

# The use of GMO Technology at Pfizer Ireland

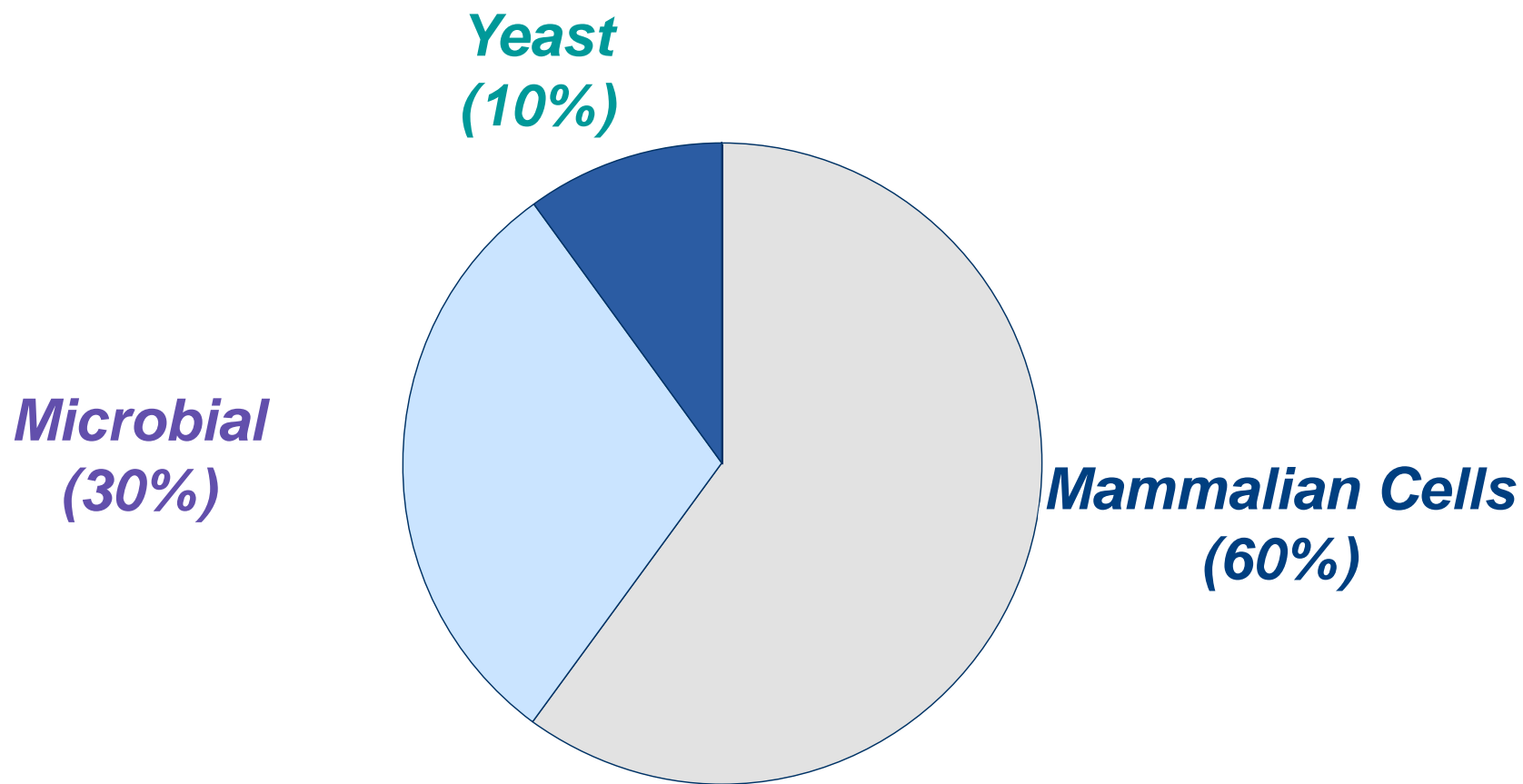
**Neysi Ibarra, PhD**

***Senior Manager, Process Development***



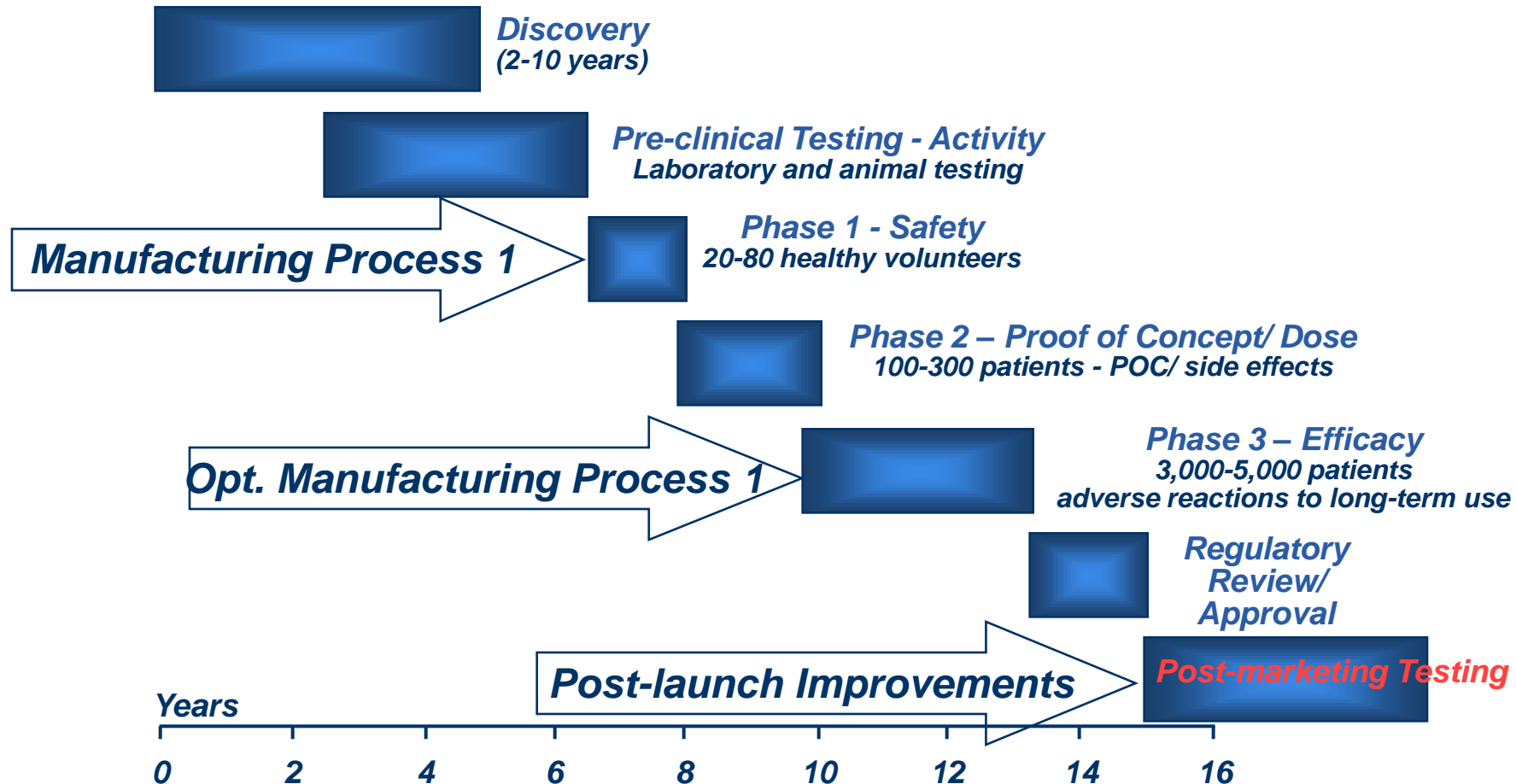
- The **use** of cell culture in the biotechnology industry
- The **complexity** of (recombinant) mammalian cells
- Brief **intro to proteins** as bio therapeutics
- Manufacturing** considerations for protein products
- Biosafety** Considerations

# The Majority of Biopharmaceutical Products on the Market are Produced using Mammalian Cells



**Mammalian cells are capable of making  
most human proteins**

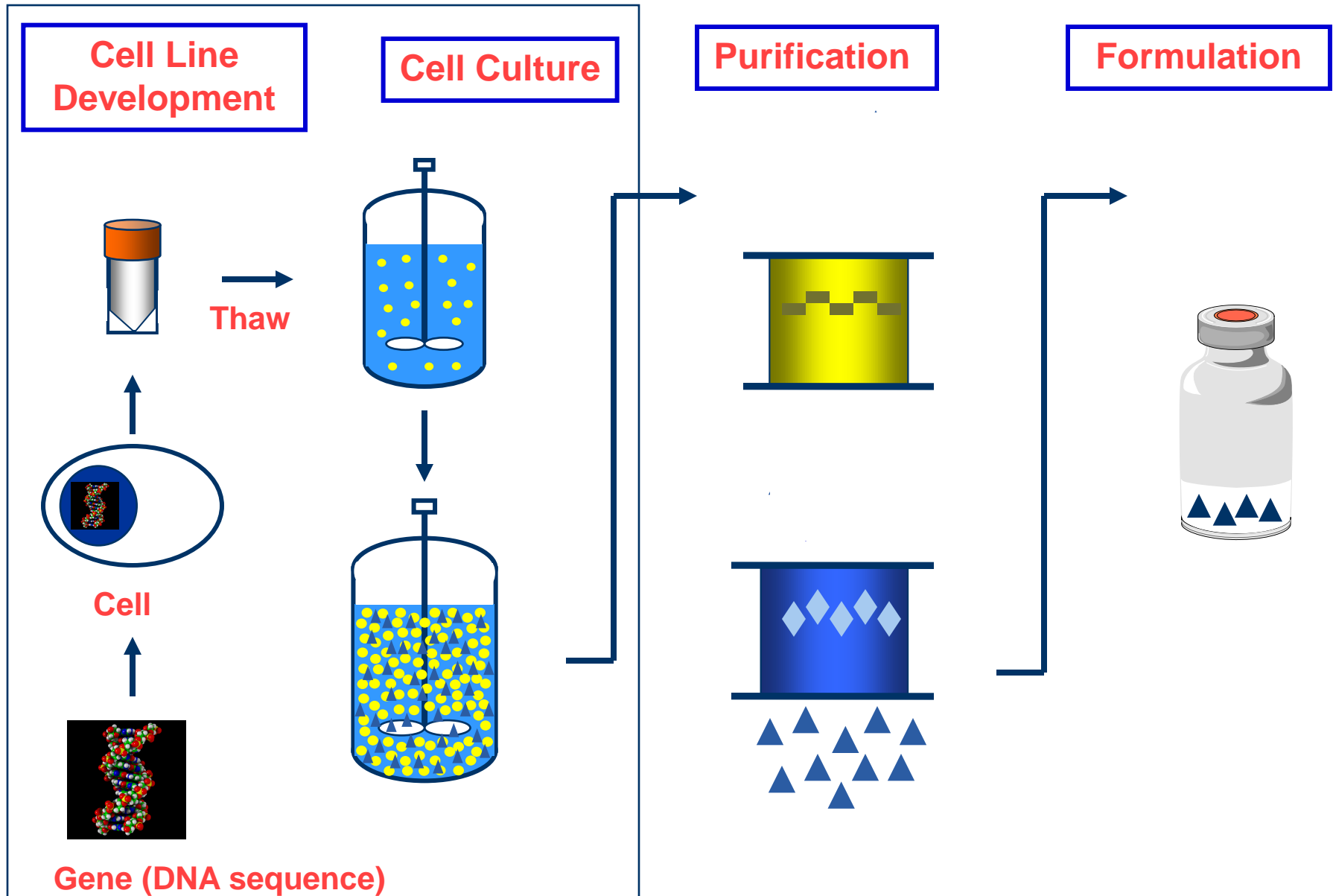
# Gene to Market - Development Timeline



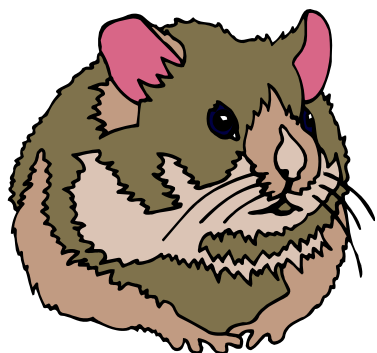
Source: PhRMA, based on data from Center for the Study of Drug Development, Tufts University. 1995



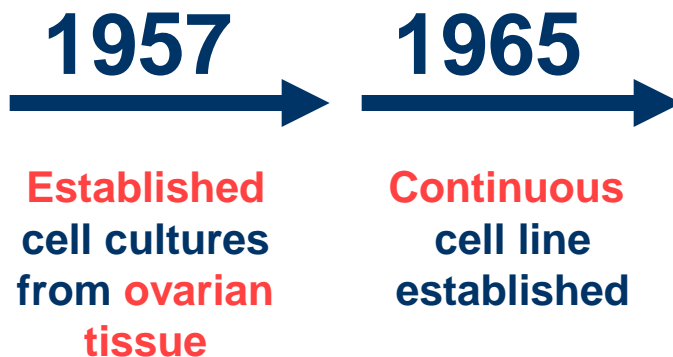
# From Gene to Drug Product: The manufacture of a protein biopharmaceutical using cell culture



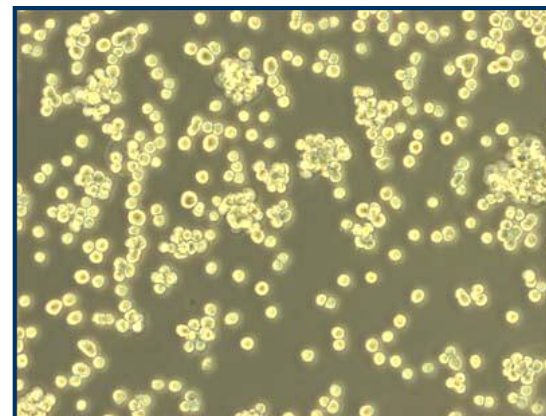
# Chinese Hamster Ovary (CHO) Cell Lines are Most Used in the Biotechnology Industry for Recombinant Protein Production



**Chinese  
Hamster**



**1979 - 2005**



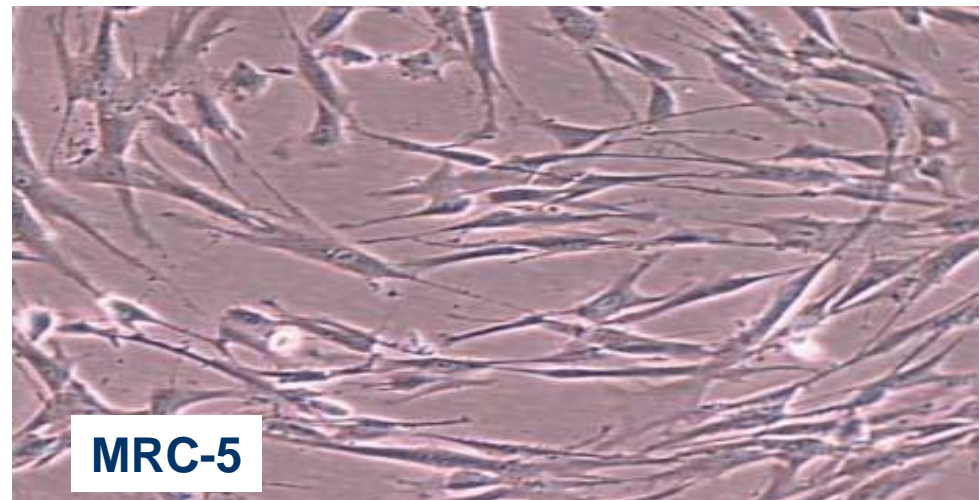
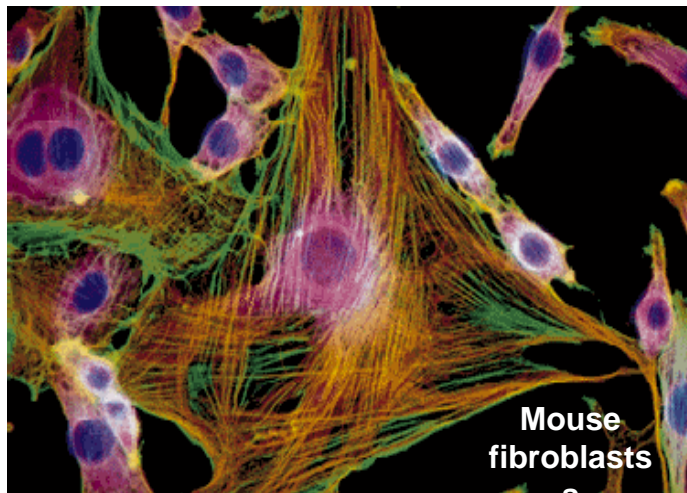
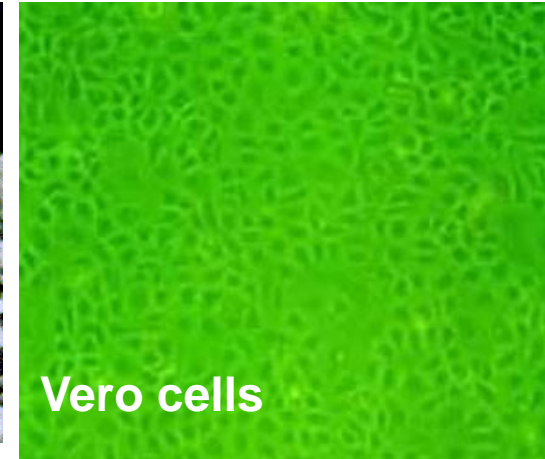
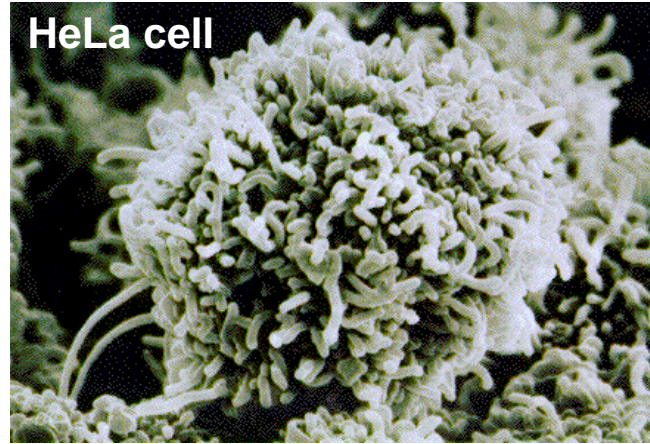
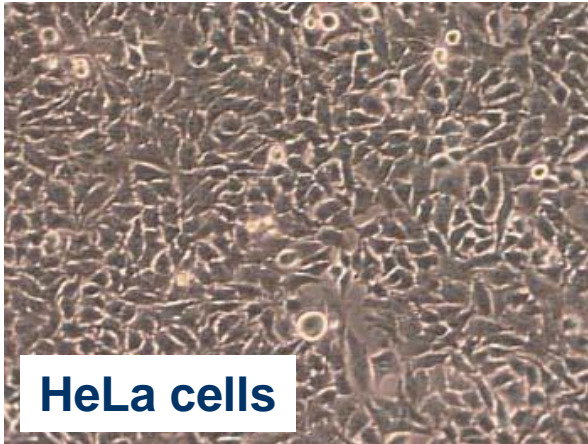
**CHO Cells**

**They look simple and  
uncomplicated here!**

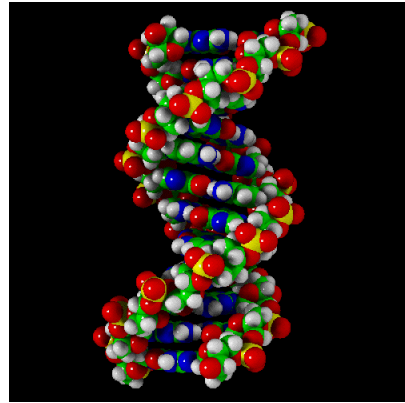
**Other cell lines used for biopharmaceutical production  
include **BHK** (Novo Nordisk), **GS-NS0** (MedImmune) &  
**SP2/0** (J&J)**



# A closer look at cell lines in culture



# Artificial insertion of foreign DNA into mammalian cells creates a recombinant cell line

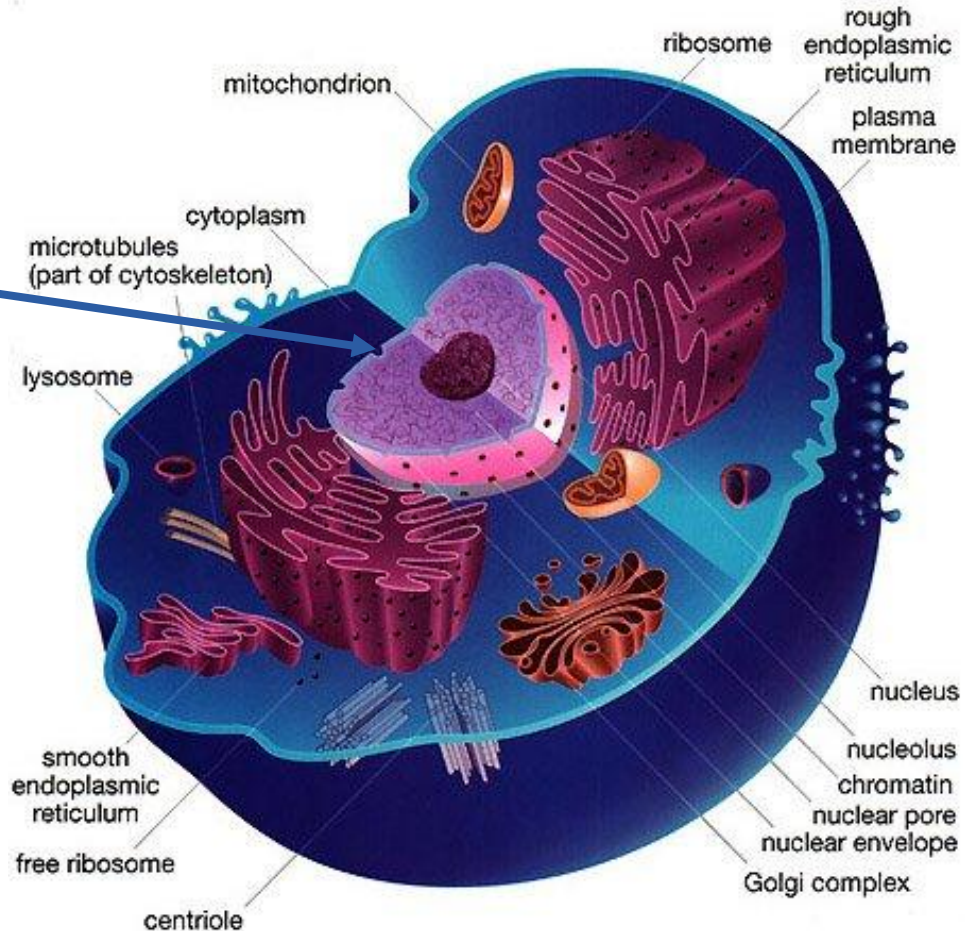


DNA

The 'foreign' **DNA** is designed to code for the protein

The **DNA** enters the **nucleus** of the cell and **integrates** into the host cell DNA

The new, recombinant cell line now can 'read' the **DNA** and **construct** the protein



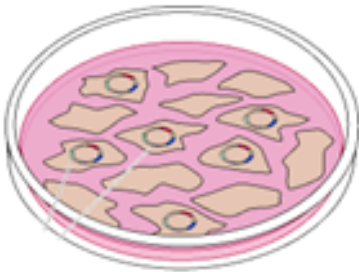
## CHO Cell



# Mammalian Cell Culture: Art or Science?

Cell lines and culture media have developed through the decades

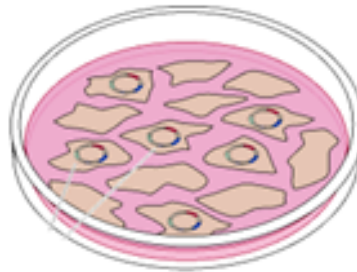
Circa 1950's



- Salts, sugars etc.
- Minced embryo
- Umbilical chord

**Finite** lifespan

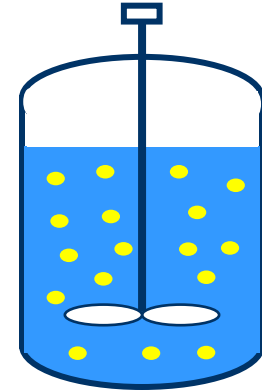
Circa 1960's



- Salts, sugars etc.
- Bovine serum

**Infinite** lifespan

Circa 2000



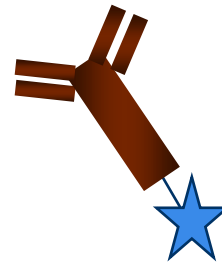
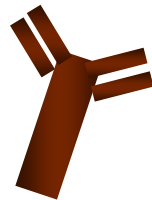
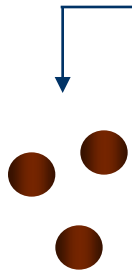
- Salts, sugars etc.
- **NO** serum or animal-derived proteins

**Infinite** lifespan

Host cells **pre-adapted** to desired production  
(**serum-free** suspension) culture conditions

# Biopharmaceutical Drugs Assume a Variety of Different Formats and Modes of Action

All examples shown are produced using cell culture technology



**Replacement  
of Defective/  
Missing  
Proteins**

**Antibodies**

**Targeted  
antibodies**

**Receptor:Fc  
fusion  
proteins**

Coagulation factors  
(for Haemophilia):

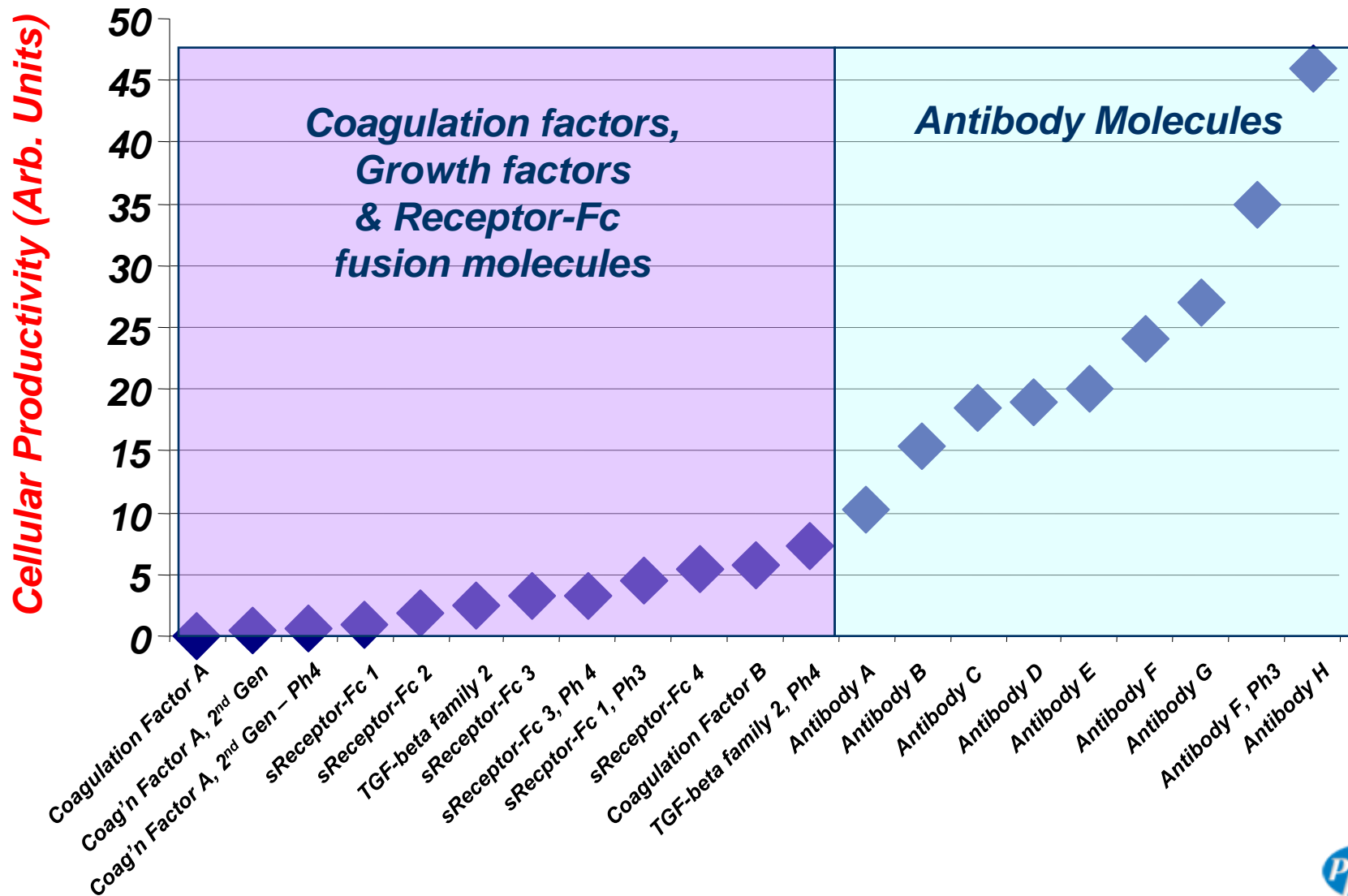
- ReFacto (Pfizer)
- BeneFIX

- Humira (Reumat. Art/Abbott)
- Herceptin (Breast Cancer/Genentech)
- Remicade (Reumat Art/J&J)
- Synagis (Respirat Dis/MedImmune)

- Mylotarg (Leukemia/Pfizer)
- Bexxar (Ab-chemotherapy for non-Hodgkin's lymphoma, Corixa/GSK)

Enbrel (Pfizer)

# Historical Experience: Non-Antibodies and Antibodies





# Antibody Heterogeneity

- Major and minor **isoforms** are observed for all recombinant mAb products
- In most cases, abundance of any one of product-related isoform is <5%
- Much of the characterization effort is focused on minor species
- Some commonly observed modifications of mAbs:

- ***N-Glycosylation (+/-)***

- ***Met oxidation***

- ***Sialic acid (+/-)***

- ***Unpaired Cys***

- ***C-Terminal Lys (+/-)***

- ***Chemical and proteolytic cleavage***

- ***Deamidation***

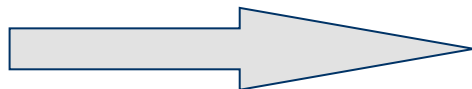
# How do we scale-up a cell culture process and maintain important performance parameters such as protein production & secretion rate?

## Develop process



Bench-scale  
(1-10 L)

## Scale-up process



Must model and pay careful attention to:

(i) Design and positioning of **impellers**, **baffles** and **spargers**

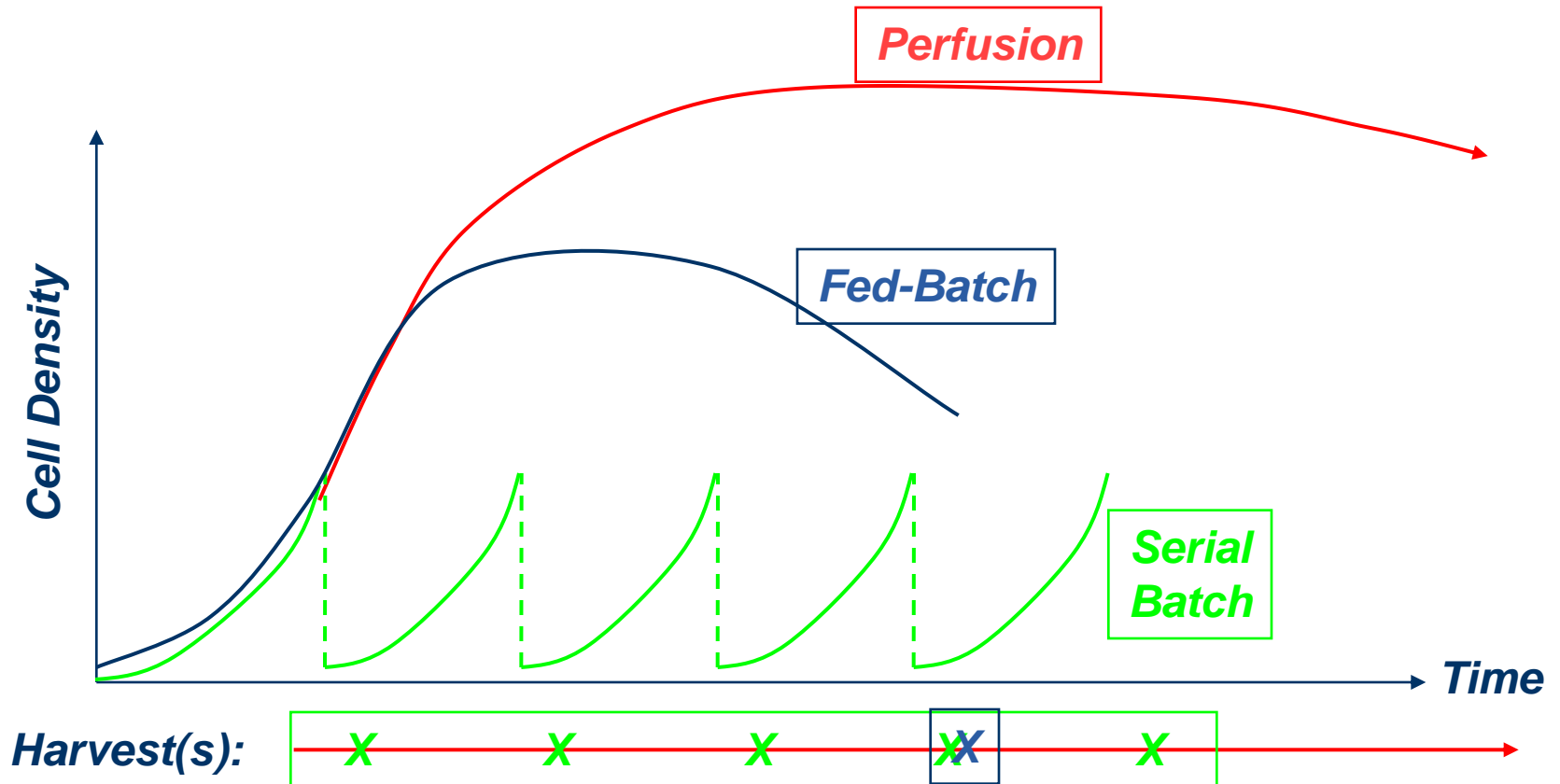
(ii) **Mass transfer** phenomena (mixing times, flow dynamics)

(iii) **Gas transfer** rates (e.g. oxygen transfer rates; OTR) etc., etc.





# Types of Bioreactor Process





# Some Biosafety Considerations

- **Protect** personnel from exposure to biohazardous materials and prevent environmental contamination.
- Provide an environment for **high quality work** while maintaining a **safe workplace**.
- **Compliance** with the necessary regulatory and corporate requirements.



*Thank you*