P.R.GOVERNMENT COLLEGE (A)

DEPARTMENT OF ZOOLOGY

CLINICAL BIOTECHNOLOGY

PRACTICAL MANUAL

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Model paper for Practical semester End Examination

Max. Marks 50 Time: 2 Hours

1. Prepare culture medium/Cell Viability Test using Trypan Blue Test/Interpret the given Gel Eletrophorogram – and Write Procedure 15M
2. Identify the following spotters: 15 M (5x3)
   A. Transgenic animal
   B. Transgenic animal
   C. Vector
   D. Genetic disorder
   E. Genetic disorder
3. Record 05 M
4. Internal Assessment 15 M

Total **50M**

Preperation of Media for animal Cell Culture

Aim

Preparation of Minimal essential growth medium.

Theory

Dulbecco modified eagle medium (DMEM) is one of the most widely used modification of eagles medium. DMEM is a modification of Basal medium eagle (BME) that contains four fold concentration of amino acids and vitamins, additionally, the formulation also includes glycine, serine and ferric nitrates. The original formulation contains 1000 mgs/l of glucose and was originally used to culture embryonic mouse cells. DMEM high glucose is a further modification of original DMEM and contains 4500 mgs glucose per liter. The various other cell lines including primary cultures of mouse & chicken cells as well as various normal & transformed cell lines.

Basic Components in Media

1. Energy sources—Glucose, fructose, amino acids
2. Nitrogen sources—amino acids
3. Vitamins: mainly water-soluble vitamins—B and C
4. Fat and fat-soluble components: fatty acids, cholesterols
5. Inorganic salts: Na^+, K^+, Ca^{2+}, Mg^{2+}
6. Nucleic acid precursors
7. Antibiotics
8. pH and buffering systems - sodium bicarbonate buffer system (3.7 g/L)
9. Oxygen
10. Hormones and growth factors

Sera in Animal Cell Media

Sera is the most important and most problematic component in animal cell media. During more than 3 decays, sera have been an essential medium component with the following functions:

Preperation of Media for animal Cell Culture

1. Provides nutrients.
2. Provides proteins that solubilize essential nutrients that do not dissolve readily.
3. Binds essential nutrients that are toxic when present in excessive amounts and releasing then slowly in a controlled manner.
4. Provides hormones and growth factors.
5. Modulates the physical and chemical properties of the medium (viscosity, rate of diffusion)—protect cells in agitated culture.
6. Has a pH-buffering function.

Dulbecco modified eagle medium (DMEM) :

Procedure

1. Suspend 13.3gms of DMEM media in 900ml tissue culture grade water with constant, gentle stirring until the powder is completely dissolved. Do not heat the water.
2. Add 3.7gms of sodium bicarbonate powder for 1litre of medium and stir until dissolved.
3. Adjust the pH to 0.2-0.3 pH units below the desired pH using 1N HCl or 1N NaOH since the pH tends to rise during filtration.
4. Make up the final volume to 1000ml with tissue culture grade water.
5. Sterilize the medium immediately by filtering through a sterile membrane filter with a porosity of 0.22 micron or less.
6. Aseptically add sterile supplements as required and dispense the desired amount of sterile medium into sterile containers.
7. Store liquid medium at 2-8°C and in dark till use.

Preperation of Media for animal Cell Culture

Preperation of Media for animal Cell Culture

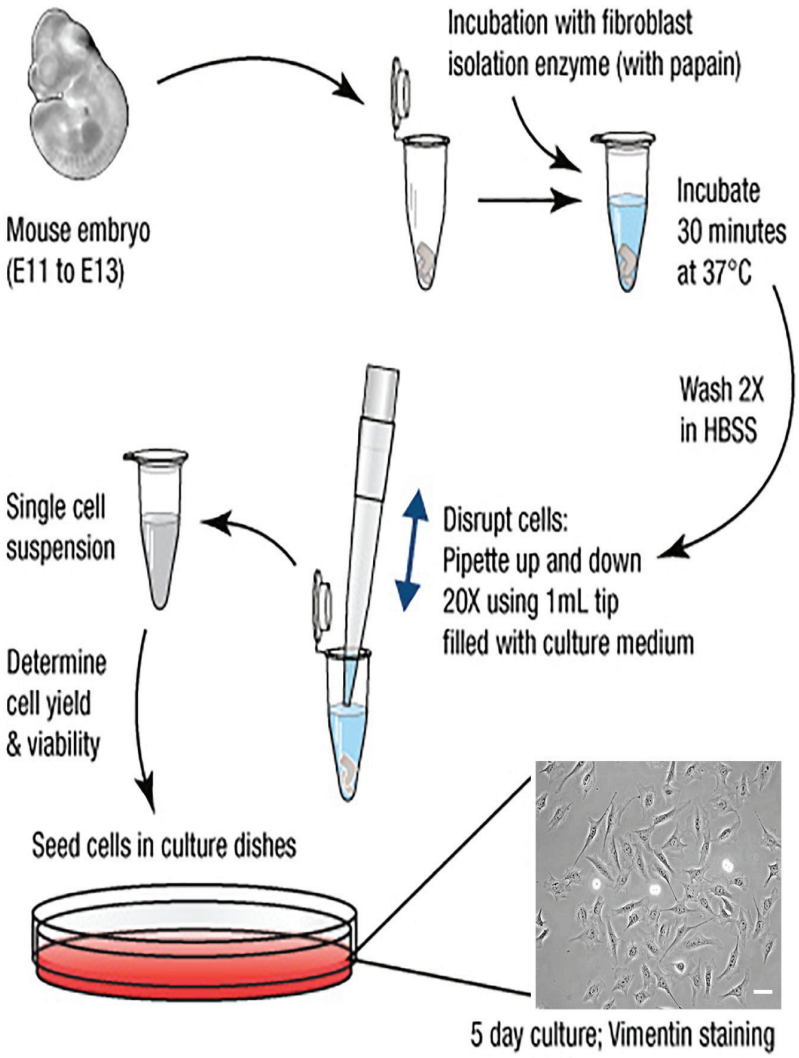
RPMI Media:

Roswell Park Memorial Institute (RPMI) media are a series of media developed by Moore et al for the culture of human normal and neoplastic cells in vitro. RPMI 1640 is the most commonly used medium in the series. A modification of McCoy's 5A medium, the medium was specifically designed to support the growth of human lymphoblastoid cells in suspension culture. Presently the medium is extensively used for a wide range of anchorage dependant cell lines. The medium needs to be supplemented with 5-20% fetal bovine serum. The medium is also known to support growth of cells in the absence of serum.

Procedure:

1. Suspend 8.4gms in 900 ml tissue culture grade water with constant, gentle stirring until the powder is completely dissolved. Do not heat the water.
2. It may be necessary to lower the pH to 4.0 with 1N HCl to completely dissolve this product. After it has dissolved completely, the pH can be raised to 7.2 with 1N NaOH prior to the addition of sodium bicarbonate.
3. Add 2.0gms of sodium bicarbonate powder or 26.7ml of 7.5% Sodium bicarbonate solution for 1 liter of medium and stir until dissolved.
4. Adjust the pH to 0.2 - 0.3 pH units below the desired pH using 1N HCl or 1N NaOH since the pH tends to rise during filtration.
5. Make up the final volume to 1000ml with tissue culture grade water.
6. Sterilize the medium immediately by filtering through a sterile membrane filter with a porosity of 0.22 micron or less, using positive pressure rather than vacuum to minimize the loss of carbon dioxide.
7. Aseptically add sterile supplements as required and dispense the desired amount of sterile medium into sterile containers.
8. Store liquid medium at 2-8°C and in dark till use.

Primary Cell Culture



Primary Cell Culture

Introduction to animal cell culture

Animal cell cultures were successfully undertaken by Ross Harrison in 1907. Several developments occurred which made cell cultures a widely available tool. First there was the development of antibiotics that made it easier to avoid contamination problems. Second, was the development of techniques such as the use of Trypsin to remove cells from culture vessels. Third, development of standardized chemically defined culture media that made cells grows easier.

When the cells are surgically removed from an organism and placed into a suitable culture environment, they will attach, divide and grow. This is called primary cultures. When the cells in primary culture vessel have grown, and filled up all the available culture, substrate they must be sub cultured to give them space for continuous growth. This is usually done by enzyme treatment or by gently scrapping the cells off the bottom of the culture vessels.

Establishment of chick embryo fibroblast primary cultures:

Embryo is very good source for processing primary culture. A primary culture is a heterogeneous culture having definite life span and limited population doublings.

Tissue explant: The removal of tissue from the organ refers to as "Tissue explant".

Aim: To initiate the primary culture of fibroblast from 9 th day old chick embryo.

Principle: Embryo from an embryonated egg is a potent source of fibroblastic cells. The embryo is carefully isolated mechanically and enzymatically disaggregated. Trypsin is the most commonly used proteolytic enzyme to disaggregate the tissue. The action of trypsin is neutralized by the addition of media, containing serum which has anti-trypsin. The cells are then pelleted and seeded to the flask at $1x10^{-6}$ cells /ml per flask and incubated at 37°C, 5% CO_2 for the formation of confluent monolayer. This gives the primary culture. The primary culture of fibroblastic cell has typical spindle shape morphology. When viewed

under a inverted microscope.

Materials required:

Embryonated chick eggs (9days old),
70% ethanol,
sterile scissors,
bent scissors,
forceps,
sterile petriplates,
pipettes,
sterile 1XPBS (Phosphate Buffer saline),
culture flask,
funnel with muslin cloth,
centrifuge inverted microscope,
canted neck flask.

Media: RPMI 1640, Penicillin-Streptomycin, trypsin

Procedure:

1. Examine 9th day embryonated egg under a bright light source for its viability (the live embryo can be seen as a shadow).

2. Place the egg in a beaker with the blunt end up and swab with 70% ethanol.

3. Puncture the top of the egg gently with a pair of sterile forceps and remove the shell thus exposing the underlining, chorioallantoic membrane (care should be taken that the shell should not fall into the egg).

4. Cut chorioallantoic membrane with a pair of sterile scissors such that the embryo gets exposed.

5. Remove the embryo gently by the neck using a sterile forceps and transfer to a petridish containing 1X PBS and wash twice or thrice to remove the yolk or blood.

6. Using forceps, remove the head, lymph and viscera. Transfer the remaining

tissues to another petridish. With 1X PBS, mix the embryo thoroughly using a pair of scissors and allow pieces to settle.

7. Add 8ml of 1:125 trypsin versene phosphate (TVP) to the minced tissue and incubate at 37°C for 15 minutes.

8. Neutralize the trypsin by adding an equal volume of RPMI1640 medium with serum.

9. Filter the cells through a sterile muslin cloth (Prewetted with 1X PBS) to remove the debris and centrifuge the cell suspension at 1000rpm for 20 minutes.

10. Resuspend the pellet in fresh RPMI 1640 medium with serum and take 0.1ml to a haemocytometer and count for viability with Trypan Blue dye.

11. Then take the cells in canted neck flask with $1X10^{-6}$ cells/ml flask and incubate at 37°C in a CO_2 incubator.

12. Observe the following day under an inverted microscope.

Cell Counting and Viability

Aim: To ensure the population of cells required for the culture works by cell counting method and its viability by vital staining methods

Introduction

Haemocytometer (also known as hemocytometer) is a glass slide with two counting chambers etched in a surface area of 9mm square. Each chamber is divided into nine 1.0mm square. It has raised sides which keep the cover slip 0.1mm above the chamber floor so that the total volume of each square becomes 0.0001ml(1.0mm x 0.1mm or 0.1mm^2 or 10^2 cm^3, L x W x H).

Principle

Staining of cells identifies viable cells. Stains generally used are Trypan Blue, Erythrosin B and Nigrosin. Nuclei of damaged or dead cells take up the stain whereas the viable cells do not do so.

Requirements

Cell suspension, Spirit lamp, Hemocytometer, Microscope, Micropipette PBS, Tryphan Blue 0.4%

Methodology

1. Take the Hemocytometer and place it on the flat surface of the work bench. Place the cover slip on the counting chamber.

2. Mix 20µl cells that have been well mixed prior to sampling with an equal volume of tryphan blue.

3. Apply to a hemocytometer by pipetting from the edge of the cover slip and permitting diffusion by capillary action.

4. Make sure that there is no air bubble and there is no overfilling beyond the ruled area.

5. Leave the counting chamber on the bench for 2-3 minutes to allow the cells to settle.

6. Place the counting chamber on the stage of the microscope between the clips to the hold slide so that the counting chamber can be moved (if the microscope is provided with a moving stage).

7. Switch to low power (10x) objective, adjust the light (less light needed,

Cell Counting and Viability

hence close the aperture or lower the condenser) and focus on the wall of the counting chamber.

8. Then slowly move the stage towards the middle of the slide until the ruling area visible, sharpen the focus and locate the large square in the centre.

9. Locate the large square in the centre with 25 small squares. Place in the middle of the field of vision and examine the distribution of viable cells on the entire area. It must be uniform orelse refill the chamber with cell suspension.

10. Carefully switch to high power objective (40 x) and move the chamber so that the smaller upper left corner square (with 16 smaller squares) is completely in the field of vision.

11. Count the number of unstained cells seen on the small square (0.2x0.2=0.04sq mm) of the upper left corner which is divided into 16 smaller squares to facilitate counting.

12. Repeat the counting with three other corner squares.

13. Make a total of all the cells counted in 4 squares. Repeat the same on the other side of the chamber and make an average of the two chambers.

Result

The concentration of cells in the original suspension in cells/ml =

$$\frac{\text{No. of cells counted}}{\text{No. of Grids counted}} \times 10^4 \times 2$$

----------/ml

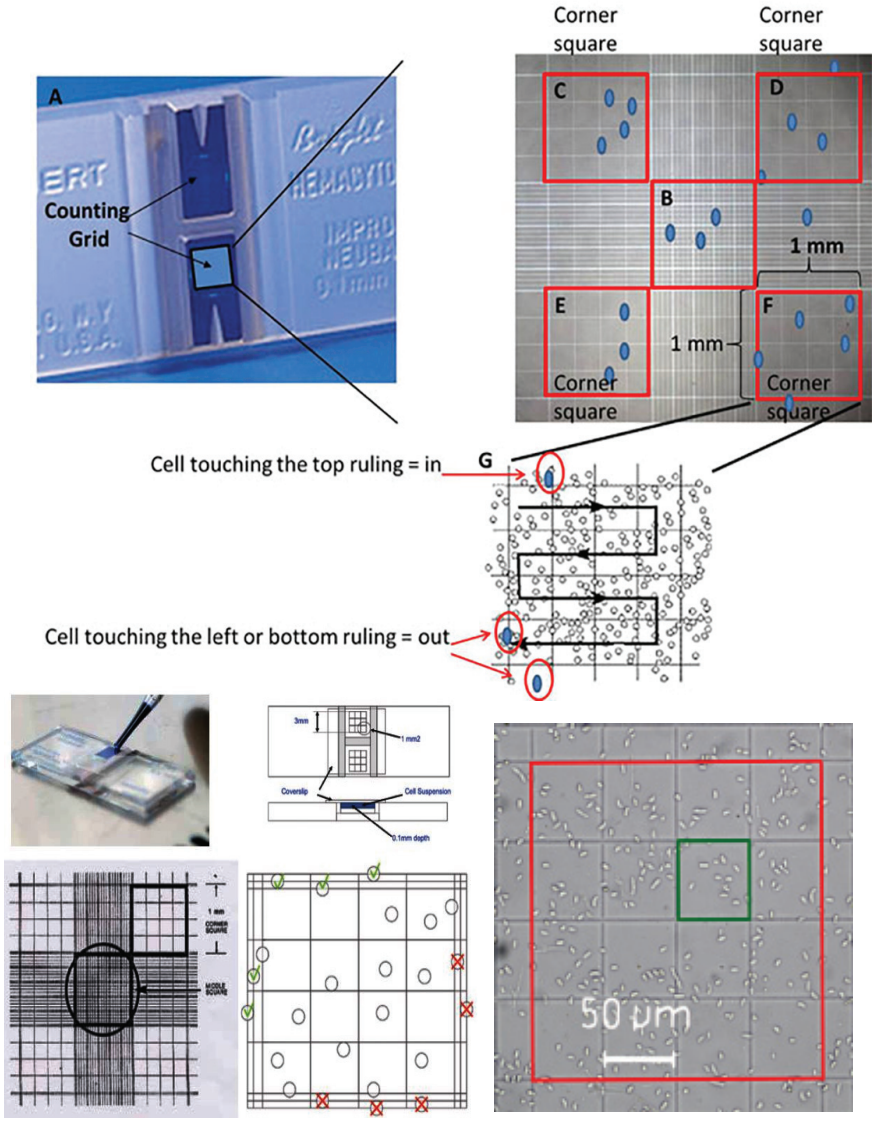
1) Starting with chamber 1 of the hemocytometer, count all the cells in the 1 mm center square and four 1 mm corner squares . Non- viable cells will stain blue. Keep a separate count of viable and non-viable cells.

NOTE: Count cells on top and left touching middle line of the perimeter of each square. Do not count cells touching the middle line at bottom and right sides (see Diagram II).

2) Repeat this procedure for chamber 2.

NOTE: If greater than 10% of the cells appear clustered, repeat entire procedure making sure the cells are dispersed by vigorous pipetting in the original cell suspension as well as the Trypan Blue- cell suspension mixture. If less than

Cell Counting and Viability



Cell Counting and Viability

200 or greater than 500 cells (i.e., 20-50 cells/square) are observed in the 10 squares, repeat the procedure adjusting to an appropriate dilution factor.

3) Withdraw a second sample and repeat count procedure to ensure accuracy.

4) Cell Counts

Each square of the hemocytometer, with cover-slip in place, represents a total volume of 0.1 mm^3 or 10^{-4} cm^3. Since 1 cm^3 is equivalent to approximately 1 ml, the subsequent cell concentration per ml (and the total number of cells) will be determined using the following calculations:

CELLS PER ml =

the average count per square x dilution factor x 10^4 (count 10 squares)

Ex: If the average count per square is 45 cells x 5 x

10^4 = 2.25 x 106 cells/ml.

TOTAL CELLS =

cells per ml x the original volume of fluid from which cell sample was removed.

Ex: 2.25 x 10^6 (cells/ml) x 10 ml (original volume) =

2.25 x 10^7 total cells.

5) CELL VIABILITY (%) =

total viable cells (unstained) ק total cells (stained and unstained) x 100.

Ex: If the average count per square of unstained (viable) cells is 37.5,

the total viable cells =

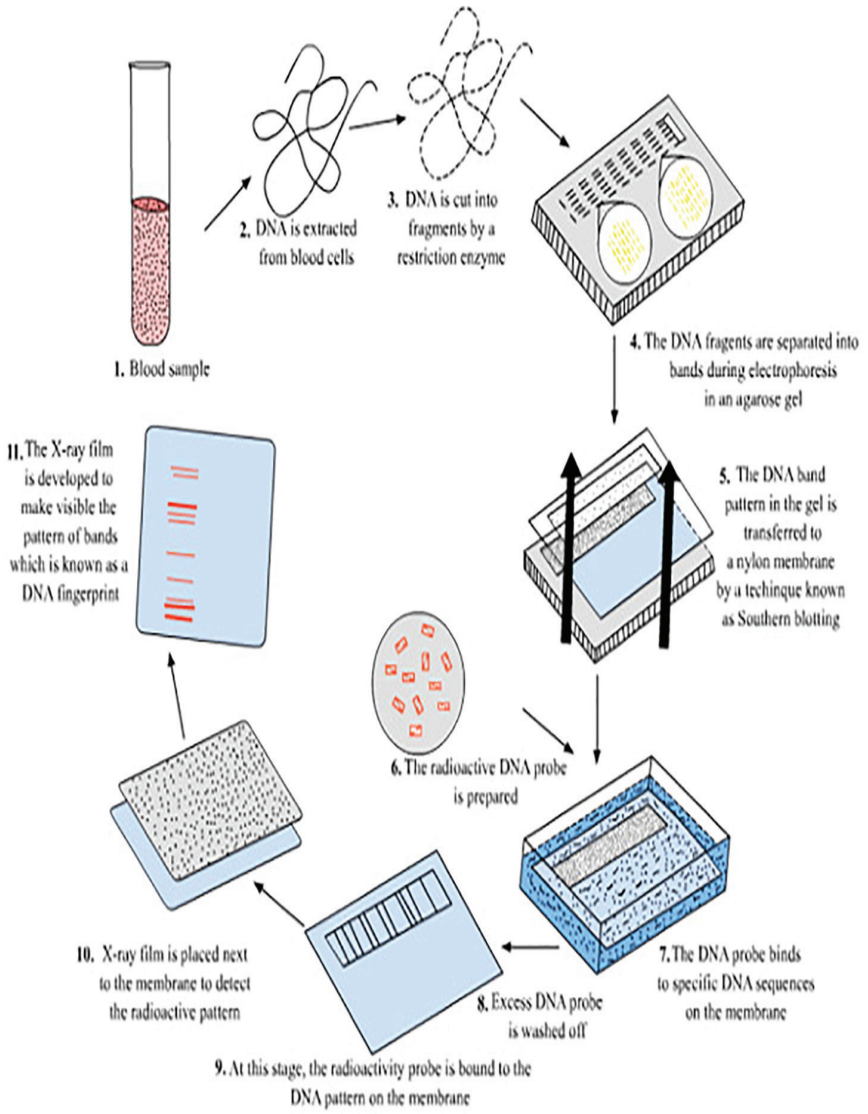
[37.5 x 5 x 104] viable cells/ml x 10 ml (original volume) = 1.875 x 10^7 viable cells.

Cell viability (%) =

1.875 x 10^7 (viable cells) ק 2.25 x 107 (total cells) x 100 =

83% viability.

DNA fingerprinting



DNA fingerprinting

DNA fingerprinting involves a number of intensive and important steps in order to fully complete and develop and DNA fingerprint of a father, a suspect or a person involved in an immigration problem.

1. The process of DNA fingerprinting starts with isolating DNA from any part of the body such as blood, semen, vaginal fluids, hair roots, teeth, bones, etc.

2. Polymerase chain reaction (PCR) is the next step in the process. In many situations, there is only a small amount of DNA available for DNA fingerprinting. Because of this, in a test tube, DNA replication is must occur to make more DNA. The DNA and the cells will undergo DNA replication in order to make more DNA to be tested.

3. After the DNA is isolated and more copies of the DNA have been made, the DNA will be tested. The scientist will treat DNA with restriction enzymes (an enzymes that cuts DNA near specific recognition nucleotide sequences known as restriction sites).

This will produce different sized fragments which are known as restriction fragment length polymorphisms (RFLPs).

These fragments can then be observed doing an experiment called gel electrophoresis which separates DNA based on fragment sizes.

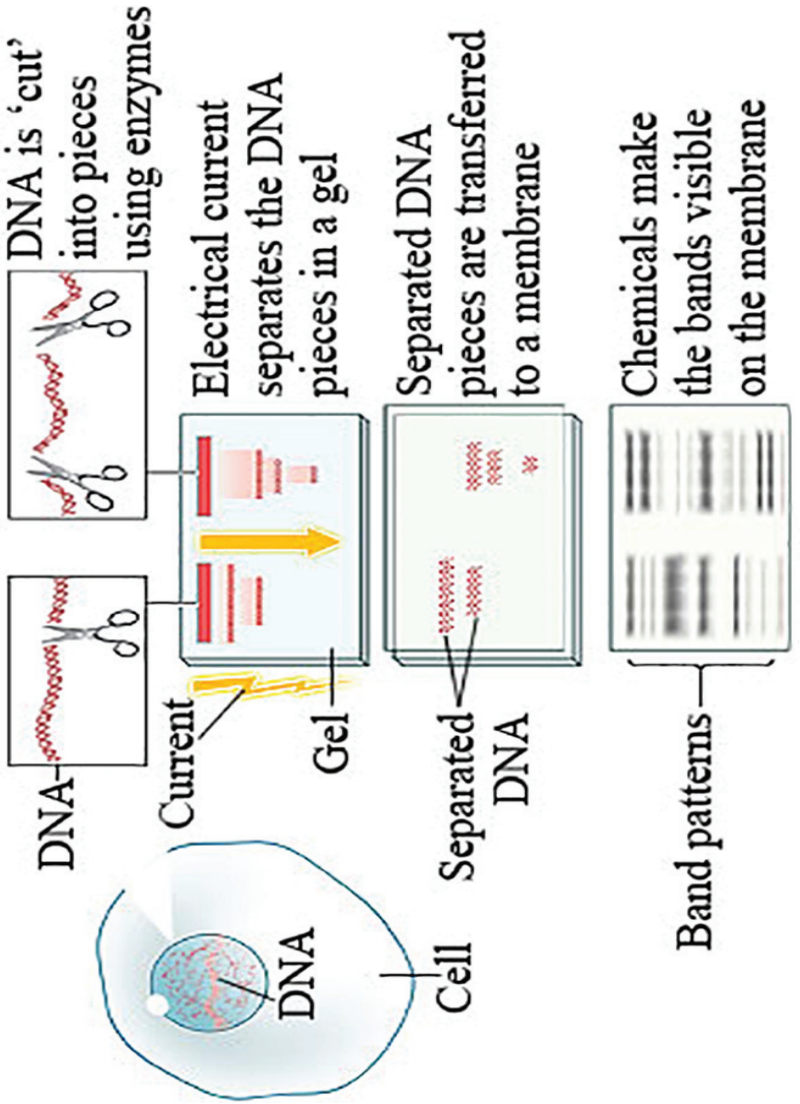
4. Gel electrophoresis is the next step in this process of DNA fingerprinting. During gel electrophoresis, an electrical current is applied to a gel mixture, which includes the samples of the DNA.

The electric current causes the DNA strands to move through the gel. This separates the molecules of different sizes.

The fragments of separated DNA are sieved out of the gel using a nylon membrane (treated with chemicals that allow for it to break the hydrogen bonds of DNA so there are sing strands).

5. The DNA (single stranded) is cross-linked against the nylon using heat or a UV light.

DNA fingerprinting



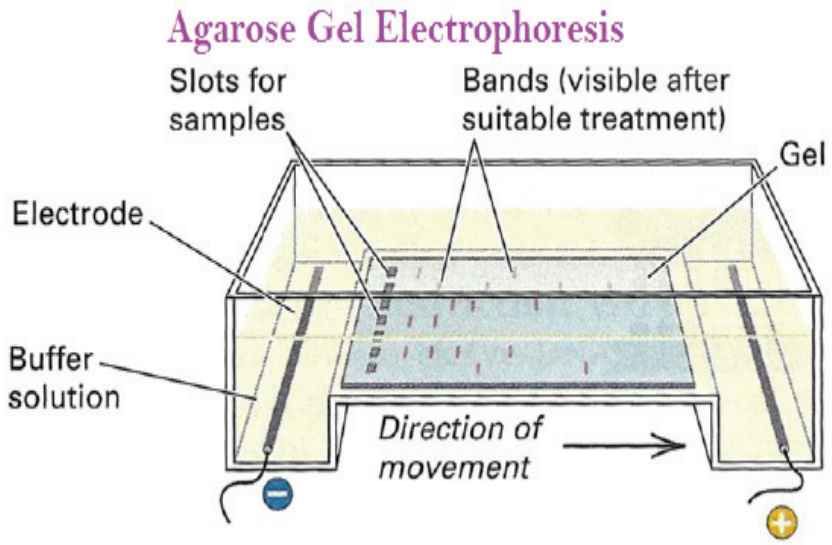
DNA fingerprinting

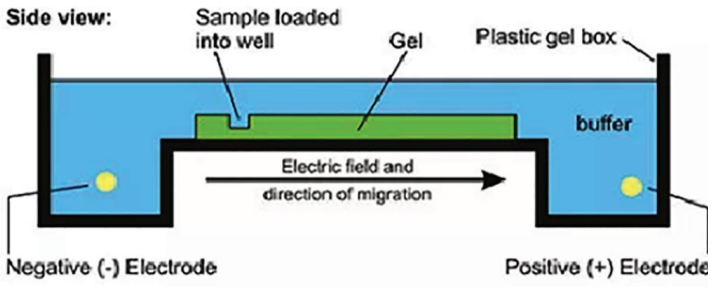
6. The probe shows up on photographic film because the strands of DNA decay and give off light. In the end it leaves dark spots on the film which are also known as the DNA bands of a person. What make up the fingerprint are the unique patterns of bands. The pattern of bands are different because we are all different and unique (other than identical twins).

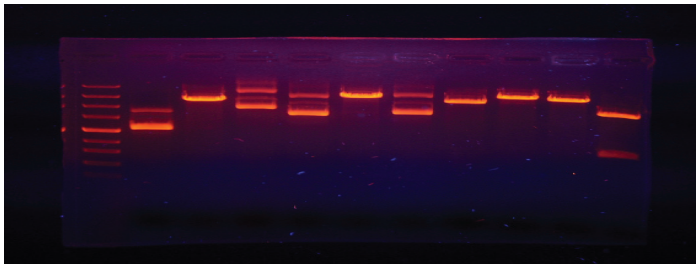
7. Once the filter is exposed to the x-ray film, the radioactive DNA sequences are shown and can be seen with the naked eye. This creates a banding pattern or what we know as DNA fingerprints. This technique is called southern blotting.

These fingerprints can be used to determine which hair strand belongs to which person for example.

DNA fingerprints of children should be similar to the their parents' fingerprints, although they may not be the same. Some bands will match one parent and other bands can match the other parent. With the bands of both of those parents, they make the bands and the identity of the child.







Aim: To separate DNA by Agarose gel electrophoresis

Principle: Electrophoresis is a technique used to separate charged molecules. DNA is negatively charged at neutral pH and when electric field is applied across the gel. DNA migrates towards the anode.

Migration of DNA through the gel is dependent upon:

1. Molecular size of DNA
2. Agarose concentration
3. Confirmation of DNA
4. Applied current

Matrix of agarose gel acts as a molecular sieve through which DNA fragments moov on application of electric current. Higher concentration of agarose gives firmer gels.

Spaces between cross linked molecule are less and hence smaller DNA fragments easily crawl through these spaces. As the length of the DNA increases, it becomes harder for the DNA to pass through spaces, while lower concentration of agarose helps in movements of larger DNA fragments as the space between the cross linked molecules is more. The progress of gel electrophoresis is monitored by observing the migration of a visible dye through the gel.

Procedure:

Preparation of 1% Agarose Gel

1. Prepare 1X TAE by biluting appropriate amount of 50X TAE buffer. (for one experiment. Approximately 200 ml of 1X TAE is required. Make up 4 ml of 1X TAE to 200 ml with distilled water.
2. Weigh 0.5 g of agarose and add to 50 ml of 1X TAE this gives 1% agarose gel.
3. Boil till agarose dissolves completely and a clear solution results.
4. Meanwhile place the combs of electrophoresis set such that it is approximately 2cm away from the cathode.
5. Add 2µl ethidium bromide to molten agarose , when temperature is around 50^0 C. mix and cast the gel.

Agarose Gel Electrophoresis

As shown in the Illustration -DNA bands are seen separated on a gel. The length of the DNA fragments is compared to a marker containing fragments of known length.

Sample A: contains one DNA fragment of 1000 base pairs length

Sample B Contains two DNA fragments of approximately 800bp and200bp

Sample C contains three DNA fragments of approximately 600bp, 300bp and 100bp.

Agarose Gel Electrophoresis

6. Pour the agarose solution in the central part of tank when the temperature reaches approximately 60ᵻ% C. do not generate air bubble. The thickness of the gel should be around 0.5 to 0.9 cm. keep the gel undisturbed at room temperature for the agarose to solidify.
7. Pour 1X TAE buffer into the gel tank till the buffer level stands at 0.5 to 0.8 cm. above the gel surface.
8. Gently lift the combs, ensuring that that wells remain intact.
9. Connect the power cord to the electrophoretic power supply according to the to the convention (red: anode, black: cathode.)
10. Load the sample in the wells in the desired order.
11. Set the voltage to 50Vand switch on the power supply.
12. Switch off the power when the tracking dye form the well reaches ¾ th of the gel. This takes approximately one hour.
13. After electrophoresis, DNA sample can be visualized under UV light. They appear fluorescent. No distaining is required in this case.

Result and Analysis

After electrophoresis DNA bands can be visualized under UV light and they appeared as orange fluorescence.

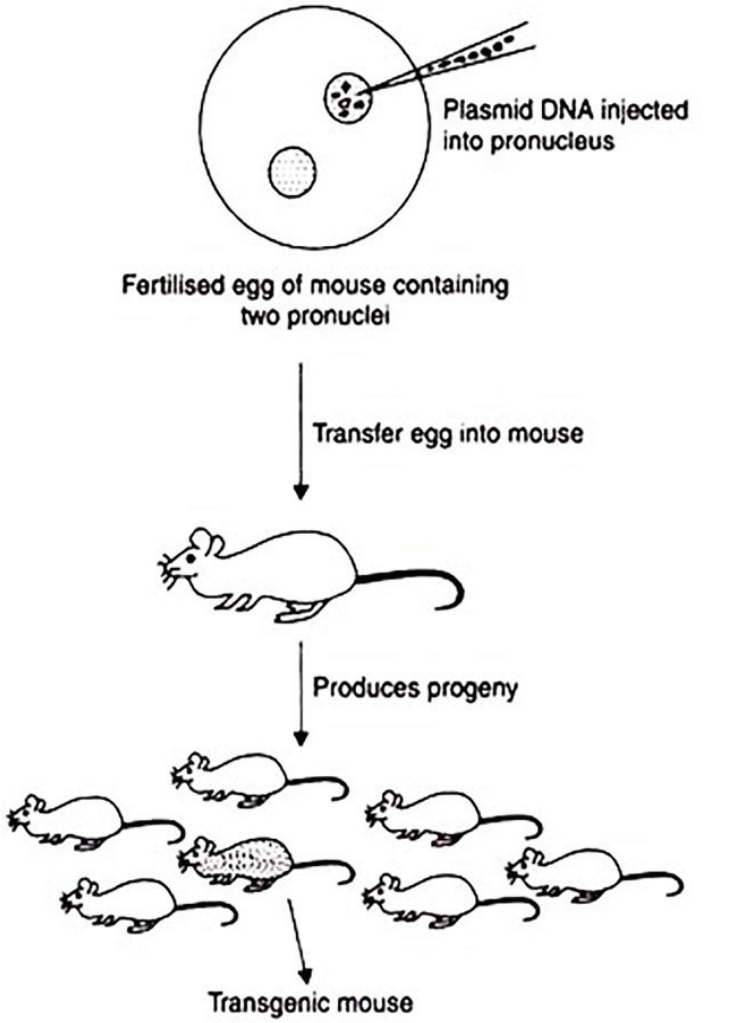
Visualising the results

To visualise the DNA, the gel is stained with a fluorescent dye that binds to the DNA, and is placed on an ultraviolet transilluminator which will show up the stained DNA as bright bands.

If the gel has run correctly the banding pattern of the DNA marker/size standard will be visible.

It is then possible to judge the size of the DNA in your sample by imagining a horizontal line running across from the bands of the DNA marker. You can then estimate the size of the DNA in the sample by matching them against the closest band in the marker.

Transgenic animals - Mouse



Production of transgenic mice (explanation in text).

Transgenic animals - Mouse

Transgenic Animals

Transgenesis is the process of introducing an exogenous gene – called transgene- into a living organism so that the organism will exhibit a new property and transmit that property to its offspring. A transgene is the name given to the introduced DNA. The term transgenesis was coined by Gordon and Ruddle in 1981.

Animals produced through transgenesis are called Transgenic animals. (GM organisms) Transgenic animals are genetically modified organisms with a new hereditary character. Transgenic animals are produced by 3 different methods 1. DNA microinjection, 2. Retrovirus-mediated gene transfer 3. Embryonic stem cell-mediated gene transfer

In 1974 Beatrice Mintz and Rudolf Jaenisch created the first genetically modified animal by inserting a DNA virus into an early-stage mouse embryo

Transgenic Mouse

1 Mice that have been genetically engineered to carry such foreign genes in their chromosomes are called transgenic mice. RD Palmiter and RL Brinter (1982) isolated gene for growth hormone in human being. This gene was ligated with plasmid pBR322 to produce rDNA. It was transferred to the zygote of a mouse *invitro*. The embryo was implanted in the uterus of a foster mouse. Then the new born mouse was found to be transgenic which contained a gene from humans. Such mice grew some 2-3 times faster than the control mice and were twice the size of the controls. It is referred as *super mouse*.

2. To generate transgenic mice, cloned DNA is microinjected into a pro-nucleus of the fertilized egg. The eggs are then transferred to foster mothers and allowed to develop to term.

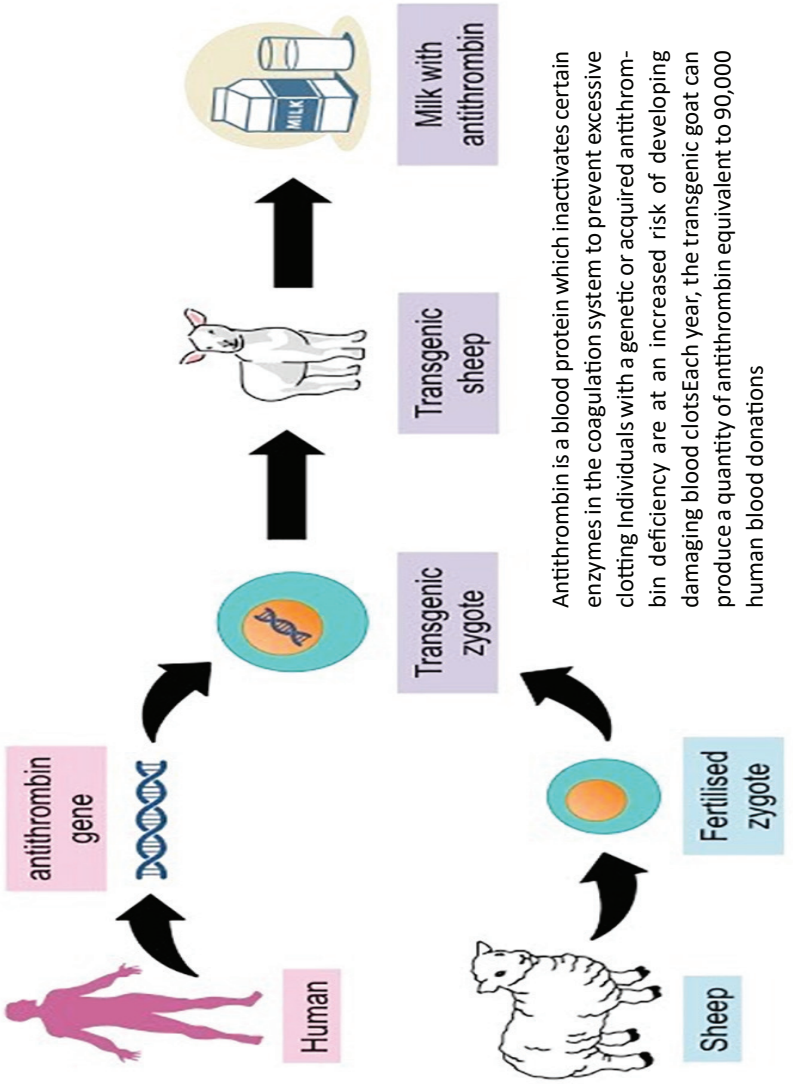
3. Among the progeny mice, about 10% will have the foreign DNA integrated into the genome of the fertilized egg, and therefore, in all cells of the adult mice.

4. Since the foreign DNA is also present in the germ line cells of the progeny mice, these mice are mated to breed new progeny mice which would inherit the foreign DNA.

5. Genetically modified mice are used extensively in research as models of human disease.Mice are a useful model for genetic manipulation and research, as their tissues and organs are similar to that of a human and they carry virtually all the same genes that operate in humans.

6. They have been used to study and model obesity, heart disease, diabetes, arthritis, substance abuse, anxiety, aging and Parkinson disease.[11]Transgenic mice generated to carry cloned oncogenes and knockout mice lacking tumor suppressing genes have provided good models for human cancer.

Transgenic goat



Transgenic goat

Transgenic goat/sheep

1. Transgenic animals were first developed in 1985 and were useful in the production of biopharmaceuticals shortly thereafter.

2. Goat is considered ideal transgenic animal that produces milk and has short generational time of 18 months.

3. The world's first cloned transgenic goats have been born as part of a research program conducted by LSU Agricultural Center and Genzyme Transgenic Corp.

4. Goats produce about 800 liters of milk per year. Using goats has been shown to dramatically increase the yield of the active protein by more than 10 times that from the cell culture model.

5. Transgenic goat is produced just like any other transgenic animal. In this process a desired gene containing DNA fragment of human being is isolated and introduced into one cell embryo.

6. The embryo is then transferred into a surrogate female animal, resulting in a transgenic offspring.

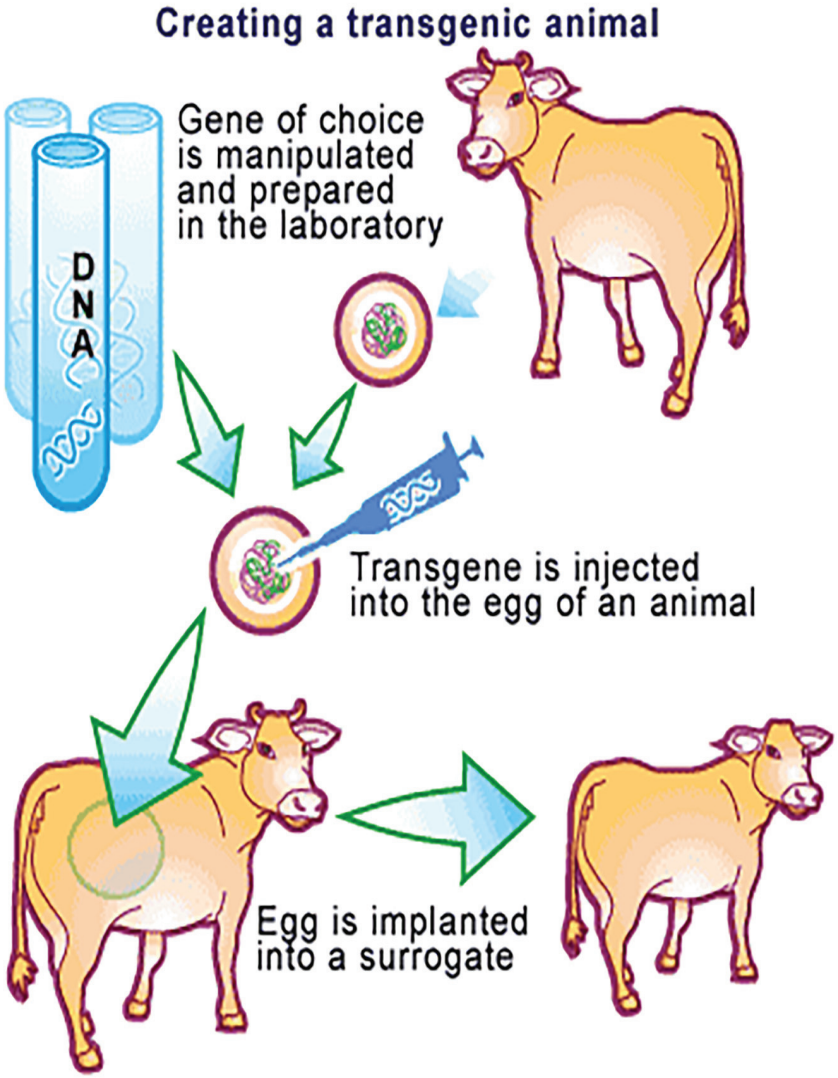
7. If, after maturing and being induced to produce milk, the animal expresses the desired protein in the milk in sufficient quantity and quality, it can be included in the producing pool of animals.

8. Transgenic goat: transgenic goats are used to produce, Human anti thrombin 3 against thrombosis, glutamic acid decarboxylase for Type I diabetes, Pro 542 to treat HIV infection etc. in their milk

9. Also extraction of spider silk like protein from transgenic goat's milk proves to be a mile stone.

10. Transgenic goats were designed to produce human breast milk which contains more bacteriocidal agents. For babies of mothers who aren't present, or can't nurse them, milk from these transgenic goats could provide the next-best alternative.

Transgenic cow



Transgenic cow

1. The first transgenic cow Rosie (in 1997) produced human protein-enriched milk. The milk contain the human alpha-lactalbuim, which is nutritionally more balanced than cow milk.

2. Transgenic cow is produced just like any other transgenic animal. In this process a desired gene containing DNA fragment of human being is isolated and introduced into one cell embryo.

3. Ag. Research's produced transgenic cows had extra bovine (cow) kappa casein genes inserted in their genome. This resulted in increased kappa casein in their milk as the transgenic

4. Casein is particularly important in cheese making. The milk from casein transgenic cows contained double the levels of kappa casein

5. This research proved to the scientists that transgenic technologies could be used to alter milk composition in cows. In the future, modified milk from transgenic cows could be used to:

6. Lactoferrin is a valuable iron-binding protein that plays a role in preventing bacterial infections. The human lactoferrin gene has been expressed in transgeneic cows.

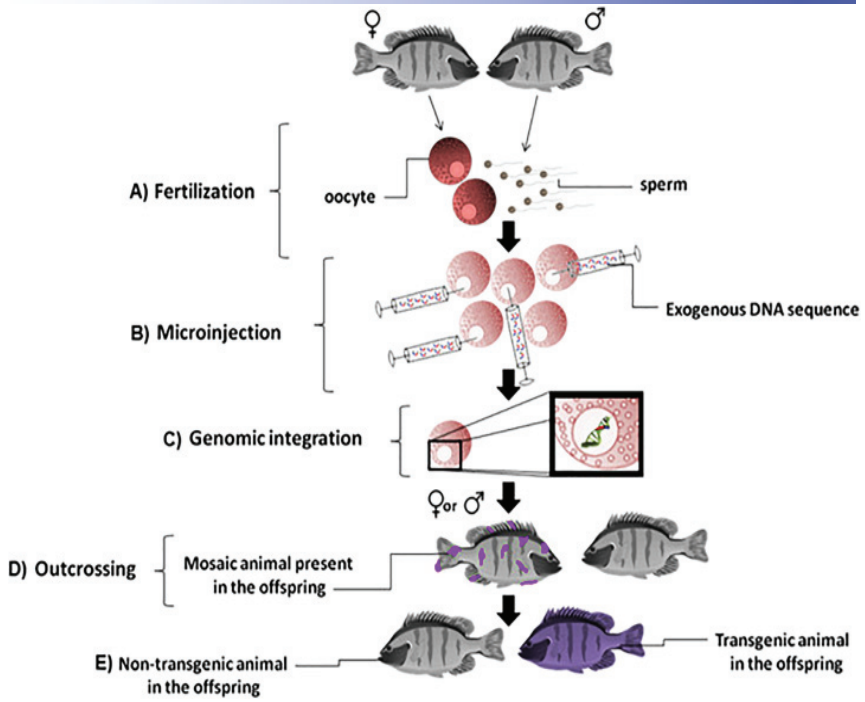
7. The cows were genetically engineered by biotech company SAB Biotherapeutics to produce human antibodies, proteins that fight pathogens. These antibodies could one day treat infectious diseases like Ebola, influenza, and Zika — and their potential to address global outbreaks

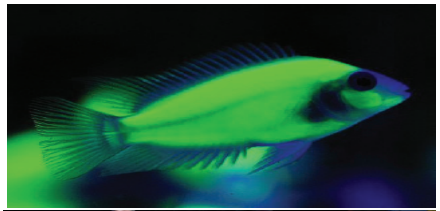
8. Transgenic cows exist that produce more milk or milk with less lactose or cholesterol.

9. Transgenic cows can be modiefied to produce human milk, improved diary products and other life saving medicines.

10. Prevent animal diseases, such as mastitis. Assist milk processing into dairy products.

Transgenic fish





Transgenic fish

Transgenic fish

1. A transgenic fish is one that contains genes from another species.

2. Transgenic fish is produced just like any other transgenic animal. In this process a desired gene containing DNA fragment of human being is isolated and introduced into one cell embryo.

3. Development of transgenic fish has focused on a few species including salmon, trout, carp, tilapia and a few others

4. GM fish are used for scientific research and as pets, and are being considered for use as food and as aquatic pollution sensors.

5. Two species of fish, zebrafish and medaka, are most commonly modified because they have optically clear, rapidly develop, and the 1-cell embryo is easy to see and microinject with transgenic DNA.

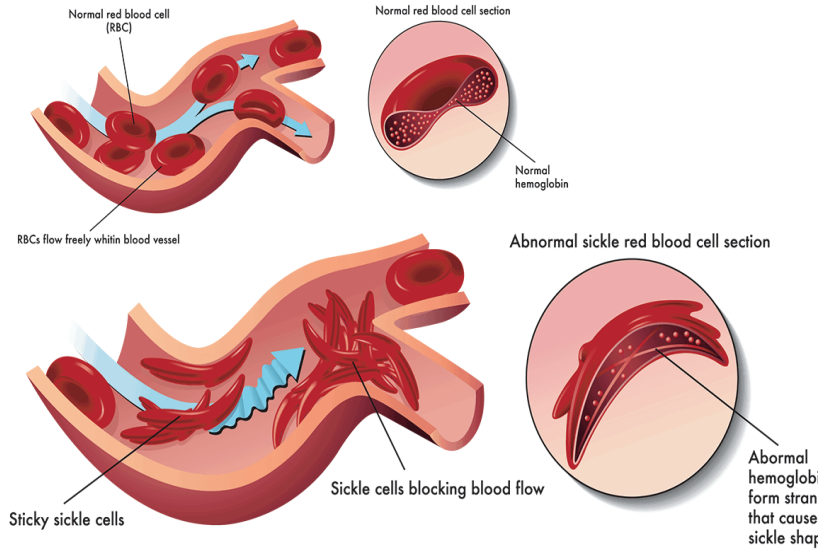
6. GM fish are used as pet animals. The GloFish is a patented brand of genetically modified (GM) fluorescent zebrafish with bright red, green, and orange fluorescent color. Although not originally developed for the ornamental fish trade, it became the first genetically modified animal to become publicly available as a pet when it was introduced for sale in 2003.

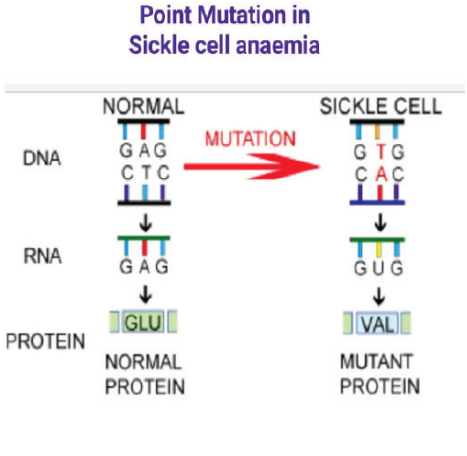
7. Genetically modified fish have been developed to give high production by using growth hormone gene. This has resulted in dramatic growth enhancement in several species, including salmon, trout and tilapia etc. Aqua Bounty, a biotechnology company working on bringing a GM salmon to market, which can weigh double and mature in half the time the non Gmsalmon.

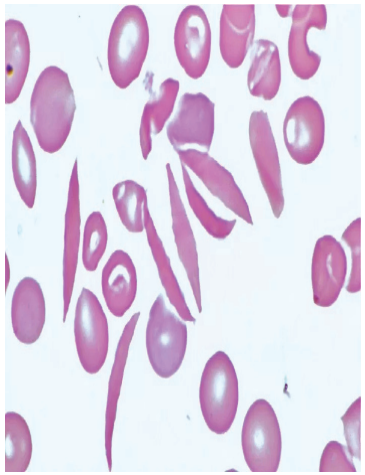
8. Several academic groups have been developing GM zebrafish to detect aquatic pollution. It will change its color in the presence of pollutants, to be used as environmental sensors

9. Transgenic common Carp: Thomas T. Chen, director of the Biotech¬nology Centre at the University of Connecticut, Storrs, transferred into common carp the growth hormone DNA from rainbow trout. The offspring of the first generation of transgenic fish grew 20 to 40% faster than their unmodified siblings.

Genetic disorders - Sickle cell anemia







Genetic disorders - Sickle cell anemia

Genetic disorders

A genetic disorder is caused by an altered gene or set of genes.They are devided as 1. Single gene or Mendelian disorders which are caused by change in the single gene. ex. Colour Blindness, Haemophilia, Sickle cell anaemia, Phenylke-tonuria, Cystic fibrosis, Thalasemia etc.

2. Chromosomal disorders are caused by change in the structure of a chromosome or in the number of chromosomes. ex. Down's syndrome, Klinefelter's syndrome, Turner's syndrome etc.

Sickle cell anemia

1. Sickle cell anaemia is an inherited blood disorder in which red blood cells develop abnormally.

2. Sickle cell anaemia is caused by a mutation in a gene called haemoglobin beta (HBB), located on chromosome 11. It is a recessive genetic disease, which means that both copies of the gene must contain the mutation for a person to have sickle cell anaemia. Heterozygous individuals are carriers.

3. The HBB gene codes for haemoglobin, a protein in red blood cells? that carries oxygen around the body . A mutation in HBB results in a change in one of the bases in the DNA sequence from an Adenine to a Thiamine. This then changes the amino acid in the haemoglobin protein from **glutamic acid to valine.** This causes the body to produce a new form of haemoglobin called HbS.

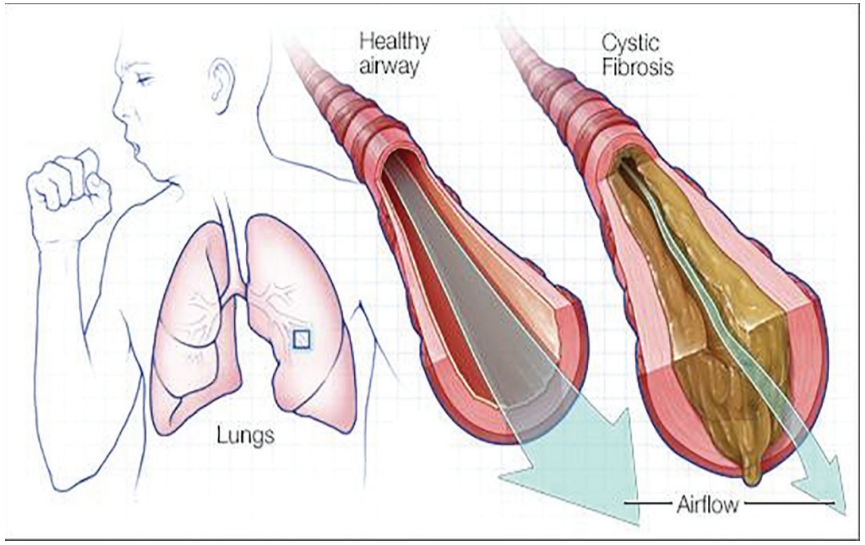
4. HbS causes the red blood cells to develop abnormally and become sickle-shaped. They become stuck in the blood vessels, causing blockages.

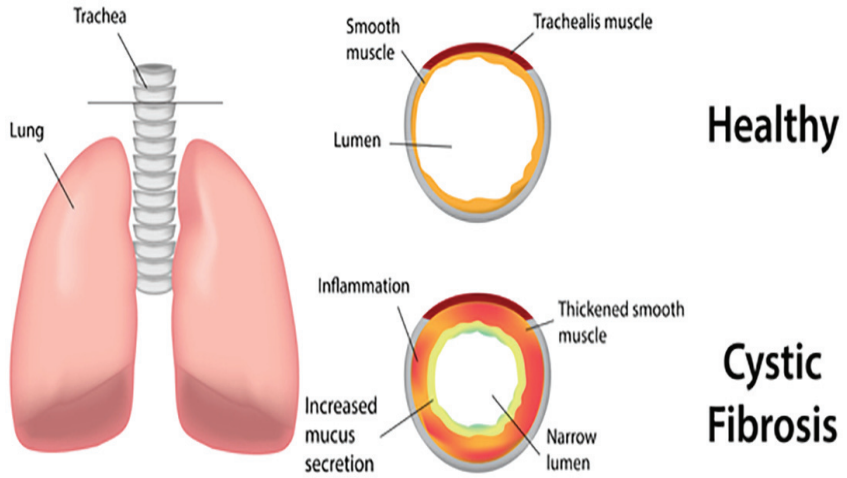
4. The symptoms of sickle cell anaemia vary considerably from person to person. Pain develops when sickle-shaped red blood cells block the flow of blood to the chest, abdomen and joints. Damage to liver, spleen, lungs, heart and finally brain stroke may come. High body temperature of 38°C or above with difficulty breathing and severe pain are common

5. Sickle cell anaemia is diagnosed using a blood test which detects the presence of the abnormal HbS haemoglobin in the red blood cells.

6. Regular blood transfusions can help reduce the risk of complications, such as stroke, by up to 90 per cent.

Cystic fibrosis





Cystic fibrosis

Cystic fibrosis

1. Cystic fibrosis is an inherited disease caused by mutations (changes) in a gene on chromosome 7, one of the 23 pairs of chromosomes that children inherit from their parents.

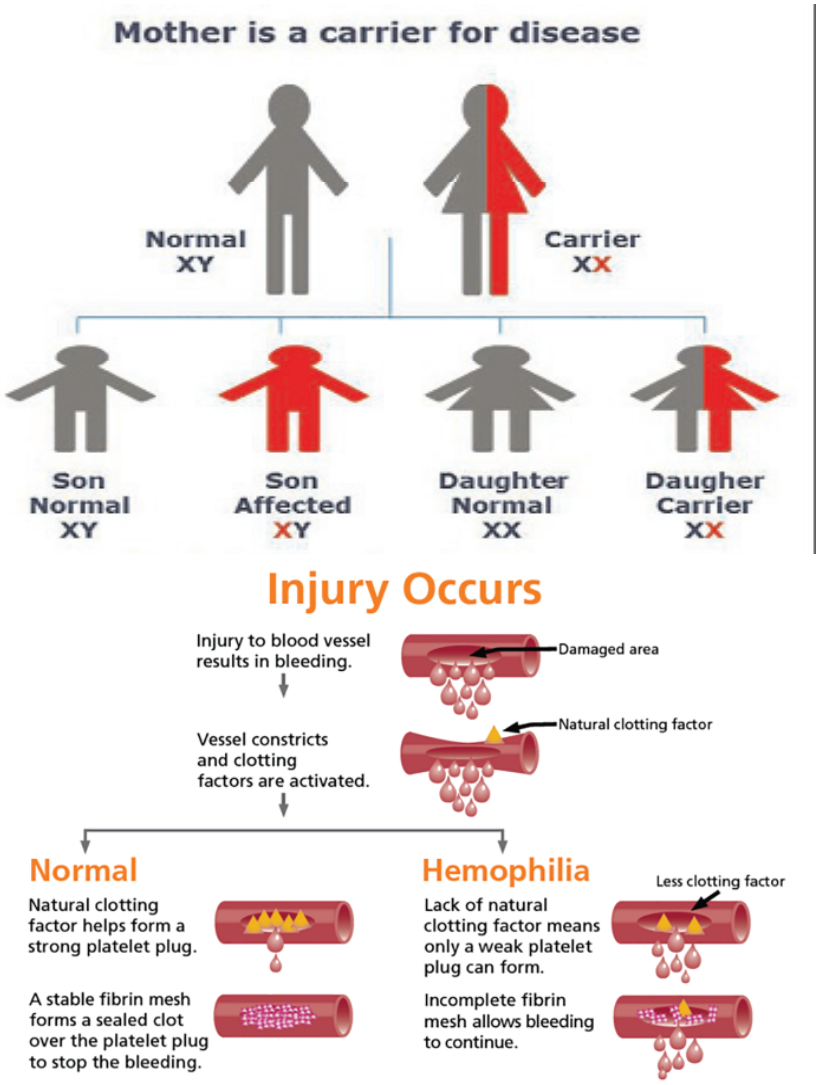
2. CF occurs because of mutations in the gene that makes a protein called CFTR (cystic fibrosis transmembrane regulator).

3. Cystic fibrosis (CF) is an inherited disease that causes the body to produce mucus that's extremely thick and sticky. The two organs most affected are the lungs and pancreas, where the thick mucus causes breathing and digestive problems.

4. CF can also affect the liver, the sweat glands, and the reproductive organs. Frequent lung infections or pneumonia, persistent wheezing, persistent cough with thick mucus, bulky, light-colored, foul-smelling bowel movements or diarrhea are some of the symptoms of cystic fibrosis

5. Cystic fibrosis has no cure. However, treatments have greatly improved in recent years. Treatment may include nutritional and respiratory therapies, medicines, exercise, and more.

Hemophilia



Hemophilia

Hemophilia

1.Hemophilia is a sex-linked recessive disease, which is transmitted from an unaffected carrier female to some of the male offsprings. The gene for haemophilia is located on X-chromosome.

2. It is a rare genetic disorder in which your blood doesn't clot normally because it lacks sufficient blood-clotting proteins (clotting factors) Due to this, patient continues bleeding even on a minor injury because of defective blood coagulation.

3. There are two types of haemophilia. Both have the same symptoms. Haemophilia A is the most common form and is due to having reduced levels of clotting factor VIII (8). Haemophilia B, also known as Christmas Disease, is caused by having reduced levels of clotting factor IX (9).

4. Small cuts usually aren't much of a problem. The greater health concern is deep bleeding inside your body, especially in your knees, ankles and elbows.

5. That internal bleeding can damage your organs and tissues, and may be life-threatening.

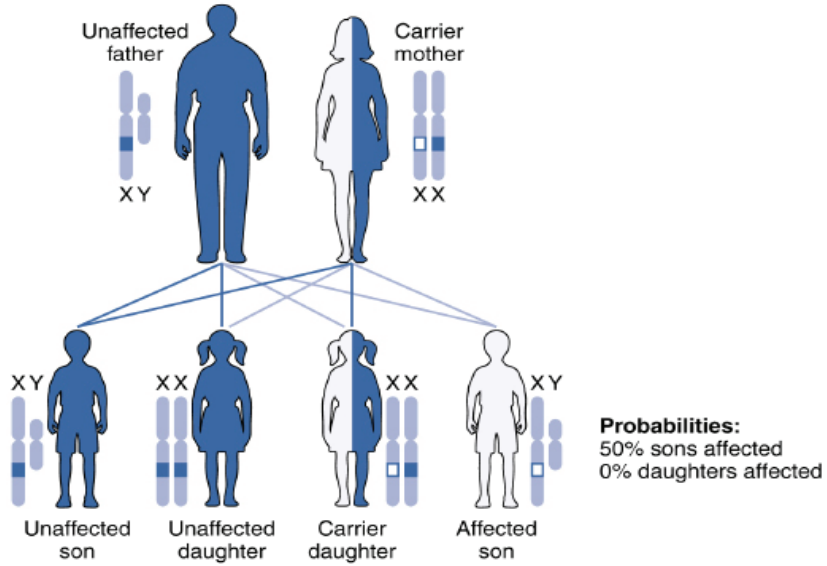
6. Symptoms include - unexplained and excessive bleeding from cuts or injuries, or after surgery or dental work, Unusual bleeding after vaccinations, Blood in urine or stool, Nosebleeding, Convulsions or seizures

7. There is no long-term cure. Treatment and prevention of bleeding episodes is done primarily by replacing the missing blood clotting factors usually produced through Recombinant DNA technology.

8. Haemophilia has featured prominently in European royalty and thus is sometimes known as 'the royal disease

9. In 2013, the U.S. Food and Drug Administration (FDA) approved Rixubis, laboratory produced blood factor IX - created with recombinant DNA technology, for patients with hemophilia B

Colour Blindness



Colour Blindness

1. It is a sex-linked recessive disorder, which results in defect in either red or green cone of eye. it leads to the failure in discrimination between red and green colour. The gene for colour blindness is present on X-chromosome.

2. Males are more likely to be color blind than females, as the genes responsible for \color blindness are on the X chromosome. As men have only one X chromosome he is susceptible to colour blindness more. A heterozygous female has normal vision, but is a carrier and passes on the disorder to some of her sons.

3. Colour blindness like any other inheritance show crisscross inheritance ie. from father to daughter to son.

4. Photoreceptors in the retina are called rods and cones.Cones are responsible for Colour vision. Threre are three types of cones responsible for sensing different colours.

5. Total colour blindness called Monochromacy is caused to the missing of all three Cone pigments. Red-green color blindness is the most common form, followed by blue-yellow color blindness and total color blindness.

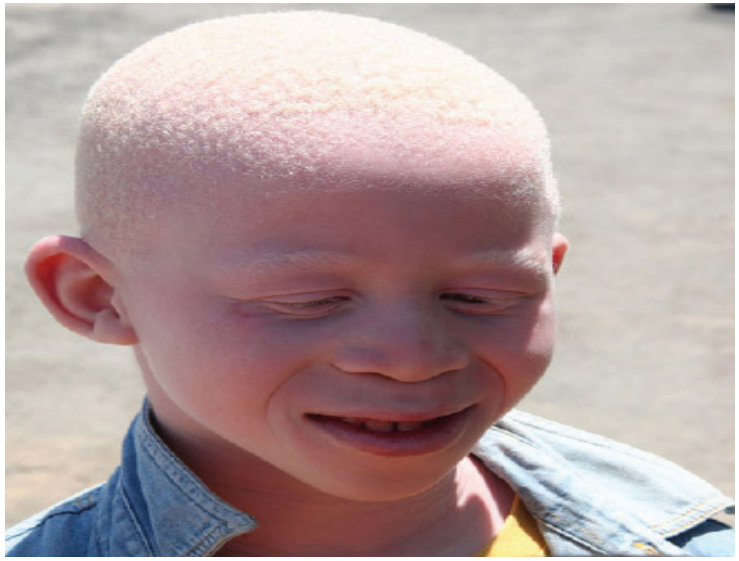
6. Absence of any one of the retinal cone pigments leads to colour blindnes. If red cone pigment is absent, that person will have difficulties in distinguishing between blue & green and red & green colours. If Blue cone receptors are absent, Blue colour appears greenish.

7. In some rare cases chromosome 7 also plays in causing colour blindness.

8. Colour blindness can be corrected by using special lenses to enhance color perception, which are colour filters available in either contact lens or eyeglass lens form.

Albinism

Oculo cutaneous albinism



Ocular albinism

Albinism

1. Albinism is a congenital disorder characterized in humans by the complete or partial absence of pigment melanin in the skin, hair and eyes.

2. Albinism is associated with a number of vision defects, such as photophobia (fear of light), nystagmus (involuntary movements of eye ball) and amblyopia (Cloudy vision)

3. Albinism results from inheritance of recessive gene alleles known as OCA (Oculocutaneous Albinism)

4. Albinism is caused due to absence or defect of tyrosinase, a copper-containing enzyme involved in the production of Melanin which gives pigmentation to the skin

5. Lack of skin pigmentation makes for more susceptibility to sunburn and skin cancers.

6.While an organism with complete absence of melanin is called an albino,

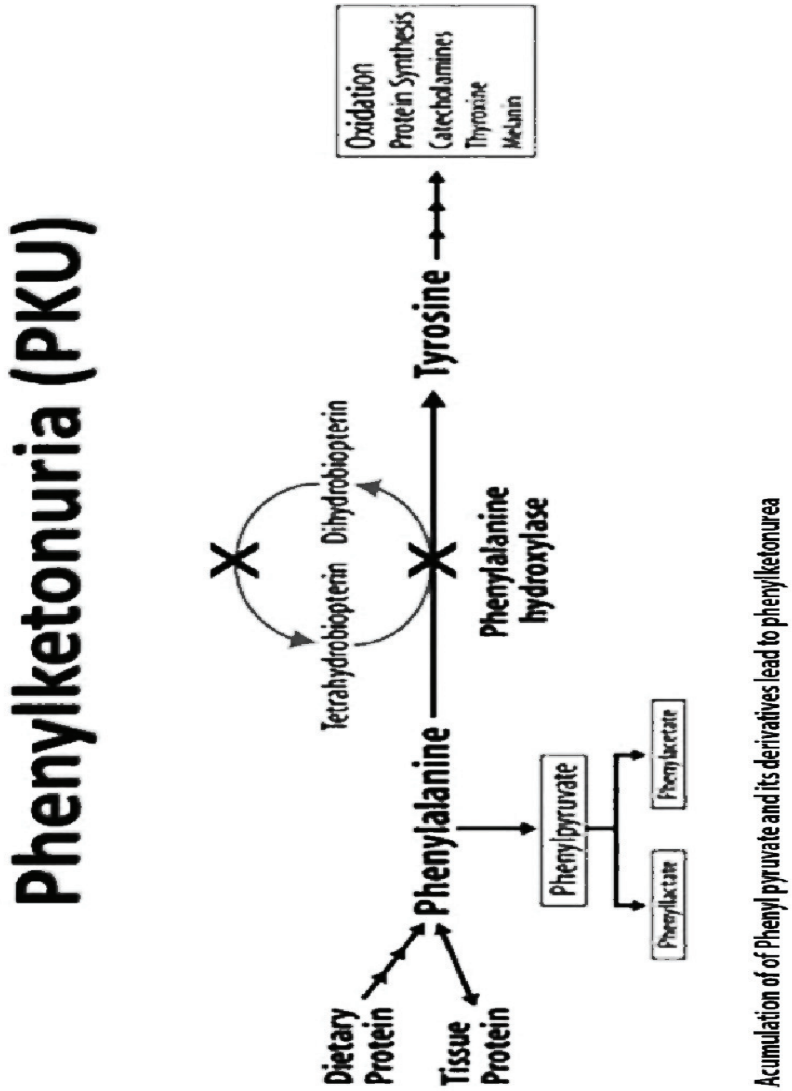
7. Ocular Albinism (only eyes are albino) causing gene is located on the X chromosome and it shows X linked inheritance.

8. The Oculocutaneous albinism (eyes and skin albinism) genes are found on chromosome number 4, 11 and 15.

9. There is no cure for albinism. Symptoms can be treated

10. Melanin normally protects the skin from UV (ultraviolet) damage, so people with albinism are more sensitive to sun exposure. There is an increased risk of skin cancer.

Phenylketonuria (PKU)



Phenylketonuria (PKU)

Phenylketonuria (PKU)

1. Phenylketonuria (PKU) is an inborn error of metabolism that results in decreased metabolism of the amino acid phenylalanine.

2. Babies with this disease have a missing enzyme called phenylalanine hydroxylase, which is needed to break down an essential amino acid phenylalanine into tyrosine in liver. This phenylalanine is accumulated and gets converted into phenyl pyruvic acid and other derivatives leading to PKU

3. Untreated, PKU can lead to intellectual disability, seizures, behavioral problems, and mental disorders.

4. It may also result in a musty smell and lighter skin.

5. A baby born to a mother who has poorly treated PKU may have heart problems, a small head, and low birth weight.

6. Phenylketonuria is a genetic disorder inherited from a person's parents.

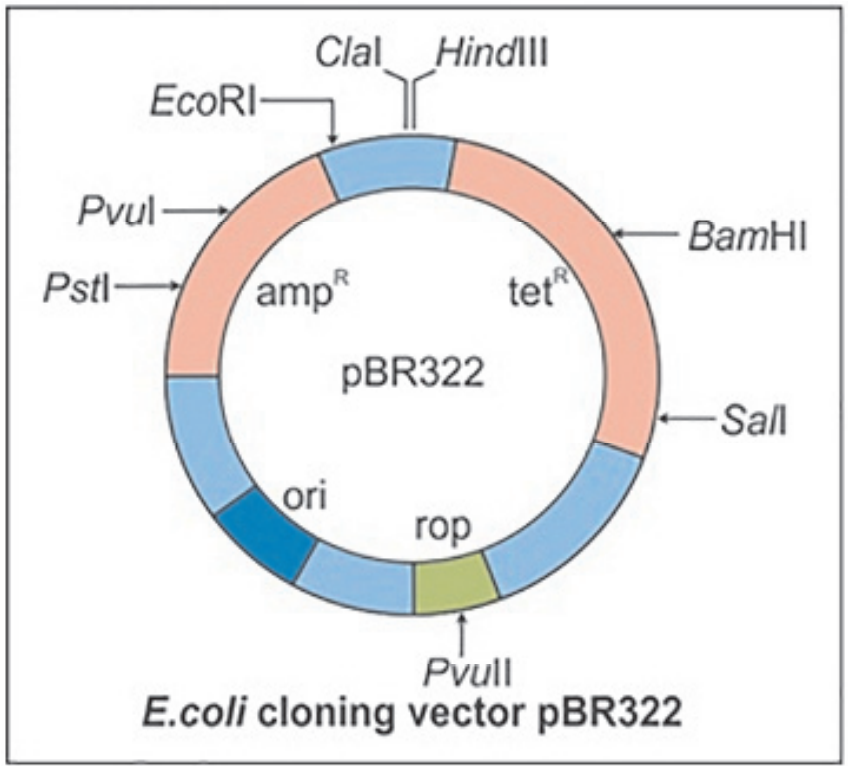
7. It is due to mutations in the PAH gene, which results in low levels of the enzyme phenylalanine hydroxylase

8. This results in the buildup of dietary phenylalanine to potentially toxic levels.

9. It is autosomal recessive, meaning that both copies of the gene must be mutated for the condition to develop. The effected chromosome is 12th chromosome

10. PKU is not curable. The main treatment for PKU is a low-protein diet that completely avoids high-protein foods (such as meat, eggs and dairy products)

pBR322



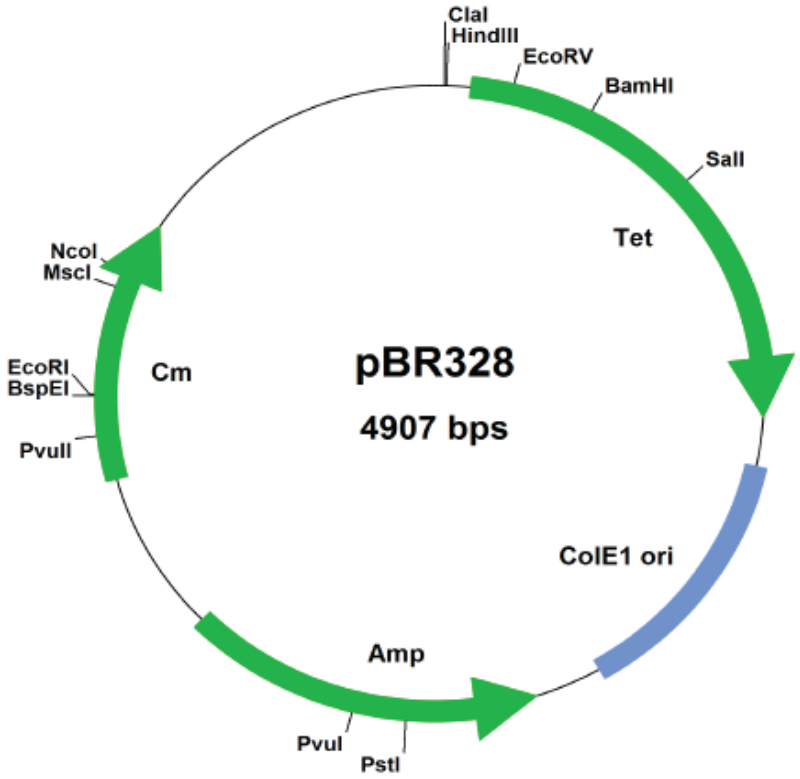
E.coli cloning vector pBR322

pBR322

pBR322

1. pBR 322 was one of the first artificial cloning vector.
2. It is very widely used cloning vector. Till now
3. pBR 322 was created by Bolivar and Rodriguez in 1977.
4. In the pBR the P stands for plasmid, and BR stands for Bolivar and Rodriguez.
5. It is 4.36 kb double stranded cloning vector.
6. pBR322 is 4361 base pairs in length.
7. pBR322 contains two antibiotic-resistance genes
8. a) one is the ampicillin-resistance gene coding for ג-lactamase . b) the tetracycline-resistance gene.
9. The plasmid vector contains 20 unique recognition sites for restriction enzymes. a) six of the theses sites are located within the gene coding for tetracycline resistance . b) two sites are located within the promoter of the tetracycline resistance gene. C) three sites are located the ג-lactamase gene.

pBR328



pBR328

1. pBR328 has been derived from plasmid pBR322 and 325.

2. A region b/w the ampicillin and tetracycline resistance gene has been deleted.

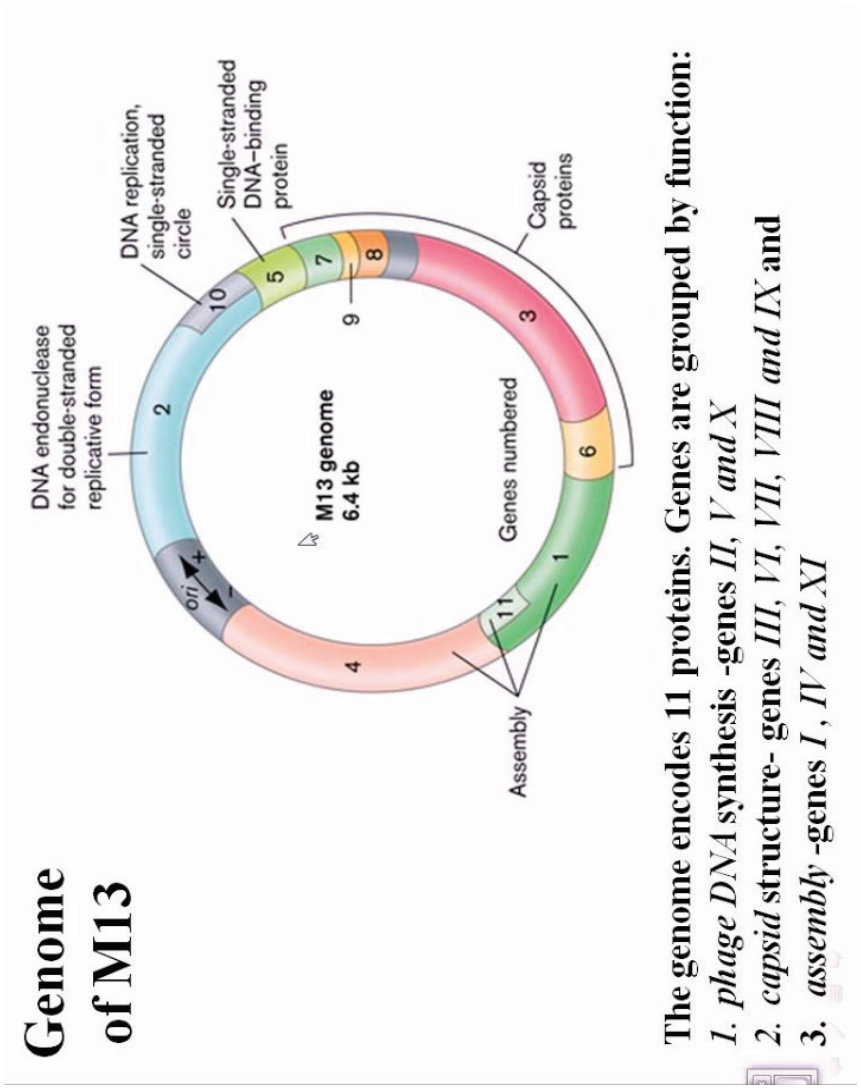
3. This has resulted in the deletion of the bom site and unlike pBR322 and pBR325, pBR328 cannot be mobilized.

4. Therefore pBR328 can be used where more stringent contaminant is re-quired.

5. The deletions have also produced unique sites for PVU-II in the resistance gene.

6. pBR328 is 4.90 kbp and carries resistance genes for tetracycline, ampicillin and chloramphenicol

M13 bacteriophase



The genome encodes 11 proteins. Genes are grouped by function:

1. *phage DNA* **synthesis -genes** *II, V and X*
2. *capsid* **structure- genes** *III, VI, VII, VIII and IX* **and**
3. *assembly* **-genes** *I , IV and XI*

M13 bacteriophase

1. M13 phage is one of the filamentous bacteriophages of E.coli.

2. It is composed of circular gene, stranded DNA encapsulated in a thin flexible single protein called Ps, the major coat protein.

3. The ends of the tube are capped with minor coat proteins

4. The DNA molecule of M13 phage is single-stranded and circular. It is 6407 bases long having 10 closely packed genes. All these genes are essential for the replication of the phages.

5. Its genome is a single stranded cirucular DNA molecule about 6.4kbp long. It is packed in a tube like capsid.

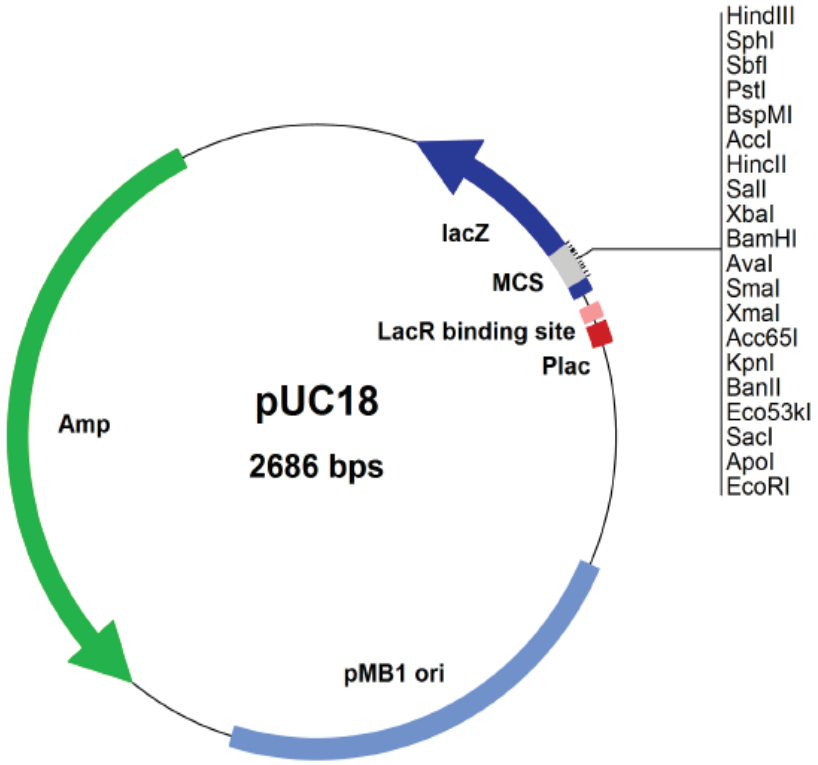
6. M13 is non lytic phage. It does not kill the host.

7. Advantages of M13 phage is they can carry very large fragments of DNA some times upto 95kbp

8. M13 phages can carry single stranded DNA which is usually used in DNA sequencing.

9. The phage coat is primarily assembled from a 50 amino acid protein called pVIII

PUC 18 vector



PUC 18 vector

1. A plasmid is a circular dsDNA molecule a few hundred or thousand base pairs in circumference.

2. Naturally-occurring plasmids are viruses of bacteria.

3. The artificial plasmid pUC18 has been genetically engineered to include (1) a gene for antibiotic resistance to Ampicillin (ampR), and (2) a gene (and its promoter) for the enzyme beta-galactosidase (lacZ).

4. The lacZ gene contains a (3) polylinker region, with a series of unique restriction sites found nowhere else in the plasmid.

5. Digestion with any one of these endonucleases will make a single cut that linearizes the circular plasmid DNA, and allow it to recombine with foreign DNA that has been cut with the same endonuclease.

6. pUC18 is a small, high copy cloning vector for replication in E. coli.

7. It has been constructed using the ampicillin resistance gene and the pMB1 origin of replication from pBR322.

8. The pMB1 of pUC18 differs from the pBR322 origin by a single point mutation and the lack of the rop gene, leading to a high copy number.

9. Additionally, pUC18 contains the lac operon of E. coli with CAP binding site, lac promoter (Plac), Lac repressor (LacR) binding site, and the 5'-terminal part of the lacZ gene encoding for the N-terminal part of -galactosidase (source – M13mp18 phage vector).

10. The vector length is 2686 bp and is isolated from E. coli strain DH5a by standard procedures.