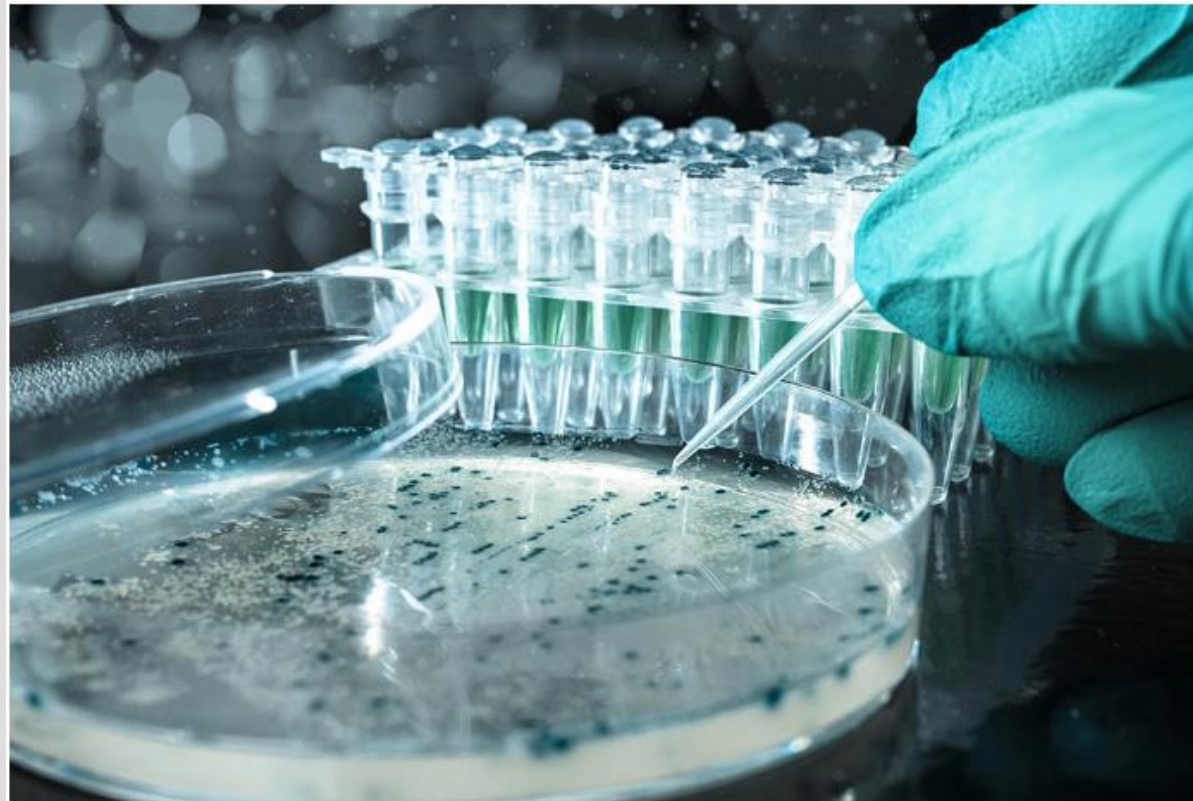


INDUSTRIAL MAMMALIAN CELL LINE DEVELOPMENT BASED ON QUALITY BY DESIGN(QBD) APPROACH.



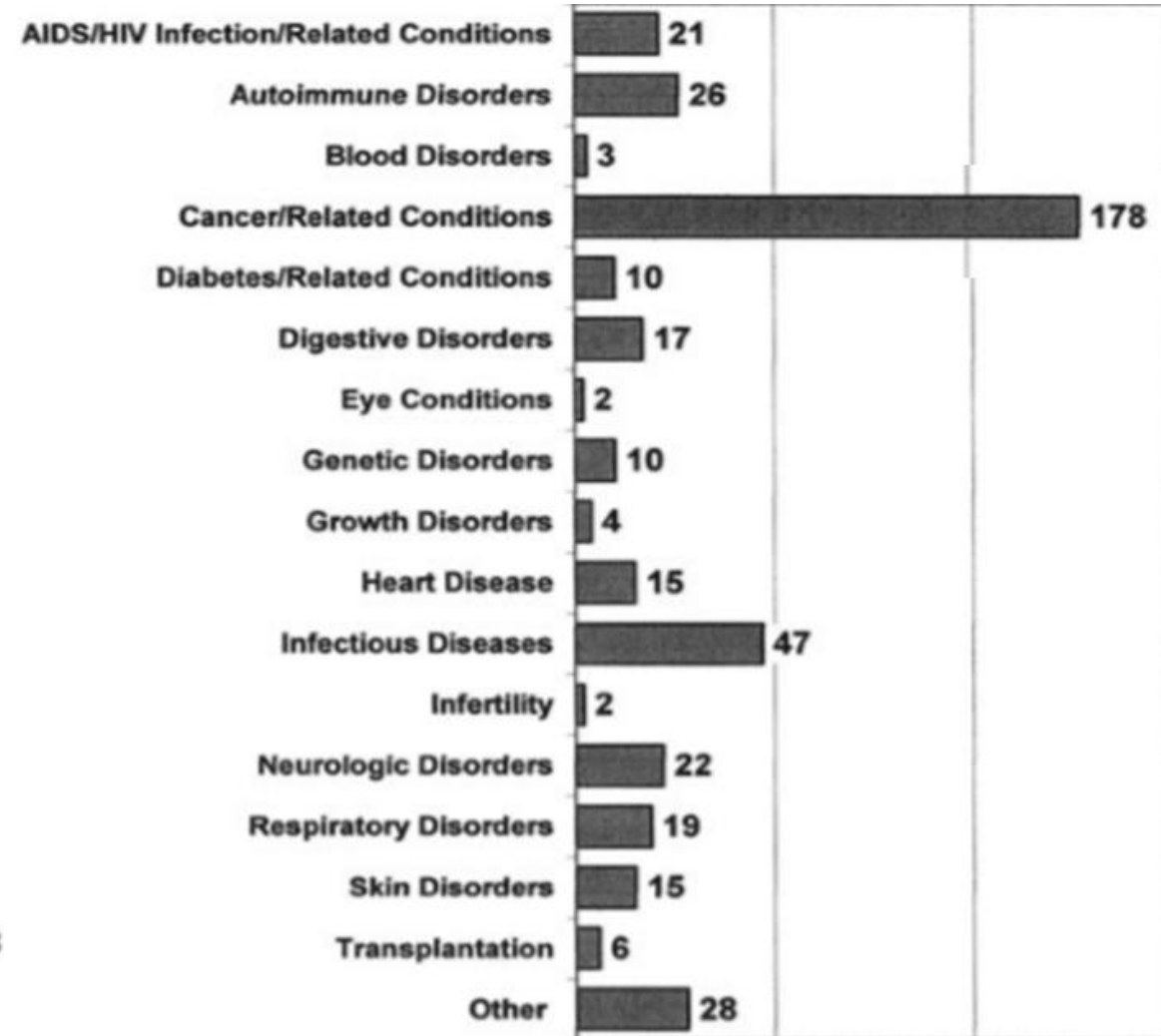
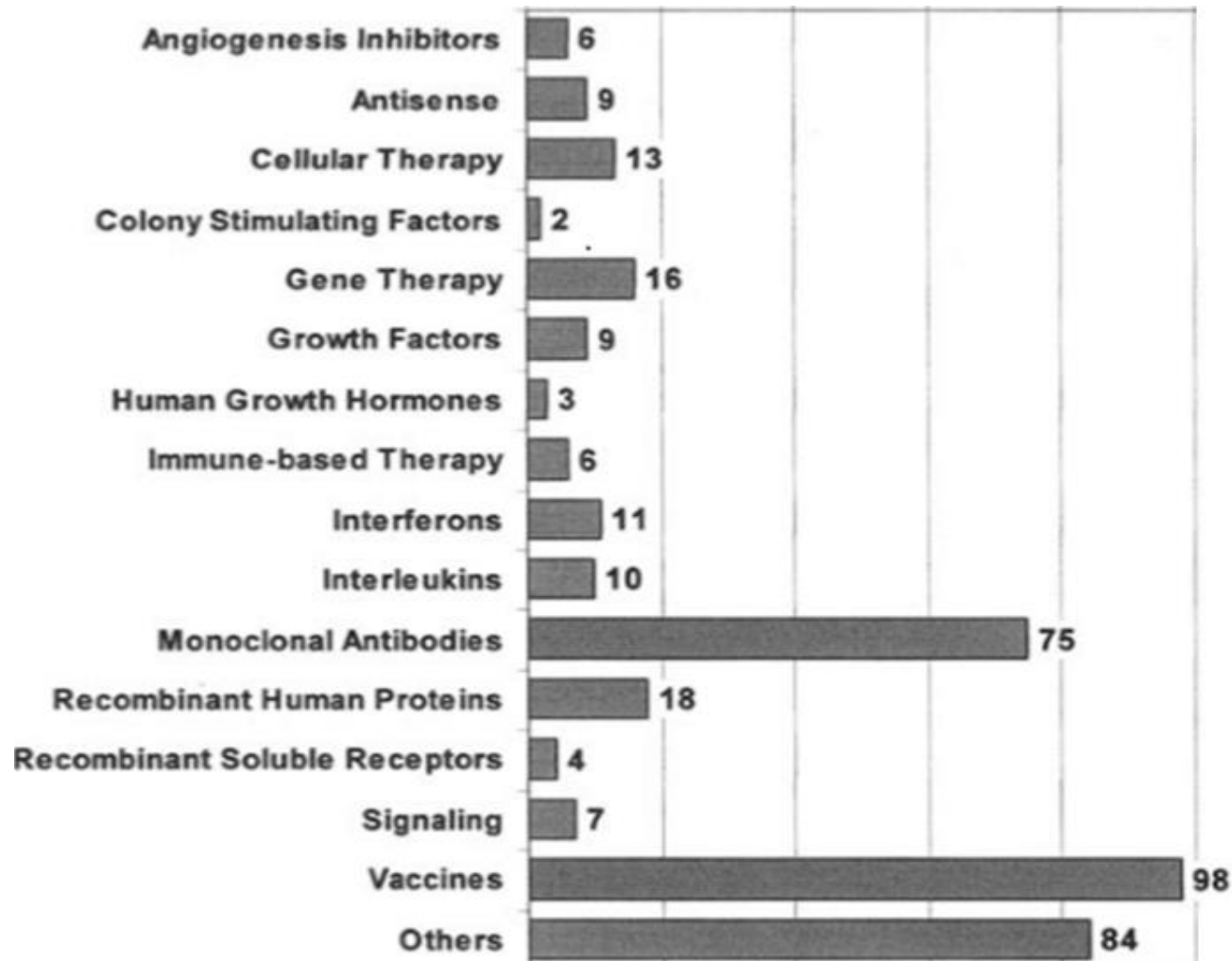
Mohammad Reza Abolhassan



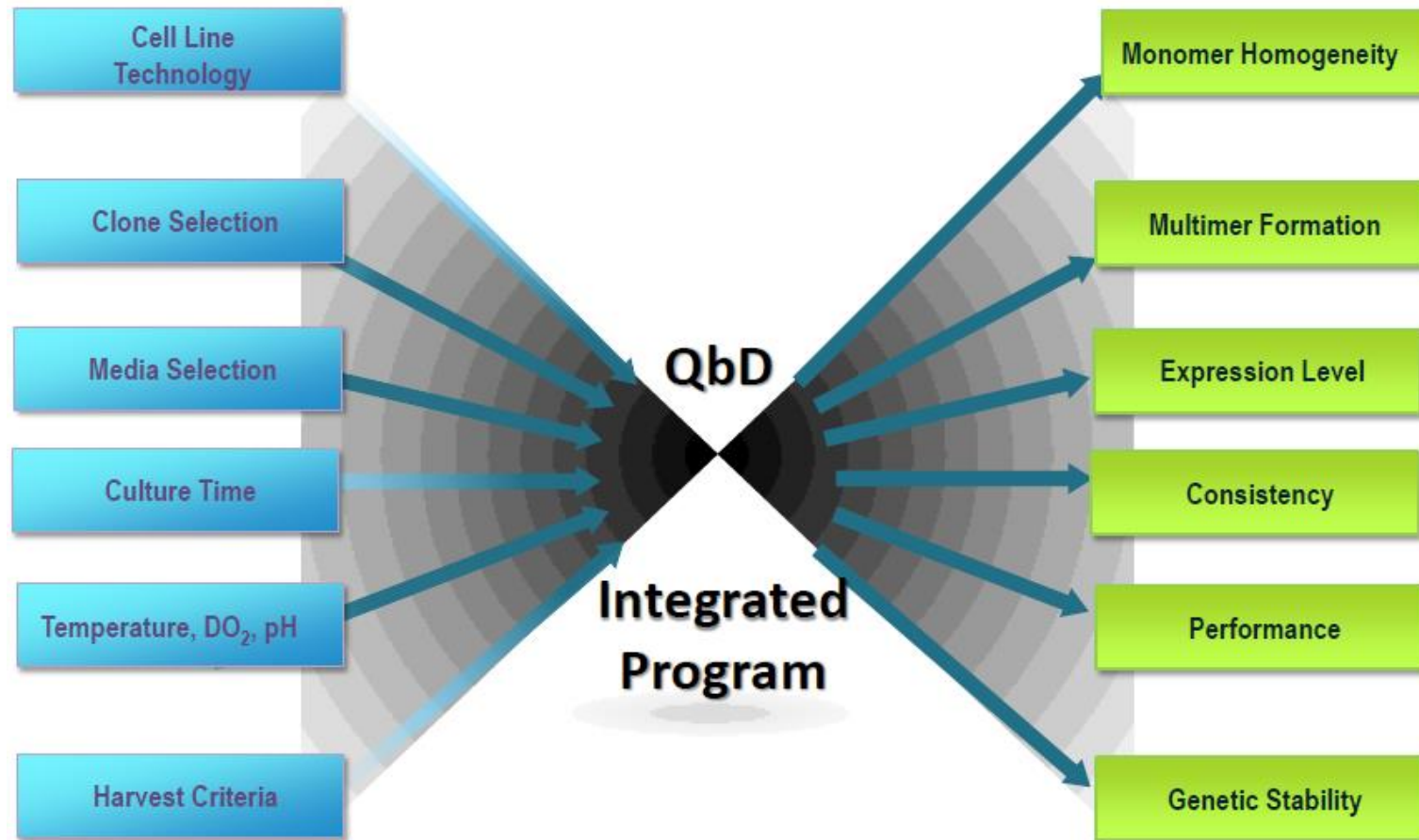
CONTENTS

- **Introduction**
- **ICH guidelines and QBD approach**
- **Host cell selection criteria**
- **Cloning and screening**
- **Cell banking**

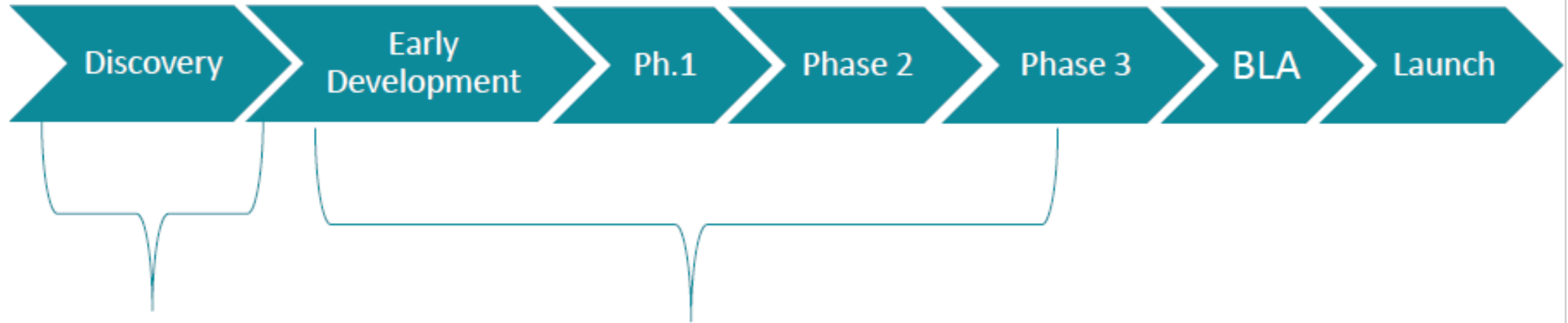
Biomedicine under development in the USA by product and therapeutic category



QBD



Quality Target Product Profile



Clinical Target Product Profile

- Molecular target
- Affinity, specificity
- Frequency of dosing
- Mode of delivery
- Relevant animal model
- Cross reactivity

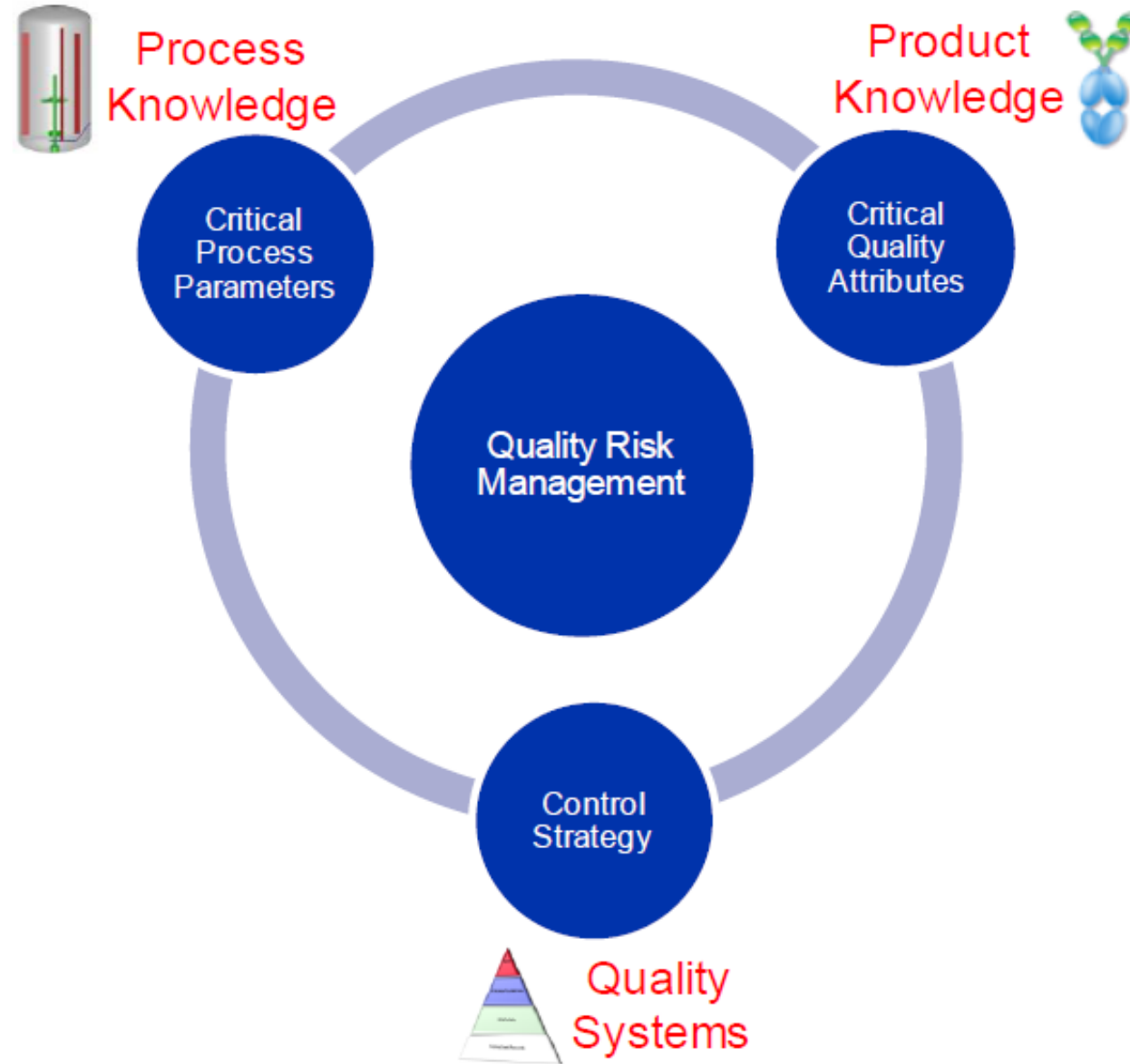
Quality Target Product Profile (QTPP)

- Post translational modifications
- Purity, safety
- Process-related impurity levels
- Stability
- Reproducible process

Product Quality Attribute

<i>Product variants</i>	<i>Process-related impurities</i>	<i>Adventitious agents</i>	<i>Composition and strength</i>
<ul style="list-style-type: none"> • Aggregation • Conformation • C-terminal lysine • Deamidated isoforms • Disulfide bonds • Fragmentation • Glycation • Glycosylation • Oxidation • Thioether link 	<ul style="list-style-type: none"> • Host cell DNA • Host cell proteins • Leached protein A • Selective agent (e.g., MTX) • Cell culture medium components (e.g., insulin) • Purification buffer components • Leachables 	<ul style="list-style-type: none"> • Viral purity • Microbial purity • Endotoxins 	<ul style="list-style-type: none"> • Product concentration • Potency • Osmolality • pH • Particulates • Clarity • Color • Volume • Excipient concentration

Quality Risk Management



General:

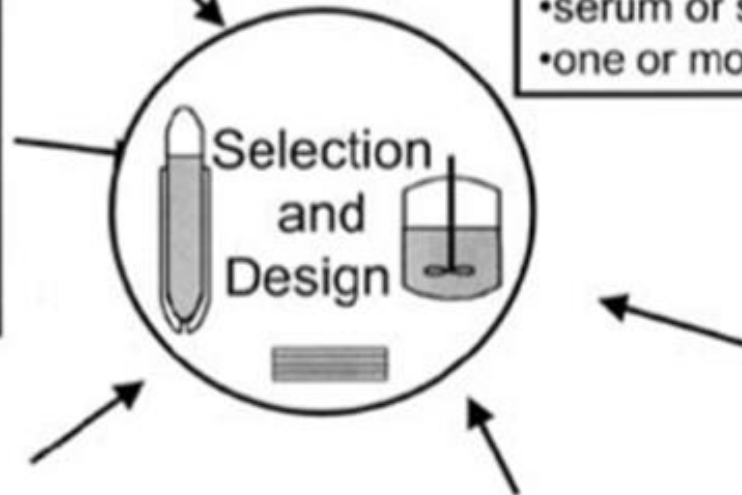
- service
- spare parts
- documentation
- media and utility supply
(steam, electricity, cooling water, gases)
- operator skills and training options

Cell related issues:

- sterility
- temperature control
- shear forces
- oxygen supply, CO₂ removal
- pH
- nutrient supply
- waste product accumulation
- suspension or adherent growth
- serum or serum/protein-free
- one or more different cell types

**Economic issues:**

- investment cost
- running cost
- space-time yields
- time-to-market
- purpose

**Process:**

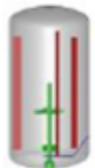
- automation
- sterilization-in-place
- cleaning-in-place
- scale-up
- monitoring and control
of critical parameters
- down-time
- reliability
- operation mode

Regulatory:

- validation
- genetic stability
- biosafety
- materials

Product related issues:

- product formation kinetics
- feed back inhibition
- degradation and truncation
- modifications
- secretory or intracellular



Process
Knowledge

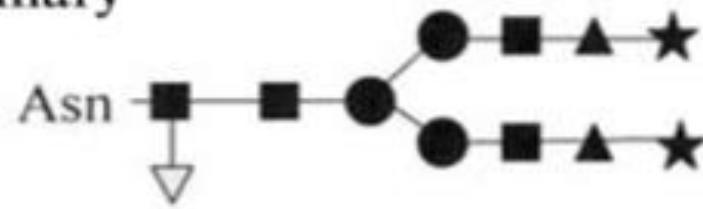
Quality Guidelines

Q5A - Q5E Quality of Biotechnological Products		^
>	Q5A(R1)	Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin
>	Q5A(R2) EWG	Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin
>	Q5B	Analysis of the Expression Construct in Cells Used for Production of r-DNA Derived Protein Products
>	Q5C	Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products
>	Q5D	Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products
>	Q5E	Comparability of Biotechnological/Biological Products Subject to Changes in their Manufacturing Process
Q9 Quality Risk Management		^
Q10 Pharmaceutical Quality System		v
Q11 Development and Manufacture of Drug Substances		v

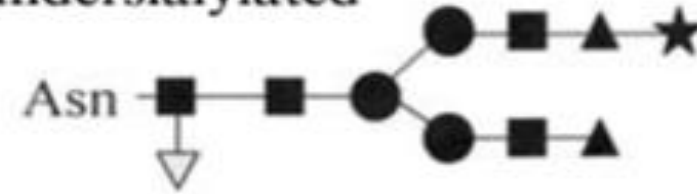
Common cell line used in bioprocessing

Cell line	Species	cell Type	Tissue isolated
W1-38	Human	Fibroblast	lung
MRC-5	Human	Fibroblast	lung
FS-4	Human	Fibroblast	Foreskin
HEK293	Human	Epithelial	Kidney
MDCK	Dog	Epithelial	Kidney
NS/SP2/0	Mouse	Lymphoid	Myeloma
CHO	Chinese Hamster	Epithelial	Ovary
BHK	Syrian Hamster	Epithelial	Kidney

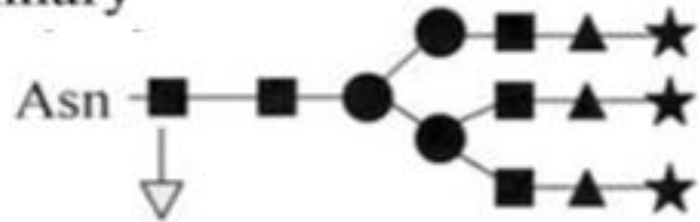
Biantennary



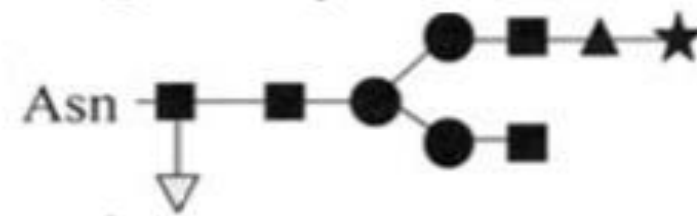
undersialylated



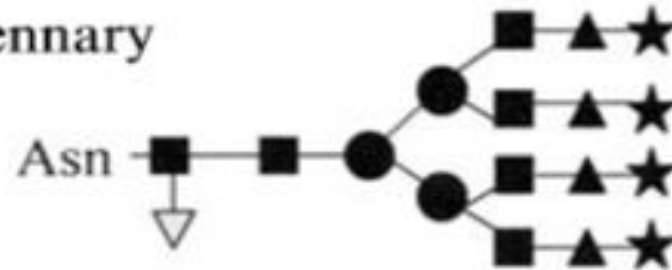
triantennary



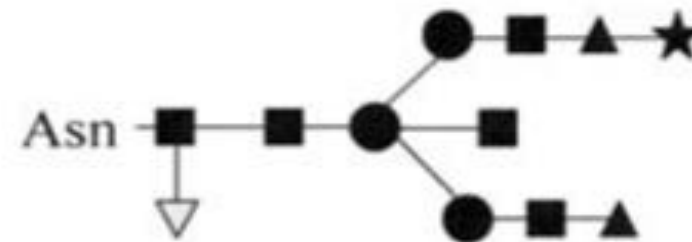
undergalactosylated



tetraantennary



bisecting GlcNAc structure



α 2,3 Sialyltransferase/
 α 2,6 Sialyltransferase



NANA

Galactose

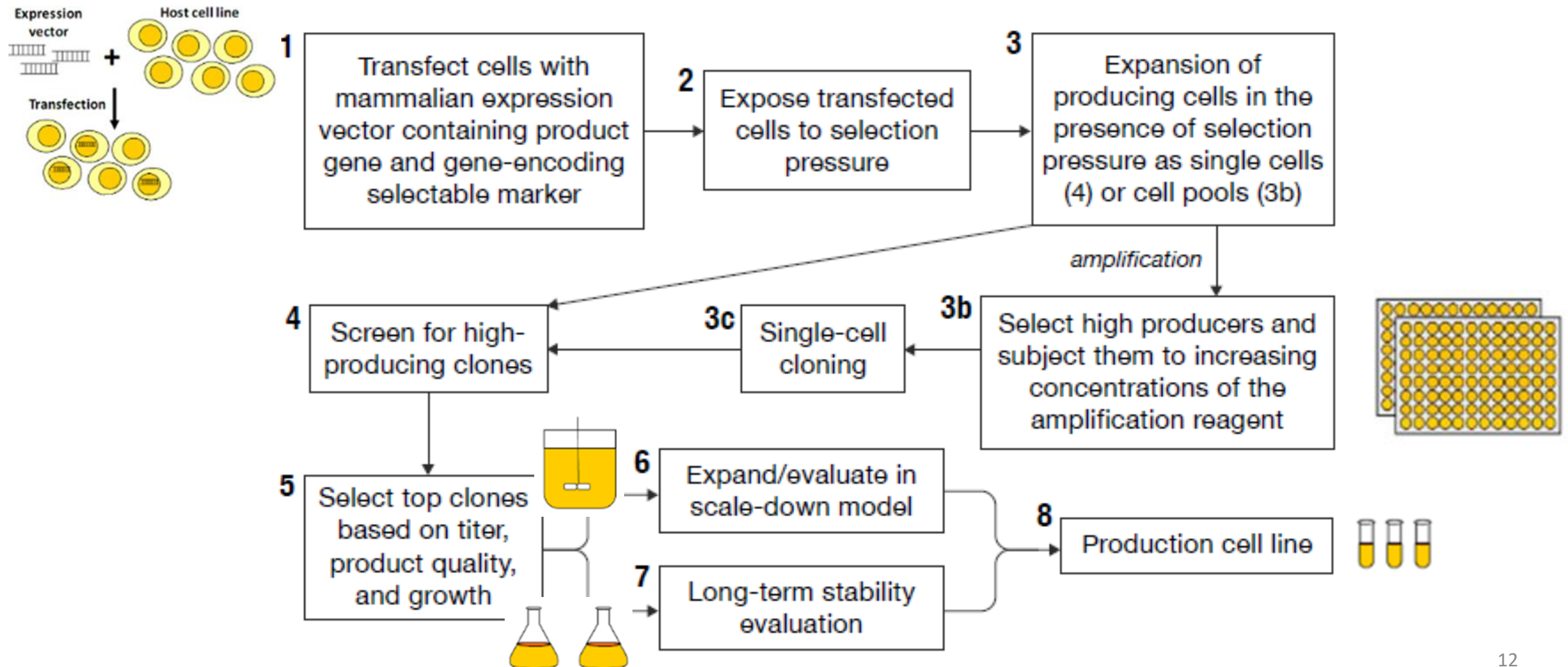
GlcNAc

Mannose

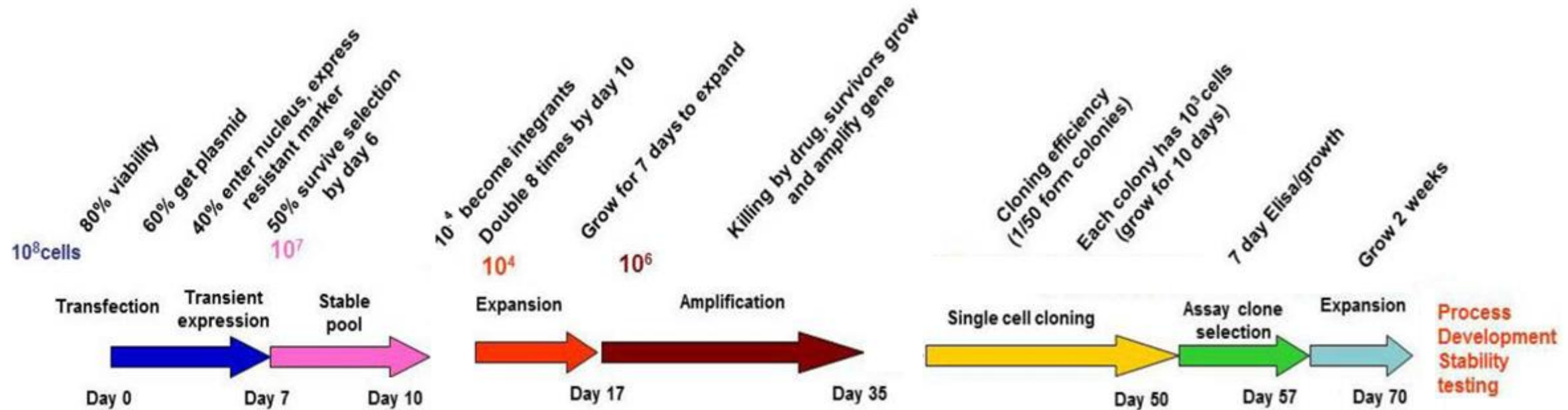


Fucose

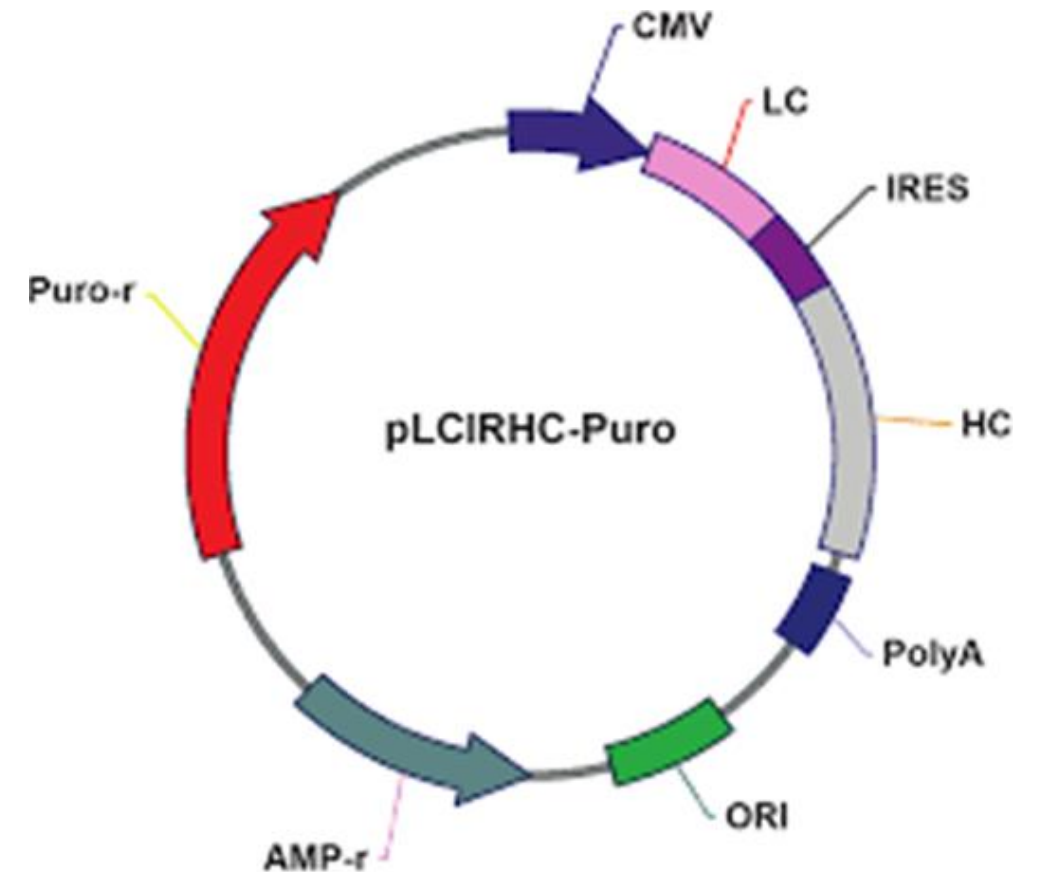
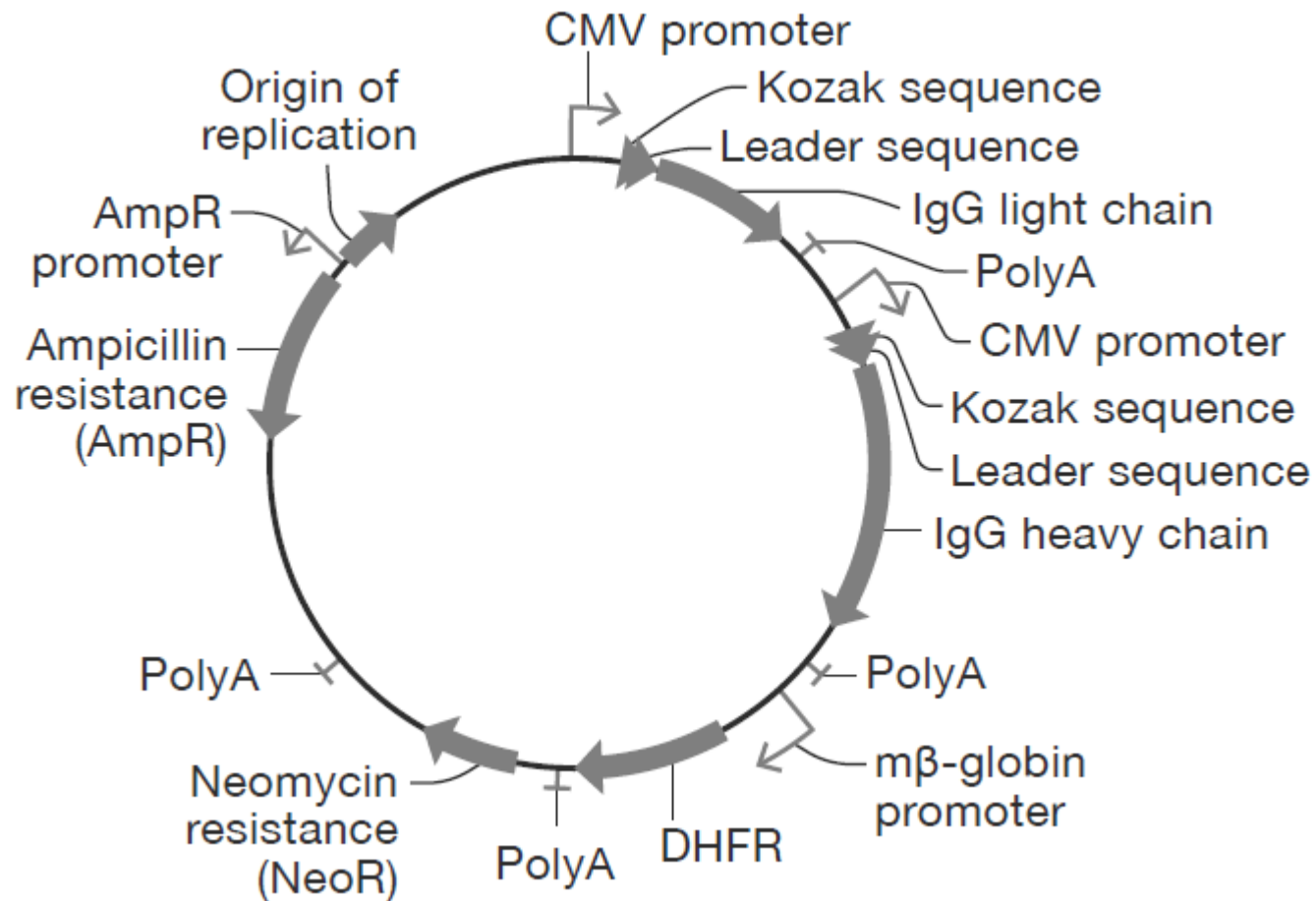
Typical steps in developing cell line



Industrial Cell Line Generation Timeline

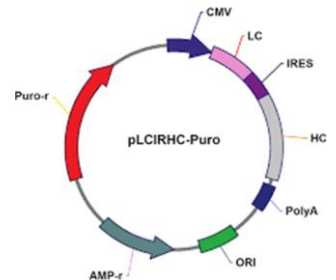


Plasmid Construct



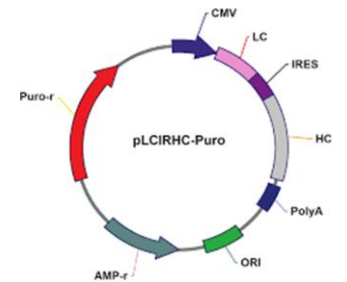
Regulatory Elements

- Enhancers
- Elements like **TATA sequences** and the **SP1** binding region (CCGCCC) occur frequently in promoters.
- **IRES sequences** are often used in molecular biology to mimic a polycistronic mRNA.
- **Kozak sequence** (CCA/GCC) is usually placed in front of the first ATG codon to improve the translational initiation of mRNA.



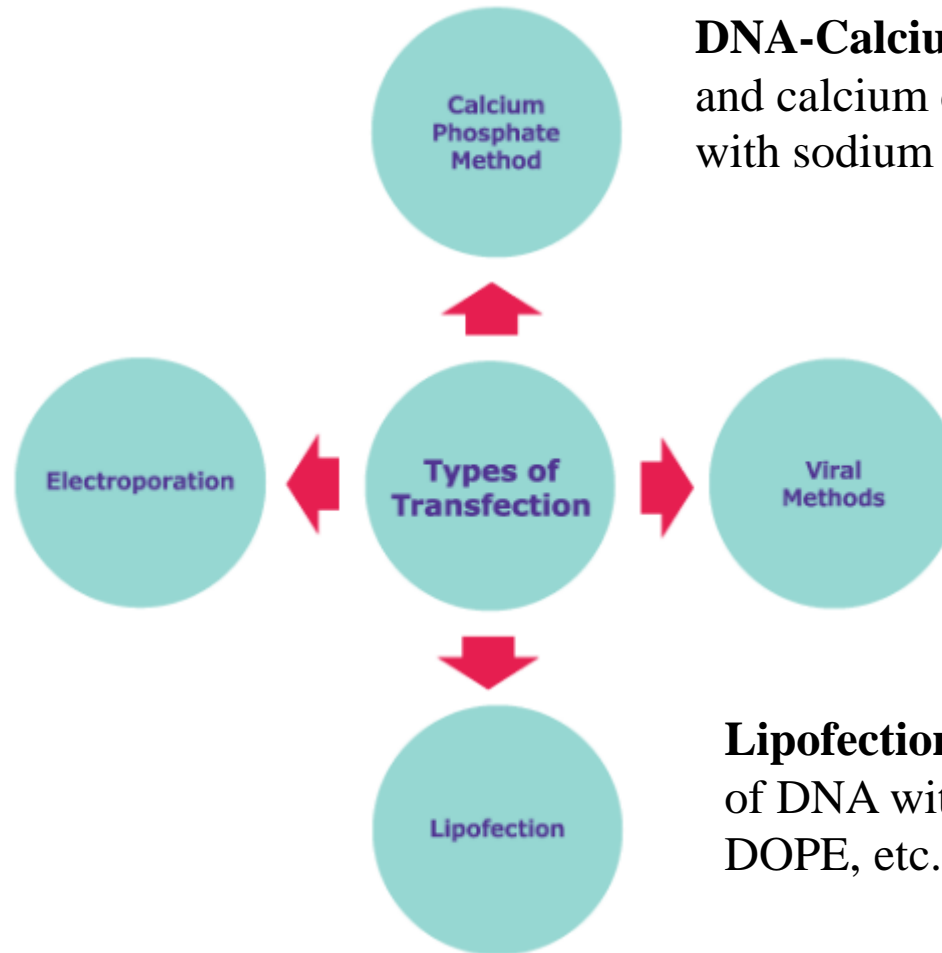
Regulatory Elements

- Viral trans and cis activating protein.
- Matrix attachment regions (**MARS**) or scaffold-attachment regions (**SARS**) to neutralize the effects of integration location or “position-dependent” effects.
- Intervening sequence (**IVS**) or splice sequences.
- Polyadenylation signals.



Transfection

Electroporation, high-voltage electropulse introduces pores in the plasma membrane, allowing entry of DNA.



DNA-Calcium Phosphate , co-precipitation DNA and calcium chloride is added into a HEPES buffer with sodium phosphate.

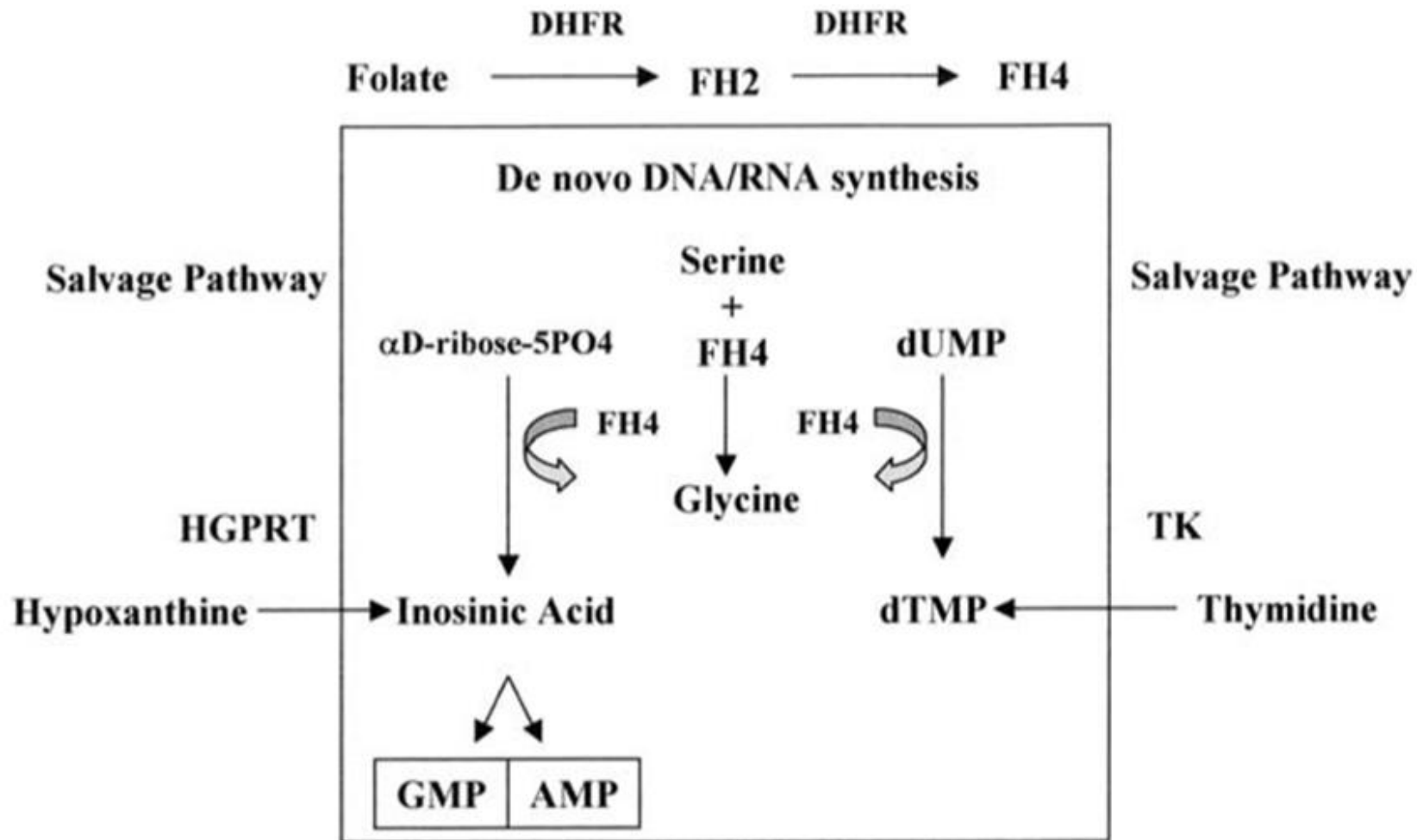
Lipofection/Lipid Mediated Gene Transfer A mixture of DNA with amphipathic compound (DOTMA, DOPE, etc).

Selective Agents

Selective Agents Commonly Used in Constructing Mammalian Expression Vectors

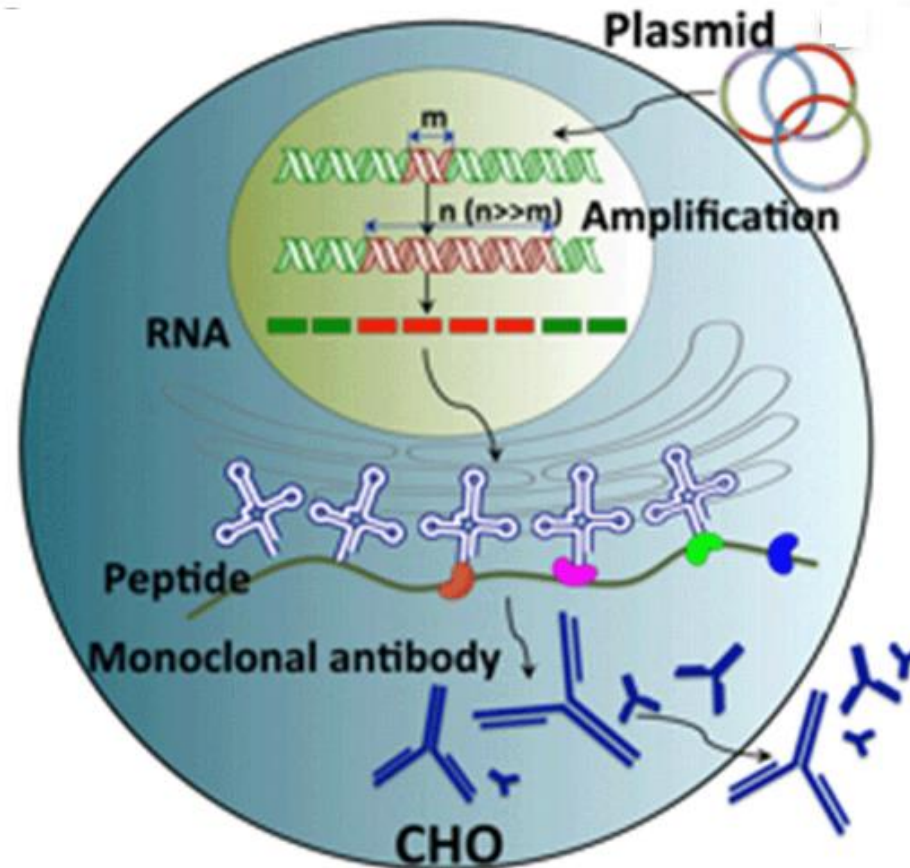
Selectable marker	Selective agent	Enzyme inhibitor used for gene amplification
<i>Dominant</i>		
Aminoglycoside phosphotransferase (aph)	Geneticin (G418)/Neomycin	
Hygromycin-B phosphotransferase (hph)	Hygromycin B	
Puromycin <i>N</i> -acetyltransferase (pac)	Puromycin	
Zeocin resistance gene (sh ble)	Zeocin	
<i>Recessive</i>		
Glutamine synthase (GS)	Glutamine-free media	Methionine sulfoximine (MSX)
Dihydrofolate reductase (DHFR)	Media lacking glycine, hypoxanthine and thymidine	Methotrexate (MTX)

Folate metabolism



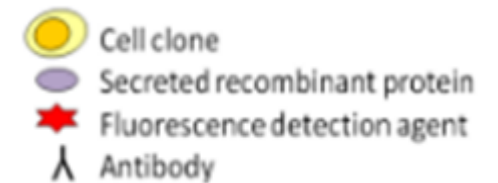
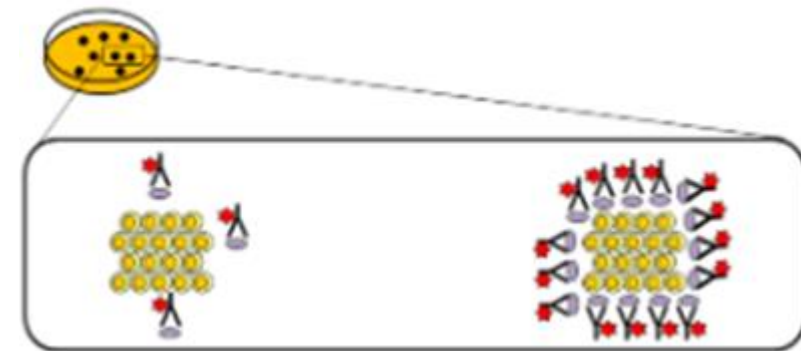
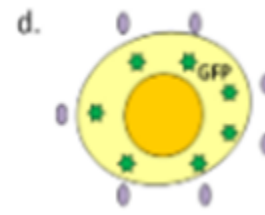
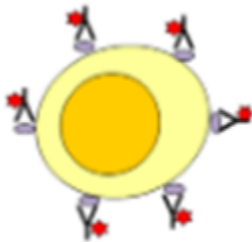
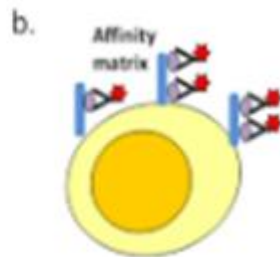
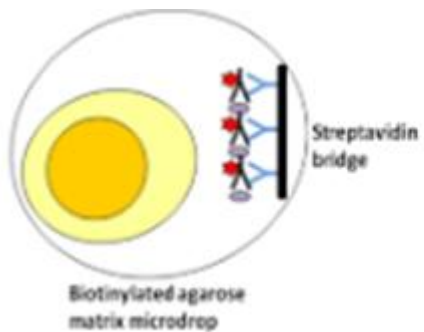
Target gene amplification

- Optimizing the concentration and repeated exposure of selective agent can improve expression of target genes.



High throughput Screening

- Fluorescence-activated cell sorting (**FACS**)
- Fluorescent MTX binding method
- Green fluorescent protein (**GFP**)
- Solid or semisolid matrix



Cell Bank Qualification

Mammalian and Non-Traditional Cell Line Characterization

- Purity
- Identity
- Stability
- Virological safety



Thank you for your attention!



References

- Cell Culture Bioprocess Engineering, Second Edition, Wei-Shou Hu, 2020
- Cell Culture Technology for Pharmaceutical and Cell-Based Therapies, Sadettin Ozturk, Wei-Shou Hu, 2005