College of Pharmacy
Fifth Stage

Pharmaceutical Biotechnology

Dr. Maytham Ahmed

Lectutre 2
Formulation of Biotechnology Products

Formulation of Biotechnology Products

Why Biopharmaceuticals are not Common

I. Newly introduced biopharmaceuticals are very **expensive.** This is partly due to:

A. The high **development costs**, combined with high initial **production costs**.

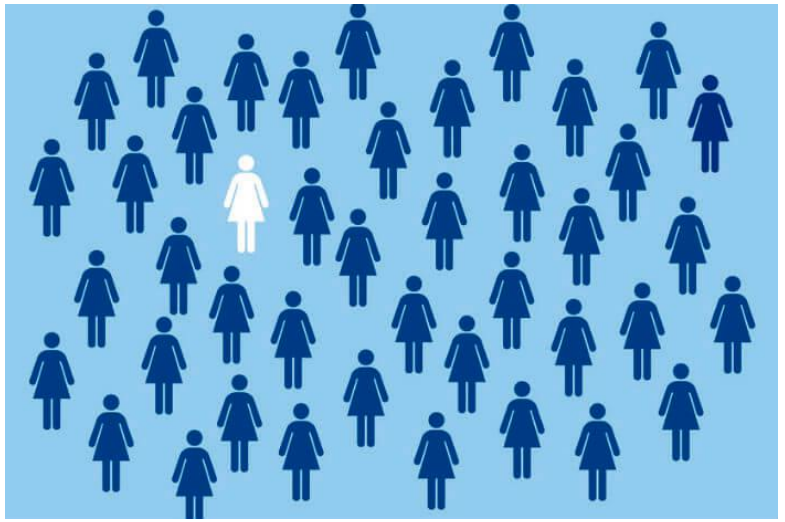
B. The relatively high price of (bio)pharmaceuticals is also due to too **many failures** during the **drug discovery** and **development process**.

Why Biopharmaceuticals are not Common

2. The **number of patients** for many marketed therapeutic proteins is relatively **small**. This has several reasons:

A. The **high price of therapeutic** proteins makes that they are used primarily for the treatment of the relative severe cases.

B. The **specificity** of many therapeutic proteins makes that they are only effective in subgroups of patients (**personalized medicine**).



Why Biopharmaceuticals are not Common

C. Some **diseases are very rare** and thus the number of patients is very small. Most of these rare diseases are **due to a genetic defect**.

- Examples are **cystic fibrosis (CF)** and **glycogen storage disease II (GSD II)**.
- **CF** is most common in Caucasians. It is clear that developing a drug for such a small patient population is commercially not very interesting.

Biopharmaceuticals vs Small Molecule Drugs

- **The main differences between Biopharmaceuticals and Small Molecule Drugs**

	Biopharmaceuticals	Small Molecule Drugs
1	Produced by living **cell cultures**	Produce by **chemical synthesis**
2	**High** molecular weight	**Low** molecular weight
3	**Complex**, heterogeneous structure	**Well-defined** structure
4	**Impossible to fully characterize** the molecular composition	**Completely** characterized

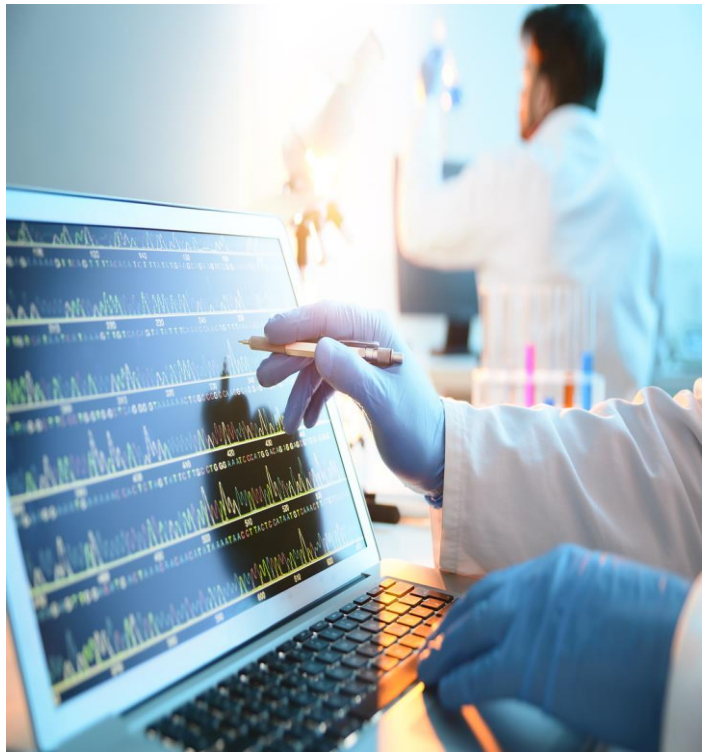
Biopharmaceuticals vs Small Molecule Drugs

- **The main differences between Biopharmaceuticals and Small Molecule Drugs**

	Biopharmaceuticals	Small Molecule Drugs
5	**Unstable**, sensitive to external conditions	**Stable**
6	Often **injected** or **infused**	Mostly **oral route**
7	Example: trastuzumab (M.Wt = 145531 Da)	Example: atorvastatin (M.Wt= 558 Da)

Products Selection of Therapeutic Protein

- This is **not straightforward** process because our knowledge is still growing about protein controlling various processes and what defect in gene or underlying protein is responsible for different diseases.
- It **sometimes a direct process** such as replacing the **endogenous protein** such as insulin for treatment of diabetic patients or erythropoietin for the treatment of anemia.

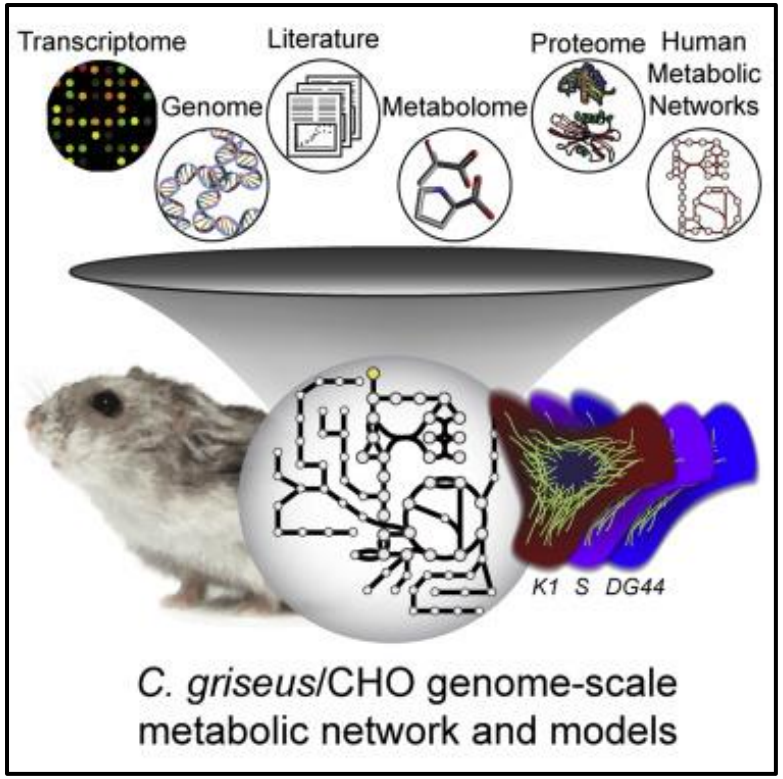


Products Selection of Therapeutic Protein

- Recombinant protein **can be produced** in E. coli, yeast, or mammalian cells.
- **Mammalian cells** are the **best choice**, in fact about 70% of marketed protein is produced in Chinese hamster ovary cells.
- Mammalian cells have **advantages: Preform all required posttranslational modification** such as glycosylation (which necessary for protein activity and stability), formation of disulfide linkage (folding of protein which necessary for stability) which **resemble most closely the human situation**.
- But these activities are nearly unavailable in bacterial and yeast hosts.

Difficulty with Mammalian Cells

1. They are **difficult to maintain** in culture compared to bacteria and yeasts.
2. **Division time** is about **24 hr** while for **E. coli** is about **30 min** and **yeast** are about **1 hr**.
3. Mammalian cells **needs more expensive** growth media.
4. Some required **growth media additives** such as bovine serum albumin may has the **risk of transferring diseases** to human such as **bovine spongiform encephalopathy** (BSE) (or what is called mad cow disease).

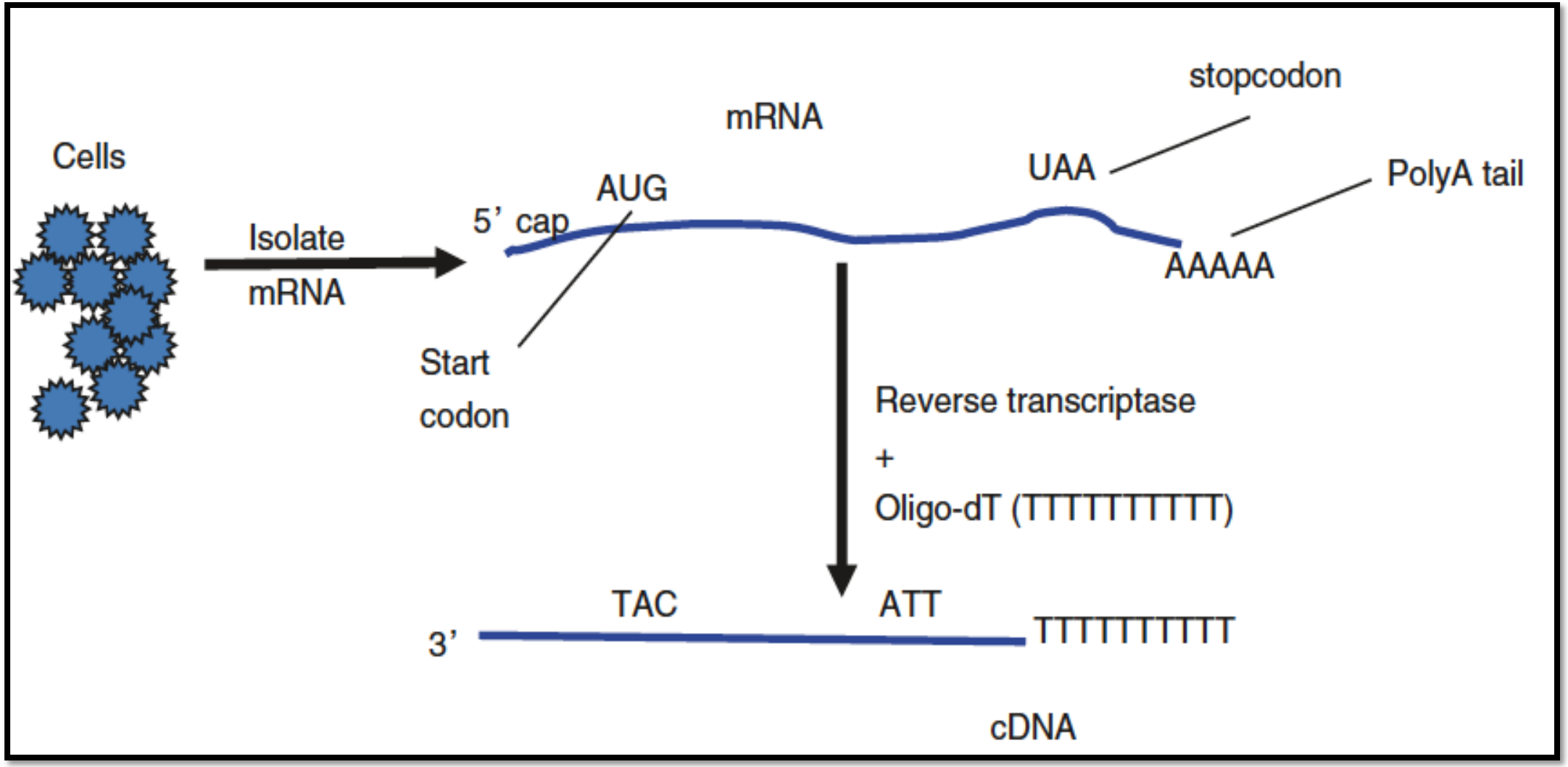


C. griseus/CHO genome-scale metabolic network and models

Formulation of Biotech Products Copy DNA (cDNA):

- The next step in formulation the biopharmaceuticals is to **obtain the actual DNA** that codes for the protein.
- This DNA is obtained by **reverse-transcribing** the mRNA sequence into **copy DNA (cDNA).**
- In this step **mRNA** that translate protein synthesis is **isolated** and then **reversed** using **enzyme reverse transcriptase** to get the original DNA and this DNA is called (cDNA).

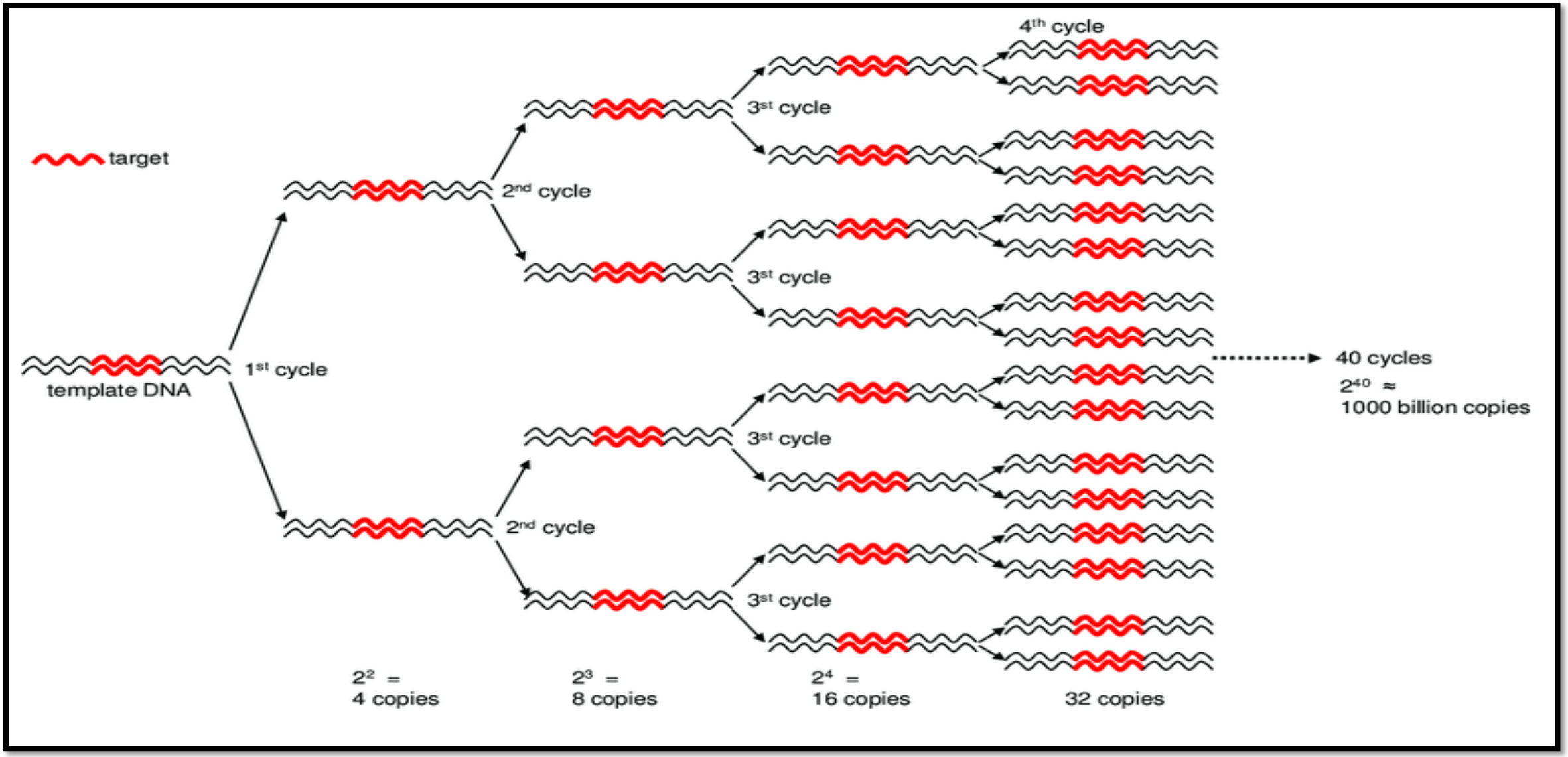
Formulation of Biotech Products Copy DNA (cDNA):



Amplifying cDNA

- The next step in formulation biopharmaceuticals is to **amplify this cDNA** using the **polymerase chain reaction (PCR).**
- The amount of DNA should **double** during each cycle.
- PCR is done for **30 cycle** and in the resulted amount of cDNA is up to **10^9** the starting amount.
- In practice this **10^9** is **never reached**. In particular at later cycles, the efficiency of the PCR reaction reduces.

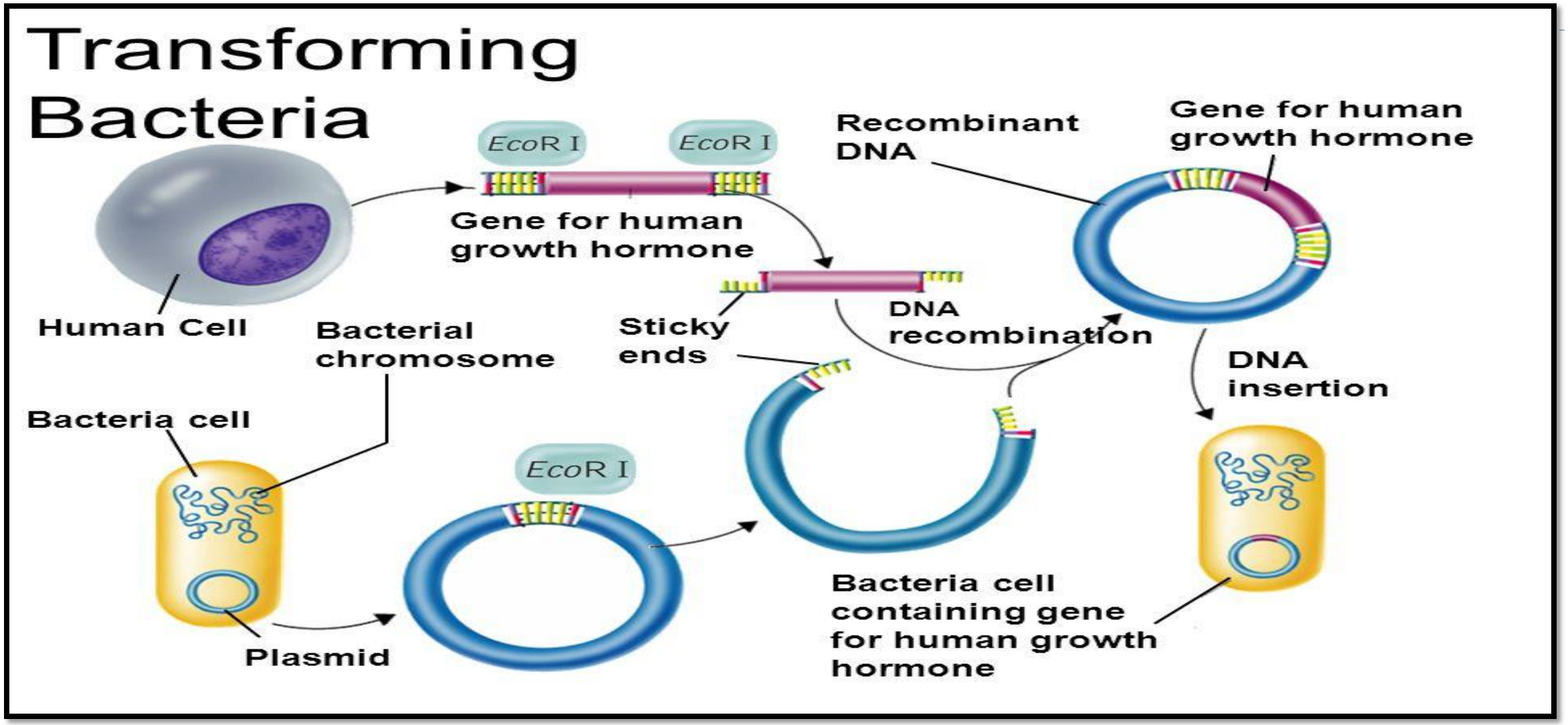
Amplifying cDNA



Introduction of cDNA into Cell

- The PCR **product** is then refined by **cloning** and introduced to the bacteria.
- Scientists have taken advantage of **plasmids** to use them as tools to clone, transfer, and manipulate genes.
- **Plasmids** that are used experimentally for these purposes are called **vectors.**
- Researchers can insert DNA fragments or genes into a **plasmid vector**, creating a so-called **recombinant plasmid**.
- This **plasmid** can be introduced into a bacterium by way of the process called **transformation**.

Introduction of cDNA into Cell



Introduction of Plasmid-DNA into Mammalian Cells

- Because of bacteria **divides rapidly**, they can be used as factories **to plasmid containing the required DNA piece** in large quantities.
- The **resulted DNA** is sequenced to make sure we will get the protein with the desired properties.
- This Plasmid-DNA can also be **transfected** into a mammalian cell to get the required protein.
- Protein is then collected and purified by a process called **downstream processing** and **affinity chromatography**.

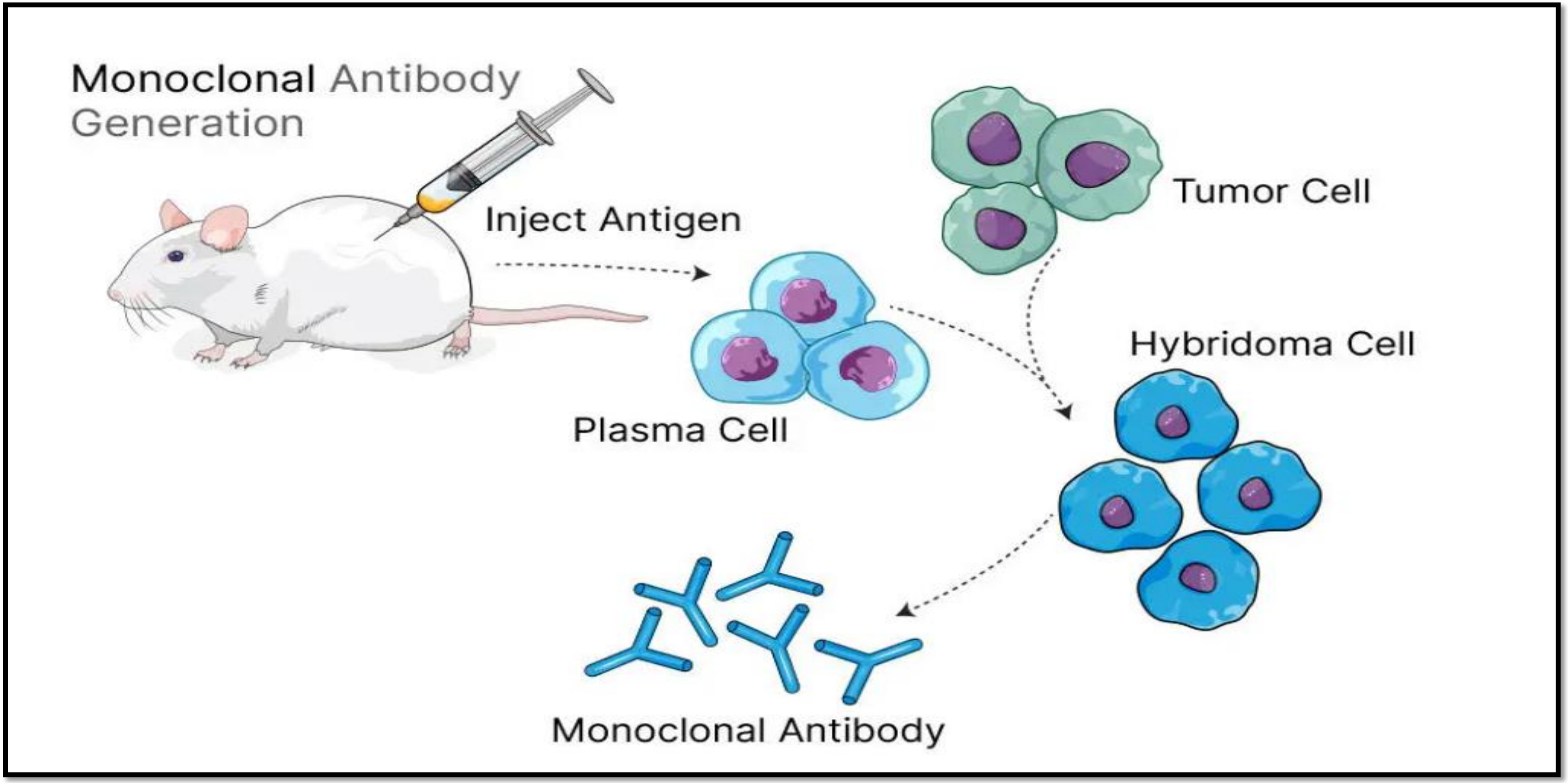
Hybridoma Technology

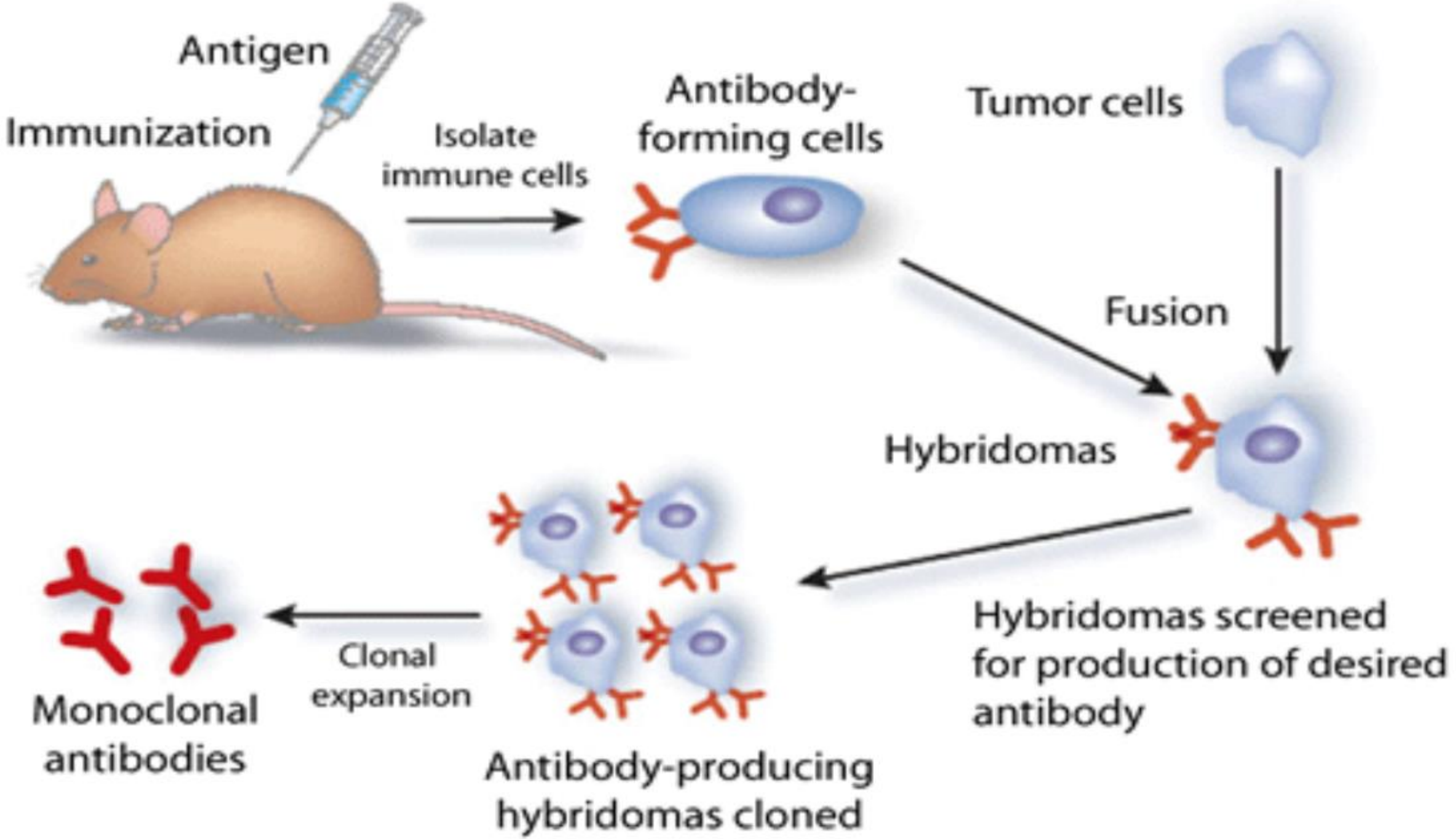
- **Hybridoma technology** is a method for producing large numbers of identical antibodies (also called monoclonal antibodies). This process starts by injecting a mouse (or other mammal) with an antigen that provokes an immune response. A type of white blood cell, the B cell, produces antibodies that bind to the injected antigen. These antibody producing B-cells are then harvested from the mouse and, in turn, fused with immortal myeloma cancer cells (myeloma cells are cancerous plasma cells; plasma cells are derived from activated B cells), to produce a hybrid cell line called a **hybridoma**, which has both the antibody-producing ability of the B-cell and the longevity and reproductivity of the myeloma.

Hybridoma Technology

- The hybridomas can be grown in culture, each culture starting with one viable hybridoma cell, producing cultures each of which consists of genetically identical hybridomas which produce one antibody per culture (monoclonal) rather than mixtures of different antibodies (polyclonal). The myeloma cell line that is used in this process is selected for its ability to grow in tissue culture and for an absence of antibody synthesis. In contrast to polyclonal antibodies, which are mixtures of many different antibody molecules, the monoclonal antibodies produced by each hybridoma line are all chemically identical.

Hybridoma Technology




Antigen
Immunization
Isolate immune cells
Antibody-forming cells
Tumor cells
Fusion
Hybridomas
Hybridomas screened for production of desired antibody
Clonal expansion
Monoclonal antibodies
Antibody-producing hybridomas cloned


Thank You