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A Review On – Genetic Technologies

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Abstact:

The rapid developments in the field of genetic engineering have given a new motivation to biotechnology. Molecular cloning is the collection of experimental procedures required to isolate and expand a specific fragment of DNA into a host organism in order to create a large number of identical copies.

Genetics is the scientific study of inherited variation. Human genetics is the scientific study of inherited human variation. This field has been energized in recent years by the Human Genome Project. Scientists expect that the project will lead to the development of new drugs targeted to specific genetic disorders. Increasingly, modern genetics involves genetic engineering; a technique used to manipulate genes and has produced many advances in medicine.

The development of techniques used to transfer foreign DNA into new host cells proved successful and nowadays is commonly implemented in different areas of knowledge, such as microbiology, biochemistry and immunology. The genetic technologies involved the different technique. these are recombinant DNA technologies, cloning, PCR (polymerase chain reaction), in-vitro fertilization. Every technology involved the very complex process. Now a days the genetic technologies is very important. various type of diseases can be treated by using the gene therapy.

Keywords: Genetic Technologies, DNA, Biotechnology, Genome

• Introduction:

The genetic units, which are communicated from one generation to the subsequent generation are called genes. A gene is an important biological unit like atom which is the important physical unit. Mendel was the first scientist who proposed genes as particulate units and called them hereditary elements or factors. But the idea of gene has undergone a significant variation since Mendel's time. The genetic blueprint confined in the nucleotide sequence can determine the phenotype of an individual.

The specifying physical and chemical bases of the DNA molecule in 1953 by Watson and Crick, followed by the complete explanation of its structure and mechanisms of repetition and gene expression, allowable the development of recombinant DNA technology. The progress of techniques used to transfer foreign DNA into original host cells demonstrated effective and currently is frequently useful in different areas of knowledge, like microbiology, biochemistry and immunology. Furthermore, recombinant DNA technology allowable the identification and sequencing of vital genes, and provided a set of techniques developed with the resolve to operate creating hybrid organisms accomplished to express molecules useful for human beings' health" with to work organism and produce hybrids capable of expressing useful molecules for human health. One example is the genome removal and molecular cloning technique that are broadly used in basic and useful research. Genetic engineering is a respected tool for (i) the study of genomes, (ii) the structure of genetically modified organisms that can produce proteins of biotechnological benefits or (iii) the development of transgenic organisms through new properties [4]

- **History:**

In the 40s, Tatum and Beadle established that genes are responsible for coding proteins. In the same decade Avery, McLeod and McCarty demonstrated that the DNA is the material that causes bacterial conversion [3]. In the 50s, Hershey-Chase experiment helped to approve that DNA is the genetic material. Finally, Watson and Crick in 1953 established a three-dimensional structure model for the DNA, which generated new understandings into the genetic code and how proteins are manufactured. Throughout the 60s, Nirenberg and collaborators through various experiments showed the genetic code and Jacob and Monod studies clarified the process of protein synthesis in bacterial cells giving rise to modern molecular biology.[4]

The identification of both small circular DNA molecules, called plasmids, ringing antibiotic resistance genes and restriction endonucleases/DNA ligase enzymes originated in bacterial cells were the "ingredients" that permitted the process of cloning, as confirmed in the work of Janet Mertz and Ronald Davis in 1972. Cutting a piece of DNA carrying a gene from one organism with the restriction endonucleases enzyme and introducing it into a plasmid that can be imitation by a host organism (i.e., bacteria) using the DNA ligase, it was possible to create a recombinant second organism expressing the protein of concentration, improved because of its capacity to grown-up in a medium containing a specific antibiotic marker. In the same year, Berg and collaborators performed a similar experiment where genes of E. coli and bacteriophages were inserted in the genome of the SV40 virus, which then were intelligent to produce recombinant molecules [5].

- **Modern Concept of Gene**

A gene can be defined as a polynucleotide chain, which is a part of DNA. It is a functional unit monitoring a specific trait such as eye colour. Beadle and Tatum decided by various experiments that gene is a segment of DNA that codes for single enzyme. They proposed one gene-one enzyme hypothesis. But as some genes code for proteins that are not enzymes, the definition of gene was altered to one gene-one protein hypothesis.

Genome-wide editing is not a new pitch, and in fact, research in this field has been active since the 1970s. The actual history of this technology happening with pioneers in genome engineering. The first significant step in gene editing was accomplished when researchers established that when a segment of DNA counting homologous arms at both ends is introduced into the cell, it can be combined into the host genome through homologous recombination (HR) and can order wanted changes in the cell [1]

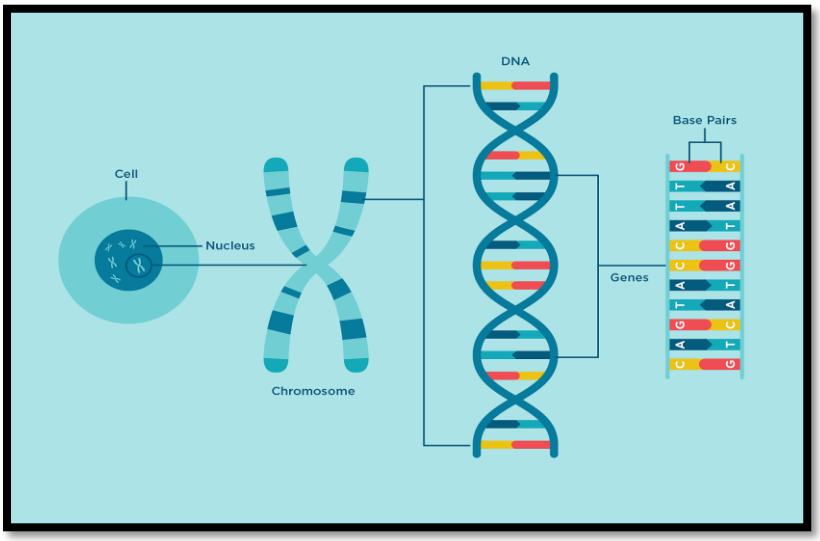

Fig : Basic Structure of gene

Genetics is the study of heredity, the development in which a parent permits sure genes onto their children." Children inherit their biological parents' genes that express precise traits, such as some physical characteristics, natural talents, and genetic disorders. The number of human genes is about 20,000-25,000. Different genes can vary in length and cover thousands of bases.

Genetics is the scientific study of inherited dissimilarity. Human genetics is the scientific study of inherited human difference. This field has been animated in recent years by the Human Genome Project. Scientists assume that the project will main to the progress of new drugs targeted to specific genetic ailments. Progressively, modern genetics involves genetic engineering; a technique used to operate genes and has formed many advances in medicine.

- ## Principles Of Genetics:

• The area where genes work is the cell.

• Each cell's function an organism is determined by the genetic information programmed in DNA.

• In eukaryotes, DNA resides within membrane-bound structures in the cell.

• In prokaryotes, DNA hangs easily within the cell body.

• DNA is packaged into structures known as chromosomes inside a cell.

• Each chromosome in a cell contains many genes, and each gene is situated at a particular site, or locus, on the chromosome.

• Chromosomes frequently occur in corresponding pairs called homologues.

• The number of homologous chromosomes in the human body hold 23 pairs of chromosomes.

An animal cell typically contains some types of membrane bound organs, or organelles.

• The nucleus directs activities of the cell and transmits genetic information from generation to generation.

• The mitochondria produce energy for the cell.

• Proteins are manufactured by ribosomes, which are destined to the rough endoplasmic reticulum or hang free in the cytoplasm.

• The Golgi apparatus changes, packages, and distributes proteins.

• Lysosomes stock enzymes for digesting food.

• The entire cell is enfolded in a lipid membrane that selectively certifications materials to pass in and out of the cytoplasm.[2]

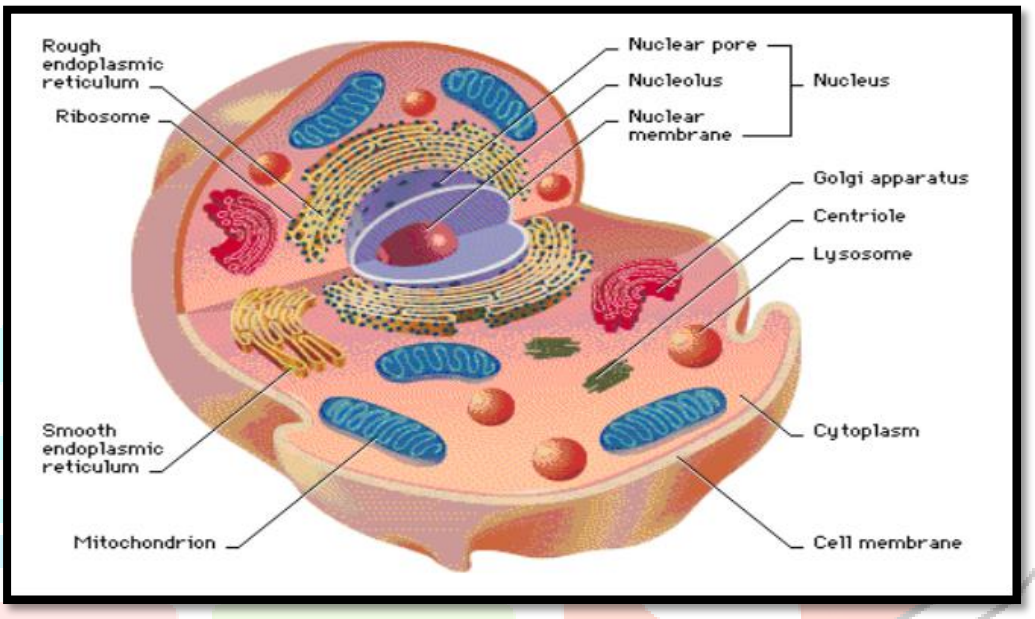

Fig: Animal cell

- **Cell Division and Reproduction:**

Organisms use two types of cell division to confirm that DNA is passed down from cell to cell during reproduction. Simple one-celled organisms reproduce by a process called mitosis. During mitosis a cell pairs its DNA before dividing into two cells and allocating the DNA evenly to each subsequent cell. Organisms that reproduce sexually produce superior cells called gametes, or egg and sperm. Through sexual reproduction, an egg and sperm bond to form a zygote, in which the full number of chromosomes is restored.

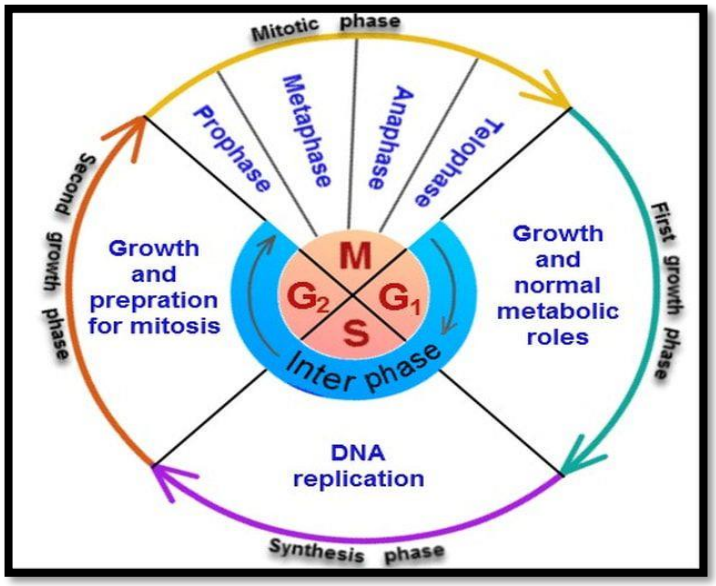

Fig: human cell cycle

Humans use two types of cell division to confirm that DNA is passed down from cell to cell during reproduction. During mitosis a cell pair its DNA before dividing into two cells. In meiosis, the chromosomes in a gamete cell are condensed by half.

- **Genetic Technologies:**

1. Recombinant DNA Technology:

The eldest of all the genetic engineering techniques uses plasmids or vectors to arrive the genetic material into the host cell. Viruses and bacteria are typically used as vectors. Bacteria cover a small size circular plasmid in it. In recombinant DNA technology wanted gene of interest forms a ring when introduced into the plasmid. Bacteria start multiplying and make many copies of plasmid along with its own genetic material. It is transferred to the host cell where it finds nucleus and proclamations gene of interest around. This gene of interest which turns as foreign genetic material associations with the genetic material of the host cell to display its properties. Mixture of human insulin acquired place through this technology.

Recombinant DNA (rDNA) molecules are DNA molecules designed by laboratory approaches of genetic recombination to carry together genetic material from several sources, generating sequences that would not else be found in the genome. Recombinant DNA was first reached in 1973 Herbert Boyer, of the University of California at San Francisco, and Stanley Cohen, at Stanford University, who used E. coli restriction enzymes to insert foreign DNA hooked on plasmids. [12]

The knowledge used for constructing artificial DNA through the grouping of dissimilar genetic materials (DNA) from different sources is devoted to as Recombinant DNA Technology. Recombinant DNA technology is widely called as genetic engineering.

The recombinant DNA technology developed with the discovery of restriction enzymes in the year 1968 by Swiss microbiologist Werner Arber.

Introducing the desired gene into the genome of the host is not as informal as it sounds. It involves the collection of the wanted gene for administration into the host followed by a selection of the flawless vector with which the gene has to be joined and recombinant DNA designed.

- **Basics of Recombinant DNA Technology[rDNA]:**

rDNA stands for recombinant DNA. Before we get to the "r" part, we essential to appreciate DNA. Individuals of you with a background in biology probably know about DNA, but a lot of Chemist haven't seen DNA meanwhile high school biology. DNA is the guardian of the all the information needed to reconstruct an organism. All DNA is made up of a base containing of sugar, phosphate and one nitrogen base. There are four nitrogen bases, adenine (A), thymine (T), guanine (G), and cytosine (C). The nitrogen bases are originated in pairs, with A & T and G & C paired together. The sequence of the nitrogen bases can be organized in an unlimited way, and their structure is called as the well-known "double helix". The sugar used in DNA is deoxyribose. The four nitrogen bases are the similar for all organisms. The sequence and number of bases is what creates multiplicity. DNA does no haptically make the organism; it one makes proteins. The DNA is copied into mRNA and mRNA is translated into protein, and the protein then arrangements the organism. By changing the DNA sequence, the way in which the protein is designed changes. This mains to either a dissimilar protein, or an inactive protein.

Tools of Recombinant DNA technology:

The tools mainly include the following:

1.The enzymes which include the restriction enzymes:

It helps to cut; the polymerases help to synthesize and the ligases help to bind. The restriction enzymes used in recombinant DNA technology play a main role in defining the location at which the wanted gene is inserted into the vector genome. They are two types, viz. Endonucleases and Exonucleases. The Endonucleases cut inside the DNA strand while the Exonucleases remove the nucleotides from the ends of the strands. The restriction endonucleases are sequence-specific which are typically palindrome sequences then cut the DNA at specific points. They examine the length of DNA and make the cut at the exact site called the restriction site. This gives increase to sticky ends in the sequence. The desired genes and the vectors are cut by the similar restriction enzymes to get the complementary sticky notes, therefore making the work of the ligases informal to bind the desired gene to the vector.

2.The vectors:

Help in carrying and integrating the wanted gene. These form a very important part of the tools of recombinant DNA technology as they are the final vehicles that transmit forward the wanted gene into the host organism. Plasmids and bacteriophages are the most common vectors in recombinant DNA technology that are used as they have very high copy number. The vectors are made up of an origin of duplication- This is a arrangement of nucleotide from where the duplication starts, a selectable marker found genes which show resistance to confident antibiotics like ampicillin; and cloning sites – the sites recognized by the restriction enzymes where wanted DNAs are introduced [9]

I. Plasmid:

Plasmids are double-stranded extra chromosomal and generally circular DNA sequences that are capable of duplication using the host cell's reproduction machinery. Plasmid vectors minimalistically consist of an of replication that permits for semi-independent replication of the plasmid in the host. Plasmids are originated broadly in many bacteria, for example in *Escherichia_coli*, but may also be create in a few eukaryotes.[6]

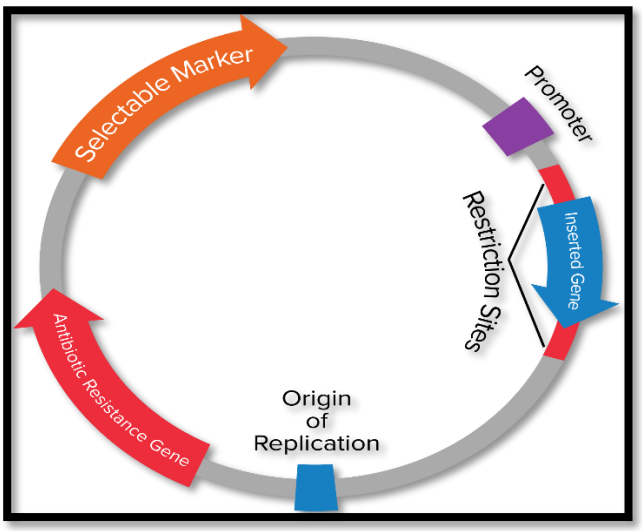

FIG: plasmid

II. Cosmid:

A cosmid is a type of hybrid plasmid that holds a Lambda phage *cos* sequence. They are frequently used as a cloning vector in genetic engineering. Cosmid can be used to build genomic libraries. They were first designated by Collins and Hohn in 1978. Cosmids can comprise 37 to 52 (normally 45) kb of DNA, limits created on the normal bacteriophage packaging size. A cosmid is a type of hybrid plasmid that contains a Lambda phage cos sequence. Cosmids (cos sites + plasmid = Cosmids) DNA sequences are initially from the lambda phage. They are frequently used as a cloning vector in genetic engineering.[7]

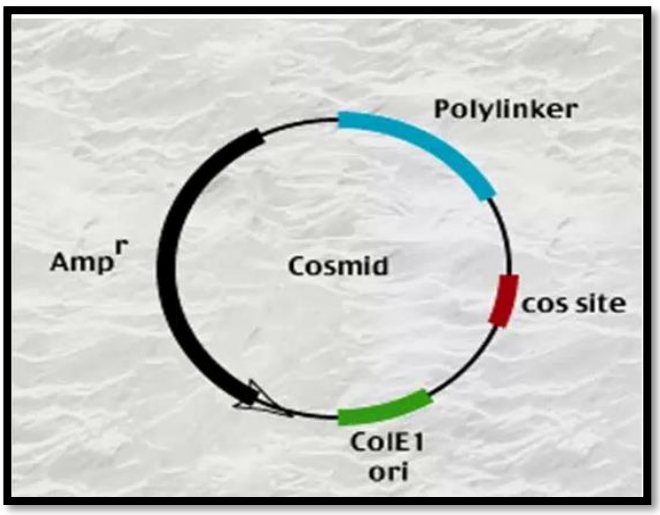

Fig: cosmid

III. Bacteriophage:

Bacteriophages are a collection of viruses that used bacterial cells as host and reproduce by infecting bacterial cells. Similar all other viruses' bacteriophages are also covering of a protein coat that delivers defenses to the genome. Most of the phases are DNA viruses that code for several genes accountable for virus repetition. A major problem of using plasmids as a cloning vector is the size of the gene of interest is small. However, bacteriophages help to overwhelmed this problem, as larger genes can be transported by using bacteriophage as a cloning vector.[8]

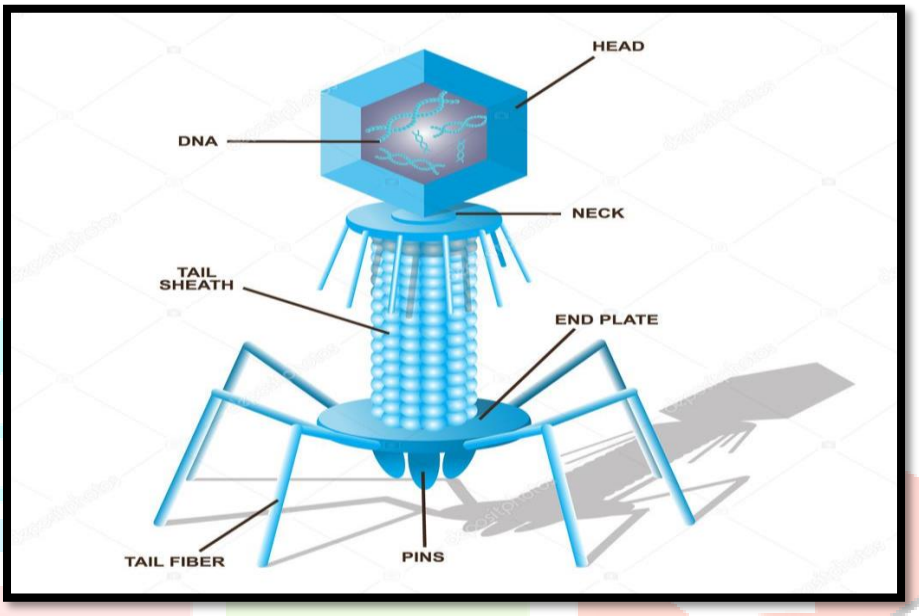

Fig: Bacteriophage

3.Host organism:

In this, the recombinant DNA is presented. The host is the eventual tool of recombinant DNA technology which takings in the vector engineered with the wanted DNA with the help of the enzymes.

- **Steps in Recombinant DNA Technology [17]**

i. Collection and isolation of DNA insert

ii. Selection of appropriate cloning vector

iii. Summary of DNA-insert into vector to procedure rec DNA molecule

iv. rec DNA molecule is presented into a suitable host.

v. Selection of changed host cells. vi. Expression and multiplication of DNA-insert in the host.

(i) Selection and isolation of DNA insert:

First step in rec DNA technology is the selection of a DNA segment of interest which is to be cloned. This desired DNA segment is then isolated enzymatically. This DNA segment of interest is called as DNA insert or foreign DNA or target DNA or cloned DNA.

(ii) Selection of suitable cloning vector:

A cloning vector is a self-replicating DNA molecule, into which the DNA insert is to be joined. A appropriate cloning vector is designated in the subsequent step of rec DNA technology. Maximum generally used vectors are plasmids and bacteriophages.

(iii) Introduction of DNA-insert into vector to form r-DNA molecule:

The target DNA or the DNA insert which has been take out and cleaved enzymatically by the selective restriction endonuclease enzymes in step (i) are now ligated by the enzyme ligase to vector DNA to form a rec DNA molecule which is frequently known as cloning-vector-insert DNA construct.

(iv) r-DNA molecule is introduced into a suitable host:

Appropriate host cells are particular and the rec DNA molecule so formed in step (iii) is introduced into these host cells. This procedure of entry of rec DNA into the host cell is called transformation. Typically, selected hosts are bacterial cells such as E. coli, though yeast, fungi may also be applied.

(v) Selection of transformed host cells:

Transformed cells are individuals host cells which have taken up the r-DNA molecule. In this stage the converted cells are separated from the non-transformed cells by using several approaches making use of marker genes.

(vi) Expression and Multiplication of DNA insert in the host:

Lastly, it is to be confirmed that the foreign DNA inserted into the vector DNA is communicating the desired character in the host cells. Likewise, the transformed host cells are increased to get sufficient number of duplicates. If wanted, such genes may similarly be transferred and expressed into alternative organism [9]

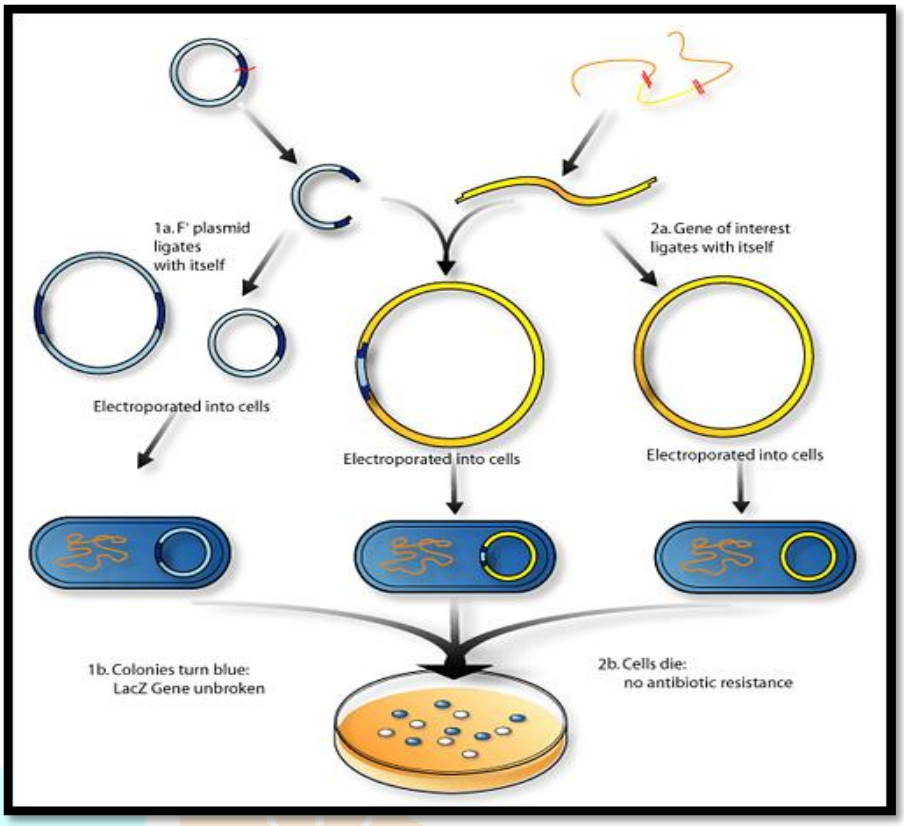

Fig. Process of r-DNA

- **Applications Of r-DNA Technologies:**

- Production of Transgenic Plants
- Production of Transgenic Animals
- Production of Hormones
- Production of Vaccines
- Biosynthesis of Interferon
- Production of Antibiotics
- Application in Enzyme Engineering
- Prevention and Diagnosis of Diseases
- Gene Therapy
- Applications in forensic science

2.Cloning:

DNA cloning is the procedure of production multiple, identical copies of a specific piece of DNA. In a characteristic DNA cloning procedure, the gene or other DNA piece of interest is first injected into a circular piece of DNA known a plasmid.

Principle of Gene Cloning A fragment of DNA:

It comprising the desired gene to be cloned, is combined into a appropriate vector, to produce a recombinant DNA molecule. The vector turns as a vehicle that transports the gene into a host cell generally a bacterium,

while other types of living cell are similarly in use in which this vector reproduces, producing several identical duplicates not only of itself but also of the gene that it conveys. The separation in host cells, copies of the recombinant DNA molecule and this approved to the progeny and supplementary vector replication takes place. The clones of matching cells are produced afterwards the large number of cell divisions. Each new cell in the colony holds one or extra copies of the recombinant DNA molecule; the gene combined in the recombinant molecule is nowadays said to be cloned.[11]

In biology, cloning is an asexual production of hereditarily identical cells, organisms, or copies of DNA. Matching gene and DNA piece that are used in genetic engineering can be generated by gene cloning. In addition to gene cloning, there is also multiplicative cloning which is distinct as the skill to produce a novel discrete that have the same genetic material of the donor of the nucleus. Therapeutic cloning also enables scientists to rapidly generate a matured cell of a specific nature. Reproductive and therapeutic cloning is a straight result of current research and discoveries on how the cell cycle is measured [12]

- **Requirements for Gene Cloning (Cell-based)**

1. DNA piece comprising the wanted genes to be cloned.
2. Restriction enzymes and ligase enzymes.
3. Vectors to carry, keep and replicate cloned gene in host cell.
4. Host cell in which recombinant DNA can replicate

- **Gene cloning involves following 7 essential steps**:

1. Isolation of specific DNA fragment comprising gene of interest which is to be cloned.
2. Insertion of isolated DNA into a appropriate vector to form recombinant DNA.
3. Introduction of recombinant DNA into a appropriate organism called as host.
4. Selection of transformed host cells and identification of the clone holding the gene of interest.
5. Reproduction and Expression of the introduced Gene inside host.
6. Isolation of various gene copies/Protein expressed by the gene.
7. Purification of the isolated gene copy/protein

A. Isolation of the DNA fragment or gene

• The actual first step is isolation of target DNA or gene fragment to be cloned. A gene of curiosity is a fragment of gene whose creation interests us. For example, gene encoding for the hormone insulin.

• The wanted gene may be isolated by using restriction endonuclease enzyme, which cut DNA at exact recognition nucleotide sequences identified as restriction sites towards the inner region producing dulled or sticky ends.

• Occasionally, reverse transcriptase enzyme may also be used which produces complementary DNA strand of the desired gene using its mRNA.

B. Selection of suitable cloning vector

• The vector is a transporter molecule which can transmit the gene of interest (GI) into a host, duplicate there along with the GI making its multiple copies.

• The cloning vectors are incomplete to the size of insert that they can carry. Depending on the size and the application of the insert the appropriate vector is designated.

• Diverse range of vectors obtainable for gene cloning are plasmids, bacteriophages, bacterial artificial chromosomes (BACs), yeast artificial chromosomes (YACs) and mammalian artificial chromosomes (MACs).

• Though, the most usually used cloning vectors contain plasmids and bacteriophages (phage λ) alongside all the other obtainable vectors.

C. Essential Characteristics of Cloning Vectors:

The cloning vectors are transporter of gene (DNA molecules). These vectors molecules have insufficient common features such as:

• They essential be self-replicating inside host cell.

• They must possess a single restriction site for restriction enzymes.

• Overview of gene must not affect with self-replication of the vector.

• They must possess some selectable marker gene such that can be used for later identification of recombinant cell/ transformed cell.

• They should be simply isolated from host cell

D. Formation of Recombinant DNA:

• The plasmid vector must cut open by the same restriction enzyme that is used for isolation of form DNA fragment.

• The DNA fragment and plasmid vector should mix organized.

• In the presence of DNA ligase, base coupling of DNA fragment and plasmid vector must take place.

• The subsequent DNA molecule is a hybrid of two DNA molecules the DNA and the vector. In the language of genetics this is called recombination.

• Consequently, this new hybrid DNA molecule is also called a recombinant DNA molecule and the technology is mentioned to as the recombinant DNA technology.

E. Transformation of recombinant vector into suitable host:

- The recombinant produced is transformed into suitable host cell, bacterial cell generally.
- This is done may be for the following reasons:
- To replicate the recombinant DNA molecule in direction to get the several copies of the DNA
- To allow the appearance of the DNA such that it produces its wanted protein product.
- Some bacteria are natural transformers; they do the uptake the recombinant vector mechanically. For example: Bacillus, Haemophilus, Helicobacter pylori, which are naturally competent bacterial cells.
- On the other hand, some bacteria need the combination by artificial methods such as Ca++ ion treatment, electroporation, PEG etc.

F. Isolation of Recombinant Cells:

- The alteration generates both transformed and non-trans- formed host cells.
- The choice of altered cells involves filtering the useful ones only.
- The selectable indicator gene of plasmid used as vector is used for selection of recombinant cell from non-recombinant cell.
- For examples, pBR322 most mutual plasmid vector covers two selectable marker gene (Ampicillin resistant gene and Tetracycline resistant gene). When pst1 RE is used it knock out Ampicillin resistant gene from the plasmid, so that the altered cell become susceptible for the presence of Ampicillin.

G. Multiplication of Selected Host Cells:

- After selecting and separation the transformed host cells by the screening methods; it develops essential to provide them best parameters to grow and multiply for generation of changed colonies in pertiplates.
- In order to achieve this transformed host cells are announced into fresh culture media.
- The host cells divide and re-divide sideways with the duplication of the recombinant DNA accepted by them and so generating multiple copies of desired gene.
- If the purpose is gaining numerous copies of DNA, then simply replication of the host cell is permitted

. • For gaining the product of attention like protein, hormones, secondary metabolites, favourable environments must be provided so that the DNA implanted in the vector expresses the product of attention.

H. Isolation and Purification of the Product:

- The last step includes segregation of the increased DNA involved with the vector or to isolate the product of interest prearranged by DNA.
- This is approved by purification processes of the isolated gene copy/protein.[10]

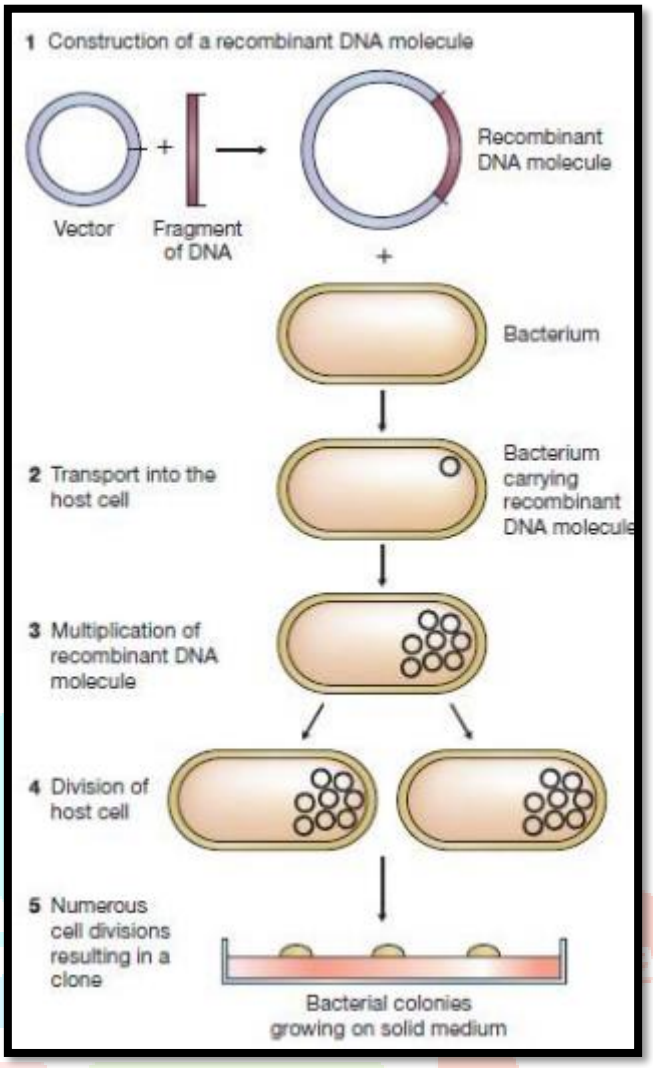

Fig: process of genetic cloning

- **Application Of Genetic Cloning:**

- The main advantage of gene cloning is that it gives numerous copies of a gene or DNA we wish to study.
- Regulate the function of an unidentified gene
- Finding the genes in a genome order
- A specific gene can be isolated and its nucleotide order determine
- Protein /enzyme /RNA function can be investigated
- Mutation (sudden heritable change) can be identified, e.g., gene defects related to specific diseases
- Organism can be engineered for specific determinations, e.g., Insulins production

3.PCR Technique:

"Opening with a single molecule of the genetic material DNA, the PCR can generate 100 billion similar molecules in an afternoon. The reaction is easy to perform. It requires no more than a test tube, a few simple reagents, and a source of heat''. This method was established by Kary Mullis in the mid 80's. [13] He was rewarded with the Nobel prize in chemistry in 1993. PCR is used to amplify a small gene or DNA to produce many reproductions of it. It can

also be termed as "Molecular Photocopier''. This technique is actually helpful in amplifying even very small amount.[14].

- **Definition of PCR:**

It is a genetic technique that happens in vitro which permits the enzymatic synthesis of large quantities (amplification) of a targeted region of DNA in exponential manner. DNA is manufactured in the same manner as that seen in vivo (in the cells) using a DNA polymerase (enzymes that cells use to replicate their DNA)3

- **Principle of PCR:**

Polymerase chain reaction (PCR) is a influential and broadly used technique that has greatly advanced our ability to analyse genes. Genomic DNA present in cells holds many thousands of genes. This makes it difficult to insulate and analyse any individual gene. [15]

PCR method has numerous applications apart from genetic examination like in field of DNA fingerprinting, Diagnosis of disease such as AIDS, development of genetically modified crops, gene mapping, etc.

Fig: Thermal cycler

The basic principle behind PCR is repeated heating and cooling cycle through which the piece of DNA goes through that lead to its amplification into large DNA concentration inside a particular machine called as Thermal cycler.[16]

- **Essential Components of PCR**

The following are the essential components of PCR

- Thermal cyclers (thermocyclers)
- Target DNA (DNA template)
- Two primers (forward and reverse primers)

- Taq polymerase (thermos aquaticus)
- Buffers
- Deoxy nucleotide triphosphates (d NTP's)
- Monovalent \bivalent cation
- Nucleotides (A\T\G\C)

Thermocyclers:

PCR reaction is carried out in 0.2-0.5 ml volume thermo cyclers. It heats and cools the reaction tubes to complete the temperature required. Many thermo cyclers have heating lids to avoid compression at the top of the reaction tube. Past thermo cyclers absence a heating lid instead of which a small ball of wax was placed inside the tubes priorly.

Target DNA and nucleotides:

It consists of the DNA to be enlarged. The segment represents a small part of a large and multifaceted mixture of a specific DNA of a genome. The shape of DNA is a double coiling structure which consists of nucleotides that wind around each other in a coiling shape. PCR requires a template molecule (i.e.) DNA\RNA. The 4 nucleotide mechanisms are like 4 bricks or building blocks used to construct genome molecules. The nucleotide bases are adenine, thymine, cytosine and guanine which also needs a small amount primer.

Two primers:

They are onward and reverse primers which are usually 16-30 nucleotides in length. Primers limit the DNA order to be imitation and results in the amplification of a particular DNA sequence. Primers are short, artificial DNA strands not more than 50 nucleotides, which regulates the beginning and the end of the section to be amplified, the polymerase synthesizes the opposite sequence from each primer. If template contains A nucleotide, enzyme adds on T nucleotide to the primer and if template contains G nucleotide, enzyme adds on C nucleotide to the primer. Two components that are considered for a primer are length of the primer and actual order of the primer. The primers depend upon-

- Primer length
- Melting point
- Specificity
- Complementary primer sequences

The length of the primer should be as short as possible. The annealing temperature should below at least 5 ℃ than the melting point temperature.

Taq polymerase:

Many micro-organisms can live in inhospitable conditions or in the presence of salt\acidic concentrations. The bacteria synthesis at the rate of 35-100 nucleotides\sec. the DNA polymerase from Thermus Aquaticus is stable at 95'C which is a thermophilic. Both the cloned gene and the native Taq obtained from Thermus aquaticus are available

commercially to serve as a standard reagent for PCR reaction. They also grow in the geysers over 110' C and can withstand heating to 94 ℃ and presence of extreme salt/acidic concentrations. They have contributed greatly for the stimulation, specificity, automation of PCR process. The bacteria synthesize at the rate of 35-100 nucleotides/sec.

D NTP'S:

These deoxy nucleotide triphosphates consist of nucleotides floating in liquid and it supplies the nucleotides to Taq polymerase enzyme to synthesize a new strand of DNA. These NTP'S consist of \Y phosphates which serve as a source of energy to PCR reaction. The concentration of d NTP's should be 20-200m. [17]

- **Steps involved in PCR:**

1.Denaturation Stages at 94-98 ^{0}c:

At this stage, the thermal cycler is heated to 94-98^{o}c which caused the denaturation or separation of double stranded DNA or the gene into single strand by the breakage of hydrogen bond between the DNA strand. These strands will now act as a template for the formation of new DNA strand. It takes 15-30 seconds.

2. Annealing stage at 45-60^{0}c:

At this stage, the thermal cycler is cooled to 45-60^{0}c which causes the annealing or binding of the short nucleotide strand of 20-30 base pair called as primer with the separated single strand of DNA obtained at the end of denaturation stage. the primer is designed as per the nucleotide sequence available at the end of gene or DNA They are designed in a set, one as forward primer and other as revers primer to amplify both the strands of DNA. primers provide free 3'hydroxyl group required in further steps by DNA polymerase enzyme. it takes 10-30 sec.

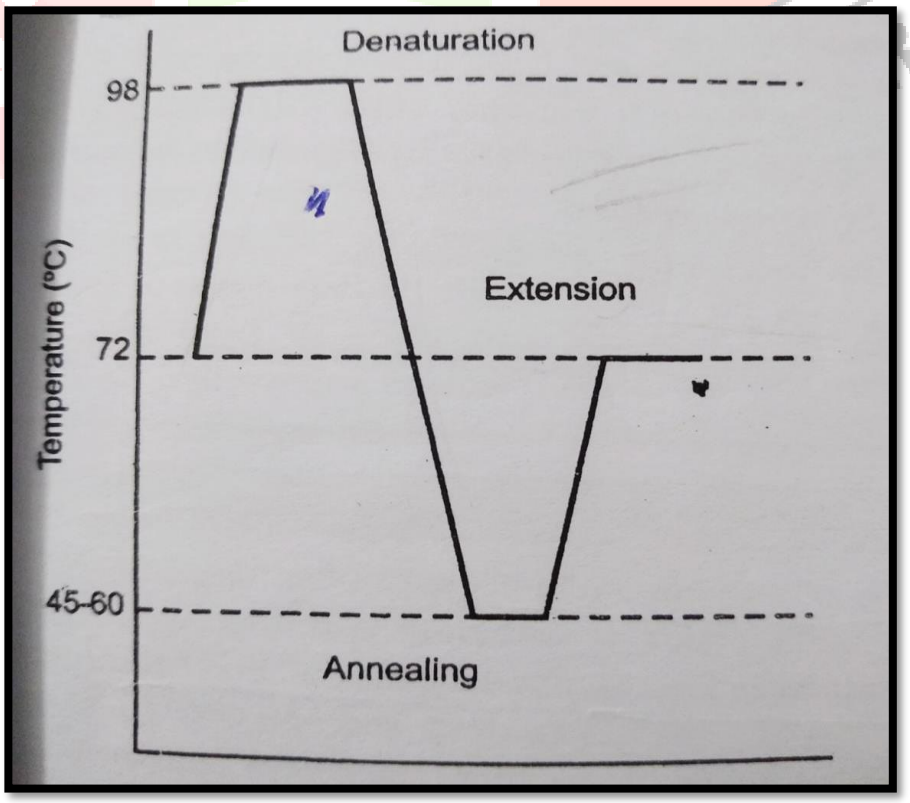

Fig. graphical representation of temperature

3.Extension stage at 72^0c

At this stage ,the thermal cycler is heated to 72^0c that causes the extension of primer which was annealed to the gene or DNA strand for the synthesis of new DNA .the synthesis of new DNA strand requires DNA polymerase enzyme which utilizes free 3' hydroxyl group provided by the primer for the synthesis of new DNA strand ,but at noted from the previous steps the thermal cycler undergoes heating to very high temperature for example ,95^0c. at this temperature ,a normal DNA polymerase could not survive .so ,a special DNA polymerase called as "Taq DNA polymerase'' is used which is resistant to denaturation even above 80^0c. Taq DNA polymerase is isolated from thermos aquaticus bacteria which lives in hot spring ,so ,at the end of extension stages a brand new strand of DNA is synthesized at 5'to 3' direction over the templets strand and a new double strand DNA is synthesized .[16]

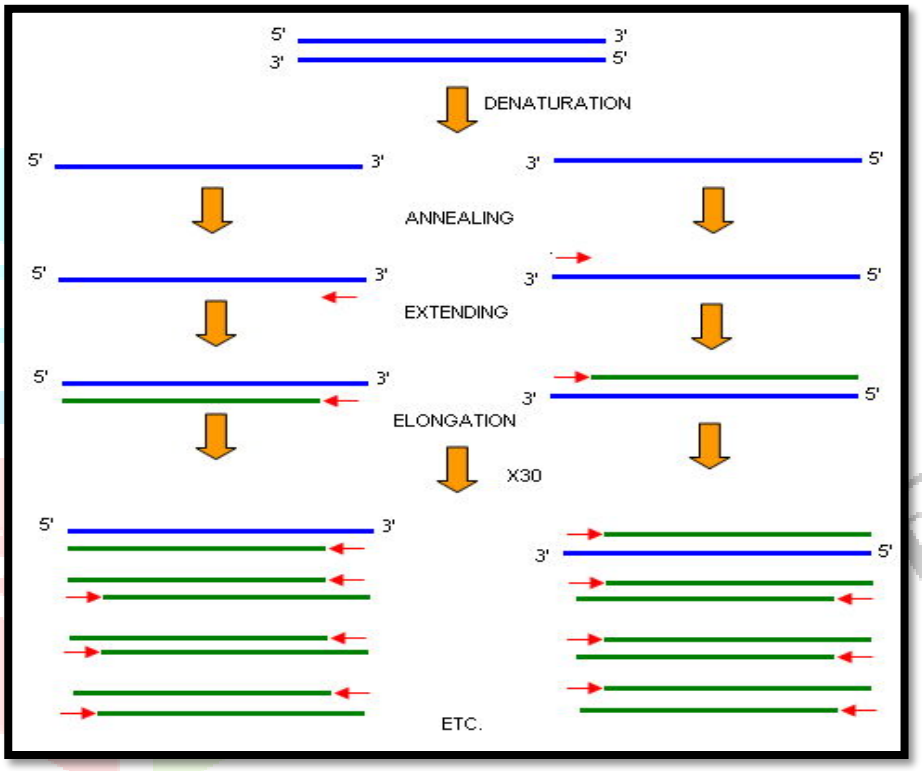

Fig: Stages of PCR amplification

Application PCR technique:

- Reverse transcriptase PCR (RT-PCR) [18]
- PCR applied to diagnosis [19]
- In nucleic acid detection assays
- In medical field
- In agricultural sciences
- In virological diagnostics
- In cancer therapy
- PCR-as biomarker
- In forensic medicine

- In bacteriology
- In PCR-fingerprinting and in the detection of microbiological gene [17]

4.In -Vitro Fertilization:

The World Health Organization (WHO) has defined sterility as a disease, if the particular couples have a desire for a child. False insights are clogging access to new research on IVF [21]. IVF is a technique to solve the female infertility in the woman which is due to the suffering of fallopian tube or there is trouble in fertilization by in vivo process. IVF is a major treatment in which an egg is fertilised by sperm outside the body. This method is used when the other fertility treatments have unsuccessful. In this process the woman's ovulation procedure is check and the egg cell or egg is removed from the woman`s ovary and then leasing sperm fertilise them in a fluid medium in a laboratory. Then the fertilised egg that is known as zygote is transferred to the woman`s uterus. Louise Brown was born as a result of natural cycle IVF. Robert G. Edwards, the physiologist who developed the treatment, was presented the Nobel Prize in Physiology or Medicine in 2010.[20]

a medical procedure whereby an egg is fertilized by sperm in a test tube or somewhere else outside the body. During IVF, mature eggs are together from ovaries and fertilized by sperm in a lab. Then the fertilized egg (embryo) or eggs (embryos) are transferred to a uterus.

- **Process of IVF:**

In IVF, egg and sperm are fertilized outside the women's body and then transferred into uterus

1.ovarian stimulation:

Stimulate ovary for healthy egg production.

2.Egg Retrieval:

Retrieval of eggs trans – vaginally

3.Fertilization of Eggs:

Combine egg and sperm in lab.

3. Embryo culture:

Embryo are cultured for 3-5 days.

4.Embryo transfer:

Transfer of embryo into uterus.

5.Pregnacy Test:

Confirm pregnancy with test

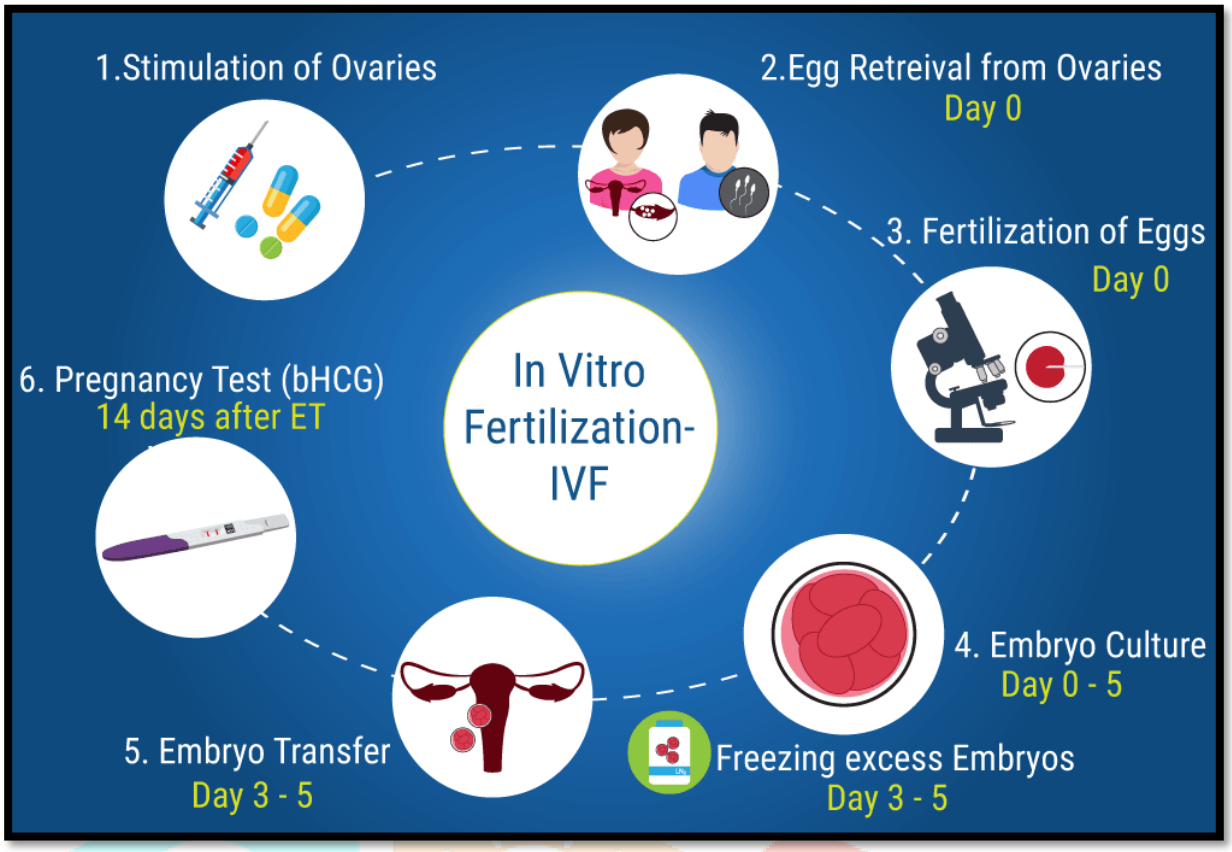

Fig: IVF process

- **The following matter is to be taken into consideration**:
- Patient should be conscious about drugs and the IVF technique Fertility medication or hormones are set to encourage the follicle production before IVF procedure patient must aware about those fertility hormones or drugs and related side effects using in IVF treatment.
- Patient should attentive how fertilisation takes place process either has been accomplished naturally or by using ICSI and resultant effects.
- Patient should aware about risk with an IVF procedure such as great pain called as ovarian hyper stimulation and side effect of anaesthesia that is nausea and irregularly bleeding.
- Patient should aware about the success rate of IVF process [22] .
- **Drugs used in IVF and Their Effects:**

In IVF procedures medicines are generally used to make body for treatment so that increase the possibility of more healthy eggs is free from ovaries but have serious side effects also on both child and mother both. They are

- Increased rate of multiple birth
- Increased rate of miscarriage
- Burning flashes, vomiting
- Headache or blurred vision
- Depression and mood swings
- Ovarian cysts and pelvic discomfort from overstimulation of the ovaries.

Medicines are generally used are Clomiphene citrate, Follicle Stimulating, Bromocriptine, Cabergoline and Gonadotropins Releasing Hormone etc [23]

- **Possible Risk Associated with IVF Treatment:**

Multiple pregnancies

Multiple pregnancies are a superior risk looks in IVF which carries important risks to both the mother and the babies as compared to singleton pregnancies. Due to multiple pregnancies medical difficulties are increased and include an increased high blood pressure and ante partum haemorrhage (bleeding before the onset of labour). There is also a risk of growth obstruction in infants and greater risk of early birth in twins [24].

Maternal risks related with multiple pregnancy

- Miscarriage
- Haemorrhage
- Pregnancy induced high blood pressure
- Diabetes
- Anaemia
- Polyhydramnios (excessive amounts of amniotic fluid that surrounds the foetus)
- Prolonged hospitalization resulting in higher cost of medical care [25].

- **Conclusion:**

The use of genetic engineering has equally advantages and problems. While the risk of tragedy caused by the mismanagement of genetic engineering is tremendously high, but at the same time the possible benefits of happening in this field in a safe and responsible way are surprising. Usage of cloning must be careful to prevent some moral and controversial issues.

In order to achieve potential benefits of genetic engineering the individual need is to progress perfect tools and techniques. Once it has been finalized then all of the difficulties related with food production examination be solved, the world environment can be re-established, and human health and lifestyle will improve elsewhere imagination. No uncertainty that there are practically no restrictions to what can be accomplished through responsible genetic engineering.

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