Transgenic Plants

The big five successful traits

- ✓ Insect Resistance
- ✓ Delayed Fruit Ripening
- ✓ Nutritional Enhancing
- ✓ Herbicide Resistance
- ✓ Virus Resistance

INSECT RESISTANT TRANSGENIC PLANTS

BT ENDOTOXINS AND THEIR GENES:

- **Bt toxins = Cry meaning crystalline (reflecting the crystalline appearance of δ -endotoxin)**
- **The cry gene of B. thuringiensis produces a protein, which forms crystalline inclusions in the bacterial spores.**
- **Crystal proteins : Responsible for the insecticidal activities of the bacterial strains.**
- **Cry genes : either code for a 130 kDa or a 70 kDa protein**

Initially classified into four distinct classes, based on their host range.

- **CryI : Active against Lepidoptera**
- **CryII : Active against Lepidoptera and Diptera**
- **CryIII : Active against Coleoptera**
- **CryIV : Active against Diptera**
- **Subsequently, CryV and CryVI groups were added; primarily nematicidal in action**

TOXIC ACTION OF CRY PROTEINS

- **Cry proteins → ingested by insects → dissolved in the alkaline juices present in the midgut lumen.**
- **The gut proteases process them hydrolytically : release the core toxic fragments.**
- **The toxic fragments of Cry proteins have 3 domains:**
- **Domain I : Functions in pore and ion channel formations**
- **Domain II : Involved in receptor recognition,**
- **Domain III : Binds the receptor.**

- **Toxic fragments : bind to specific high-affinity receptors present in the brush border of midgut epithelial cells**

- **As a result, the brush border membranes develop pores, : nonspecific in nature**

- **Permits influx into the epithelial cells of ions and water,**

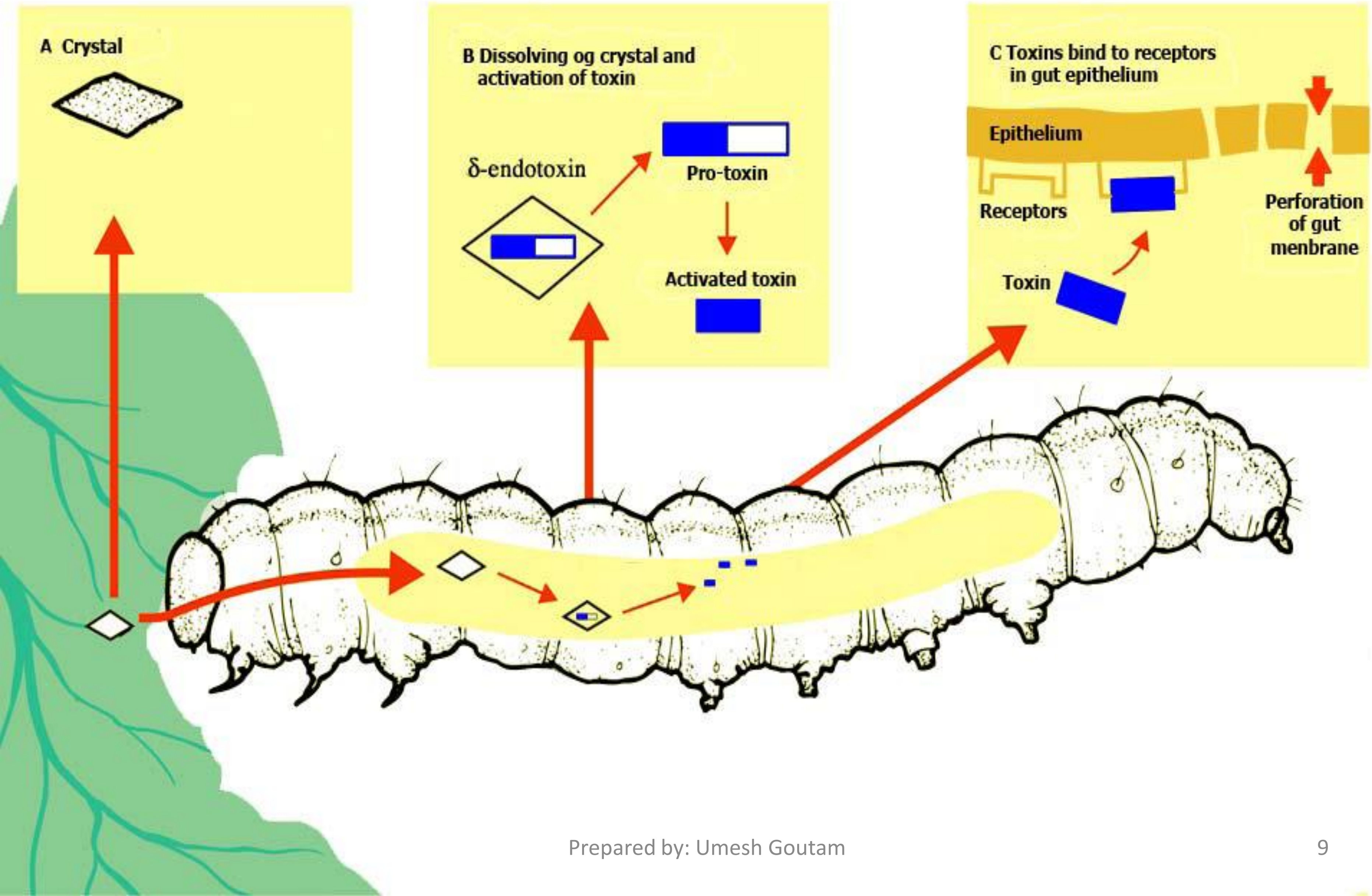
- **As a result, the gut is rapidly immobilized, swelling, and lyses of epithelial cells**

- **Larva stops feeding, and the gut pH is lowered by equilibration with the blood pH.**

- This lower pH enables the bacterial spores to germinate
- Bacterium can then invade the host, causing lethal septicaemia.
- Reason for Cry toxin Specificity:

  Presence of specific receptors in the midgut epithelium

Mechanism of toxicity of Crystal proteins



Virus Resistant Transgenic Plants

Transgenic Approach

- **PDR: pathogen-derived resistance**
- **Pathogen genomic sequences** are **engineered** into **host plant's genome** in such a way that sequence may express at

an inappropriate time, amount or form during infection cycle to induce resistance in plant.

Approaches used to engineer plants for virus resistance:

(I) Coat protein gene,

(II) cDNA of satellite RNA,

(III) Defective viral genome,

(IV) Antisense RNA approach, and

(V)Ribozyme-mediated protection

- **Strategies based on genes derived from the pathogenic viruses themselves.**
- **Disease resistance generated by employing pathogen genes = Pathogen-derived resistance (PDR).**

Virus Coat or Capsid Protein Gene

- TPs having virus coat protein gene linked to a strong promoter : In crop plants, e.g., tobacco, tomato, alfalfa, sugarbeet, potato, etc.

- The first TP of this type was tobacco

- Contained the coat protein gene of tobacco mosaic virus (TMV) strain U1.

- Plants were inoculated with TMV U1: symptoms either failed to develop or were considerably delayed.

- Less accumulation of virus than in the control plants in both inoculated and systemically infected leaves.

- Expression of a virus coat protein gene confers resistance to the concerned virus

- **The effectiveness of coat protein (CP) gene in conferring virus resistance : affected by both the amount of coat protein produced in transgenic plants and by the concentration of virus inoculum.**

- **Resistance generated by CP : Blocking of the process of uncoating of virus particles**

- **Necessary for viral genome replication as well as expression.**

cDNA of Satellite RNA

- **Some RNA viruses have small RNA molecules= satellites**
- **Depend on the viral genomes for their replication and transmission, but are not necessary for viral functions.**
- **Gets packaged with viral genome to cause infection elsewhere**
- **Satellites either increase or decrease the severity of disease produced by the virus carrying it.**
- **Presence of sat-RNA leads to reduction in severity of disease symptoms: used for developing resistance against specific viruses.**

- The cDNA copies of satellites that reduce disease severity have been integrated into host genomes

- Expression of the satellites : reduce disease symptoms as well as virus accumulation

- For example, tobacco plants expressing the satellites of cucumber mosaic virus (CMV) showed reduced disease symptoms when infected with CMV

Defective Viral Genomes

- **Defective or deleted genomes of some RNA and DNA viruses disrupt the replication of complete genomes of those viruses with which they are associated.**

- **Plants expressing such viral sub-genomic DNAs show decreased disease severity due to the concerned viruses.**

- **Ex: African cassava mosaic virus (ACMV) genome**

- **ACMV Consists of two ss- DNA molecules designated as A- and B-DNAs.**

- **In addition, a 50% deleted B-DNA is also found associated with ACMV particles.**

- **Tobacco plants containing this deleted B-DNA integrated in their genomes showed reduced systemic spread when they were infected with ACMV**

Antisense RNA Approach

Antisense RNA molecules : interact with mRNA molecules by base-pairing to form double-stranded RNA.

IDEA EXPLOITED FOR BROAD SPECTRUM RESISTANCE

- **Inhibit expression of a gene responsible for a non-essential host cell enzyme, required for virus replication : Get resistance against a broad spectrum of viruses**

- **Enzyme S-adenosyl homocysteine hydrolase (SAHH) was chosen for manipulation.**
- **Responsible for several methylation reactions including 5'-cap mRNA methylation**
- **S-adenosyl- methionine is used as methyl donor.**
- **If SAHH is inhibited, there will be under-methylation at the 5' end of viral mRNA leading to inhibition of viral replication.**
- **SAHH inhibitions : used as antiviral agents**

Ribozyme-Mediated Protection

- **Ribozymes = RNA molecules that exhibit enzyme activities.**
- **The strategy consists of:**

(i) producing a ribozyme specific to a part of the target virus genome,

(ii) to produce cDNA of this ribozyme and

(iii) to integrate it into the host plant genome.

Transgenic tobacco plants expressing ribozymes against TMV showed some resistance to TMV infection

Commericialization of virus resistant plants

- **Papaya: Transgenic lines (SunUp and Rainbow) developed at Cornell University**
- **Potato: Transgenic lines having**

Bt gene and resistance against PLRV and PVY

Delayed Fruit Ripening

- **Allow for crops, such as tomatoes, to have a higher shelf life.**

- Tomatoes generally ripen and become soft during shipment to a store.

- Tomatoes have been engineered to produce less ethylene so they can develop more taste before ripening, and shipment to markets

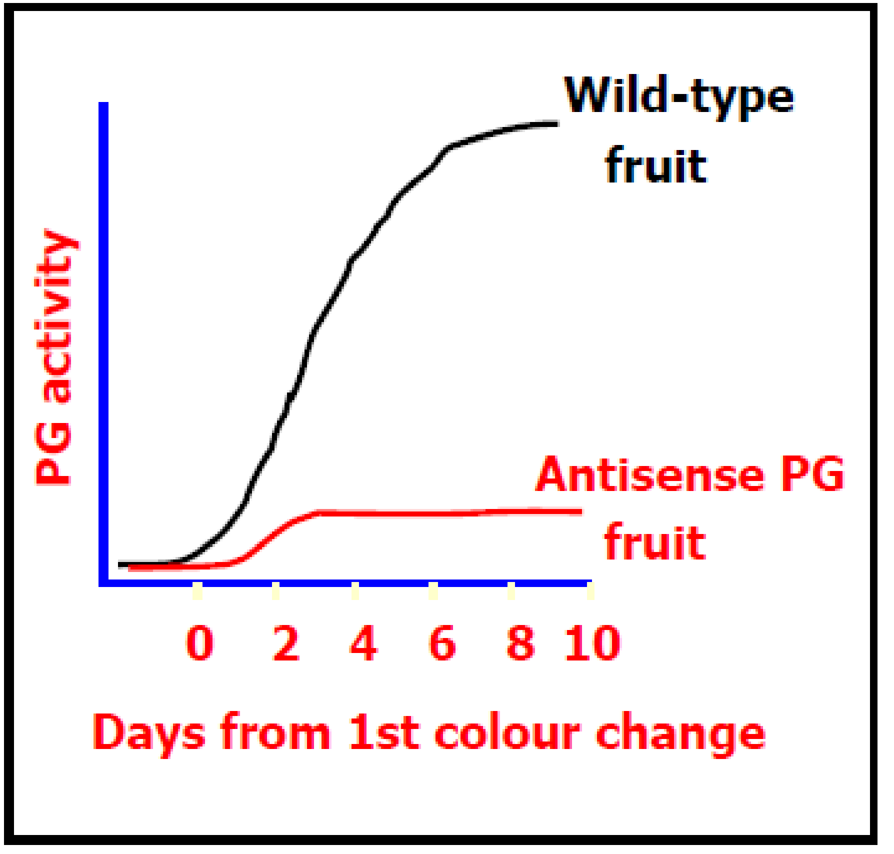
Altering Fruit Ripening with Antisense RNA

Polygalacturonase (PG) is an enzyme that breaks down pectin in ripening fruit walls.

Plants with an antisense PG transgene produce less PG.

Walls soften more slowly.

Many genes manipulated in the same way to answer basic questions: what is the role of hormones in ripening; what do particular enzymes do in fruit walls?



Nutritionally Enhanced Plants

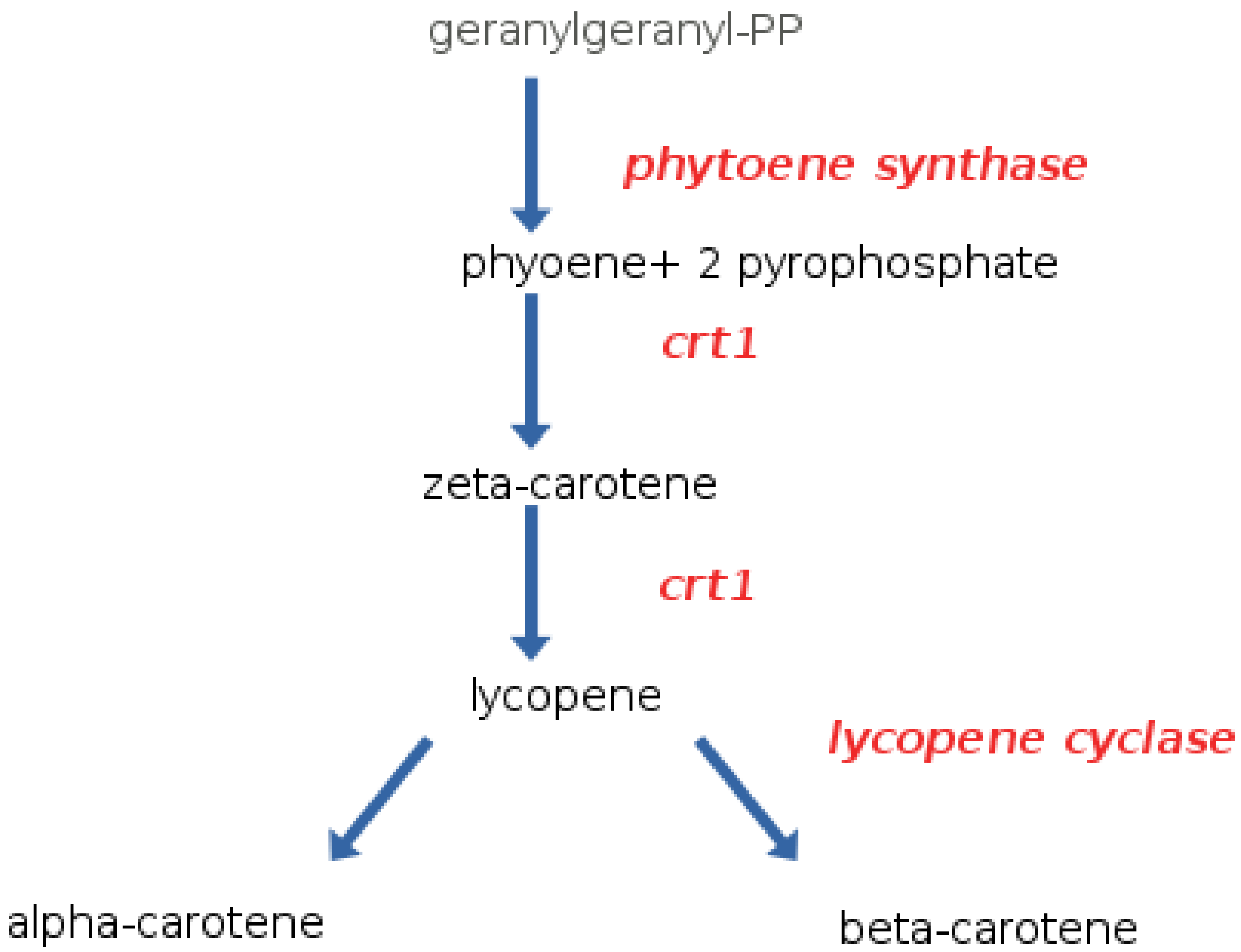
- Golden Rice was genetically engineered to produce high levels of beta-carotene, which is a precursor to vitamin A. Vitamin A is needed for proper eyesight.

- Other enhanced crops include iron-enriched rice and tomatoes with three times the normal amount of beta-carotene

- Golden rice was created by transforming rice with two beta-carotene biosynthesis genes:

- *psy* (phytoene synthase) from daffodil (*Narcissus pseudonarcissus*)

- *crtI* (carotene desaturase) from the soil bacterium *Erwinia uredovora*


geranylgeranyl-PP
phytoene synthase
phyoene+ 2 pyrophosphate
crt1
zeta-carotene
crt1
lycopene
lycopene cyclase
alpha-carotene
beta-carotene

Golden Rice

Transgenic technology produced a type of rice that accumulates beta-carotene in rice grains. Once inside the body, beta-carotene is converted to vitamin A.

"Normal" rice

"Golden" rice

What happened to the Flavr Savr tomato?

- Produced by blocking the polygalacturonase (PG) gene, which is involved in spoilage.
- Plants were transformed with the anti-sense PG gene, which is mRNA that base pair with mRNA that the plant produces, essentially blocking the gene from translation.
- First genetically modified organism to be approved by the FDA, in 1994.

HERBICIDE RESISTANT TRANSGENIC PLANTS

GLYPHOSATE ACTION

- **Glyphosate is a broad-spectrum herbicide**

- Active ingredient in Roundup herbicide

- **Inhibits a key enzyme** (***EPSP synthase***) in an amino acid pathway

- **Enzyme EPSPS is involved in aromatic amino acid**
- **biosynthesis in plants.**

- **Plants die because they lack the key amino acids**

- **A resistant EPSP synthase gene allows crops to survive spraying**

Roundup Sensitive Plants

Shikimic acid + Phosphoenol pyruvate

↓ **+ Glyphosate**

Plant EPSP synthase ✗

3-Enolpyruvyl shikimic acid-5-phosphate (EPSP) ✗

↓

Aromatic amino acids ✗

Without amino acids, plant dies

Roundup Resistant Plants

Shikimic acid + Phosphoenol pyruvate

+ Glyphosate

Bacterial EPSP synthase

RoundUp has no effect; enzyme is resistant to herbicide

3-enolpyruvyl shikimic acid-5-phosphate (EPSP)

With amino acids, plant lives

Aromatic amino acids

Roundup Ready™ Soybeans

Transgenic animals

INTRODUCTION

- A transgenic animal is one that carries a foreign gene that has been inserted into its genome.

- Foreign genes are inserted into the germ line of the animal, so it can be transmitted to the progeny.

- Transgenic technology has led to the development of fishes, live stock and other animals with altered genetic profiles which are useful to mankind.

- First transgenic animal was a ‘***Supermouse***’ created by Ralph Brinster (U Pennsylvania) and Richard Palmiter (University of Washington) in 1982.

- It was created by inserting a human growth hormone gene in mouse genome.

- The offspring was much larger than the parents.

- Mouse – common transgenic experiment.

- Other animals include pig, goat, cow, sheep, fish etc.

PRODUCTION OF TRANSGENIC ANIMALS – THE METHODOLOGY

- **Step 1 – Construction of a transgene**

Transgene made of 3 parts:

1) Pomoter
2) Gene to be expressed
3) Termination sequence

- **Step 2 – Introduction of foreign gene into the animal**

1) Pronuclear microinjection method

2) Embryonic stem cell method.

- The transgene containing solution is injected into the male pronucleus using a micropipette.

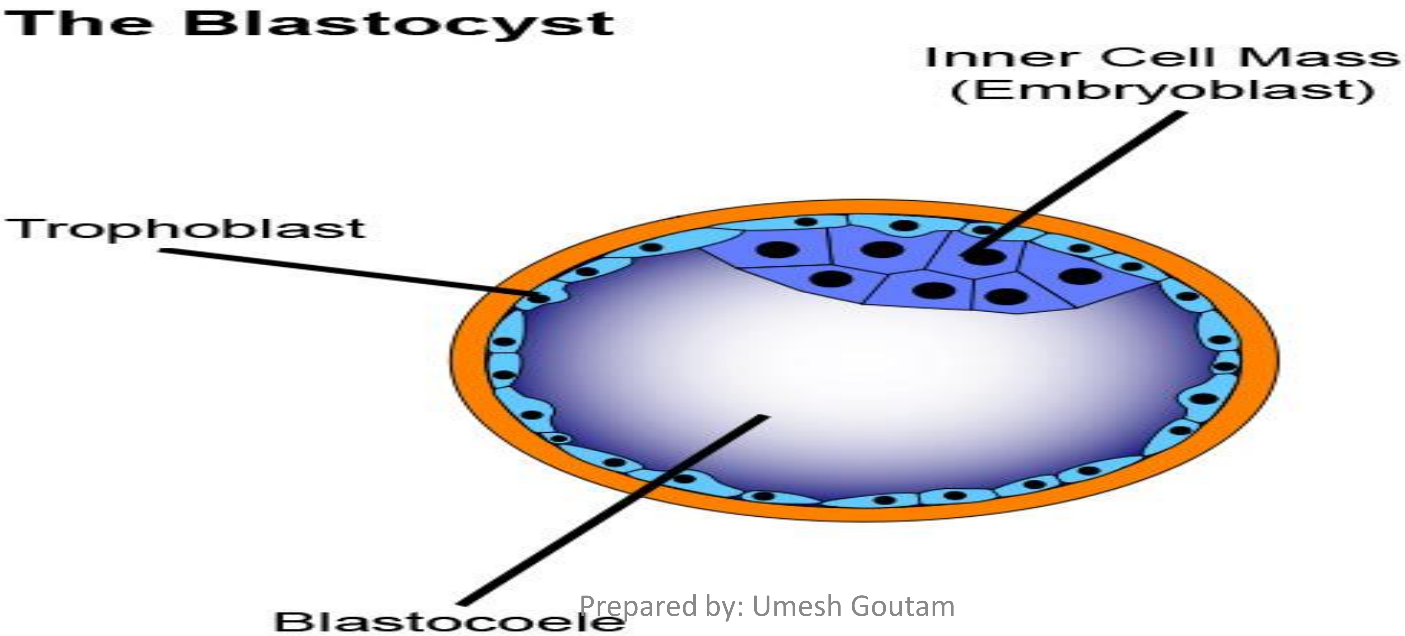
- Eggs with the transgenes are kept overnight in an incubator to develop to a 2 cell stage.

- The eggs are then implanted into the uterus of a pseudo - pregnant female

EMBRYONIC STEM CELL METHOD

- Transgenic animals can be created by manipulating embryonic stem cells.

- ES cells are obtained from the inner cell mass of a blastocyst.

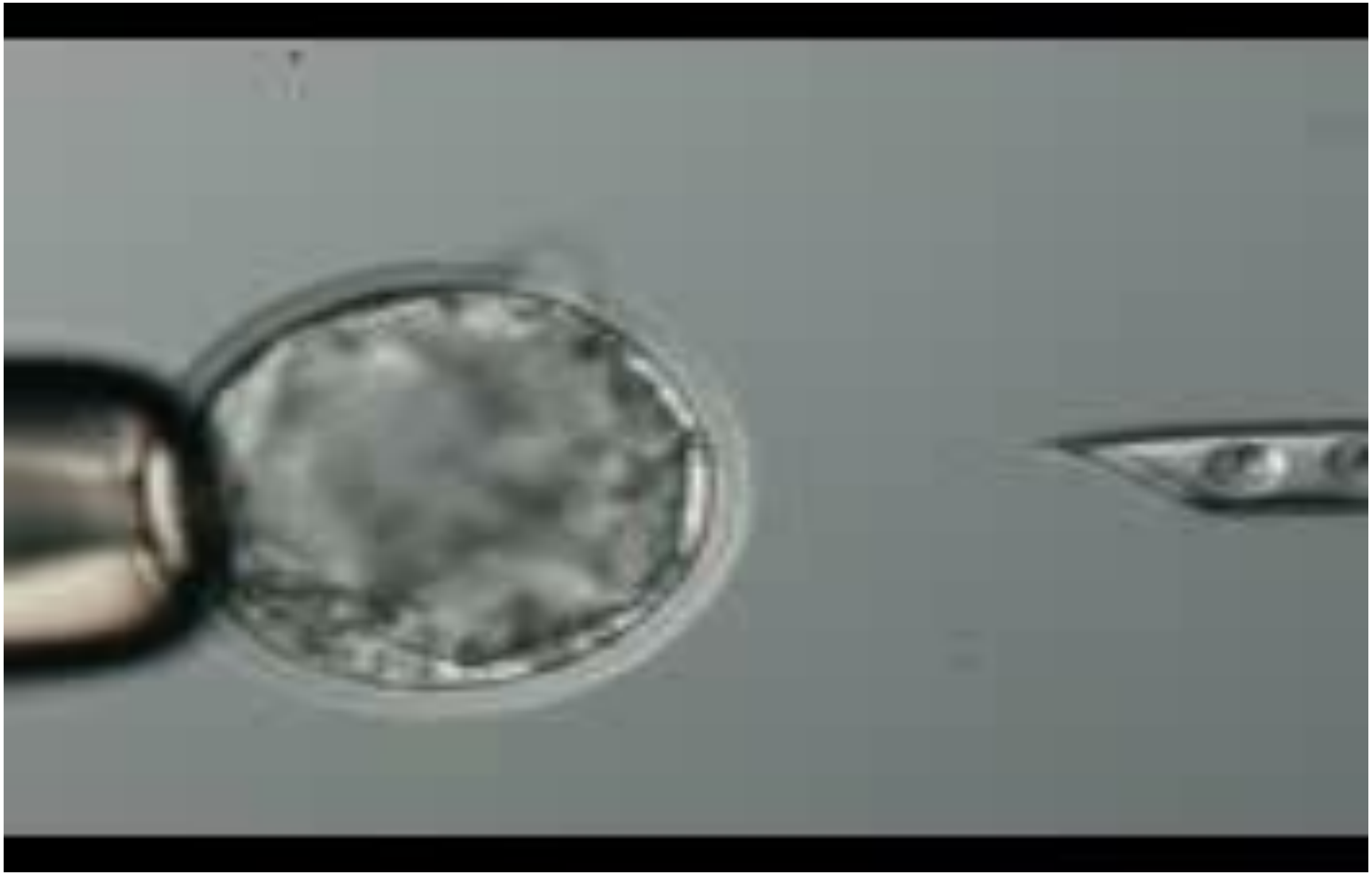


- Transgene is incorporated into the ES cell by
  - Microinjection
  - By a retro virus
  - By electroporation

- Transgenic stem cells are grown in vitro.

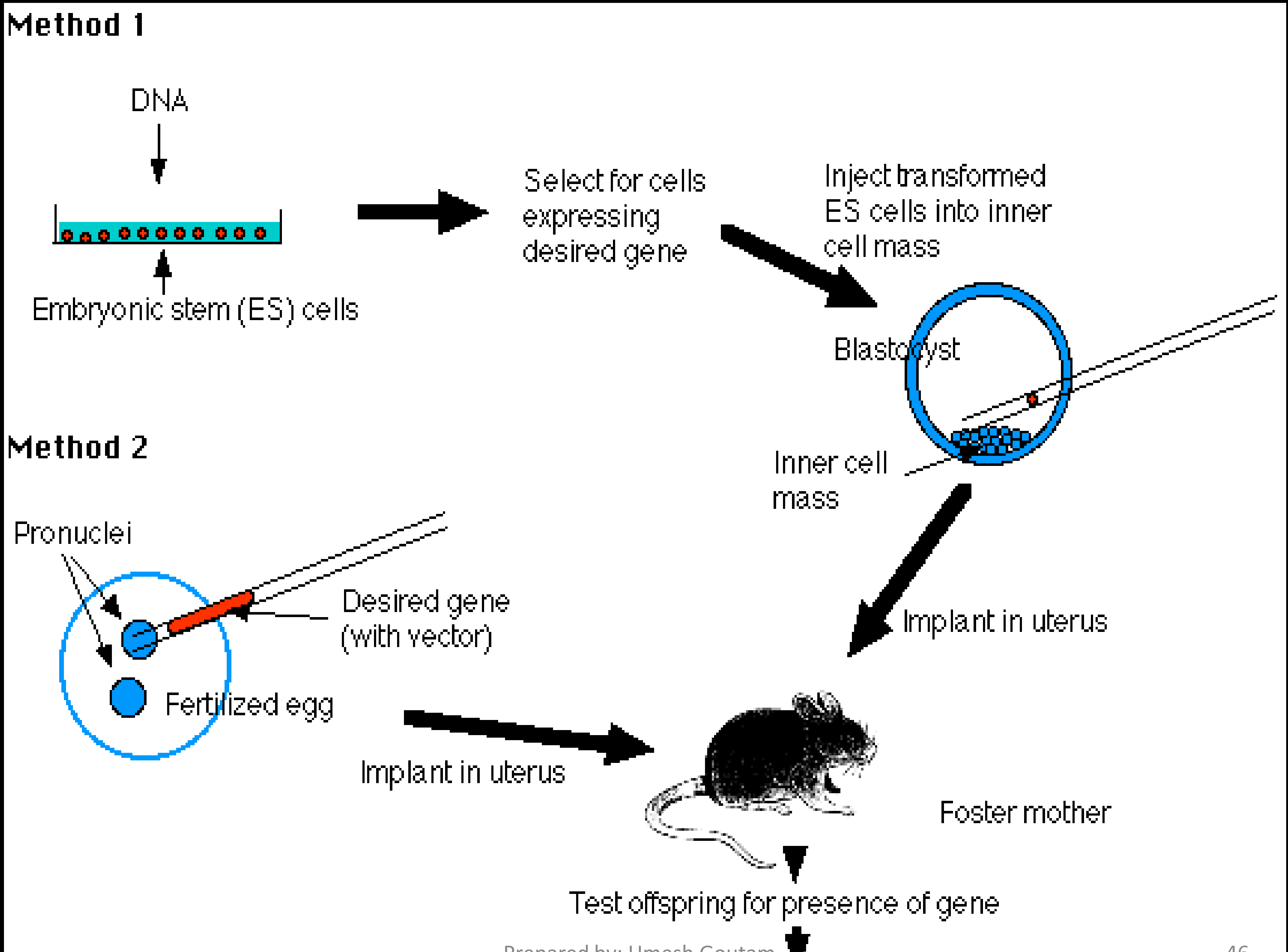
- Then they are inserted into a blastocyst and implanted into a host’s uterus to grow normally.

BLASTOCYST MICROINJECTION



- **Step 3: Screening for transgenic positives**
  - Transgenic progenies are screened by PCR to examine the site of incorporation of the gene

- **Step 4: Further animal breeding is done to obtain maximal expression.**


Method 1
DNA
Embryonic stem (ES) cells
Select for cells expressing desired gene
Inject transformed ES cells into inner cell mass
Blastocyst
Inner cell mass
Method 2
Pronuclei
Desired gene (with vector)
Fertilized egg
Implant in uterus
Implant in uterus
Foster mother
Test offspring for presence of gene

PROBLEMS

- Multiple insertion – too much proteins
- Insertion into an essential gene – lethality
- Insertion into a gene leading to gene silencing
- Insertion into a different area can affect the gene regulation

SOME EXAMPLES OF TRANSGENIC ANIMALS

TRANSGENIC FISH

- **Superfish**
  - Increased growth and size
  - Growth hormone gene inserted into fertilized egg.
  - Transgenic salmon grows about 10 – 11 times faster than normal fish.

- **Glo fish**
  - GM freshwater zebra fish (*Danio rerio*)
  - Produce by integrating a fluorescent protein gene from jelly fish into embryo of fish.

TRANSGENIC MOUSE

- **Alzheimer's mouse**
  - In the brain of Alzheimer's patients, dead nerve cells are entangled in a protein called amyloid.
  - Mouse made by introducing amyloid precursor gene into fertilized egg of mice.

- **Oncomouse**
  - Mouse model to study cancer
  - Made by inserting activated oncogenes.

- **Smart mouse**
  - Biological model engineered to overexpress NR2B receptor in the synaptic pathway.
  - This makes the mice learn faster like juveniles throughout their lives.

- Alba, the EGFP (Enhanced Green Flurescent protein)
- Created in 2000 as a transgenic artwork.

TRANSGENIC MONKEY

- ANDi was the first transgenic monkey, born in 2000.

- An engineered virus was used to insert the harmless gene for green fluorescence protein (GFP) into ANDi's genome.

Buffalo calf

In 2010: Garima

2013: Mahima

IMPORTANCE OF TRANSGENIC ANIMALS

- Medical importance
  - Disease model
  - Bioreactors for pharmaceuticals

- Agricultural importance
  - Disease resistant animals
  - For improving quality and quantity of milk, meat, eggs and wool production

- Industrial importance
  - Toxicity sensitive transgenic animals to test chemicals.
  - Spider silk in milk of goat

ISSUES RELATED TO TRANSGENIC TECHNOLOGY

- Blurring the lines between species by creating transgenic combinations.

- There may be health risks associated with transgenics.

- There may be long term effects on the environment when transgenic animals are released into the field.

- Various bioethicist argue that it is wrong to create animals that would suffer as a result of genetic alteration.

CONCLUSION

- Transgenic technology is a field that is under constant evolution.

- Many transgenic animals have been successfully created for a variety of purposes, and the prospects are enormous.

- It holds great potential in many fields including agriculture, medicine and industry.

- With proper research and careful use the transgenic animals can go a long way in solving several problems for which