

Defining a gene function by mutational study in plant

Overview of the lecture

- ❖ Brief background on molecular biology

 - DNA, genetic materials, mechanism of transfer of genetic information

- ❖ How to elucidate the gene in the mutant plant

- ❖ Making the audience aware about the importance and power of molecular biology in enhancing the quality of our life



Plants are built with the same genetic material that we are made of



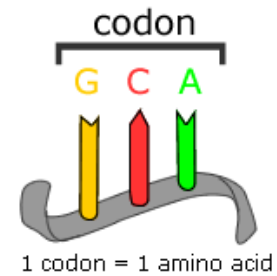
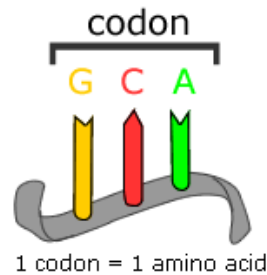
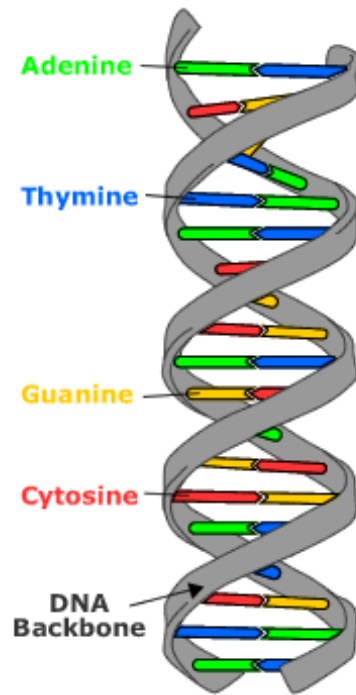
Like human or animals, they have chromosomes, where all the genetic information are stored



To process the genetic information the plants and animals essentially use the same mechanism



Finally, like human or animal the plant also respond to different stimulus such as light, temperature, gravity etc.



Deoxyribonucleic acid, or DNA, is a nucleic acid that contains the genetic instructions used in the development and functioning of all known living organisms

More information on DNA

The main role of DNA molecules is the long-term storage of information

DNA contains the instructions needed to construct other components of cells, such as proteins and RNA molecules. It is often called the blue print of life

The DNA segments that carry this genetic information are called genes

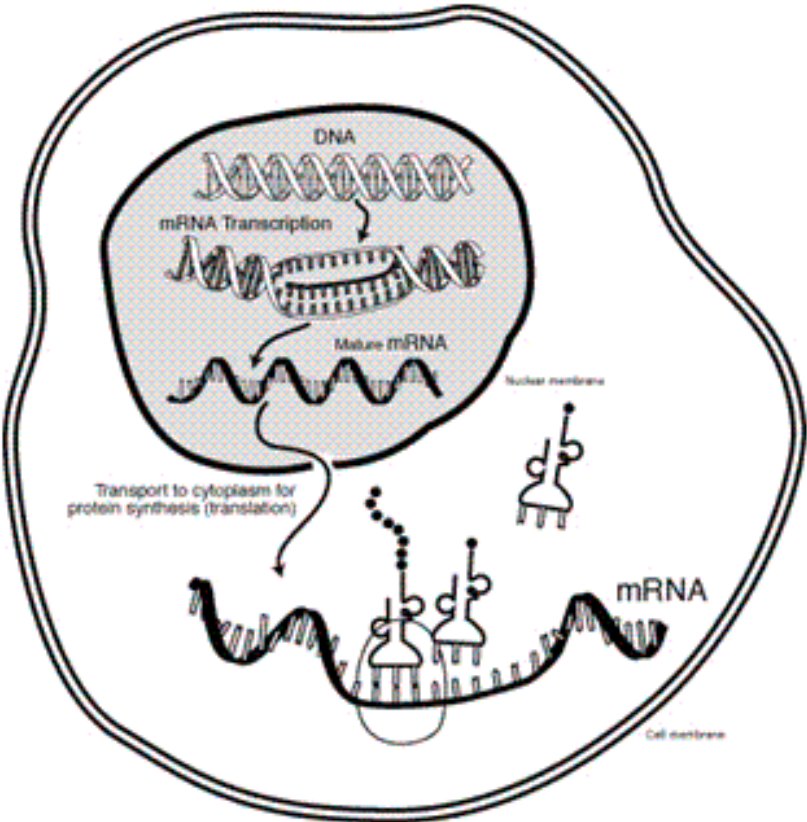
The other DNA sequences have structural purposes, or are involved in regulating the use of this genetic information

Within cells, DNA is organized into structures called chromosomes and the set of chromosomes within a cell make up a genome



Arabidopsis chromosome

Mechanism of transfer of genetic information



DNA

Genes

Chromosome

Genome

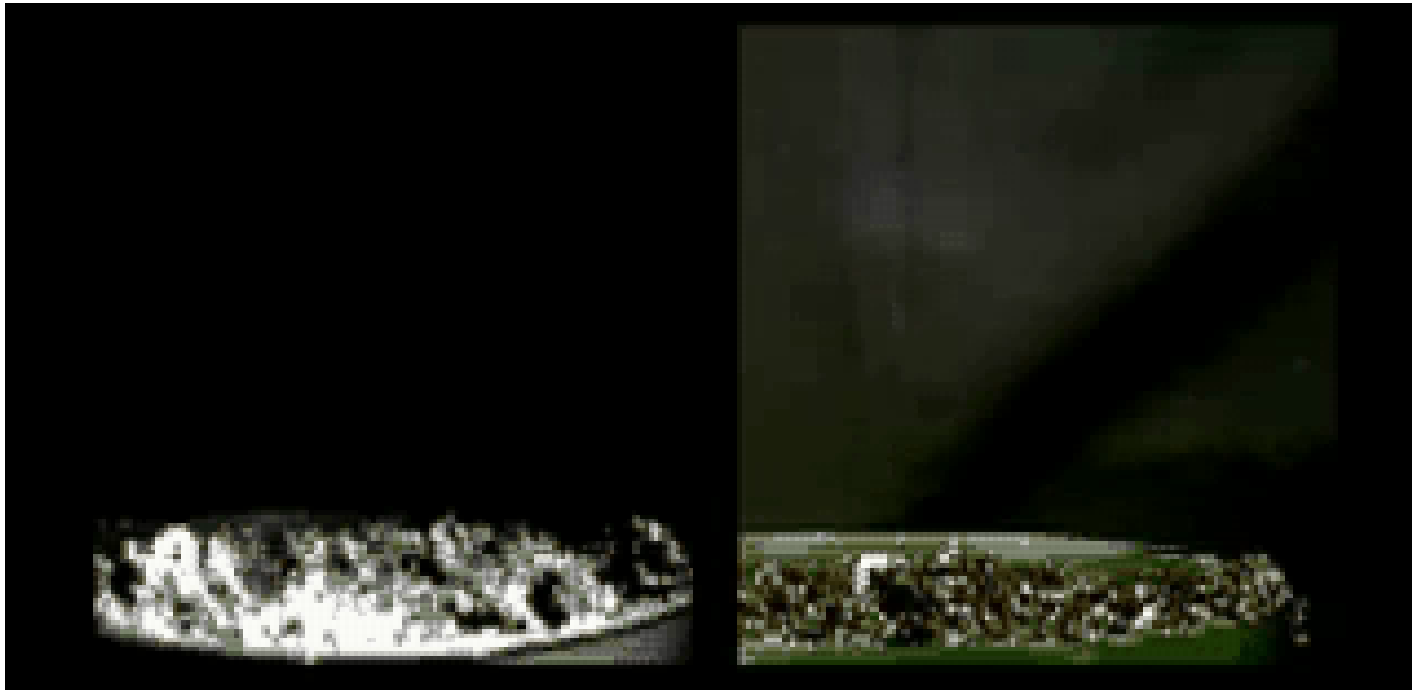
Mechanism of transfer of
genetic material

Plants response to different environmental stimulus



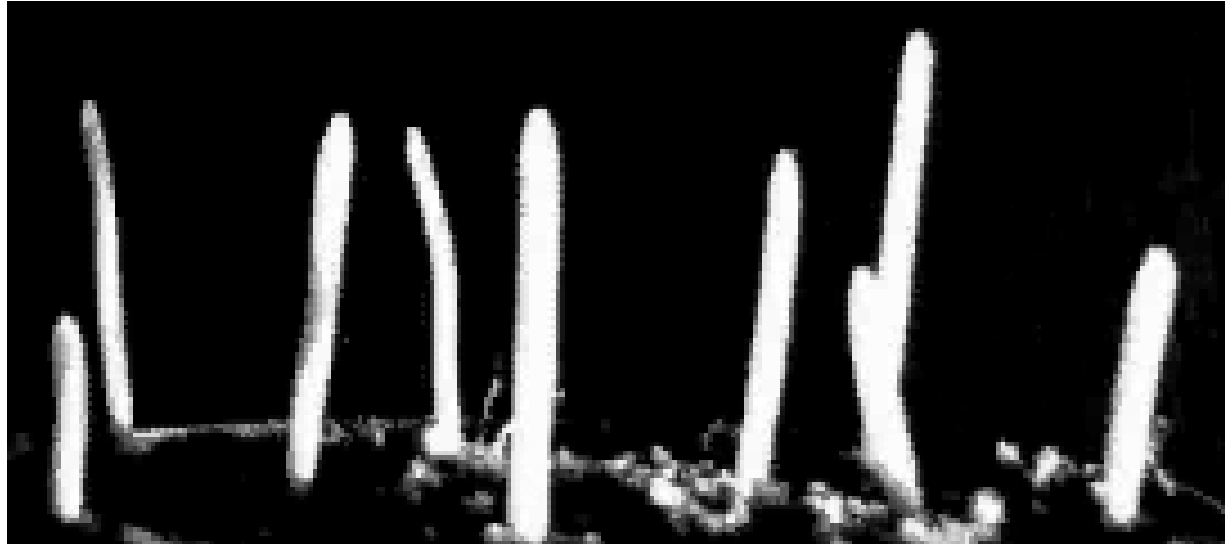
Arabidopsis germination

Courtesy: Roger Hangarter



Germination of Sunflower in light
and dark

Courtesy: Roger Hangarter



Corn phototropism

Courtesy: Roger Hangarter



Response to light

Courtesy: Roger Hangarter



Gravity response in root and shoot

Courtesy: *Roger Hangarter*



Sunflower solar track

Courtesy: Roger Hangarter

Questions:

1) What determines such responses of plants?

2) Is it possible to find out the factors that influence the plant responses to different stimulus?

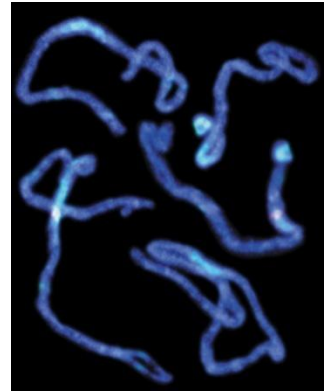
Model Plant

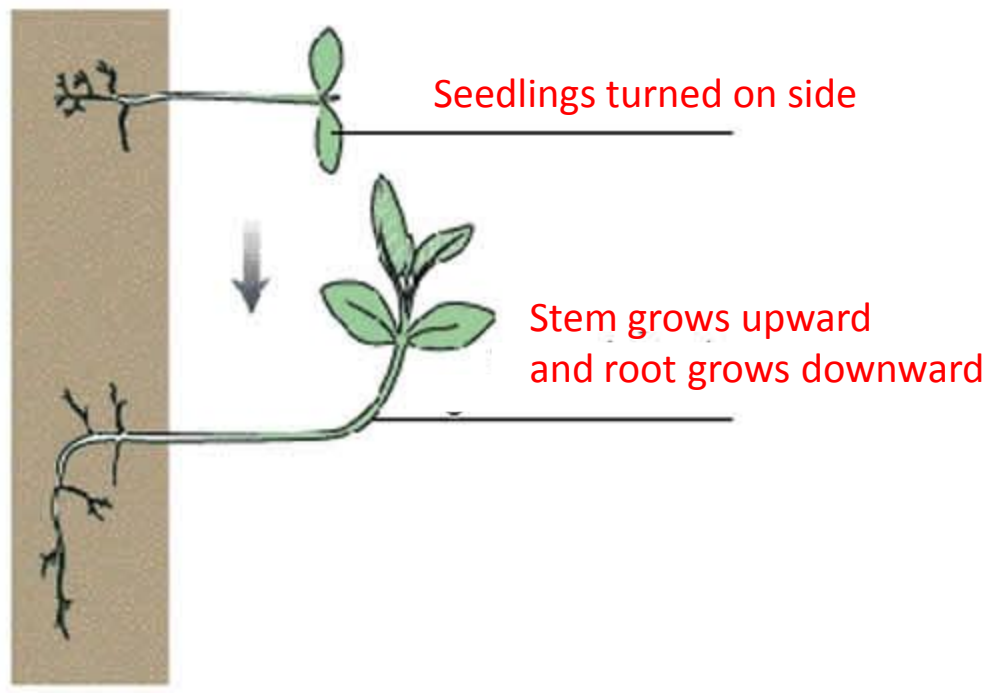
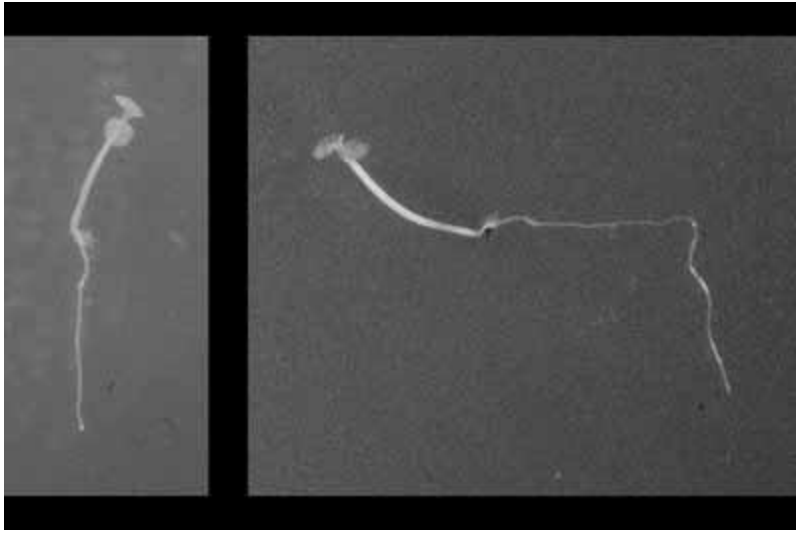
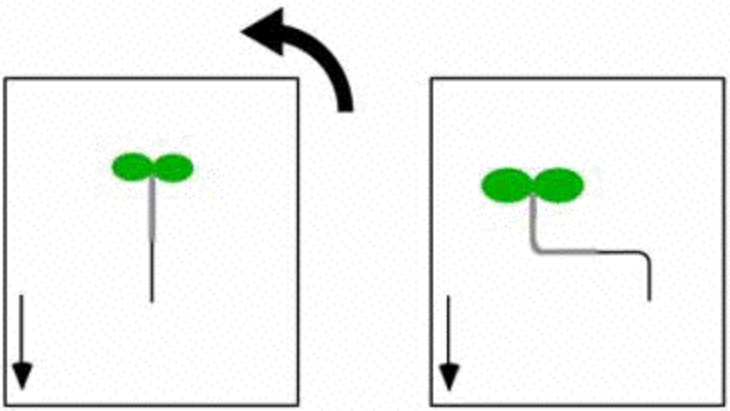


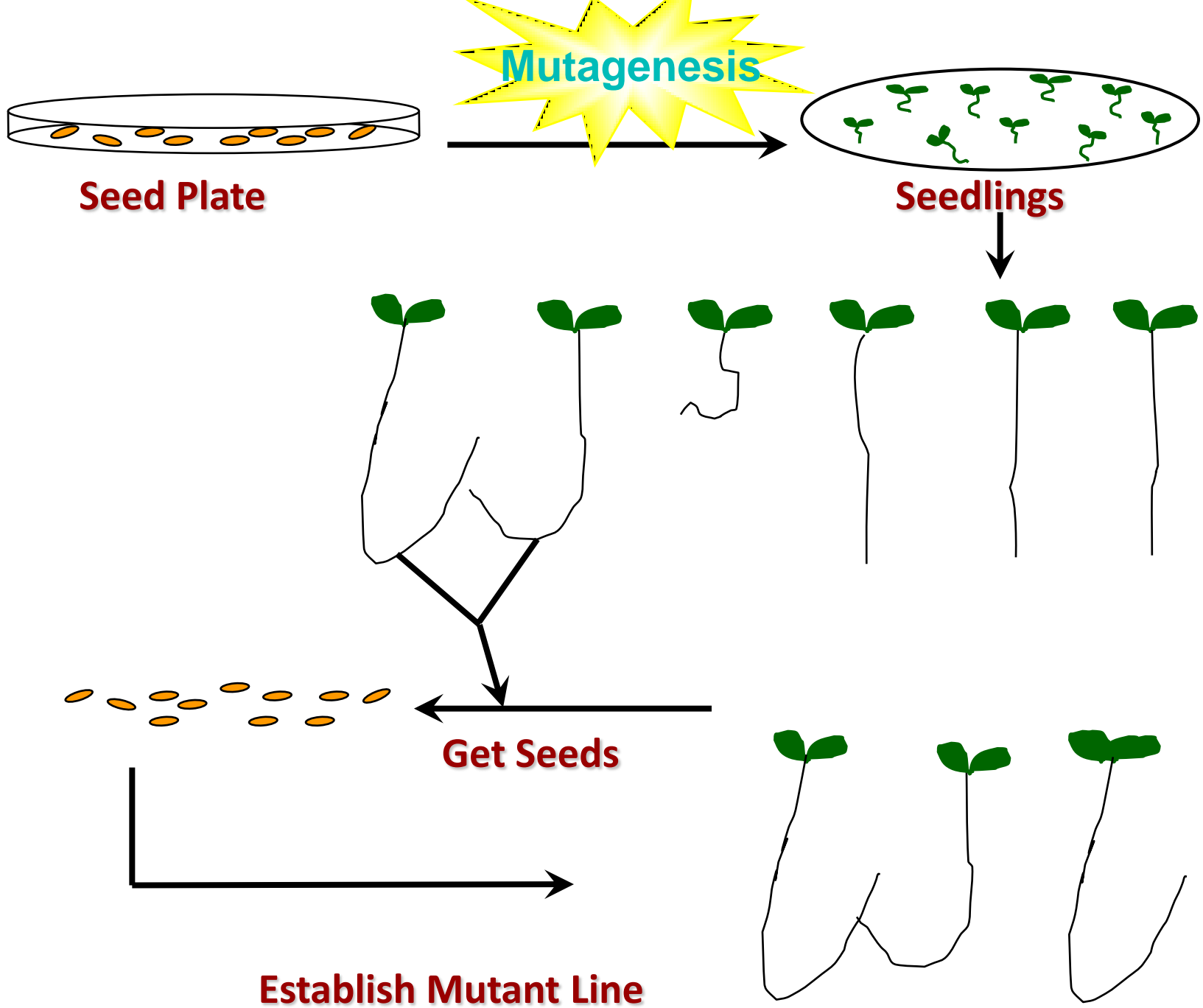
Arabidopsis thaliana

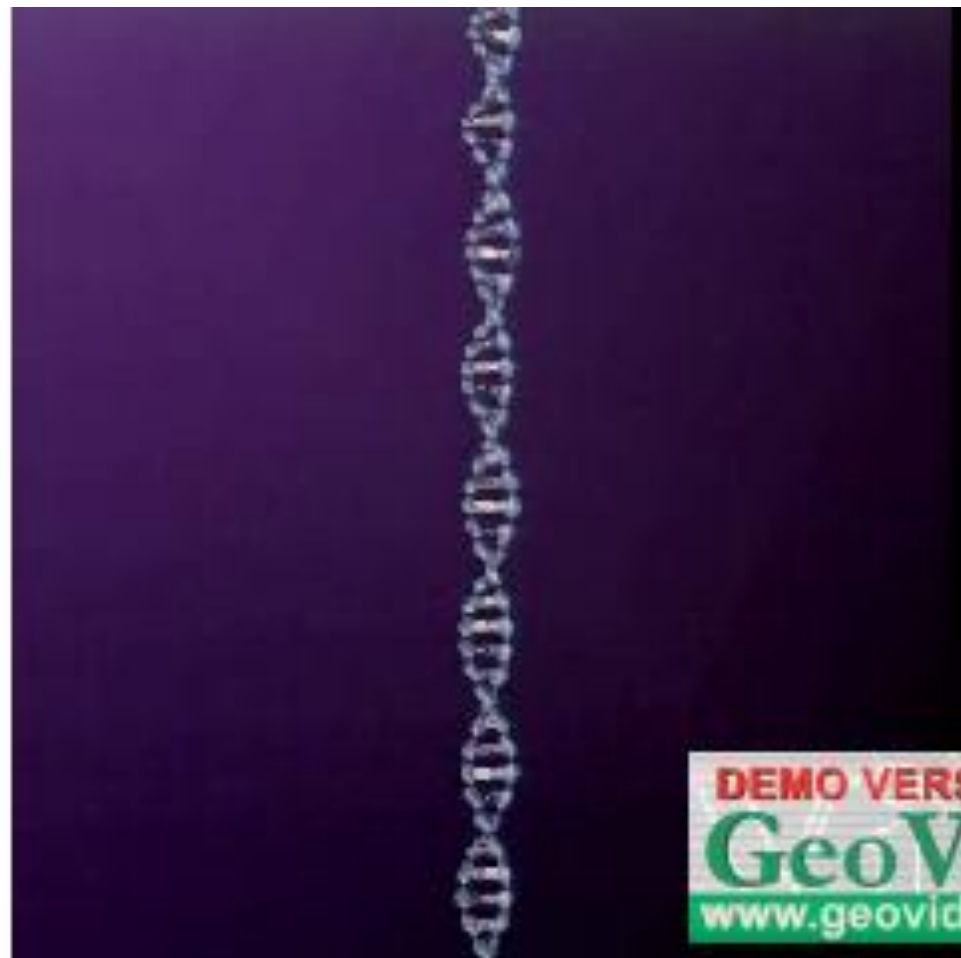
- ★ *Arabidopsis thaliana* has a small genome size
- ★ The whole genome has been sequenced
- ★ The genome of this plant is composed of 5 chromosomes which contains 125 Mb of DNA and 25,948 identified proteins
- ★ It has a shorter life cycle relative to other plants;
Takes only six weeks from germination to produce seeds
- ★ It is easy to grow. Virtually it grows everywhere
- ★ Finally transferring the gene in this plant is relatively easy compared to other plants

Arabidopsis chromosomes



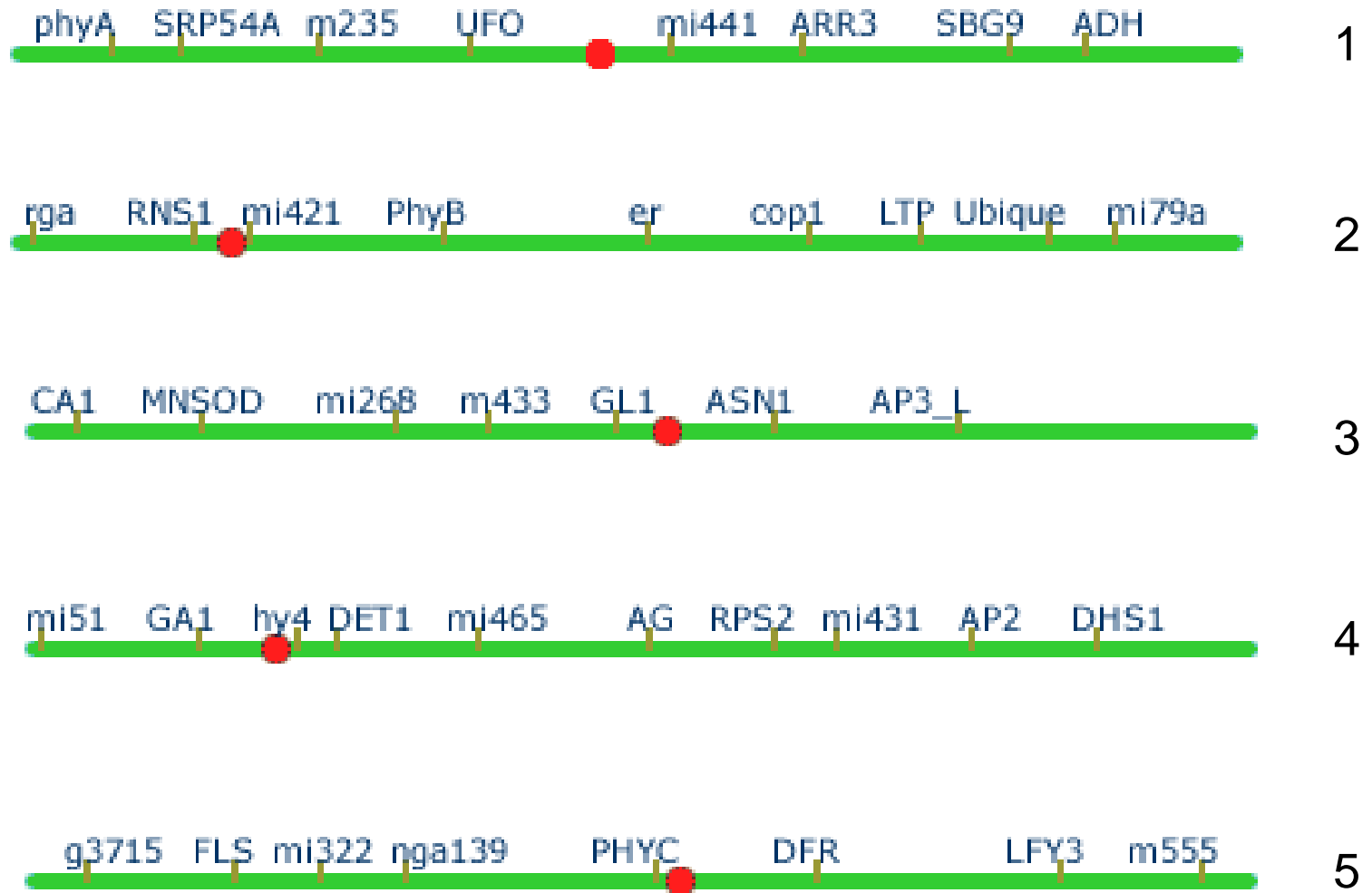






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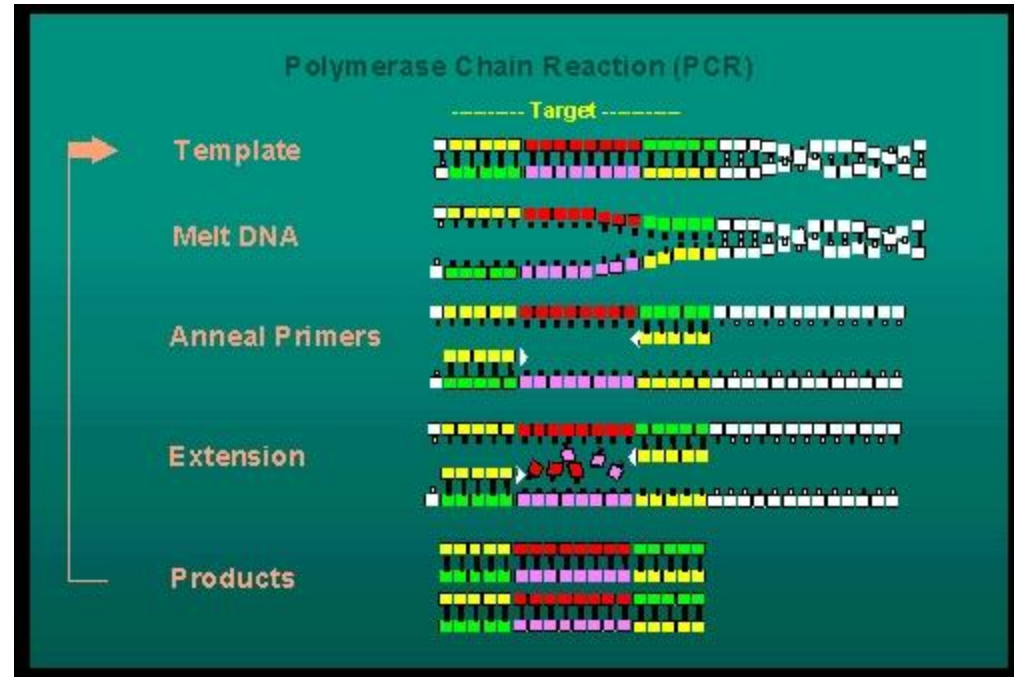
Chromosome



Polymerase chain reaction (PCR) based mapping

Polymerase chain reaction (PCR) is a molecular biology technique and used to amplify specific regions of a DNA strand. This can be a single gene, just a part of a gene, or a non-coding sequence.

It was Invented in 1983 by Dr. Kary Mullis. He later got Nobel prize for this invention



1) Identify the mutation by sequencing

EIR1- chromosome 5 Marker- CTR1 and CIW14

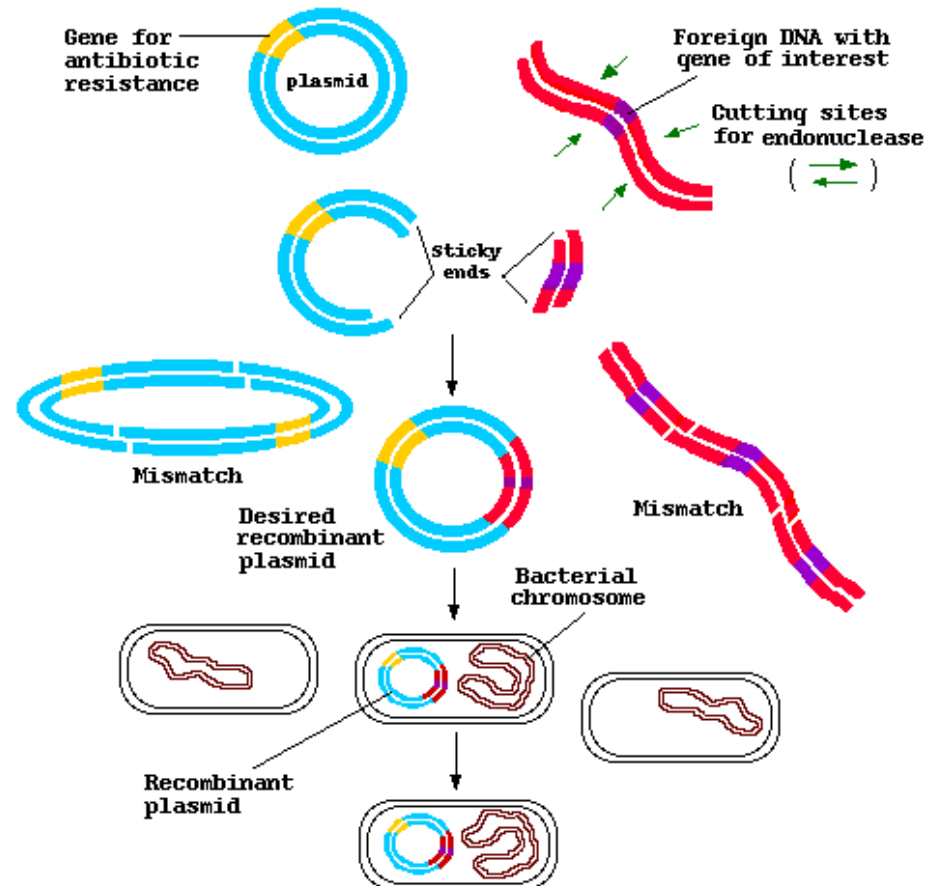
Gene ID- At5g57090

2) Complementation test

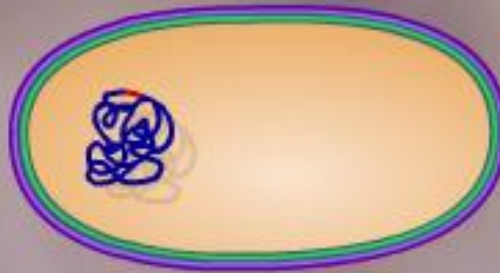
Inserting the wild-type gene in the mutant to see whether it can restore the abnormal phenotype

Steps in gene cloning

Plasmid Insertion



Steps in Cloning a Gene



Play



Pause



Audio



Text

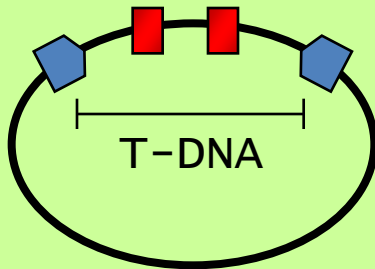
The first step in cloning a gene is to isolate the DNA from the organism that contains the desired gene.

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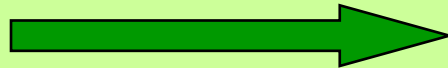
Once the gene is cloned in a vector, next step is to transfer the gene to the mutated plants

For transferring the gene, we use a natural soil bacteria, Agrobacterium and the process is called agrobacterium mediated transformation

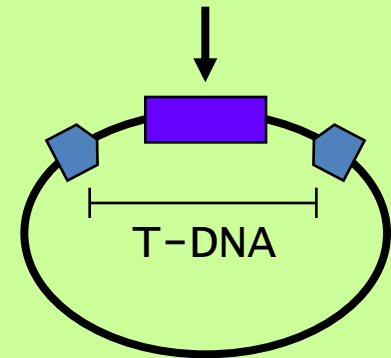
“ONC” genes



- Removal of “ONC” genes
- Replace with antibiotic resistance gene



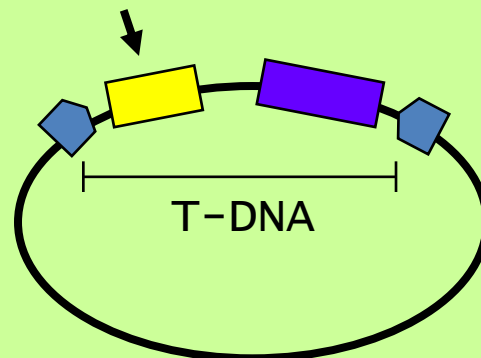
Antibiotic resistance gene



Insert chosen foreign gene into T-DNA



Foreign gene

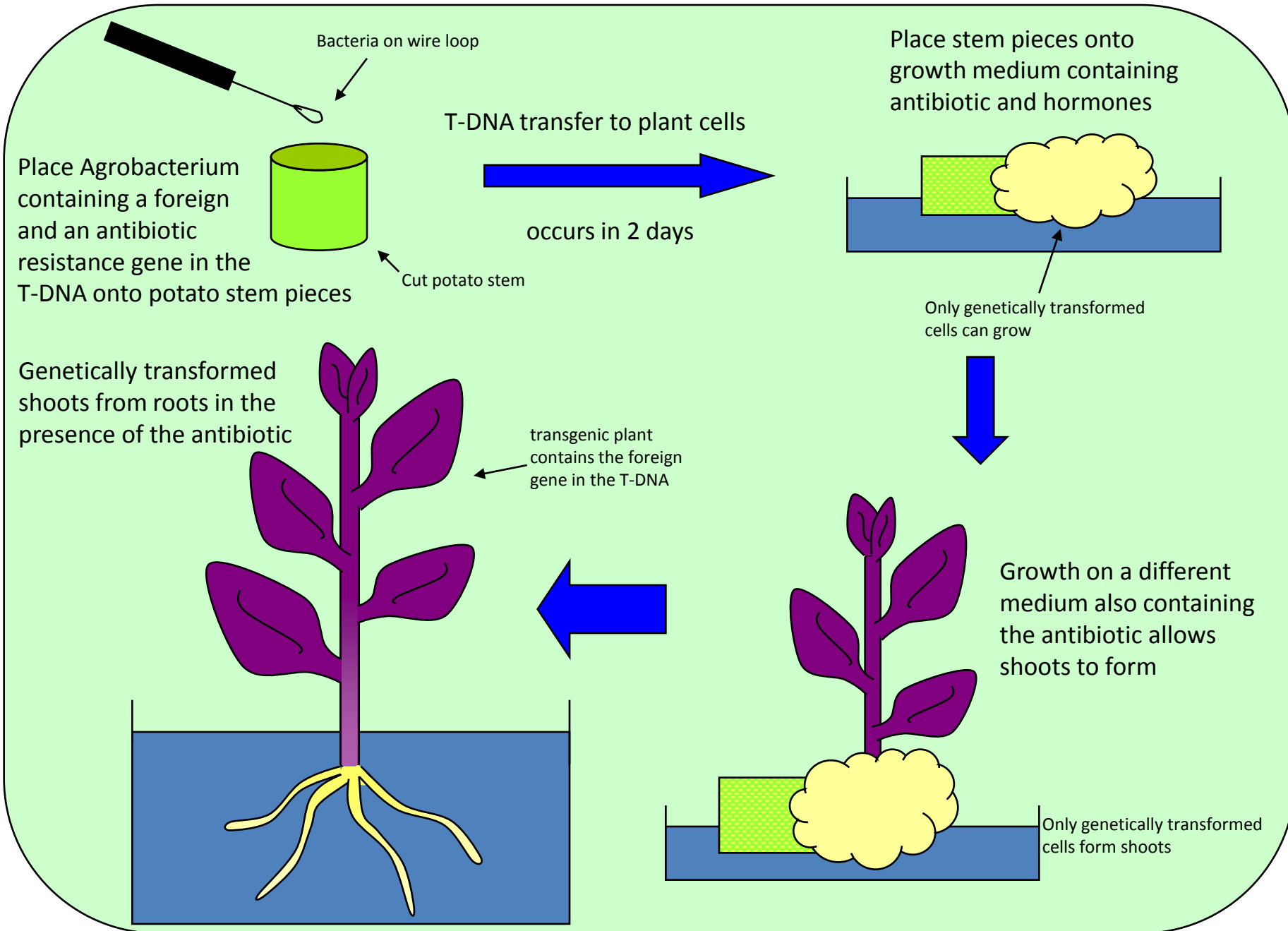


Infect wounded plant cells



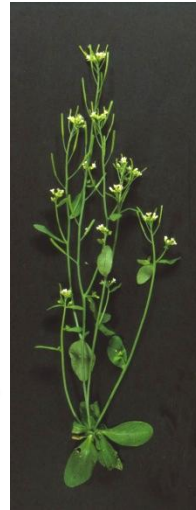
with *Agrobacterium* containing modified Ti-plasmid

T-DNA carries foreign gene into plant cells which are also resistant to the antibiotic



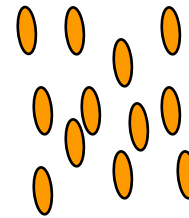
Final step: to validate the gene function we need to restore the mutant phenotype

Transfer of EIR1 gene
to mutant plants by
Agrobacterium mediated
transformation

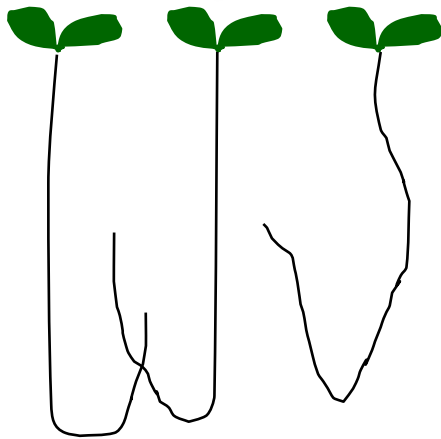


eir1 mutant

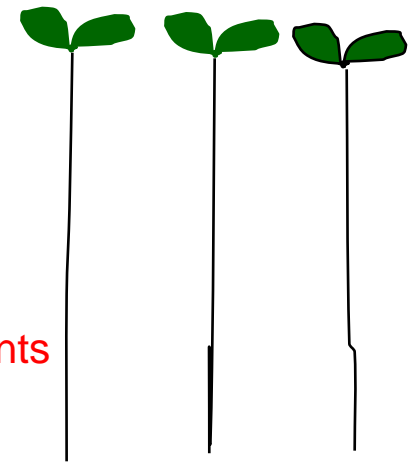
Collect seeds



Grow plants to look at
the phenotype



Complemented plants



Real life application of gene transfer technique

By using this technique we can speed up our conventional breeding system

Enhance the nutritional value of the crops

Make the crops resistant to different adverse environmental conditions such as cold, drought, salinity, flood etc.

Bio-engineer the plants to detoxify the heavy metal contaminations such as cadmium, mercury, arsenic from soil

Create the exotic flowers with different colors and fragrance

Produce vaccines in plant leaves

Take home message

The study of gene function by mutant analysis approach is a powerful technique for molecular biologists. This helps us to unravel the blue print of life and can play a great role in improving the quality of our life