Level 8 Cell Culture Processing (BIO08045)

Lecture 1 – "Introduction to Cell Culture Processing"

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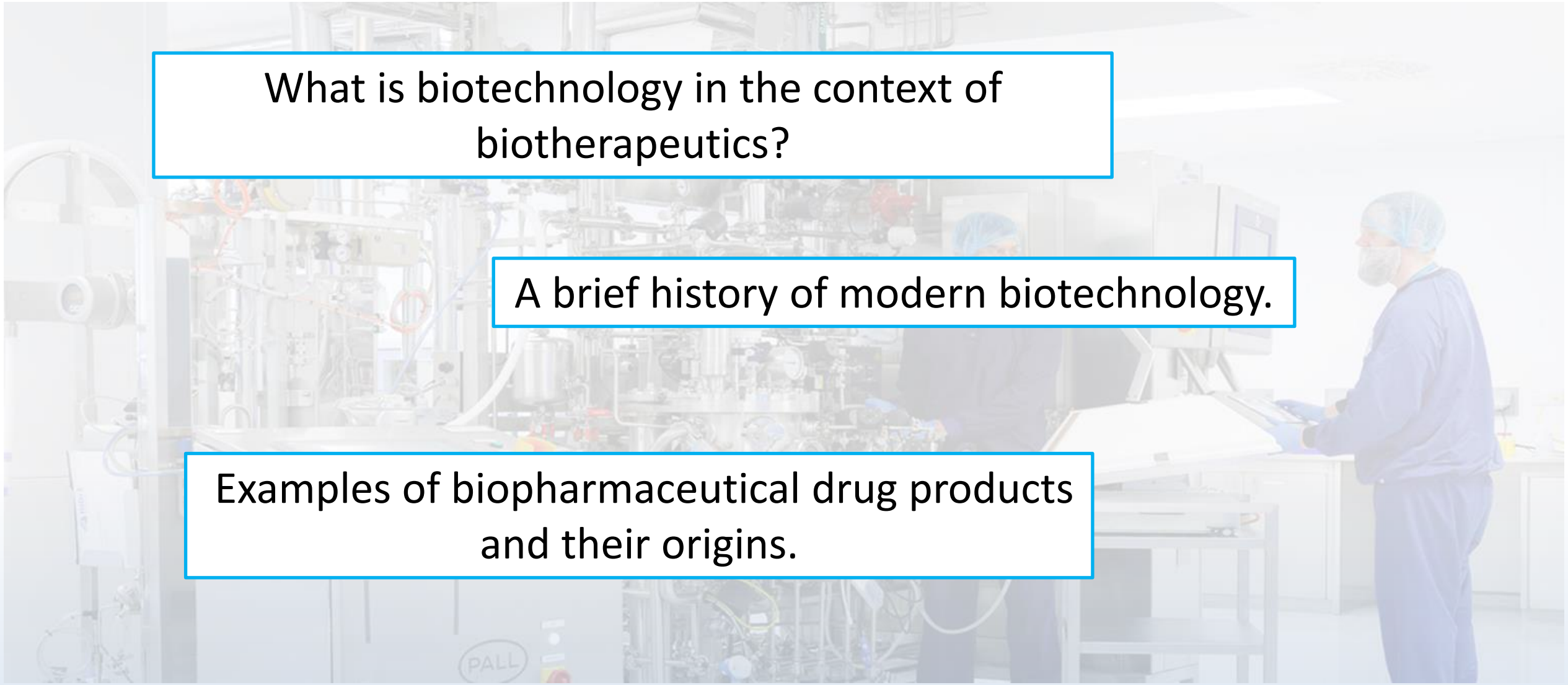
Learning Objectives



What is biotechnology in the context of biotherapeutics?

A brief history of modern biotechnology.

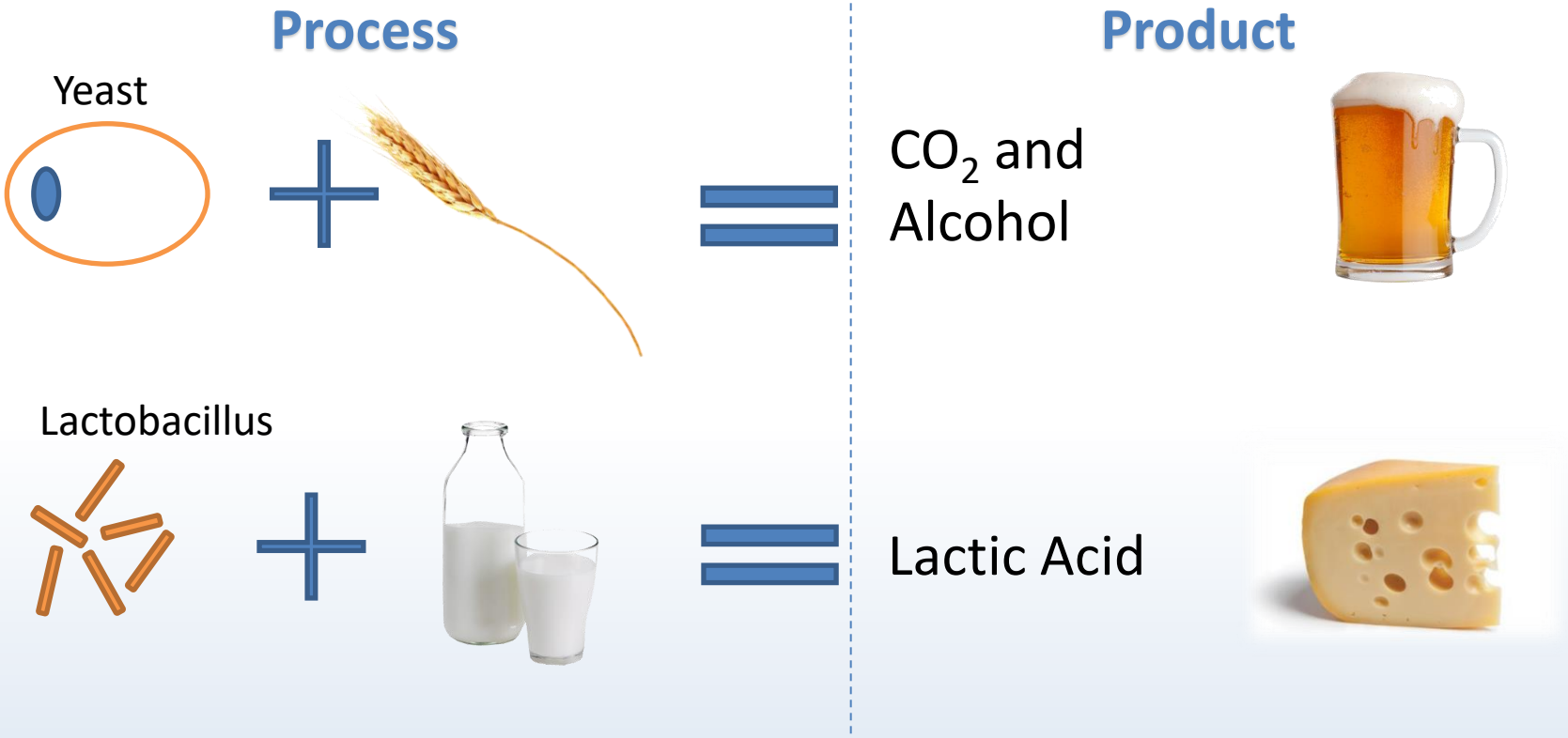
Examples of biopharmaceutical drug products and their origins.

Lecture Topics

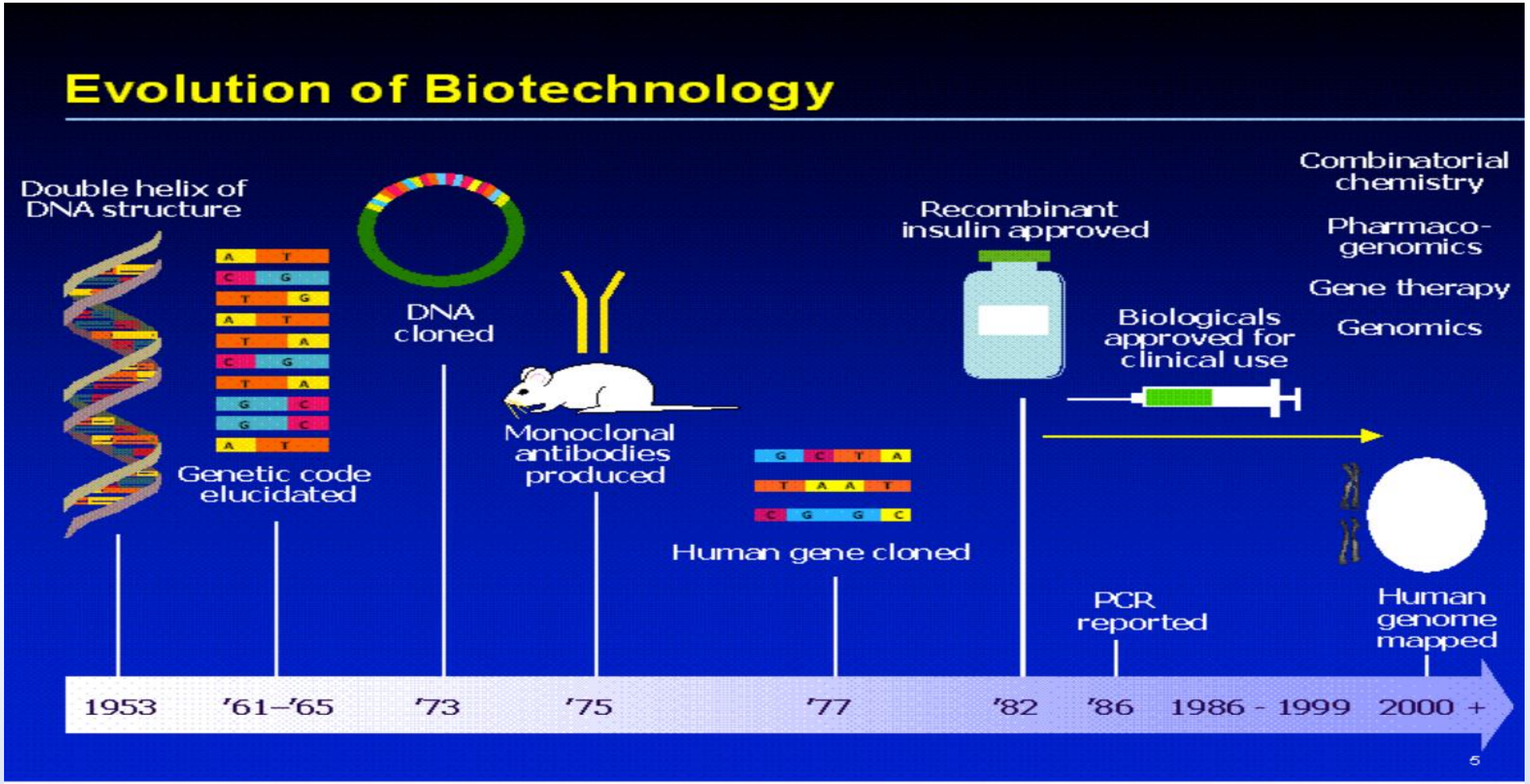
- What is Biotechnology?
- Developing Cell Lines for Bioprocessing.
- Mammalian Cell Lines for Bioprocessing.
- Monoclonal Antibodies.

What is Biotechnology?

- **Biotechnology** is any technological application that uses biological systems, living organisms or derivatives of them, to make or modify products or processes.



Evolution of Modern Biotechnology



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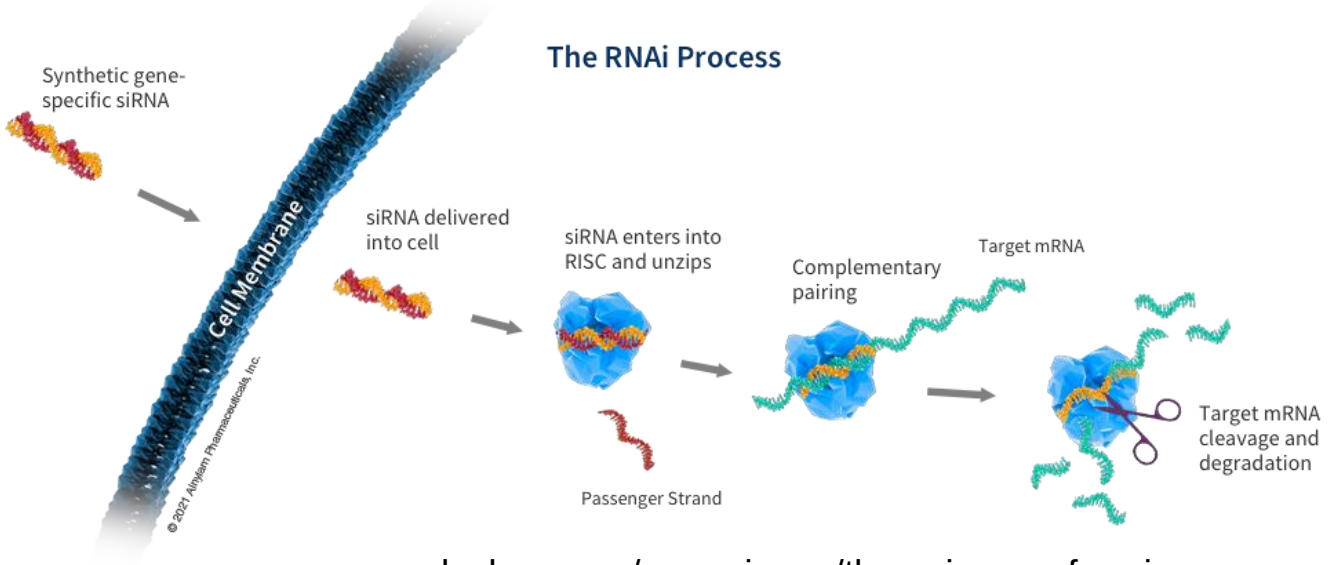

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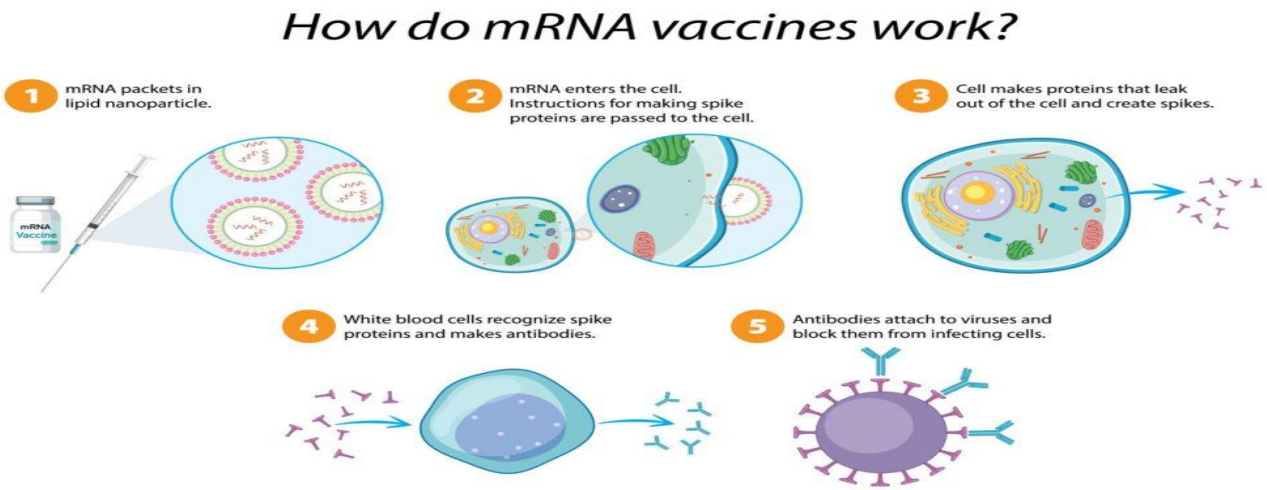

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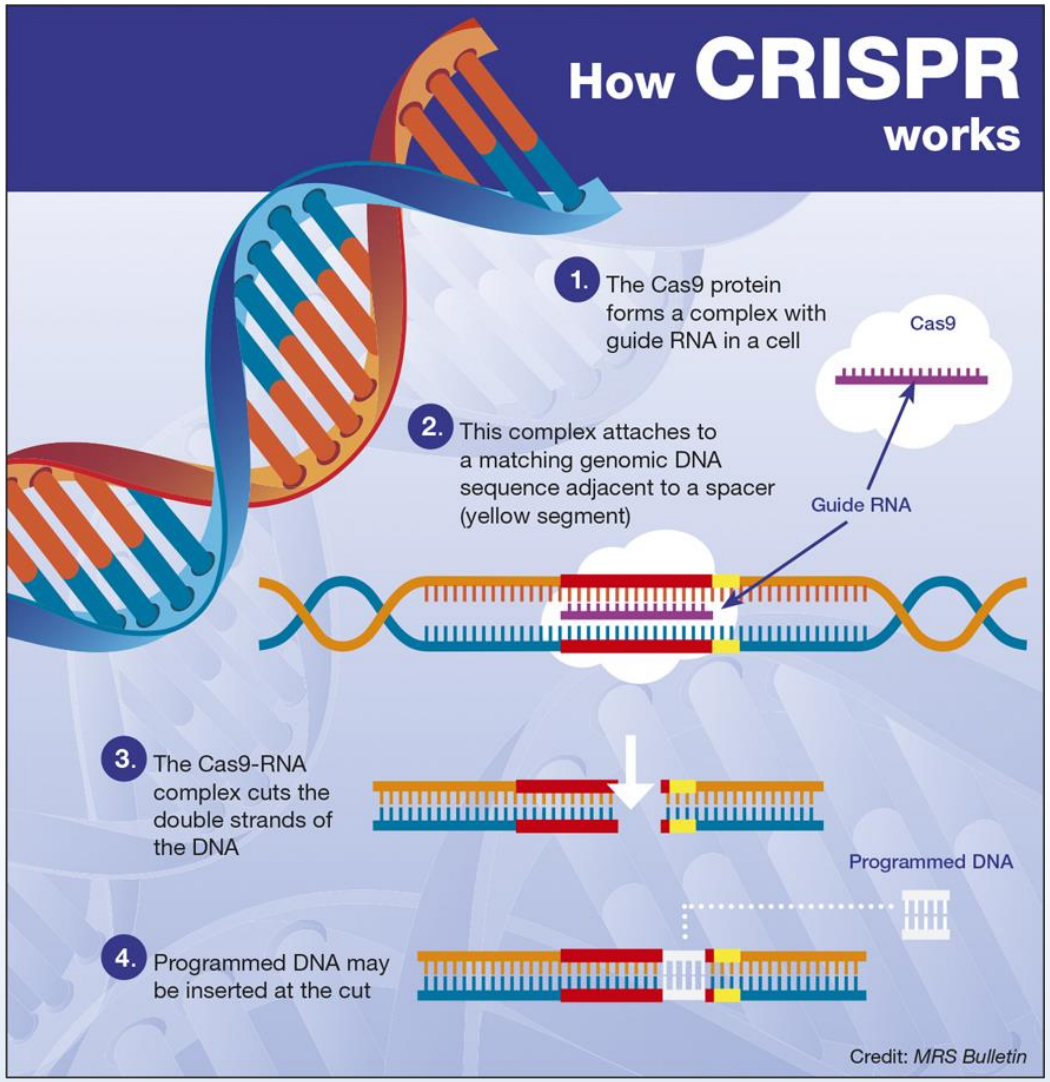


www.cambridge.org/core/journals/mrs-bulletin/news/crispr-implications-for-materials-science

2022 Sales

	Product	Active Substance	Company	Indication	Sales 2022 ($bn)
1	Comirnaty	Tozinameran	Pfizer	Covid Vaccine	55.9
2	Humira	Adalimumab	Abbvie	Arthritis, Crohn's Disease	21.2
3	Keytruda	Pembrolizumab	Merck	Oncology	20.9
4	Paxlovid	Nirmatrelvir	Pfizer	COVID-19	18.9
5	Spikevax	Elasomeran	Moderna	COVID-19 Vaccine	18.4
6	Eliquis	Apixaban	BMS	Blood clots, stroke	18.2
7	Dupixent	Dupilumab	Sanofi	Allergic Disease	17.4
8	Eylea	Aflibercept	Regeneron	Oncology	12.7
9	Biktarvy	Bictegravir/emtricitabine/ tenofovir alafenamide	Gilead	HIV/AIDS	10.3
10	Revlimid	Lenalidomide	Celgene	Oncology	10.0

= Manufactured in Ireland

Biologic

Small Molecule

Reference: Drug Discovery & Development: The 50 best-selling pharmaceuticals of 2022

2028 Predicted Sales

	Product	Active Substance	Company	Indication	Predicted Sales 2028 ($bn)
1	Keytruda	Pembrolizumab	Merck	Oncology	31.1
2	Opdivo	Nivolumab	BMS	Oncology	14.7
3	Dupixent	Dupilumab	Sanofi	Allergic Disease	14.4
4	Darzalex	Daratumumab	J&J	Oncology	14.1
5	Ozempic	Semaglutide	Novo Nordisk	Diabetes / Obesity	13.8
6	Biktarvy	Bictegravir/emtricitabine/ tenofovir alafenamide	Gilead	HIV/AIDS	11.6
7	Skyrizi	Risankizumab	AbbVie	Psoriasis/Crohn's Disease	11.3
8	Trikafta/ Kaftrio	Elexacafotr/tezacaftor/ ivacaftor	Vertex	Cystic Fibrosis	10.3
9	Comirnaty	Tozinameran	Pfizer	COVID-19 Vaccine	9.7
10	Imbruvica	Ibrutinib	J&J and AbbVie	Oncology	9.7

= Manufactured in Ireland

Biologic

Small Molecule

Reference: Evaluate Pharma World Preview 2022 Outlook to 2028: Patents and Pricing

Development of an Industrial Bioprocess

Pre-Manufacturing

Gene for therapeutic product

Gene integration

Selection

Candidate clones

Formulation of media

Optimisation of media

Characterization at small scale

Master cell selected

Choice of Reactor

Manufacturing

Master cell

Master cell bank (MCB)

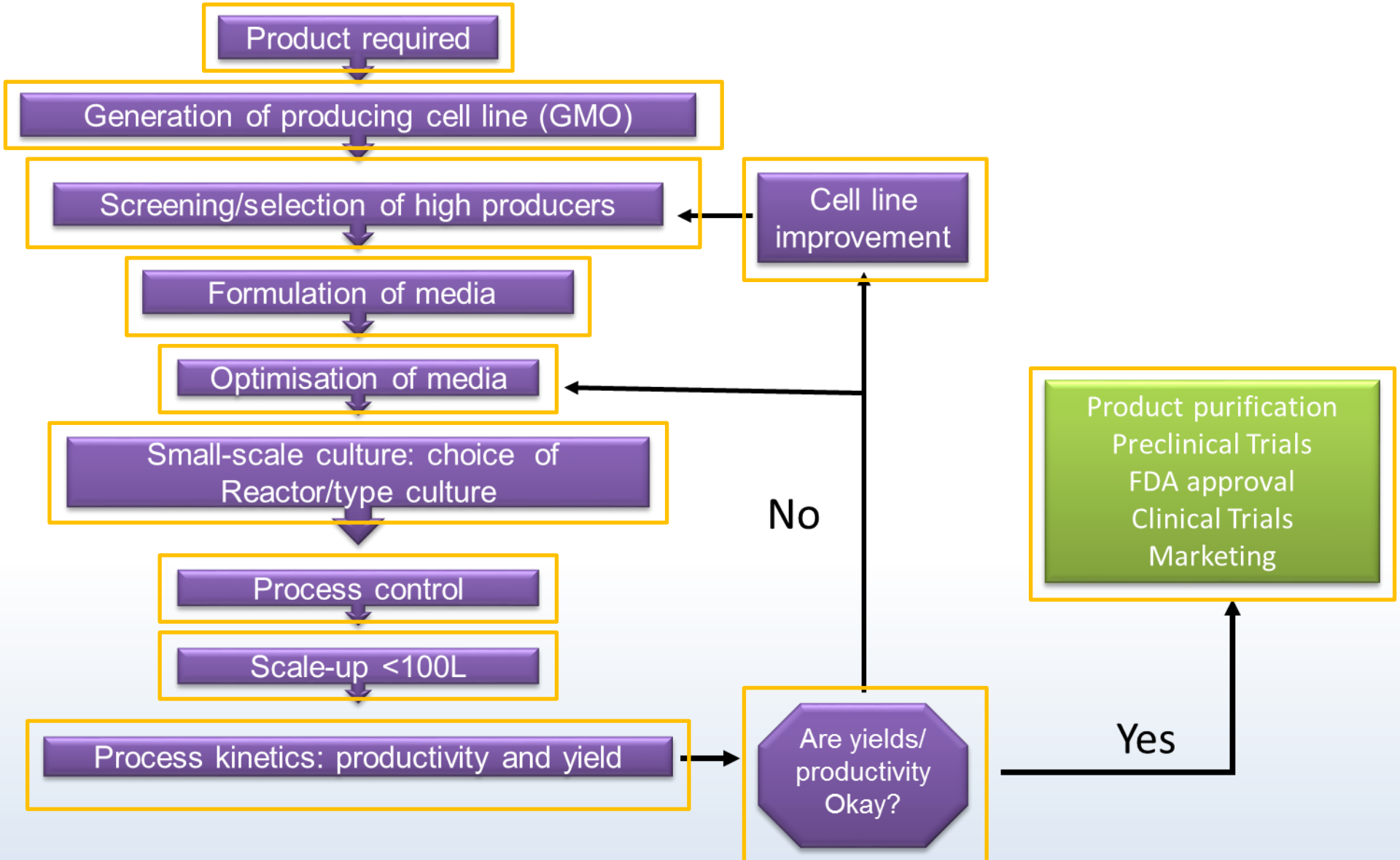
X 200

Working cell bank (WCB)

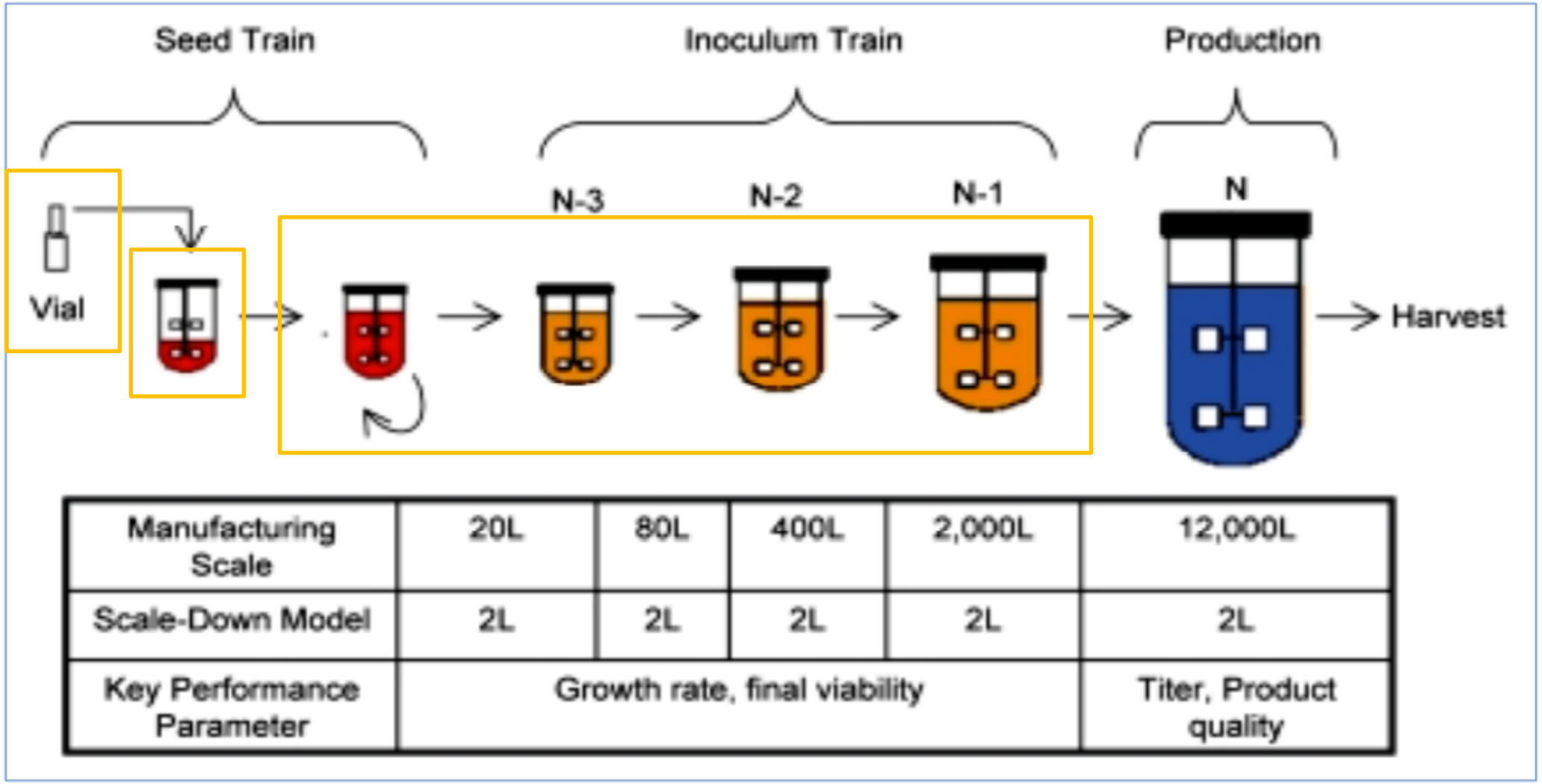
X 500

Large scale production

Development of an Industrial Bioprocess



From Cryovial to Production



Manufacturing Scale	20L	80L	400L	2,000L	12,000L
Scale-Down Model	2L	2L	2L	2L	2L
Key Performance Parameter	Growth rate, final viability				Titer, Product quality

Cell Culture Processing

Tissue Culture Flasks

Spinner Flask

Disposable Bioreactor

Cell Stock Vial

Cell Culture Scale Up

Shake Flasks

Bench top bioreactor

Stainless steel Production Bioreactor

Biopharmaceuticals

- Biopharmaceuticals are medicinal compounds produced using biotechnology: produced in microbial, plant and mammalian cells.
  - They involve incorporation of foreign DNA into an organism's genetic material to generate a genetically modified organism (GMO) producing elevated amounts of a therapeutic protein.

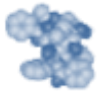
- Majority of biopharmaceuticals are proteins or glycoproteins

  (i.e. proteins with sugars) so referred to as **'Therapeutic proteins'.**

- Companies also use the term **"Recombinant Products"** as products are produced using recombinant DNA technology.

Biologics versus Small Molecule Drugs

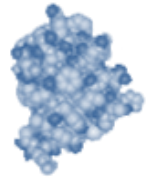
Aspirin
(180.2 Da)



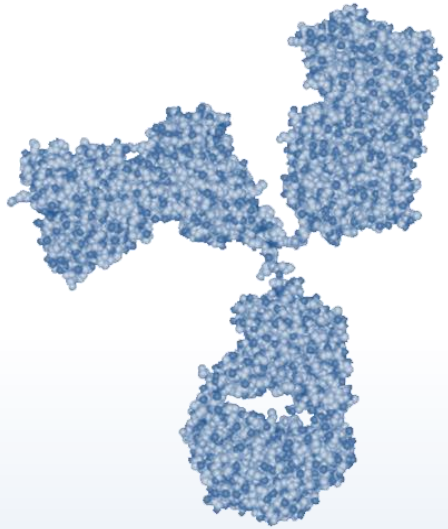
Atorvastatin
(558.6 Da)

Ibuprofen
(206.3 Da)

Small Molecules	Typical Attributes	Biologics
Oral	Administration route	Parenteral
Higher	Stability	Lower
Higher	Side effects	Lower
Systemic	Mode of action	Specific
Easier	Manufacturing	More Difficult
Lower	Immunogenicity potential	Higher
Lower	Half-life	Higher



Insulin
(5808 Da)



Infliximab
(~150,000 Da)

So Why Biologics?

- Small molecule drugs often **lack specificity** which can cause off-target interactions and side-effects.
- Biologics can be engineered to **act with extreme precision** resulting in less side effects and lower toxicity.
- Biologics **can target complex molecular processes** that small molecules cannot – can treat diseases such as cancer or autoimmune diseases.

Small molecules

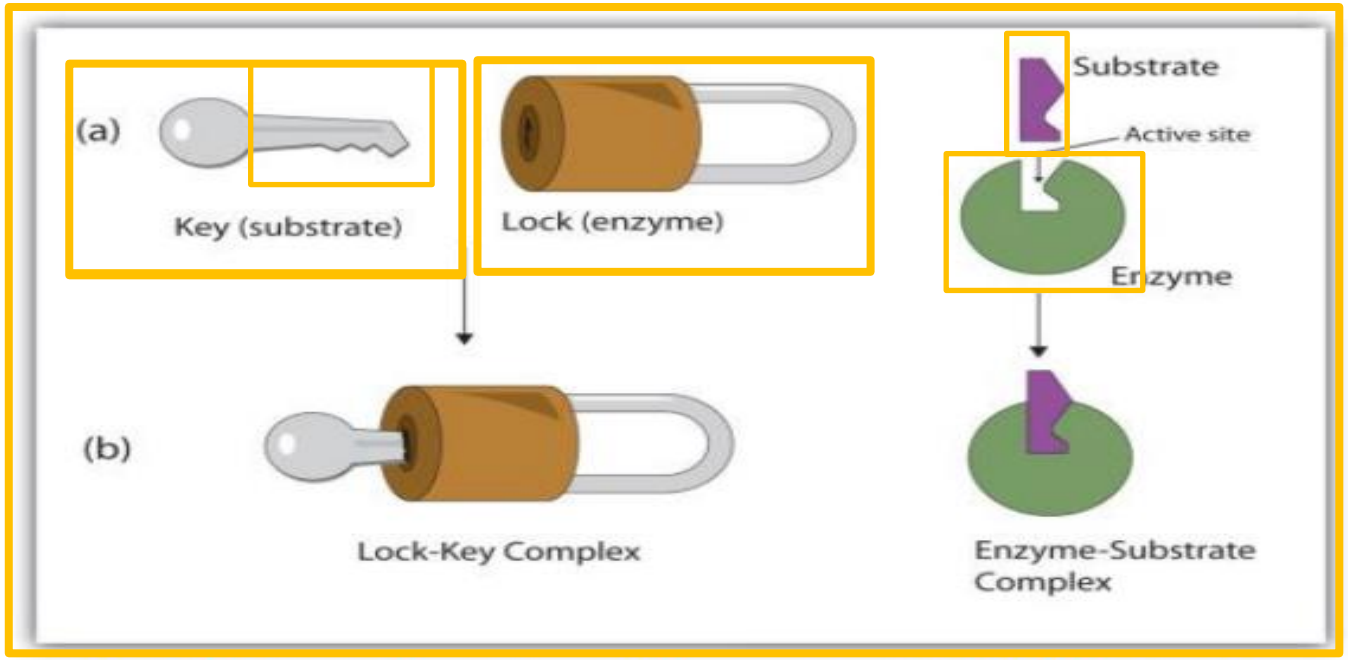
IMAGE: freepik.com

VS.

Biologics

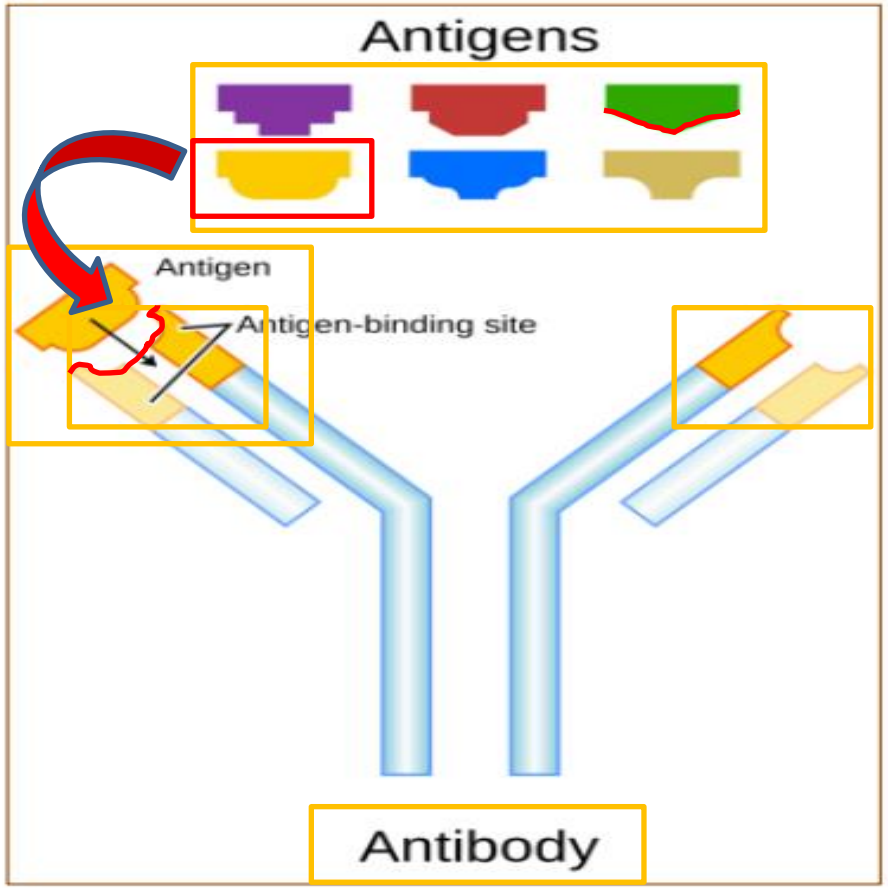
IMAGE: www.gmapswidget.com

Why Biologics? Lock and Key Model



https://biochemchica.files.wordpress.com/2013/03/lock1.jpg

http://psych.hanover.edu/classes/neuropsychology/WebNotes/Images/lockkey.gif



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Lecture Topics

- What is Biotechnology?
- Developing Cell Lines for Bioprocessing.
- Mammalian Cell Lines for Bioprocessing.
- Monoclonal Antibodies.

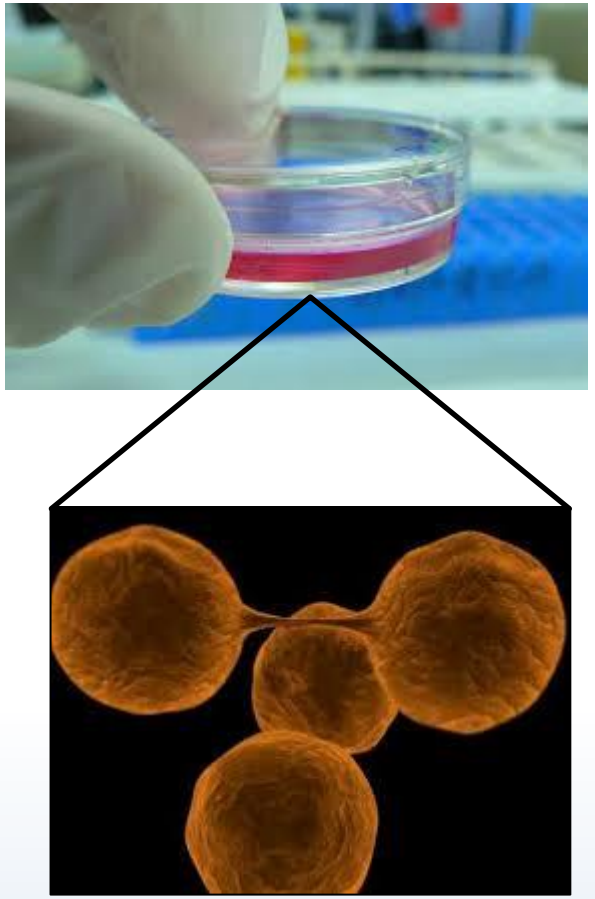
Cell Line Definition

Cell line = a culture developed from a single cell and therefore consisting of cells with a uniform genetic make-up.

Primary cell culture = derived directly from living tissue – begins with many cells ∴ more variation.

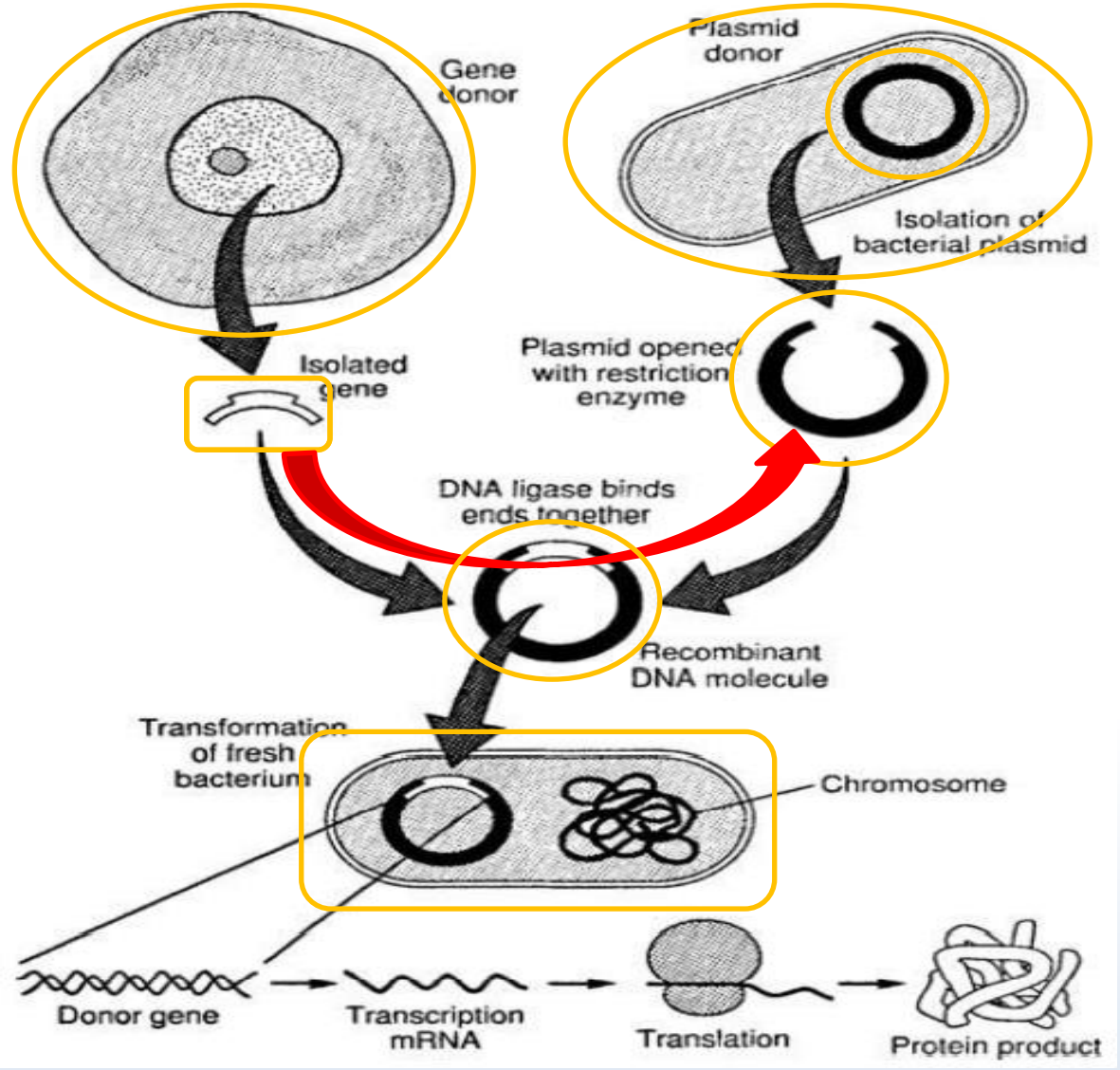
Cell lines are often immortalised and grow continuously in culture.

Immortalised cells may be cancerous, arise spontaneously or be transformed.

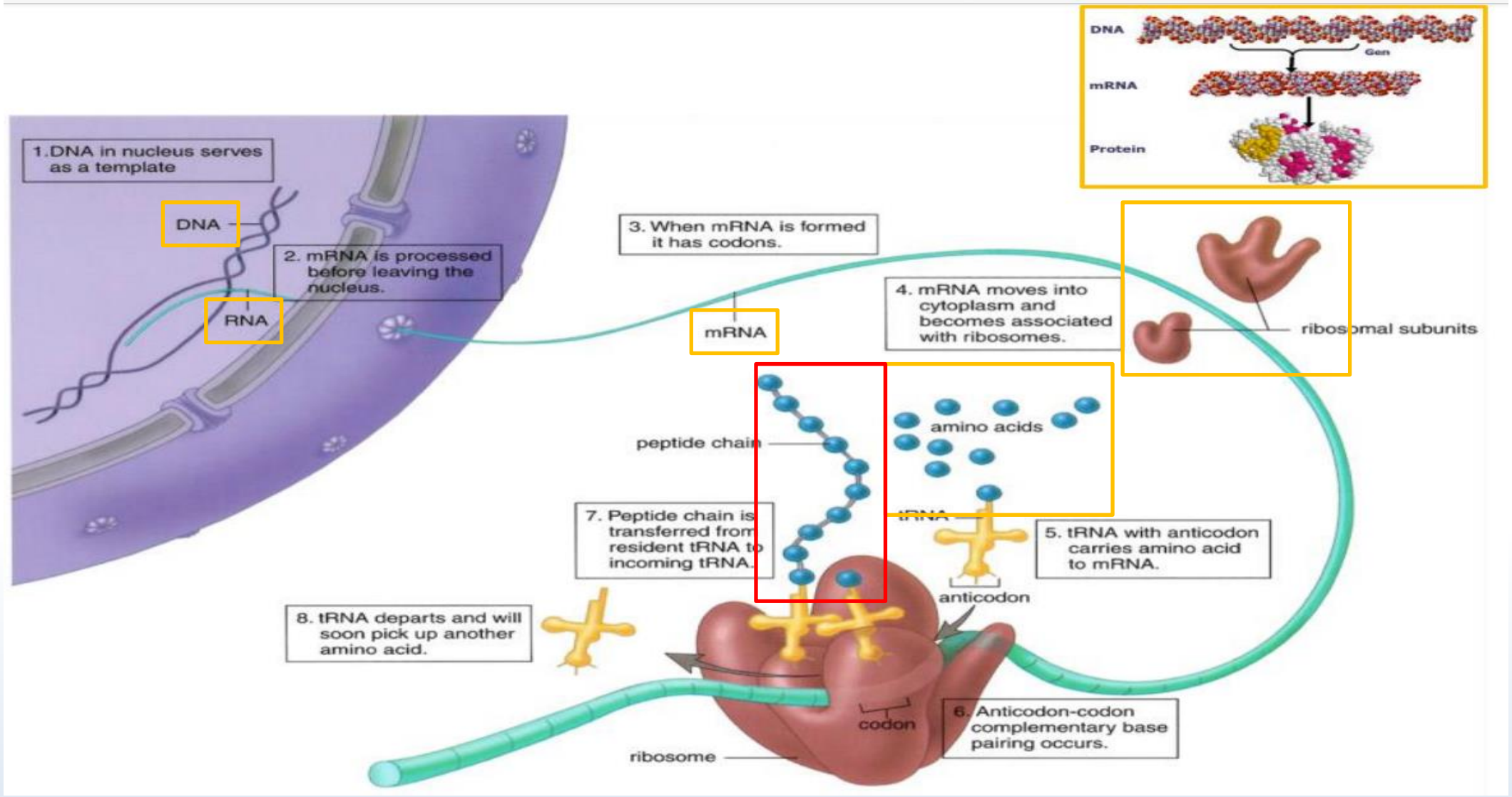


Importance of Genetic Engineering

- Plasmid is removed from bacterium and DNA is removed from cell nucleus.
- Restriction enzymes are used to cut plasmid open and remove desired gene from DNA.
- Biochemically bind desired gene into open plasmid.
- Genetically modified plasmid is reintroduced into bacterium.
- Recombinant DNA reproduces and producing cells are selected for growth.

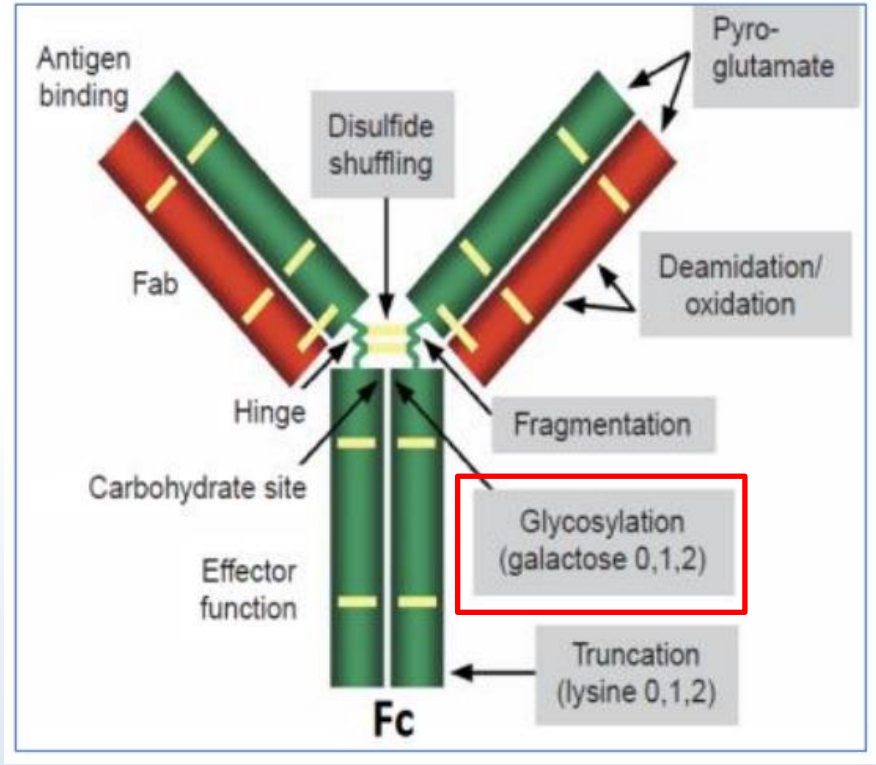


Summary of Protein Synthesis Process



Proteins: Post-translational modifications

- Chemical modification of a protein after translation (ribosomal synthesis).
- Proteins can undergo >100 different types of PTM* which can influence structural aspects and/or the functional roles of the protein.
- Modulates molecular interactions, protein localisation and stability.
- Involves:
  - Covalent addition of functional groups.
  - Proteolytic cleavage of regulatory subunits.
  - Degradation of entire proteins.



Proteins: Post-translational modifications

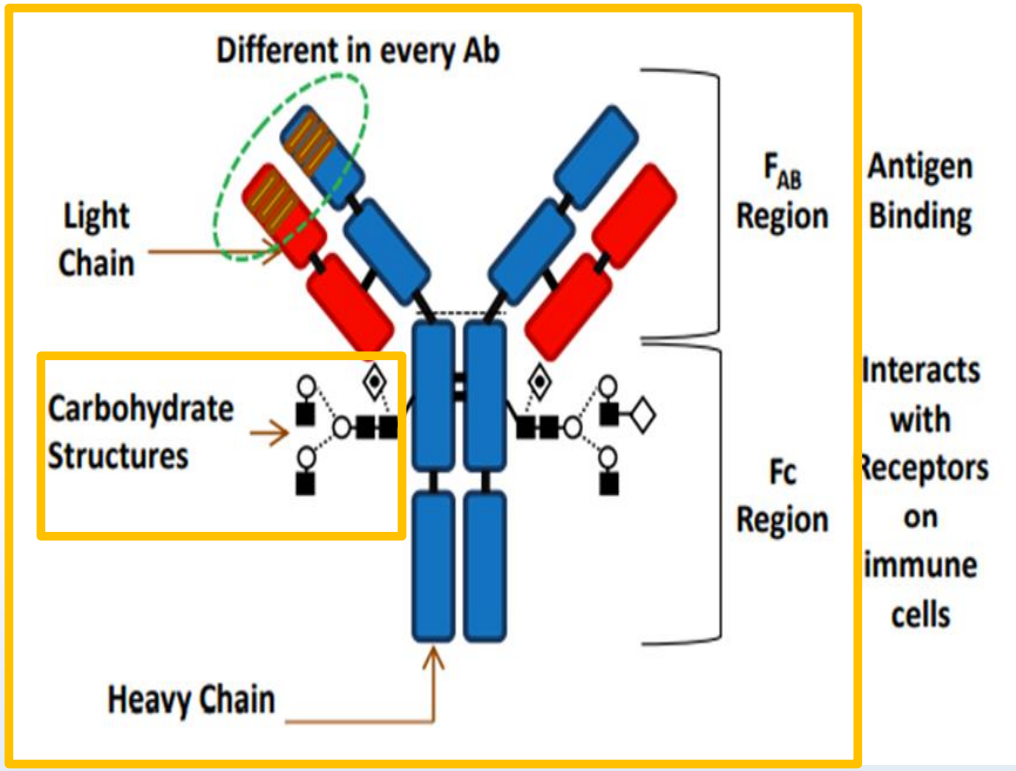
Important PTMs for biopharmaceuticals:

Glycolysation
Disulfide bond formation
Proteolytic processing
Carboxylation
Hydroxylation
Sulfation
Amidation

Most common form of PTM on biopharmaceuticals

Seen on many biopharmaceuticals.

Only seen on a small number of biopharmaceuticals.



Choosing a Cell Type

For Simple Proteins

- Simpler Fermentation
- Scalable
- Lower Cost of Goods

- Post-translational Modifications that are not human-like

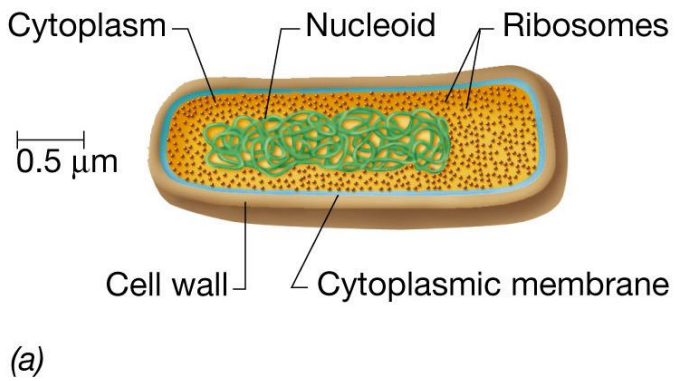
VS

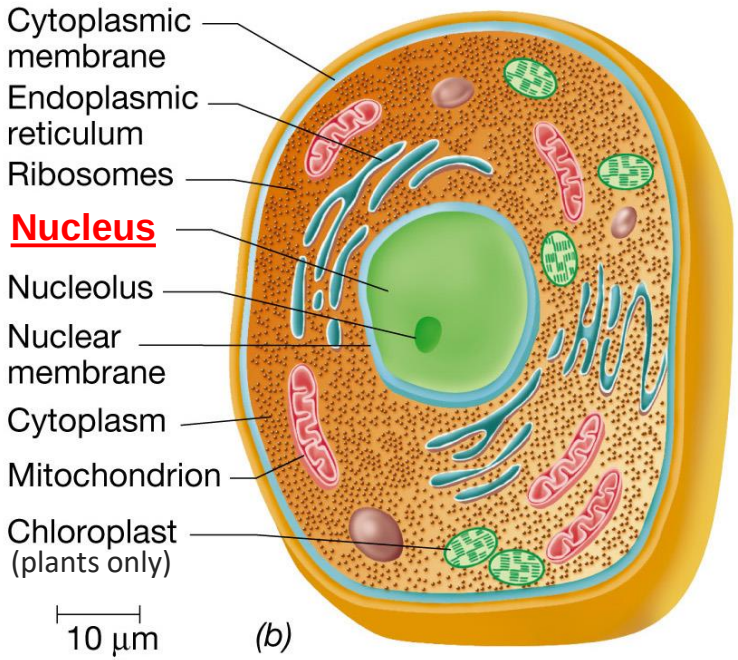
For Complex Proteins

- Complex Culture
- Less Scalable
- High Cost of Goods

- Human-like PTMs

Prokaryotic Vs Eukaryotic





Prokaryotic cell e.g. *E. coli*

Eukaryotic cell e.g. Mammalian Cell

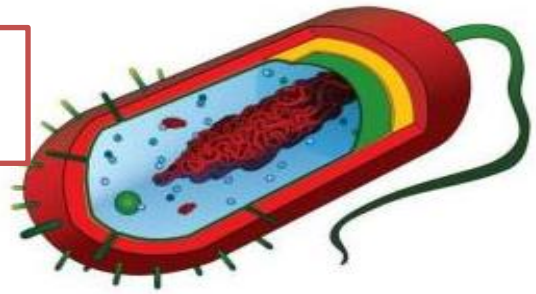
Protein synthesis begins in the cell's **nucleus**
(or the nucleoid for prokaryotic cells)

Prokaryotic Vs Eukaryotic

Prokaryote
Small & simple
No nucleus
No membrane-bound organelles
Intracellular expression
Doubling time approx. 20mins
No complex PTMs
May have cell wall
Easier to grow and shear resistant

Eukaryote
Large & complex
Nucleus
Membrane-bound organelles
Extracellular expression
Doubling time approx. 24hours
Complex PTMs
Usually no cell wall - fragile
More difficult to grow and shear/osmotic stress sensitive

Prokaryote e.g. E. coli



Eukaryote e.g. CHO

Examples of Industrial Cell Lines

Human

-HEK293 (Human Embryonic Kidney)

- Per C.6

Hamster

-CHO (Chinese Hamster Ovary)

- BHK (Baby Hamster Kidney)

Mouse

-NS0 (Non-secreting Myeloma)

- SP2/0 (Non-secreting Hybridoma

Monkey

- Vero (Green Monkey)

Yeast

- S. cerevisae

- P. pastoris

Bacteria

- E. coli

Insect

- Sf 9/21 (Fall Army Worm)

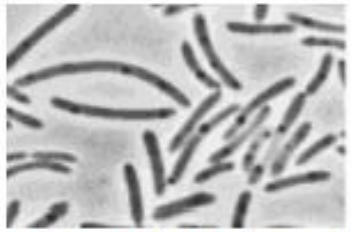
Plant

-Carrot

Bacteria As Host Cells

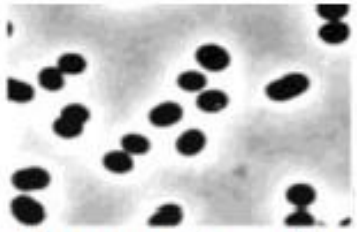
ADVANTAGES
Simple system, easily manipulated.
Short generation times (i.e. grow quickly).
Large yields at low cost.
Some can secrete protein into the culture medium (e.g. Bacillus subtilis).

DISADVANTAGES
High level of expression but may not fold properly.
Protein not secreted can form insoluble bodies.
Cannot modify protein i.e. glycosylation (addition of sugar groups) so protein may be biologically inactive.



Cylindrical (rod / Bacillus)

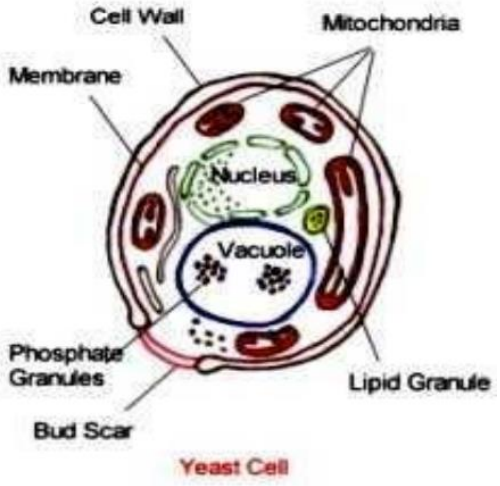
Spiral



Spherical (coccus / cocci)

Yeast As Host Cells

- Yeast cells are single cell eukaryotes which are a type of fungi.
- They are small in size, ~2µm and have a cell wall, membrane and organelles.
- Used in brewing, baking and biopharmaceutical production.



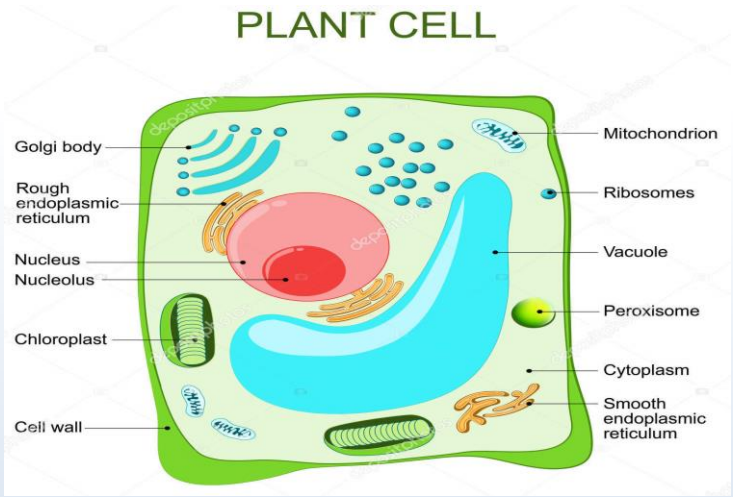
ADVANTAGES
Well developed genetic tools.
Simple eukaryote & resemble mammalian cells.
Grow as quickly & cheaply as bacteria.

DISADVANTAGES
Protein yields can be low.
Foreign proteins can be toxic to yeast.
Can get hyperglycosylation (multiple sugars added).

Plant Cells As Host Cells

ADVANTAGES
Production of pharmaceuticals (e.g. taxol), biodegradable plastics.
Production of vaccines : food based vaccines.
Alter nutritional content of plants, e.g. decrease fat content, increase vitamin.
Introduction of herbicide and pest resistance genes.

DISADVANTAGES
Procedure for getting DNA into plants cells very complex.
Plants cell in culture difficult to maintain and grow.
Not possible to do large-scale culture of plant cells.



Lecture Topics

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Why Mammalian Cells?

	Mammalian Cells	Bacteria/Yeast
Correct protein folding	✓	✗
Protein secretion	✓	✗
PTMs	✓	✗
Large, complex protein production	✓	✗
Good regulatory track record	✓	✓
Fast growth and cheap media	✗	✓
Simple purification protocols	✗	✓
Animal component free media	✗	✓
Simple to characterize	✗	✓

Criteria for Cell Line Selection

Before manufacture ever begins, some questions need to be asked;

Product type?	• Simple recombinant protein • Monoclonal antibody • Fusion protein
Genetic Stability?	• Stability of transfection • Long-term genetic stability
Growth and productivity in large scale culture?	• Ease of selection of high producers • Adaptation to protein-free suspension culture • Apoptosis, proliferation rate, max cell number
Safety issues?	• Potential for endogenous viral contamination • Use of animal products in media (potential contamination)

Common Examples of Industrial Cell Lines

Abbreviation	Full Name	Origin	History	Companies
CHO	Chinese Hamster Ovary	Fibroblast from hamster ovary	Used for mutation research in 50s	Genentech, BMS, Lilly, Janssen, Pfizer
NS0	Mouse non-secreting myeloma	Mouse lymphocyte	Mouse lymphocyte	Alexion, Biogen
Per.C6	Human	Human retinal cell	Fully human PTMs	Crucell, DSM, Merck

How are Cell Productivity Improvements Achieved?

- Generation of recombinant cell lines with high specific productivities
- Formulation of media to support high density cell cultivation
- Understanding of bioprocess conditions for cell cultivation
- Sustained viability of cell lines in high-density batch and fed-batch cultures

Parameter	1986	Today
Protein Pg/cell/day	10	90

Parameter	1986	Today
Cell Density	$2x10^6$ cells/mL	$>20x10^6$ cells/mL

Parameter	1986	Today
Process duration	7 days	21 days

Parameter	1986	Today
Titres	50 mg/L	>5 g/L

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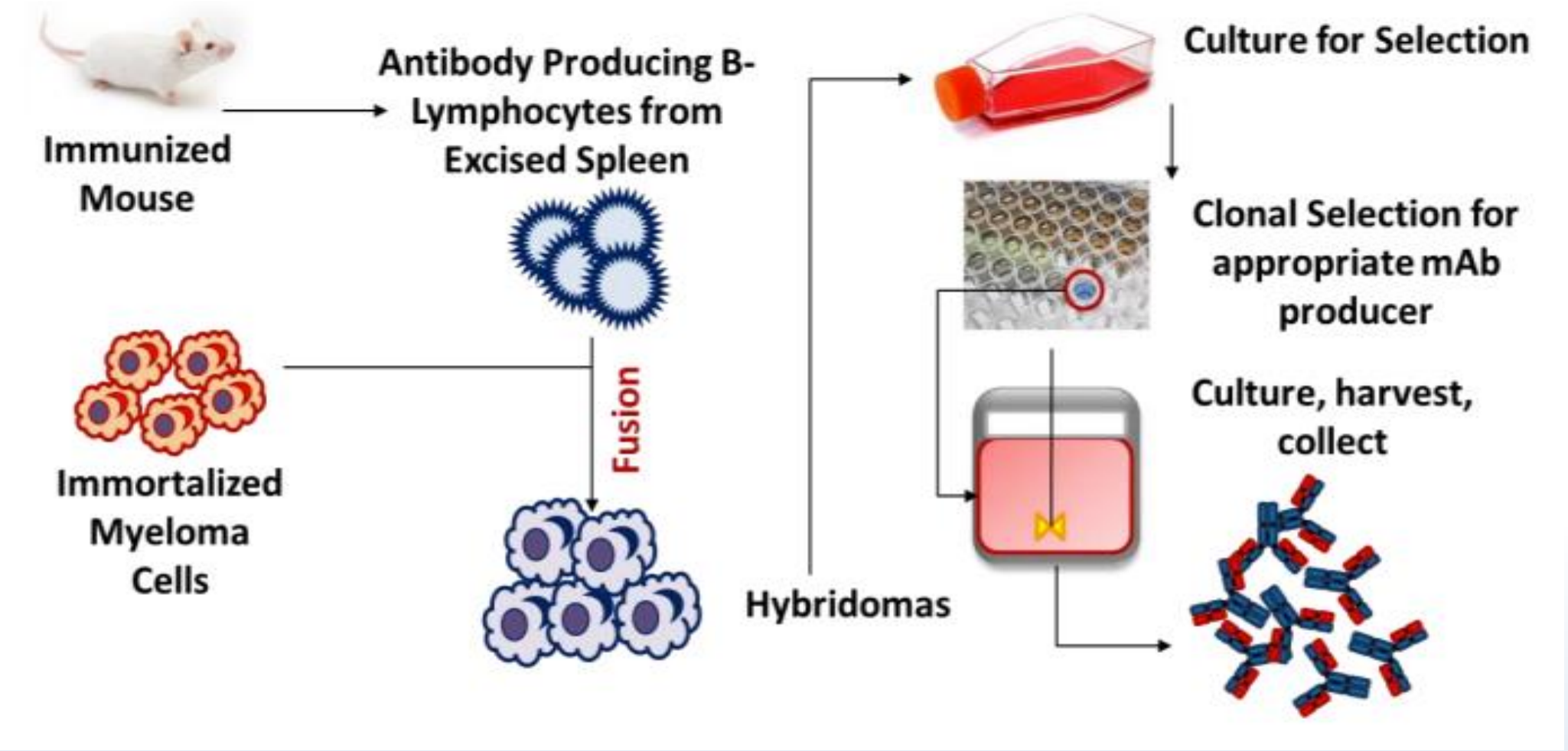
Monoclonal antibodies (MAb)

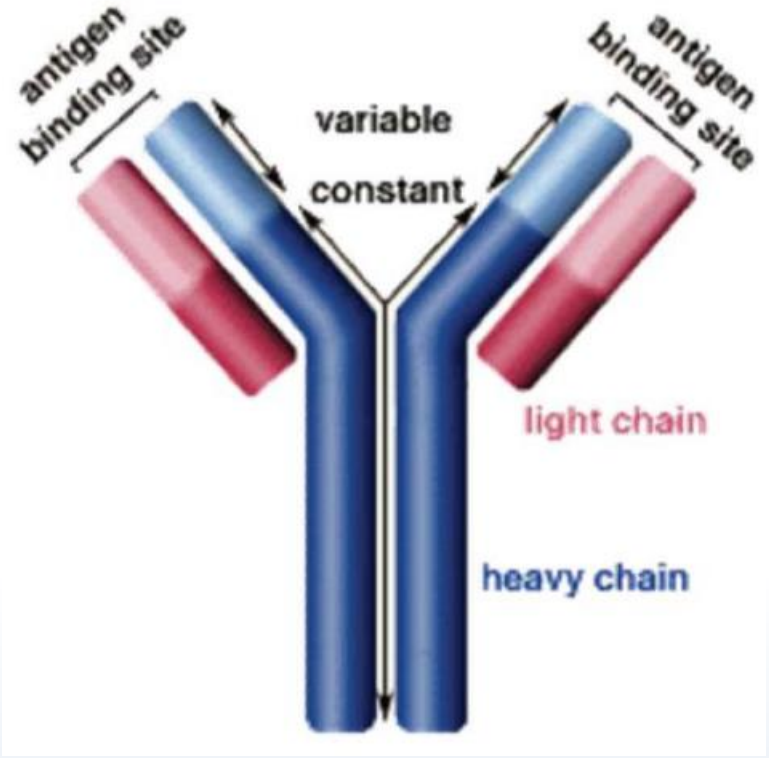
- The first monoclonal antibody in Ireland was made at Wyeth (Pfizer) Biopharma facility in 2004; this antibody was **anti Tumour Necrosis Factor (anti-TNF)**
- TNF is a protein released by immune system if it senses damage to cells in body
- In diseases, sometimes the body is fooled into believing cells are being damaged and continually releases TNF (problems caused by excess TNF production are rheumatoid arthritis, Crohn's disease, Alzheimer's disease and diabetes)
- Antibody (anti TNF) binds TNF and stops pain and swelling
- Examples of Mab's:
  - **Herceptin** produced in CHO cells for breast cancer treatment
  - **Remicade** produced in myeloma cells to treat Crohn's and arthritis
  - **Enbrel** produced in CHO cells to treat rheumatoid arthritis

Origin of Monoclonal Antibodies

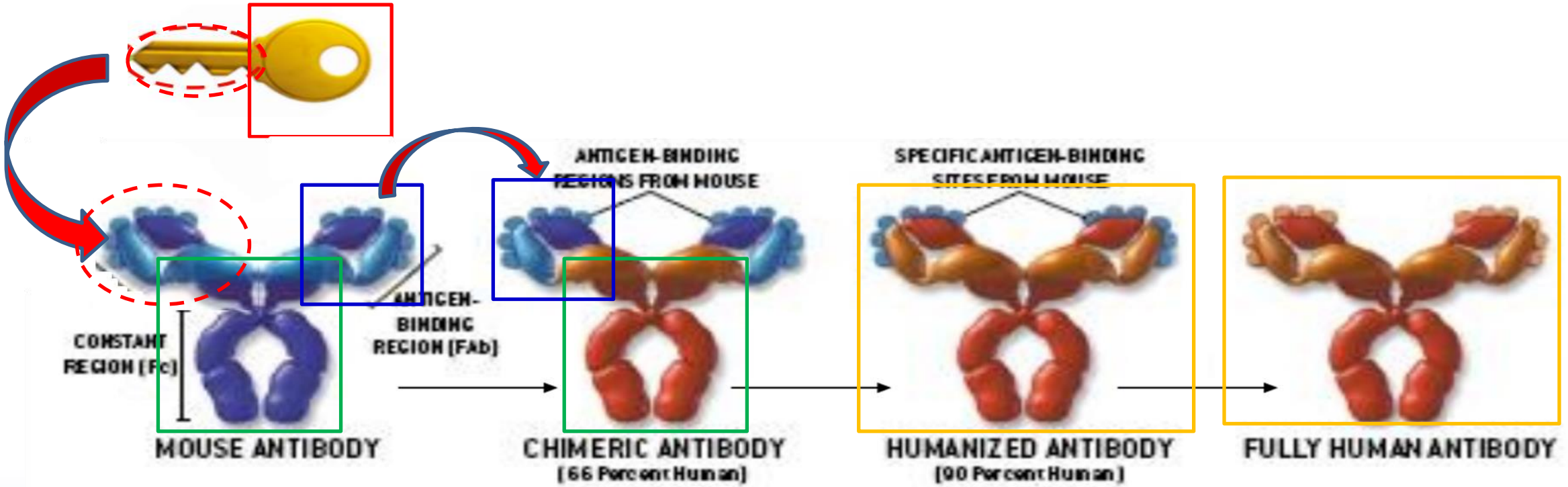


Antibodies: Structure

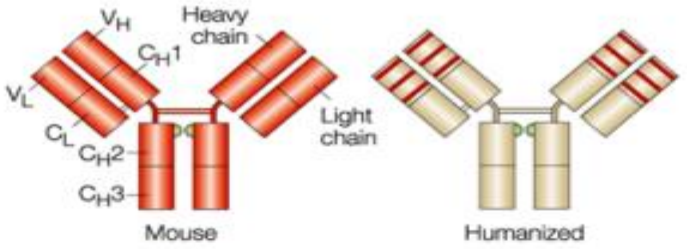
- Antibodies or immunoglobulins: 2 polypeptide chains (heavy and light chains) held together by disulfide bonds
- Each chain contains a constant and variable region
- End of each chain is specific for a particular antigen: determined by sequence of variable region: antigen binding site (ABS)
- Sequence of ABS is referred to as complementary determining regions (CDRs) changes with to different antibodies



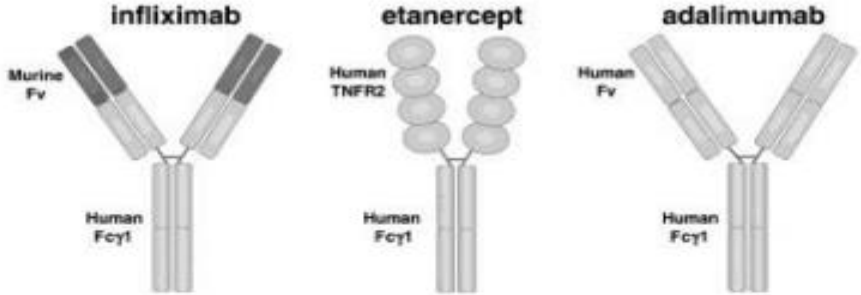
The Evolution of Antibodies As Drugs

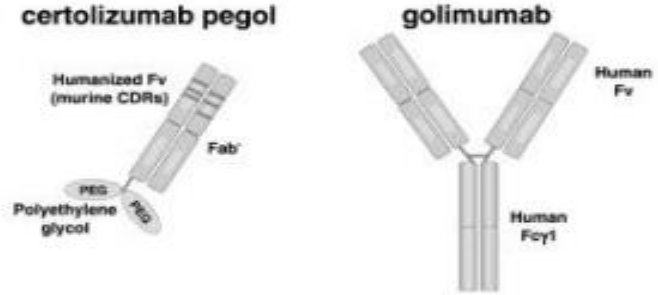


The Evolution of Antibodies As Drugs



Chimeric Antibodies- combination of one protein from different species. E.g. -mouse and human protein





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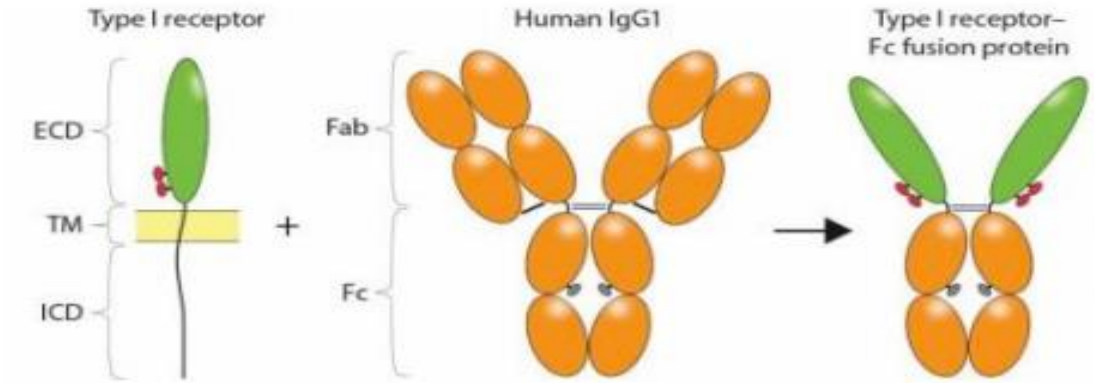

Fusion Proteins-combination of two different proteins to result in antibody with properties of both. E.g. Enbrel, Eylea, Zaltrap

Summary

- Biotechnology for the biopharma industry is about production of biologically active molecules, predominantly proteins, for use as therapeutics or vaccines.
  - Can be produced in bacteria, yeast, plant or animal cells.
  - For human use, mammalian cell lines are preferred as they post-translationally modify proteins similar to human proteins.
- Relies on the use of genetically engineered cell lines designed to produce large amounts of the target protein.
- MAbs represent one of the major types of protein produced as a biotherapeutic.
- Originated in mouse systems and now humanised for improved compatibility with the human recipient.

Questions?

Sample Questions

- Glycosylation is a critical post-translational modification for many biotherapeutic proteins - explain why?
- Originally, monoclonal antibodies were produced in mice. Why are these no longer used and what steps have been taken to overcome their limitations?
- For biomanufacturing purposes, what types of cells may be used as “cell factories”? Discuss their relative pros and cons.
- Which cell types are generally preferred for production of products for use in humans and why?