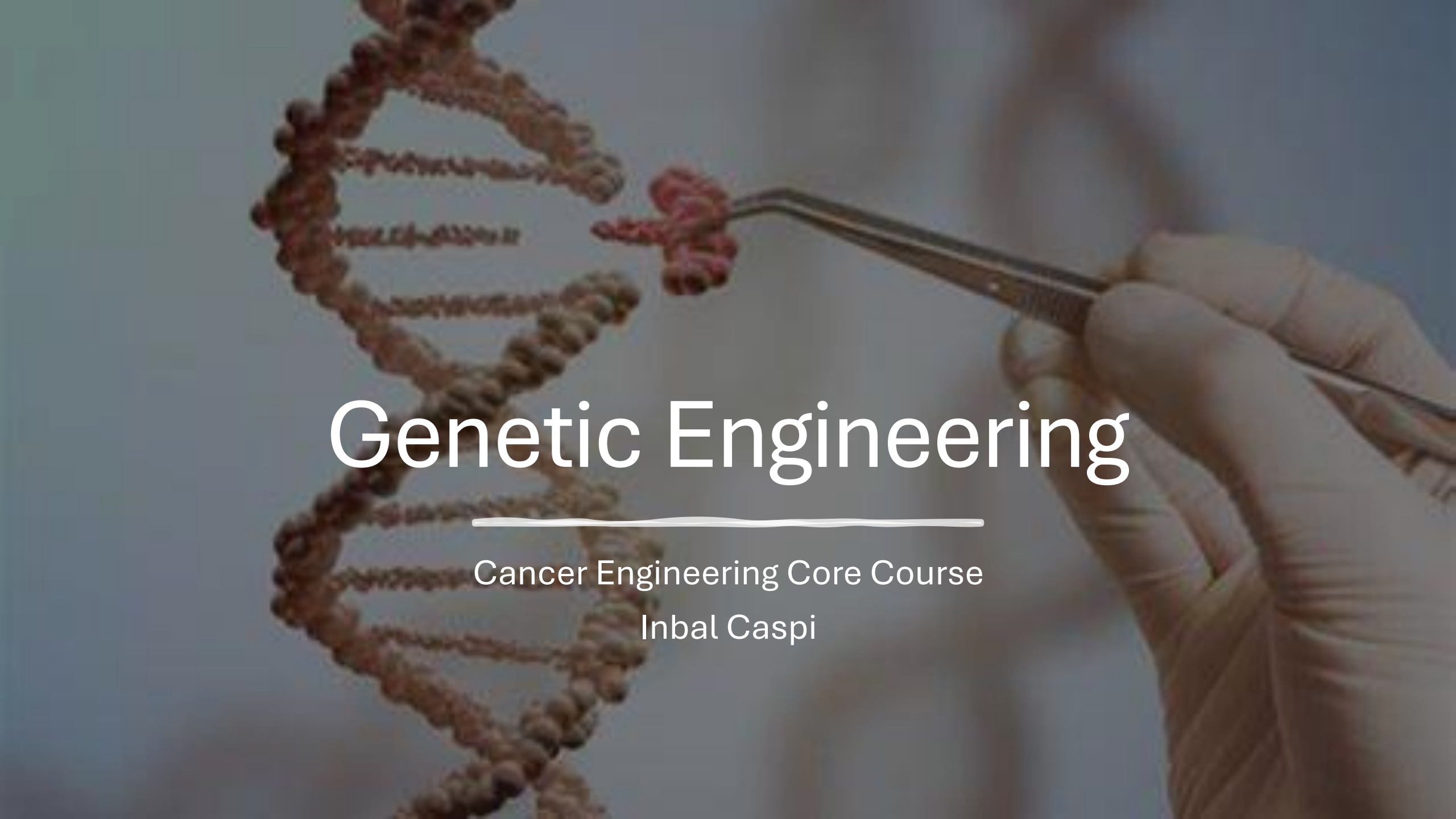


Genetic Engineering

Cancer Engineering Core Course

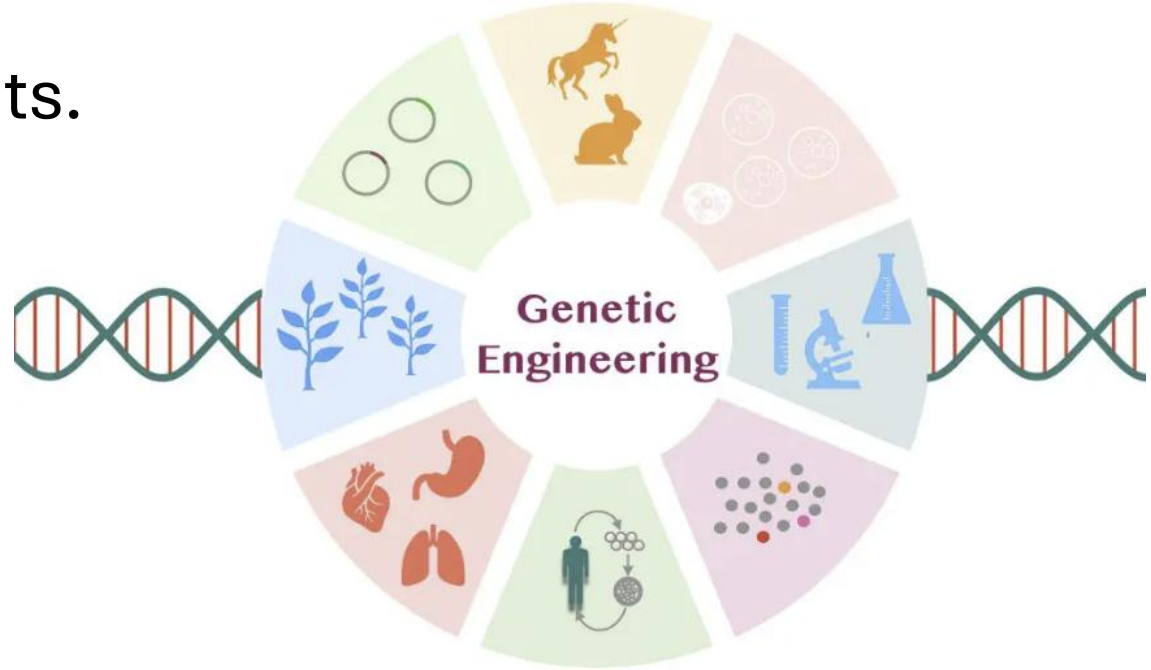
Inbal Caspi

Schedule

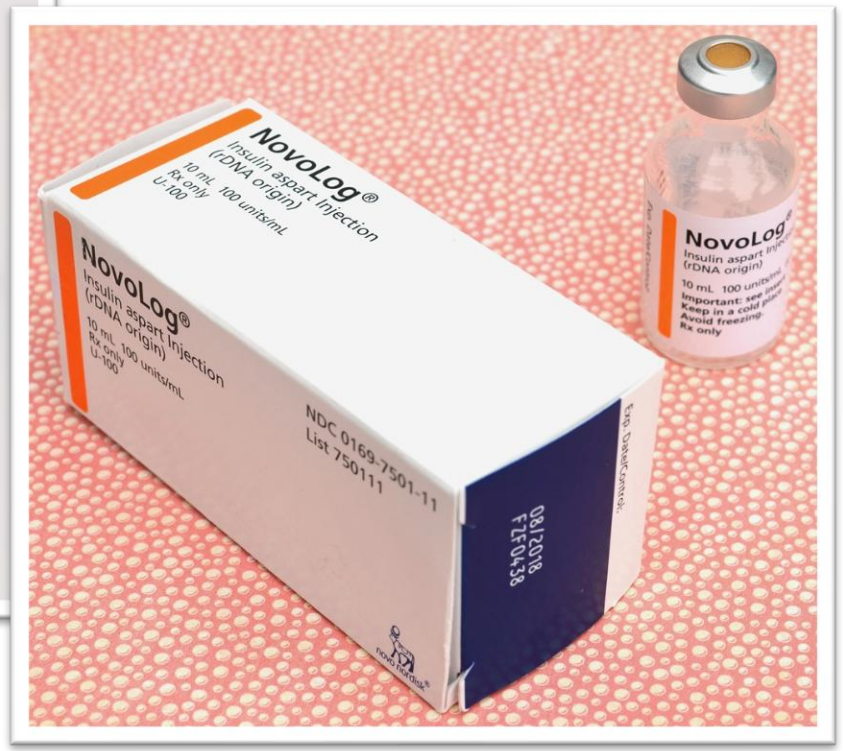
GENETIC ENGINEERING		
Problem set 2, Imaging Section, Distribute 11/10/2025 and due 11/17/2025		Monday, November 10, 2025
Introduction to Genetic Engineering 1	Caspi, Inbal	Monday, November 10, 2025
Introduction to Genetic Engineering 2	Caspi, Inbal	Tuesday, November 11, 2025
Deep dive into Tools for Genetic Engineering CRISPR/CAS9, ZFN and TALEN	Choi, Junhong	Wednesday, November 12, 2025
Transgenic Animal Models	Ventura, Andrea	Thursday, November 13, 2025
Somatic genome editing and chromosomal engineering	Ventura, Andrea	Friday, November 14, 2025
Computational Analysis of Genetic Screens	Norman, Thomas	Monday, November 17, 2025
Engineering stem cell models of human biology	Caspi, Inbal **9:30-12:30**	Tuesday, November 18, 2025
Multicellular platforms from organoids to tissue biology.	Lee, Joo-Hyeon **1:30-4:40**	day, November 18, 2025
Human Genetics	Dey, Kushal	nesday, November 19, 2025
Genetic Screening Core	Garippa, Ralph	Thursday, November 20, 2025
Protein Engineering	Yang, Xinbo	Friday, November 21, 2025
CART cells	Perica, Karlo	Monday, November 24, 2025
Hands on group project	Caspi, Inbal	Tuesday, November 25, 2025
No Class - Thanksgiving Break		Wednesday, November 26, 2025
No Class - Thanksgiving Break		Thursday, November 27, 2025
No Class - Thanksgiving Break		Friday, November 28, 2025
Tour of Robotic Surgery/Cell Therapies	(Tour of facility begins at 7:00 am)/Studer, Lorenz	Monday, December 1, 2025
Stem Cell Therapy Clinical Trials	Tabar, Viviane	Tuesday, December 2, 2025
Biotherapies core tour	Wang, Xiuyan **tours from 10:00-11:00**	Wednesday, December 3, 2025
Tissue engineering	Panagiotakopoulos, Magdalini	Thursday, December 4, 2025
Project Preparation/Discussion	Caspi, Inbal	Friday, December 5, 2025
Entrepreneurship		
Entrepreneurship	Mohan, Anu/Ehlers-Surur, Imke	Monday, December 8, 2025
Entrepreneurship	Mohan, Anu/Ehlers-Surur, Imke	Tuesday, December 9, 2025
Entrepreneurship	Mohan, Anu/Ehlers-Surur, Imke	Wednesday, December 10, 2025
Entrepreneurship	Mohan, Anu/Ehlers-Surur, Imke	Thursday, December 11, 2025
Entrepreneurship	Mohan, Anu/Ehlers-Surur, Imke	Friday, December 12, 2025
Group Project presentations	Kushal Dey/Thomas Norman	Thursday, January 16, 2025
Problem set 3, Genetic Engineering Section, Distribute 1/2/2025 and due 1/9/2025		

What Is Genetic Engineering?

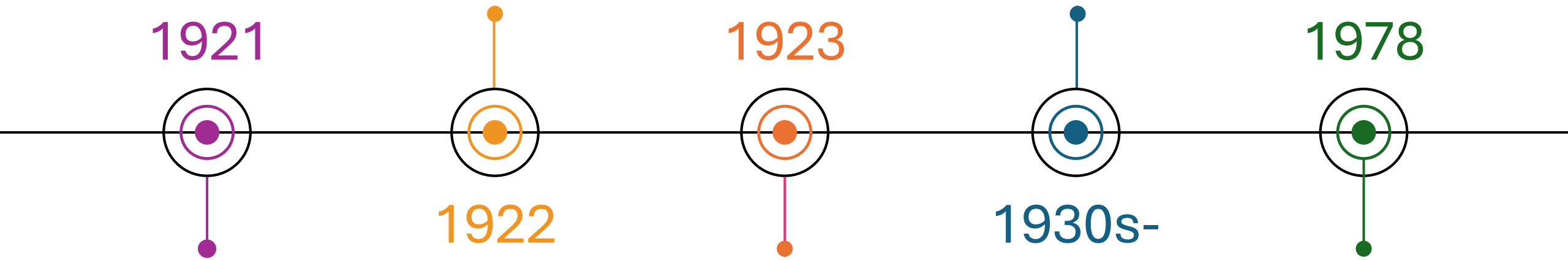
- Gene modification activities
- Manipulation, modification, and recombination of DNA.
- Modifying an organism’s genetic material to introduce changes or traits.



How Do We Treat Diabetes?



Genetic Engineering of Insulin



1921

Frederick Banting figures out how to remove insulin from a dog's pancreas

John Macleod refines insulin from cow pancreas

1922

First patient (14 years old) is treated with insulin
Previously, diabetes was a death sentence – there was no treatment or cure

1923

Eli Lilly begins production of commercial insulin

Banting and Macleod receive Nobel Prize

1930s-1970s

Insulin from cow and pig pancreas continues to be used for decades

1978

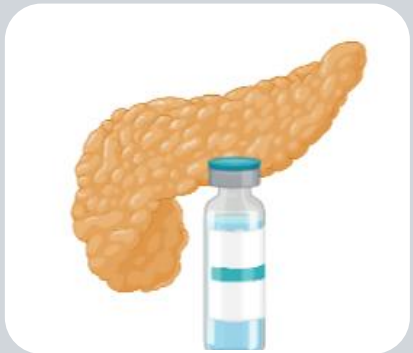
First genetically engineered synthetic human insulin produced in *E. coli* at Genentech

Humulin brought to market by Eli Lilly in 1982

Recombinant Drugs

Gold

$127

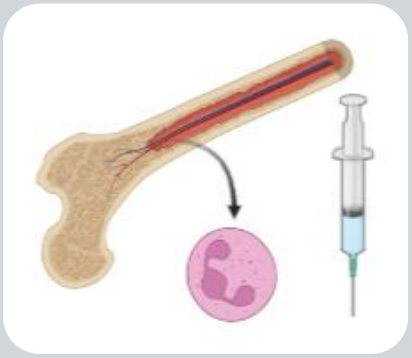


Insulin

$8800

Human Growth Hormone

$580,000



Granulocyte Colony Stimulating Factor

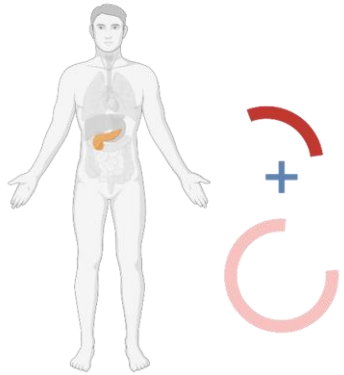
$1 million+

More Recombinant Drugs

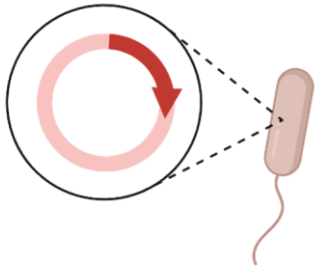
Drug (Brand)	Molecule / Mechanism	Indications	Cell Factory
Filgrastim (Neupogen)	Cytokine — stimulates growth of white blood cells	Acute lymphocytic leukemia	*E. coli*
Interferon alfa-2a	Recombinant interferon - antiviral	AIDS-related Kaposi's sarcoma, chronic hepatitis C, leukemia	*E. coli*
Hepatitis B Vaccine (Recombivax)	Subunit viral vaccine from HBsAG	Hepatitis B	*S. cerevisiae*
Trastuzumab (Herceptin)	Recombinant human monoclonal antibody	Breast cancer, stomach cancer	CHO
Etanercept (Enbrel)	Recombinant soluble fusion protein — TNF inhibitor	Rheumatoid arthritis	CHO
Adalimumab (Humira)	Recombinant human monoclonal antibody	Arthritis, plaque psoriasis, Crohn's disease, ulcerative colitis	CHO

Genetic Engineering and Bacterial Transformation

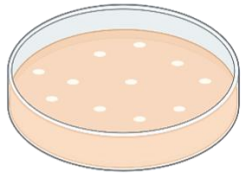
1. Clone human insulin gene into plasmid



2. Transform bacteria with plasmid

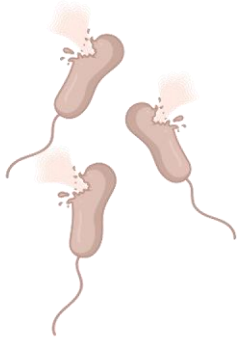


3. Select transformed bacteria

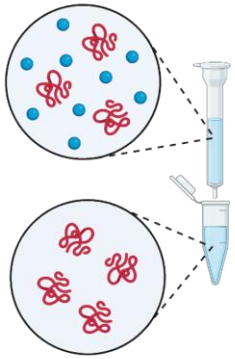


4. Culture bacteria and induce gene expression

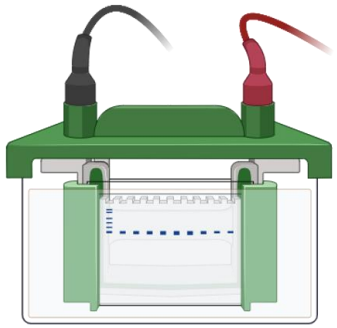
5. Lyse cells



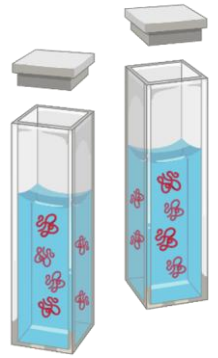
6. Purify protein with chromatography



7. Analyze purification with SDS-PAGE

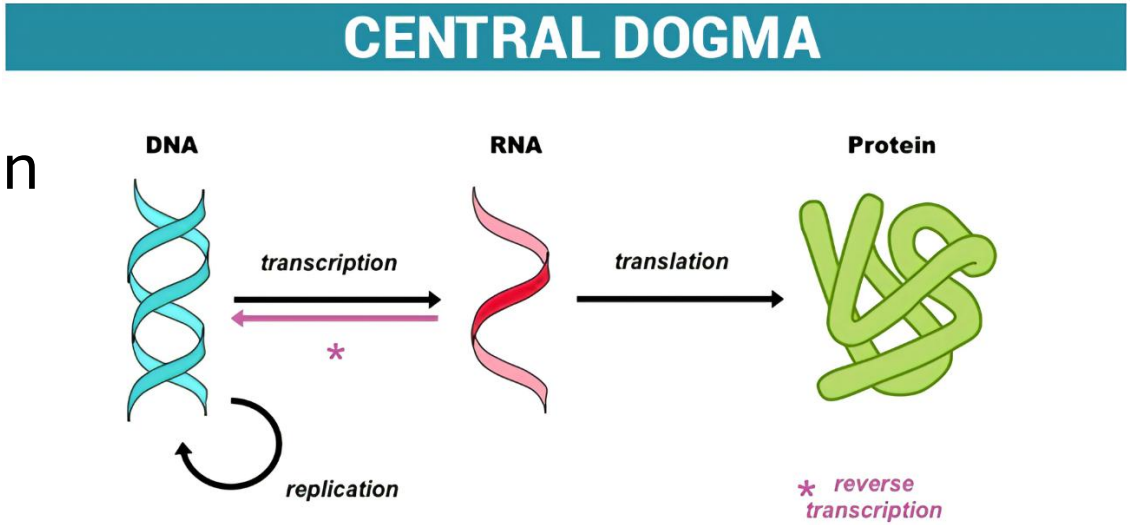


8. Assay protein activity



Why Can We Express A Human Gene Within Bacteria?

- Shared genetic principles
- Conserved transcription and translation machinery



Potential challenges

- Lack of intron splicing
- Differences in codon usage
- Limited post-translational modifications
- Improper protein folding

Milestones in Genetic Engineering

"It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material."

1953

The discovery of the structure of DNA by Watson and Crick

1973

Development of recombinant DNA technology by Stanley Cohen and Herbert Boyer

1974

The first genetically modified animal was a mouse created by Rudolf Jaenisch

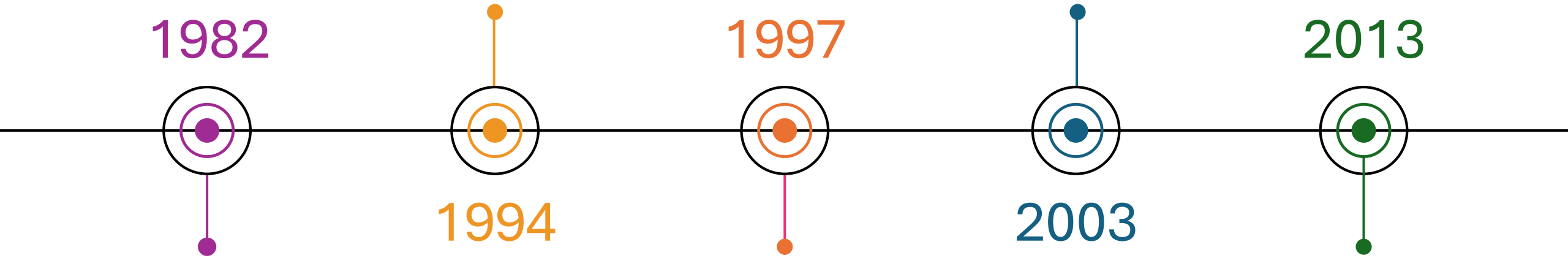
1978

The first genetically modified organism, a bacterium capable of producing human insulin, is created.

1982

First genetically modified crop produced: tobacco plants with an inserted antibiotic resistance gene

Milestones in Genetic Engineering



1982

First genetically modified crop produced: tobacco plants with an inserted antibiotic resistance gene

1994

Approval of the first genetically engineered food product: a tomato with an enhanced shelf life

1997

Dolly the sheep becomes the first cloned mammal

2003

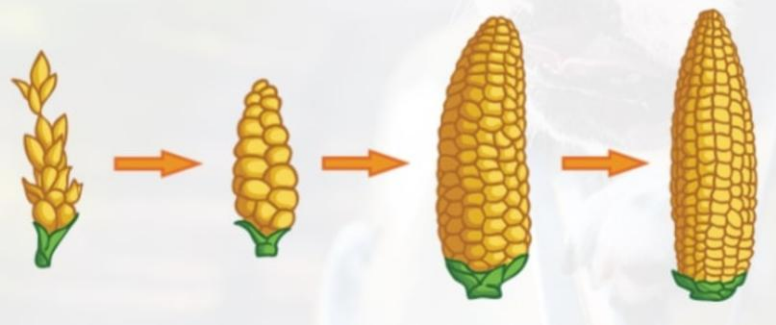
The Human Genome Project is completed

2013

The first use the genetic editing system, CRISPR – Cas9 in human and animal cells by George Church and Feng Zhang

Nonspecific Genetic Engineering

- **Selective breeding** – people selected specific traits in species of animals and plants and bred individuals with those traits over generations.
- **Induced mutagenesis** - radiation and chemical mutagens were used to increase the likelihood of genetic mutations in experimental model organisms (forward genetics).
- **Reverse genetics** - determining the function of a gene by analyzing the phenotypic effects of specific mutations.

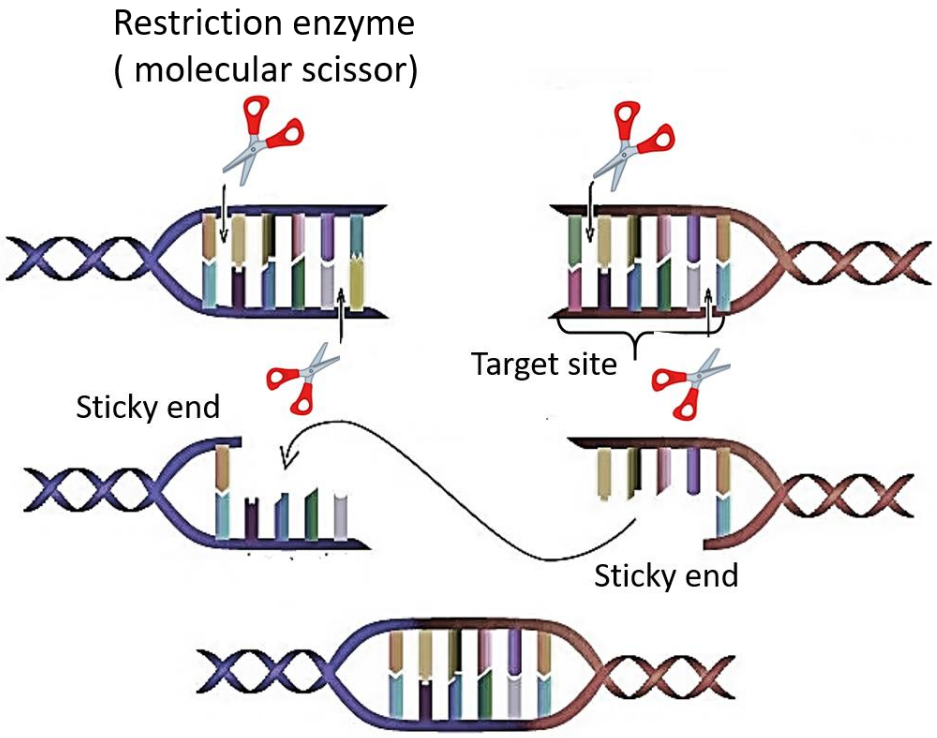


Terminology

- **Genome engineering** – the field concerned with modifying and designing DNA sequences.
- **Genome editing** – a genome engineering technique that introduces site-specific modifications into genomic DNA.
- **Gene editing** - a genome engineering technique that introduces site-specific modifications in a single gene.

Editing Tools – 1. Restriction Enzymes

- Cutting DNA at specific sites called **recognition sequences**.
- Endonucleases – enzymes that cut within a DNA strand.

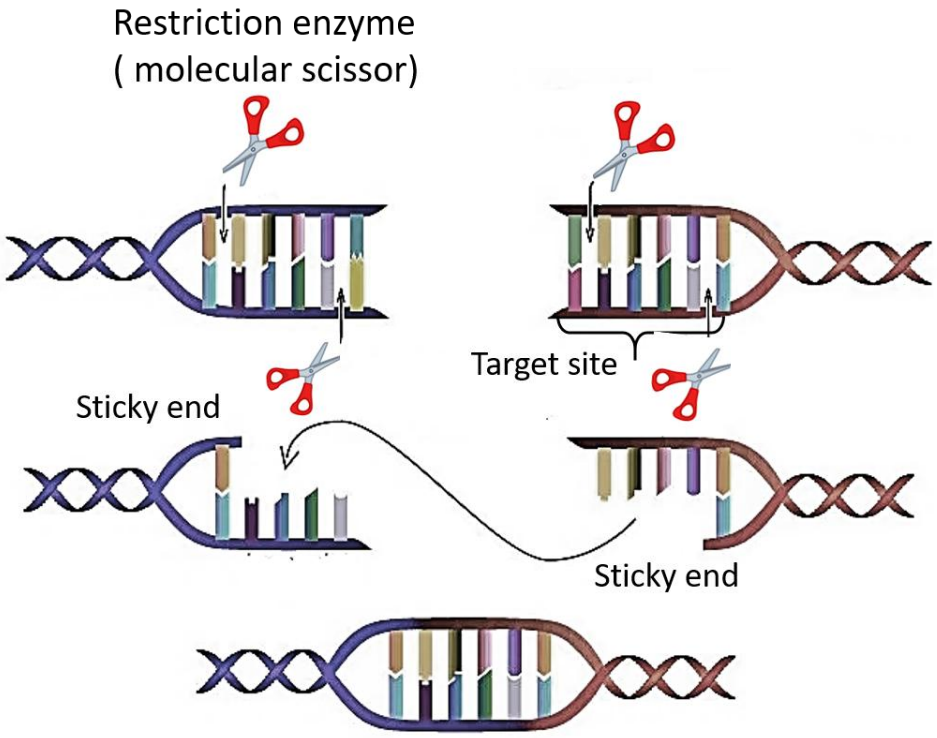


Editing Tools – 1. Restriction Enzymes

Type	Characteristics
I	The first restriction enzymes to be discovered. These enzymes are large, have combined restriction/modification functions and recognise asymmetric recognition sequences (RS). Cleavage sites (CS) are random sites some 1 000 bp from the RS. Of limited use in gene manipulation.
II	The most useful type for routine use. Loosely grouped into several subtypes, depending on specific characteristics. Type IIP (often just called type II) are the most common. These recognise an inverted palindrome RS and cut within it. Other types recognise asymmetrical RS and/or may cut a few base-pairs from the site, or may cut both ends of the RS. One of the most useful subtypes is type IIS. These enzymes have two domains, one for recognition and one for cleavage. They recognise an asymmetric RS and cut a small number of base-pairs from this. They have become a key part of a cloning method called Golden Gate cloning (see Section 8.4.5).
III	Type III are large combined restriction–modification systems and cut about 20 bp from the RS. Require two inverted RS and often do not cleave DNA fully.
IV	Cleave modified regions such as methylated DNA.
V	The Cas (CRISPR-associated protein) enzyme system is considered a form of restriction enzyme that utilises guide RNAs to cleave DNA. Most well known as the CRISPR-Cas9 editing system (see Chapter 12).
Artificial	Enzymes can be engineered by combining a DNA recognition domain with a functional nuclease domain. This approach opens up the design of specific recognition sequences that can be used for very precise manipulation of gene sequences. Examples include zinc-finger nucleases, TALENs and the CRISPR-Cas9 system (see Section 5.2.1 and Chapter 12).

Editing Tools – 1. Restriction Enzymes

- **Nomenclature** is based on the genus and species names of the organism from which the enzyme is derived. The **first letter of the genus** name and the **first two letters of the species** name are used.
  - Eco - enzyme from Escherichia coli
  - Bam - enzyme from Bacillus amyloliquefaciens

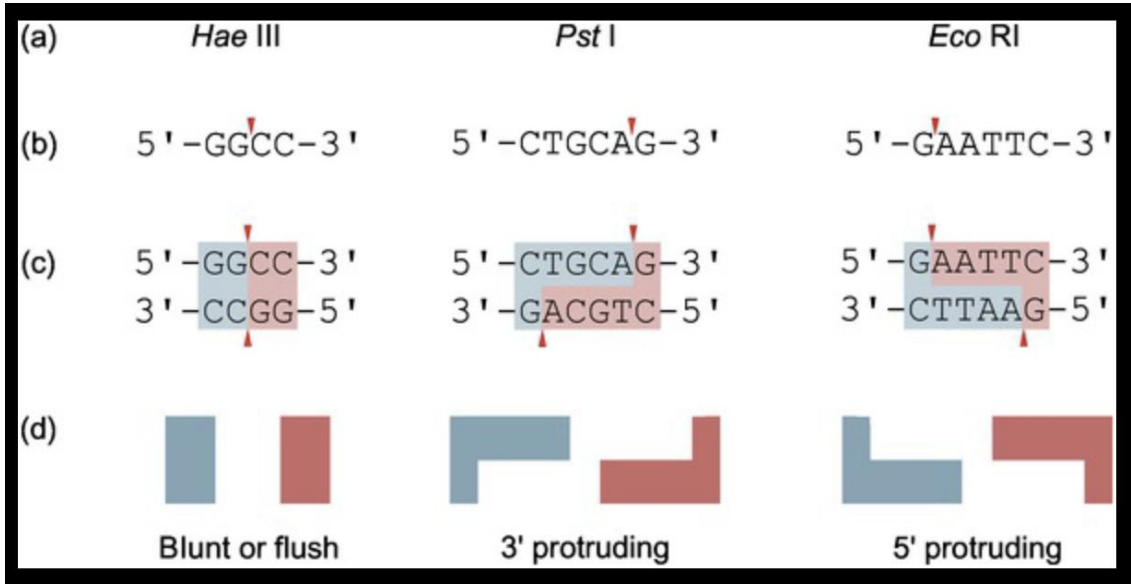


Recombinant DNA

Editing Tools – 1. Restriction Enzymes

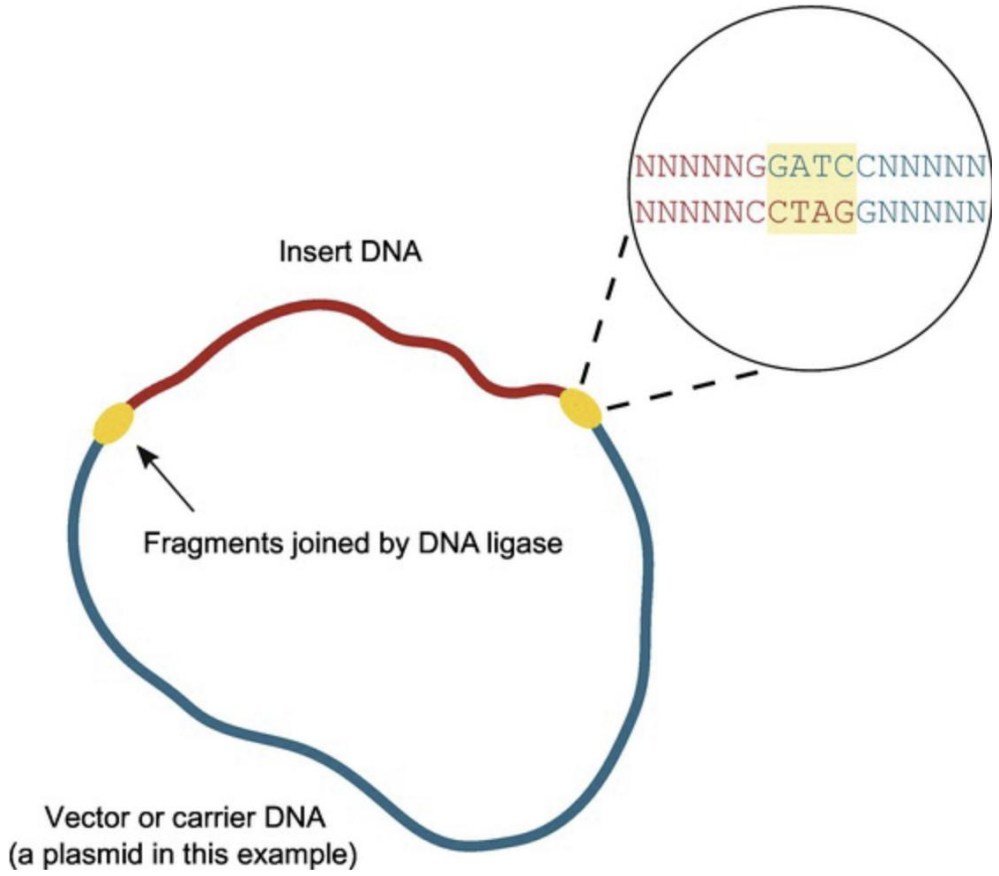
- Moat recognition sequences are 4,5,6 base-pairs in length.
- Expected frequency of RS = 4^n

  n=number of base-pairs
- RS are palindrome (read the same forward and backwards).
- Types of cutting products:
  - Blunt ends
  - Protruding 3' ends (sticky)
  - Protruding 5' ends (sticky)



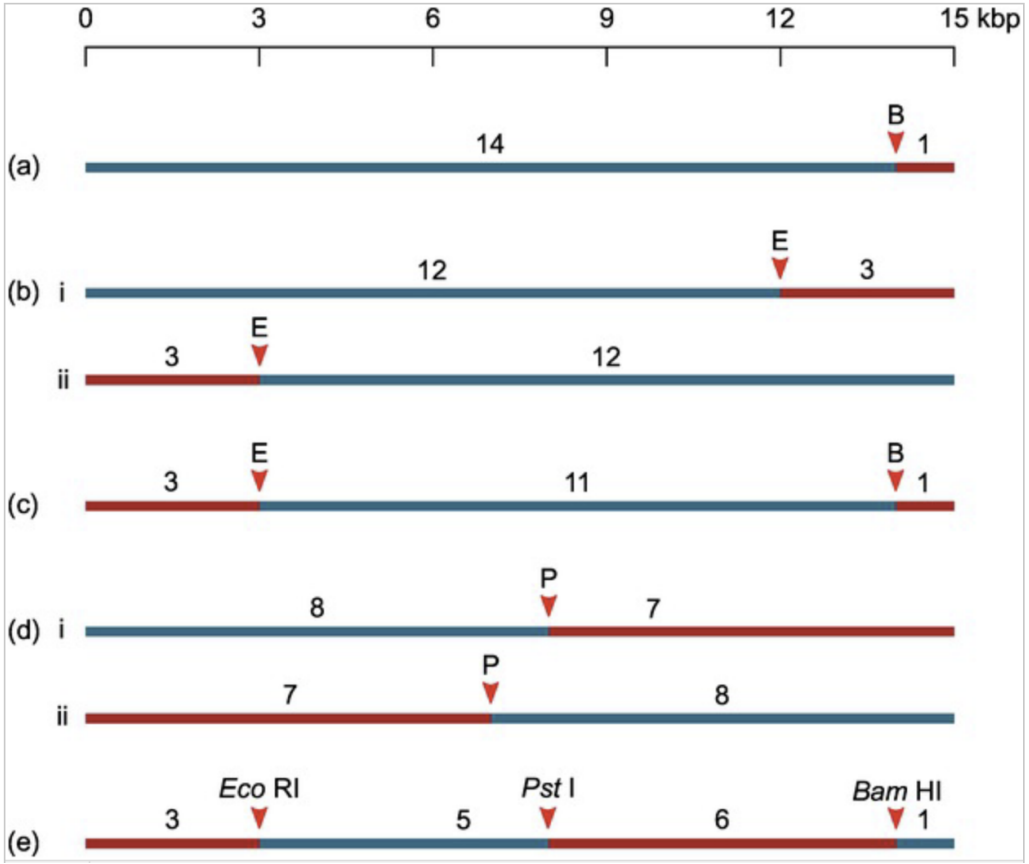
Editing Tools – 1. Restriction enzymes

- Protruding sequences can base-pair with complementary sequences generated by the same enzyme.
- Recombinant DNA can be produced by cutting two different DNA samples with the same enzyme and mixing the fragments together.
- **Restriction mapping** – mapping all known restriction sites of desired restriction enzymes on the DNA sequence.



Editing Tools – 1. Restriction enzymes

- Gene mapping tools: SnapGene, NEBcutter
- Predict enzyme cut sites in plasmids or genes
- Advantages:
  - Saves time and avoids trial-and-error in the lab
  - Allows in silico cloning and design
  - Visualizes fragments, orientation, and compatibility
- Limitations:
  - Real-world results may vary (methylation, incomplete digestion)
  - Requires accurate input sequence
- *In silico* mapping improves planning and precision



Editing Tools – 2. DNA Modifying Enzymes

Nuclease Enzymes

- Cut DNA by breaking the phosphodiester bonds.
- **Exonucleases -** degrade DNA from the 5' or 3' ends.
- **Endonucleases** - cut within the DNA strand (e.g., restriction enzymes).
- About 30 types exist, each with different modes of action.
- Some are engineered for precision genome editing:
  - Zinc-Finger Nucleases (ZFNs)
  - TALENs
  - CRISPR-Cas9

Editing Tools – 2. DNA Modifying Enzymes

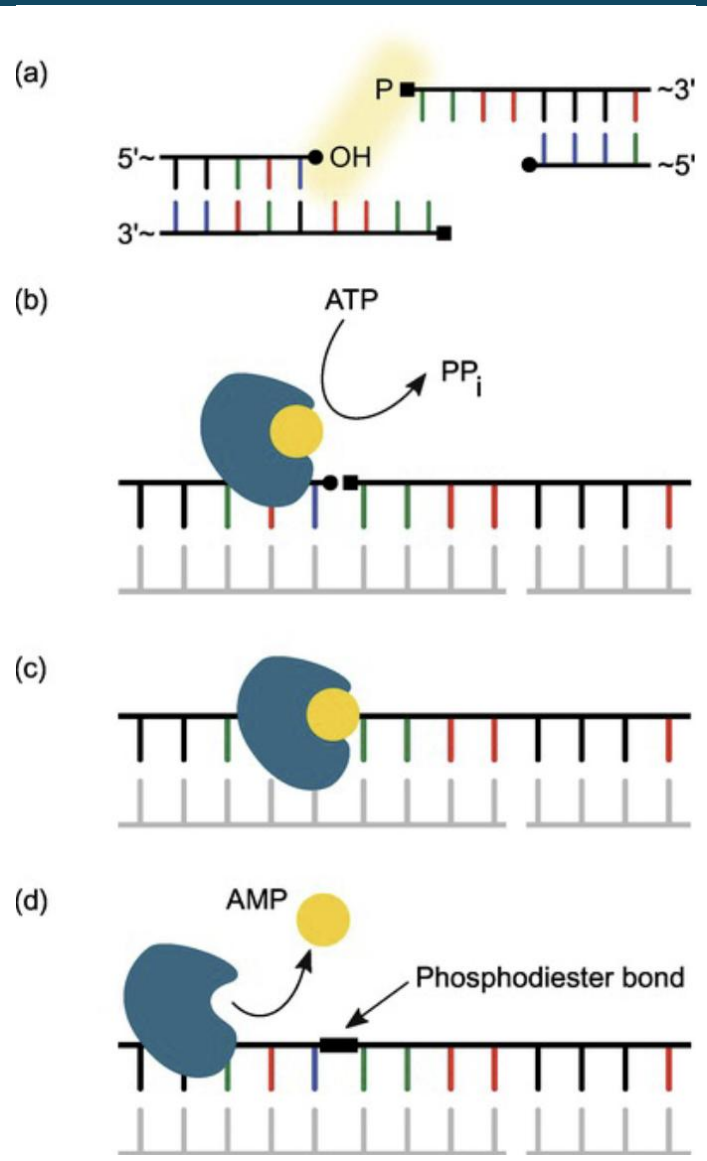
Polymerase enzymes

- Synthesize copies of nucleic acid molecules.
- Template-dependent – can be DNA-dependent or RNA-dependent, depending on the template used.
- **Reverse Transcriptase (RT)** – an RNA-dependent DNA polymerase used to make **complementary DNA (cDNA)** from an RNA template.

Editing Tools – 2. DNA Modifying Enzymes

DNA Ligase

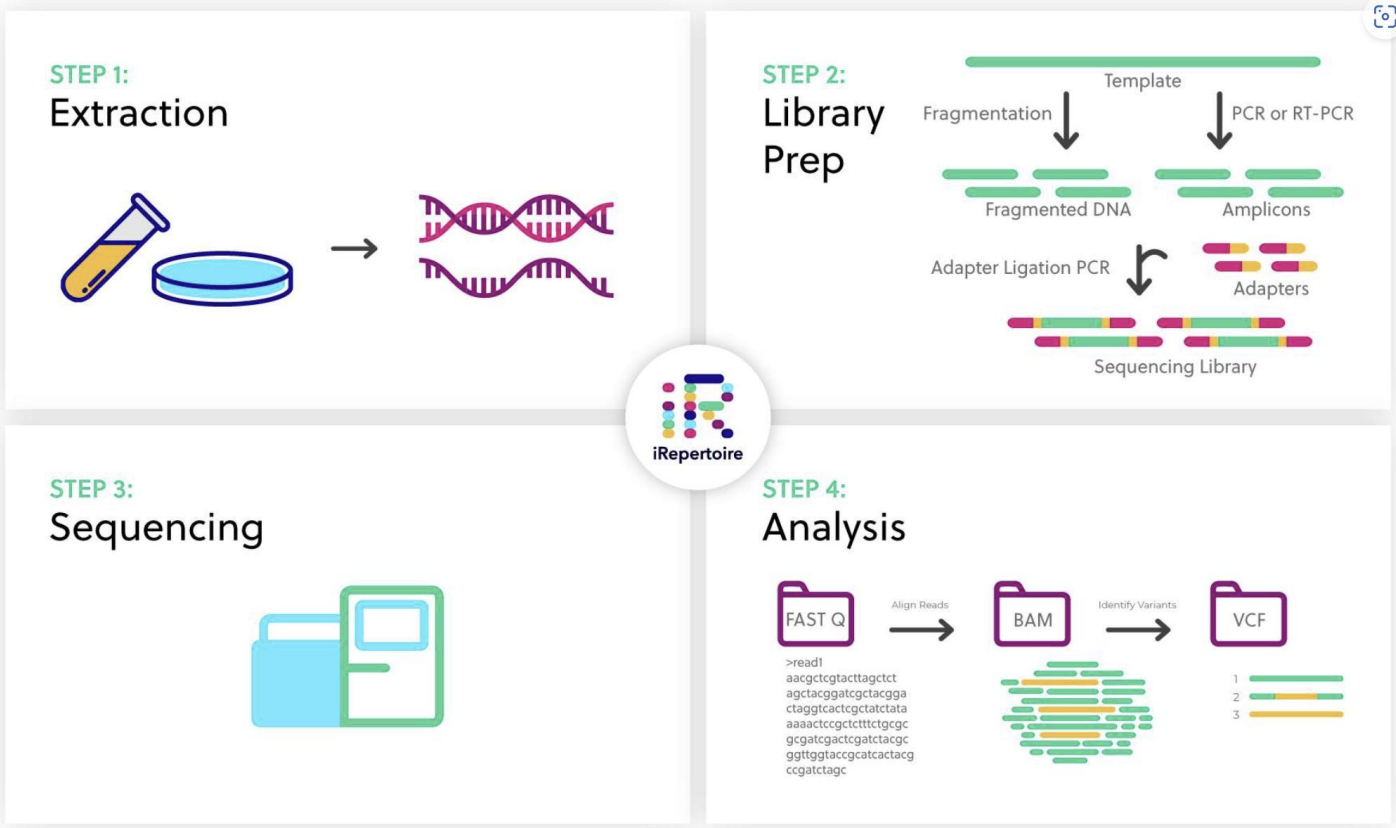
- Repairs broken phosphodiester bonds during DNA replication or recombination.
- In genetic engineering, it is used to seal nicks during cloning.



Editing Tools – 3. Next-Generation-Sequencing

- High-throughput sequencing (HTS)- large scale DNA sequencing using NGS techniques
- Steps:

1. Extraction
2. Library prep
3. Sequencing
4. Analysis



Host Cells

- **Prokaryotic** hosts are easy to handle, available, and accept a wide range of vectors.
- **Eukaryotic** yeast cells share many of the characteristics of bacteria such as ease of growth and availability of mutants.
- **Higher eukaryotic** cells are typically used to alter the genetic makeup of the organism by creating a transgenic cell.

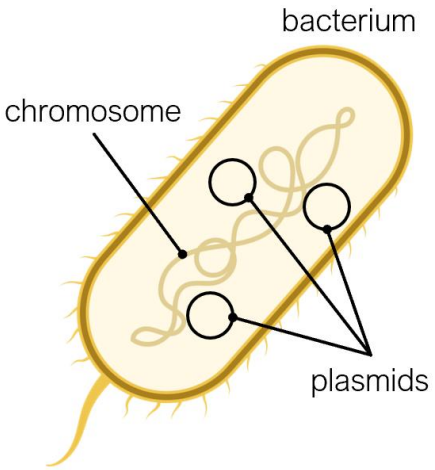
Vectors

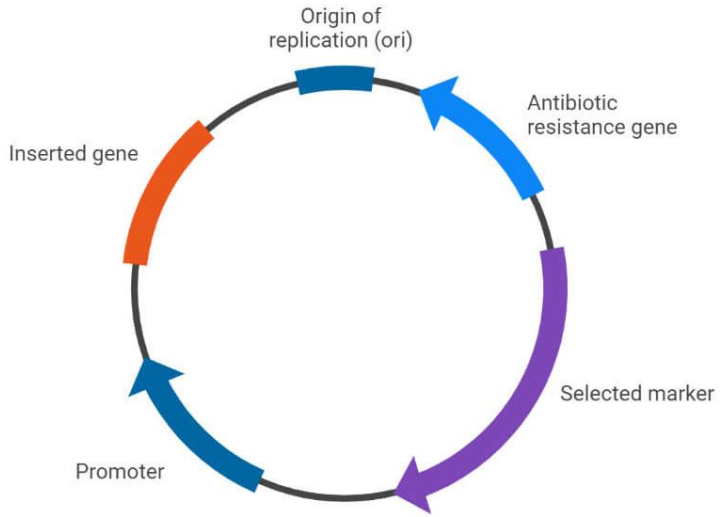
Commonly used cloning vectors:

1. **Plasmids** are circular, autonomously replicating DNA molecules widely used as cloning vectors.
2. **Bacteriophages**, also known as phages, are viruses that infect bacteria.
3. **Cosmids** are hybrid vectors that combine the characteristics of both plasmids and bacteriophages. They are more stable than regular plasmids.
4. **Bacterial artificial chromosomes (BACs)** are large cloning vectors used for cloning DNA sequences in bacterial cells. They can hold DNA segments up to 350 kb.
5. **Yeast artificial chromosomes (YACs)** are vectors used for cloning DNA fragments larger than 1 megabase (1 Mb). They are commonly used in genome mapping and sequencing projects.

Plasmid Vectors

- Naturally occurring extrachromosomal circular DNA molecules that often carry antibiotic resistance genes.
- Cloning vectors are plasmids that have been modified to carry new genes.
  - Small DNA molecules, easy to manipulate
  - Origin of replication – allows plasmid maintenance during cell division
  - Selectable marker – only cells containing the plasmid survive on selective media
  - Multiple cloning site – contains unique restriction sites for easy DNA insertion

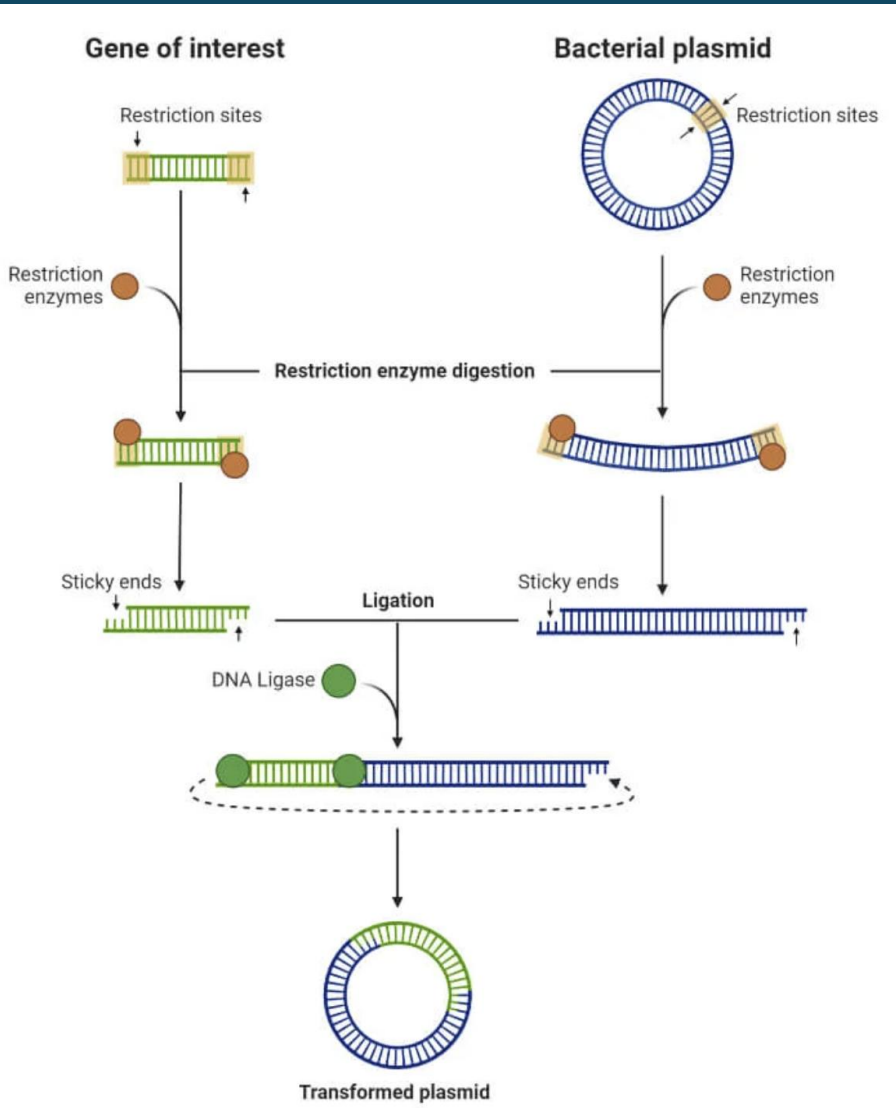




Plasmid Vectors

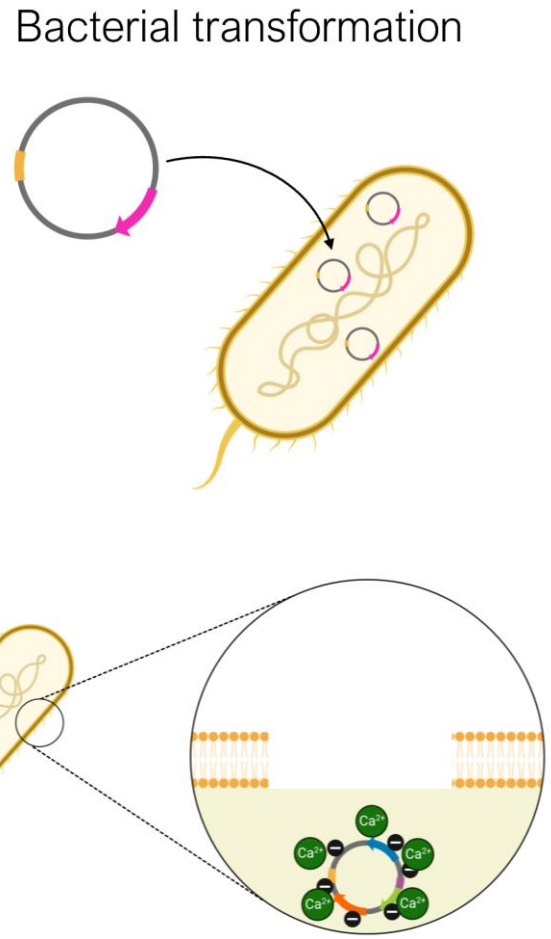
Cloning

- Isolate the gene of interest and cut it with restriction enzymes.
- Cut the plasmid with same restriction enzymes.
- Insert the gene into the plasmid using DNA ligase.



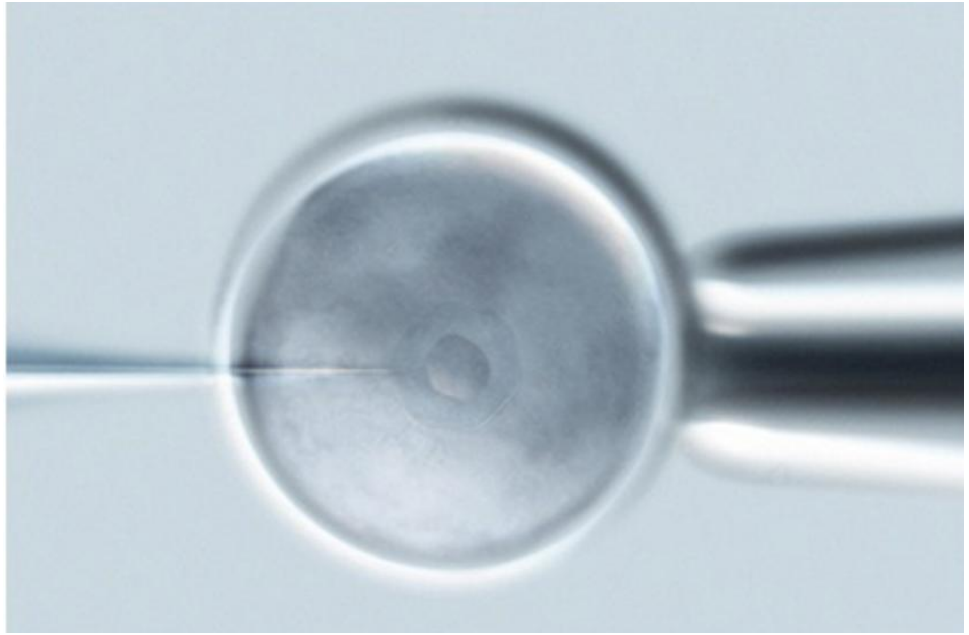
Gene Delivery

- Genetic transformation - when a cell takes up DNA and expresses the genes on that DNA.
- **Competent cells** are made able to take up exogenous DNA
  - $CaCl_2$ and heat shock
    - Positive charge of Ca^{+2} ions neutralizes negative charge of DNA phosphates and of membrane phospholipids.
    - Heat-shock increases of cell membrane permeability, promoting plasmid uptake.



Gene Delivery

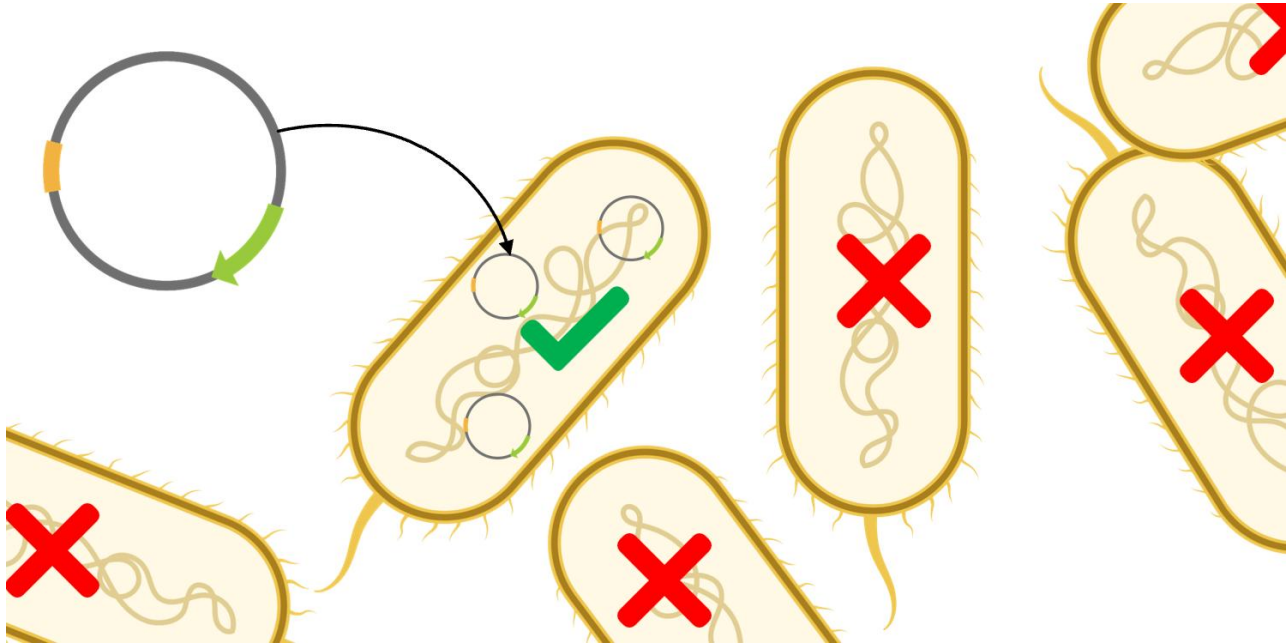
- Electroporation - host cells are exposed to an electric field, which creates temporary pores in the cell membrane.
- Microinjection - inject the DNA directly into the nucleus.



Gene Delivery

Selection

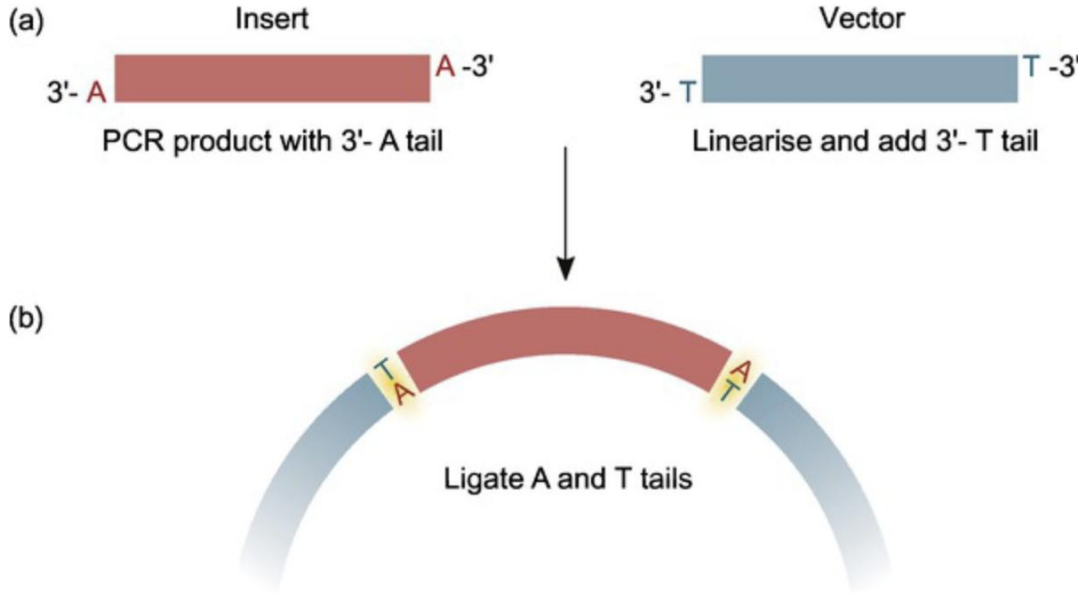
- Only cells with the plasmid will grow on antibiotic.



Alternative Cloning

PCR Cloning

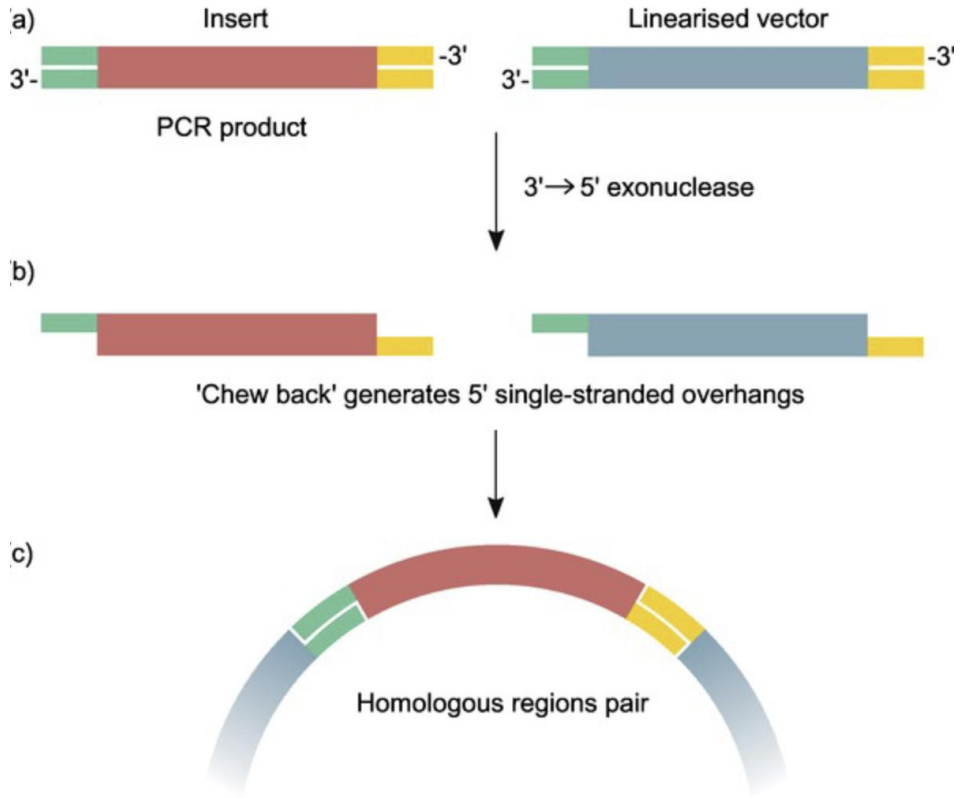
- Involves the direct ligation of DNA fragments obtained through PCR amplification into a vector without the need for cutting the insert using restriction enzymes.
- In TA cloning, Taq polymerase adds an adenine (A) residue to the 3′ ends of PCR products, creating “A-tailed” DNA fragments. These fragments are directly ligated with “T-tailed” vectors having thymidine (T) residues at their ends using DNA ligase.



Alternative Cloning

Ligation-Independent Cloning (LIC)

- Specific short sequences are added to the ends of a DNA insert to match the sequences on a vector.
- The 3' ends of the DNA fragments are trimmed using enzymes with 3' to 5' exonuclease activity, creating cohesive ends between the DNA insert and the vector.
- The vector and insert are combined. The resulting plasmid contains four single-stranded DNA nicks, which are repaired by the host during transformation.
- This method maintains the original sequence integrity without introducing any additional elements.



Bioinformatics

- The discipline of collecting and analyzing biological data.
- Interactive and predictive applications.
- Bioinformatics benefits:
  - Access to a large network of databases
  - Continuous upload of new data
  - Storage of large datasets
  - Open access to most of the sites and databases
  - Access to the most recent versions of software

Site/page
European Bioinformatics Institute (EBI)
EBI training site
The Wellcome Sanger Institute
National Center for Biotechnology Information (NCBI)
NCBI education site
Swiss Institute of Bioinformatics (SIB)
The Expasy resource portal of SIB
The Bioinformatics Organisation

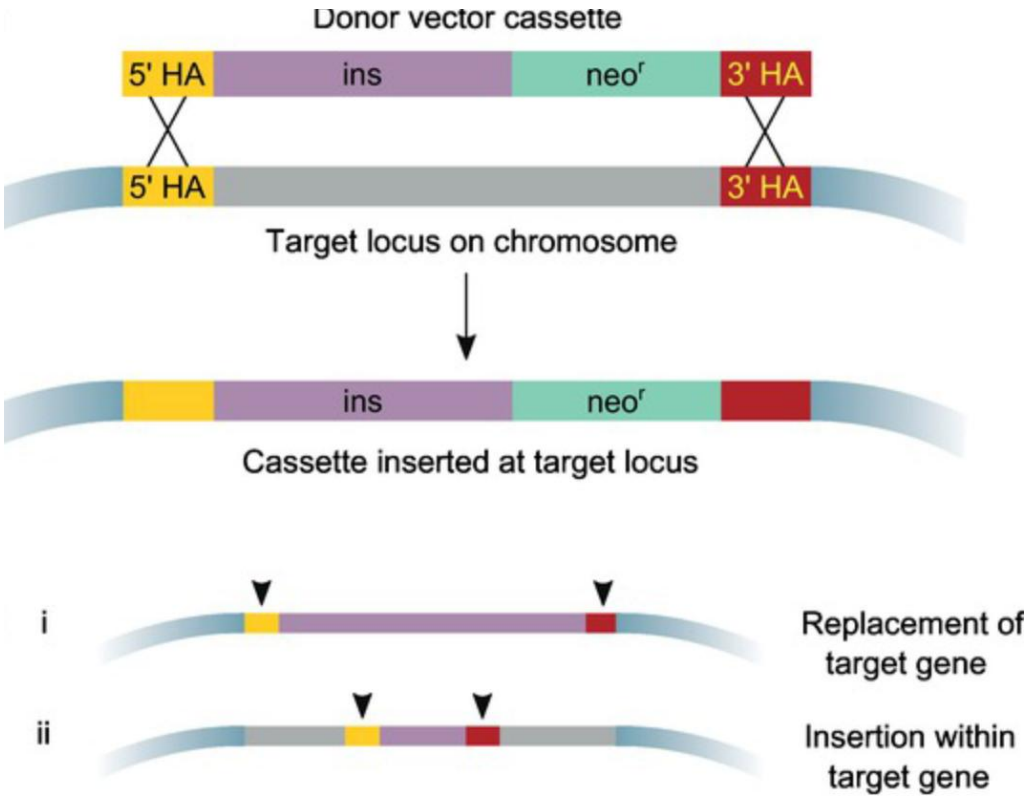
Gene Editing

Gene editing techniques have common requirements:

- Identify the target gene.
- Use a specific mechanism to introduce the change.
- Ensure the process is efficient.
- Minimize off-target effects.

Gene Editing – Gene Targeting

- **Homologous Recombination (HR)** – an exchange of genetic information between identical or similar nucleic acid molecules.
- A targeting cassette can be used to replace or insert genetic material within a gene:
  - A donor vector carries the gene of interest.
  - A selectable marker.
  - Homology arms (3’ and 5’) target the gene sequence for recombination.



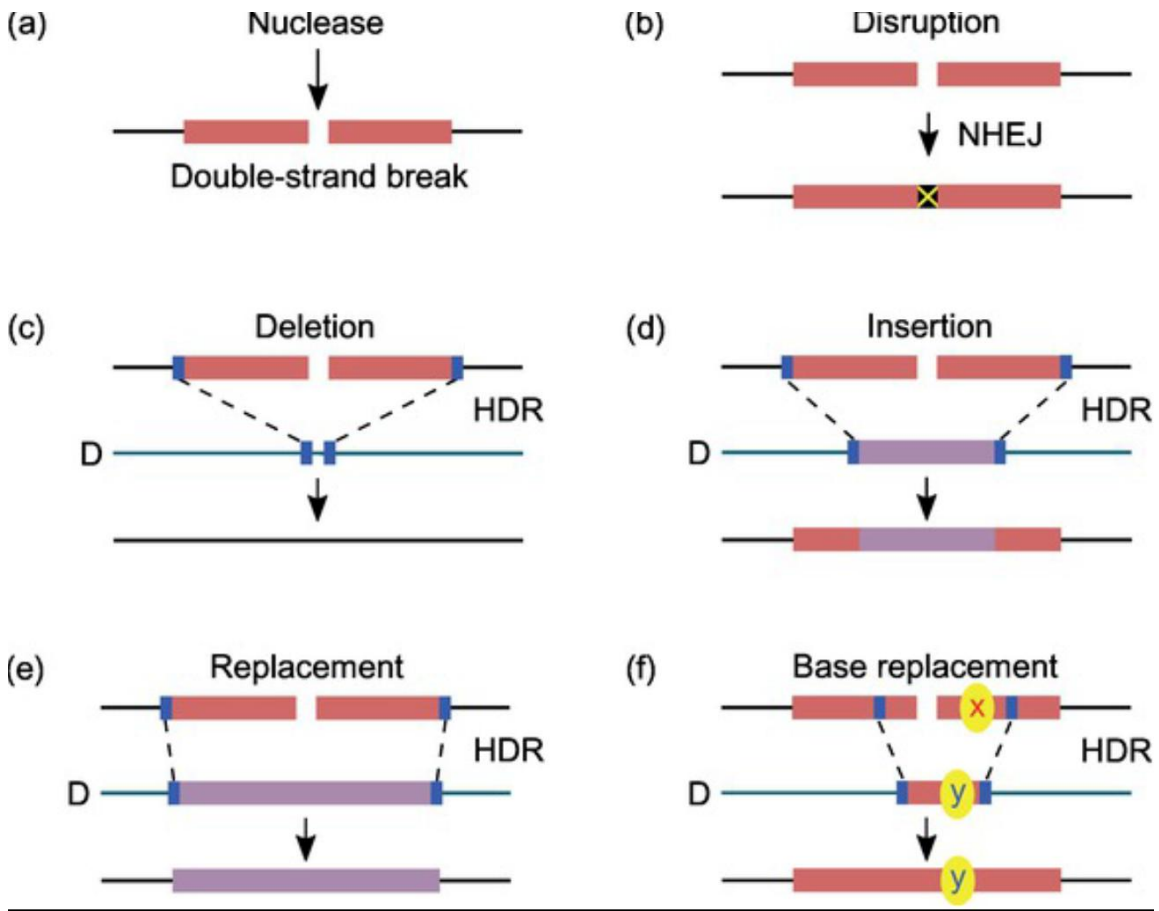
Gene Editing – Gene Targeting

- Knockout (KO) – a method for gene silencing by removal of the gene from the genome or its inactivation through mutagenesis. Causes loss of function.
- Knock-in (KI) – a method of substitution of genetic material that introduces a gene into the genome.

Gene Editing – Gene Targeting

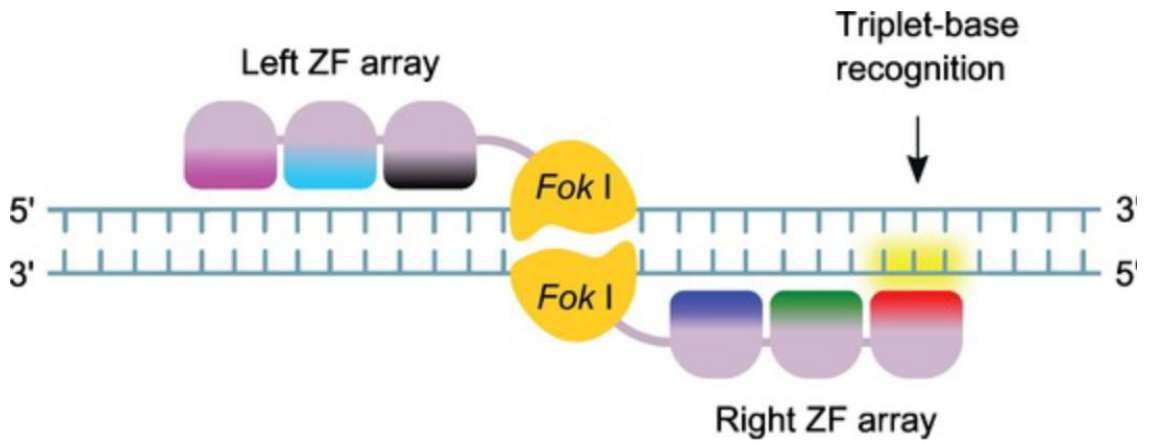
Outcomes for the repair of double-strand breaks (DSB):

- (a) A double-strand break is generated in a gene sequence using a nuclease.
- (b) Repair by non-homologous end joining (NHEJ).
- (c)–(f) Introducing a donor vector sequence (D) to drive homology-directed repair (HDR):
- (c) KO using a donor with homologous arms (blue).
- (d) KI by insertion withing an existing gene, using a donor carrying a gene sequence flanked by the HA.
- (e) KI by replacement of the entire endogenous gene.
- (f) Single base replacement.



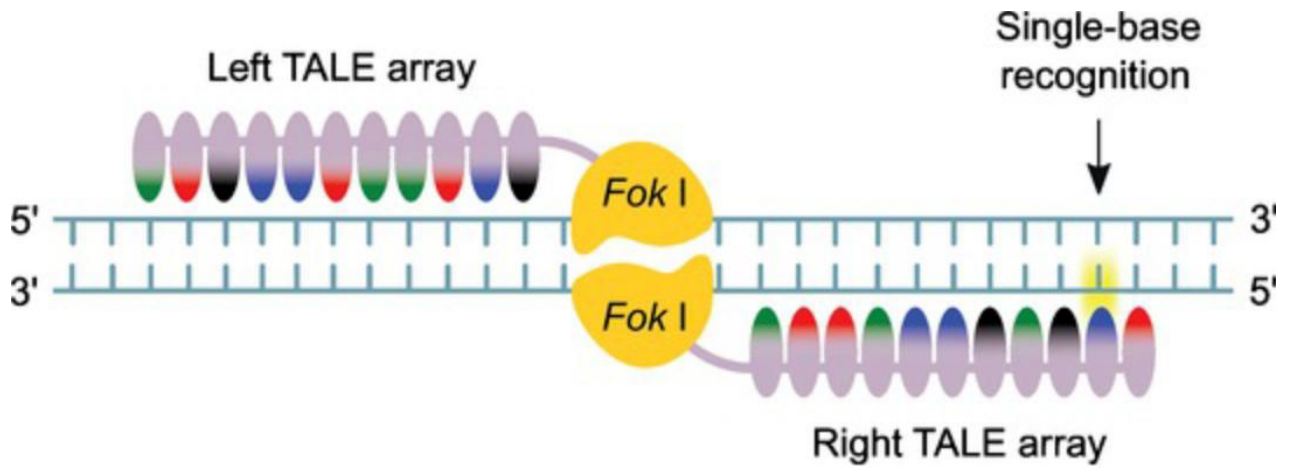
Gene Editing – Zinc Finger Nucleases (ZFN)

- Artificially engineered restriction enzymes for custom site-specific genome editing.
- **Hybrid heterodimeric enzyme** - each subunit contains several zinc finger domains and a FokI endonuclease domain.
- Binds DNA as a dimer to generate a DSB.
- **Context-dependent specificity** – depends on the context in the adjacent zinc fingers and DNA, not only on the target sequence.
- Many non-specific cleavages (toxic).



Gene Editing – Transcription activator-like effector nucleases (TALENs)

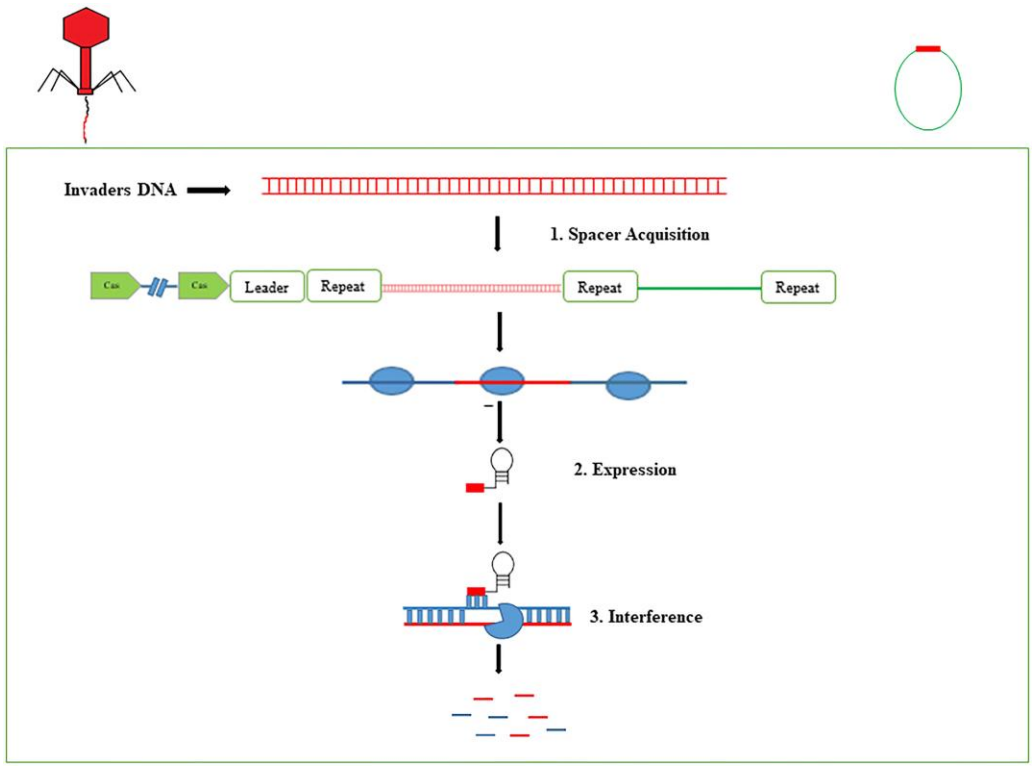
- Artificial chimeric protein fuses a non-specific FokI restriction endonuclease domain to a DNA-binding domain.
- The DNA-binding domain derived from transcription activator-like effectors (TALE).
- One module recognizes just one nucleotide.
- More efficient in producing DSBs.
- Less toxic.

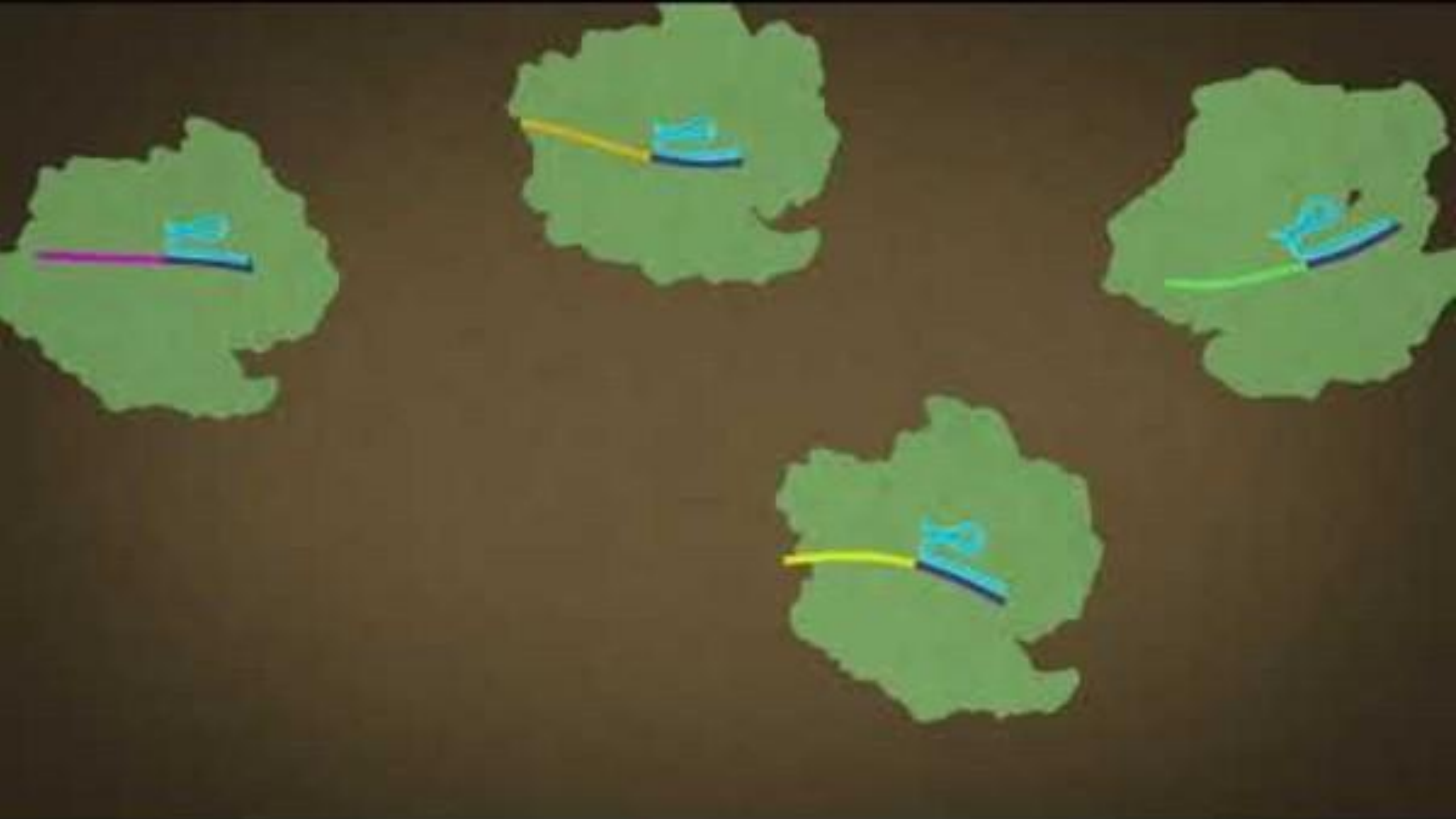


Gene Editing – CRISPR/Cas9

- Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) / CRISPR-associated protein 9 (Cas9)
- An adaptive immune system found in bacteria and archaea.
- Mechanism of CRISPR-Cas immunity:

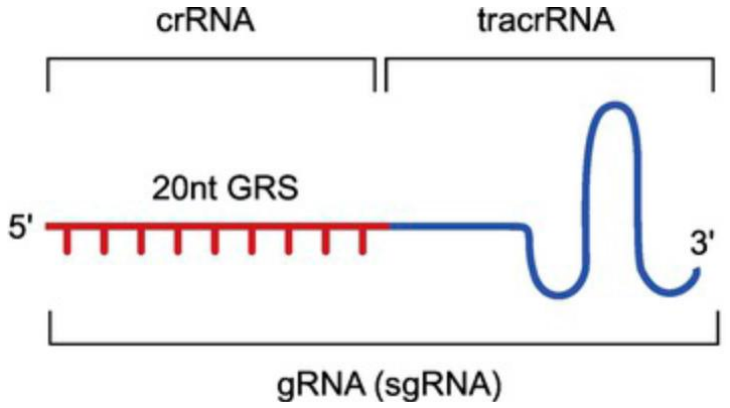
1. Spacer acquisition - specific fragments of viral DNA are integrated into the CRISPR array in host DNA as a unique spacer (red box) interspaced between repeats (blue box).
2. **crRNA** - RNA polymerase transcribes **CRISPR-associated RNA (crRNA).**
3. Interference – a specific match between crRNA spacer and target sequence leads to cleavage of foreign genetic elements (blue and red strips).

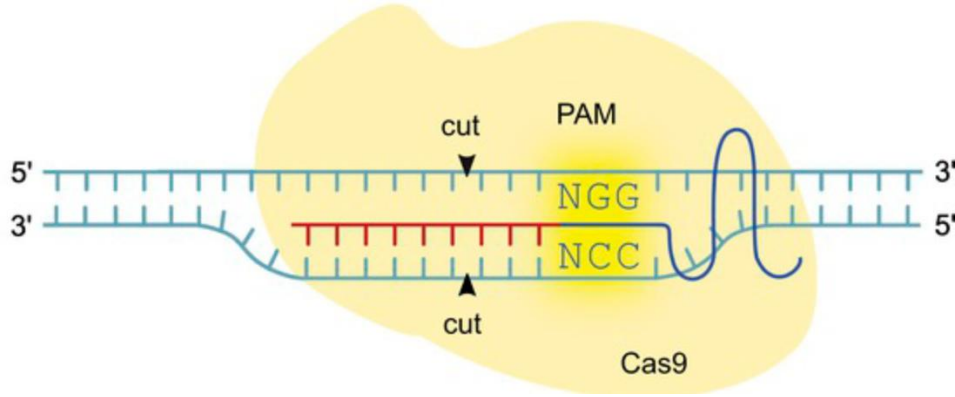




Gene Editing – CRISPR/Cas9

- **crRNA – CRISPR RNA** is a 20-nt sequence complementary to the target DNA sequence.
- **tracrRNA - trans-activating CRISPR RNA**. A short RNA molecule, essential for nuclease activity.
- **sgRBA - single guide RNA** is a complex of tracrRNA and crRNA.
- **PAM - protospacer adjacent motif.** A short 5'-NGG-3' sequence found downstream of Cas9 recognition site in the target DNA.
- DNA recognition site allows for easy and cheap design.
- Easy to predict off-targets.



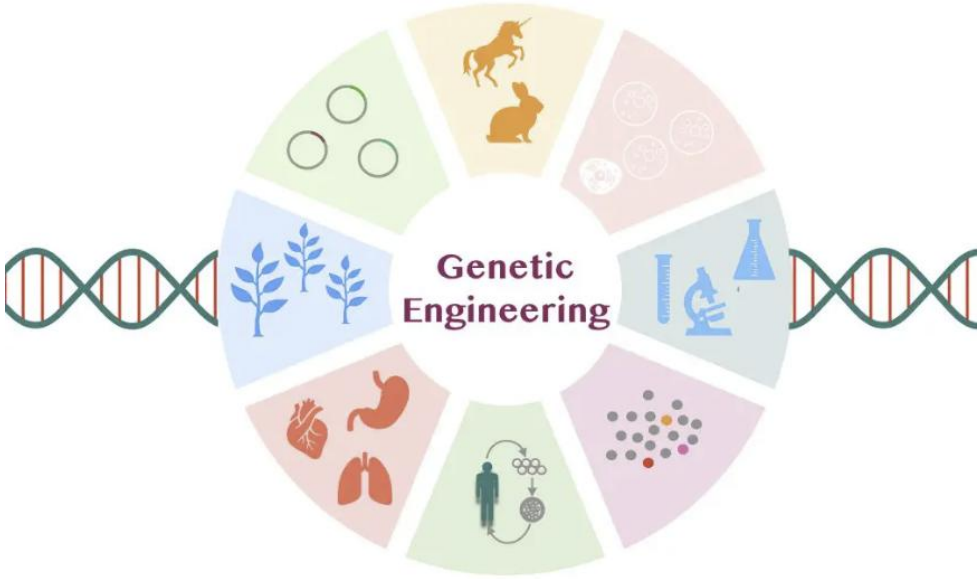


How to Present a Paper

- The student presenter will prepare the slides for the paper. They will introduce the background of the paper, and all necessary information.
- Each figure will then be explained by a different student (or more than one, if the figure is long).
- Fo each figure please include in you explanation:
- What is the purpose of the main figure (main point).
- What is the purpose of each subfigure.
- How did they try to address that purpose. How was the experiment performed (what method or methods were used – know how to explain the technique, why was this method used, is there a better one).
- What is the result of each subfigure.
- What is the overall conclusion of the figure.

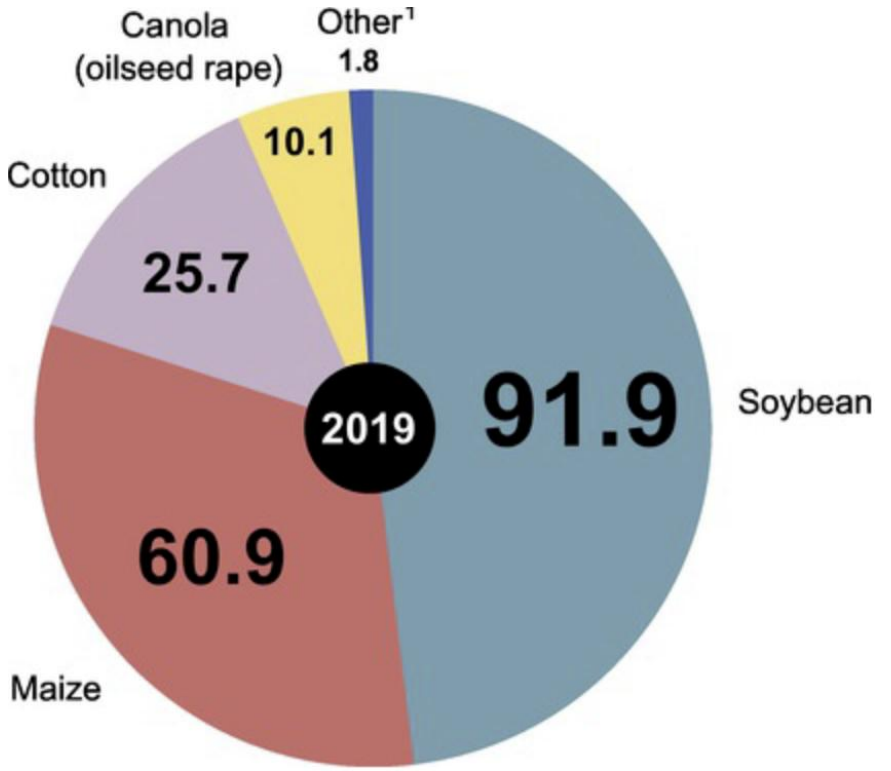
Applications

- **Transgenic organism** – an organism that carries foreign DNA from another species. The change is permanent and inherited.



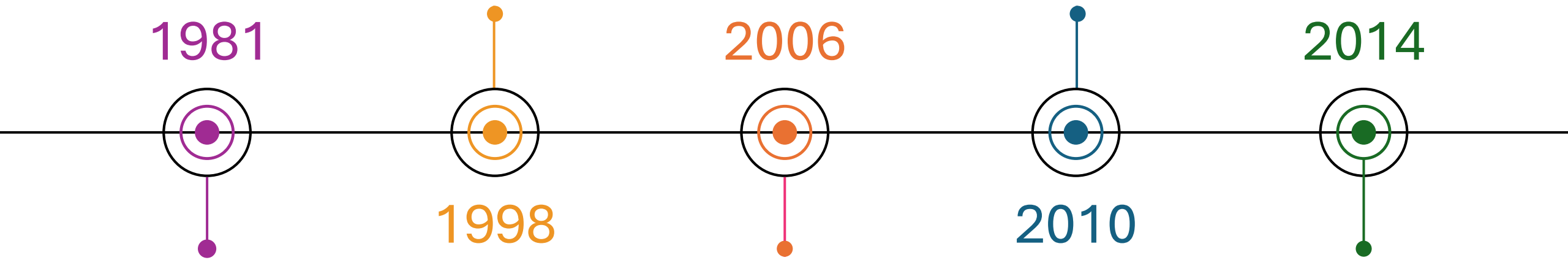
Applications - Agriculture

- Improved crop varieties with higher yields.
- High nutritional value, extended shelf life, improved stress tolerance, increased disease and pest resistance, removal of undesirable traits.
- **GMO – genetically modified organism**
- No need for sexual compatibility between species.
- Difficulties:
  - Method for introducing the manipulated gene.
  - Knowledge of the system's molecular genetics, especially for polygenic traits.



Global areas of the main GM crops grown in 2019

Milestones in Stem Cells Research



1981

First mouse ESCs successfully isolated and cultured by Martin Evans and Gail Martin

1998

First mouse ESCs successfully isolated and cultured by James Thomson

2006

Shinya Yamanaka reprogrammed mouse fibroblasts into Induced Pluripotent Stem Cells (iPSCs)

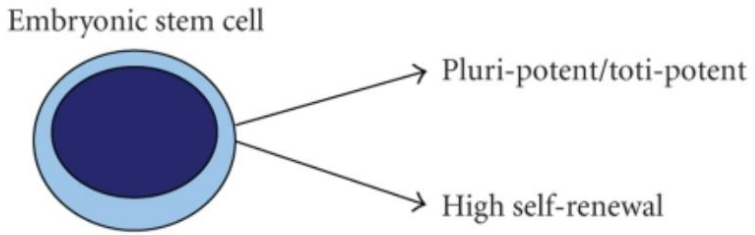
2010

First hESC-Based Clinical Trial for spinal cord injury

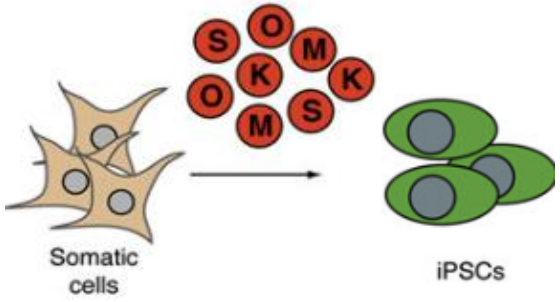
2014

First iPSC-Derived Cell Transplant in Humans. iPSC-derived retinal cells transplanted into a patient in Japan

Stem Cells



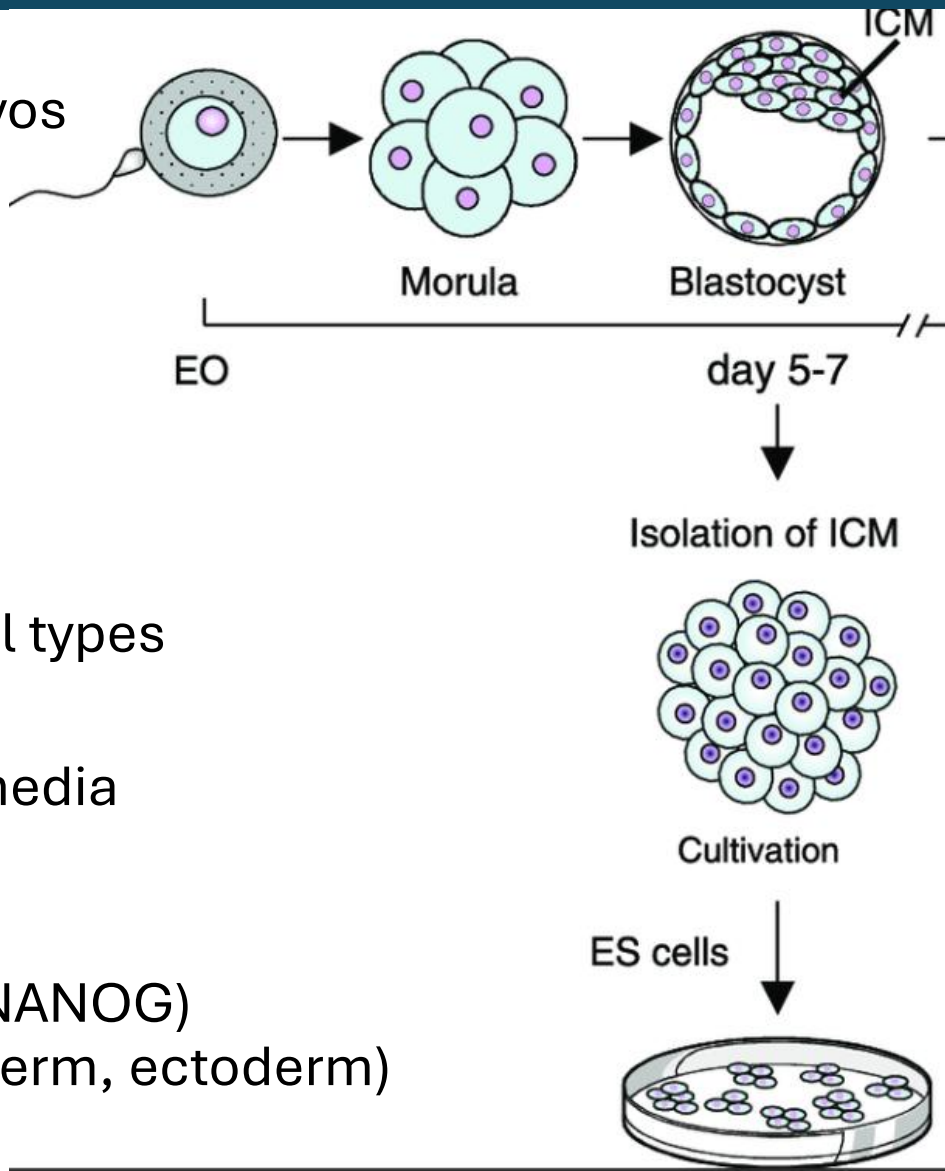
- **Embryonic Stem Cells (ESCs)**
  - Source: Early-stage embryos
  - Differentiation: Pluripotent – can become *any* cell type.
  - Cell Division: Unlimited self-renewal.
- **Adult (Somatic) Stem Cells**
  - Source: Found in specific tissues after birth.
  - Differentiation: Multipotent – can develop into a *limited range* of cell types (from that tissue).
  - Cell Division: Can self-renew, but more limited than ESCs.
- **Induced Pluripotent Stem Cells (iPSCs)**
  - Source: Reprogrammed adult cells (e.g., skin or blood).
  - Differentiation: Pluripotent – same potential as ESCs.
  - Cell Division: High self-renewal capacity, similar to ESCs.



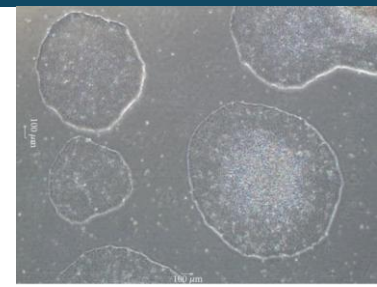
How Are Human Embryonic Stem Cells Obtained?

- Derive pluripotent stem cells from early-stage human embryos
- **Main Steps:**

1. **Source of Embryos**
   - Excess embryos donated from IVF fertility clinics
   - Donors provide informed consent for research use
2. **Isolation of the Inner Cell Mass (ICM)**
   - Embryos at the **blastocyst stage** (~5–7 days old)
   - ICM contains pluripotent cells that can form all body cell types
3. **Culture and Expansion**
   - ICM cells placed on supportive feeder layer or defined media
   - Cells grow into a stable **hESC line**
4. **Validation of Pluripotency**
   - Check expression of pluripotency markers (e.g., OCT4, NANOG)
   - Confirm ability to form 3 germ layers (endoderm, mesoderm, ectoderm)

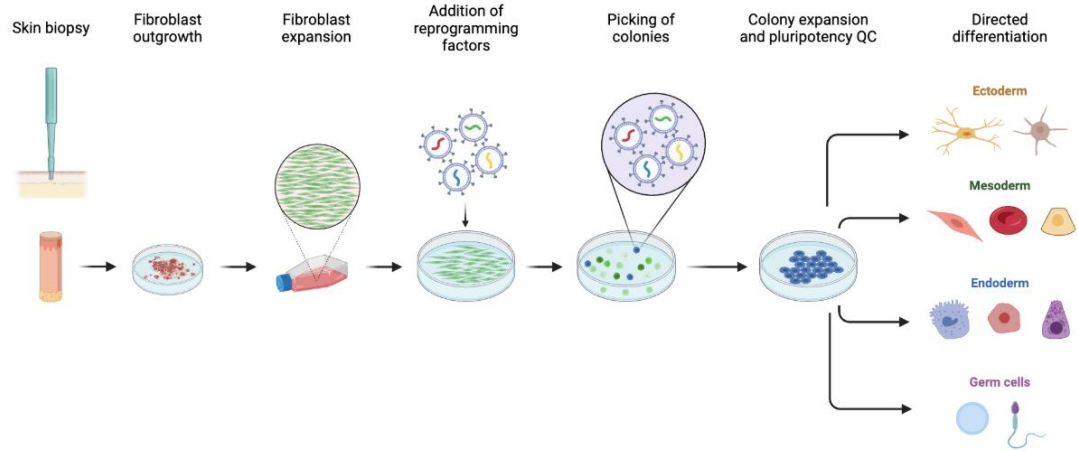


How To Create Induced Pluripotent Stem Cells?



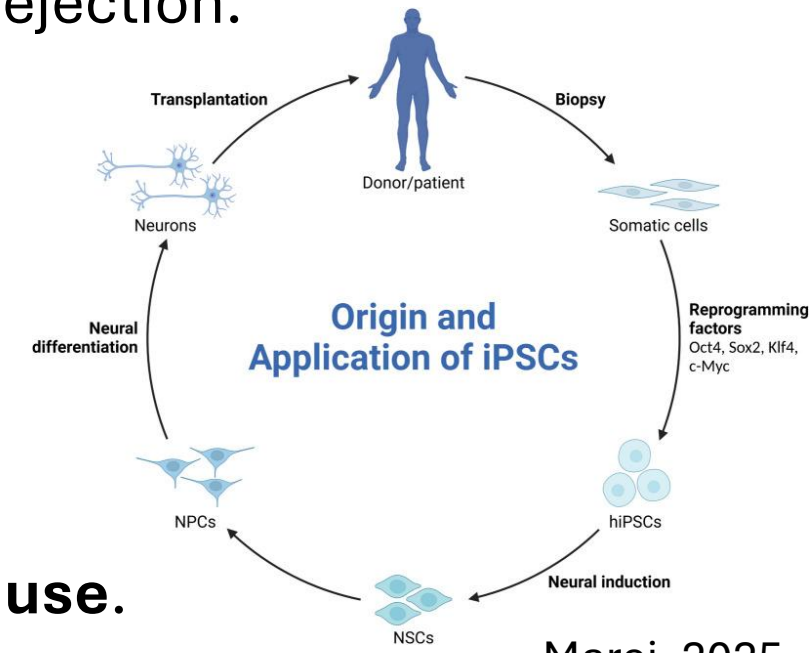
- Reprogram an adult somatic cell back into a pluripotent stem cell.
- **Main Steps:**

1. **Collect Patient Cells**
2. **Reprogramming –** Expression of the "Yamanaka factors": **OCT4, SOX2, KLF4, c-MYC.** Delivered via integrating or non-integrating methods (e.g., Sendai virus, mRNA, episomal vectors)
3. **Reprogramming -** Cells gradually change identity, until they obtain stem cell-like morphology.
4. **iPSC colony selection and expansion -** Pick colonies with correct morphology and expand into stable iPSC lines.
5. **Quality control of iPSCs:**
   - Pluripotency markers expression (NANOG, TRA-1-60)
   - Normal karyotype
   - Ability to differentiate into 3 germ layers.



Stem Cells - iPSCs

- iPSCs have demonstrated enormous promise in drug discovery, disease modeling, and possible clinical uses.
- **Benefits of iPSCs:**
  - Ethically acceptable – no embryo destruction required.
  - Enable **patient-specific therapies** and reduced immune rejection.
  - Derived from accessible sources like **skin or blood cells**.
  - Provide a **limitless supply** of pluripotent cells.
- **Challenges to Clinical Use:**
  - Risk of **genetic mutations** during reprogramming.
  - **Tumorigenicity**, especially with oncogenes like *c-Myc*.
  - Inefficient and **costly production.**
  - Requires further optimization for **large-scale therapeutic use**.



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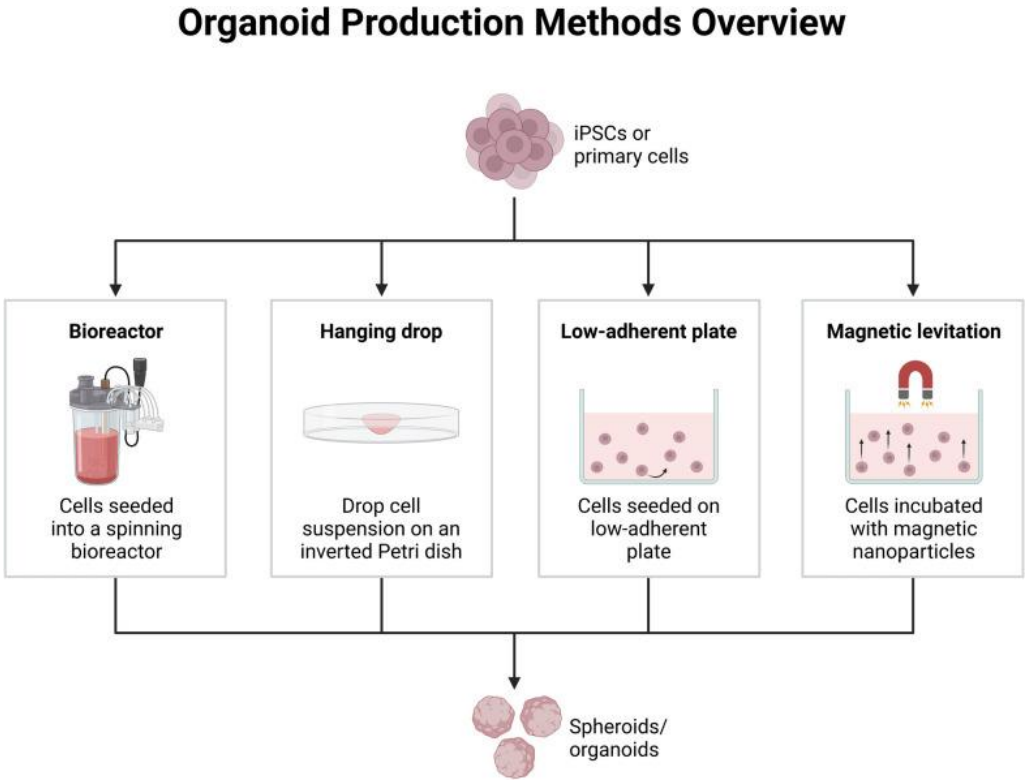
hESCs vs iPSCs – How Do They Compare?

Feature	hESCs	iPSCs
Source	Derived from donated IVF embryos	Reprogrammed from patient's somatic cells
Ethical Considerations	Involves embryo use	No embryo involvement
Immune Rejection Risk	↑ Higher (not patient-matched)	↓ Low (patient-specific)
Genetic Background	Healthy unless engineered	Naturally carries patient's mutation
Use for Disease Modeling	Requires genetic editing to create disease model	Directly models the patient's disease
Use for Cell Therapy	Allogeneic (donor → patient)	Autologous (patient → patient)
NIH Funding Restrictions	Only hESC lines approved on the NIH registry can be used with federal funding	No special NIH restrictions
Advantages	Highly stable pluripotency; long history of research	Personalized, avoids rejection, disease-relevant
Limitations	Ethical concerns; immune mismatch; funding restrictions	Time-intensive to generate & differentiate

Stem Cells - Organoids

- Organoids are 3D, self-organizing cell clusters mimicking real organ structure.
- Organoids replicate human physiology more accurately than 2D cultures
- **Challenges to Clinical Use:**
  - Lack of immune and vascular components
  - Variable results due to inconsistent differentiation protocols
  - Ethical and regulatory hurdles for clinical use



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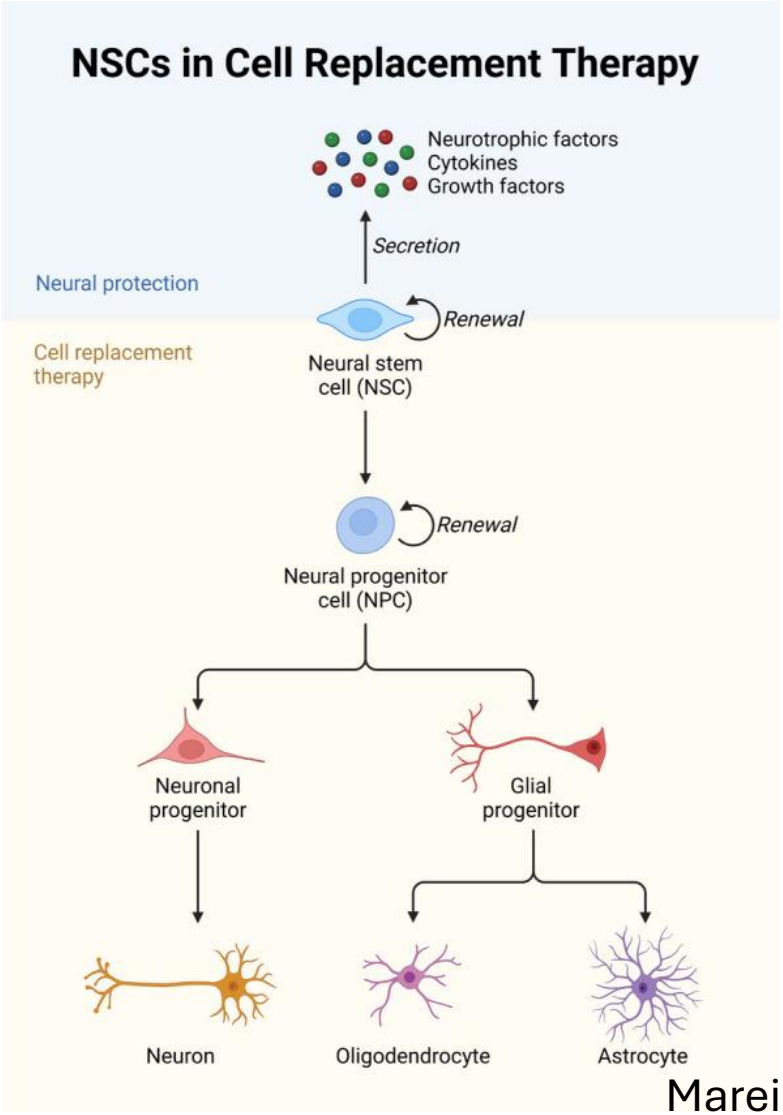
Stem Cells in Regenerative Medicine

- Better understanding of disease and cell plasticity.
- Key tools: iPSCs, single-cell omics, CRISPR-Cas9.
- Personalized and regenerative medicine.
- Applications: tissue repair, organ regeneration, disease therapy.
- Strong potential in neuro and cardiac disorders.



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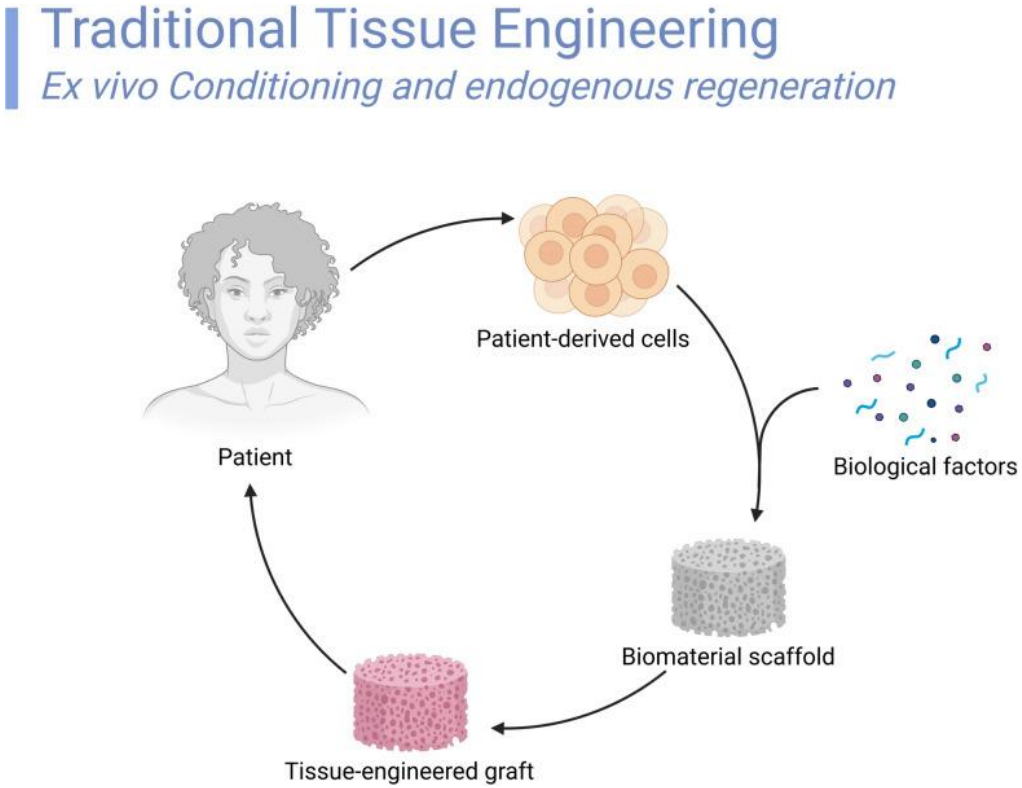
Stem Cells in Regenerative Medicine

- Stem cell-based organ transplantation and tissue engineering.
- **Benefits:**
  - SC can differentiate into many cell types.
  - Patient-specific tissues.
  - Cut reliance on organ donors.
- **Challenges:**
  - Complex organs (heart, liver, kidney) are difficult to grow in full.
  - High cost, scalability, and tumor risk remain concerns.
  - Differentiation protocols still need optimization for full functionality.



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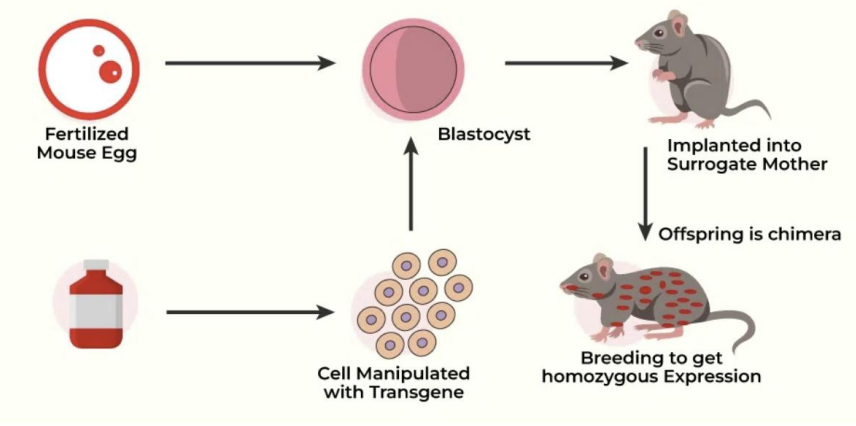
Applications – Transgenic Animals

- Generation of transgenic animals
- **Transgene (Tg)** - a foreign gene.
- Used in research and biotechnology
- Introduction of genes into embryos:

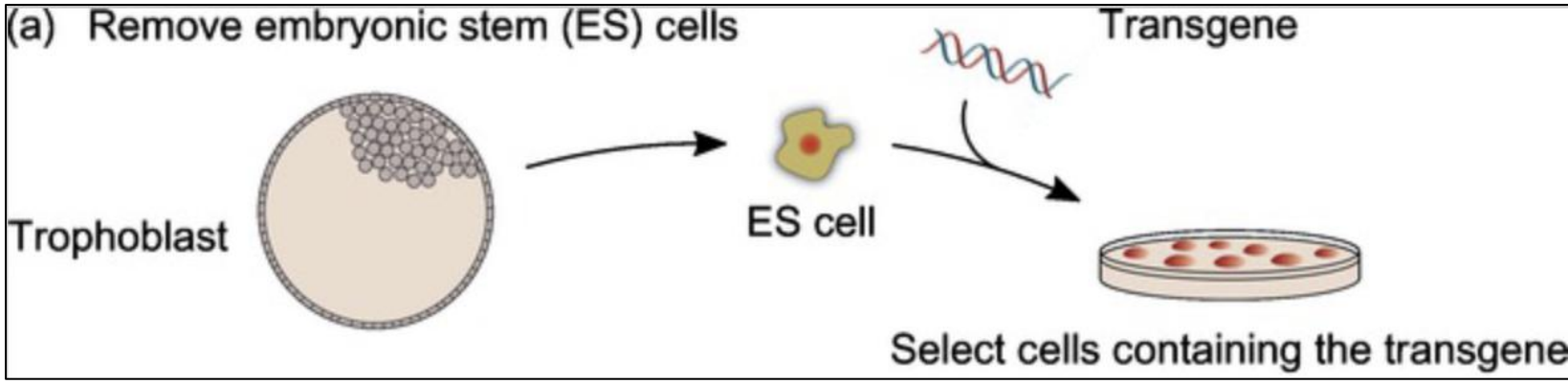
1. Direct transfection or retroviral infection of embryonic stem cell (ESCs).
2. Retroviral infection of early embryos followed by their transplantation.
3. Direct microinjection of DNA into oocytes, zygotes, or early embryonic cells.
4. Sperm-mediated transfer.
5. Transfer into unfertilized ova.
6. Physical techniques such as electrofusion.



Applications – Transgenic Animals

Step 1: Engineer the Embryonic Stem (ES) Cells

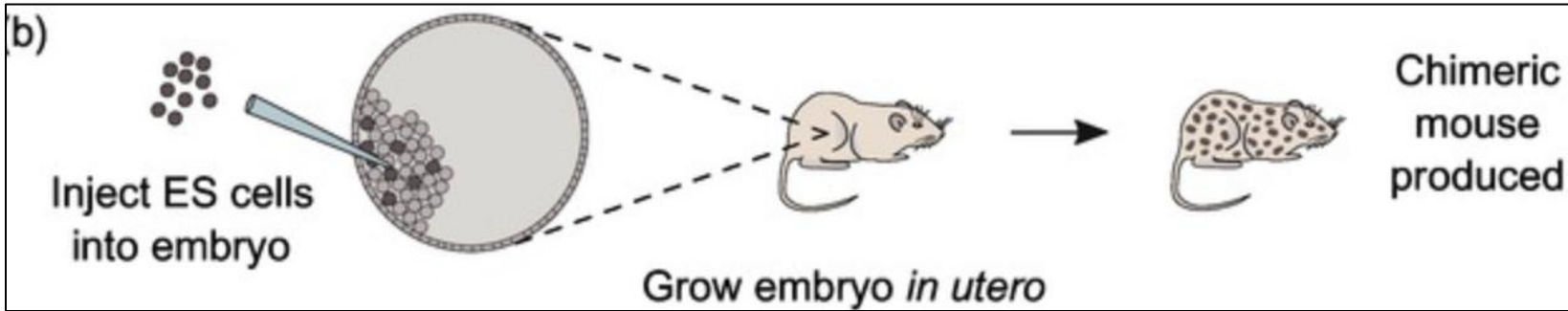
- Create ES cells that carry the desired genetic modification
- Design the targeting construct (how to insert the gene, selectable marker, conditional expression.
- Electroporate ES cells
- Select and screen for correctly modified ES cell clones
- Verify genomic integrity (karyotype, sequencing)



Applications – Transgenic Animals

Step 2: Inject ESCs into a blastocyst

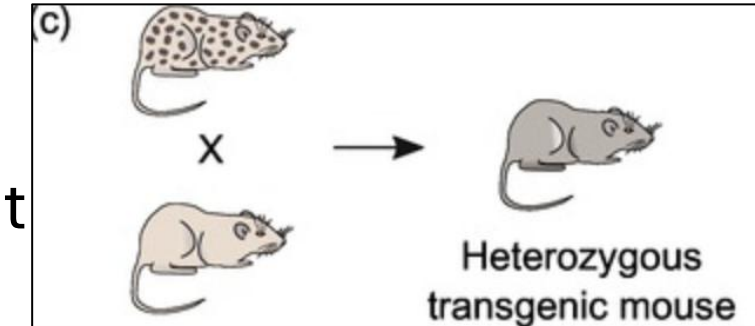
- Inject modified ES cells into the ICM (blastocyst - E3.5) of a host blastocyst from another strain (often coat-color contrasted).
- Injected blastocysts are placed into the uterus of a **pseudopregnant recipient female.**
- **Chimera mice born.** The pups have tissues derived from **both** the host ICM and the injected ES cells.
- **Coat-color chimerism** (spotted/patchy in the cartoon) gives a **visual estimate** of ES-cell contribution.



Applications – Transgenic Animals

Step 3: Breed chimera to obtain heterozygous transgenic offspring

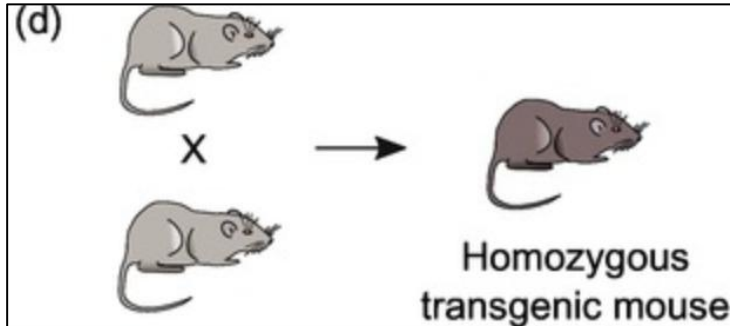
- Achieve germline transmission of the modified allele
- Test for germline transmission - Mate the chimera with wild-type mice of the host strain.
- Screen offspring for Tg gene by PCR.
  - If positive pups appear: the targeted allele reached the germline. These pups are heterozygous (one targeted allele from ES-cell lineage, one wild-type allele from the host parent).
  - If none: that chimera's germline derived from the host ICM; try a different chimera.
- Why heterozygous? Because only one chromosome set in the gamete carried the targeted allele; the other parent contributes the wild-type chromosome.



Applications – Transgenic Animals

Step 3: Breed heterozygotes to produce homozygous transgenic line

- Establish a stable line with the modified gene in all cells
- Intercross heterozygotes (F1 × F1)
- Expected ratios: 1 WT : 2 Het : 1 Homo
- Genotype the litter to identify homozygote mice.
- If the homozygous state is embryonic-lethal, you'll recover only WT and heterozygotes; use conditional (Cre-loxP) or tissue-specific strategies to study function.



Applications – Transgenic Animals

- Tg animals are used for developmental studies, functional gene analysis, disease modeling, and drug development.
- ANDi is the first non-human primate to be created using genetic modification in 2001.
- The rise of CRISPR/Cas9, TALENs, and base editing has enabled precise gene modifications in rhesus and cynomolgus monkeys, including disease-associated gene knockouts and germline transmission of edits.

Applications – Medicine

Xenotransplantation – the use of tissues and organs from non-human source for transplantation.

- Gene editing of the pig's genome:

- KO of 3 genes that cause severe reactions.
- A pig gene was modified to control the growth of the pig's heart.
- 6 human genes were KI to increase acceptance by the immune system.

In this photo provided by the University of Maryland School of Medicine, Lawrence Faucette sits with wife, Ann, in the school's hospital in Baltimore, Md., in September 2023, before receiving a pig heart transplant. Deborah Kotz/University of Maryland School of Medicine/AP

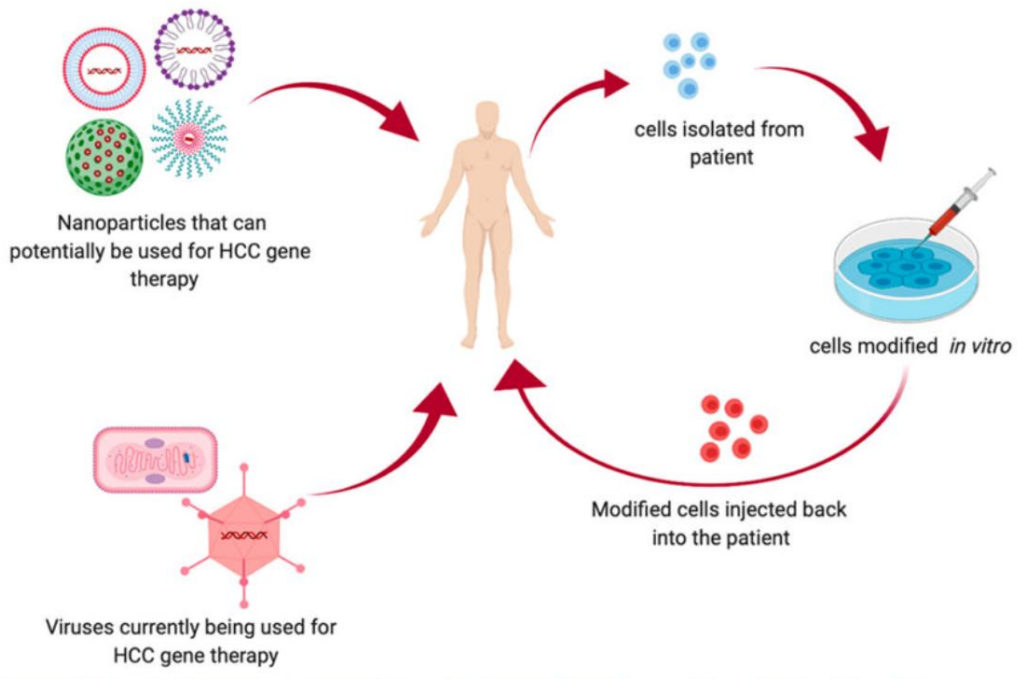
the first person to receive a genetically modified pig heart (2022)

Applications – Medicine

Gene therapy

- Requirements for gene therapy:

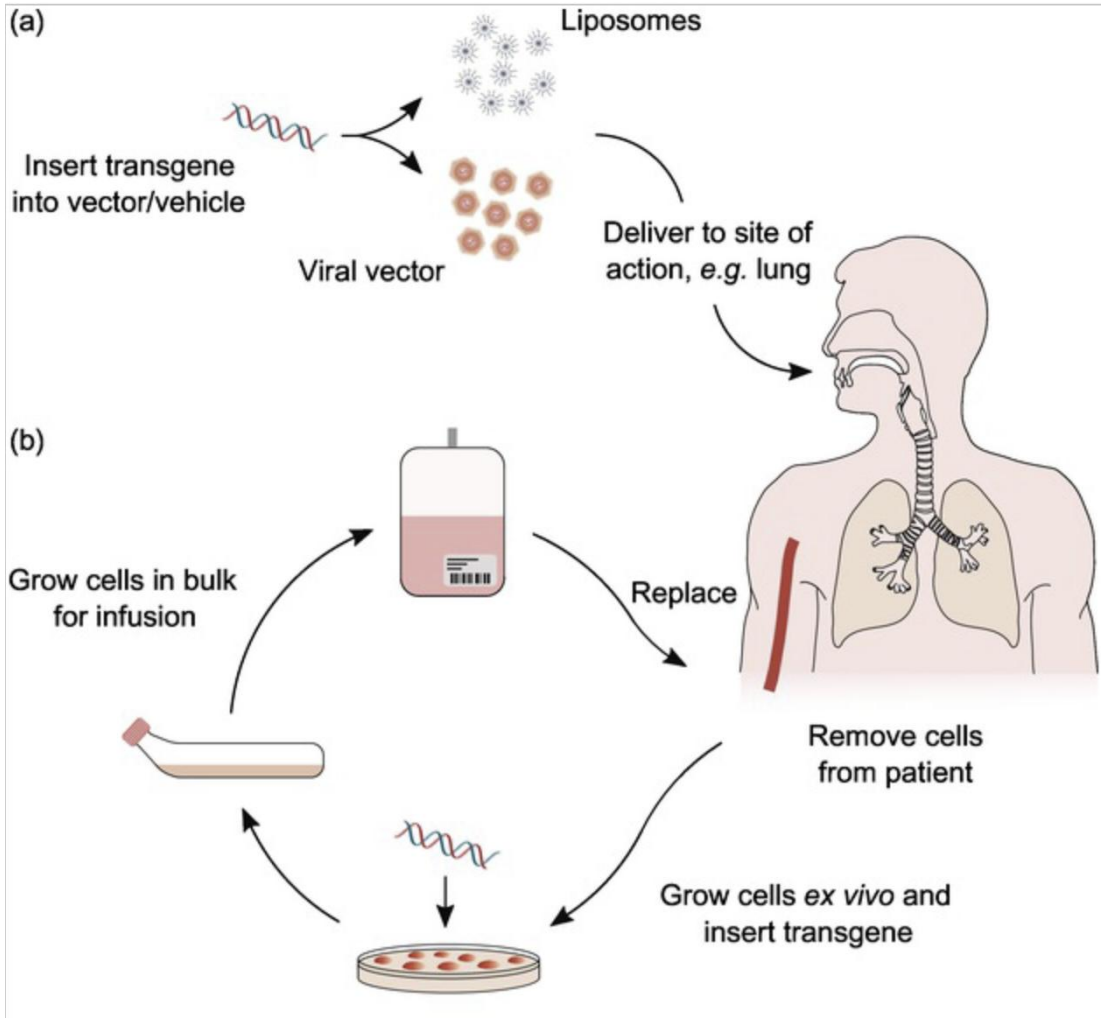
- The gene has been characterized, cloned, and is available.
- A delivery system is available.
- The inserted gene must be expressed in the target cells. The endogenous gene is either replaced (KI) or silenced (KD), if the gene defect is not dominant.



Applications – Medicine

Gene therapy

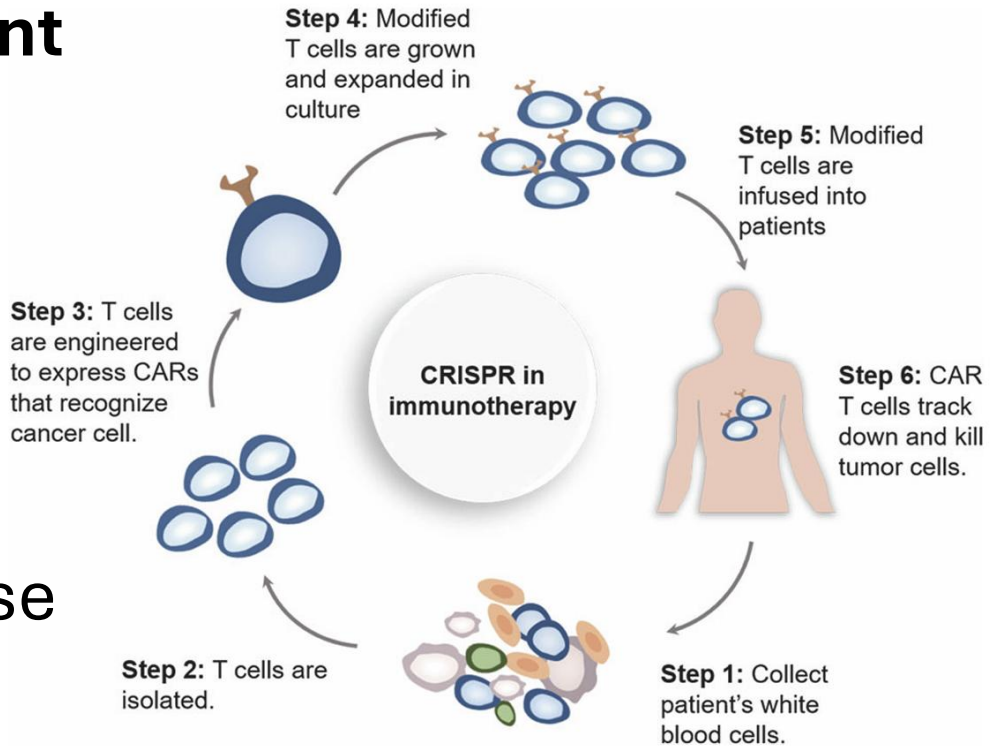
- *Ex vivo* gene therapy - cells are removed from a patient, altered and replaced.
- *In vivo* gene therapy - treating the affected tissues within the body.
- Viral delivery (retrovirus, lentivirus, AAV, HSV)
- Non-viral delivery (liposomes)



Applications – Medicine

CAR T-Cell Therapies in Cancer Treatment

- A T-cell engineered to express Chimeric Antigen Receptors (CAR) on their cell surface.
- Used to target cancer cells for targeted therapy.
- CRISPR-Cas9 has promoted more precise engineering of the CAR T-cells.



Applications – Medicine

Advanced Gene Editing: Base and Prime Editing

Base Editing

- Makes single nucleotide changes (A→G or C→T) without cutting both DNA strands.
- Reduces risk of double-strand breaks and off-target effects.

Prime Editing

- Enables targeted insertions, deletions, or substitutions without double-strand breaks.
- Combines Cas9 nickase with reverse transcriptase for versatile edits.

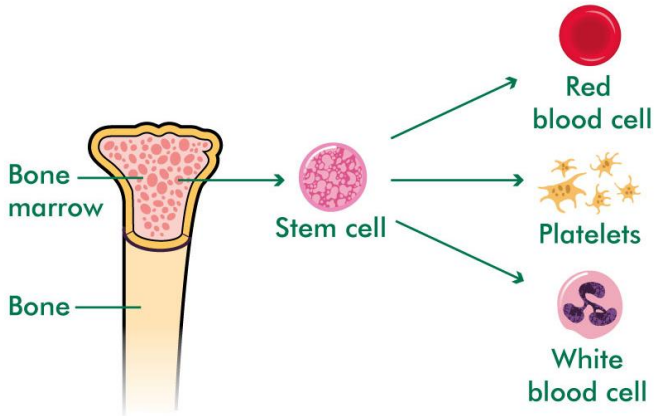
Applications

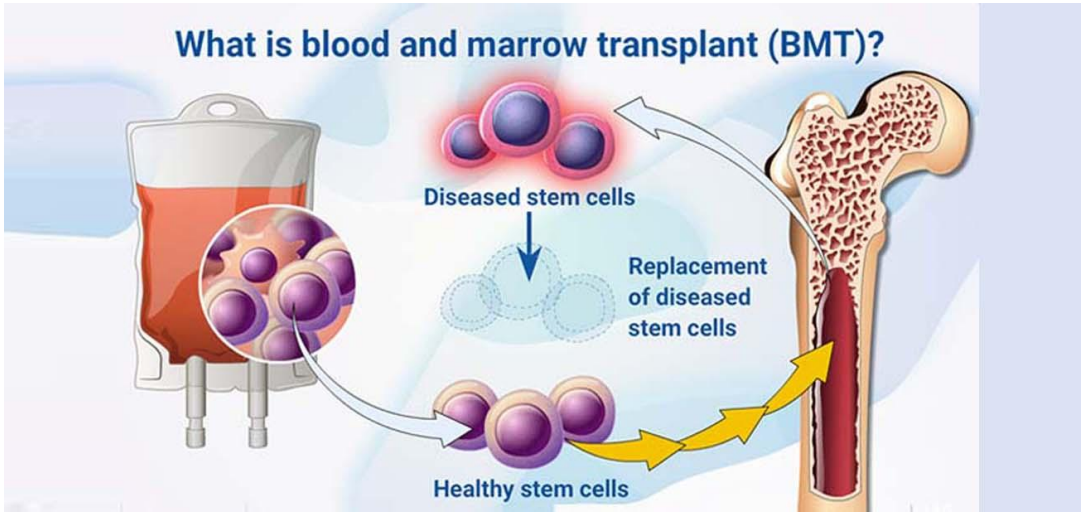
- Enhances safety and precision in CAR T-cell engineering.
- Potential for correcting disease-causing mutations in monogenic disorders.
- Being explored for in vivo therapies (e.g., liver, blood, eye diseases).

Applications – Medicine

Sickle cell anemia

- A blood disorder in which erythrocytes have an elongated (sickle) shape.
- Caused by a single nucleotide mutation in the β-globin gene, leading to a valine substitution for glutamic acid in hemoglobin.
- Misshapen cells can block blood flow, causing pain and organ damage.






CNN

Ethical Problems

- What are the potential risks and concerns?
- What benefits can gene editing offer to society?

Ethical Problems

Pros	Cons
Enhanced characteristics in organisms	Ethical concerns
Increased crop yields	Potential risks and unintended consequences
Improved disease resistance	Potential for genetic mutations to spread uncontrollably
Potential for curing genetic diseases	Long-term effects on ecosystems and biodiversity
Potential for breakthroughs in medicine, agriculture, and environmental conservation	