Formulation of Biotech Products

Why Biopharmaceuticals are not Common

1. 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.

- The few products that actually reach the market have to **compensate** for all the expenses made for failed products.

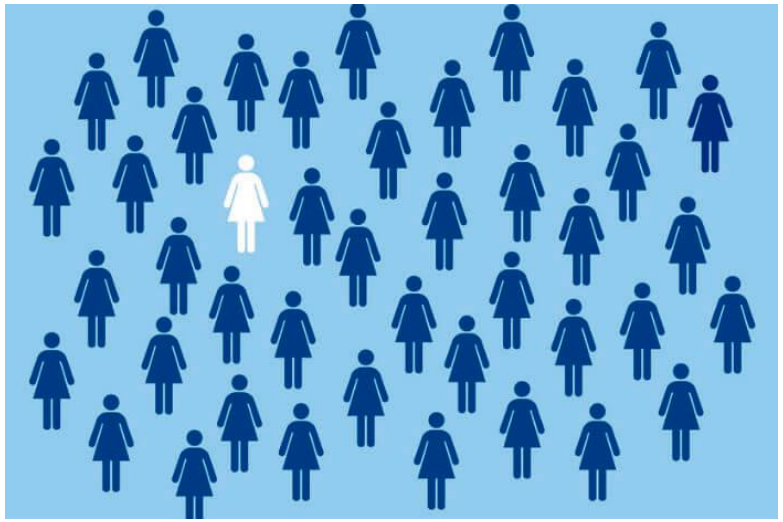
Why Biopharmaceuticals are not Common

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**).

- This is in particular true for the **monoclonal antibodies** used to treat cancer patient for example: the antibody **trastuzumab (Herceptin)** is only approved for breast cancer patients with high expression levels of the HER2 receptor on the tumor cells (**20% of breast cancer cases**).



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 Drug Molecules

- **The main differences between Biopharmaceuticals and small drug molecule**

Biopharmaceuticals	**Small molecule drugs**
Produced by living **cell cultures**	Produce by **chemical synthesis**
High molecular weight	**Low** molecular weight
Complex, heterogeneous structure	**Well-defined** structure
Strongly **process-dependent**	Mostly **process-independent**
Impossible to fully characterize the molecular composition and heterogeneity	**Completely** characterized

Biopharmaceuticals vs small Drug Molecules

- **The main differences between Biopharmaceuticals and small drug molecule**

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

Formulation of Biotech 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.

Formulation of Biotech Products
Selection of Expression Host

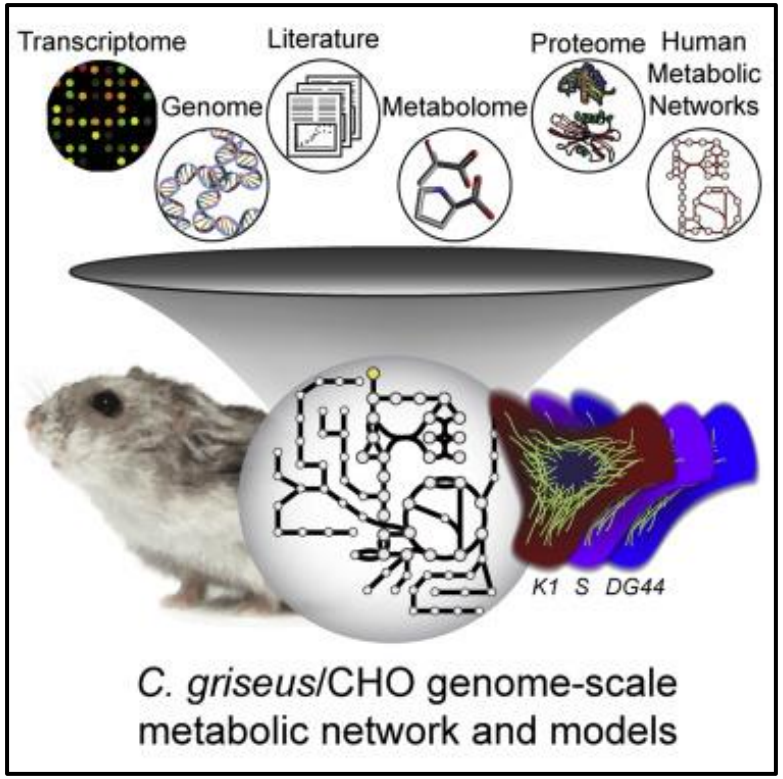
- Recombinant protein **can be produced** in E. coli, yeast, or mammalian cells.
- **Mammalian cells** are the **best choice**, in fact about 70% of marketed protein is produced in Chinese hamster ovary (CHO) cells.
- Mammalian cells have **advantages:**

1. They are able to **grow in suspension**.
2. **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).

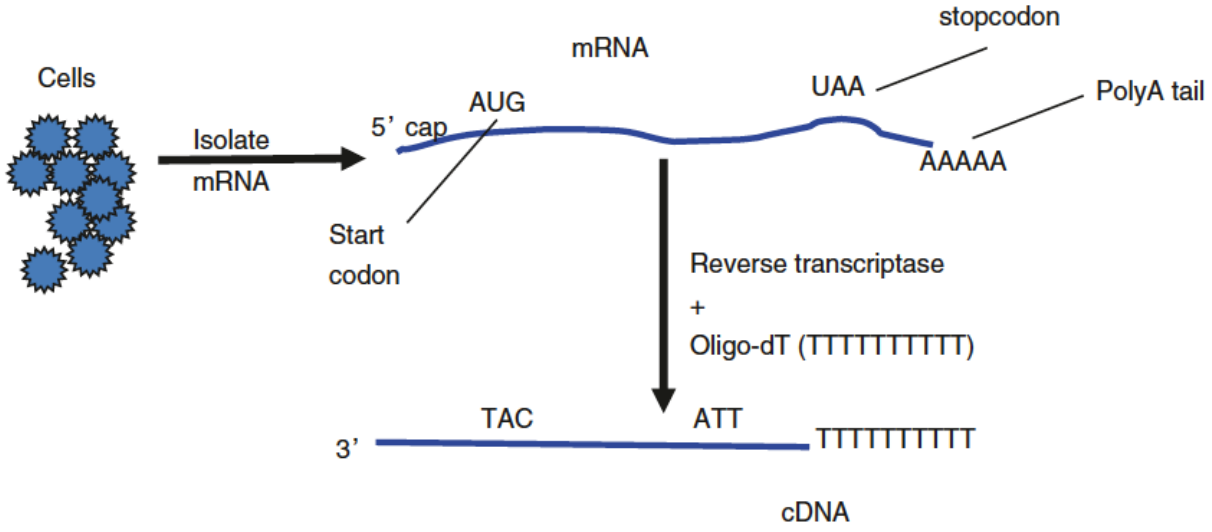
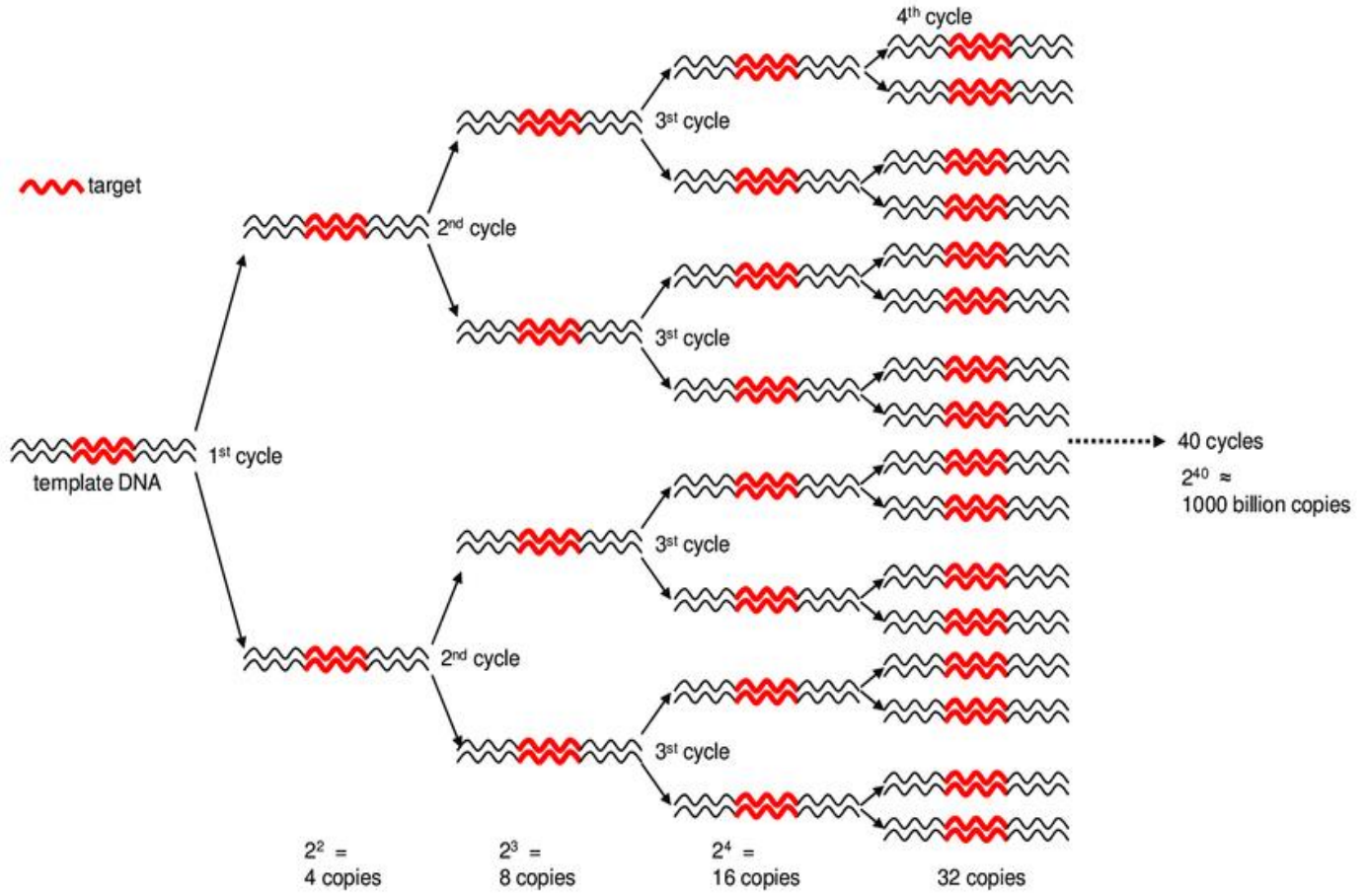


Figure 1.6 ■ Reverse transcriptase reaction

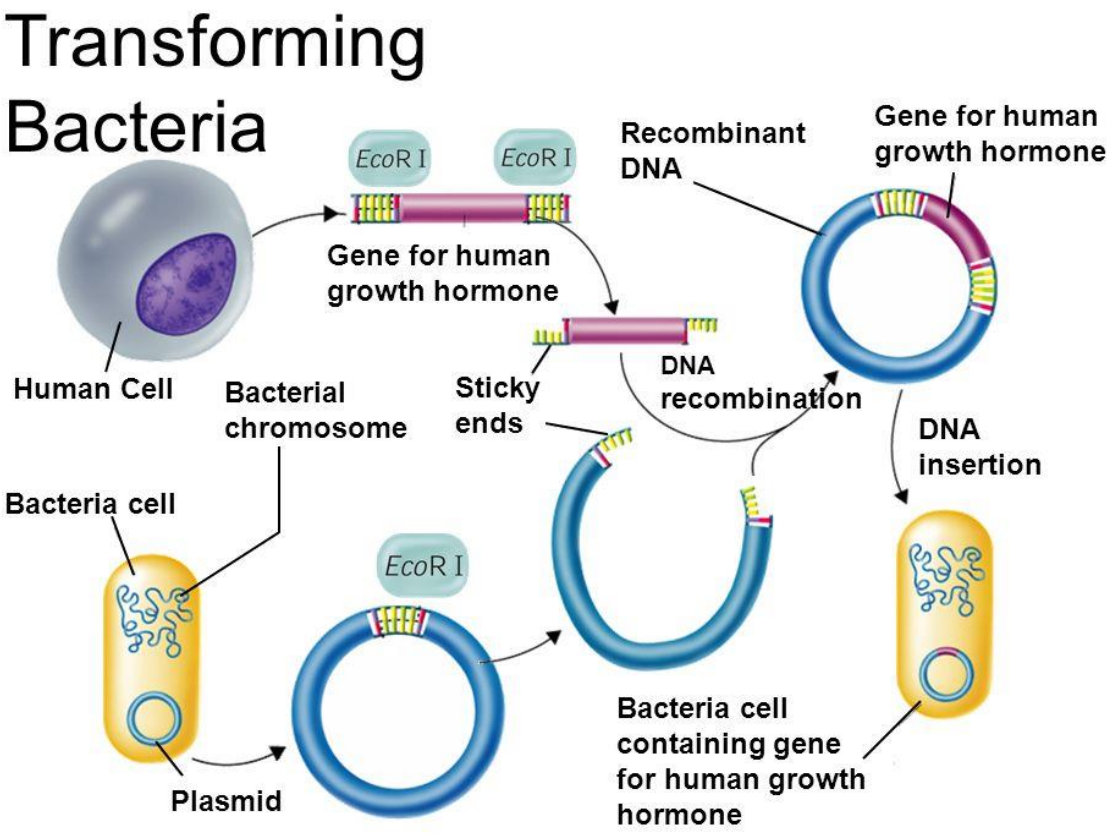
Formulation of Biotech Products
Amplifying cDNA

- 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.



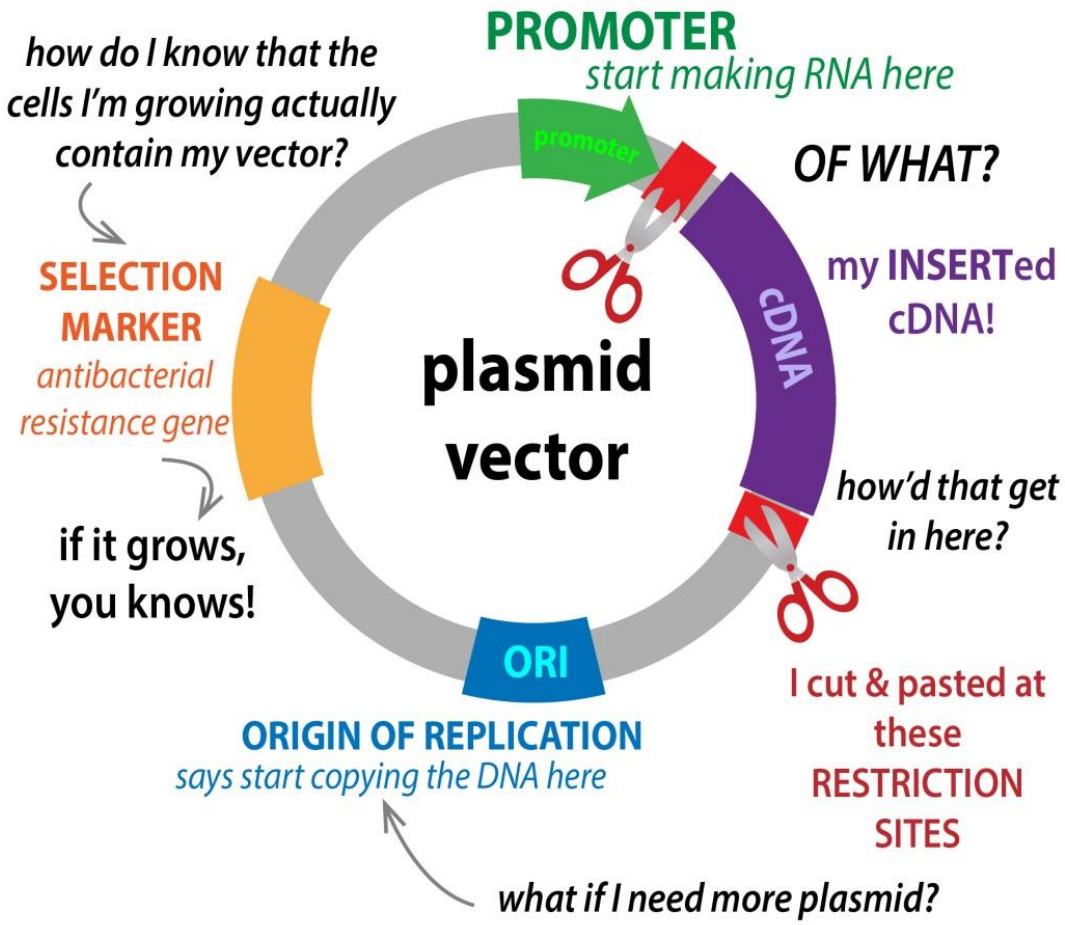
Formulation of Biotech Products
Introduction of cDNA into Cell

- 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**.



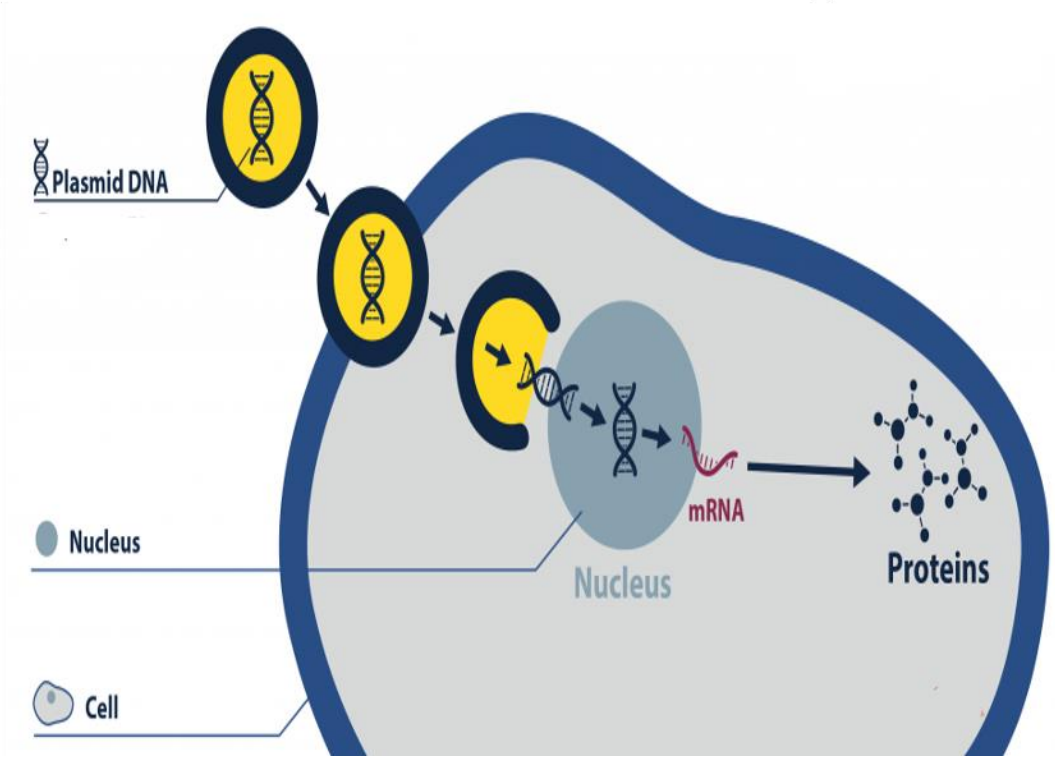
Plasmid

- **Plasmid vector** is introduced into bacterial cells.
- These cells is grown into an agar with antibiotics.
- Since the **plasmid is responsible for antibiotic resistance**, only cells that contain the plasmid will survive and the other will die.



Formulation of Biotech Products
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**.

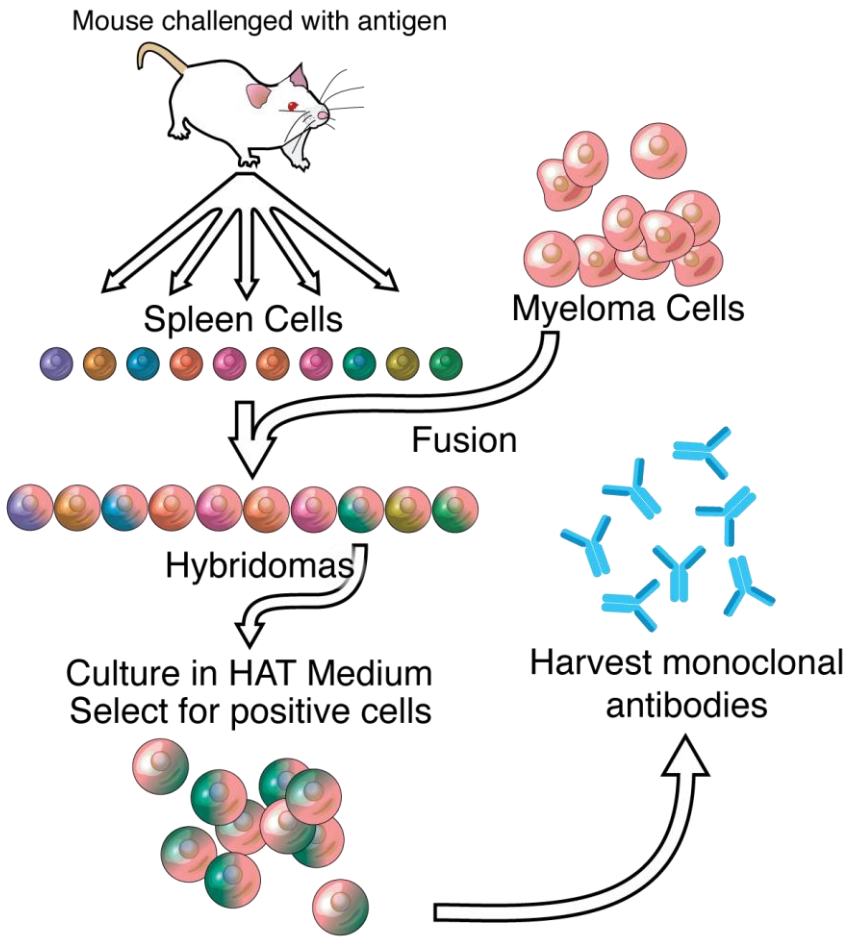


Monoclonal Antibodies (mAB)
Hybridoma Technology

- Many marketed therapeutic proteins are **monoclonal antibodies**.
- The classic way to make a monoclonal antibody **starts by** immunizing a laboratory animal with a purified human protein against which the antibody should be directed.
- This technique for producing mAB is called **hybridoma technology**.
- These monoclonal antibodies (mAB) are used as a treatment mainly for cancers and for some other diseases.
- Examples are **tocilizumab (Actemra)** used to treat rheumatoid arthritis and it was used to treat cytokine storm associated with covid-19 as it **attacks interleukin 6 receptor**.
- Other example is **Bevacizumab (Avastin)** which is used for colorectal cancer and some eye diseases.

Hybridoma technology

- mAB is made by injecting **antigen** into **an animal** and then collect the **B lymphocyte** that produce the antibody.
- These **B cells is infected** or infused with human cancer B (**myeloma cells**) cells (which are immortal but not able to produce the antibody).
- So, we will get a new **hybrid cell** that are able to produce antibody and are immortal.
- The antibody produced are **genetically similar** and **called monoclonal antibody**.
- This way of production **is slow and somewhat expensive** and nowadays genetic engineering is used to produce large quantities of monoclonal antibody.



Definitions for Biotechnology Terms

Antibody	A component of the body's immune response. A Y-shaped protein, it is secreted in response to an antigenic stimulus.
Antigen	Any substance, almost always a protein, not normally present in the body that when introduced to the body stimulates a specific immune response.
Cloning	The replication of a DNA sequence from one organism to create an exact genetic copy.
Codon	A string of exactly three mRNA bases that code for a specific amino acid during translation of mRNA into DNA.
Proteomics	The study of proteins. Proteomics has three major goals: to identify and quantify all the proteins expressed in an organism, to determine the structure and function of each protein and to study the protein-protein interactions that affects how one protein interacts with other proteins to control cellular processes.