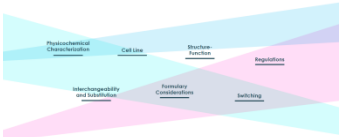


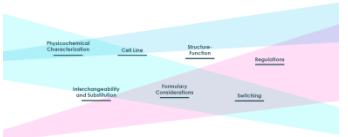
Assistant Professor Musaed Abdullah Alkholief, PhD, Saudi Arabia

- Assistant Vice-Rector for Planning and Development, King Saud University, Saudi Arabia
- Assistant Professor of Pharmaceutics, College of Pharmacy, King Saud University, Saudi Arabia

GaBI Scientific Meetings

First GCC Stakeholder Meeting on **Approval Process, Interchangeability/Substitution and Safety of Biosimilars**

20 November 2017, Holiday Inn Izdihar Riyadh, Saudi Arabia



Biosimilar cell line development

Assistant Professor Musaed Abdullah Alkholief, PhD

20 November 2017

GaBi
GENERICS AND BIOSIMILARS INITIATIVE
Building trust in cost-effective treatments

SPS Saudi Pharmaceutical Society الجمعية الصيدلية السعودية
PPCI Pro Pharma Communications International

Biosimilar Cell Line Development

Musaed Alkholief, PhD

Assistant Vice-Rector for Planning and Development
Director of Nanomedicine Research Unit
Assistant Professor of Nanobiotechnology, College of Pharmacy
King Saud University

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Saudi Pharmaceutical Society
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جامعة الملك سعود
King Saud University

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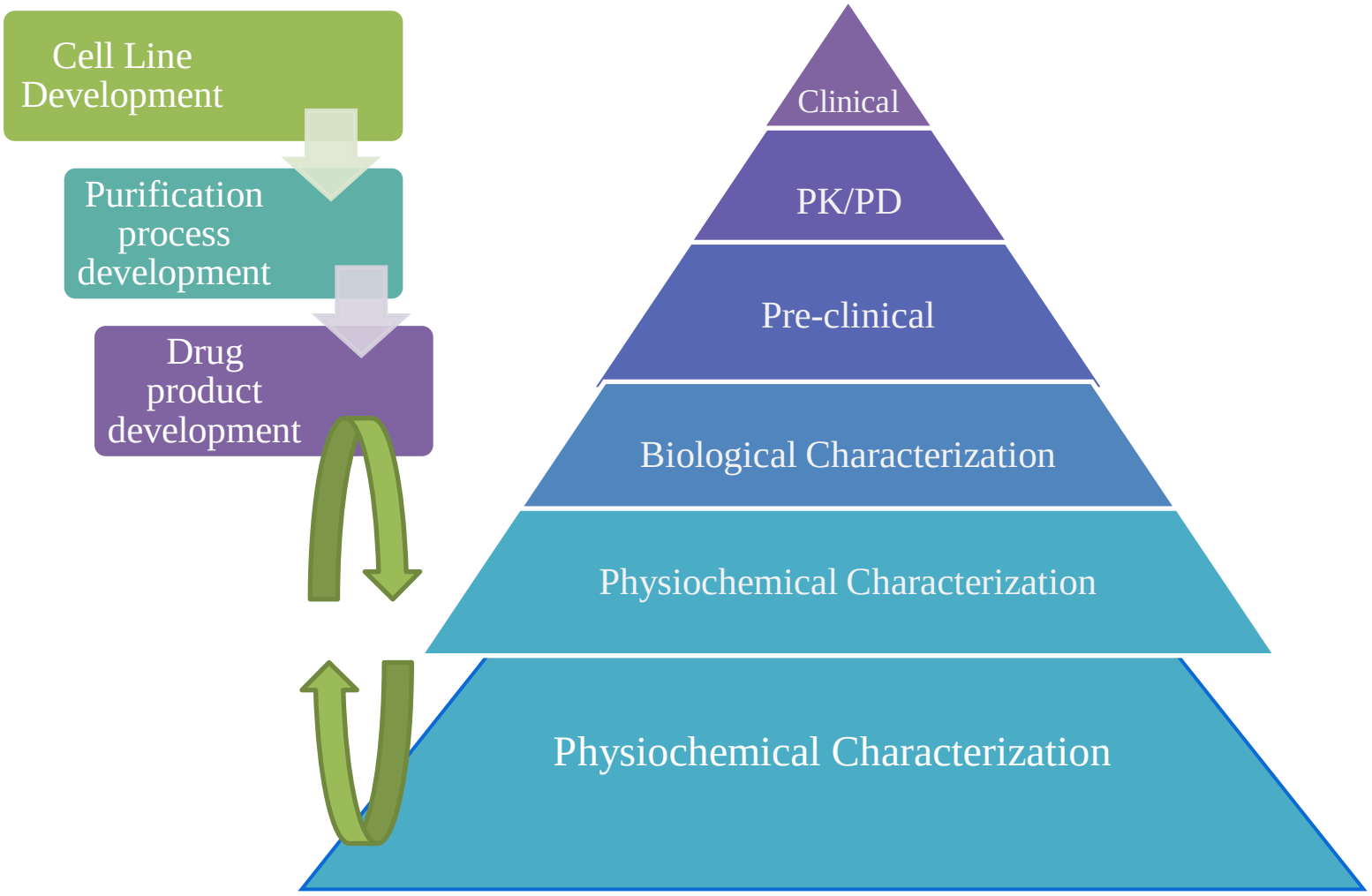
GENERICS AND BIOSIMILARS INITIATIVE

sps

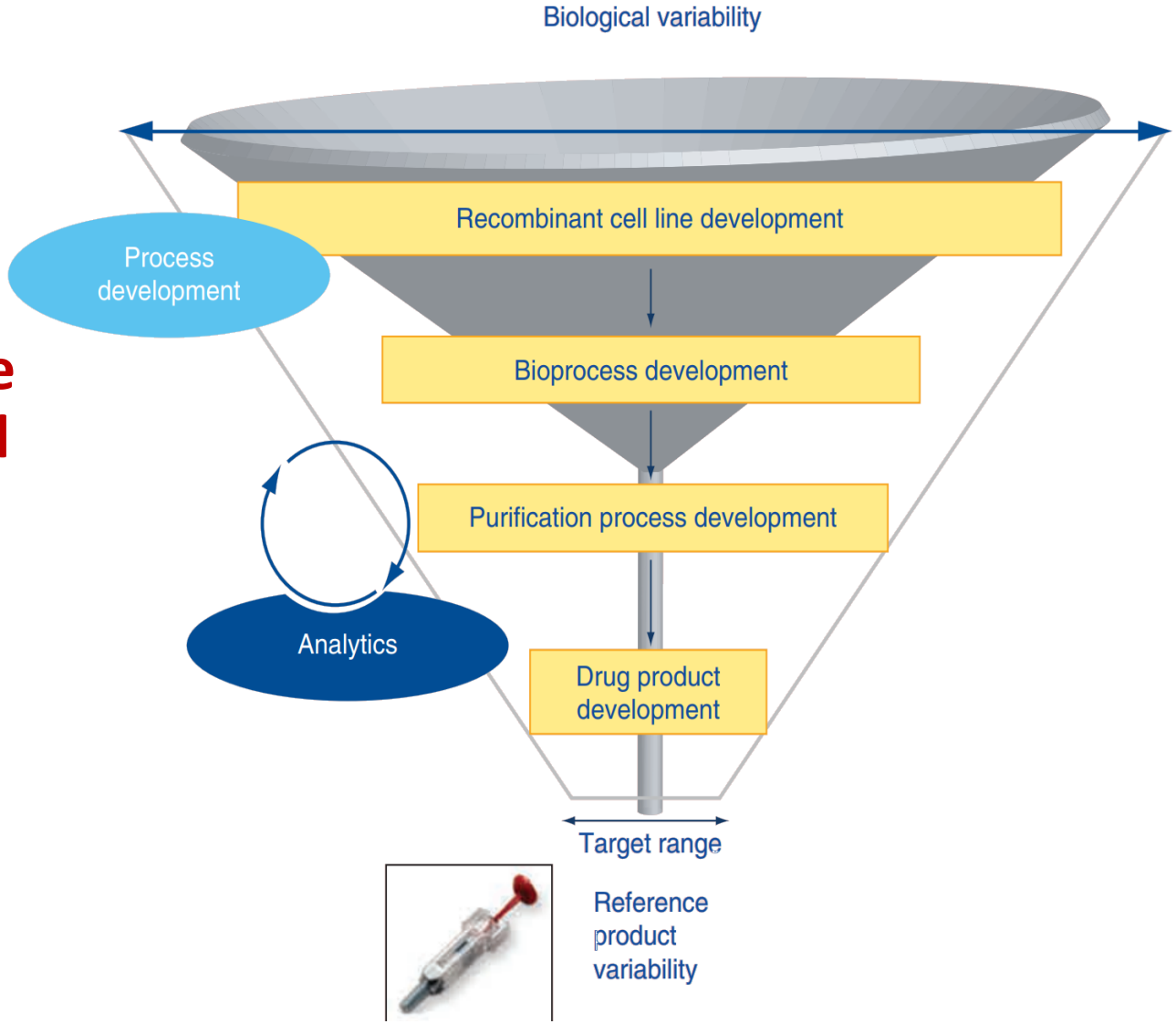
Saudi Pharmaceutical Society
الجمعية الصيدلية السعودية

Overview and Significance

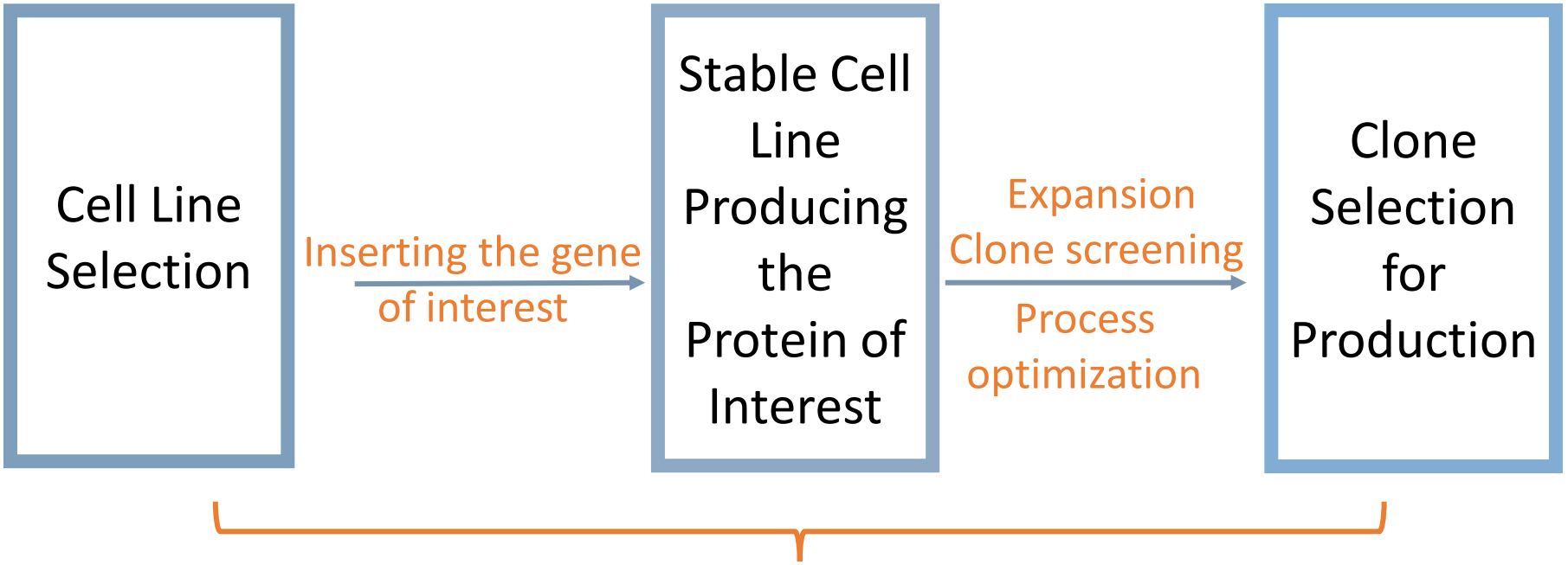


Overview and Significance

Consistency is the key for successful cell line development

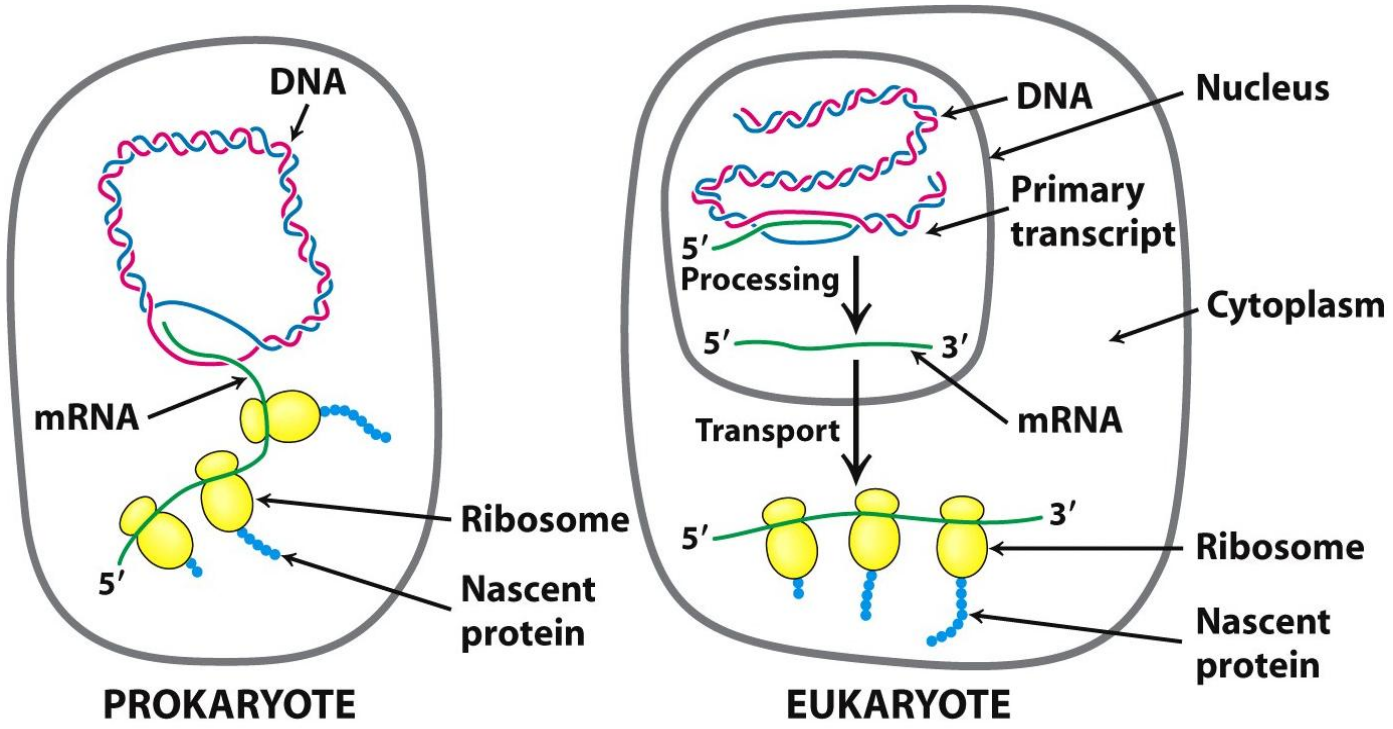


Outline


Cell Line Selection
Inserting the gene of interest
Stable Cell Line Producing the Protein of Interest
Expansion
Clone screening
Process optimization
Clone Selection for Production
Sources of Variability
Host Cell Line Engineering

Cell Line Selection

Why do we need cells to produce biosimilars?



Cell Line Selection

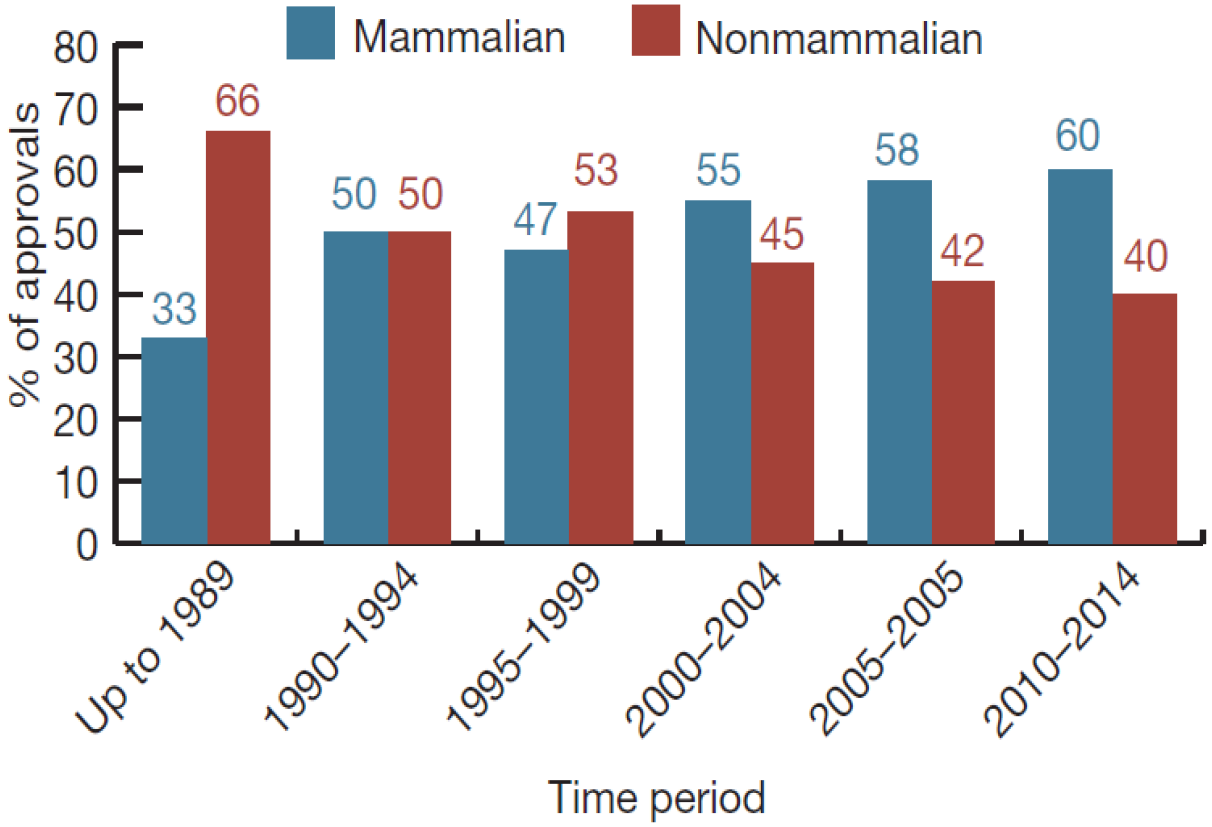
Types of cells that could be used:

Non-mammalian:

- E.Coli
- Yeast

Mammalian:

- Animal
- Human



Source: Biopharmaceutical benchmarks, 2014. Gary Walsh

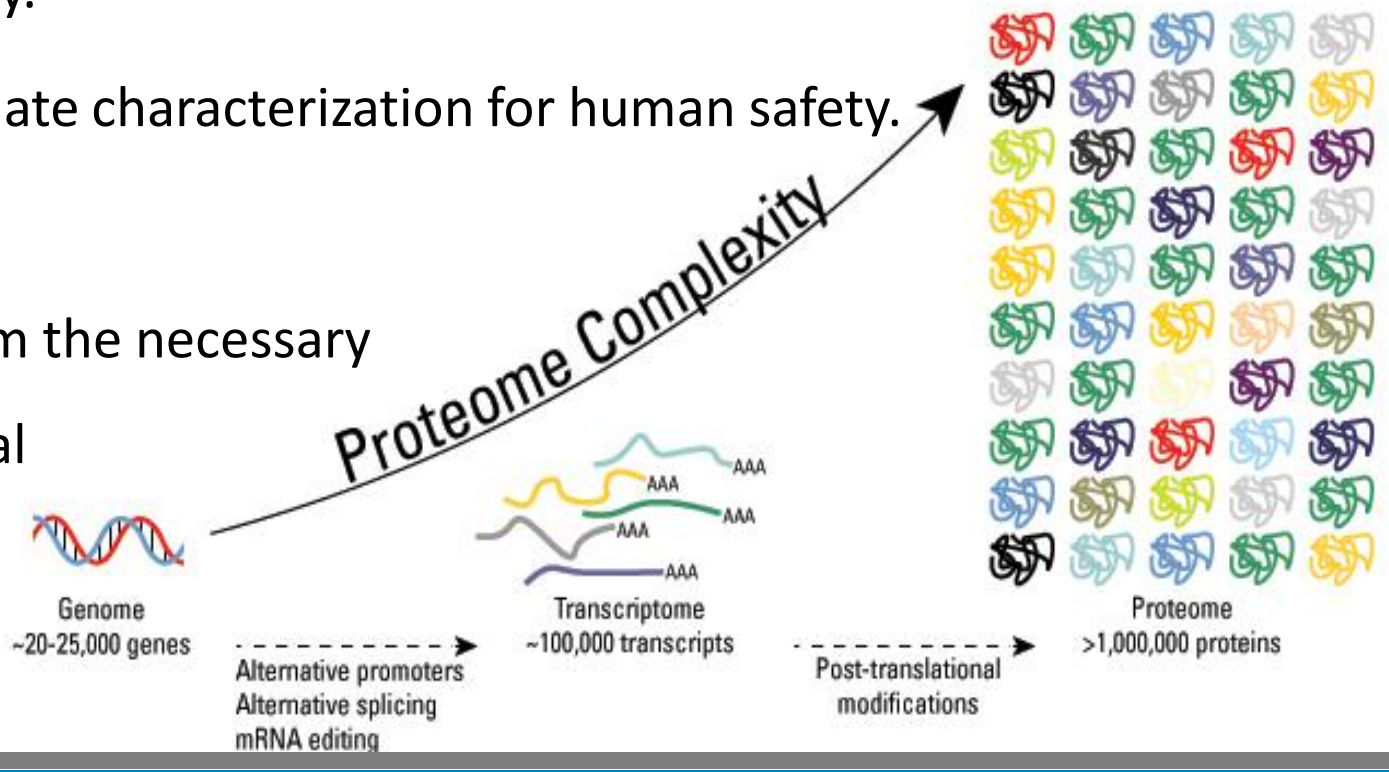
Cell Line Selection

	Non- Mammalian	Mammalian
Cell growth	**Rapid**	**Slow**
Complexity of growth media	**Simple**	**Complex**
Cost of growth media	**Cheap**	**High**
Expression level	**High**	**Low to moderate**
Protein folding	**Refolding required**	**Yes**
Post translational modification	**Only for Yeast**	**Yes**

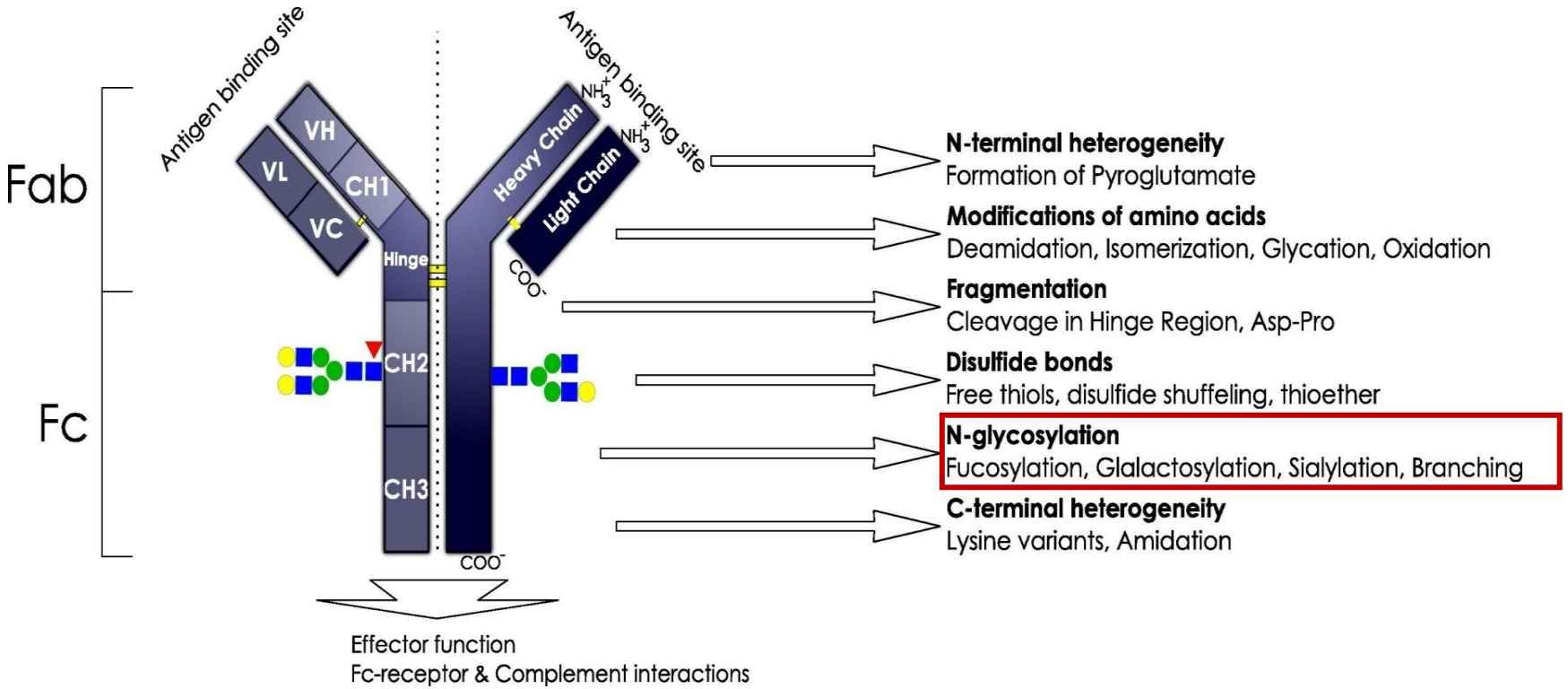
Cell Line Selection

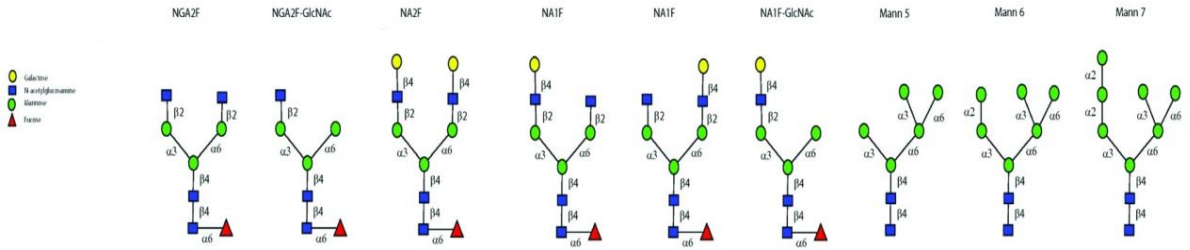
Selection Criteria:

- High level production over long bioreactor incubation times while maintaining high cell viability, density, and genetic stability.
- Allow appropriate characterization for human safety.
- Scalable.
- Able to perform the necessary post-translational modifications.



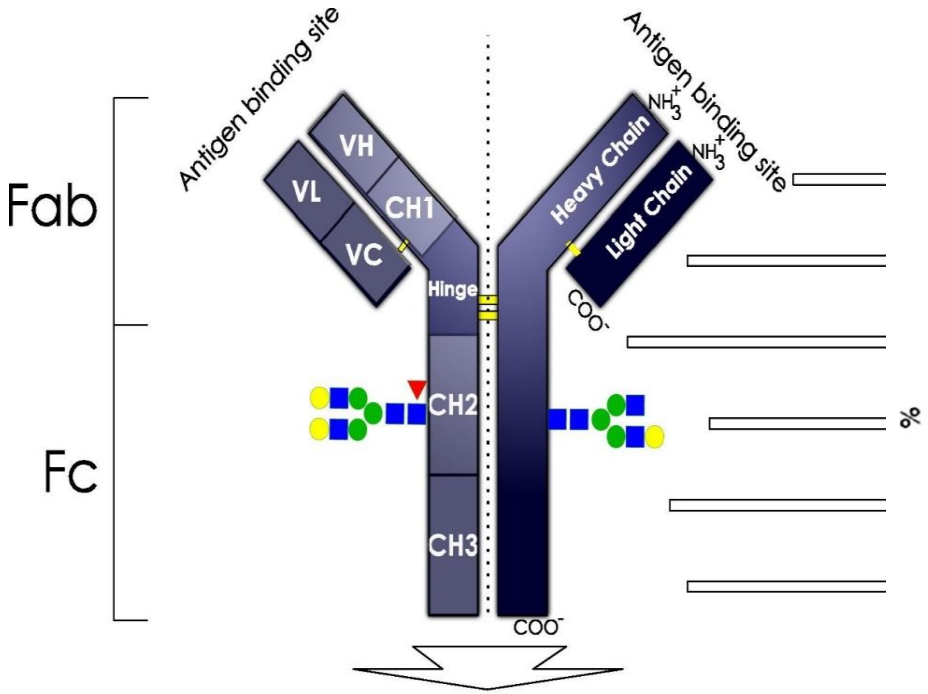
Cell Line Selection

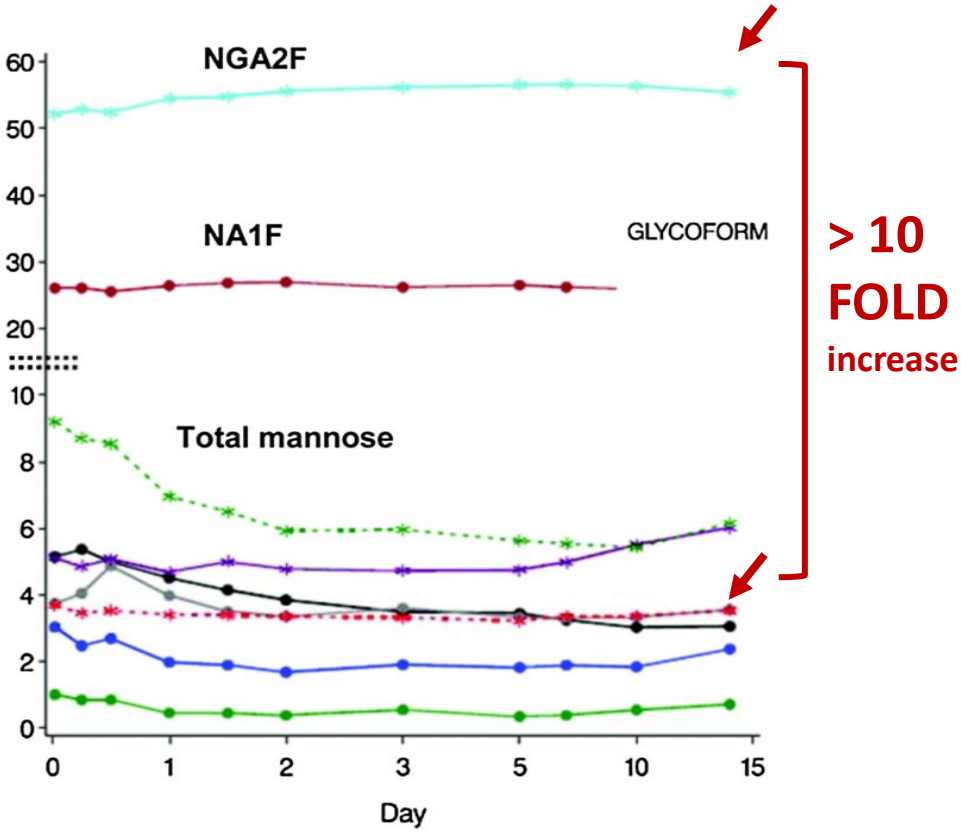


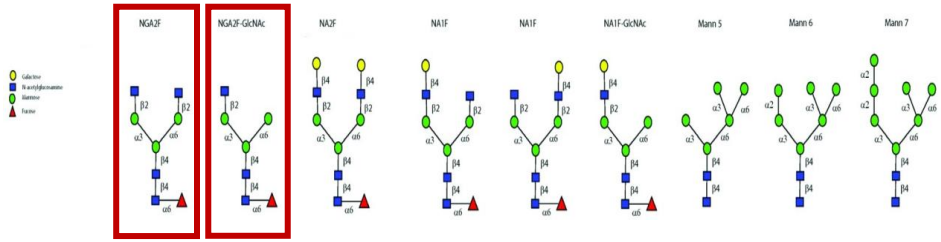


Source: N-glycosylation heterogeneity and the influence on structure, function and pharmacokinetics of monoclonal antibodies and Fc fusion proteins. Eur J Pharm Biopharm. 2016 Mar.

Cell Line Selection





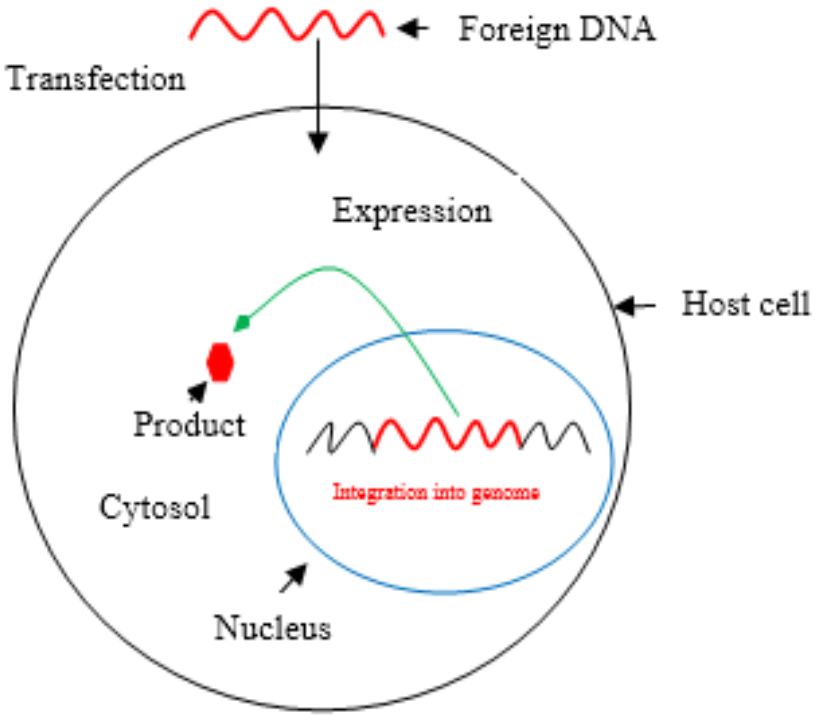


Source: N-glycosylation heterogeneity and the influence on structure, function and pharmacokinetics of monoclonal antibodies and Fc fusion proteins. Eur J Pharm Biopharm. 2016 Mar.

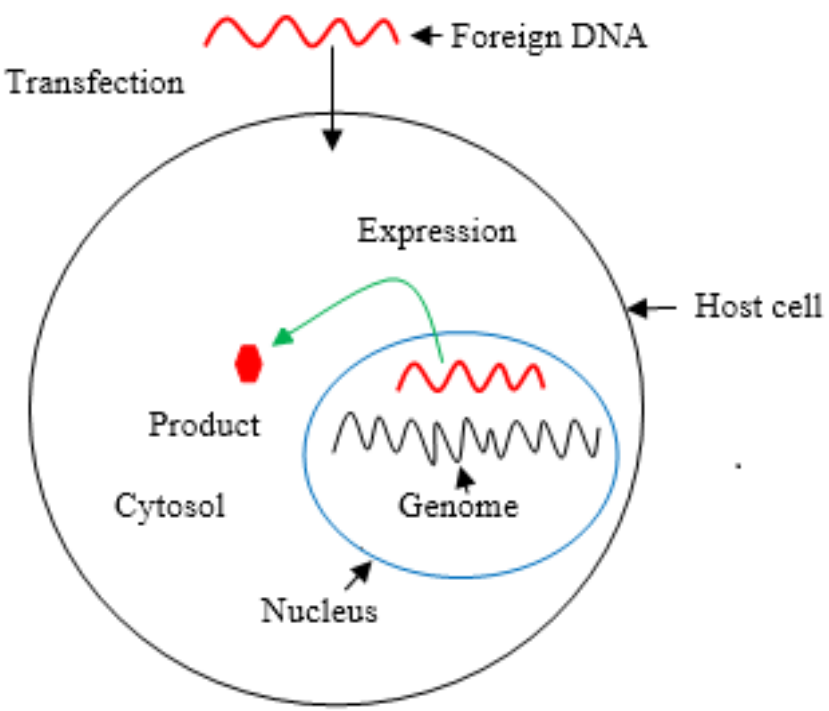
Stable Cell Line Development

The goal: selecting a cell line that stably expresses the protein of interest

Stable expression



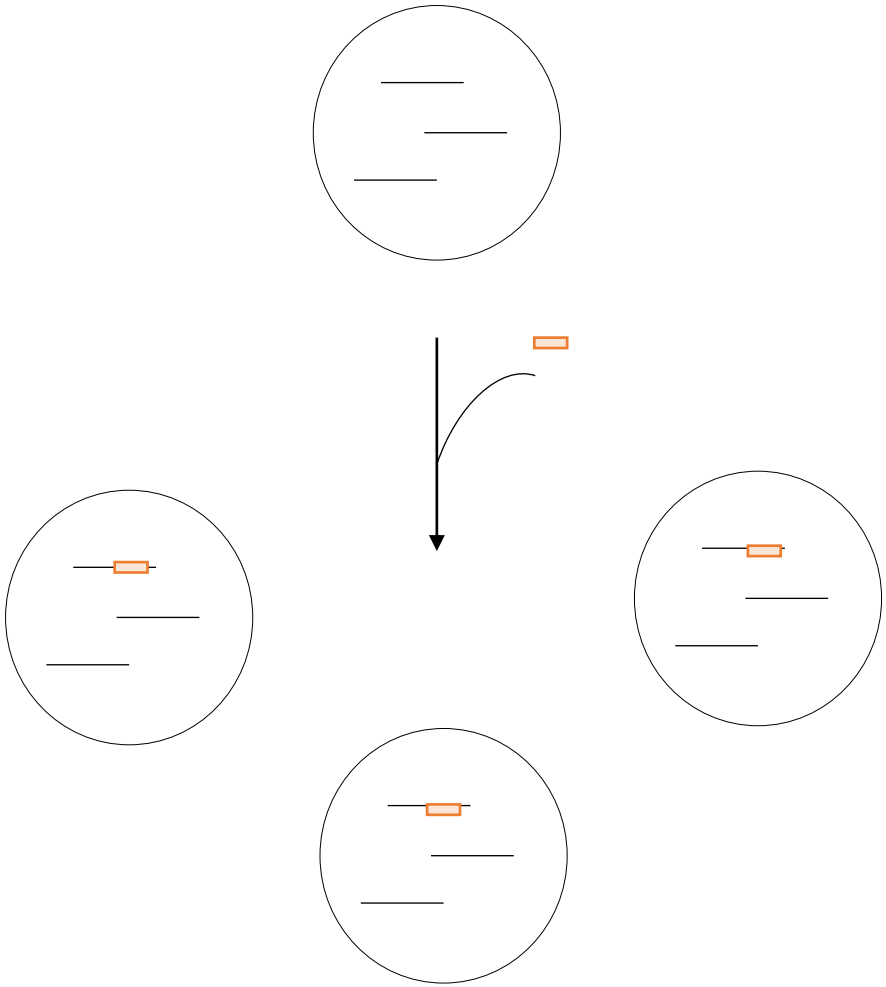
Transient expression



Stable Cell Line Development

Random integration:

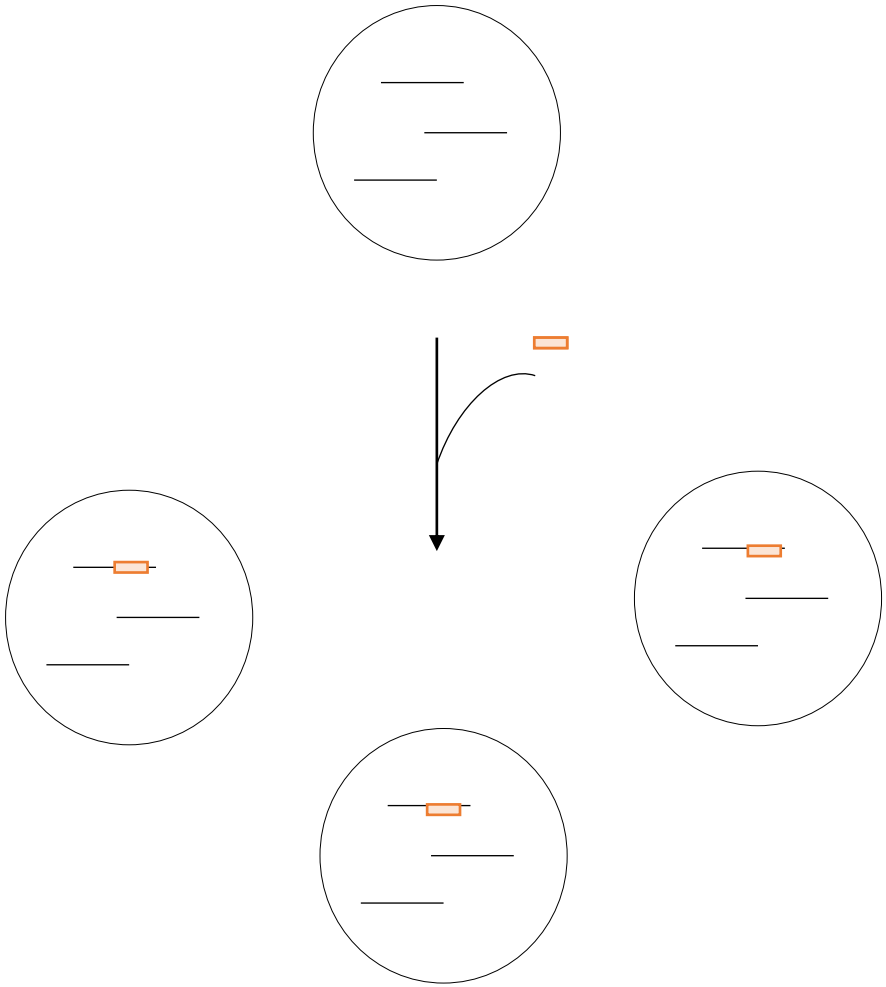
- High diversity in expression and stability.
- Requires intensive screening.



Stable Cell Line Development

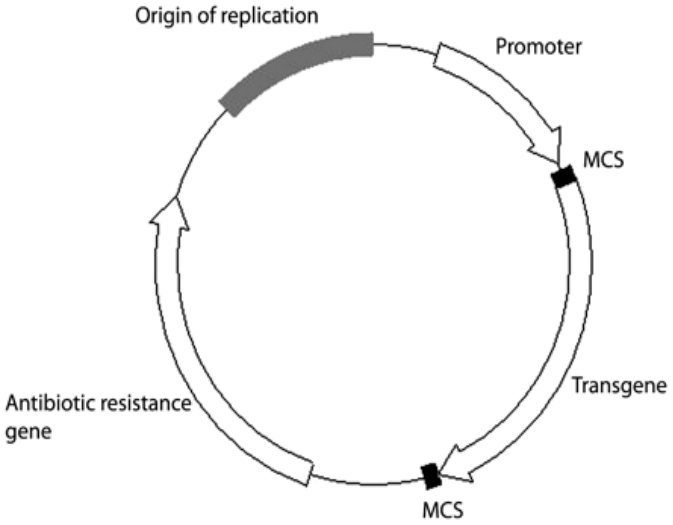
Site specific integration:

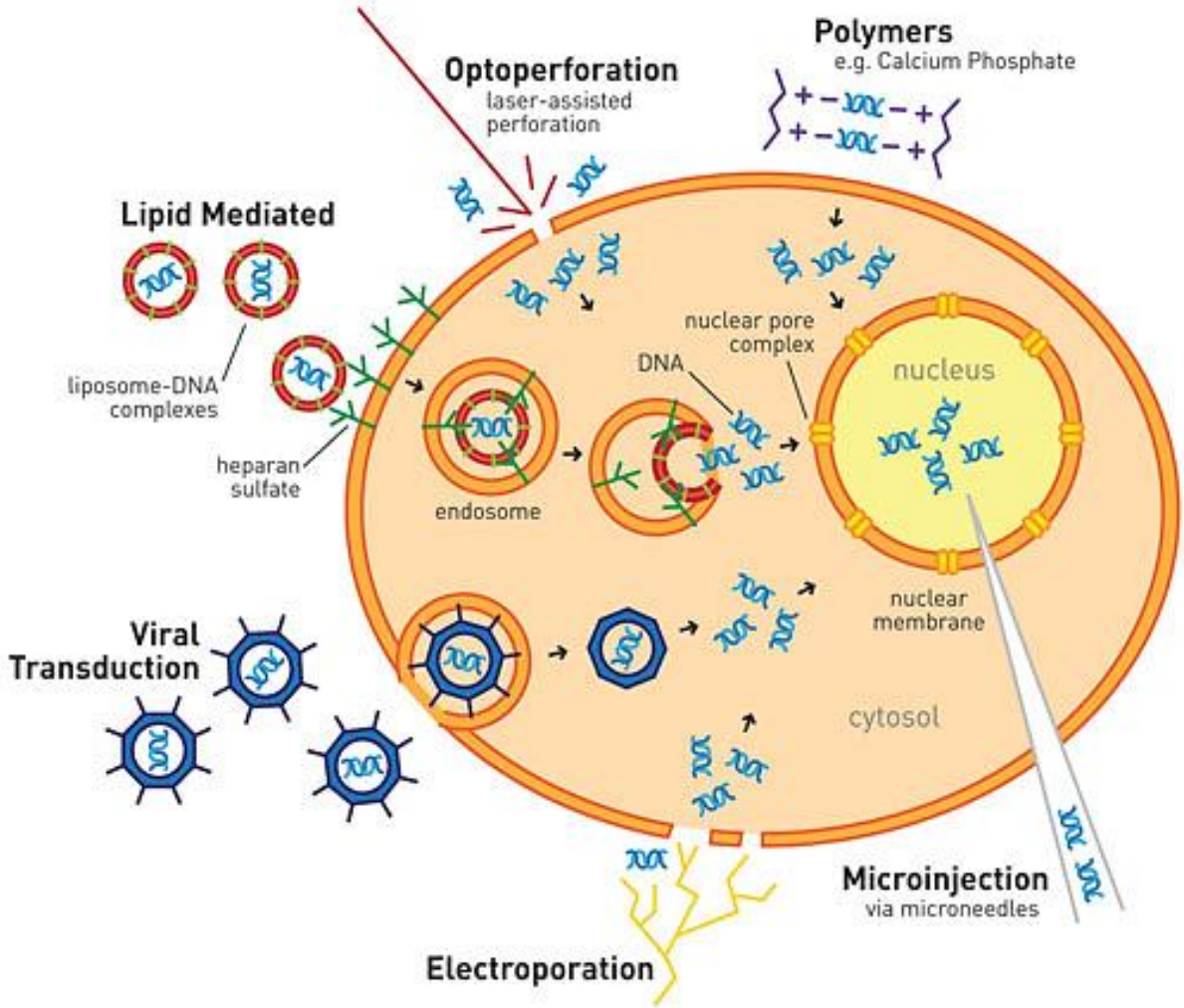
- High predictability in expression and stability.
- Requires less intensive screening.
- Enables shorter development time.



Stable Cell Line Development

Tools:



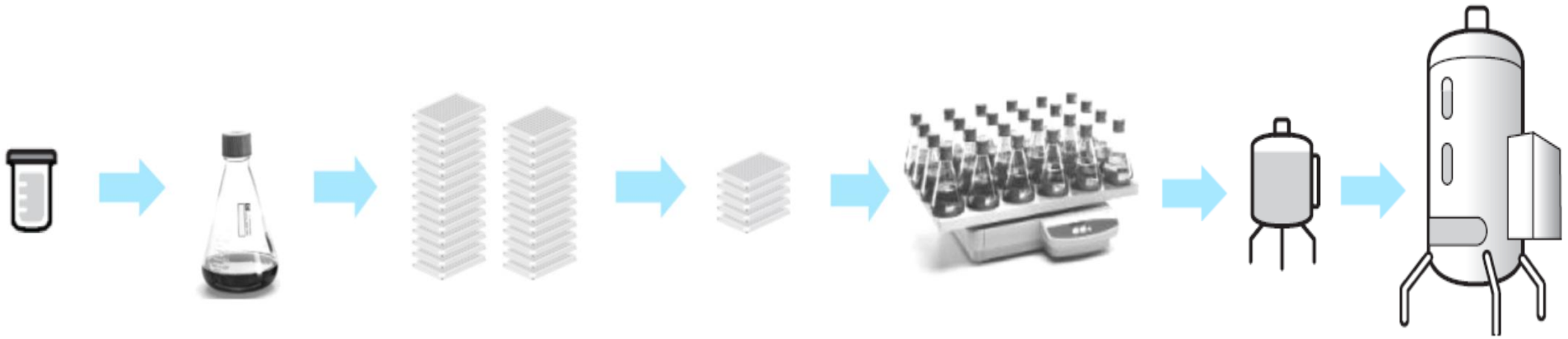


Source: Plasmid-mediated gene therapy for cardiovascular disease. Cardiovasc Res. 2011 Sep
Source: Genetic Engineering and Biotechnology news.

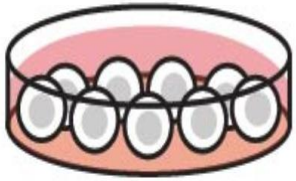
Clone Selection for Production

Expansion

Clone screening and further expansion



Process optimization

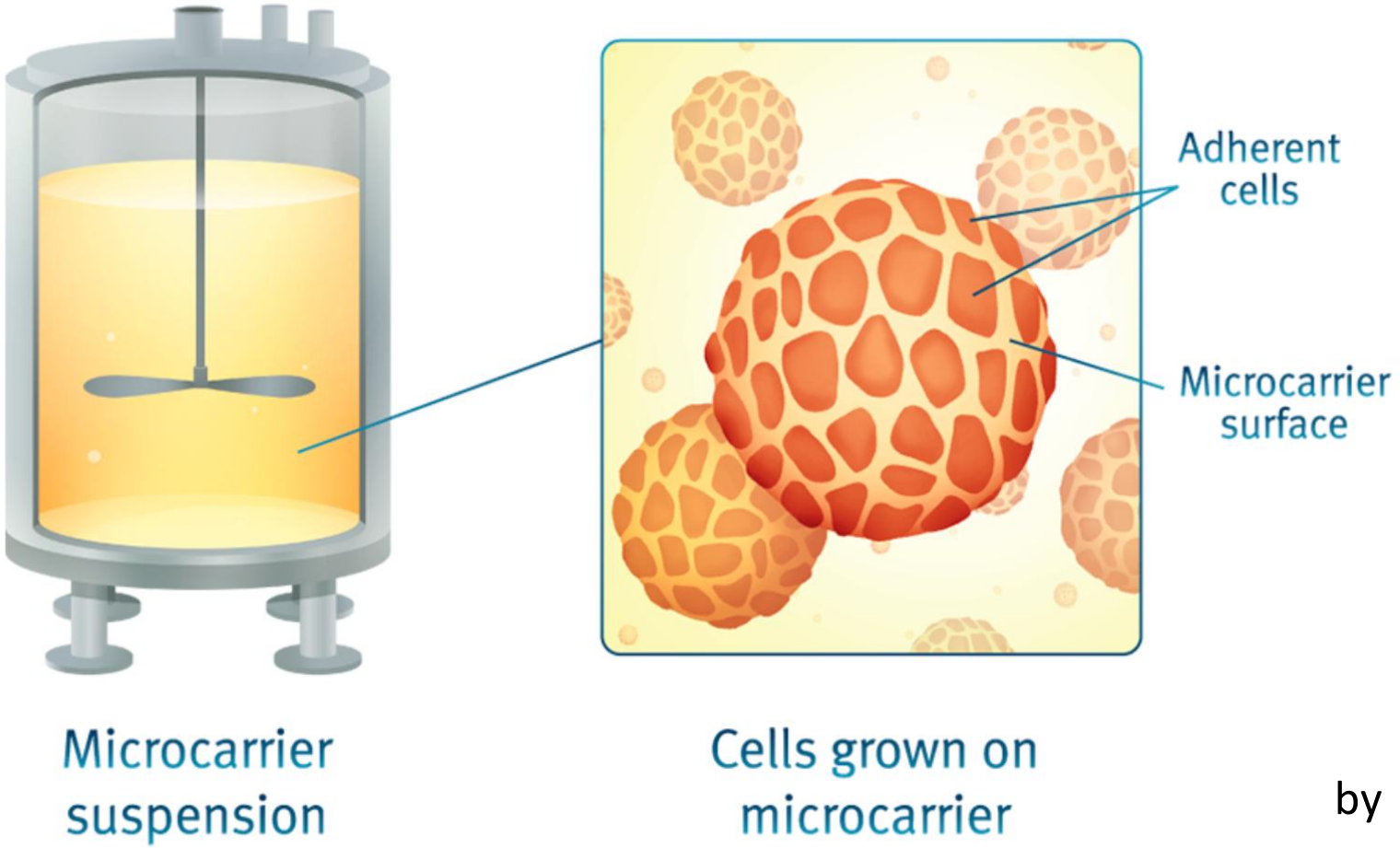


Adherent Cells

Suspended cells

Source: Maxcyte.com

Clone Selection for Production

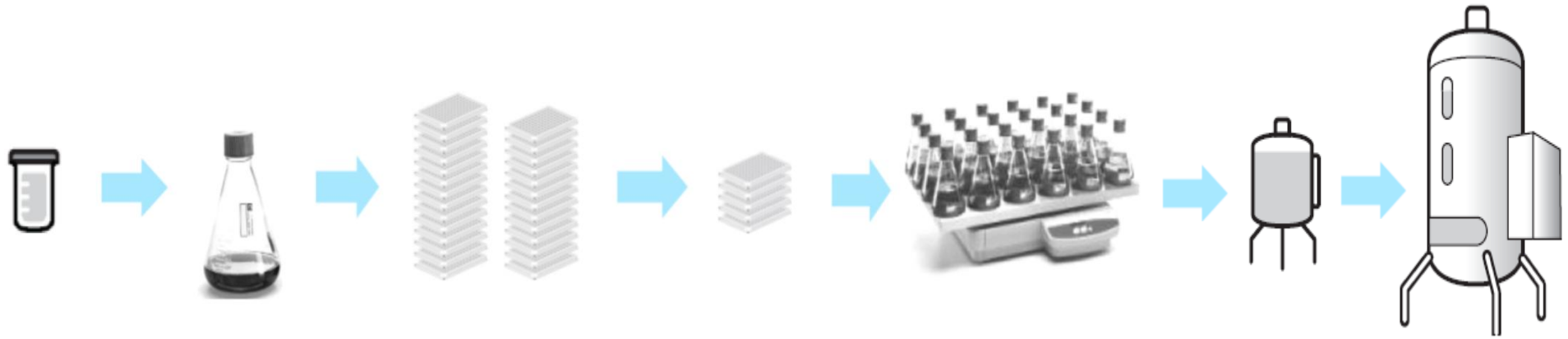


by chemometec

Clone Selection for Production

Expansion

Clone screening and further expansion



Process optimization

Serum-rich Protein-rich media

Serum-free Protein-free media

Optimum performance

Inconsistent quality
Regulatory Challenging

Sub-optimal performance

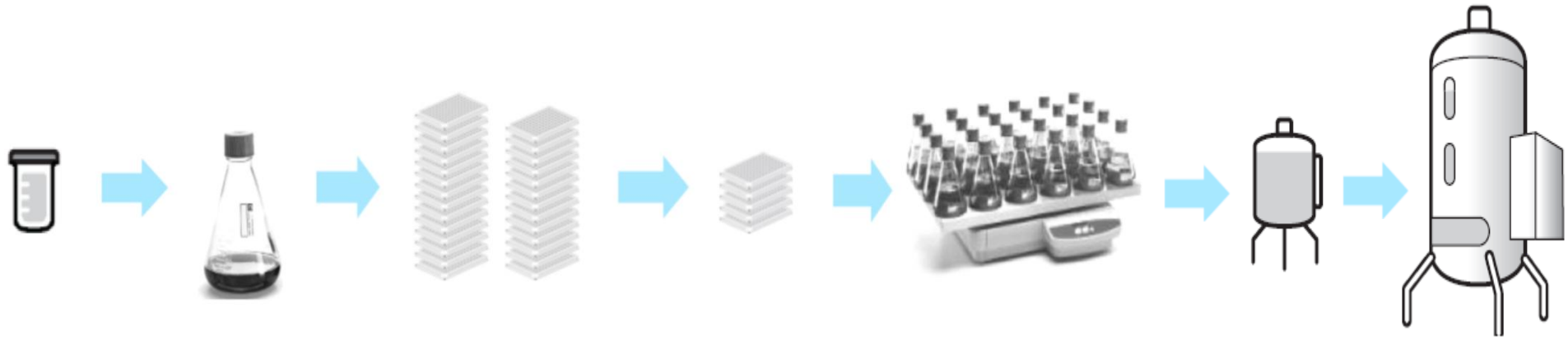
Consistent quality
Regulatory accepted

Source: Maxcyte.com

Clone Selection for Production

Expansion

Clone screening and further expansion

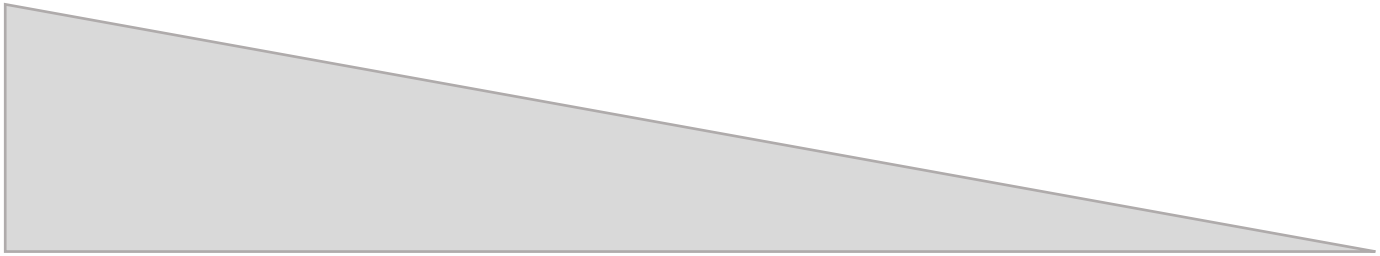


Process optimization

Hundreds of clones **One clone**

Source: Maxcyte.com

Clone Selection for Production



Hundreds of clones

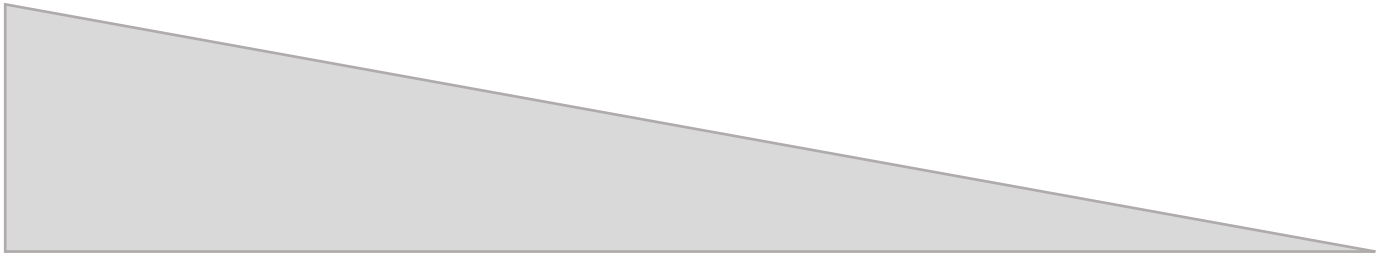
- Robust cell growth
- High productivity

Selection criteria

One clone

- Robust cell growth
- Cell-specific and volumetric productivity
- Clone stability
- Glycosylation profiles
- Development of charge variants
- Aggregate formation
- Protein sequence heterogeneity

Clone Selection for Production



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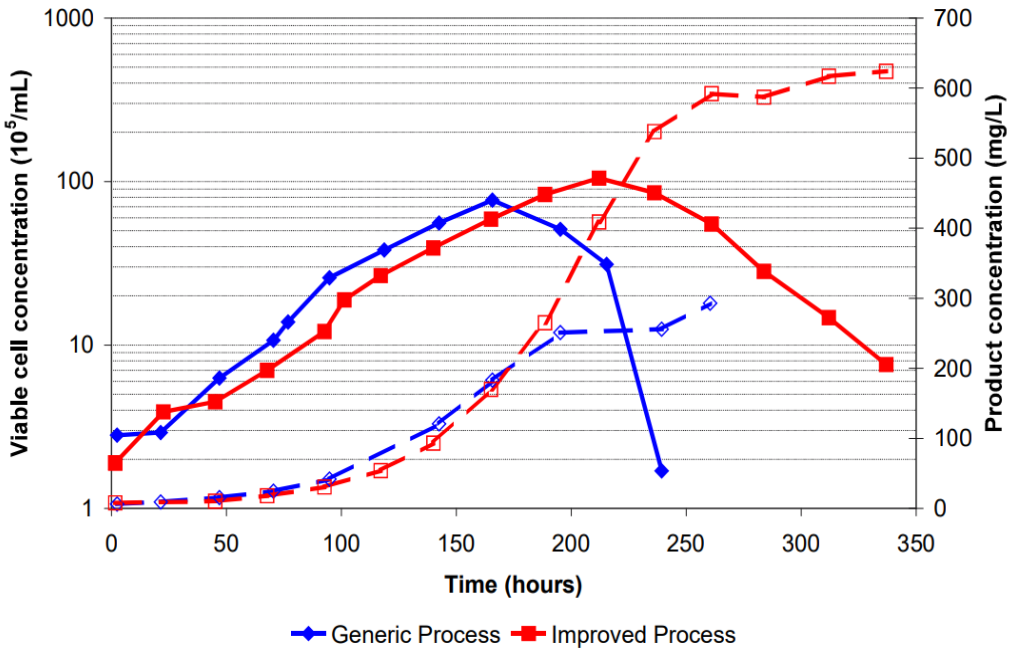

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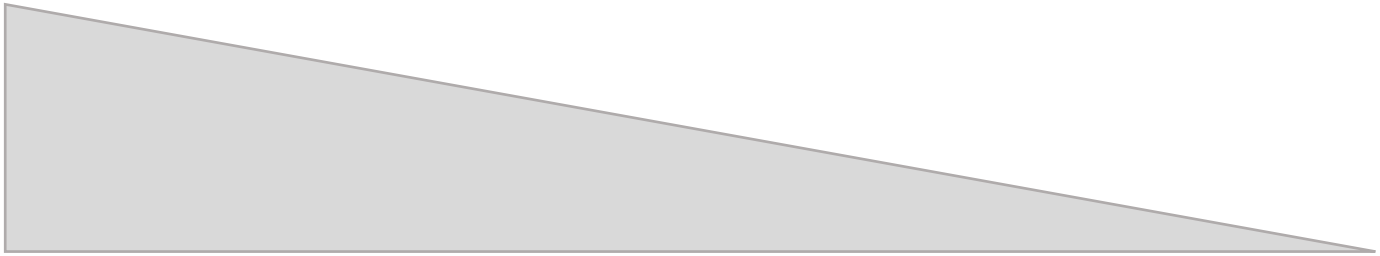
Source: Development of Mammalian Cell Bioreactor Processes: Increasing Productivity and Process Robustness . J. Wayne 2010

Clone Selection for Production



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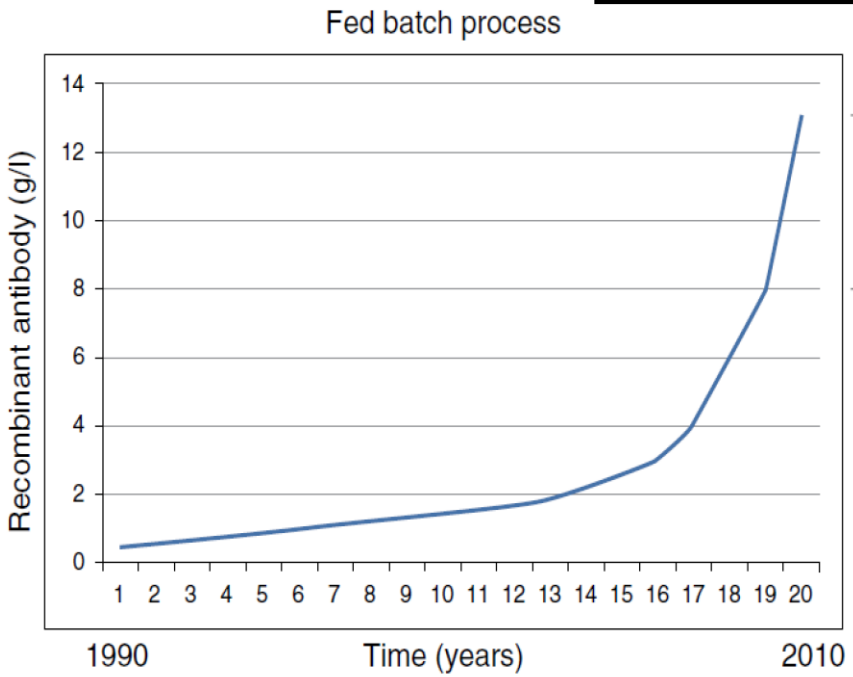
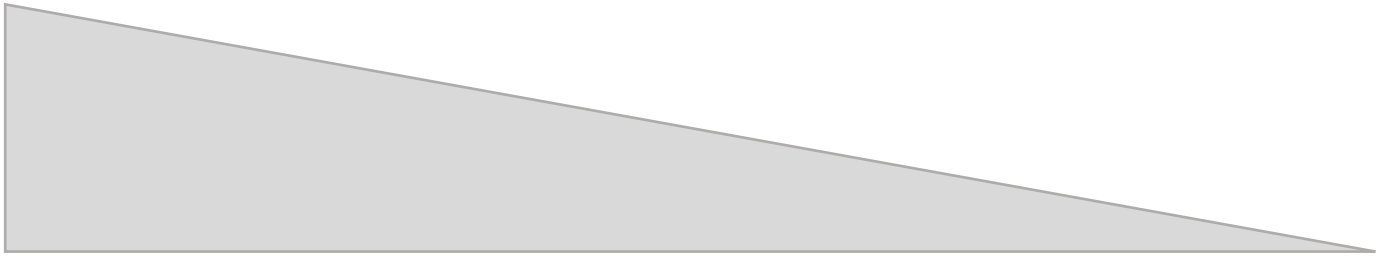


Source: Biosimilars of Monoclonal Antibodies: A Practical Guide to Manufacturing, Preclinical, and Clinical Development. R Sodoyer 2017.

Clone Selection for Production



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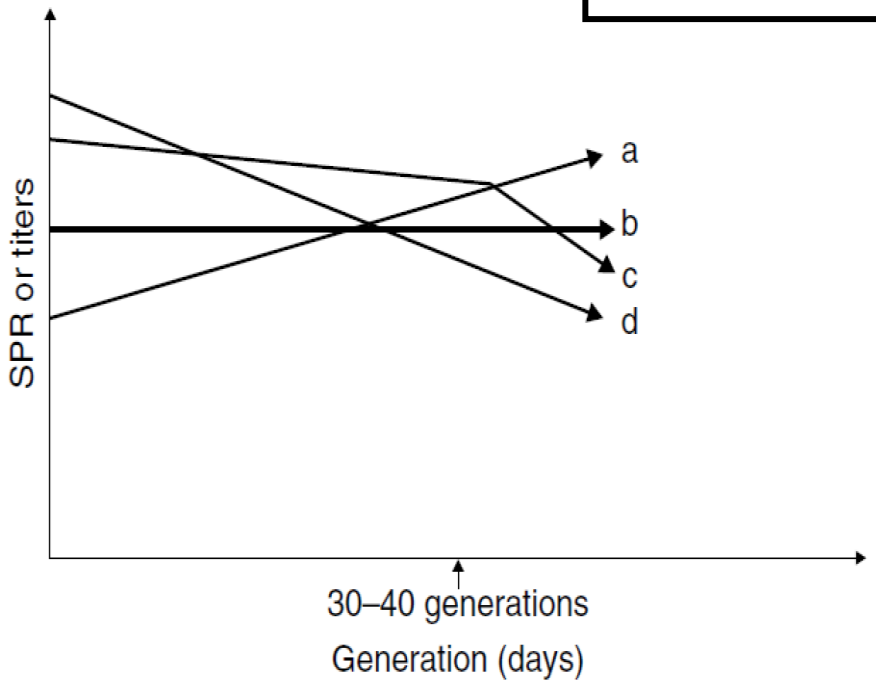

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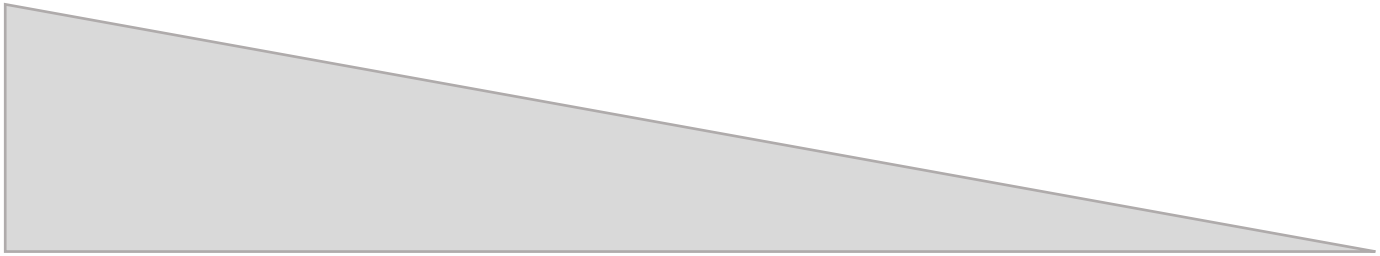

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Selection criteria

One clone

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- Glycosylation profiles
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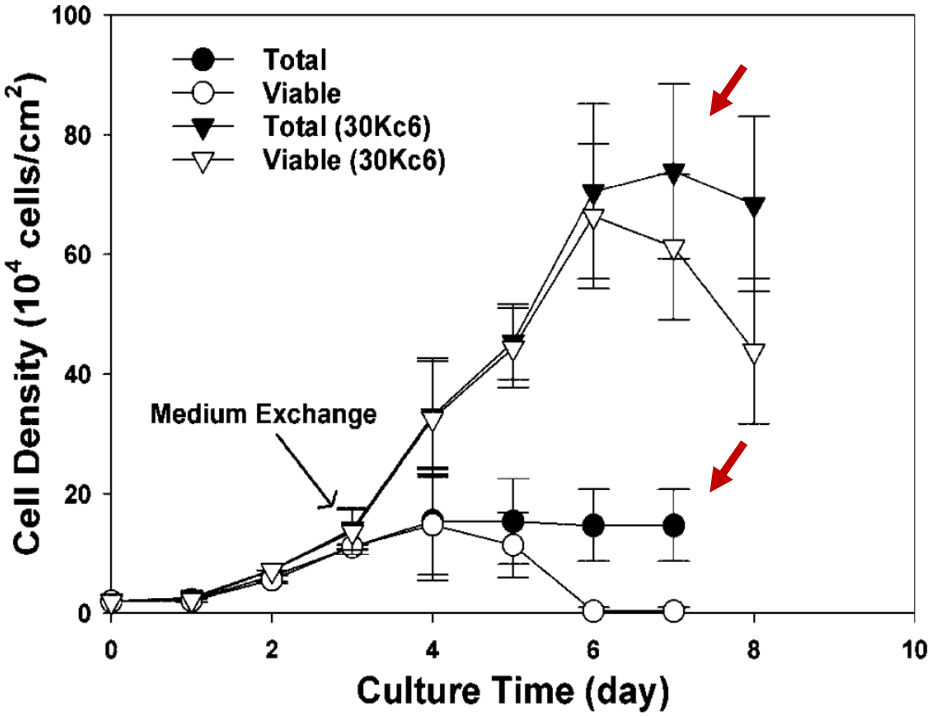
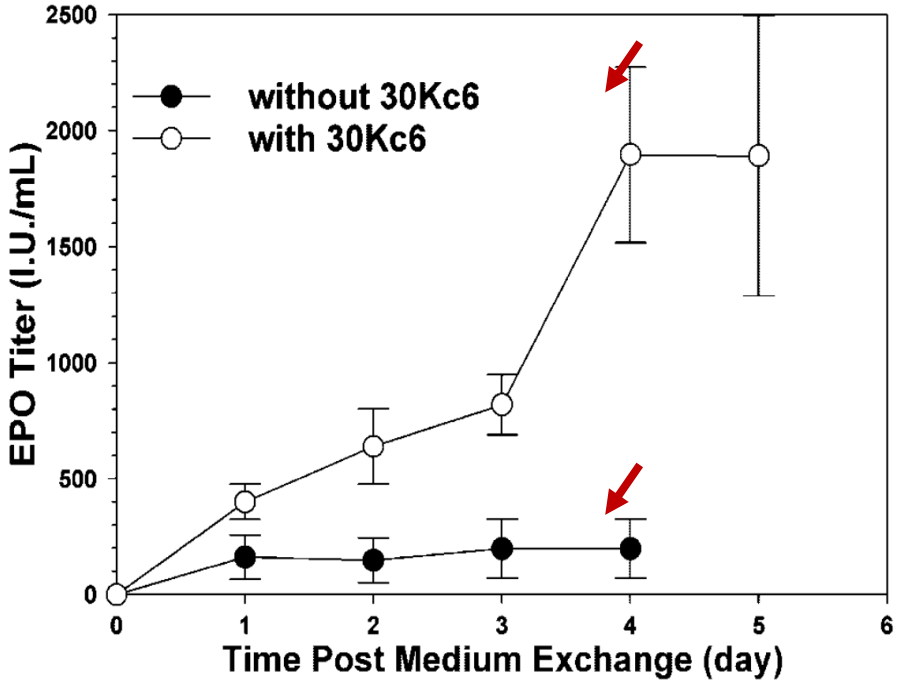
- Protein sequence heterogeneity

Host Cell Line Engineering

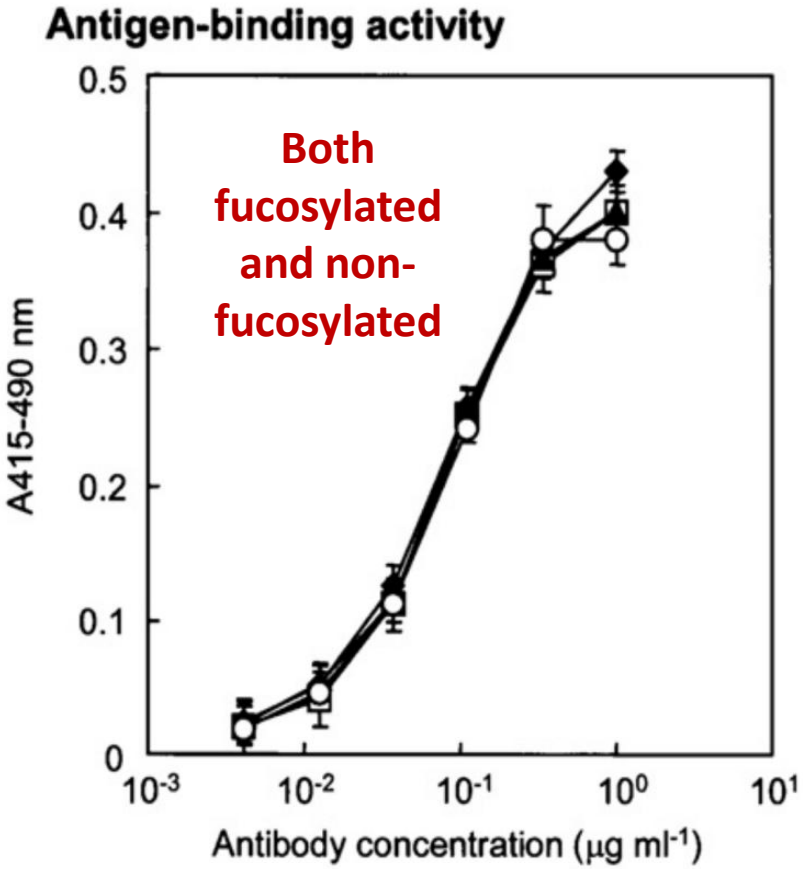
1- Overexpressing anti-apoptotic genes:

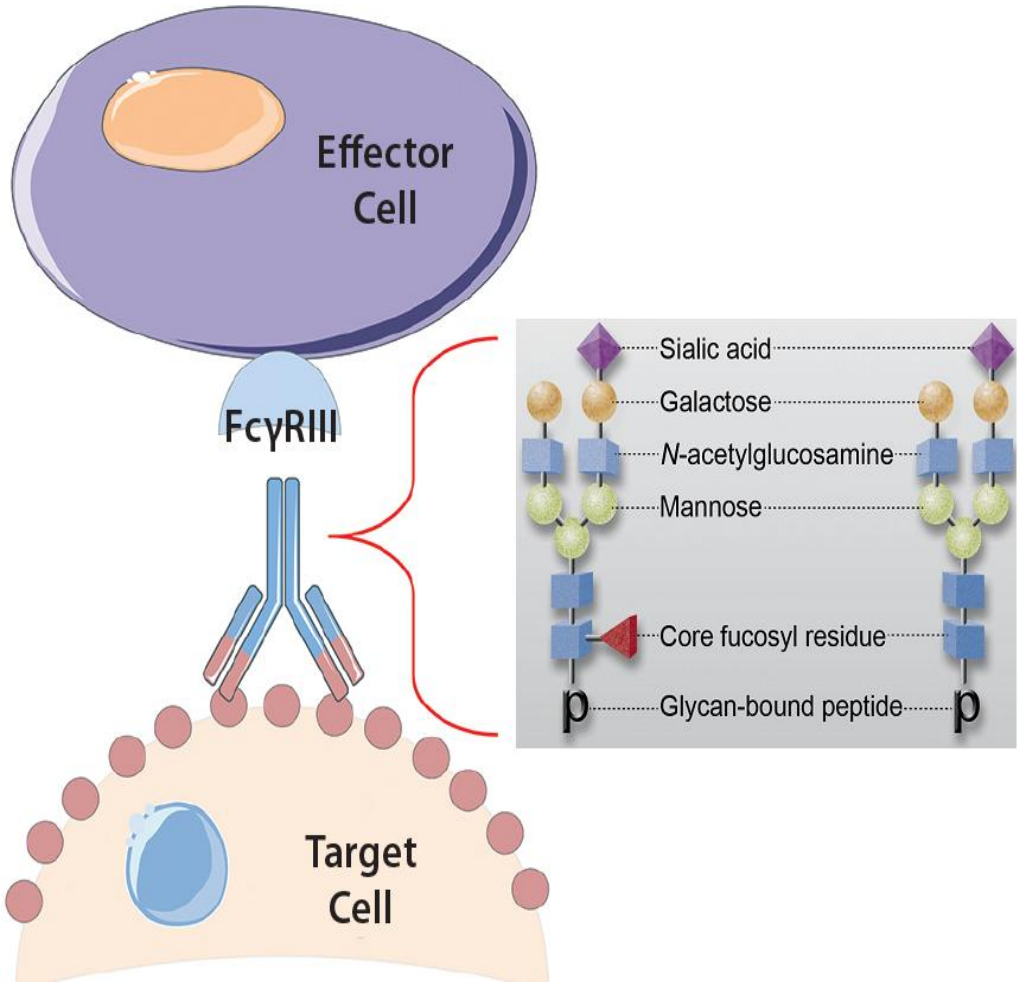




Host Cell Line Engineering

2- Altering the glycosylation profile:





Source: Production of therapeutic antibodies with controlled fucosylation. MABs 2009

Host Cell Line Engineering

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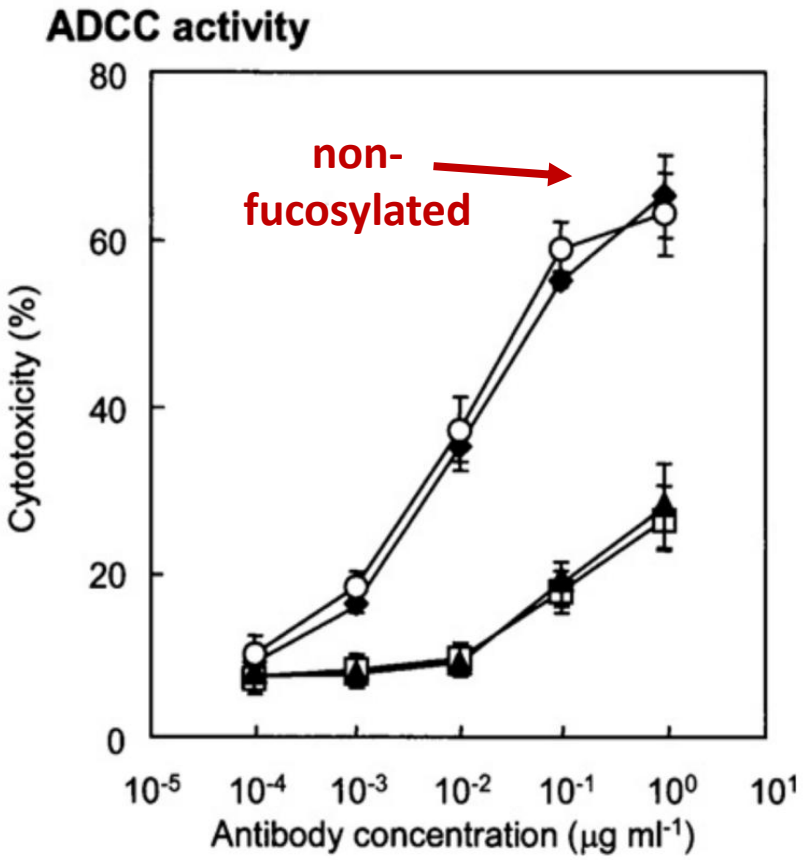

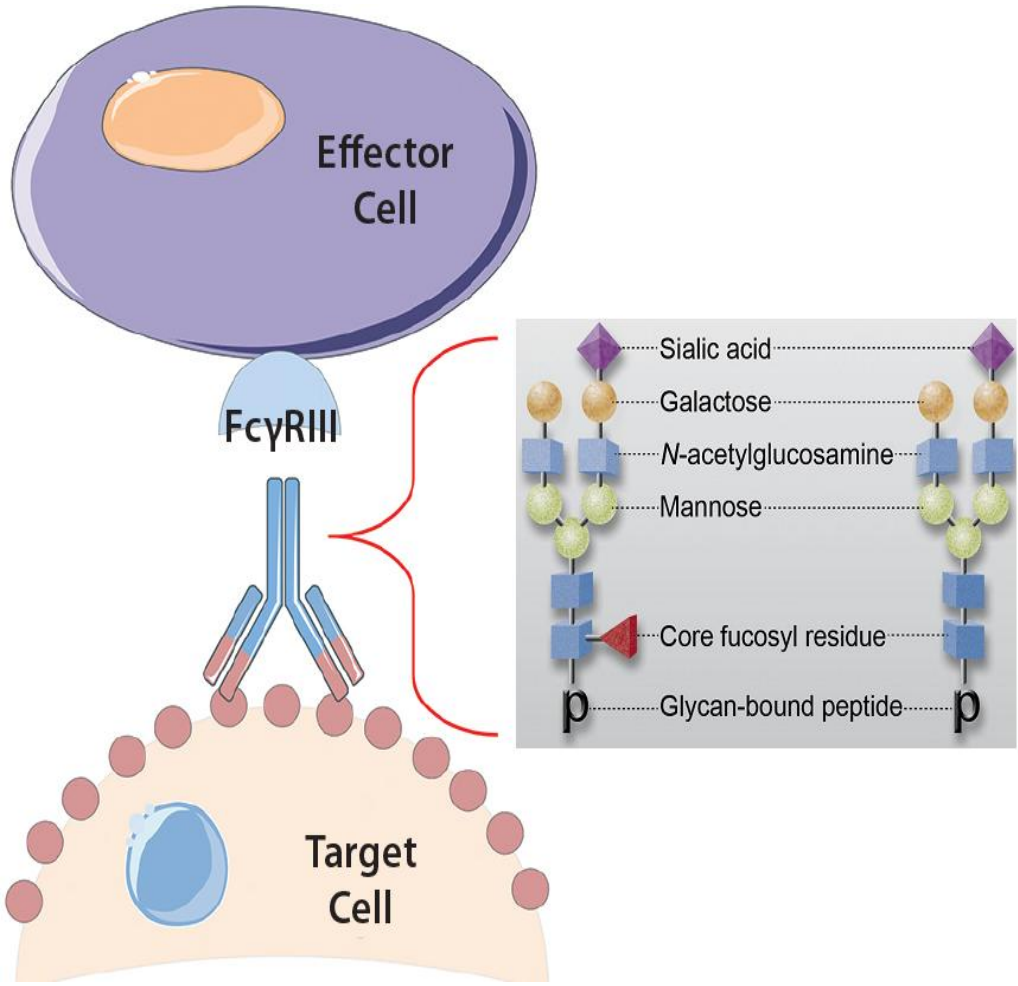

Concluding Remarks

- Cell line and cell culture:

- The most critical component in biosimilar manufacturing processes.
- The greatest source of variability.
- Always accompanied with strict analytical tests.

Thank you!