Cellular and Molecular Biology I

BIO-205-11

Camille Goemans - 2024

Summary

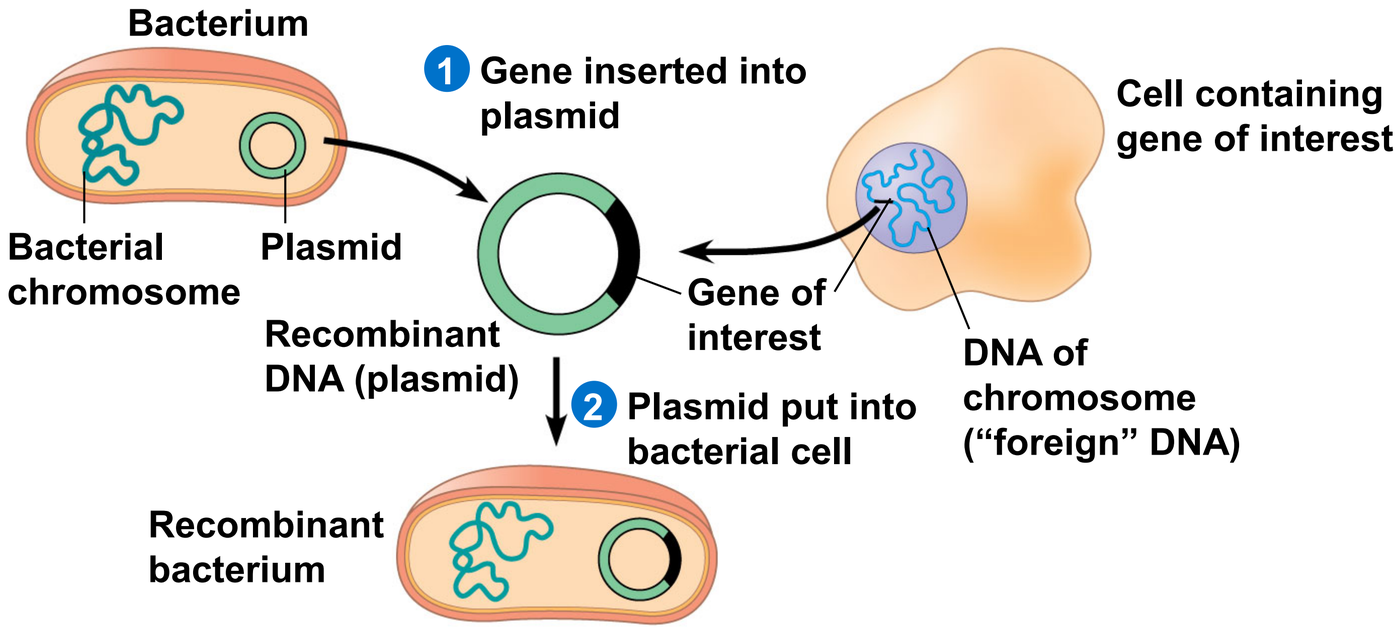
- Studying proteins
    - Protein interactions
    - Real-life examples
- Studying DNA
    - DNA sequencing
    - DNA extraction
    - DNA amplification

Plan

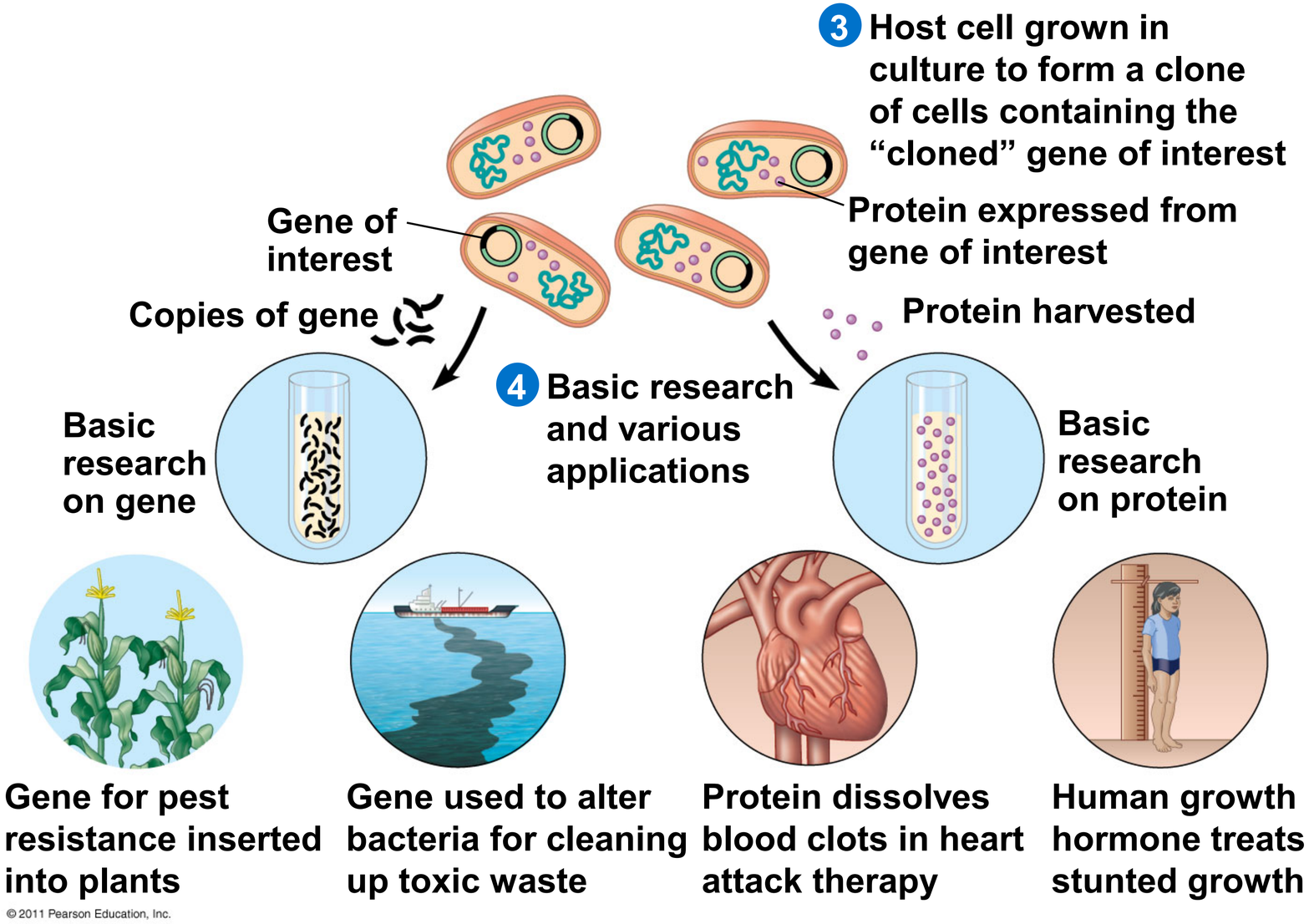
- Studying DNA
    - **DNA cloning**
    - Cloned organisms and stem cells
    - Practical applications of DNA-based technology

DNA cloning

- **Plasmids** are small circular DNA molecules that are present in bacteria and replicate separately from the bacterial chromosome
- We can insert **DNA into plasmids** to produce recombinant DNA
- This is then **inserted into bacteria** to make copies of the plasmid, to express a protein, etc.

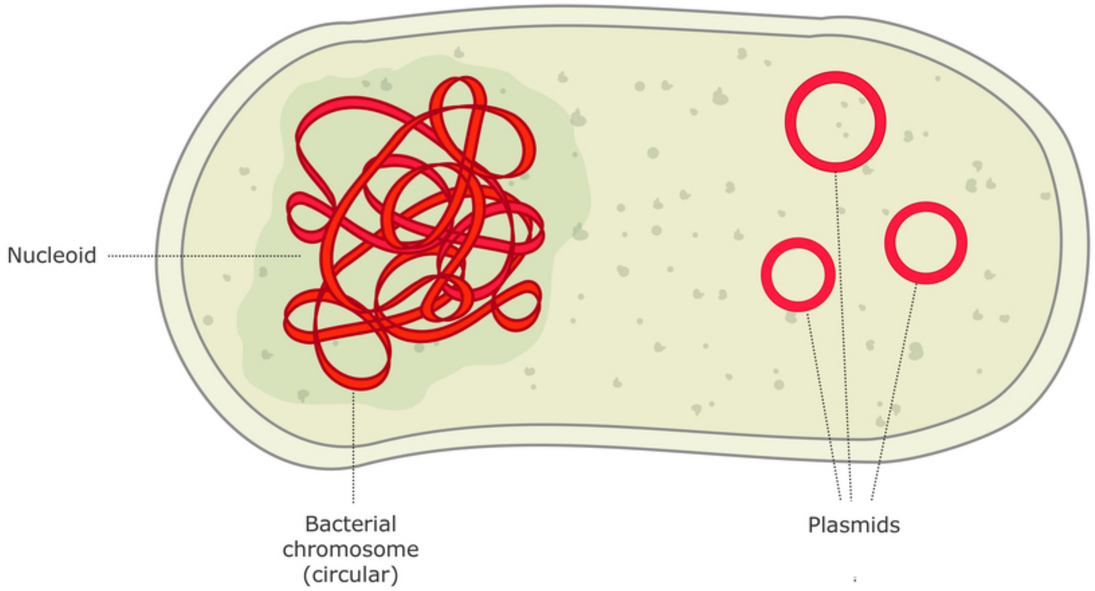


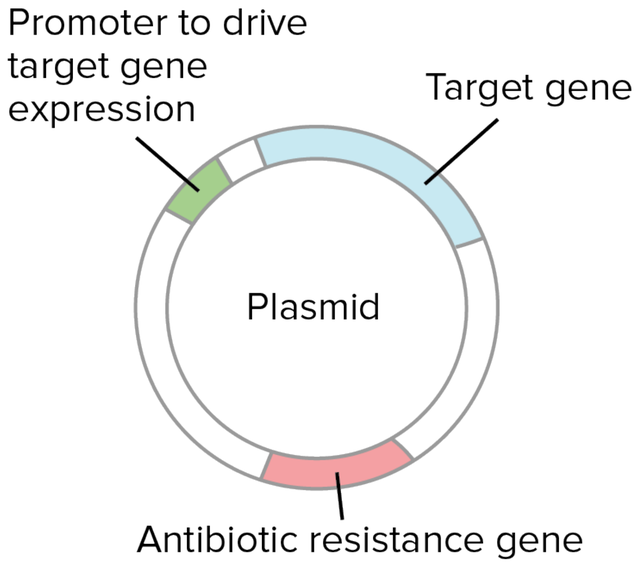
DNA cloning



Preparing plasmids

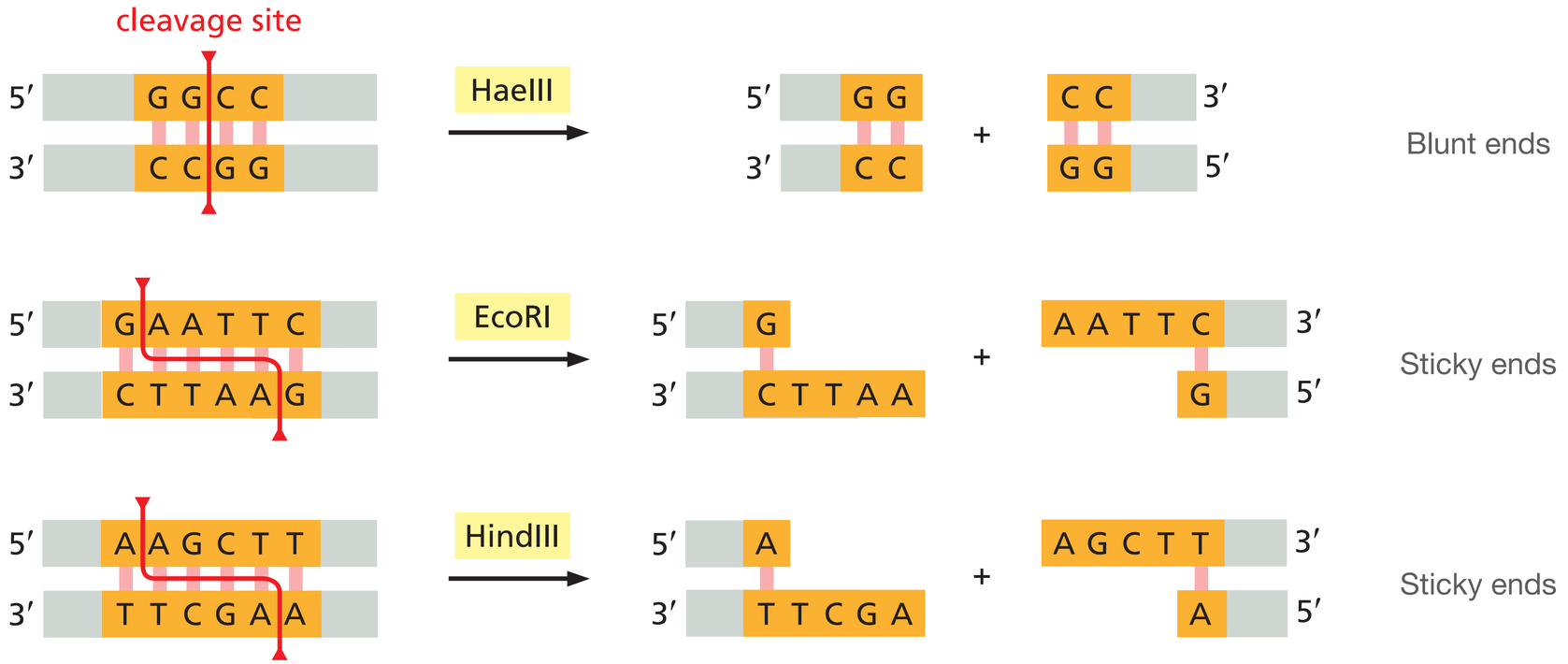
- **Cloning** genes using **bacteria** and **plasmids** (self-replication circular ds-DNA)





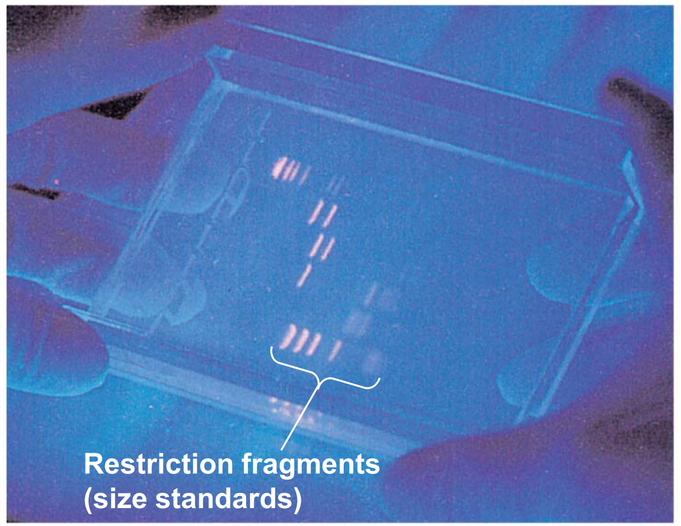
Cutting DNA

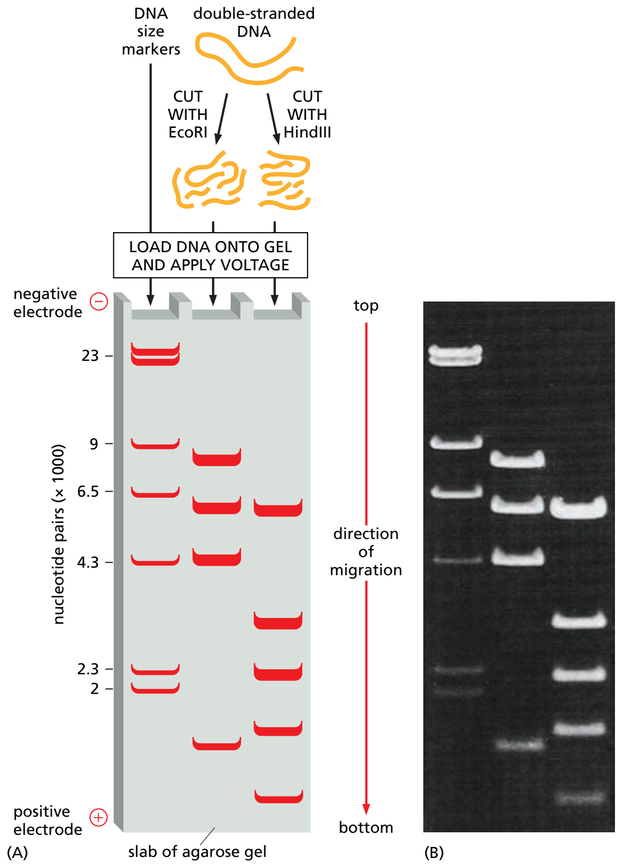
- **Restriction nucleases** are enzymes that cut DNA at specific sequences
  - Purified from bacteria where they serve as **foreign DNA defense systems**



Visualizing DNA

- **Gel electrophoresis** separates DNA fragment by size
  - As for proteins, but each nucleotide already carries a **negative charge** on the phosphate group
  - Agarose gel
  - Use of an **intercalating agent** that **binds DNA** and fluoresces under **UV**





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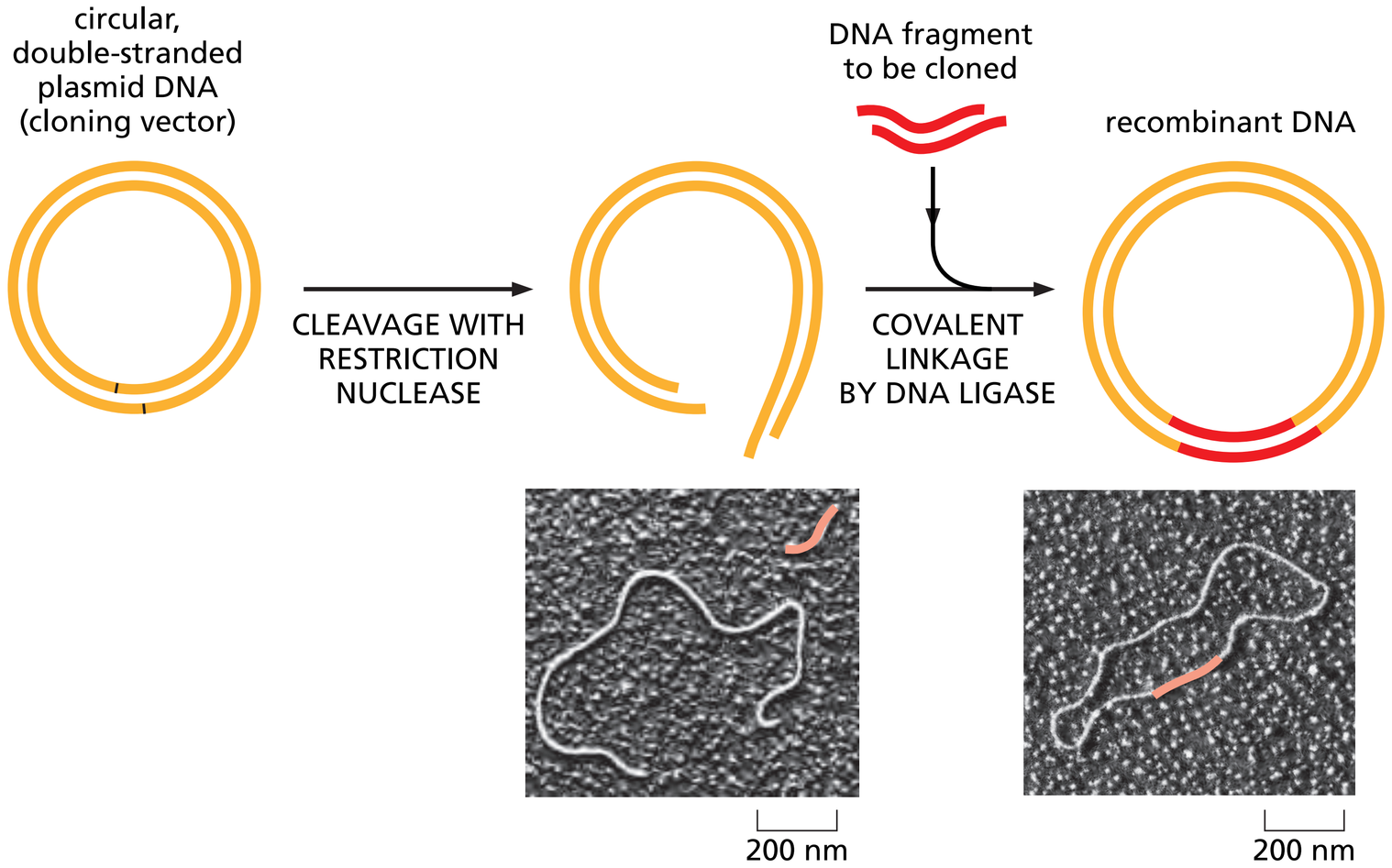
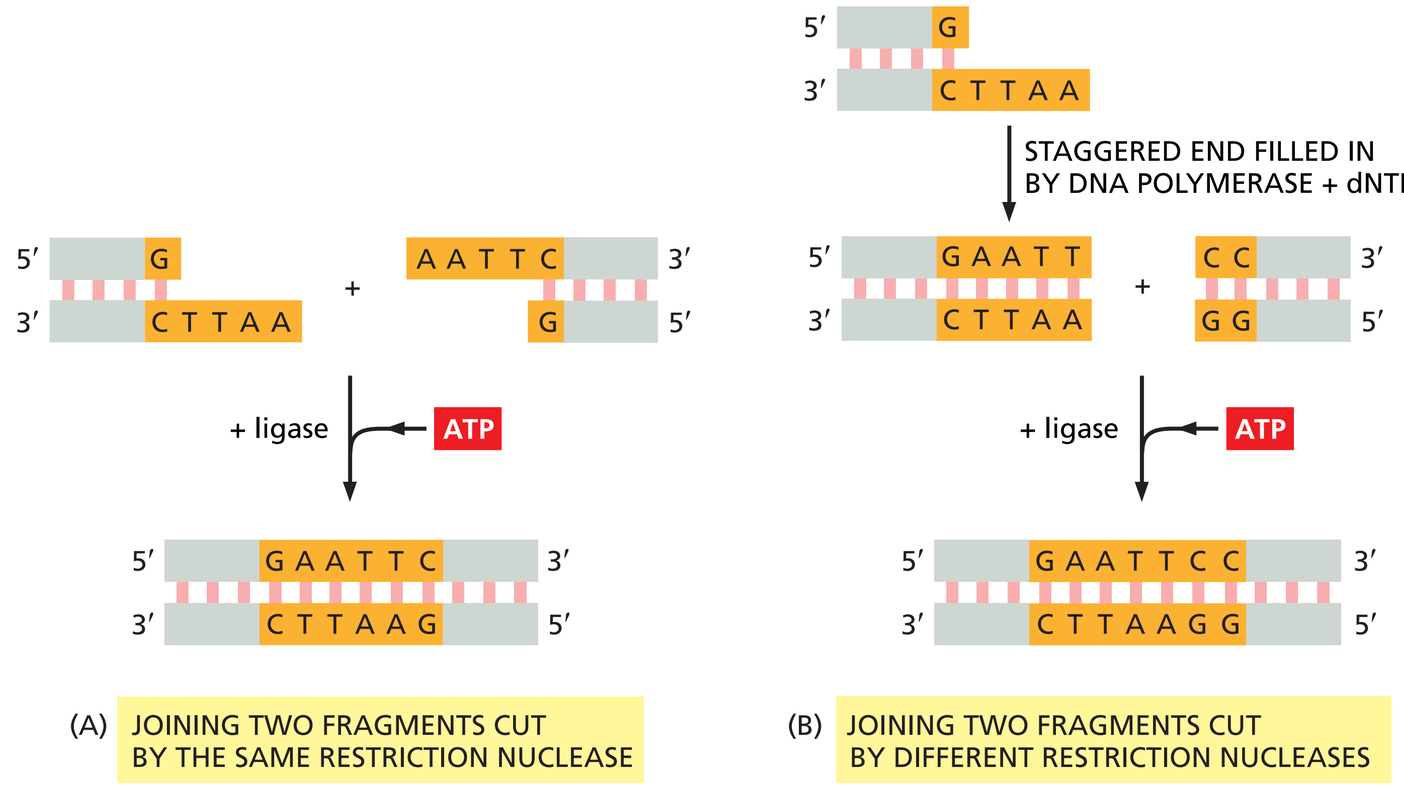


Preparing plasmids

- **Preparing the plasmid** using restriction enzymes



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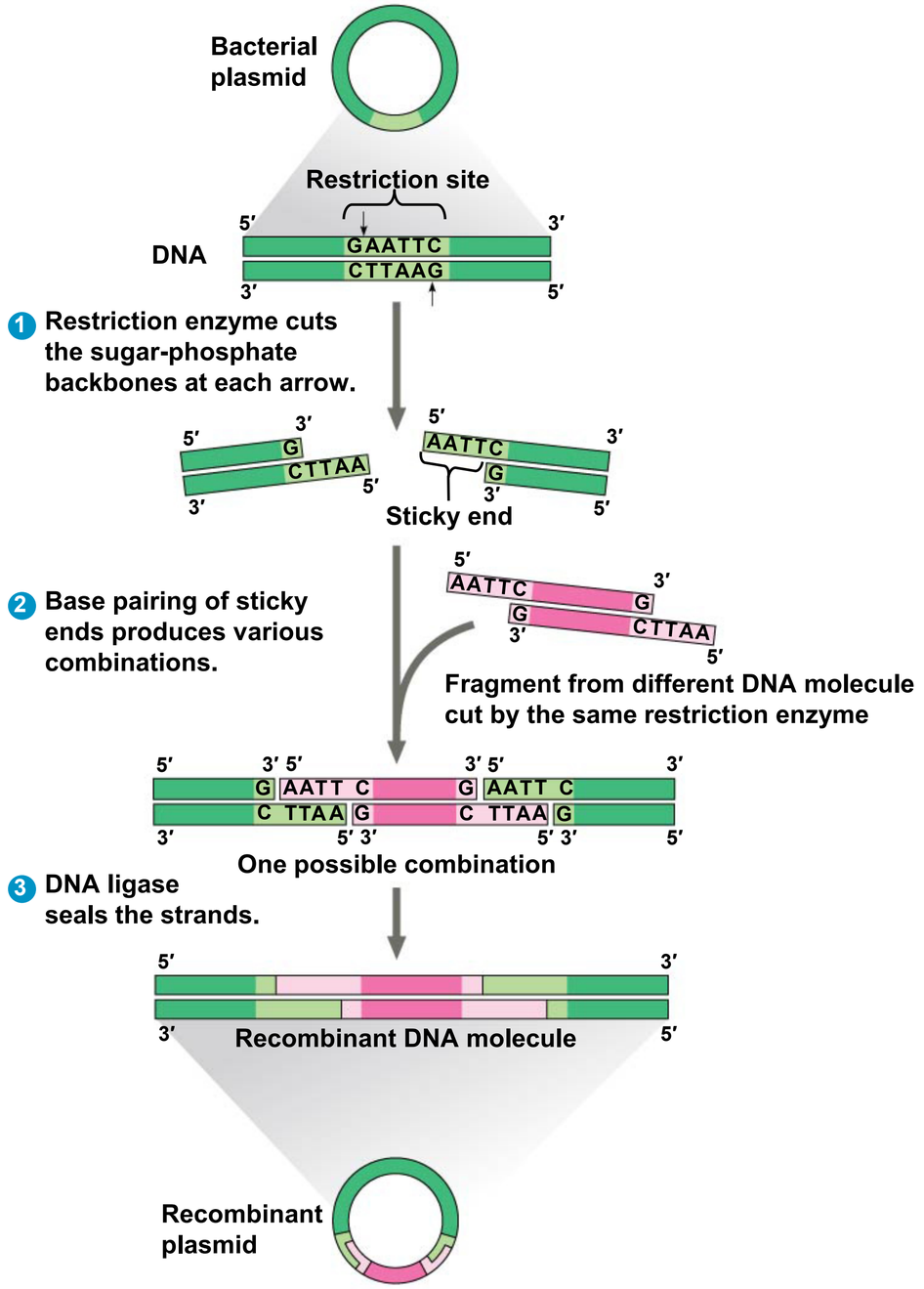
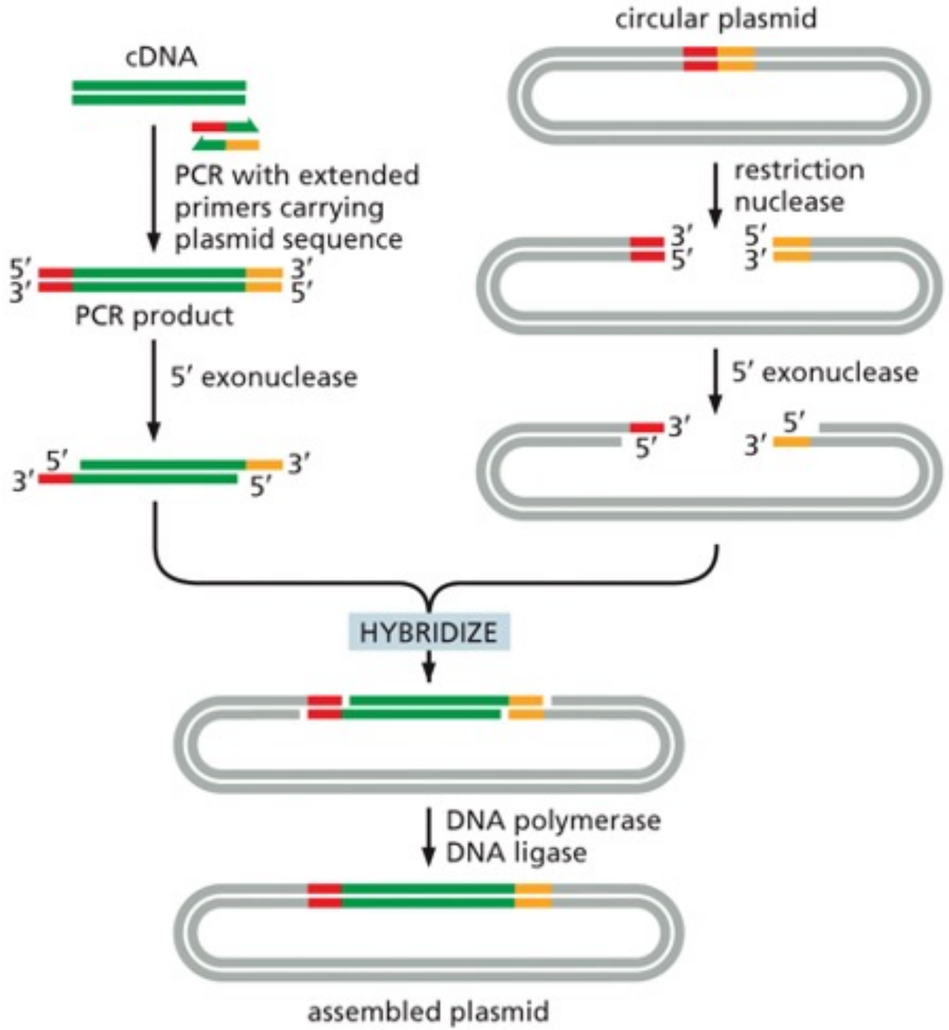


Preparing plasmids

- **Preparing the plasmid** using Gibson assembly

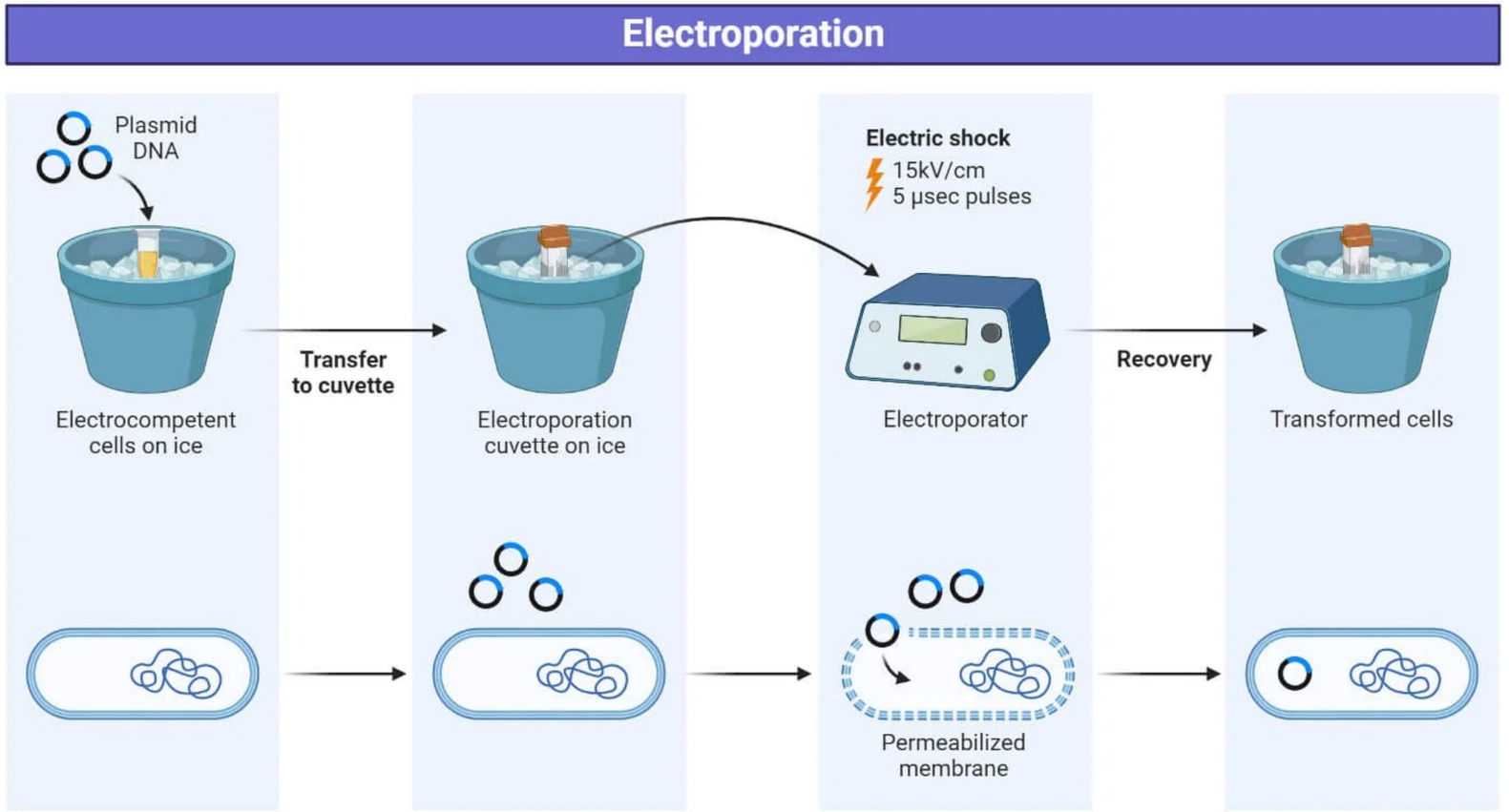


Preparing plasmids

- In cloning, the plasmid is called a **cloning vector**
- It can carry **foreign DNA** into a host cell and **replicate** there

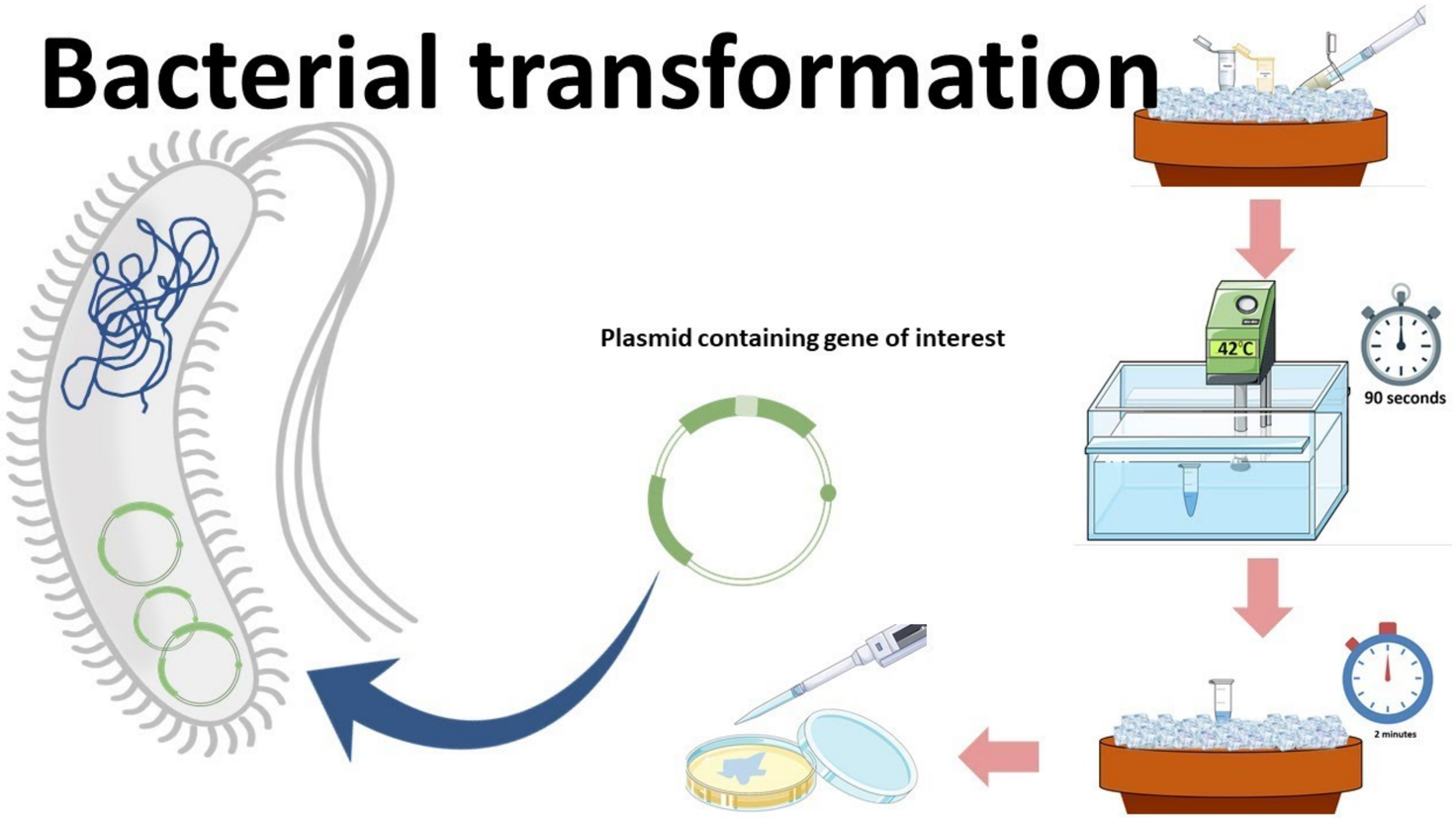
Transforming plasmids into bacteria

- using electroporation



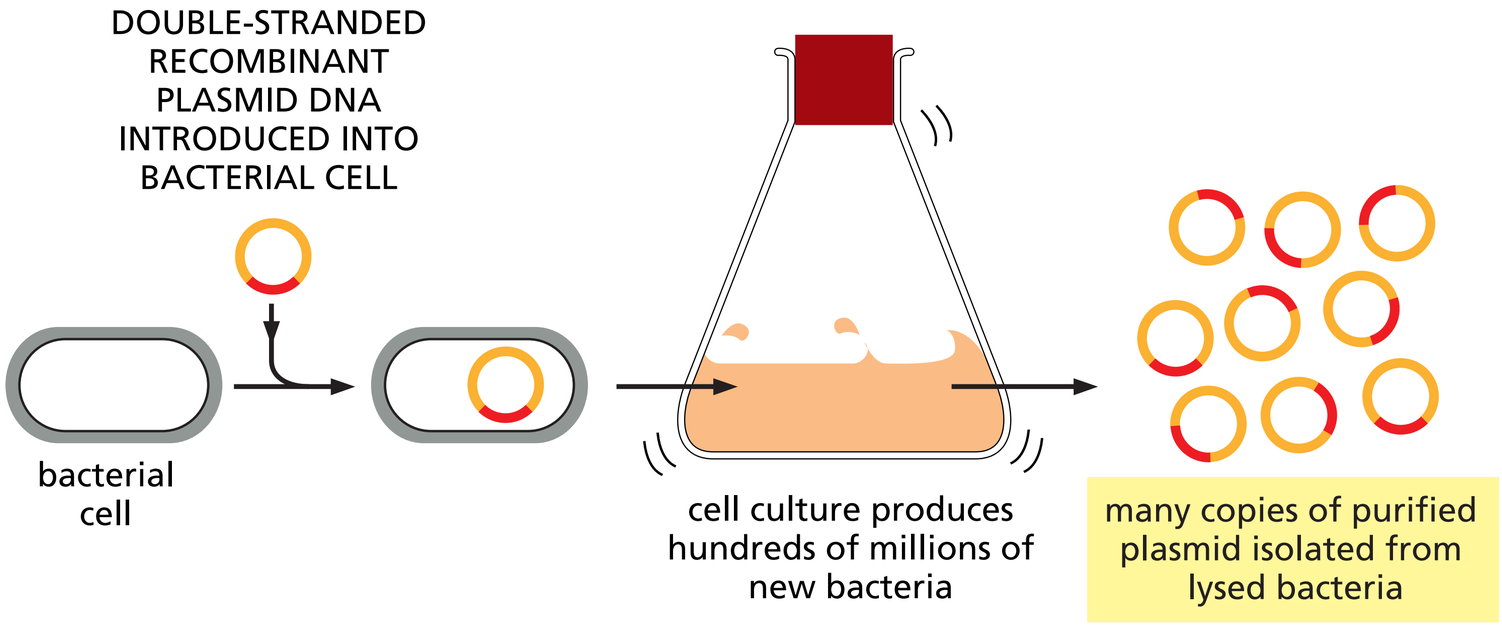
Transforming plasmids into bacteria

- using heat shock



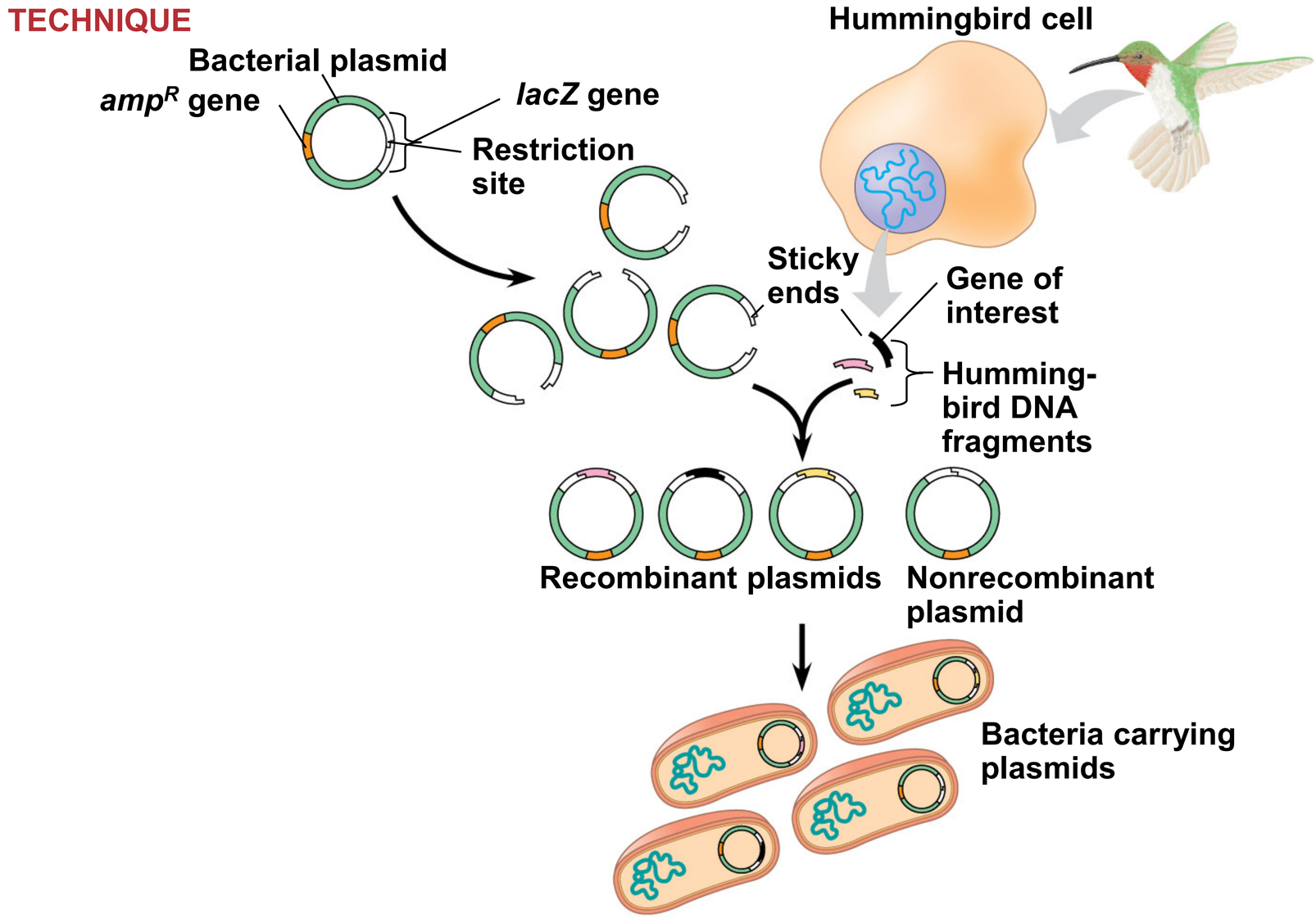
Growing the transformed bacteria

- **Growing** the bacterial culture containing the plasmid of interest

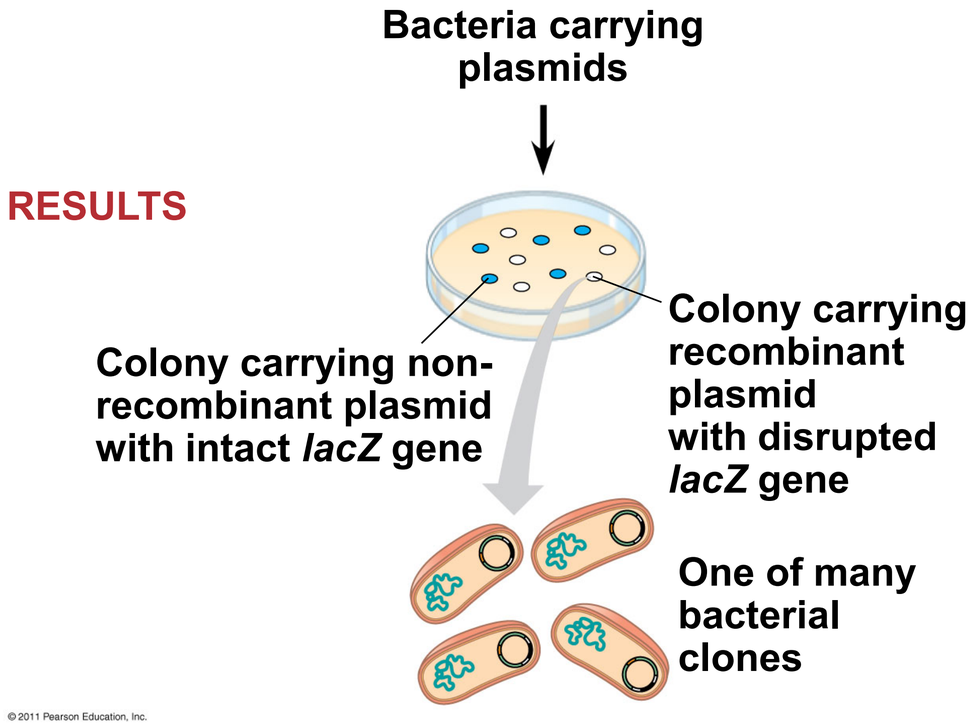


- Purify the plasmid
- Express a protein of interest
- Overexpress a protein for purification
- . . .

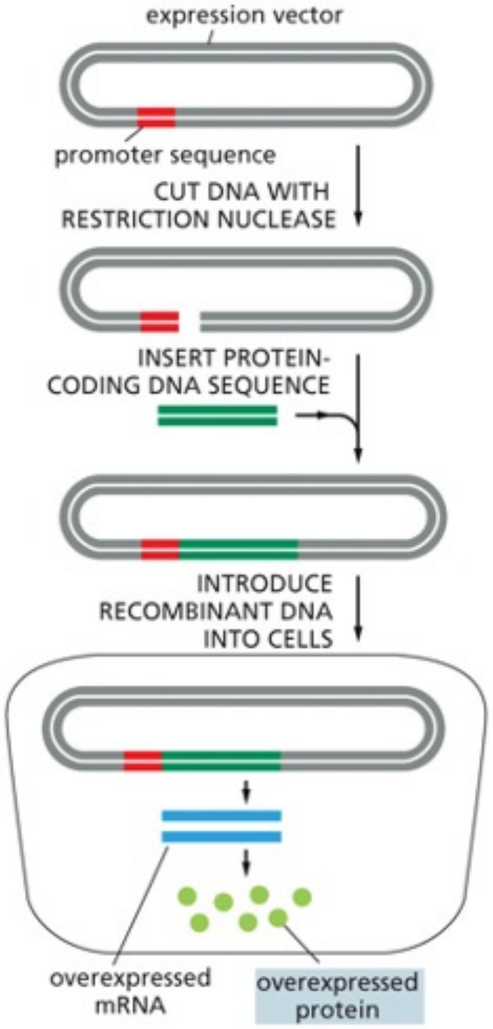
Cloning in bacteria

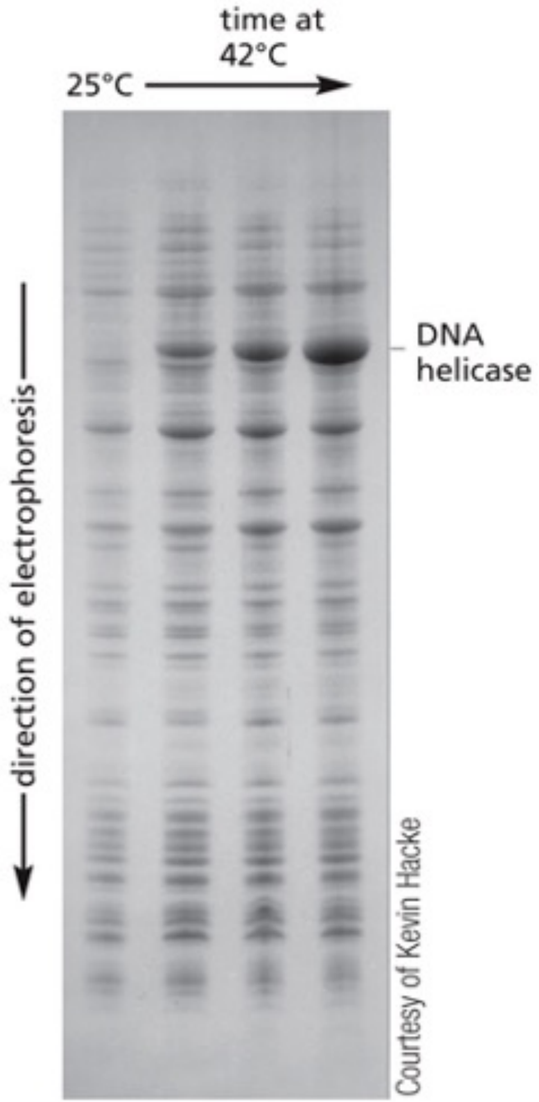


Cloning in bacteria



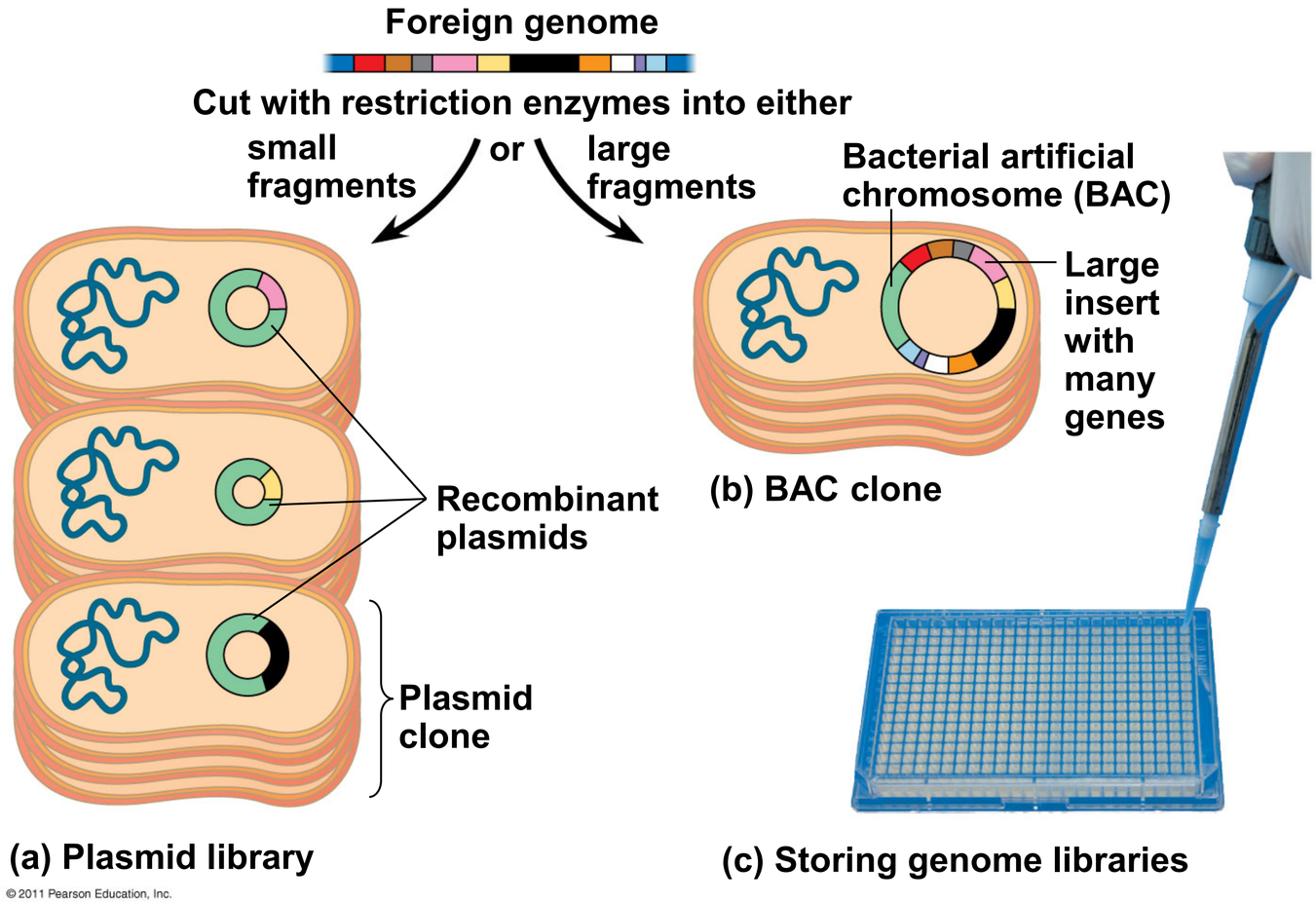
Protein overexpression





DNA libraries

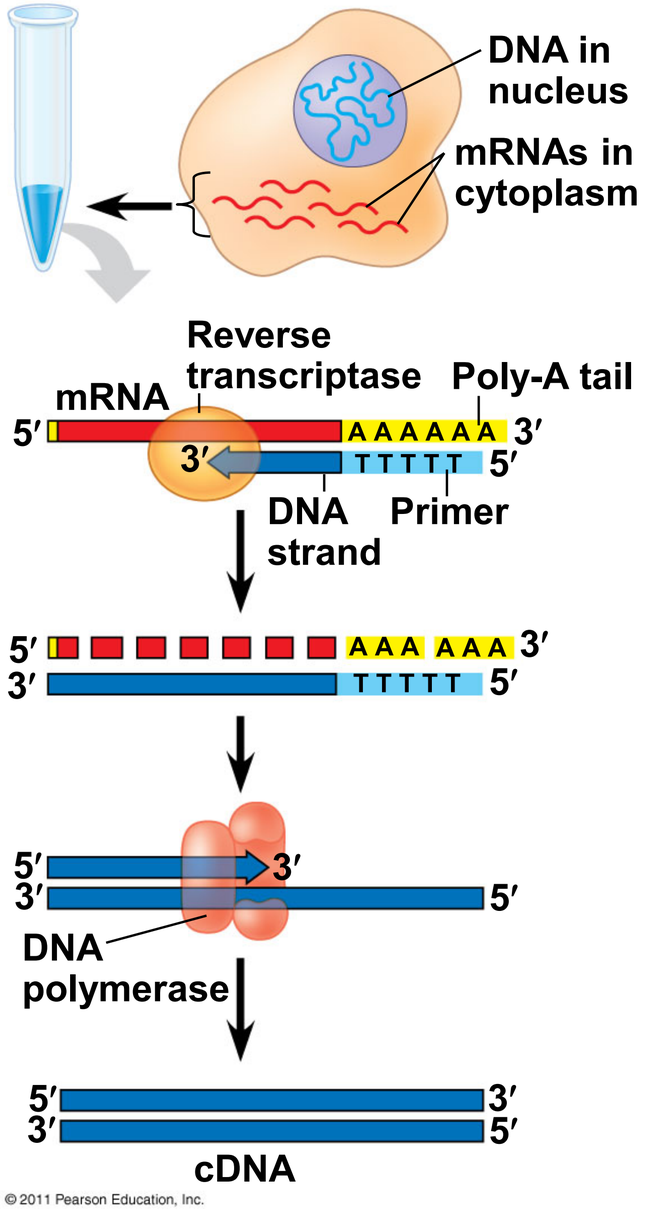
- A **genomic library** is a collection of bacteria each carrying a plasmid with one DNA fragment coming from an entire genome
- The library aims at covering the **whole genome**
- A **bacterial artificial chromosome** (BAC) is a large plasmid that can carry large DNA inserts



DNA libraries

- A **cDNA library** is made by cloning DNA made in vitro by reverse transcription of all the mRNA produced in the cell

- It represents **only one part of the genome**, the part being transcribed



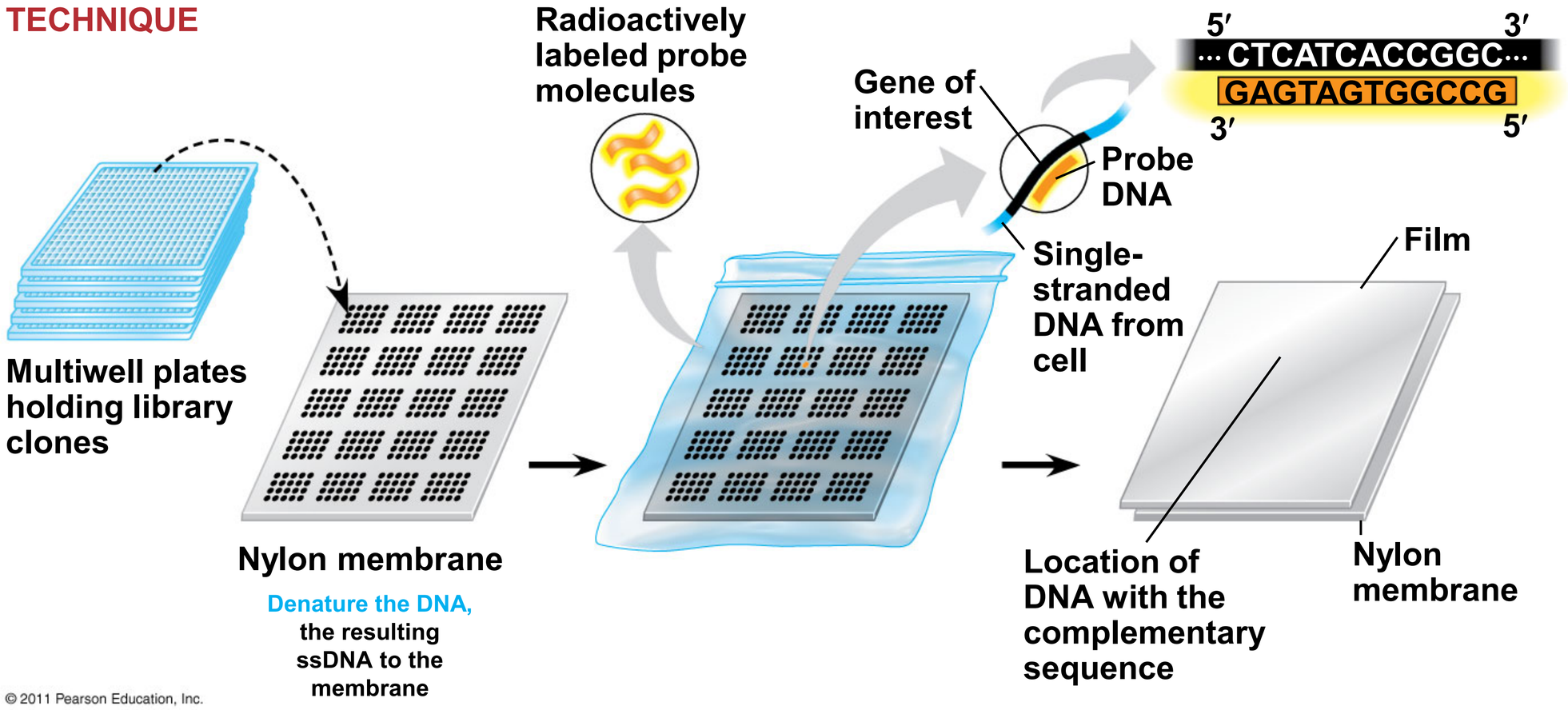
DNA libraries

- Libraries can be screened to identify clones carrying a gene of interest using **nucleic acid probes**
- This based on nucleic acid **hybridization**

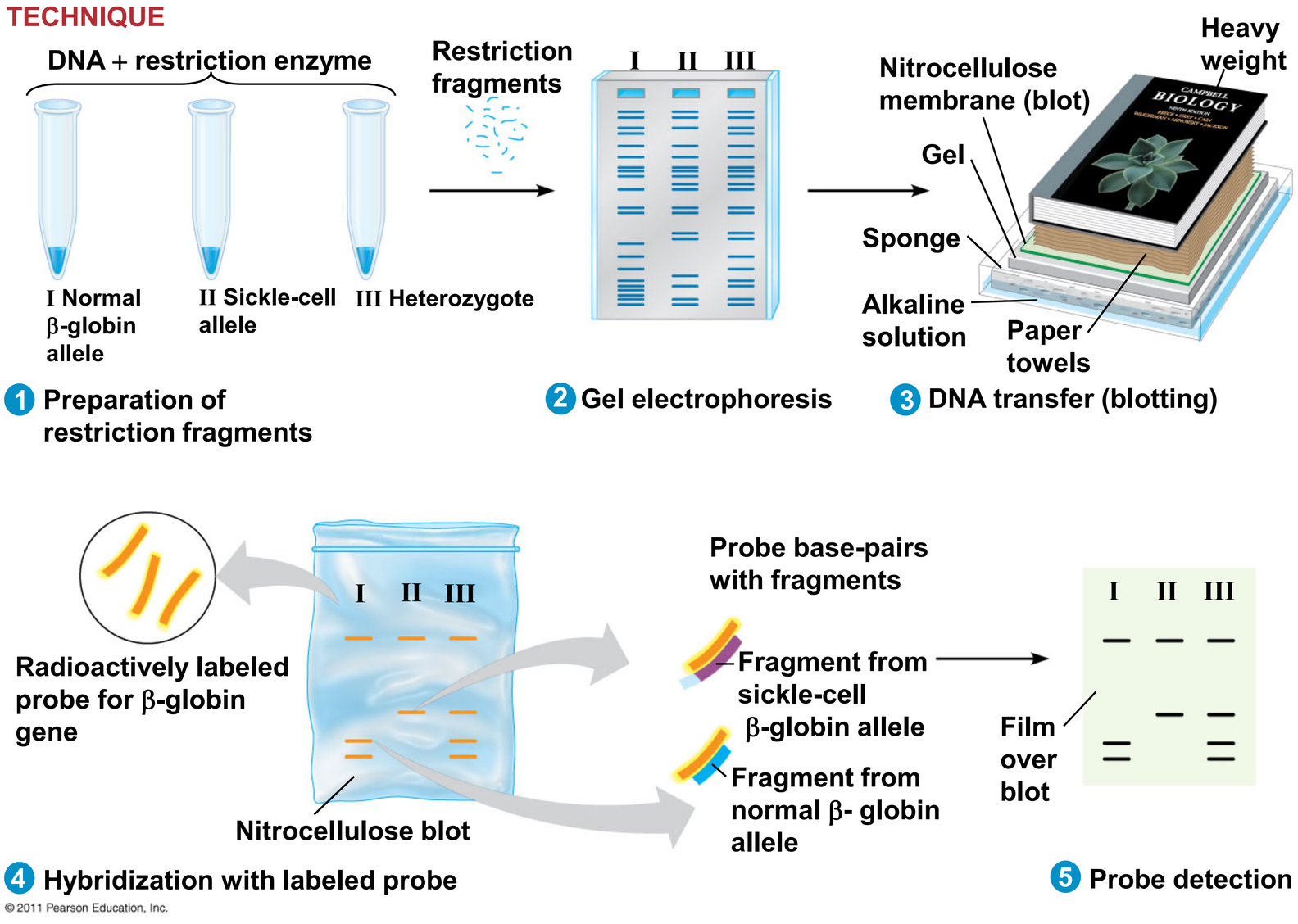
Sequence in the gene of interest

Probe

DNA libraries



Southern Blotting



Expressing eukaryotic genes

- After cloning a gene, its **protein product** can be produced in **large amounts**
- They can be expressed in **bacteria or eukaryotic cells**

- What are the **difficulties** when expressing a eukaryotic protein in bacterial cells?
    - Differences in **promoters** -> use of expression vectors with strong promoters
    - Presence of **introns** -> use of cDNA
    - Protein **glycosylation**

Eukaryotic cloning systems

- Eukaryotic genes may **not be expressed properly** in bacteria (introns, post-translational modifications, …)
- Researchers have then to use eukaryotic cells, like **yeasts**
- In some cases, yeasts do not have proteins required to modify a mammalian protein properly, in which case **insect cells** or **cultured mammalian cells** are used

- Recombinant DNA is introduced by **electroporation** or by **injection** (with microscopically thin needles)
- Once inside the cell, DNA is incorporated by **natural recombination**

Plan

- Studying DNA
    - DNA cloning
    - **Cloned organisms and stem cells**
    - Practical applications of DNA-based technology

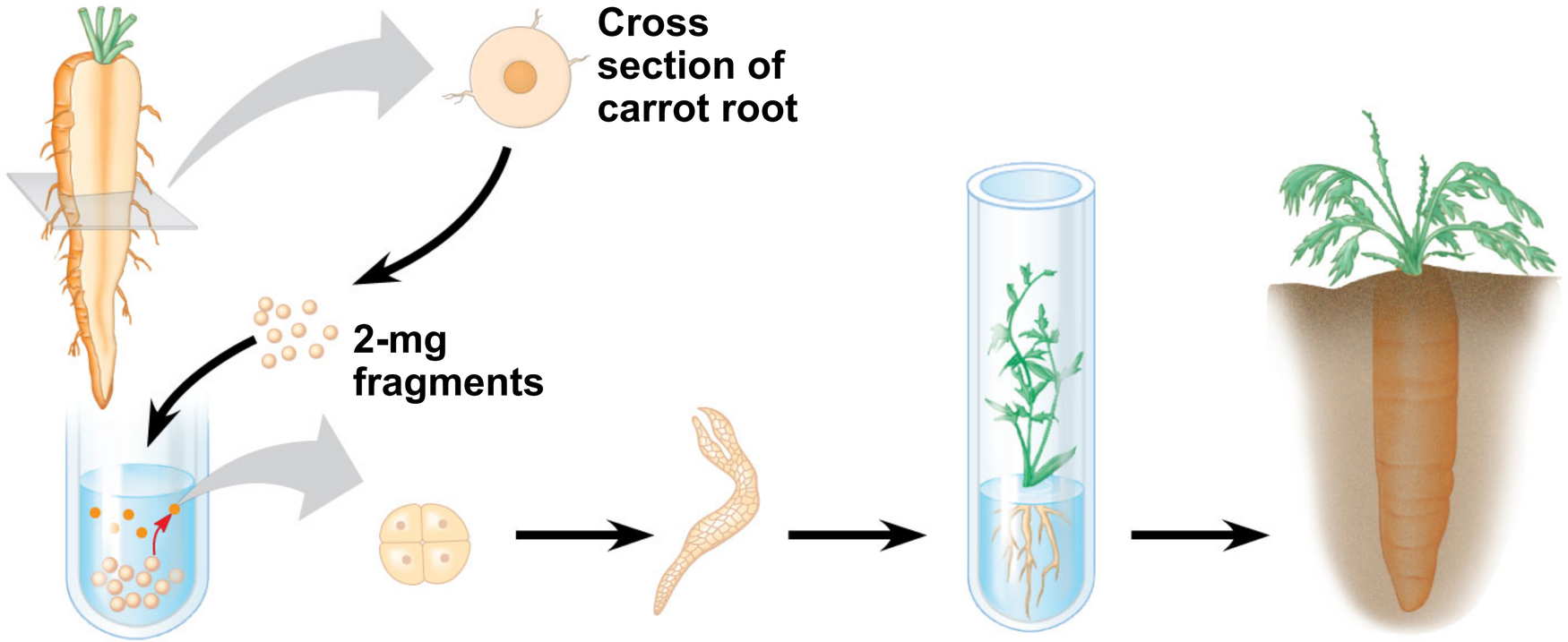
Use of cloned organisms and stem cells

- Here cloning refers to the production of organisms genetically **identical to the “parent”** that donated the single cell
- A **stem cell** is not specialized and can reproduce itself indefinitely or **differentiate into specialized cells** under certain conditions

Cloning plants

- In plants, cells can **de-differenciate** and give rise to **all cell types**
- A cell that can give rise to a **complete new organism** is called **totipotent**
- Plant cloning is extensively used in **agriculture**

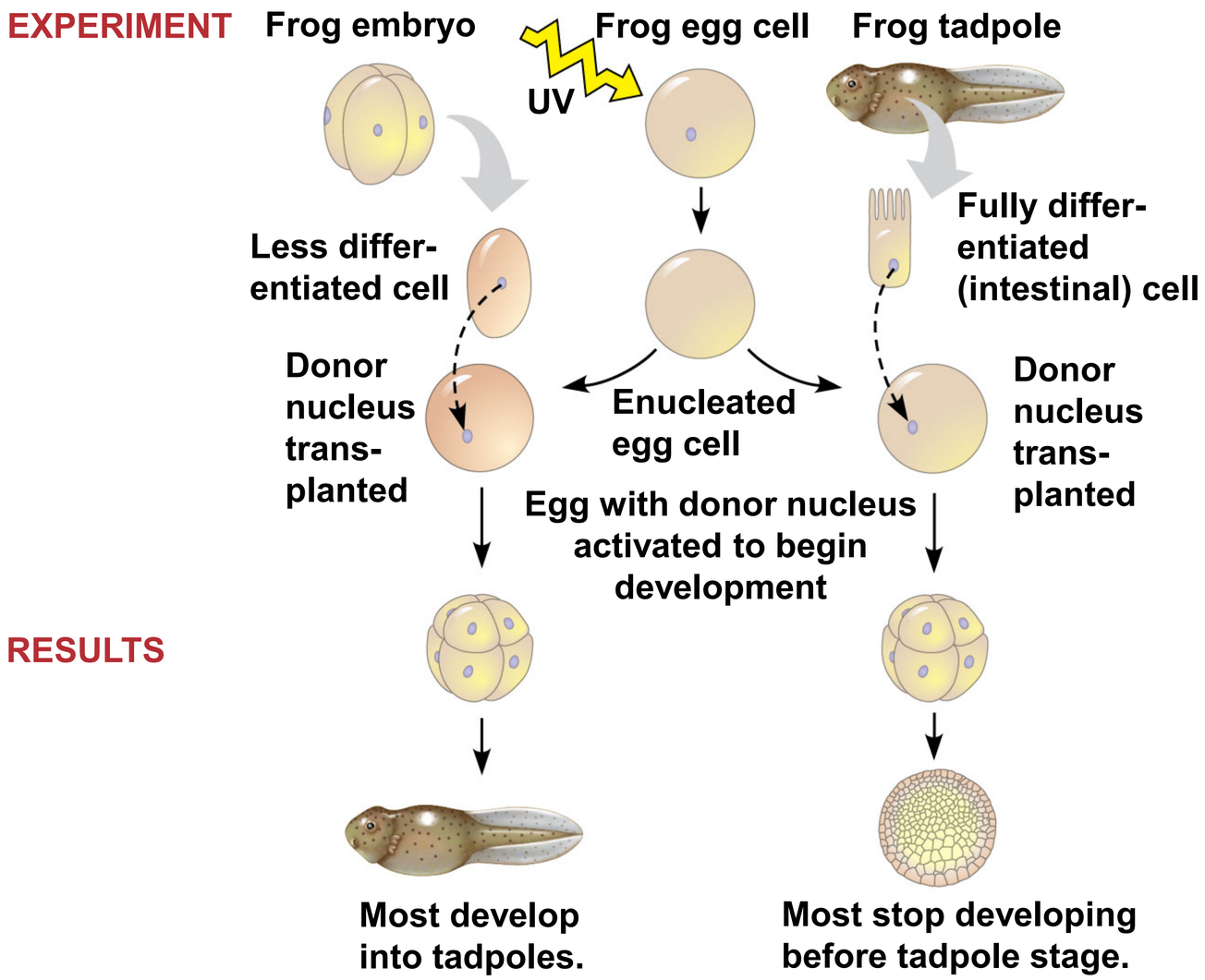
Cloning plants



Cloning animals: nuclear transplantation

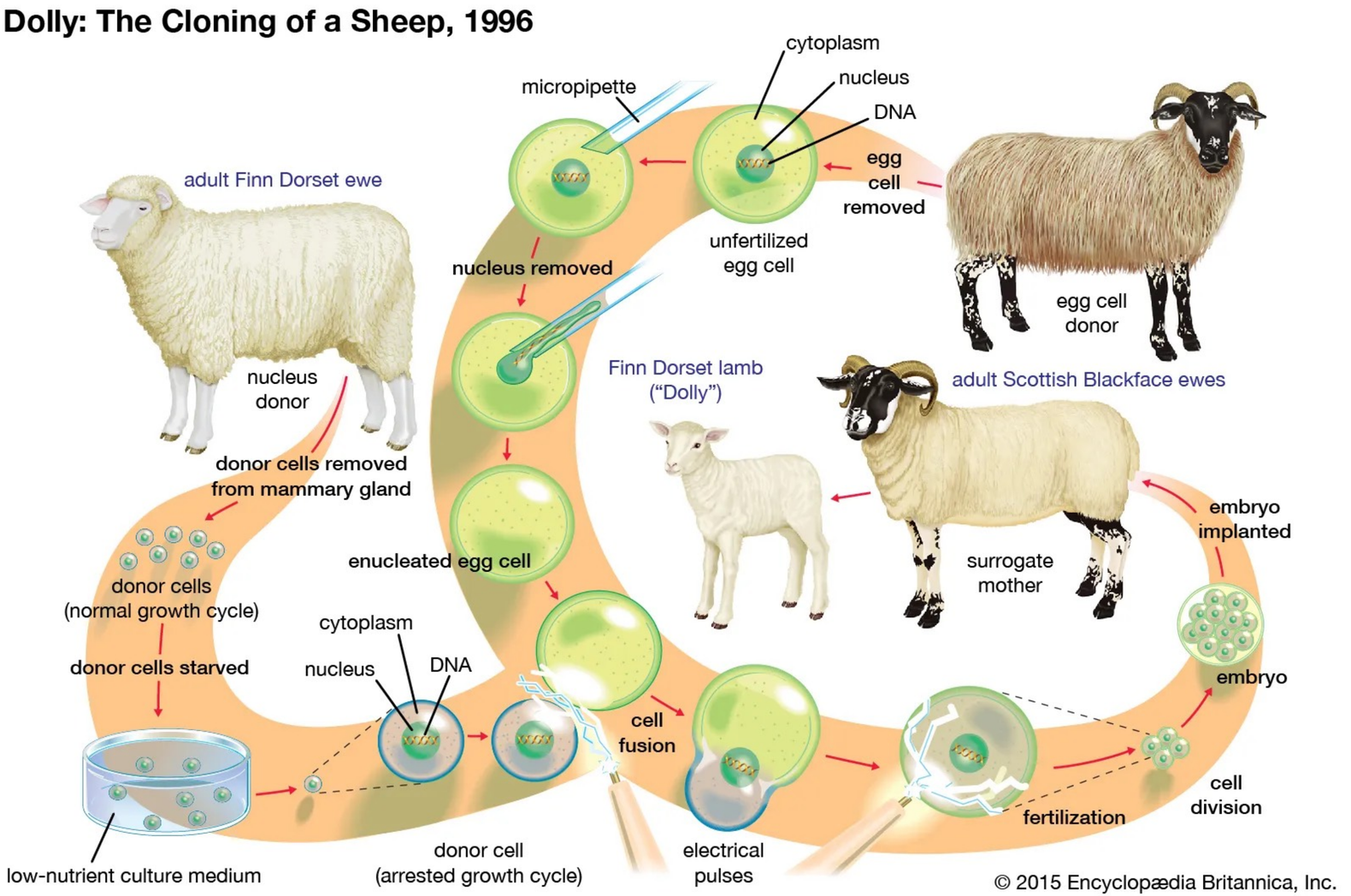
- The nucleus of an **unfertilized egg** is replaced with the **nucleus of differentiated cell**

- In frog embryos, this can support the **normal development of the egg** (depending on how **old** the donor nucleus is)



Cloning animals: mammals

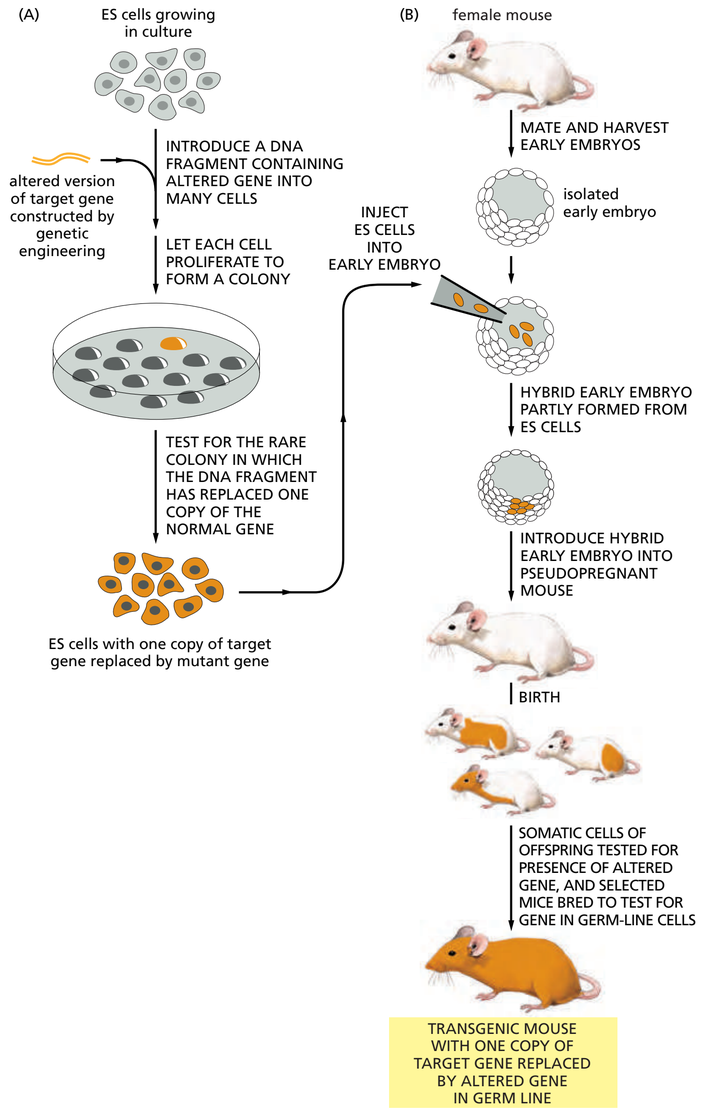
- Cloning of **Dolly**, a lamb cloned from an adult sheep
- **Premature death** and arthritis - potential incomplete reprogramming of the original transplanted nucleus
- Since then, many **mammals** were succesfully cloned (many exhibiting defects, epigenetics)



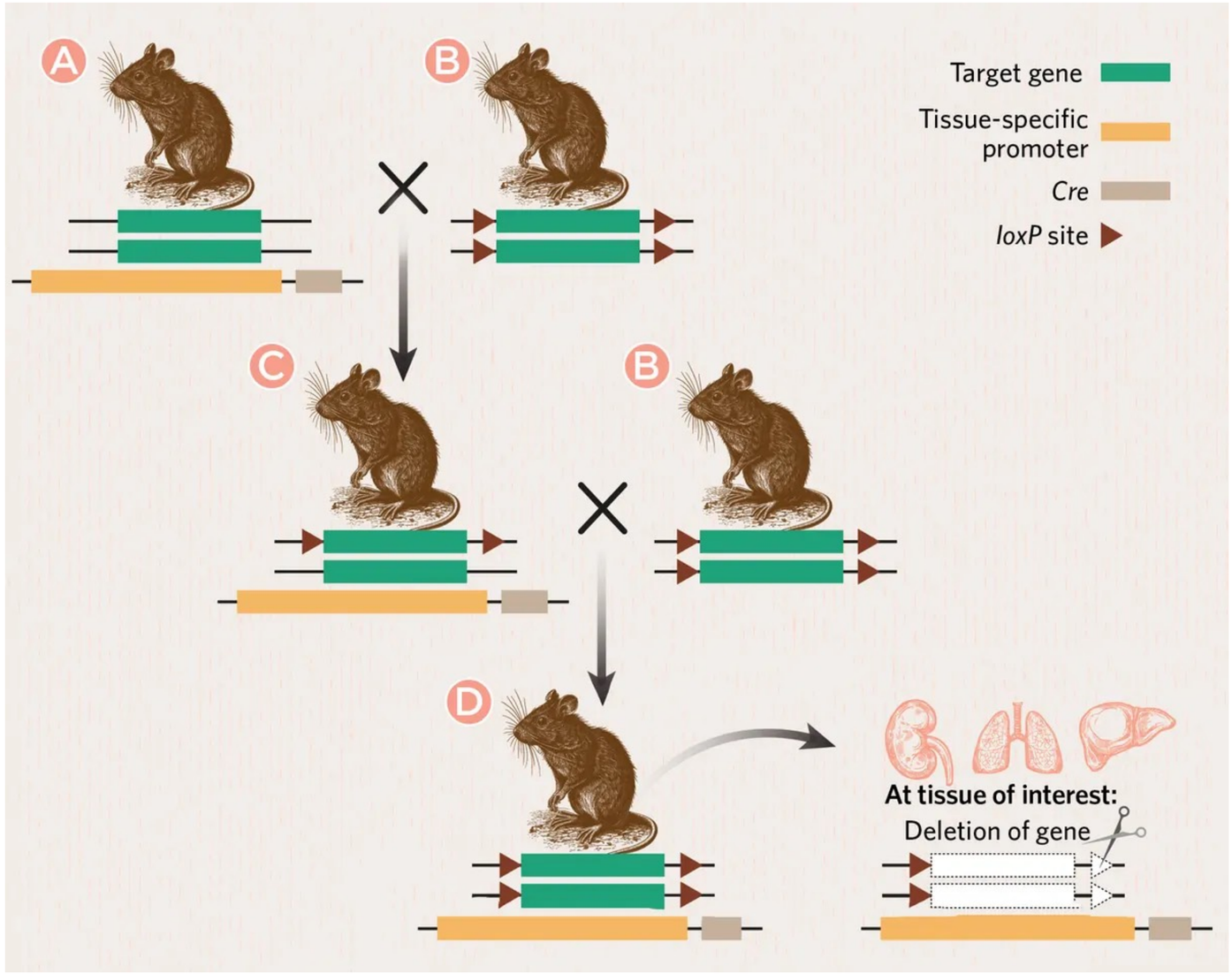
Engineering animals: transgenic organisms

- **Transgenic organisms** are organisms that have been genetically engineered
- **A transgene** is a foreign gene that has been added

Often inserts at random;
Needs lots of screening



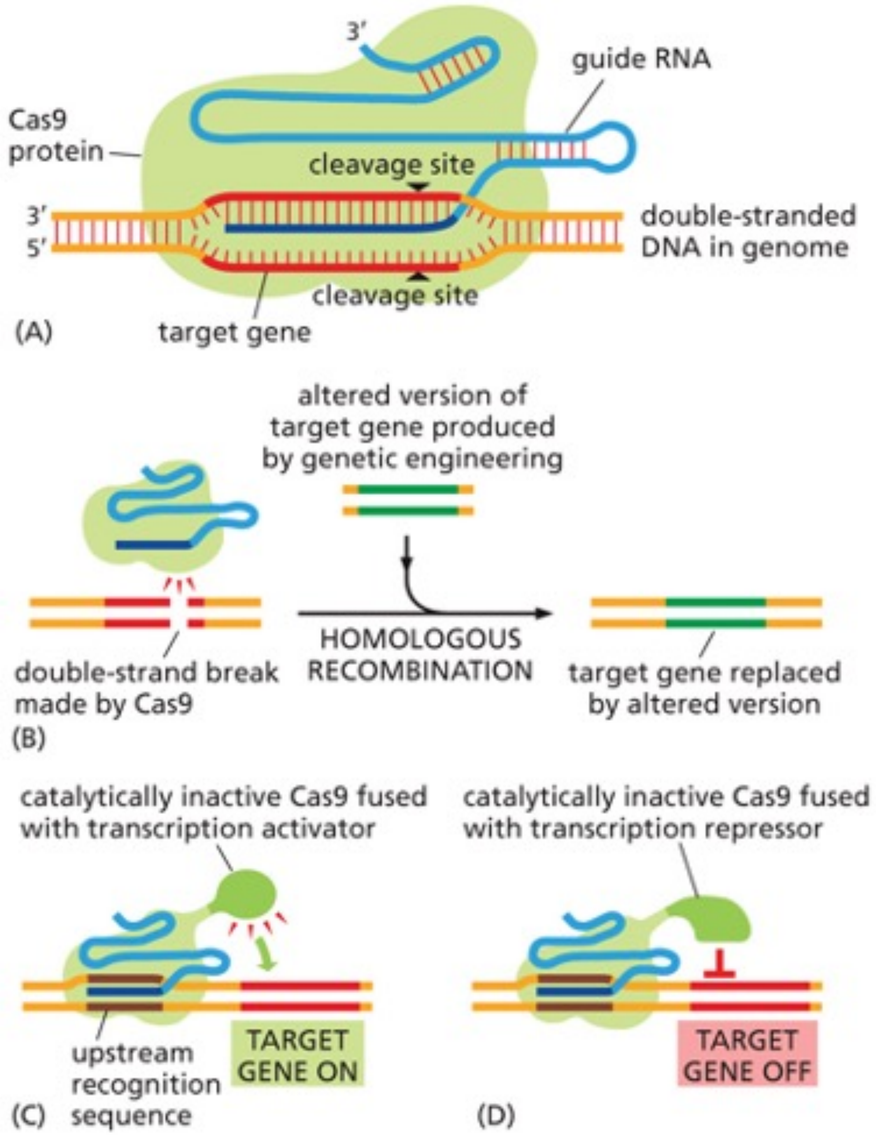
Engineering animals: the CRE-Lox system



https://www.the-scientist.com/infographic-the-cre-lox-system-explained-71500

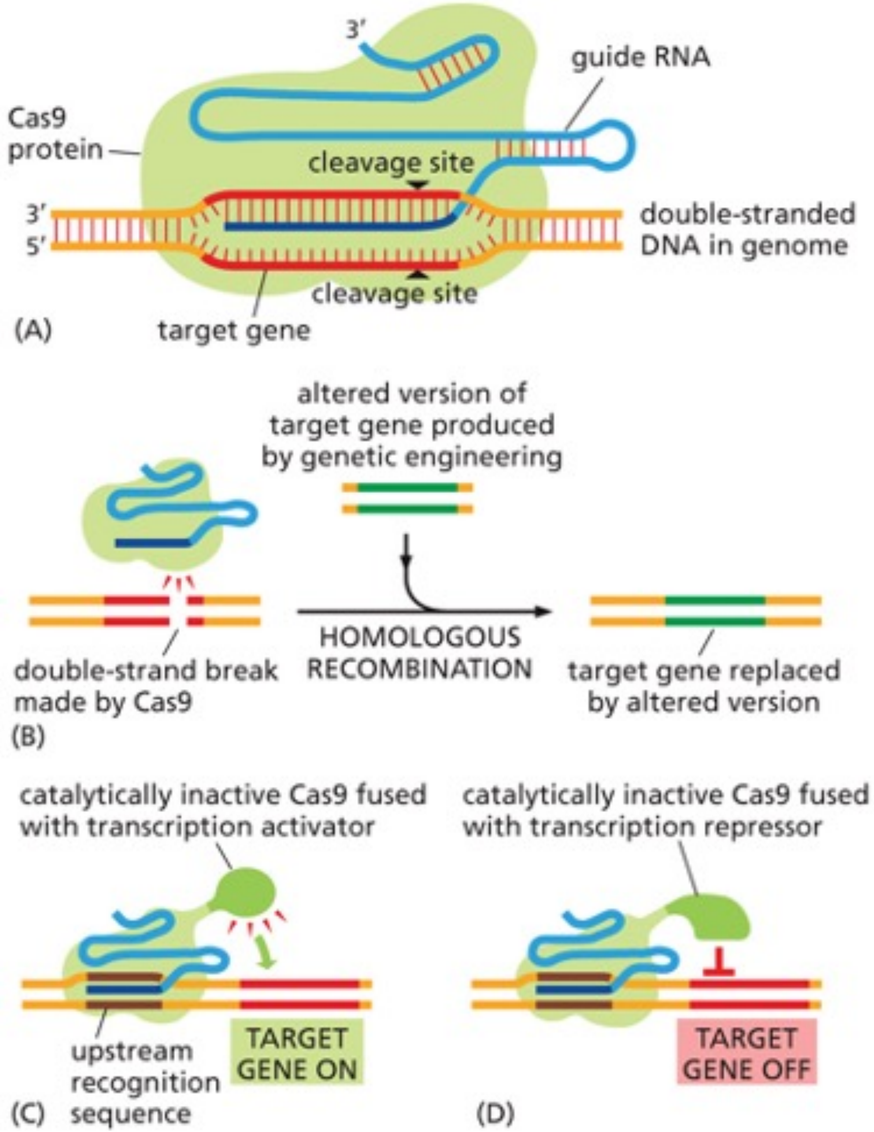
Engineering animals: the CRISPR system

- **Cas9 and guide RNA** are expressed in culture cells
- They associate and mediate **double-strand break** of the chosen DNA region
- This is repaired by non-homologous end joining leading to errors and often a **gene deletion phenotype**
- An **altered target gene** can be provided to be added by homologous recombination
- Can also be added to **turn genes on and off** by fusing Cas9 with transcription regulators



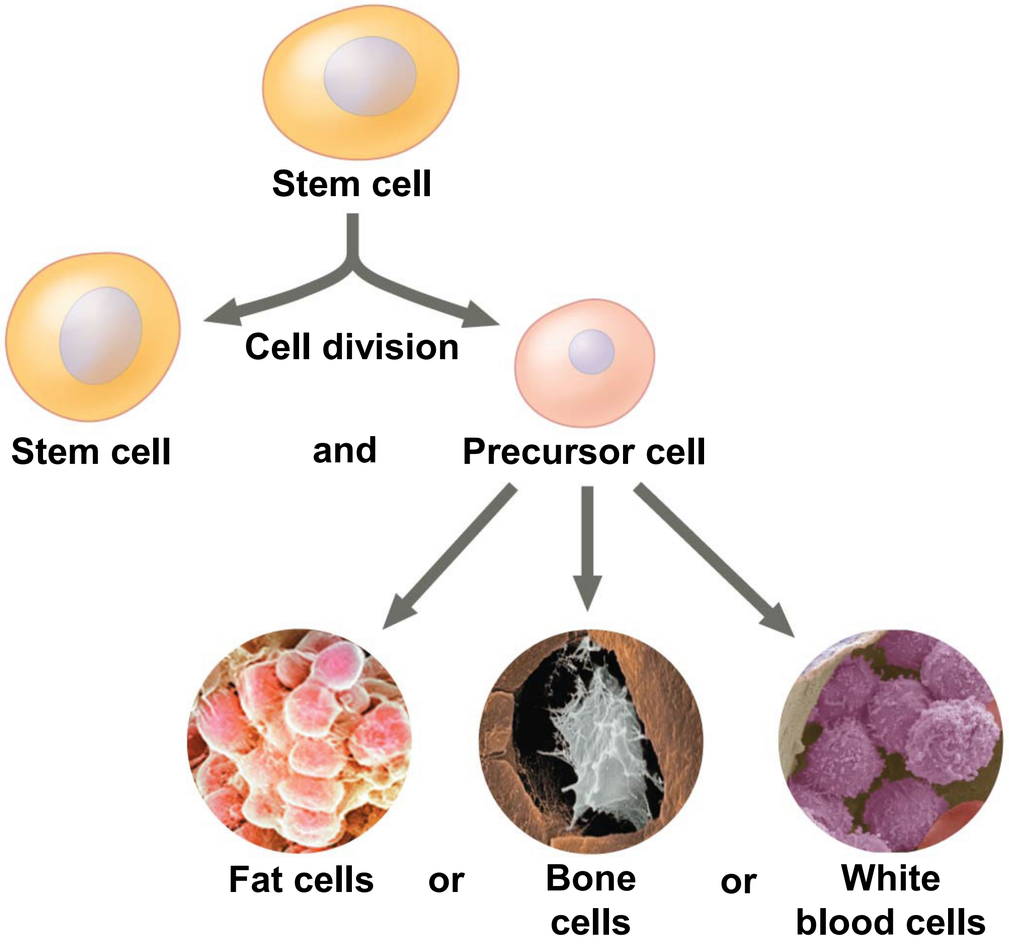
Engineering animals: the CRISPR system

- **easy** to design guide RNAs
- **numerous genes** can be controlled simultaneously (one Cas9, different guide RNAs)
- can virtually be adapted to **all organisms**



Stem cells (ES)

- **Stem cells** are unspecialized, reproduce themselves indefinitely and differentiate into specialized cells upon specific signals
- Stem cells from early embryos are called **embryonic stem cells (ES)** and can differentiate into **all cell types**
- In **adult** bodies, stem cells replace **non-reproducing specialized cells**
- ES are **pluripotent,** they can differenciate in many cell types
- The aim is to supply cell for the **repair of diseased organs**



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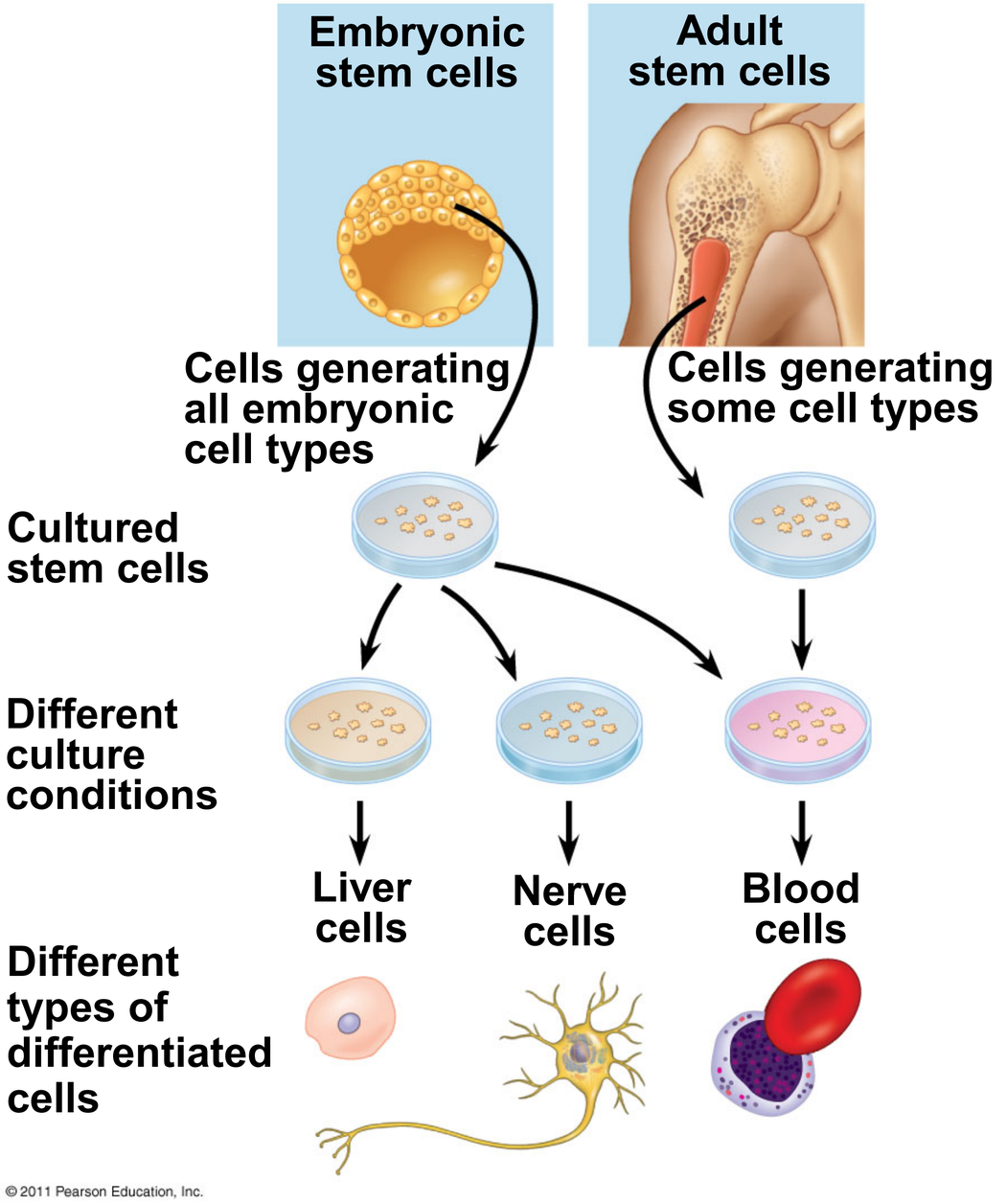
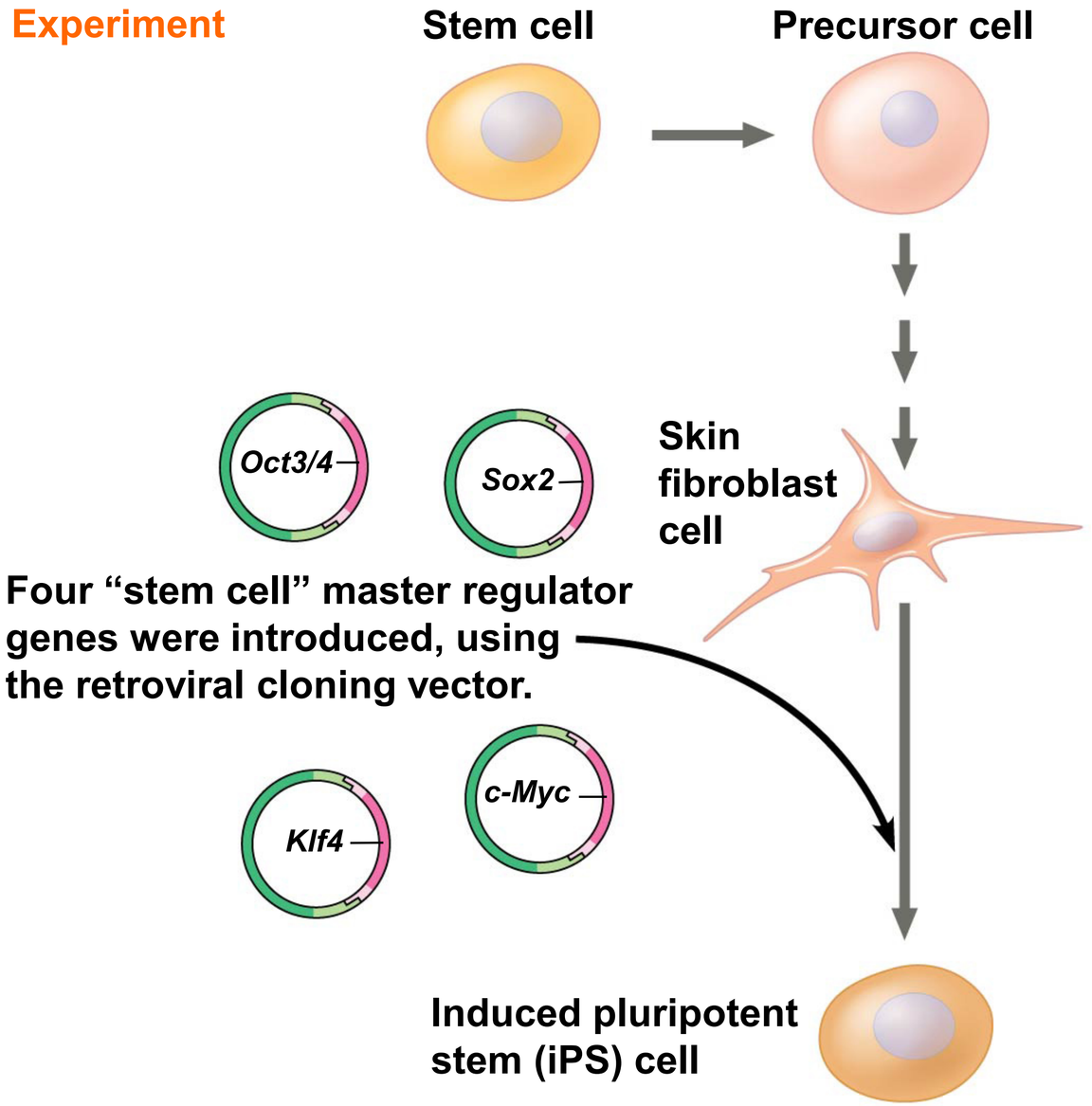


Stem cells (iPS)

- **Induced pluripotent stem cells (iPS)** are differentiated cells that are reprogrammed to act as ES
- Retroviruses are used to induce extra copies of **4 master regulators** to produce iPS
- They can be used as **models** to study certain diseases



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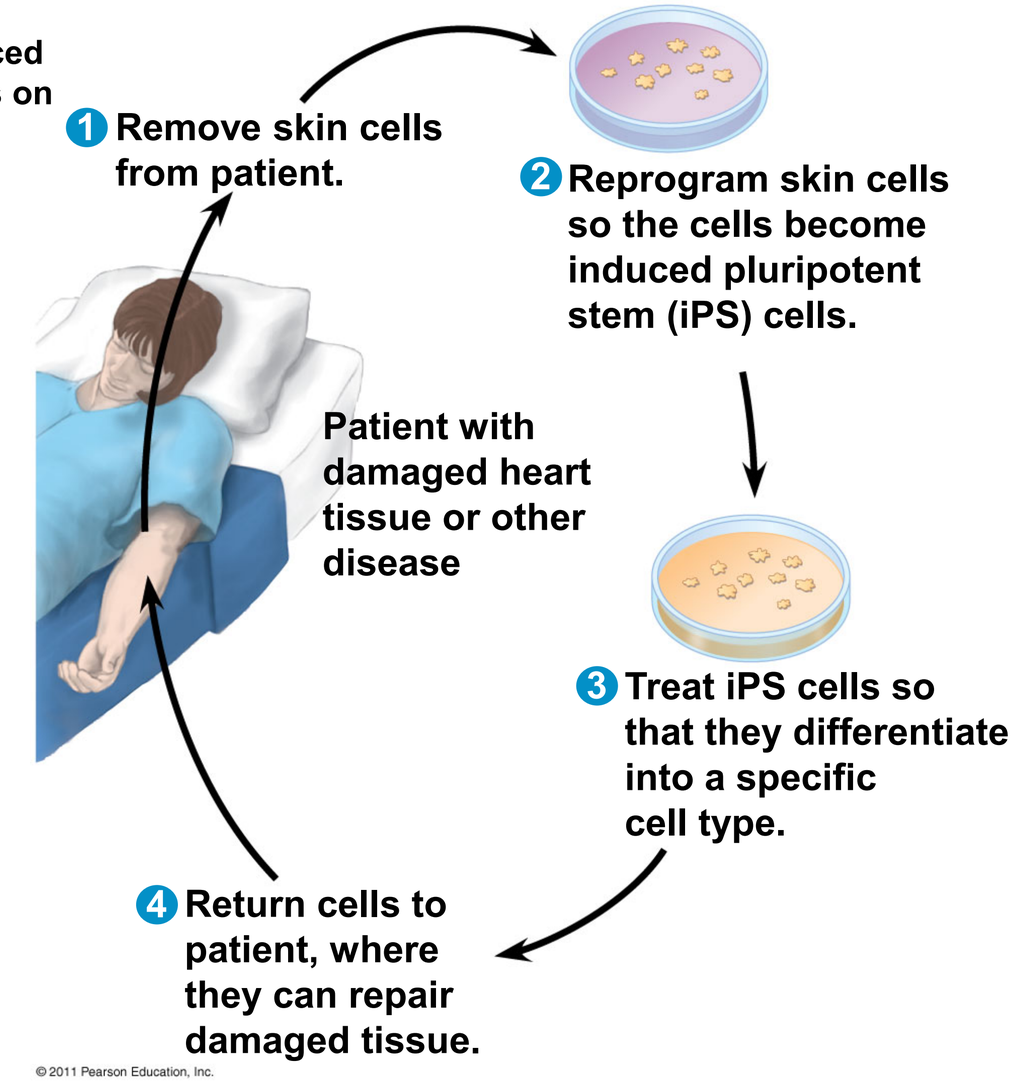
Impact: The Impact of Induced Pluripotent Stem (iPS) Cells on Regenerative Medicine

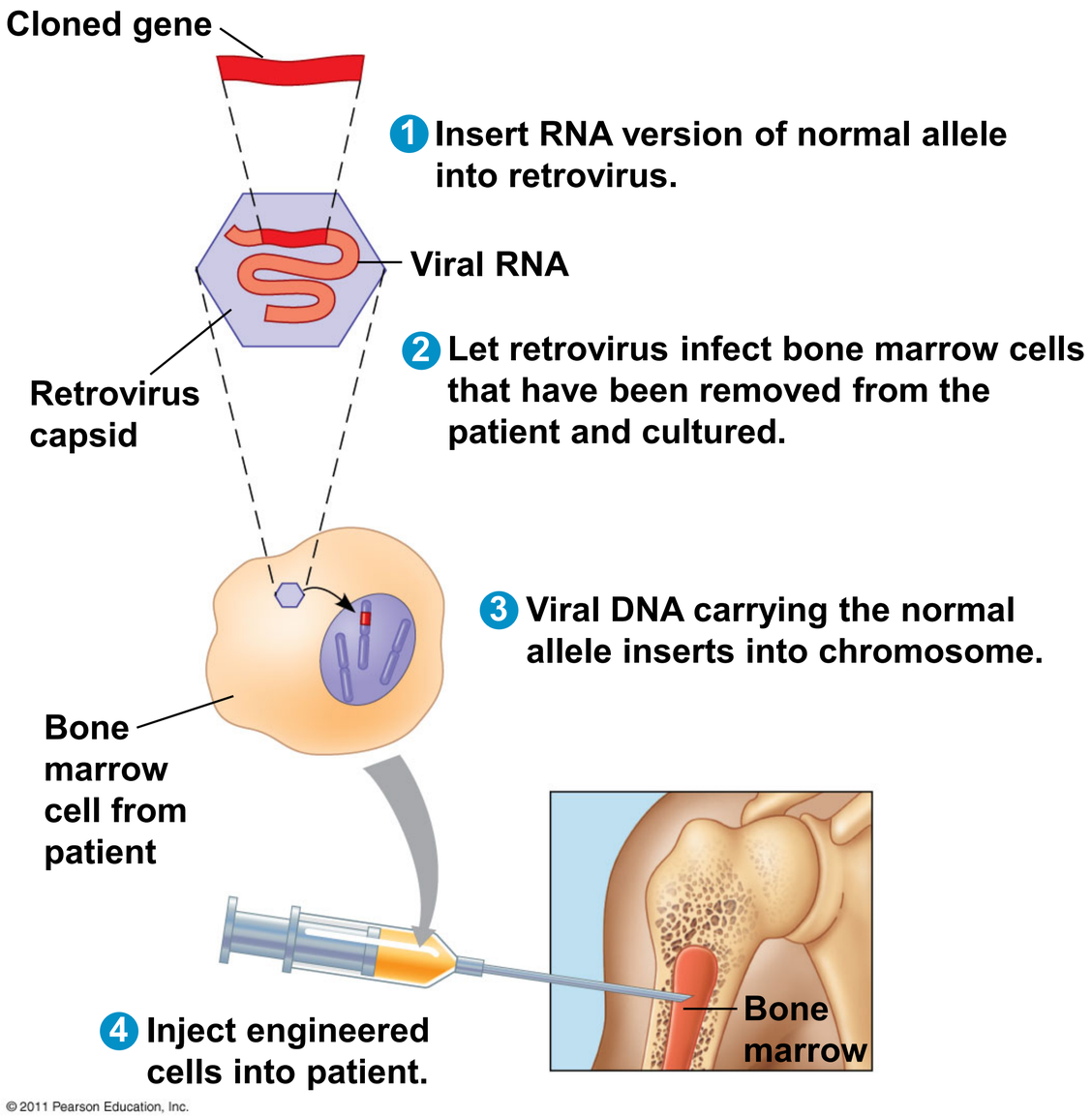


Plan

- Studying DNA
    - DNA cloning
    - Cloned organisms and stem cells
    - **Practical applications of DNA-based technology**

Practical applications of DNA-based technology

- Medical application
  - identify genes/mutations involved in **genetic diseases** which are target for **treatment or prevention**
  - **diagnostic** by PCR for disease-causing mutation
  - **gene therapy**, i.e. repairing a “bad” gene - technical and ethical questions



Practical applications of DNA-based technology

- Pharmacy
  - **transgenic animals** as **pharmaceutical factories**, producing large amounts of rare substances

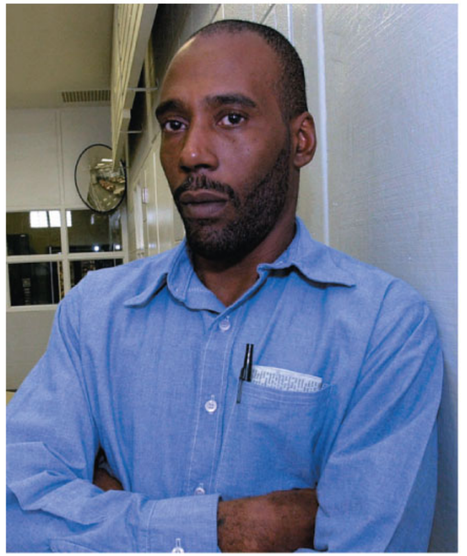
  ➡ Goat produces anti-thrombin in their milk, which helps patients with genetic disease lacking this enzyme and that have blood clots in their vessels

Practical applications of DNA-based technology

- Forensic science
  - Each individual has a **unique DNA sequence** (=genetic profile) that can be obtained from body tissues or fluids
  - DNA testing can **identify individuals** with a high degree of certainty
  - As of 2013, more than **300 innocent people** have been released from prison as a result of old DNA analysis

Practical applications of DNA-based technology

(a) This photo shows Washington just before his release in 2001, after 17 years in prison.



Source of sample	STR marker 1	STR marker 2	STR marker 3
Semen on victim	17,19	13,16	12,12
Earl Washington	16,18	14,15	11,12
Kenneth Tinsley	17,19	13,16	12,12

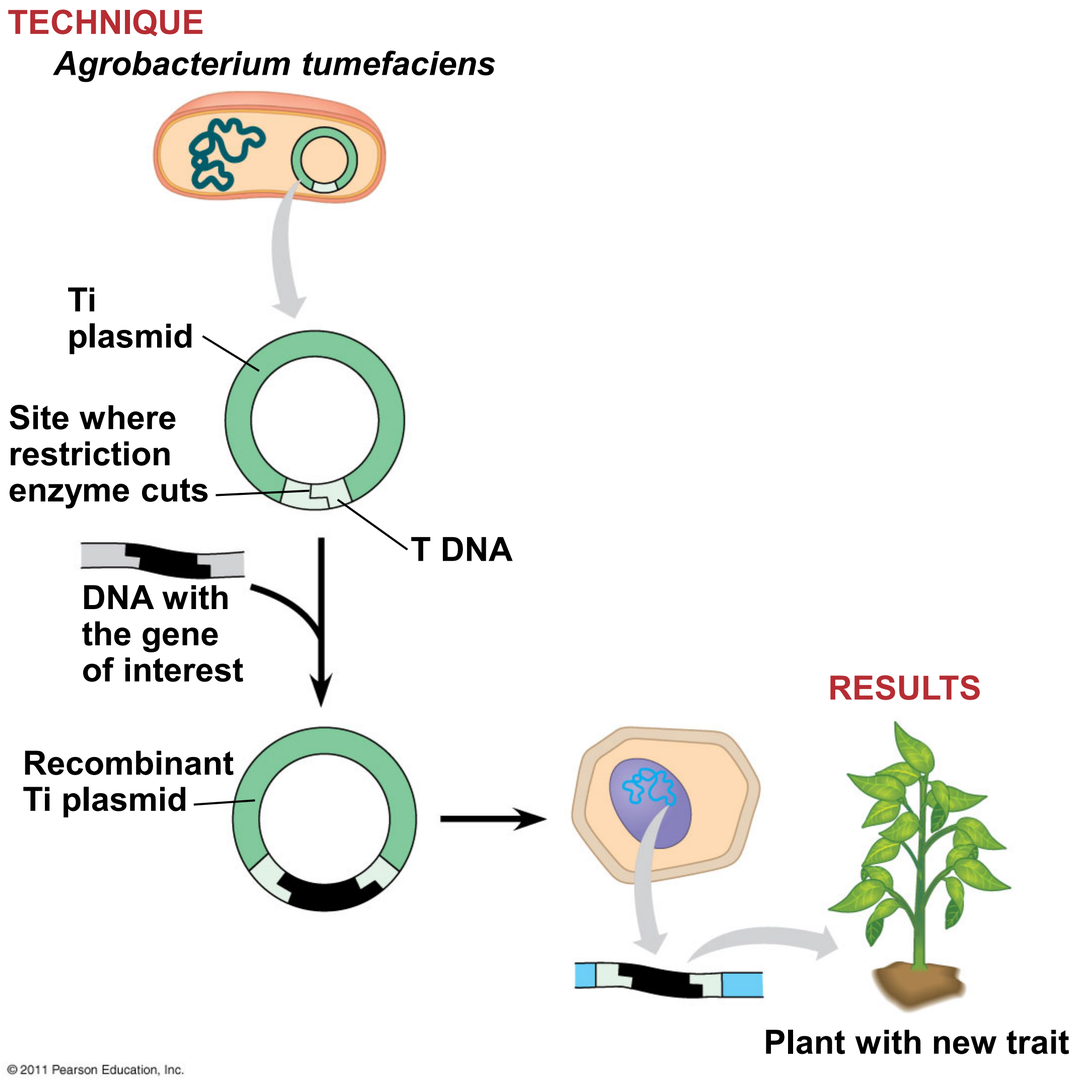
(b) These and other STR data exonerated Washington and led Tinsley to plead guilty to the murder.

Practical applications of DNA-based technology

- Environmental science
  - Genetic engineering to modify the **metabolism of micro-organisms** (degradation of toxic waste, mineral extraction, …)

Practical applications of DNA-based technology

- Agriculture
  - Improve **productivity** and **food quality**
  - Herbicide resistance, resistance to pests, resistance to salinity, improved nutritional value, …



Practical applications of DNA-based technology

Potential benefits must be weighed against **potential hazards** of creating harmful products or procedures

Summary

- Studying DNA
    - DNA cloning
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Have a nice day!