



Introduction to Genetic Engineering

- Definition: Genetic engineering (or genome editing) → direct manipulation of an organism's DNA using biotechnology. Genetic engineering = changing the DNA of an organism in the lab.
Purpose: Improve human health, agriculture, and knowledge of biology.
Core idea: Modify DNA → alter traits, cure diseases, create new products.
- How it's done:
Change one base pair (A-T or C-G).
Delete a DNA region.
Add a new DNA segment.
Transfer a gene from one species to another (to give new traits).
Ethics & regulation: Field has always evolved with strong emphasis on safety, responsibility, and ethical debates.
- Applications:
Medicine: e.g., cancer therapies.
Industry: e.g., brewing yeasts.
Agriculture: GM plants and livestock.
Research: studying genes, creating models.
Progress Over Time:
Earlier → cloning & lab analysis.
Now → advanced synthetic biology → deeper understanding + new biomedical tools.

Field	Focus / Definition	Main Activities	Applications	Example
Genetics	Study of genes, heredity, variation.	Gene mapping, inheritance studies.	Medical genetics, breeding.	Mendelian genetics.
Genetic Engineering	Direct manipulation of DNA using rDNA tools.	Adding, deleting, editing genes.	GM crops, gene therapy, drug production.	CRISPR gene editing, Bt crops.
Biotechnology	Broad use of biology + technology for products/processes.	Fermentation, bioprocessing, tissue culture.	Medicine, food, agriculture, environment.	Insulin production, biofuels.

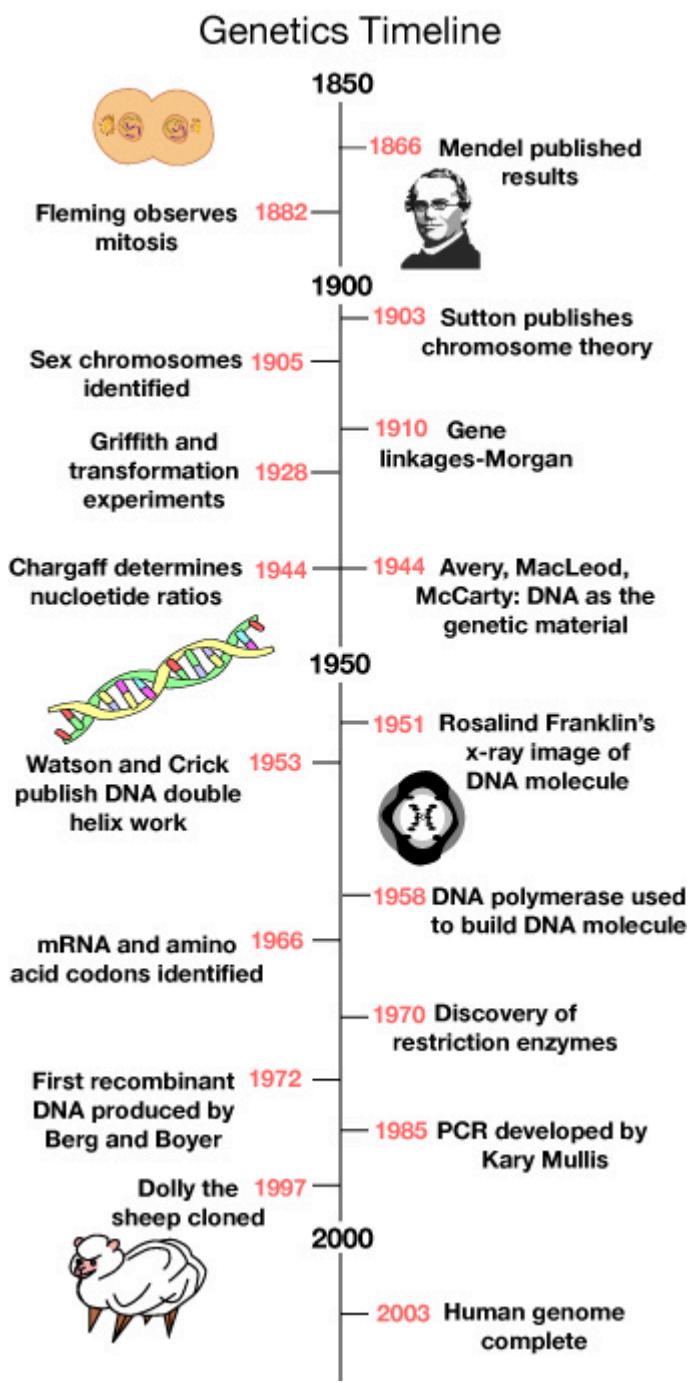
Bioinformatics	Use of computers + data science for biology.	Genome analysis, protein modeling.	Drug design, personalized medicine.	Human Genome Project analysis.
Microbiology	Study of microorganisms (bacteria, fungi, viruses, protozoa).	Isolation, culture, fermentation.	Medicine, food industry, environment.	Antibiotics, probiotics.
Molecular Biology	Study of molecules of life (DNA, RNA, proteins).	Gene expression, protein synthesis.	Cancer research, diagnostics.	PCR, DNA sequencing.
Cell Biology	Study of cell structure & function.	Microscopy, cell culture.	Cancer research, regenerative medicine.	Stem cell research.
Immunology	Study of immune system.	Antibody production, vaccines.	Disease prevention, autoimmunity research.	COVID-19 vaccine, monoclonal antibodies.
Biochemistry	Study of chemical processes in organisms.	Enzyme activity, metabolism studies.	Nutrition, medicine, drug development.	Krebs cycle, enzyme-linked assays.
Pharmacology	Study of drug action on living systems.	Testing, dose-response studies.	Drug discovery, toxicology.	Aspirin mechanism, cancer drugs.
Neuroscience	Study of nervous system & brain.	Brain mapping, neural signaling.	Neurology, psychology, AI links.	Alzheimer's research.
Environmental Biology	Study of organisms & environment interactions.	Ecology, pollution studies.	Conservation, waste management.	Climate change impact studies.

Marine Biology	Study of ocean organisms & ecosystems.	Field surveys, conservation studies.	Fisheries, marine conservation.	Coral reef studies.
Agricultural Science	Application of biology to crop & livestock improvement.	Plant breeding, pest control.	Food security, sustainable farming.	Hybrid seeds, GM crops.
Forensic Science	Use of biology in crime investigation.	DNA fingerprinting, toxicology.	Criminal justice.	DNA profiling in courts.



Importance of Studying Its History

- Helps understand current techniques (like CRISPR) and their potential.
- Shows scientific progress from discovery of DNA → modern therapies.
- Reveals ethical concerns and regulations that shaped today's biotechnology.



Early History (Pre-1960s)

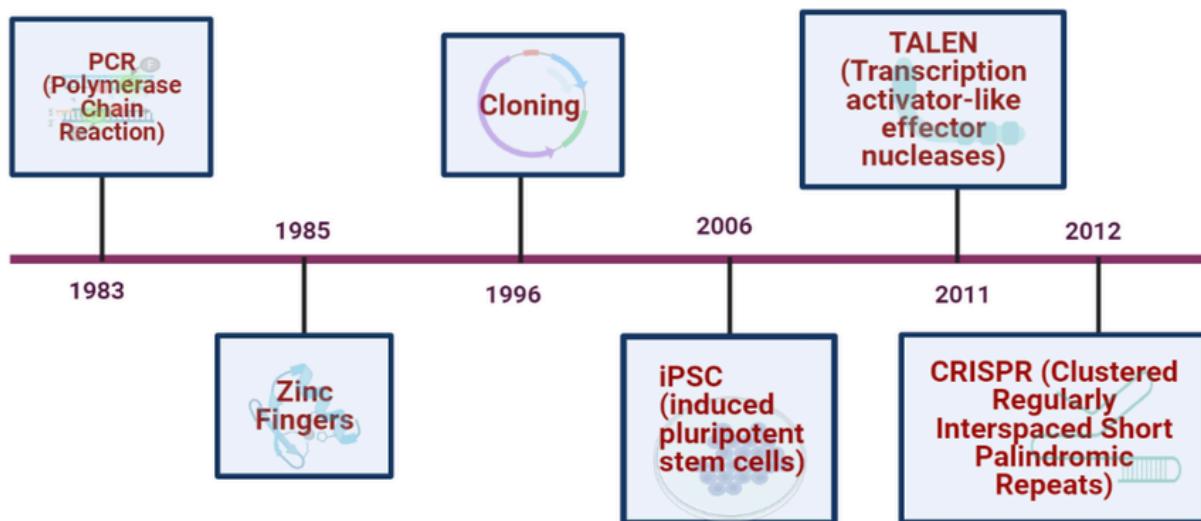
1953 – Discovery of the Double Helix

- Scientists: James Watson & Francis Crick (with Rosalind Franklin's X-ray diffraction images).

- Significance: Identified DNA's double helix structure → foundation of modern genetics.
 - Note: Franklin's contribution was vital but often under-recognized.
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1958 – First DNA Synthesized in Test Tube

- Scientist: Arthur Kornberg.
- Achievement: Isolated DNA polymerase enzyme and synthesized DNA in vitro.
- Significance: Proved DNA could be artificially created, opening a path to gene editing.
- Award: Nobel Prize in Physiology/Medicine (1959).



Timeline

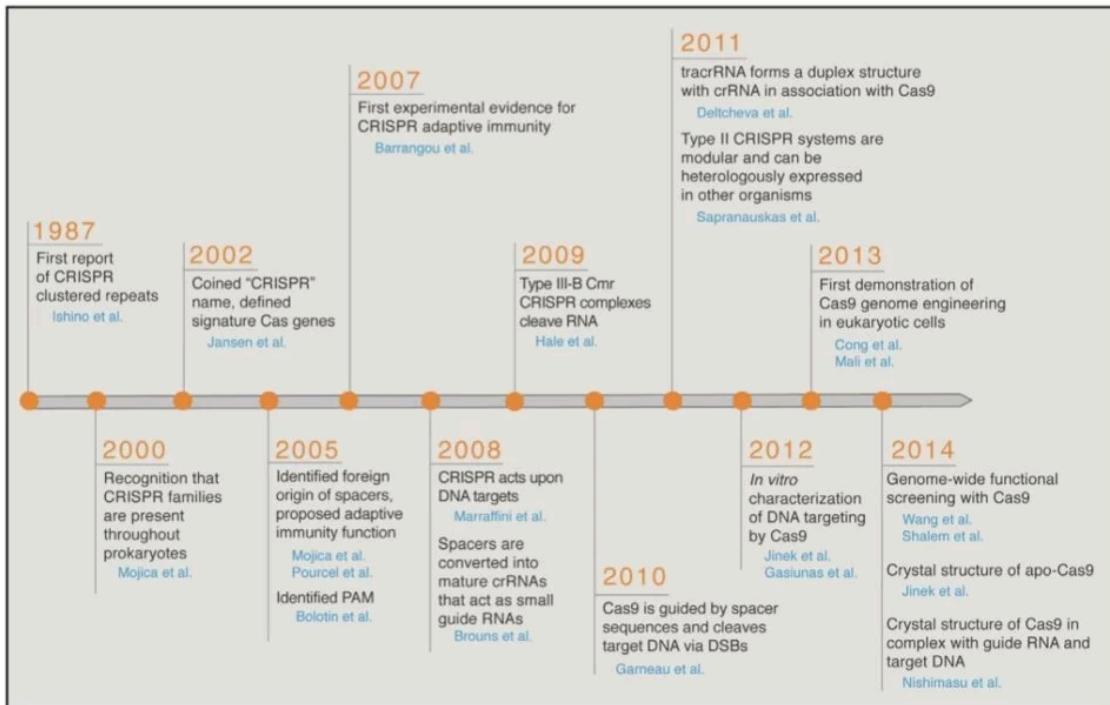


Figure: Hsu, Lander, Zhang: Development and Applications of CRISPR-Cas9 for Genome Engineering; Cell 157, June 5, 2014



Timeline of Genetic Engineering



1960s – Linking DNA & Visualizing Cells

1962 – Green Fluorescent Protein (GFP)

- Scientists: Osamu Shimomura; later Martin Chalfie & Roger Tsien.
- Discovery: Isolated GFP from jellyfish *Aequorea victoria*.
- Use: Tag proteins → visualize gene expression in live cells.
- Award: Nobel Prize in Chemistry (2008).

1967 – Discovery of DNA Ligase

- Function: Enzyme that joins DNA fragments by catalyzing phosphodiester bonds.

- Significance: Enabled splicing and recombinant DNA experiments.

1968 – Discovery of Restriction Enzymes

- Scientist: Werner Arber (hypothesis), later Daniel Nathans & Hamilton Smith.
 - Function: Enzymes cut foreign DNA (like bacteriophage) but protect host DNA using methylation.
 - Impact: Molecular "scissors" → critical for DNA manipulation.
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🧬 1970s – Birth of Recombinant DNA

1970 – Purification of Type II Restriction Enzymes

- Scientist: Hamilton Smith.
- Discovery: HindII enzyme → first site-specific DNA cutter.

1971 – Gene Splicing (Paul Berg)

- Created recombinant DNA from two viruses → proved DNA fragments could be joined.
- Nobel Prize in Chemistry (1980).

1972 – Recombinant DNA (Cohen & Boyer)

- First successful rDNA created in bacteria plasmid.
- Foundation of modern genetic engineering.

1974 – Moratorium on rDNA Experiments

- Reason: Ethical concerns over biosafety.
- Led to Asilomar Conference (1975) → guidelines for safe genetic research.

1975 – Hybridoma Technology (Köhler & Milstein)

- Fusion of B-cells + myeloma cells → monoclonal antibodies.
 - Revolutionized diagnostics and immunotherapy.
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1980s – Medical Applications Emerge

1981 – First Transgenic Animal

- Method: DNA microinjection (rabbit gene into mouse).
- Significance: Foundation for GM animals.

1982 – First Human Drug (Synthetic Insulin)

- Company: Genentech.
- Impact: Replaced animal-sourced insulin → large-scale human treatment.

1983 – Polymerase Chain Reaction (PCR, Kary Mullis)

- Amplifies DNA → millions of copies in hours.
- Essential for modern genetic research, forensics, diagnostics.

1985 – Zinc Finger Nucleases (ZFNs)

- Early genome-editing tool (designer nucleases).
- Allowed targeted DNA cleavage & gene modification.

1986 – First Recombinant Vaccine (Hepatitis B)

- Created using yeast cells (*S. cerevisiae*).
- Foundation for modern recombinant vaccines (HPV, etc.).

1988 – First GMO Crop (Bt Corn)

- Pest-resistant corn expressing *Bacillus thuringiensis* toxin.
 - First official GMO field trials in the U.S.
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1990s – Cloning & Human Genome

1990 – Start of Human Genome Project

- Goal: Map entire human genome (completed 2003).

1993 – CRISPR Principle Discovered

- Scientist: Francisco Mojica.
- Found repeating DNA sequences in bacteria defending against viruses.

1994 – FLAVR SAVR Tomato (Calgene)

- First GMO food approved for sale (failed commercially).

1996 – Dolly the Sheep Cloned

- First mammal cloned from adult cell (somatic cell nuclear transfer).

1999 – First Human Chromosome Sequenced

- Chromosome 22 → milestone for Human Genome Project.
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2000s – Genomics & Therapies

2001 – First Gene-Targeted Drug (Imatinib / Glivec)

- Treats chronic myelogenous leukemia (CML).

2003 – Human Genome Project Completed

- Full sequence (~20,000 genes).
- Foundation for personalized medicine.

2004 – UN Endorses Biotech Crops

- GM crops promoted for food security.

2006 – Preventive Cancer Vaccine (HPV, Gardasil)

- First vaccine preventing cancer.

2006 – Induced Pluripotent Stem Cells (iPSCs, Shinya Yamanaka)

- Adult cells reprogrammed into stem cell-like state.
 - Nobel Prize (2012).
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⚡ 2010s – CRISPR Revolution

2010 – First Synthetic Life (Craig Venter)

- Created synthetic bacterial genome → proof of man-made life.

2011 – TALENs Developed

- New genome editing tool, easier than ZFNs.

2012 – CRISPR-Cas9 as Gene Editing Tool

- Scientists: Jennifer Doudna & Emmanuelle Charpentier.
- Game-changer: Cheap, precise, easy editing of DNA.

2013 – CRISPR in Eukaryotic Cells (Feng Zhang)

- Showed CRISPR works in human/mammalian cells.

2014 – Concept of Gene Drive (Kevin Esvelt)

- Using CRISPR to bias inheritance (e.g., eradicate malaria mosquitoes).

2015 – First CRISPR-edited Human Embryo (China)

- Controversial germline editing (β -thalassemia gene).

2017 – First CAR-T Cancer Therapy Approved

- Genetically engineered T-cells to fight cancer.

2018 – First Human CRISPR Trials Approved

- Vertex & CRISPR Therapeutics → treatment for β -thalassemia, sickle cell anemia.

2019 – Prime Editing (David Liu's Lab)

- Precise gene editing without double-stranded breaks.
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2020s – CRISPR in Action

2020 – CRISPR Clinical Success

- First patients (like Victoria Gray) show relief from sickle cell & thalassemia after CRISPR therapy.

2020 – Nobel Prize for CRISPR

- Awarded to Doudna & Charpentier.

2020–Present – Rapid Progress

- Applications in:
 - Human therapies (genetic diseases, cancer).

- Agriculture (climate-resilient crops, disease resistance).
- Synthetic biology (biofuels, biomaterials).
- Ethical debates (germline editing, biosecurity).



Genetic Engineering in Crops



Definition

- Genetic engineering = directed addition of foreign DNA (gene) into an organism.
 - Purpose = give the organism a new trait (disease resistance, higher yield, etc.).
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5 Basic Steps in Crop Genetic Engineering

1. DNA Extraction

- Take DNA from an organism with the desired trait.
- Example: Bt gene (toxin against insects) taken from *Bacillus thuringiensis*.

2. Gene Cloning

- Locate and copy the specific gene from the extracted DNA.
- Use PCR to make many identical copies.

3. Gene Modification (Design)

- Adjust the gene so it will work inside the new organism.
- Replace promoters (DNA "switches") so the gene turns on in the right place (e.g., corn leaves).

4. Transformation

- Insert the gene into plant cells (via Agrobacterium or gene gun).
- Use markers (like antibiotic resistance) to select successful cells.
- Transgenic cells grow into full plants (totipotency).

5. Backcross Breeding

- Cross the transgenic plant with elite high-yield varieties.
 - Repeat breeding → stable, high-yielding line with the desired trait.
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Difference: Genetic Engineering vs. Traditional Breeding

- Genetic Engineering:
 - Directly add specific new gene(s) (can be from any species).
 - Precise: transfer only the desired gene.
 - Overcomes species barriers.
 - Traditional Plant Breeding:
 - Requires sexual mating.
 - Can only combine traits within same or compatible species.
 - Transfers many genes at once (including unwanted traits).
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Time Required

- Whole process = 6–15+ years (depends on crop, gene, resources).
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Key Takeaways

- Genetic engineering = targeted + precise.
 - Steps = DNA extraction → gene cloning → gene design → transformation → backcross breeding.
 - Allows transfer of traits across species (unlike traditional breeding).
 - Used to create transgenic crops like Bt maize (insect resistant).
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Tools of Genetic Engineering

1. **Restriction enzymes** – Cut DNA at specific sites (“molecular scissors”).
 2. **DNA ligase** – Joins DNA fragments (“biological glue”).
 3. **Electroporation** – Electric pulses make pores in cell membrane → allows gene entry.
 4. **Vectors** – “Vehicles” to deliver DNA (plasmids, viruses, artificial chromosomes).
 5. **Other tools** – Gel electrophoresis, PCR, transgenes, RNA, etc.
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Methods of Gene Transfer

- Transformation
 - Conjugation
 - Microinjection
 - Transduction
 - Liposome-mediated transfer
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Applications

Agriculture

- GM crops (e.g., Bt cotton, Bt brinjal).
- Benefits: Higher yield, pest resistance, less pesticide use, better nutrition.

Medicine & Health Sciences

- Production of insulin, vaccines, antibiotics, cancer drugs.
 - Gene therapy (fix faulty genes).
 - Model animals (lab mice) for research.
- Other Fields
- Crime scene investigation (DNA profiling).

- Animal husbandry (improved livestock).
 - Molecular biology (research).
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Ethical Issues

- Only the rich may afford advanced therapies.
 - Risk of health problems in humans.
 - GM crops → loss of traditional farming practices.
 - Alteration of life's basic units raises moral concerns.
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Conclusion

- Genetic engineering = one of humanity's greatest achievements.
 - Present in food, medicine, agriculture, research.
 - Future = more advanced molecular biology + global competition.
 - Promise: "defectless world," but must balance benefits vs ethics.
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Figure 1. Traits, such as flower and seed color, are controlled by DNA.

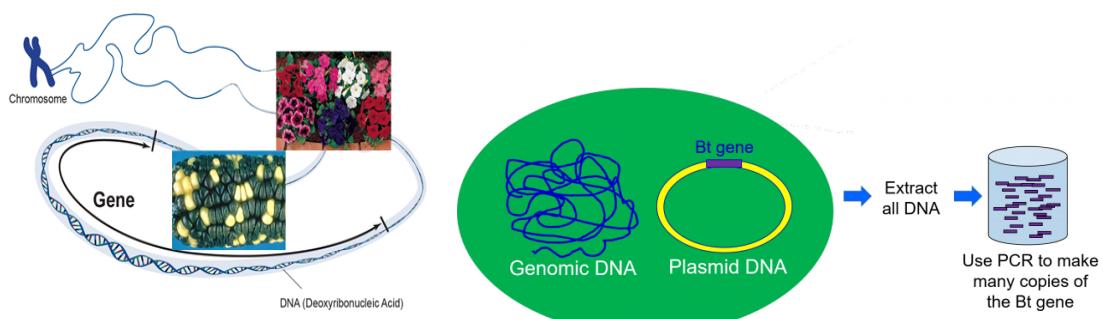
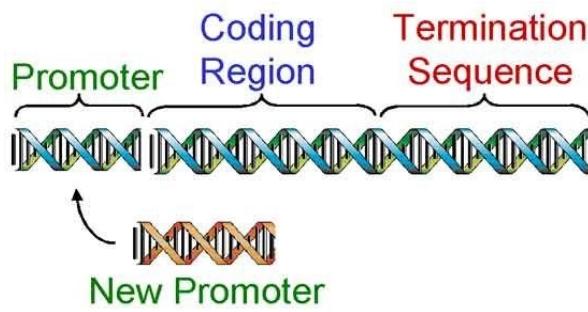


Figure 2. Using DNA from *B. thuringiensis* to clone the Bt gene.



new promoter.

Figure 3. Replacing existing promoter with

new promoter.

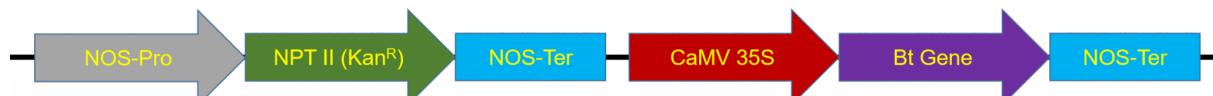


Figure 4. The basic elements of a transgene construct.

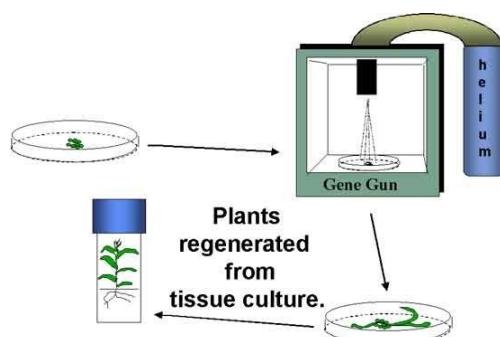


Figure 5. Using the gene gun method to transform

Agrobacterium tumefaciens
carrying the Bt gene and a
selectable marker (resistance
to the antibiotic Kanamycin)

plant cells.

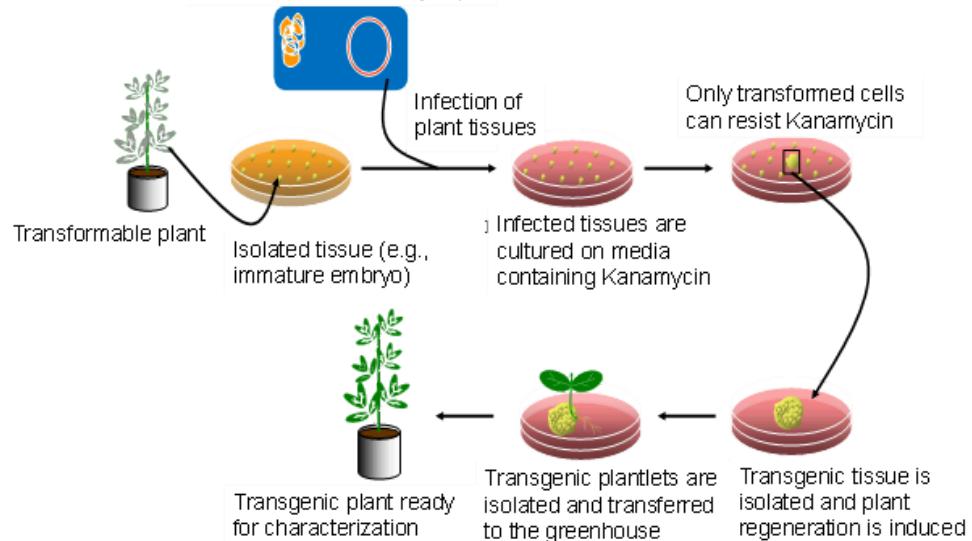


Figure 6. Transferring genes into plant cells by the Agrobacterium method. After infection of select tissue with the bacterium carrying a transgene construct with an antibiotic resistance gene as the selectable marker, the tissue is grown in a medium that contains the antibiotic that will kill all untransformed tissues or cells. Therefore, only tissues whose cells have been transformed with the transgene construct survive in the presence of the antibiotic. The surviving tissue is removed from the antibiotic and

allowed to regenerate into whole plants. Normally, transgenic plants will be monitored in a controlled environment such as a growth chamber or greenhouse before they are grown in the field.

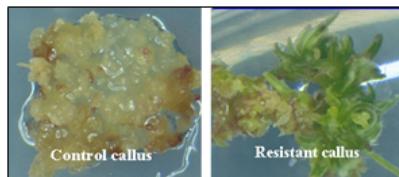
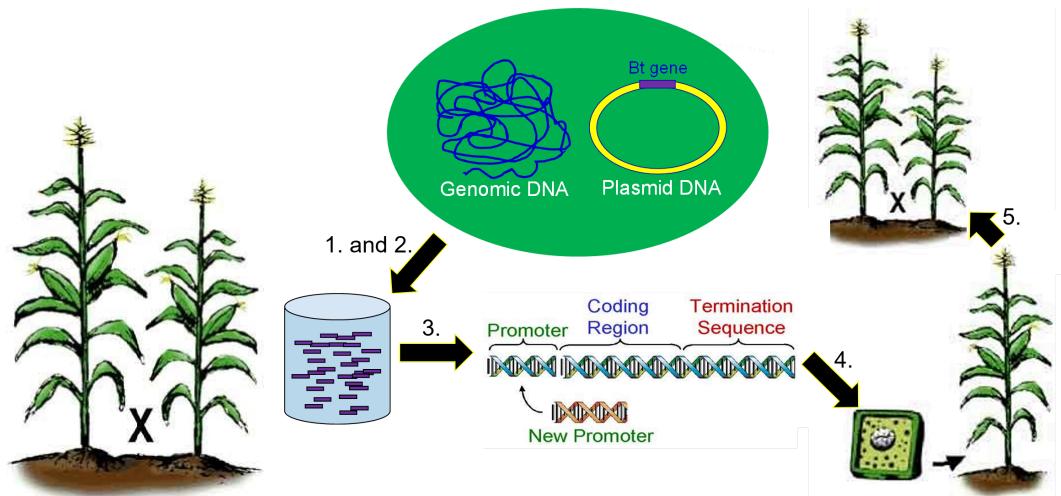


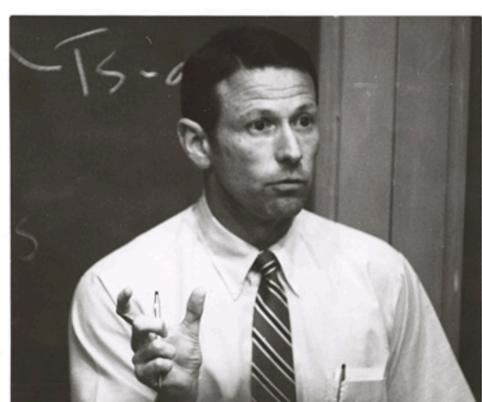
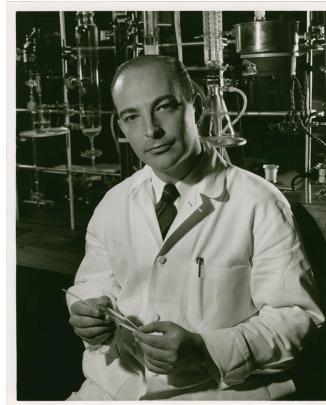
Figure 7. Selection for herbicide tolerance in buffalograss transformed with the gene that makes plants tolerant to glyphosate. The control calli lacking the glyphosate tolerance gene are killed by the herbicide that is part of the media on which the cells are grown (left panel). The calli in the right panel grew from a cell that received the glyphosate resistance gene during transformation and survived on



the media. **Figure 8.** The ratio of possible offspring when a hemizygous diploid plant is self-pollinated.



Using the backcross breeding method. **Figure 10.** Crop genetic engineering includes: 1) DNA isolation 2) gene cloning 3) gene design 4) transformation, and 5) plant breeding.



<https://www.youtube.com/watch?v=AtOUu67mkRk>

Scope of Biotechnology in India

- Rosalind Elsie Franklin was a British chemist and X-ray crystallographer.
- Arthur Kornberg was an American biochemist who won the Nobel Prize in Physiology or Medicine in 1959 for the discovery of "the mechanisms in the

biological synthesis of ribonucleic acid and deoxyribonucleic acid" together with Spanish biochemist and physician Severo Ochoa of New York University.

- Werner Arber is a Swiss microbiologist and geneticist. Along with American researchers Hamilton Smith and Daniel Nathans, Werner Arber shared the 1978 Nobel Prize in Physiology or Medicine for the discovery of restriction endonucleases.
- Daniel Nathans and Hamilton Smith, alongside Werner Arber, won the 1978 Nobel Prize in Physiology or Medicine for discovering restriction enzymes and applying them to molecular genetics. Smith purified the first restriction enzyme, which he had helped discover, and Nathans applied it to SV40 virus DNA.
- Paul Berg was an American biochemist and professor at Stanford University. He was the recipient of the Nobel Prize in Chemistry in 1980, along with Walter Gilbert and Frederick Sanger.
- The Cloning of Dolly the Sheep: Dolly was a female Finn-Dorset sheep and the first mammal that was cloned from an adult somatic cell. She was cloned by associates of the Roslin Institute in Scotland, using the process of nuclear transfer from a cell taken from a mammary gland. Dolly lived at the Roslin Institute throughout her life and produced several lambs. She was euthanized at the age of six years due to a progressive lung disease. No cause which linked the disease to her cloning was found. Dolly's body was preserved and donated by the Roslin Institute in Scotland to the National Museum of Scotland, where it has been regularly exhibited since 2003.
- Jennifer Anne Doudna ForMemRS is an American biochemist who has pioneered work in CRISPR gene editing, and made other fundamental contributions in biochemistry and genetics. She received the 2020 Nobel Prize in Chemistry, with Emmanuelle Charpentier, "for the development of a method for genome editing. In 2012, Doudna and Emmanuelle Charpentier were the first to propose that CRISPR-Cas9 (enzymes from bacteria that control microbial immunity) could be used for programmable editing of genomes, which has been called one of the most significant discoveries in the history of biology.