It of another organism. the final step will

It is then joined with the DNA of another organism.

The final step will be to allow this “ desired gene” to express itself in an appropriate host or new environment and to produce the gene product. In several cases the gene product will be isolated.

Major Steps of Genetic Engineering:

1. Isolation of the Desired Gene: Specific DNA of fragments or desired genes are to be identified. They are then isolated and purified for being transferred to an appropriate host. This DNA of interest is also called passenger DNA, target DNA or donor DNA. Isolation of DNA is done by any one of the following biochemical method. (a) Extraction of total cell DNA.

(b) Synthesis of complementary DNA [cDNA] 2. Selection and Isolation of a Vector: If a desired gene is to be introduced into a host cell, a carrier molecule that can transport the gene into the host cell is required. Such a molecule is called a cloning vehicle, carrier molecule or a vector. A vector is a self replicating molecule of DNA to which the donor gene can be linked. The vector should also be able to propagate in the host organism or cell. Commonly plasmids and the DNA of viruses are used as vectors.

A suitable vector is identified and then isolated. New techniques are now evolved to directly introduce the donor DNA into the host csll (Fig. 4). 3. Construction of Recombinant Vector: The donor DNA is inserted into the vector to produce a donor-vector hybrid DNA molecule.

This hybrid is also called recombinant DNA a chimera or a recombinant vector as it contains DNA from unrelated sources. This recombinant DNA can

now be introduced into a host cell. 4. Introduction of the Recombinant Vector into the Host Cell: The recombinant vector is introduced into a suitable host cell.

Since most bacteria and eukaryotic cells can take up naked DNA molecules from the medium under suitable conditions they are used as host cells. Such host cells are also called target cells. Inside the host cell the recombinant DNA is either linked to the host chromosome or maintained free in the cytoplam. If viruses are used as vectors, the synthetic viruses carrying the recombinant DNA infect the target cells.

The genome of such sythetic virus contains the gene of interest. This gene is present in the place of a segment of viral DNA that is not essential for its replication. Inside the target cell the recombinant vector DNA may multiply.

Thus numerous copies of the recombinant DNA are formed. This means, the desired gene it carries is also multiplied. 5. Selection and Multiplication of the Host Cells Carrying the Recombinant DNA Molecule: The cells which have successfully taken up the recombinant DNA molecule are selected. These cells are allowed to divide.

After a large number of cell division, a colony of identical host cells is produced. At the cellular level it is a clone of cells. Each cell in clone carries one or more recombinant DNA molecules. Thus the desired gene carried by the recombinant DNA is cloned.

6. Expression of the Desired Gene: The desired gene can now express itself in the body of the host organism and produces the desired trait. In many

cases of genetic engineering the desired gene will produce the gene product in the new environment which can be isolated and purified, e. g.

, synthesis of human insulin, synthesis of somatostatin etc.

Applications of Genetic Engineering:

1. R-DNA technology in nitrogen fixation. i. Introduce nif-gene into several other microbe.

ii. To increase host range iii. To transfer nif-genes from bacteria to plants. 2. Transgenic plants Tt with Herbicide Resistance. i. Atrazine resistant oil seed (Brassica- ïàðò). ii.

Atrazine is detoxified by glutathione-s- transferase. 3. Transgenic Glyphosate tolerant plants glyphose is an active components of many herbicide. 4. Genetically Engineered plants that Resists/repel pests and pathogens.

Bacillus thuringensis crop: cotton, maize, Brassica. i. NAPI-Nicogene natural insecticide effective against many pest from Nicotiana alata. 5. Genetically Engineered Protection Against viruses in transgenic Plants.

i. The presence of one virus in plants can interfere with infection by another virus or strain. This is called cross protection. ii. Coat protein. 6. Gnetically Engineered plants are used to produce Novel Compounds. i.

Transgenic Banana with haptitis virus. ii. GMO tobacco plants to produce interferon. 7. Plants that produce plastic: i. Transgenic Arabidopsis thaliana to produce polyhydroxy butyrate (PHB). 8. Transgenic plants to Improve Photosynthetic Efficiency.

Modification of RUB I SCO to favour up take co2 rather than o2. 10. To delay senescence. i. Tomato > " flavour savour" variety to delay ripening.

ii. Carnation – Antisence RNA technology for delay flower senescence. 11. Golden Rice.

i. ?-Carotin gene is inserted in rice to improve vitamin -A content.

Transformation Techniques:

The uptake of foreign DNA or transgenes by plant cell is called transformation. A variety of technique have been used to introduce transgenes into plant cells. 1. Agro bacterium mediated.

(à) Co-culture with tissue Explants. (b) In Planta transformation. 2. Agro infection: Introduction of a viral genome into plant by placing with in the T-DNA of a Ti Plasmid and using the Agro becterium containing this recombinant plasmid for co-culture with plant cell is called Agro infection. 3. Direct gene transfer.

(Biolisitic method). (a) Chemical methods [(Poly ethylene glycol) PEG], (b) Electroporation. (c) Particle gun delivery. (d) Lipo fection.

(e) Micro injection. (f) Macro injection. (g) Pollen transformation. (h) Delivery via growing pollen tubes. (i) Laser induced. (j) Fiber -mediated gene transfer.

Transgenic Plants:

The technology of Gentic Engineering is the practice of altering or disturbing the gentic blue prints of living organism. Transgenic for transfer the desirable qualities from one organism to another. Vectors: R-DNA technology

is based on the insertion of a DNA fragments (gene) into a suitable cloning vector and then producing into a suitable host to propagate the recombinant DNA. 1.

Plasmids: Circular, double standard, extrachromosomal self replicating DNA molecule. PlasmidsSize (nucleotide length in kb)PBR 3450. 7PBR 3224. 363Col E16.

36Other Vector: VectorCap ability (Kbp)1. Cosmid45-482. ÀÑ153.

ÐÀÑ154. Phagemid15-60Identification of transgenic plants by reporter gene. Certain marker genes on which no selection pressure is imposed are need to identify the transformed cells/Plants. Such gene are called reporter gene. Two types: 1. Selectable marker: Cell which persent survive under selective condition Eg.

kanamycin, Neo mycine. 2. Scorable marker: Produce different phenotype which allow an easy identification of R-DNA cell Eg. SVS, ?- galactriodase.

Marker Genes used for Gene Transfers into Plants:

Table 2: some of the market genes used for gene used for gene transfers into plants.

GeneScorable/SelectableCharateristicnos (Napoline synthase)ScorableNopaline productioncat (Chlormaphemicol acetyi transfersase)ScorableAcetylation of chloramphenicollux (Luciferase)ScorablePhosphorescencegusA (P-galactosidase)ScorableBlue colourdhfr (dihydrofolate reductase)SelectableMethotrexate resistancenptll (Neomycin phosphotranserase)SelectableKanamycin, neomycin and G 418

resistancehptIV (Hygromycin phosphotransferease)SelectableHygromycin resistancebar (PhosphinothricinSelectablePhosphinothricin resistance

Important Transgenes Encoding Valuable Polypeptides/Enzymes Expressed in Plants:

Polypeptide/EnzymeSourceApplicationsPOLYPEPTIDESEnkephalin ’HumanOpiate activity. HirudinSyntheticThrombin inhibitor.?-TrichosanthinChinese medicinal plantInhibition of HIV replication.

Epidermal growth factorHumanProliferation of specific cells. ErythropoietinHumanRegulation of erythrocyte levels. Growth hormoneTroutGrowth stimulation. Human serum albuminHumanPlasma expander. interferonHumanAnti-viral activity. ENZYMESa-AmylaseBacillus licheniformisLiquefaction of starch.(1-3, 1-4)-?-GlucanaseTrichoderma reesei; Hybrid of two Bacillus speciesBrewing.

Manganese-dependent lignin peroxidasePhanerochaete chrysosporiumBleaching and pulping of paper. PhytaseAspergillus nigerIncreased phosphate utilization from feed. XylanaseClostndiuw thermocellum; Animal feed, paper and pulp. Cryptococcus olhidus Major Risk of Transgenics: 1. Escape of Enginerred genes by gene flow or gene dispersal.

2. Non target effects or ecological effects. 3. Invasiveness or weediness of transgenics.

4. Creation of super weeds and super-virus. 5. Toxicity and allergenicity to human beings and animals.

6. Expression of undesirable phenotypic traits. 7. Erosion of Biological diversity. Risk Management: 1. First to outline the risk assessment. 2. Isolation zone.

3. Trap crop. 4. Refuge crop.

5. Male sterility. 6. Removal of flower from transgenic plants.