

## **Lecture 39: Biotechnology Applications (Part-I)**

**Introduction:** As discussed in previous lecture, biotechnology has influenced human life in many ways by invention to make his life more comfortable or easy. It has made advancement in agriculture to increase the yield, increases the value of animals by improving their breed and contributed significantly in the development and design of drugs against infectious diseases.

**Biotechnology in plant sciences-** Genetic Engineering has allowed us to produce genetically modified plants with diversified properties such as resistance against pest, drought, abiotic stress. These are few selected examples of advancement in the plant sciences due to technological contributions of biotechnology.

**Insect control-** Insect uses plants for nutrition and reduces the crop yield. There are two ways to control the effect of insects on the crop; reduction in the number of insects in the affected area or generation of plants with insect resistance.

**Sterile male insects-**The mechanism of reducing the number of insects in the affected area through the use of sterile male insect is given in Figure 39.1. In a typical insect life-cycle, male and female mate with each other to produce large number of fertilized eggs. Eggs go through a series of development stages to produce large number of baby insects to continue the life-cycle. In this approach, male insects are exposed to the radiation or other treatment in the laboratory to render them infertile. These sterile male insects are spread over the infected area. In the field, female mate with these sterile males but no offspring is produced. As a result over the course of time, the insect population will be reduced. The classical example of this approach is eradication of boll weevil, an insect responsible for the loss of cotton crop in USA.

**Insect resistant plants-**A genetically altered crop is produced to develop resistance against insects. One of the approach is to genetically modify the plant which will express a toxin to kill the insects but will be safe for human consumption. *Bacillus thuringiensis* (Bt) is a bacteria which secretes a insecticidal toxin. Spraying Bt toxin was in circulation to control the insect population. With the use of genetic engineering transgenic plants are produced which express Bt toxin in their somatic cells. When insect feeds on the plant, toxin reach to the stomach and causes internal bleeding to kill the insect.

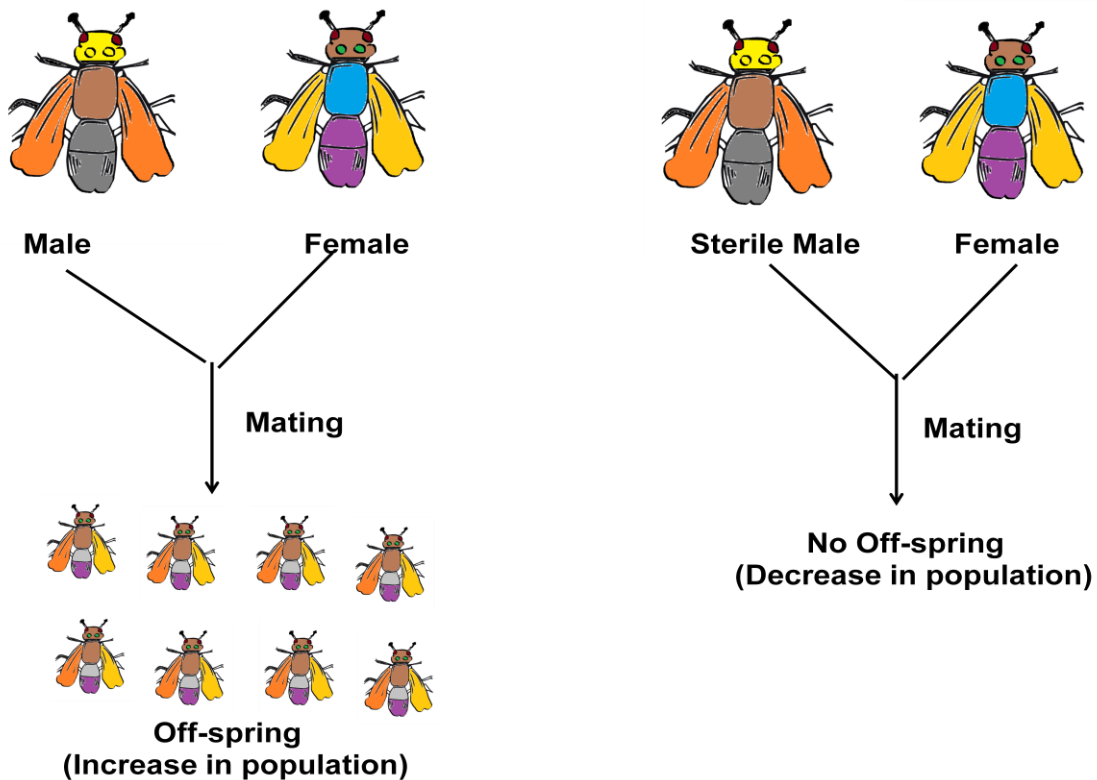


Figure 39.1: Schematic depiction of the impact of sterile male insects in controlling insect population.

**Herbicide resistant plants-** Weeds grow very fast and they compete for nutrients with the crop plant. Chemical herbicides are used in the agriculture to eradicate weeds from the fields. If weeds need to be removed from the crop, herbicide should do little or no effect on the crop plants. Herbicides are either selective towards a class of plant or non-selective to kill all plants they applied to and used more often to kill all vegetation. Glyphosate is one of the first herbicide designed to kill weeds. It interferes biosynthesis of aromatic amino acid tyrosine, phenylalanine and tryptophan by inhibiting enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSP). The enzyme catalyzes the conversion of the shikimate-3-phosphate to the 5-enolpyruvylshikimate-3-phosphate (Figure 39.2). The treated plant can not be able to produce these amino acids as well as protein needed and dies. There are two approaches, adopted to develop herbicide resistance in crop plant. (1) The genetically modified crop plant is designed with an alternate pathway to supply the aromatic amino acid to compensate the inhibition of EPSP. (2) Few bacterial strains use an alternate form of EPSP that is resistant to the glyphosate inhibition. The modified version of EPSP gene was isolated from the *Agrobacterium strain* CP4 and cloned into the crop plant to provide herbicide resistance. So far the crop plant commercially available with herbicide resistance are soy, maize, sorghum, canola and cotton.

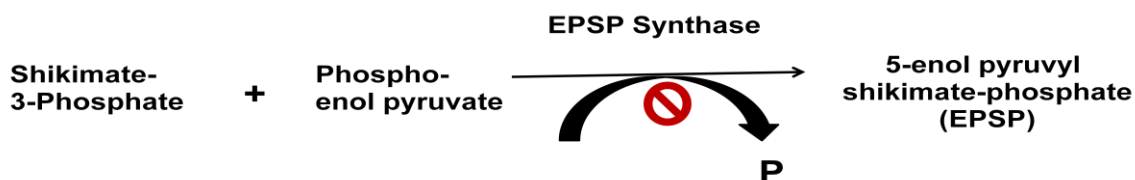


Figure 39.2: Biochemical reaction catalyzed by EPSP Synthase, a target of Glyphosate action.

**Disease resistant plants**-Plants are under continuous exposure to the pathogenic organism and the environmental conditions. Pathogenic organisms (bacteria, fungi, mycoplasma, virus) attack on plants to gain nutrients for their growth and disturb its metabolism to exhibit pathological symptoms. There are multiple approaches to develop disease resistant plant, although in few cases it is not possible to develop a disease resistant plant at all (Table 39.1).

**Selection and breeding of natural disease resistant plant species**-Few naturally occurring plant species have acquired resistance against a particular disease. These species are preferred over other species for production. In few cases plant species resistant to the disease are either susceptible to other disease or the yield is low. In both cases, it is preferred that the disease resistant plant species can be cross breeding with a high yield plant species to acquire resistance as well as high yield.

**Production of Resistance Protein**- Plants have R gene (resistance gene) which produces R protein and these virulence factors allow acquiring resistance to combat pathogens. Every R gene recognizes pathogen protein in a receptor-ligand fashion and as a result R gene product provides resistance against a particular pathogen or a family of related pathogens. R gene has the ability to modify its product to acquire resistance against new species of pathogen. A good example include barley MLO against powder mildew, wheat Lr34 against leaf rust, and wheat Yr36 against stripe rust.

Table 39.1: Selected list of disease resistant plant species.

Crop	Variety	Resistance
French Bean	1. Aigullion	Mosaic virus and anthracnose
	2. Hilda	Mosaic Virus
Broad bean	Futura	Chocolate spot
Cabbage	Stone head F1	Mildew
Carrot	Fly Away F1	Carrot fly
Cumcumber	Bush Chambion F1	Cucumber mosaic virus
Pea	Ambassador	Powdery mildew, fusarium wilt
Potato	1. Colleen	Blight and scab
	2. Osprey	Scab and eelworm
	3. Milva	Blight Resistant
Pepper	Bell Boy F1	Mosaic Virus resistant
Sweet Corn	Golden Sweet F1	Smut
Tomato	Alicante	Greenback and mildew

**Abiotic stress resistant plants**-Over-production of systemin and HypSys has been found to provide resistance in plant against salt and UV radiation. In the transgenic plants, systemin lower down the stomatal opening in comparison to the normal plants to reduce the loss of water. Whereas in higher salt solution, plants had larger stomatal opening, lower concentration of abscisic acid and proline.

**Biotechnology in animal breeding**-Biotechnology has greatly facilitates the animal breeding and improving their species with additional traits. I will discuss few examples to explain you the impact of biotechnology on animal breeding.

**Artificial insemination**-The over-all process involves the introduction of male sperm into the reproductive tract of the female animal artificially. The availability of superior breed animal is due to the artificial insemination (AI). There are several advantages of AI compare to the natural breeding.

1. The male of a high breed (commonly known as **sires**) is very costly in comparison to the semen from them.
2. Through a natural breeding process, many diseases can be transmitted to the female through mating. These possibilities are much reduced in an AI procedure.
3. The high breed animal imported from other countries needs to go through quarantine process to ensure no spreading of disease. This process is costly and time consuming.

**Steps in artificial insemination**-In an AI procedure, semen is collected from the superior breed male in a test tube. The quality of the sperm such as motility, number is checked through a microscopic observation. It is mixed with the extenders such as milk, yolk, glycerine and antibiotics and stored in liquid nitrogen for future use. Before go for final step of AI, it is important to study the estrus cycle of the female to know the exact time of ovulation. The semen is injected into the female reproductive tract to facilitate the process of fertilization. A skilled technician is required to perform most of the steps of artificial insemination.

**Embryo Transfer-** A superior breed female (commonly known as **dam**) can be able to give more offspring in a year by the process of embryo transfer. In this process, using an AI procedure superior breed male semen is used to fertilize the eggs in the female. Once the embryo is formed, it is transferred in another female (low breed) to produce offspring. The process of embryo transfer is given in Figure 39.3. The donor female is treated with the hormone to produce several eggs, the process known as **superovulation**. The egg is fertilized either by natural intercourse or by artificial insemination. The fertilized eggs are recovered from the uterus of the donor animal using a catheter. The solution from catheter is used to flush the uterus and fallopian tubes. On average 6-8 embryos are collected in each flush. The quality of the embryo is checked in the microscope and suitable embryo is implanted into the recipient animal. It is important that both donor and recipient animals must have a synchronized estrus cycle for successful embryo transfer.

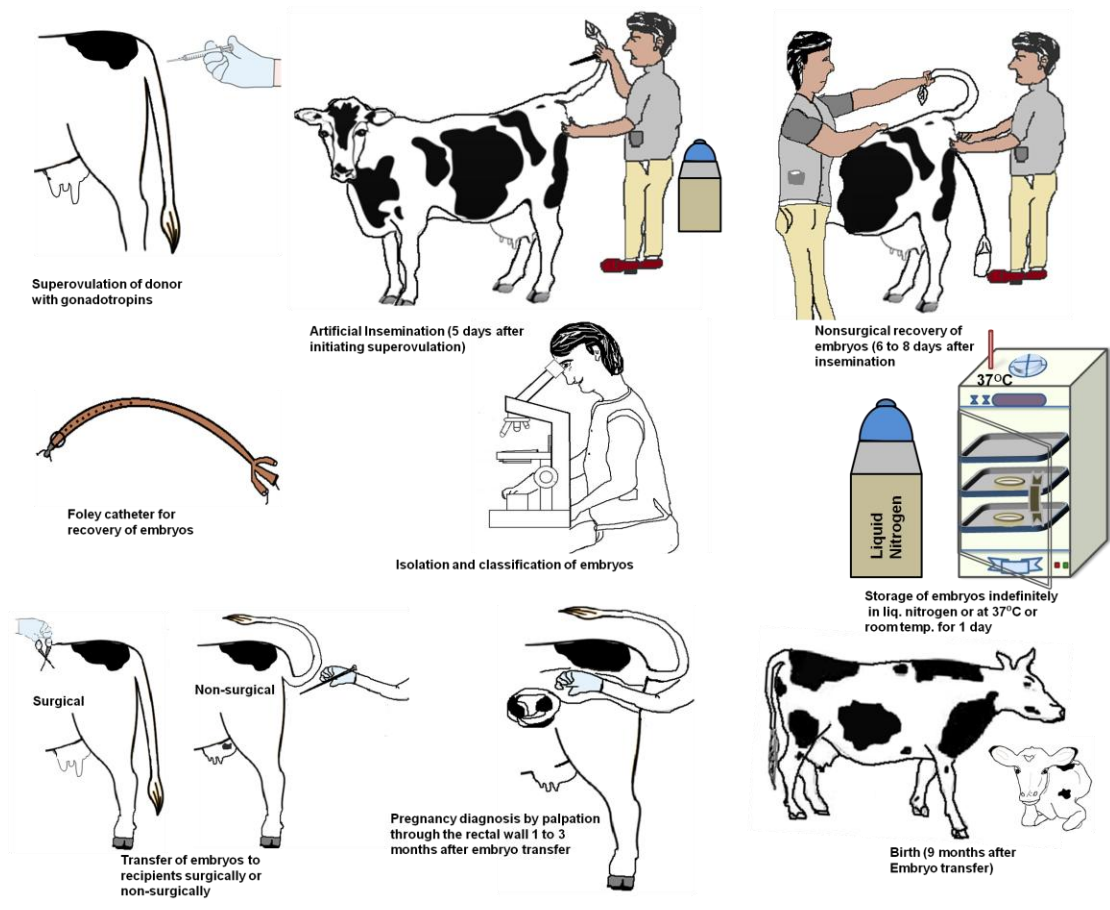


Figure 39.3: Steps of embryo transfer between donor and recipient.

**Lecture 40:  
(Part-II)**

**Biotechnology Applications**

**Biotechnology in medicine**-medicines are class of molecules used to correct the disturbances in the host physiology. They can be chemical in nature and used to inhibit aberrant enzymatic activity from the host or pathogen. In few cases, host enzymes can be supplied as a drug formulation to drive the biochemical reaction. Biotechnology has potentials in contributing into the development of the drug molecules. Besides this, biotechnology contributes many ways into the medicine field.

**(A) PRODUCTION OF THERAPEUTICALLY IMPORTANT PROTEINS**

A large number of genetic or metabolic diseases can be corrected by the supplying proteins or factors. Following the advancement in the biotechnology, many other proteins or factor produced are produced in different bacterial expression systems (Table 40.1). In an approach, gene of the enzyme or proteinous factor is cloned into the appropriate plasmid to produce recombinant clone. For example, production of human insulin. Insulin is a dimer of an A chain and B-chain linked by disulphide bonds, composed of 51 amino acids with a molecular weight of 5808. A schematic presentation of steps in insulin production is given in Figure 40.1. In this process, gene A and B is cloned into the bacterial plasmid separately to produce two recombinant clones. Peptide chain A and B is over-expressed in the e.coli and recombined together to produce functional insulin.

**Other important examples of production of proteinous factors are as follows-**

**(I) Recombinant Chymosin-** chymosin is required for manufacturing cheese. A non-pathogenic e.coli strain (K-12) is used for large scale production. The recombinant enzyme is safe to use, cost less and available in abundance.

**(II) Recombinant Human Growth hormone (HGH)-** It is produced by the pituitary gland and hormone is required to support growth and development of human. Recombinant HGH is cheap and safe to use for therapeutic applications.

**(III) Recombinant blood clotting factor VIII-** In a normal individual, blood loss from a damaged blood vessel is prevented by the formation of a clot. Blood clotting is a series of reaction involving different factors. Factor VIII is deficient in bleeding disorder such as hemophilia, and recombinant factor VIII is supplied to improve the disease condition.

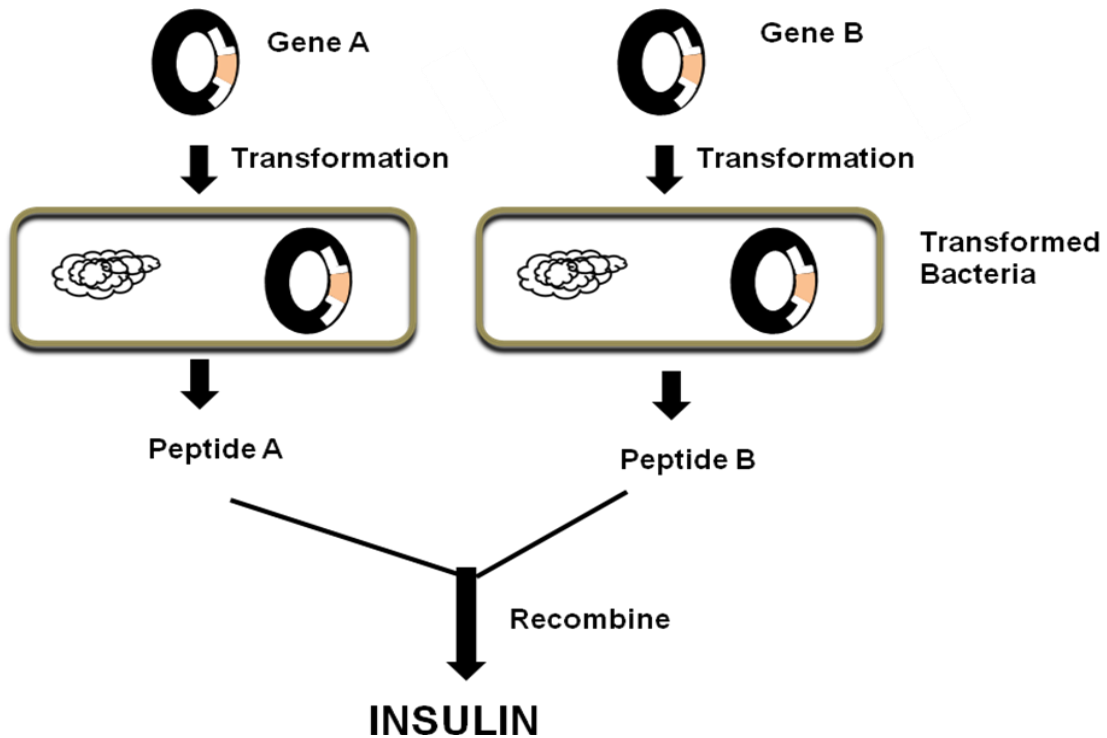


Figure 40.1: Recombinant DNA Technology to produce human insulin.



**(B) GENE THERAPY**

As discussed before, production and supply of recombinant proteins is a temporarily solution for the treatment of a disease condition. In another approach, human expression system is used to produce the proteinous factor after inserting the recombinant clone into the human cells or inside the human body. Recombinant DNA is packed into the appropriate DNA delivery system (either a virus or liposome mediated) to deliver the gene into the human cells to correct the mutated genes or encode a therapeutic protein drug to provide treatment.

Table 40.1: Therapeutics products in development.

S.NO.	Protein	Potential Application in disease
1	Factor VIII, Factor IX	Hemophilia
2	Tissue plasminogen activator	Thrombosis
3	Lactoferrin	GI tract infection
4	Human protein C	Thrombosis
5	Alpha-I antitrypsin	Emphysema
6	Fibrinogen	Wound healing
7	Pro542	HIV infection
8	Antithrombin 3	Thrombosis
9	Collagen I	Tissue repair
10	Serum albumin	Blood volume

**There are two different types of gene therapy-**

**Somatic Gene therapy-**In this therapeutic approach, the therapeutic genes is transferred into the somatic cells as per the requirement of individual to treat the functional defects. This treatment does not move to the patient’s offspring or next generations.

**Germ line gene therapy-** In this therapeutic approach, germ cells (sperm or egg cells) are transformed by the introduction of the required gene to produce the protein or correct the mutated gene. This allows the treatment move to the patient’s offspring or next generations. Although the approach seems promising in providing long term solution to treat genetic disorder, but there are several ethical, technical reasons and possible future risks.

**The technical problems associated with the gene therapy is as follows-**

**1. Short lived-** Therapeutic gene delivery into the cells gives short term effects, either by rejection of recombinant DNA or suppression of the gene expression. Due to this problem, patient needs to go for several rounds of gene therapy.

**2. Immune reaction-** virus containing gene is treated as the foreign object, and immune system is stimulated to attack the invader. It is the main reason of reduced effectiveness of gene therapy.

**3. Viral vector** used as a vector to deliver the gene causes much adverse immune reactions and toxicity in patients.

**4. Disturbance of host physiology-** if the gene integrate to a wrong place in the genome, it may cause functional defects. In few cases, it may disrupt the function of the tumor suppression genes results into the development of the tumor. For example, X-linked severe combined immunodeficiency (X-SCID) patients injected with the transformed hematopoietic stem cells led to the development of T-cell leukemia.

**(C) MONOCLONAL ANTIBODY PRODUCTION**

Antibodies are used in the adjuvant therapy to remove the infectious agents or toxic substances. Monoclonal antibodies are generated by the hybridoma technology. The complete process of hybridoma technology to produce antibody has seven steps (Figure 40.2).

**STEP 1: Immunization of mouse-**mouse is injected with the desired antigen to produce antibodies. It is performed by a series of injections over the course of several weeks.

**STEP 2: Isolation of B-Cells-** splenocytes are isolated from the spleen of the mouse.

**STEP 3: Cultivation of Myeloma cells-**Myeloma cells are grown in the serum containing complete media.

**STEP 4: Fusion of myeloma and B-Cells-** B-Cells and myeloma cells are fused using polyethylene glycol (PEG) or the Sendai virus.

**STEP 5: Screening of fused cells-** Fused cells are grown in the HAT medium (hypoxanthine-aminopterin-thymidine medium) for an extended period. Myeloma cells lack salvage pathways, and they die in the presence of the aminopterin, an inhibitor that blocks nucleotide synthesis. The fused cells grow in HAT medium due to the contribution of genetic material from B-cells. Fused hybridomas are screened to test the production of a desirable antibody against the antigen. The culture supernatant is tested for the presence of antibody in a sandwich ELISA.

**STEP 6: Multiplication-** The hybridomas producing a desirable antibody can be propagated in the medium containing IL-6 to establish hybridoma cell lines. It grows in culture medium to produce antibodies.

**STEP 7: Harvesting-** The hybridomas are transferred into large culture flasks to secrete antibodies. The culture supernatant can be collected and stored at -200C or other lower temperature for future use.

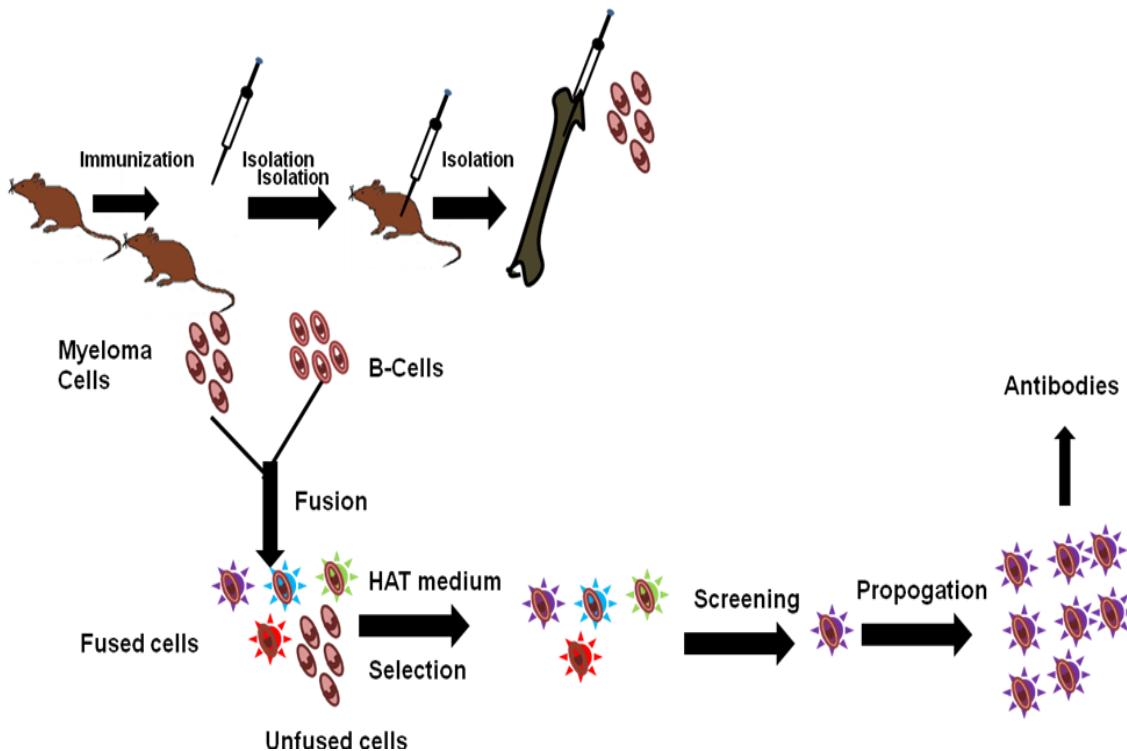


Figure 40.2: Hybridoma technology to produce monoclonal antibodies.

## **(D) VACCINE**

Vaccine is given to develop immunity against the disease in the human or other vertebrate animals. Vaccines are dead, attenuated organism or proteins derived from them. There are different strategies to enhance the immunological response to give long lasting protecting against the disease with minimum adverse effects. Different types of vaccine developed for human vaccination is as follows-

**Killed**-In this vaccine preparation, pathogenic organism is killed by chemical or UV treatment and used as an immunogen. It is mixed with the adjuvant to enhance immunological responses and long memory.

**Attenuated**-In this vaccine preparation, organism is treated with the chemical to destroy its ability to cause disease. As a result, organism grows and give stimulation to the immune system for long term immunological memory.

**Toxoid**-In this vaccine preparation, inactivated toxic compounds are used as an immunogen.

**Subunit**- In this vaccine preparation, a pure protein or antigen is given as an immunogen. It is the safest form os vaccine with minimum adverse allergic reactions.

**Conjugate**-In this vaccine preparation, bacterial coat is tagged with the immunogenic protein to induce production of immune response against the bacterial coat.

**A detail description of different types of vaccine available for vaccination is given in any immunology text book such as I. Roitt, J. Brostoff and D. Male, Immunology, 6<sup>th</sup> Ed, Harcourt Publishers, 2001.**