Define rDNA technology and rDNA.

A technique in which a gene is extracted from one organism and transferred to the DNA of another organism, of the same or another species is called rDNA technology.

25. What is genetic engineering?

Genetic engineering involves the manipulation of DNA and naturally occurring processes such as protein synthesis for a wide range of applications including the production of therapeutically important proteins.

26. Explain why cloning of Dolly, the sheep was such a major scientific breakthrough?

Dolly was the first animal to be cloned from a differentiated somatic cell taken from an adult animal without the process of fertilization. Hence cloning of Dolly is considered a major scientific breakthrough.

27. PCR is a useful tool for early diagnosis of an Infectious disease.

Elaborate.

The concept behind PCR based diagnosis of infectious diseases is simple - if the pathogen is present in a clinical specimen its DNA will be present. Its DNA has unique sequences that can be detected by PCR, often using the clinical specimen (for example, blood, stool, spinal fluid, or sputum) in the PCR mixture.

28. What are stem cells? Explain its role in the field of medicine.

Stem cells are undifferentiated cells found in most of the multi cellular animals.

Stem cells are capable of self-renewal and exhibit 'cellular potency'. Stem cells can differentiate into all types of cells that are derived from any of the three germ layers ectoderm, endoderm and mesoderm.

Their potential application in the field of medicine is the generation of cells and tissues that could be used for cell - based therapies. They also could be used to test new drugs.

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29. What are DNA vaccines?

A DNA vaccine consists of a gene encoding an antigenic protein, inserted onto a plasmid, and then incorporated into the cells in a target animal. DNA instructs the cells to make antigenic molecules which are displayed on its surfaces. This would evoke an antibody response to the free-floating antigen secreted by the cells.

30. Differentiate between Somatic cell gene therapy and germline gene therapy.

Somatic cell gene therapy	Germline gene therapy
Therapeutic genes are transferred into the somatic cells.	Therapeutic genes are transferred into the germ cells.
Genes are introduced into bone marrow cells, blood cells, skin cells etc.,	Genes are introduced into eggs and sperms.
Will not be inherited to later generations.	Heritable and passed on to later generations.

31. What is ADA and how is it caused? What is the symptom of it?

ADA deficiency or SCID (Severe combined immunodeficiency) is an autosomal recessive metabolic disorder. It is caused by the deletion or dysfunction of the gene coding for ADA enzyme. In these patients the absence of this enzyme leads to non-functioning of T-Lymphocytes. Hence T- lymphocytes cannot elicit immune responses against invading pathogens. This causes severe immunodeficiency.

32. What are transgenic animals? Give examples.

Animals that are produced by DNA manipulations are called transgenic animals or the genetically engineered or genetically modified organisms. Ex. mice, rat, rabbit, pig, cow, goat, sheep and fish

33. Explain how “Rosie” is different from a normal cow.

In 1997, Rosie, the first transgenic cow produced human protein enriched milk, which contained the human alpha lactalbumin. The protein rich milk (2.4 gm/litre) was a nutritionally balanced food for new born babies than the normal milk produced by the cows.

34. What is genetically engineered Insulin?

Genetically engineered insulin is produced using a technique called Recombinant DNA technology. This technique involves the insertion of human insulin gene on the plasmids of E. coli. The polypeptide chains are synthesized as a precursor called pre-pro

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insulin, which contains A and B segments linked by a third chain (C) and preceded by a leader sequence.

The leader sequence is removed after translation and the C chain is excised, leaving the A and B polypeptide chains.

35. How was Insulin obtained before the advent of rDNA technology? What were the problems encountered?

In the early years, insulin isolated and purified from the pancreas of pigs and cows was used to treat diabetic patients. Due to minor differences in the structure of the animal insulin as compared to human insulin, it resulted in the occurrence of allergic reactions in some diabetic patients.

36. ELISA is a technique based on the principles of antigen-antibody reactions. Can this technique be used in the molecular diagnosis of a genetic disorder such as Phenylketonuria?

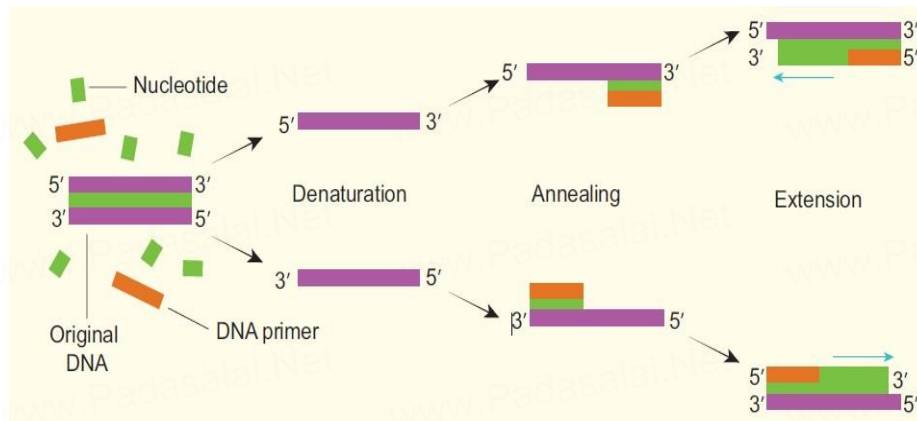
ELISA can detect antibodies produced by the body in response to the specific antigen. Phenylketonuria is not caused due to an infective agent. It is caused due to a defective gene that fails to produce an enzyme phenylalanine hydroxylase. This enzyme is important to break phenylalanine. Hence absence of this enzyme leads to accumulation of more phenylalanine. Hence ELISA is not a diagnostic tool in detecting phenylketonuria.

37. Gene therapy is an attempt to correct a Genetic defect by providing a normal gene into the individual. By this the function can be restored. An alternate method would be to provide gene product known as enzyme replacement therapy, which would also restore the function. Which in your opinion is a better option? Give reasons for your answer.

Though providing a normal gene is effective than enzyme replacement therapy, gene therapy is still in experimental stage. More over gene therapists have to ensure that the newly introduced gene is not harmful to the patient and it is expressed appropriately. Hence enzyme replacement therapy is the better option.

38. Draw the schematic representation to show the steps involved in PCR.

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5 MARK QUESTIONS:

39. How is the amplification of a gene sample of interest carried out using PCR?

Denaturation, renaturation or primer annealing and synthesis or primer extension are the three steps involved in PCR.

Denaturation:

The double stranded DNA of interest is denatured to separate into two individual strands by high temperature up to 950^c.

Renaturation or primer annealing:

Each strand is allowed to hybridize with a primer (renaturation or primer annealing). Taq – DNA polymerase is used to synthesize DNA.

Annealing is done by rapid cooling of the mixture.

Synthesis or primer extension:

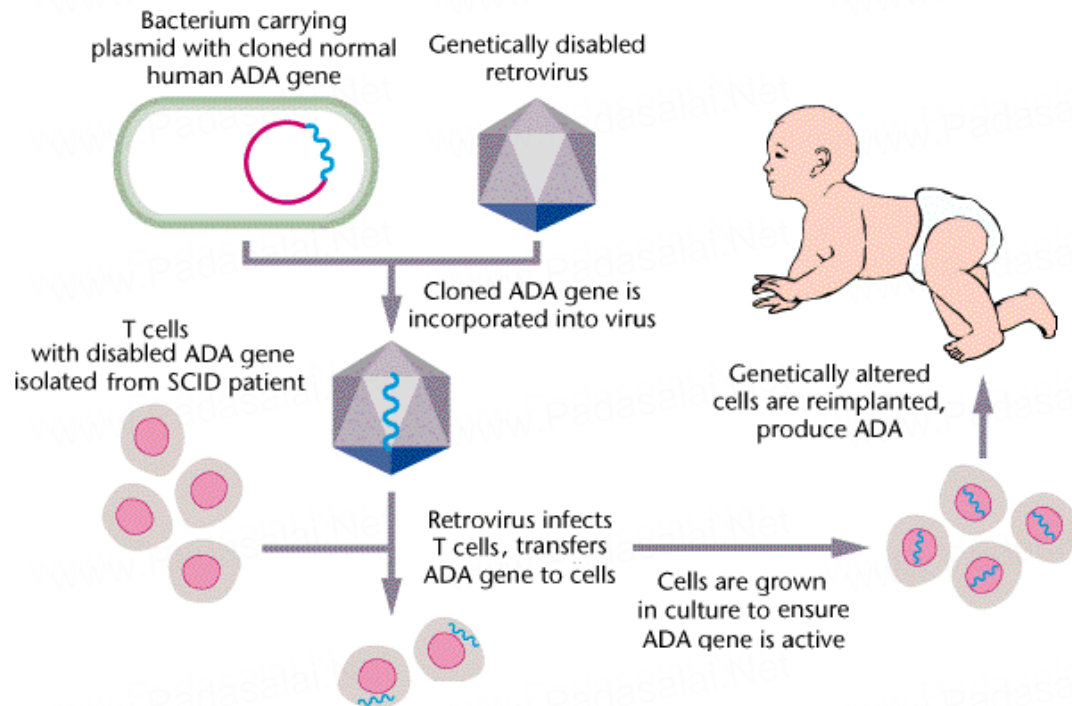
During synthesis or primer extension the temperature of the mixture is increased to 750^c allowing, Taq DNA polymerase to extend each primer by copying the single stranded template.

At the end of incubation both single template strands will be made partially double stranded.

These steps are repeated again and again to generate multiple forms of the desired DNA. This process is also called DNA amplification.

40. Explain the steps involved in gene therapy with a diagrammatic representation.

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41. Explain how ADA deficiency can be corrected?

There are different methods of correction of ADA deficiency:

Bone marrow transplantation: In some children ADA deficiency could be cured by bone marrow transplantation, where defective immune cells could be replaced with healthy immune cells from a donor.

Enzyme transplantation: In some patients it can be treated by enzyme replacement therapy, in which functional ADA is injected into the patient.

Gene therapy: During gene therapy the lymphocytes from the blood of the patient are removed and grown in a nutrient culture medium. cDNA encoding this enzyme Adenosine deaminase is introduced into the lymphocytes using a retrovirus. The genetically engineered lymphocytes are subsequently returned to the patient. Since these cells are not immortal, the patient requires periodic infusion of such genetically engineered lymphocytes.

Permanent treatment: The disease could be cured permanently if the gene for ADA isolated from bone marrow cells are introduced into the cells of the early embryonic stages.

42. What are the possible risks of GMOs?

- Harming non-target species such as soil organisms, non-pest insects, birds and other animals.
- Disrupting biotic communities including agro ecosystems.
- Irreparable loss or changes in species diversity or genetic diversity within species.

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- Creating risks for human health.
- The release of GMOs into the environment could also have far reaching consequences.

43. What are recombinant vaccines? Explain the types.

Recombinant DNA technology has been used to produce new generation vaccines, called recombinant vaccines.

Different types of recombinant vaccines include subunit recombinant vaccines, attenuated recombinant vaccines and DNA vaccines.

Subunit recombinant vaccines:

Vaccines that use components of a pathogenic organism rather than the whole organism are called subunit vaccines; recombinant DNA technology is very suited for developing new subunit vaccines. It includes components like proteins, peptides and DNAs of pathogenic organisms. The advantages of these vaccines include their purity in preparation, stability and safe use.

Attenuated recombinant vaccines:

This includes genetically modified pathogenic organisms (bacteria or viruses) that are made non-pathogenic and are used as vaccines. It is now possible to genetically engineer the organisms (bacteria or viruses) and use them as live vaccines and such vaccines are referred to as attenuated recombinant vaccines.

DNA Vaccines:

A DNA vaccine consists of a gene encoding an antigenic protein, inserted onto a plasmid, and then incorporated into the cells in a target animal. DNA instructs the cells to make antigenic molecules which are displayed on its surfaces. This would evoke an antibody response to the free-floating antigen secreted by the cells.

44. Say about the applications of PCR.

- The differences in the genomes of two different organisms can be studied by PCR.
- PCR is very important in the study of evolutions, more specifically phylogenetics.
- As a technique which can amplify even minute quantities of DNA from any source, like hair, mummified tissues, bones or any fossilized materials.
- PCR technique can also be used in the field of forensic medicine.
- A single molecule of DNA from blood stains, hair, semen of an individual is adequate for amplification by PCR.

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- The amplified DNA is used to develop DNA fingerprint which is used as an important tool in forensic science.
- Thus, PCR is very useful for identification of criminals.
- PCR is also used in amplification of specific DNA segment to be used in gene therapy.

45. Explain how dolly was produced.

- ❖ The mammary gland/udder cells (somatic cells) from a donor sheep (ewe) were isolated and subjected to starvation for 5 days.
- ❖ The udder cells were unable to undergo normal growth cycle, entering a dormant stage and became totipotent.
- ❖ An ovum (egg cell) was taken from another sheep (ewe) and its nucleus was removed to form an enucleated ovum.
- ❖ The dormant mammary gland cell/udder cell and the enucleated ovum were fused.
- ❖ The outer membrane of the mammary cell was ruptured allowing the ovum to envelope the nucleus.
- ❖ The fused cell was implanted into another ewe which served as a surrogate mother.
- ❖ Five months later dolly was born.
- ❖ Dolly was the first animal to be cloned from a differentiated somatic cell taken from an adult animal without the process of fertilization.

46. Mention the advantages and disadvantages of cloning.

Advantages:

- ✓ Offers benefits for clinical trials and medical research.
- ✓ It can help in the production of proteins and drugs in the field of medicine.
- ✓ Aids stem cell research.
- ✓ Animal cloning could help to save endangered species.

Disadvantages:

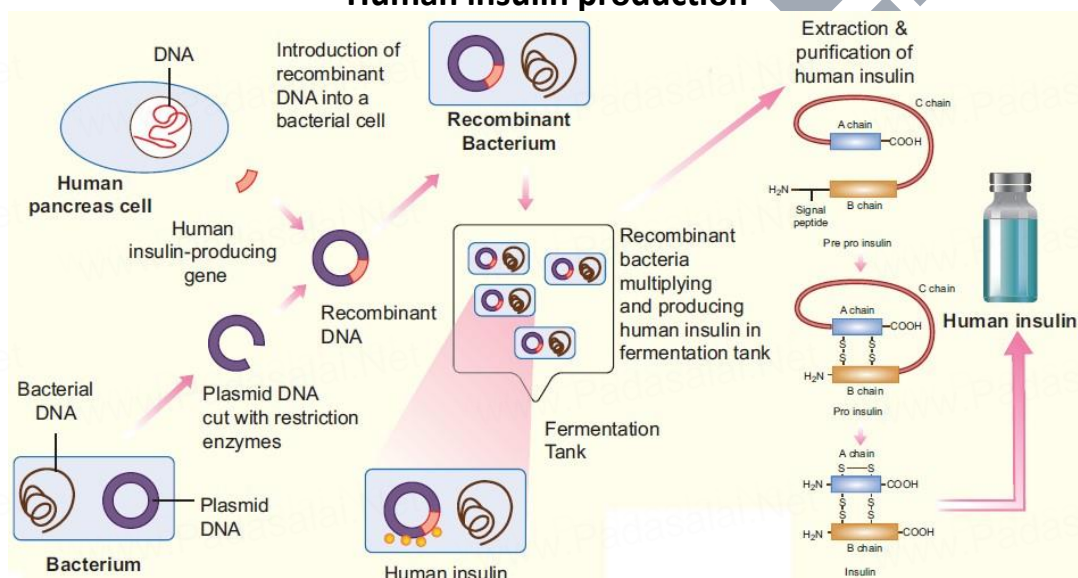
- ✓ It is a threat to biodiversity.
- ✓ Tedious and very expensive process.
- ✓ It can cause animals to suffer.
- ✓ Cloned animals were affected with disease and have high mortality rate.
- ✓ Human health may be affected through consumption of cloned animal meat.
- ✓ Cloned animals age faster and are less healthy.
- ✓ Genetic disorders occur in animals.
- ✓ Cloned animals fail to produce a viable offspring.

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47. Explain how recombinant Insulin can be produced.

- ✓ This technique involves the insertion of human insulin gene on the plasmids of E. coli.
- ✓ A small portion of the plasmid DNA from a bacterium is cut using restriction enzyme.
- ✓ Human insulin producing gene is removed and fused with plasmid DNA.
- ✓ Now this recombinant DNA is inserted into a bacterial cell.
- ✓ The recombinant bacterium multiplies in a fermentation tank.
- ✓ From this recombinant bacterium human insulin gene is extracted and purified.

Human insulin production



- ✓ The polypeptide chains are synthesized as a precursor called pre-pro insulin, which contains A and B segments linked by a third chain (C) and preceded by a leader sequence.
- ✓ The leader sequence is removed after translation and the C chain is excised, leaving the A and B polypeptide chains.

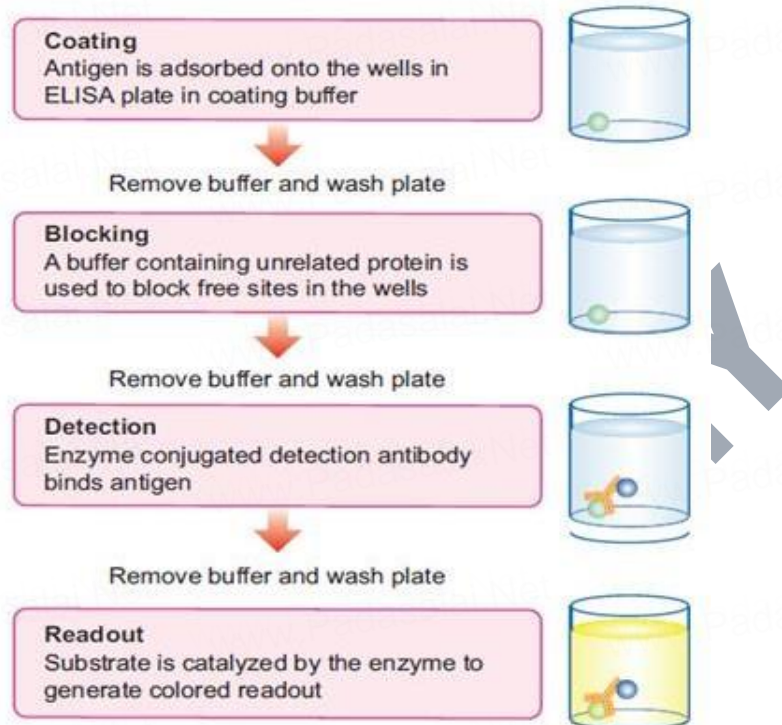
48. Explain the steps involved in ELISA.

- During diagnosis the sample suspected to contain the antigen is immobilized on the surface of an ELISA plate.
- The antibody specific to this antigen is added and allowed to react with the immobilized antigen.
- The anti-antibody is linked to an appropriate enzyme like peroxidase.
- The unreacted anti-antibody is washed away and the substrate of the enzyme (hydrogen peroxidase) is added

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with certain reagents such as 4 chloro naphthol.

Steps involved in Enzyme Linked Immuno Sorbent Assay



- The activity of the enzyme yields a coloured product indicating the presence of the antigen.
- The intensity of the colour is directly proportional to the amount of the antigen.

49. What is the role of PCR in clinical diagnosis?

- DNA of a pathogen has unique sequences that can be detected by PCR, often using the clinical specimen (for example, blood, stool, spinal fluid, or sputum) in the PCR mixture.
- PCR is also employed in the prenatal diagnosis of inherited diseases by using chorionic villi samples or cells from amniocentesis.
- Diseases like sickle cell anaemia, β -thalassemia and phenylketonuria can be detected by PCR in these samples.
- cDNA from PCR is a valuable tool for diagnosis and monitoring retroviral infections e.g., Tuberculosis by Mycobacterium tuberculosis.
- Several virally induced cancers, like cervical cancer caused by Papilloma virus can be detected by PCR.
- Sex of human beings and live stocks, embryos fertilized invitro can be determined by PCR by using primers and DNA probes specific for sex chromosomes.
- PCR technique is also used to detect sex linked disorders in fertilized embryos.

50. List the uses of transgenesis.

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- Transgenesis is a powerful tool to study gene expression and developmental processes in higher organisms.
- Transgenesis helps in the improvement of genetic characters in animals. Transgenic animals serve as good models for understanding human diseases which help in the investigation of new treatments for diseases.
- Transgenic models exist for many human diseases such as cancer, Alzheimer's, cystic fibrosis, rheumatoid arthritis and sickle cell anaemia.
- Transgenic animals are used to produce proteins which are important for medical and pharmaceutical applications.
- Transgenic mice are used for testing the safety of vaccines.
- Transgenesis is important for improving the quality and quantity of milk, meat, eggs and wool production in addition to testing drug resistance.

ALL THE BEST!

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