Leap In Transposase Platform

It's what you paste …

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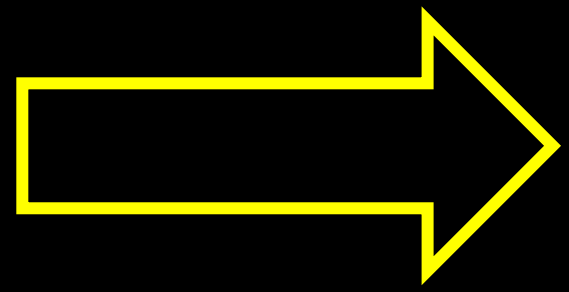
Transposase – Transposon: all you need to know

Cut  Paste

Leap-In Transposase CLD platform

- Expression construct integrity maintained
- Rapid, robust and representative bulk pool generation
- Robust, high titer and extremely stable clones

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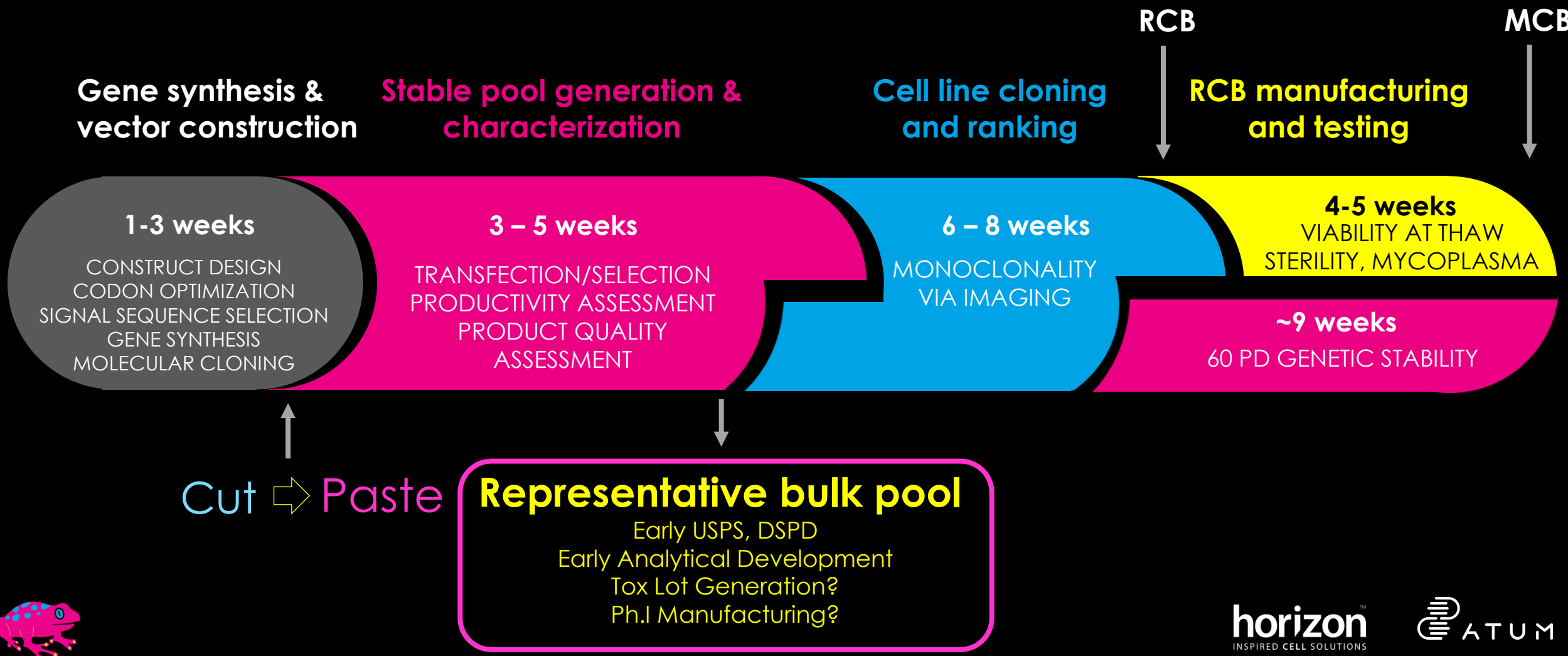
transfection to RCB in ~10-12 weeks



robust market adoption

- Launched ~4 years ago
- Offered as a service by ATUM: >150 projects delivered
- ~40 active licensees: 5 of top 7 pharma
- 20 regulatory filings in ~3 years: Including Ph.II/III

IT WORKS

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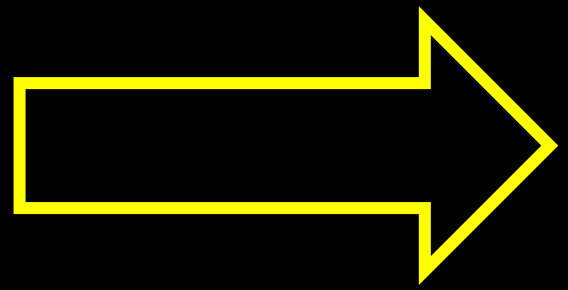
fundamental mechanism

Cut  Paste

… what you paste matters …

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moving beyond the routine

- Reduction of target gene expression

the miLPN platform

Use Leap In Transposase platform to reduce gene expression

miCHO-GS

- K1 derived
- GS deficient
- GMP Cell Bank

miFuc

- Reduced fucoyslation
- Increased ADCC
- Vector based
- Host cell agnostic

miLPN

- Custom projects

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the miLPN platform

Use Leap In Transposase platform to reduce gene expression

miCHO-GS	miFuc	miLPN
• K1 derived • GS deficient • GMP Cell Bank	• Reduced fucoyslation • Increased ADCC • Vector based • Host cell agnostic	• Custom projects

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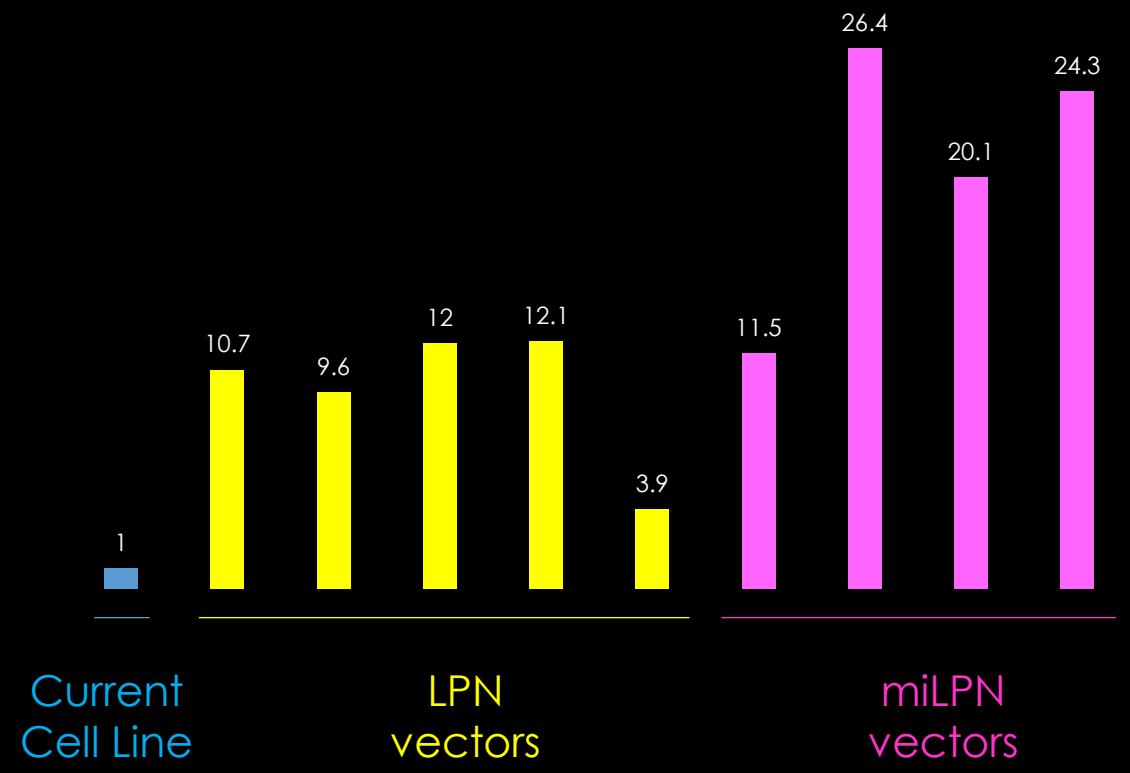
miLPN platform: custom project

Overview

Approved cytokine therapeutic
Low expressor

Cytokine may inhibit expression/cell growth via interaction with endogenous CHO receptor

Use miLPN technology to express cytokine and reduce endogenous receptor expression on host cell



Rentschler Biopharma

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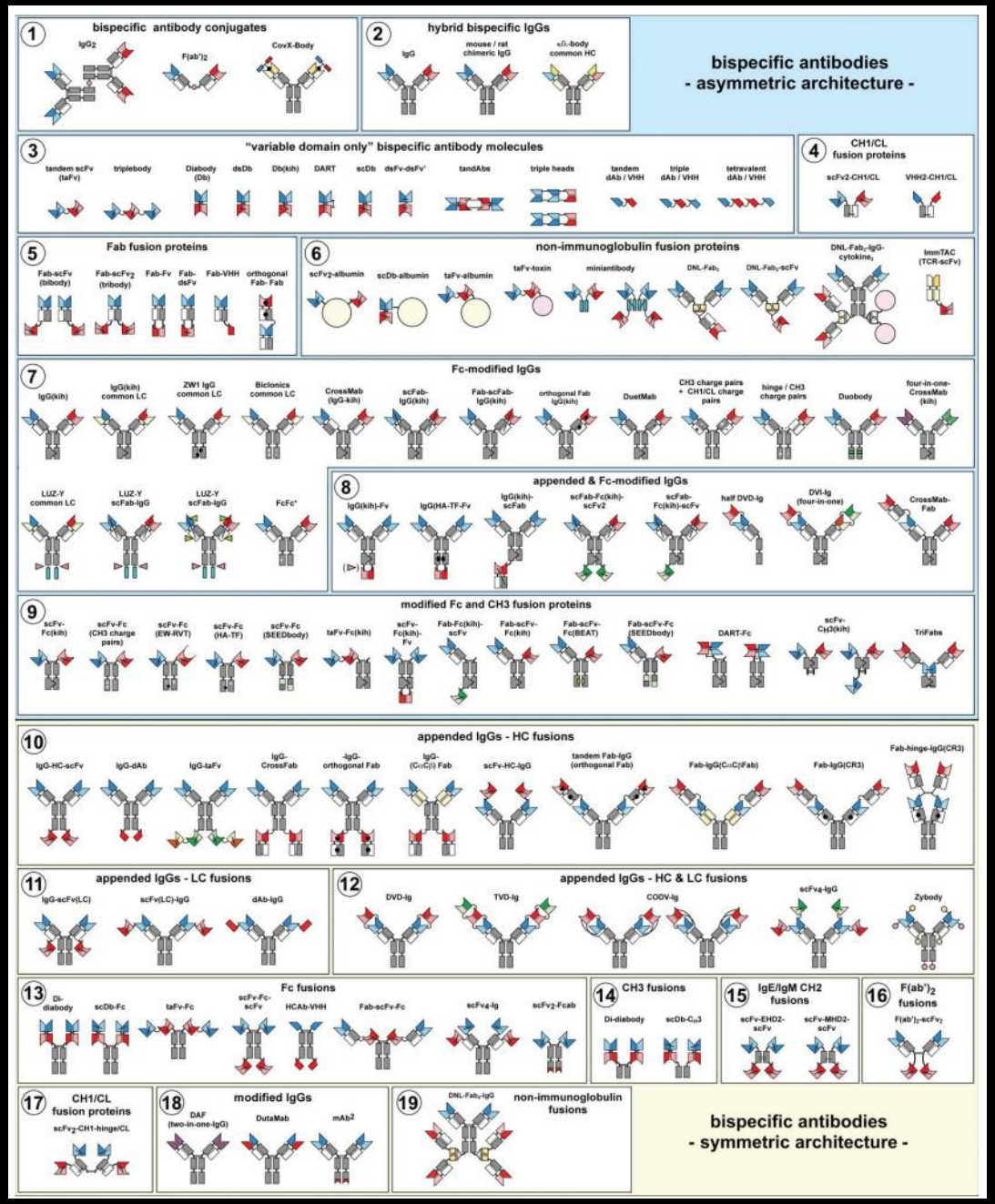
moving beyond the routine

- Reduction of target gene expression
- Chain ratio balancing for titer and product quality



Brinkmann and Kontermann; 2017

3 chains and more

The "zoo" of bispecifics

Chain ratio balancing is key

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considerations for chain ratio balancing

Sequence

- Codon choice
- mRNA 2º structure
- Poly-A signal
- 5'/3' UTR choice
- mRNA stability
- Ribosomal entry/processivity
- Splice site donor/acceptor
- Signal sequences
- Etc.

Vector

- Promoter choice
- Order of expression cassettes
- Number of expression cassettes
- Spacing of expression cassettes
- Directionality of expression cassettes
- Size of vectors
- Single vector or multiple vectors
- Choice of insulators
- Etc.

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case study: 3-Chain bispecific mAb

- Known to be difficult
  - Low titer
  - Poor assembly
- 14 vector configurations
  - Varying expression ratios
  - Varying expression levels
- Leap In Transposase based pool selection
- Analytical assessment
  - Total titer
  - Chain expression: Relative and Amount
  - % Bispecific

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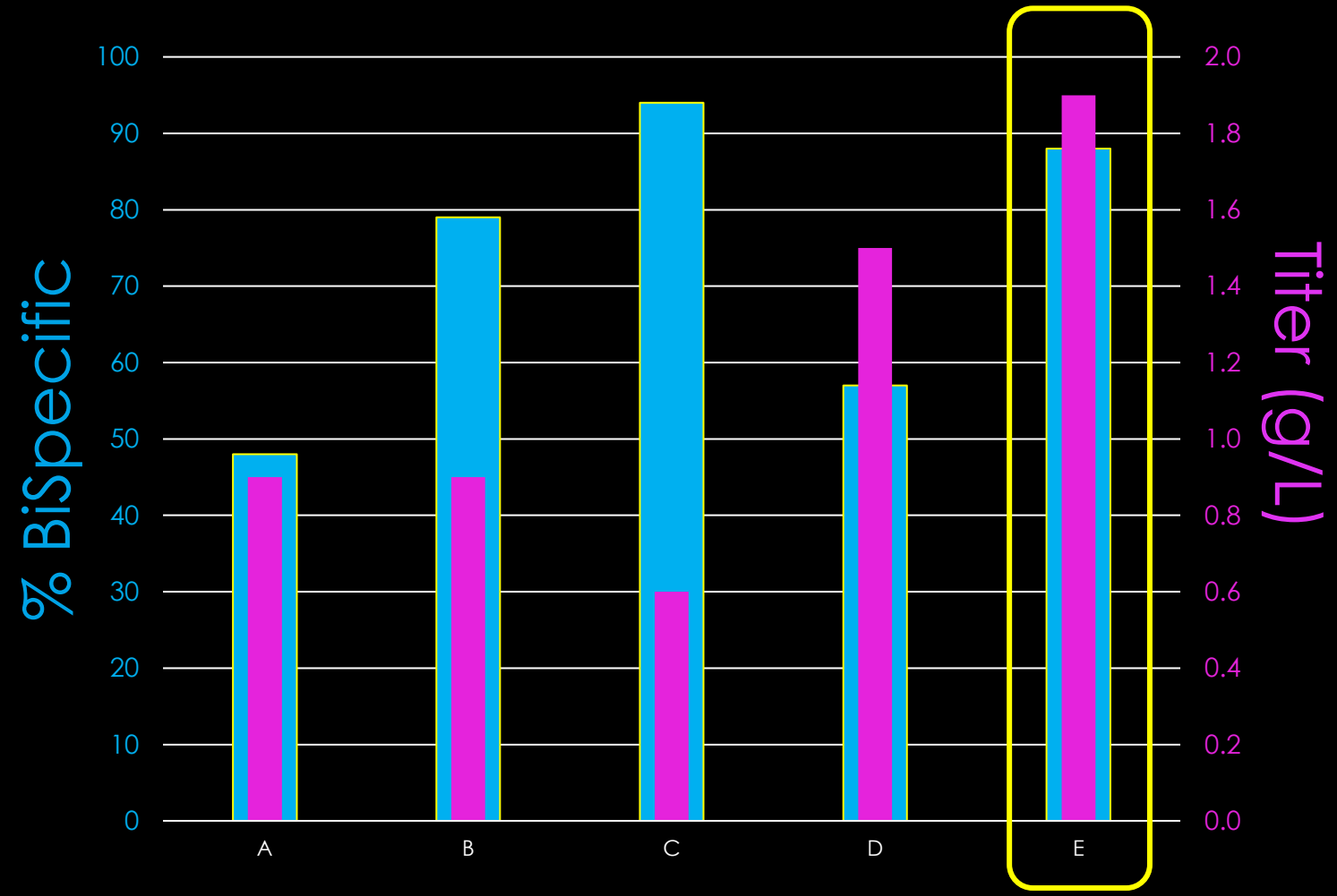
case study: 3-Chain Bispecific mAb - bulk pools



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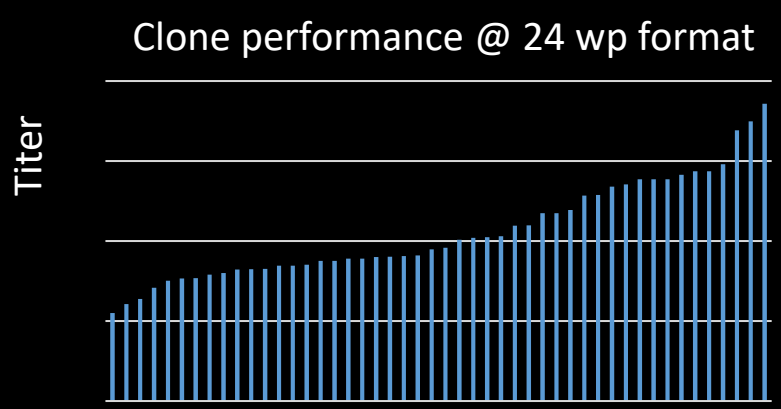
case study: 3-Chain biSpecific mAb - clones

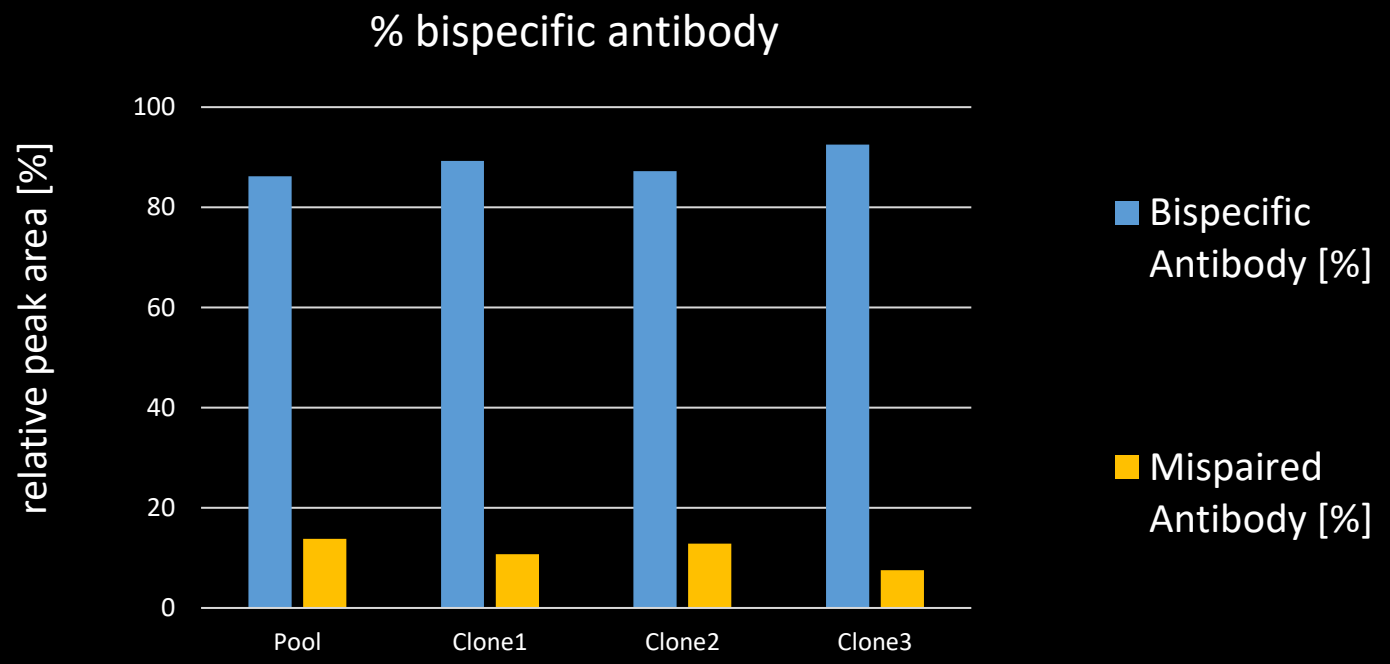
Pool E



Pool and clone productivity

Pool*	derived clones*
1.9 [g/L]	up to 5.5 [g/L]

**Day 12 standard fed-batch*



Good pools predict good clones

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moving beyond the routine

- Reduction of target gene expression
- Chain ratio balancing for titer and product quality
- Bulk cell pools for clinical trial manufacturing?

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cell pools for speeding timeline to IND

Advantages

Reduced timelines to IND

Reduced cost to IND

Risks

Low titer

Expression and product quality stability

Pool product quality ≠ Clone product quality

Pool Requirements

Clone like expression titer

Expression and product quality stability

Comparable product quality to derivative clones

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robust high titer stable bulk CHO pools

Protein	Volumetric productivity
IgG1	7.7 g/L
IgG1	7.9 g/L
IgG1	5.5 g/L
IgG1	5.3 g/L
IgG4	5.0 g/L
IgG4	5.0 g/L
Fc Fusion	3.5 g/L
3 ORF Bispecific	~8 g/L
3 ORF Bispecific	~7 g/L
3 ORF Bispecific	~3 g/L

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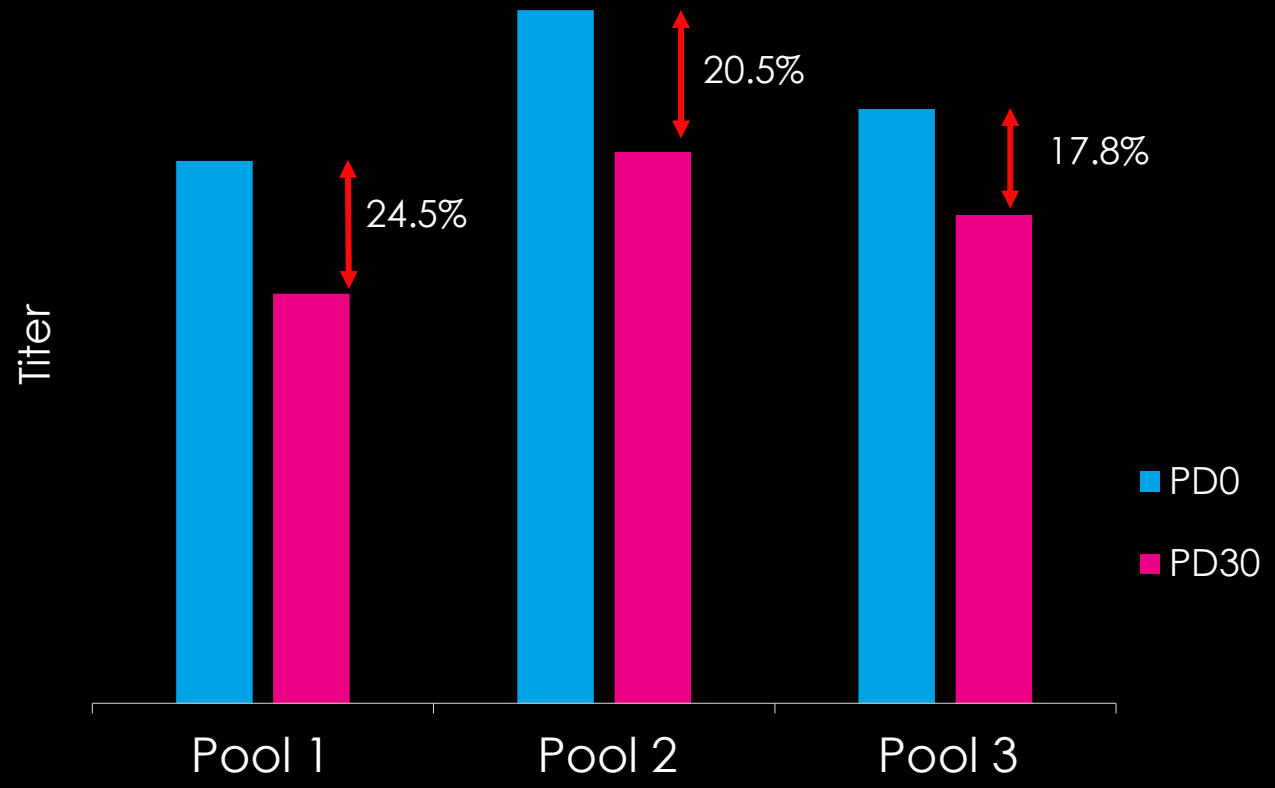
bulk pools: expression stability



- ~80% productivity maintained
- Acceptable criteria for expression stability for Ph.I manufacture

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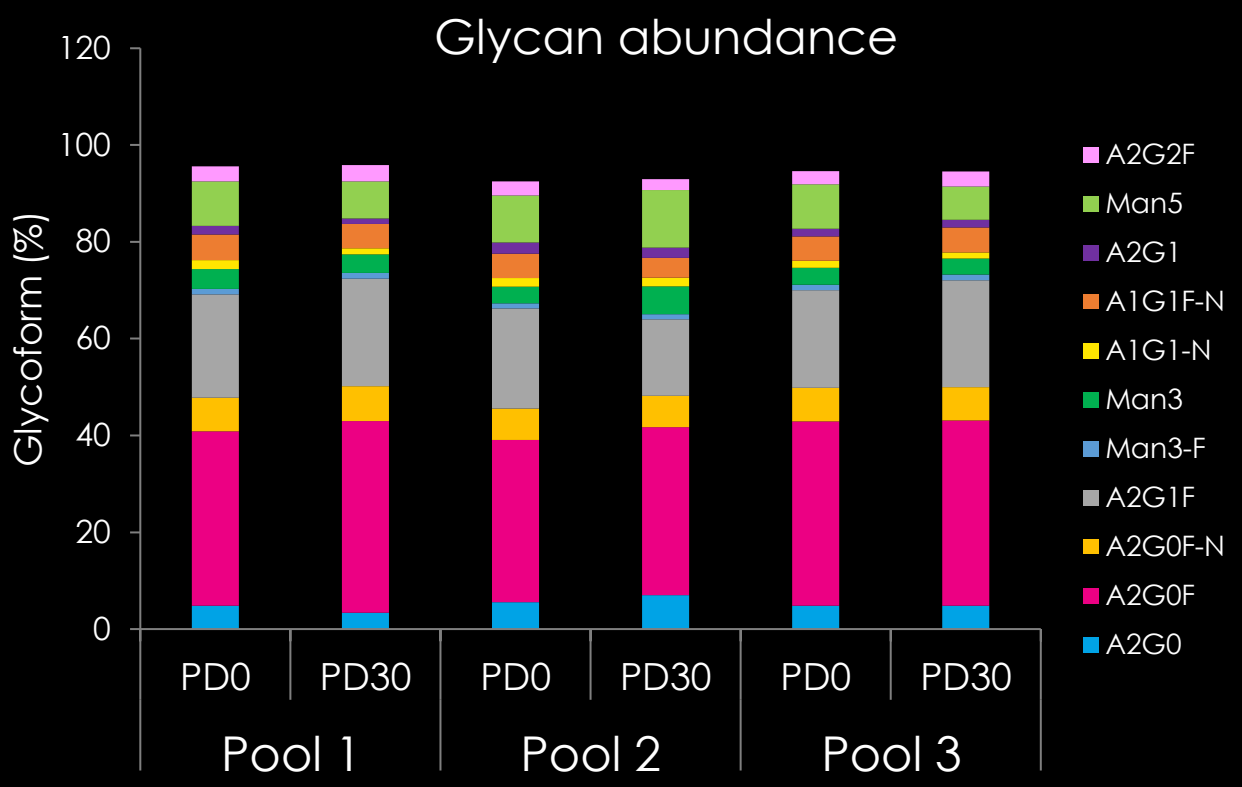
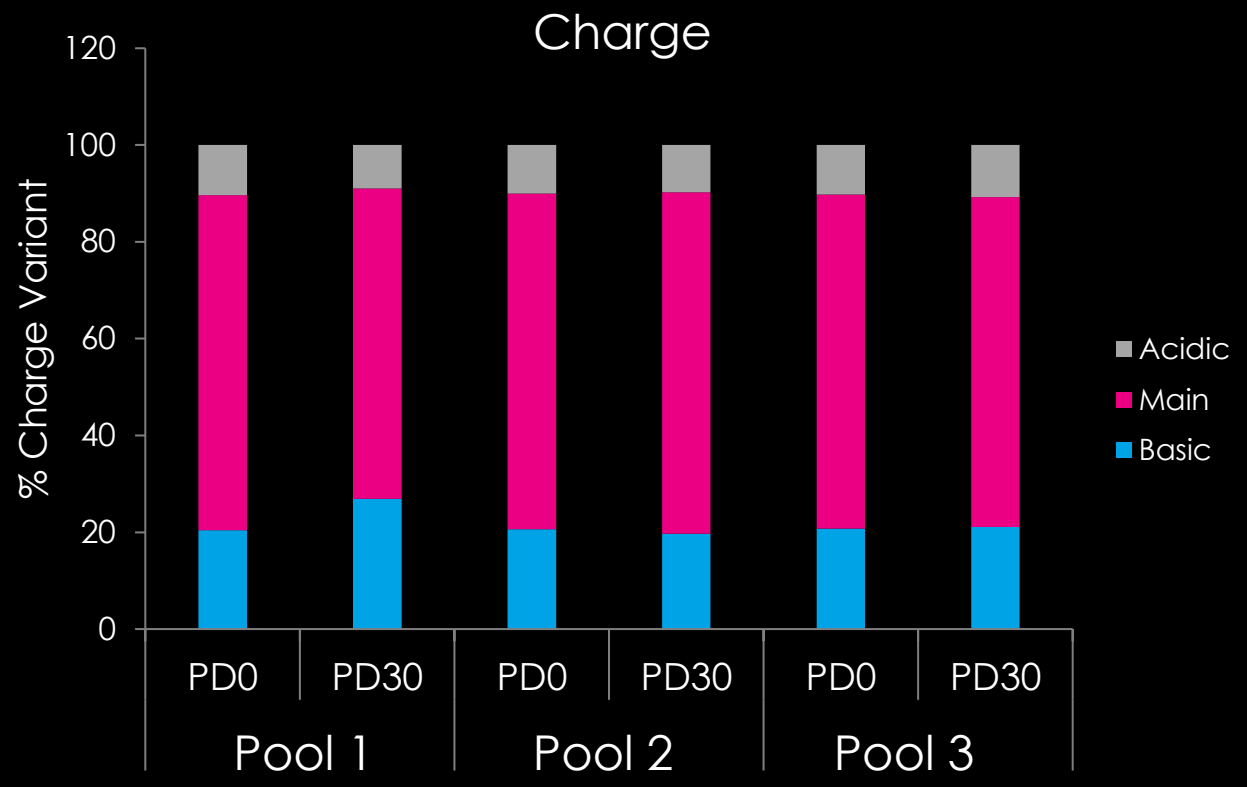
Bulk pools: product quality stability





Consistent product quality of stable pools

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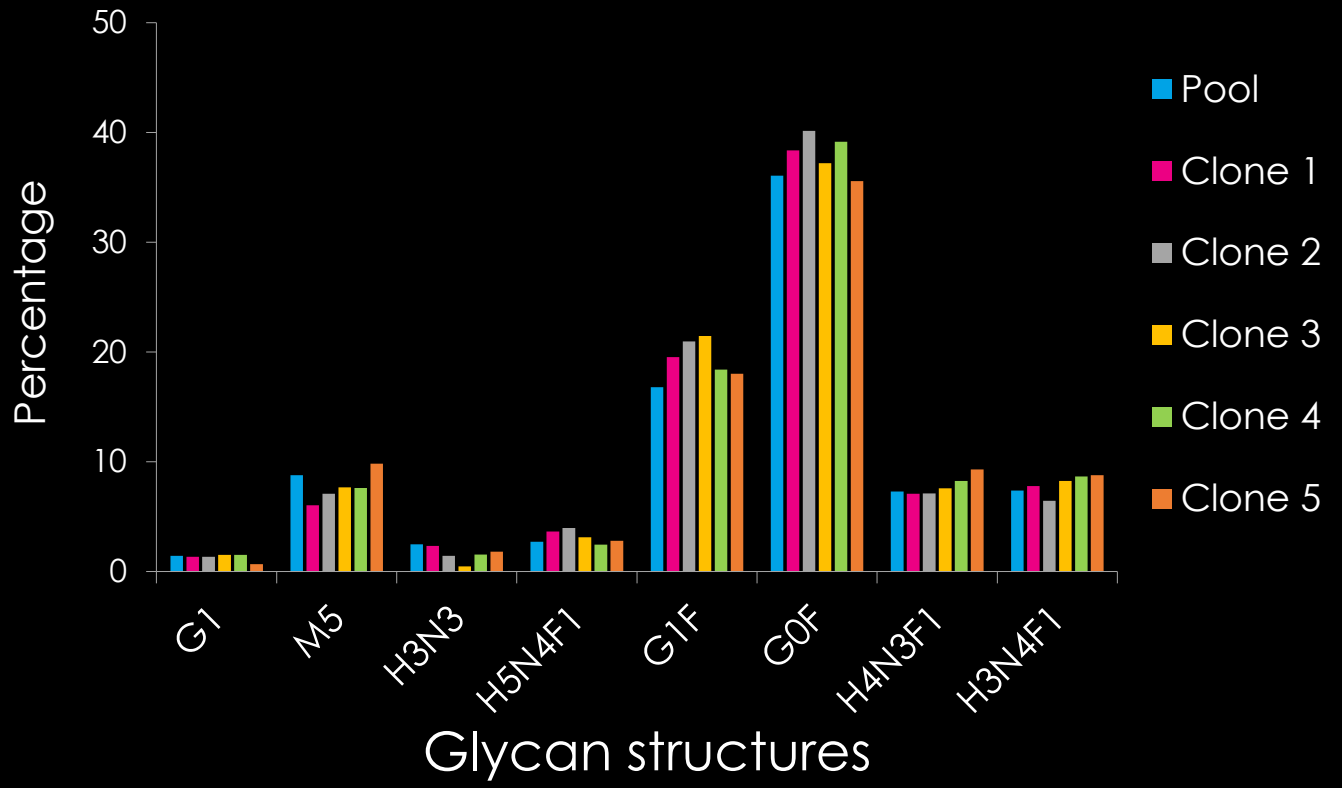
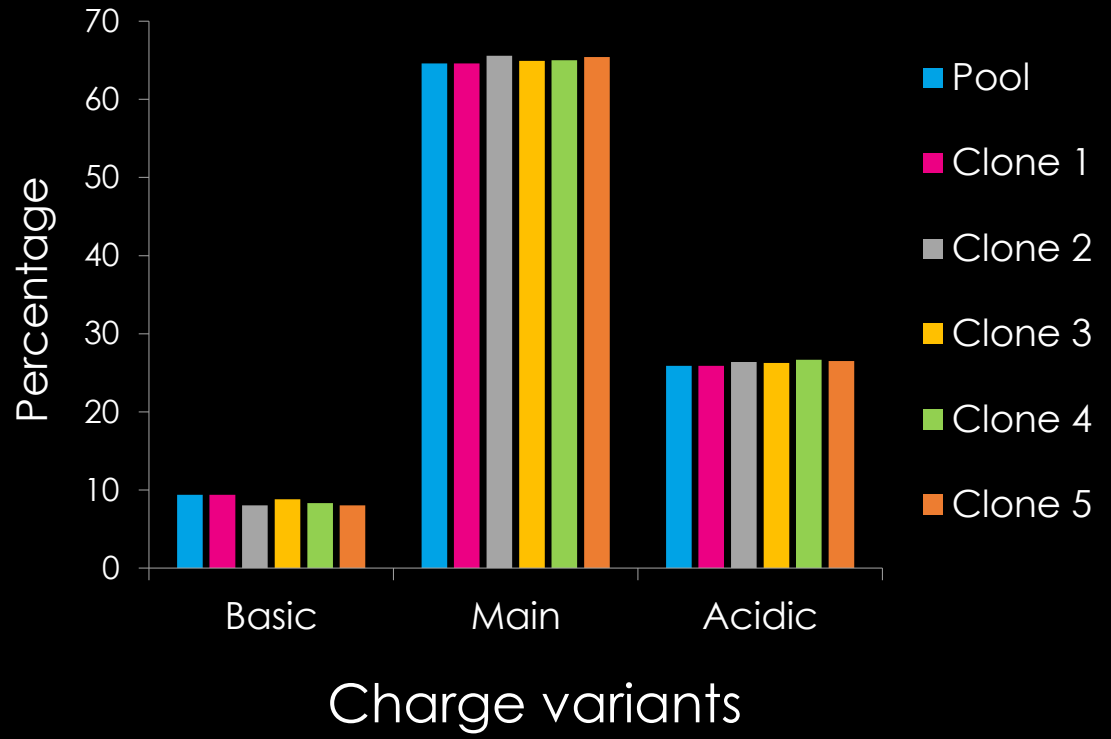
bulk pools to clones: product quality

Glycans: Pool vs Clones



Charge variants: Pool vs Clones



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bulk cell Pools for speeding timeline to IND

Advantages

Reduced timelines to IND

Reduced cost to IND

Risks

Low titer

Expression and product quality stability

Pool product quality ≠ Clone product quality

Pool Requirements

Clone like expression titer

Expression and product quality stability

Comparable product quality to derivative clones

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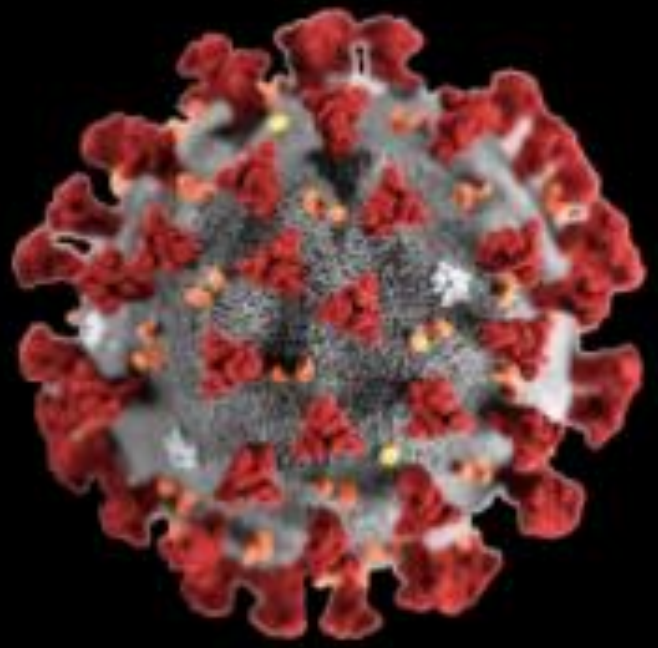
REGULATORY RISK

antibodies For COVID 19 treatment



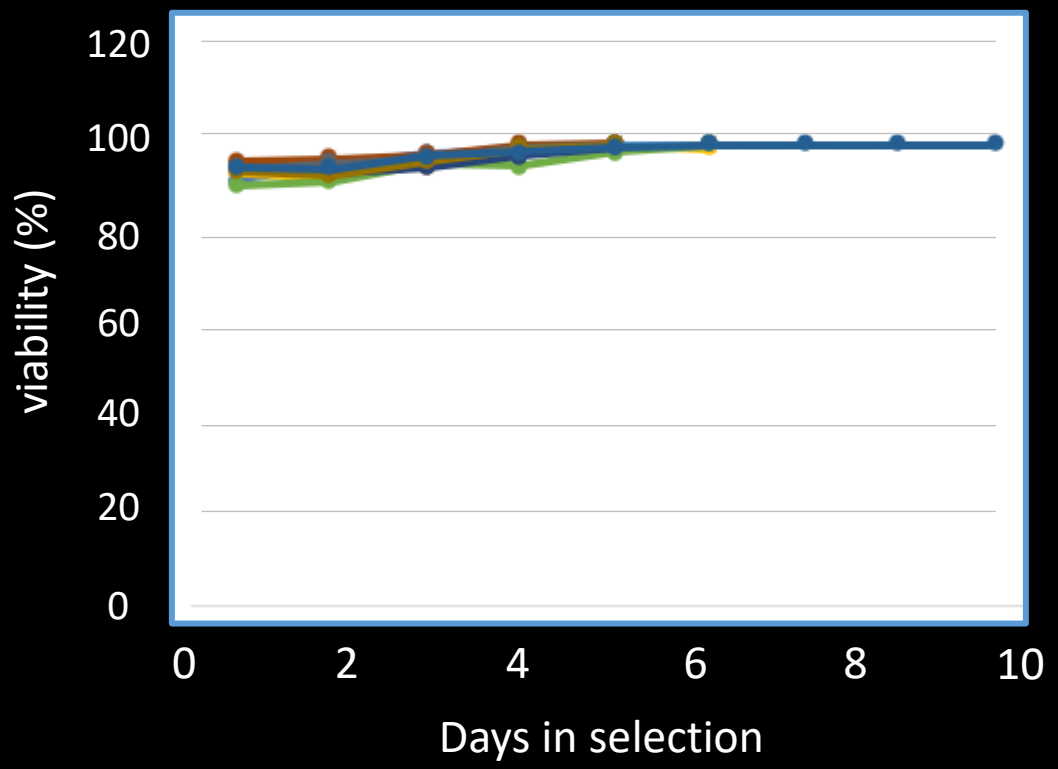
- Eleven candidate therapeutic mAb’s
- Desire to initiate human trials ASAP
- Rapid progress: sequence to Ph.I
- Use cell pools for GMP manufacturing

rapid cell line development: bulk pools

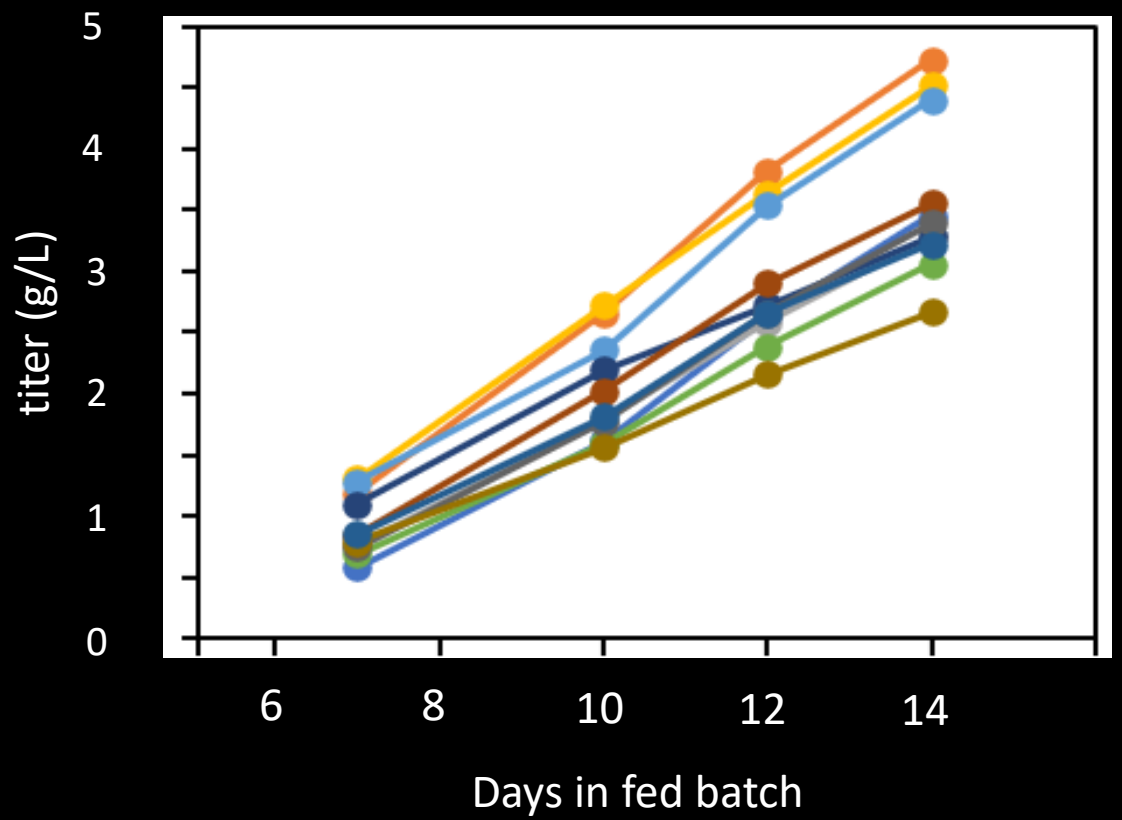
- Two vector sets for each of 11 mAb's
- Create Leap-In Transposase® derived pools
- Test expression in platform fed-batch format
- Freeze RCB's for transfer to CDMO

COVID 19: ATUM accelerated timeline: 1

Vector-2



Vector-2



Selection in ~3 days

Titer: 2.5 – 4.8 g/L

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COVID 19: ATUM Accelerated Timeline: 1

Gene synthesis → Plasmid prep → Pool Selection → Pool Banking → Bank Testing → Fed Batch → Ship to CDMO

38 days

IND filed

CDMO: Intensified fed batch process

>12 g/L

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COVID 19: ATUM accelerated timeline: 2

Rapid cGMP Manufacturing of COVID-19 monoclonal antibody using stable CHO cell pools

Rita Agostinetto[1], Jessica Dawson[2], Angela Lim[2], Mirva Hejjaoui-simoneau[3], Cyril Boucher[3], Bernhard Valldorf[4], Adin Ross-gillespie[3], Joseph Jardine[5], Devin Sok[5], Dennis Burton[5], Thomas Hassell [6], Hervé Broly[7], Wolf Palinsky[3], Philippe Dupraz[3], Mark Feinberg[6], and Antu Dey[8]

[1]Merck Serono SpA
[2]EMD Serono Biotech Center Inc
[3]Ares Trading SA
[4]Merck KGaA
[5]The Scripps Research Institute
[6]International Aids Vaccine Initiative
[7]Merck Serono SA-Corsier-sur-Vevey
[8]Greenlight Biosciences Inc

Preprint on Authorea.com

Pools — 6.0 g/L

↓

200L — Preclinical Safety

↓

2000L — Phase I

"‥ Enabled manufacturing of early clinical trial material within 4.5 months …"

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COVID 19: ATUM accelerated timeline: 3

Towards Maximum Acceleration of Monoclonal Antibody Development: Leveraging Transposase-Mediated Cell Line Generation to Enable GMP Manufacturing within 3 Months using a Stable Pool

Valerie Schmieder[1], Juergen Fieder[1], Raphael Drerup[2], Erik Arango Gutierrez[2], Carina Guelch[3], Jessica Stolzenberger[4], Mihaela Stumbaum[5], Volker Steffen Mueller[6], Fabian Higel[6], Martin Bergbauer[7], Kim Bornhoefft[8], Manuel Wittner[9], Petra Gronemeyer[10], Christian Braig[11], Michaela Huber[12], Anita Reisenauer-Schaupp[13], Markus Michael Mueller[14], Mark Schuette[15], Sebastian Puengel[1], Benjamin Lindner[1], Moritz Schmidt[1], Patrick Schulz[1] and Simon Fischer[1,*]

1: Cell Line Development, Bioprocess Development Biologicals, Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany

Journal of Biotechnology, 2022

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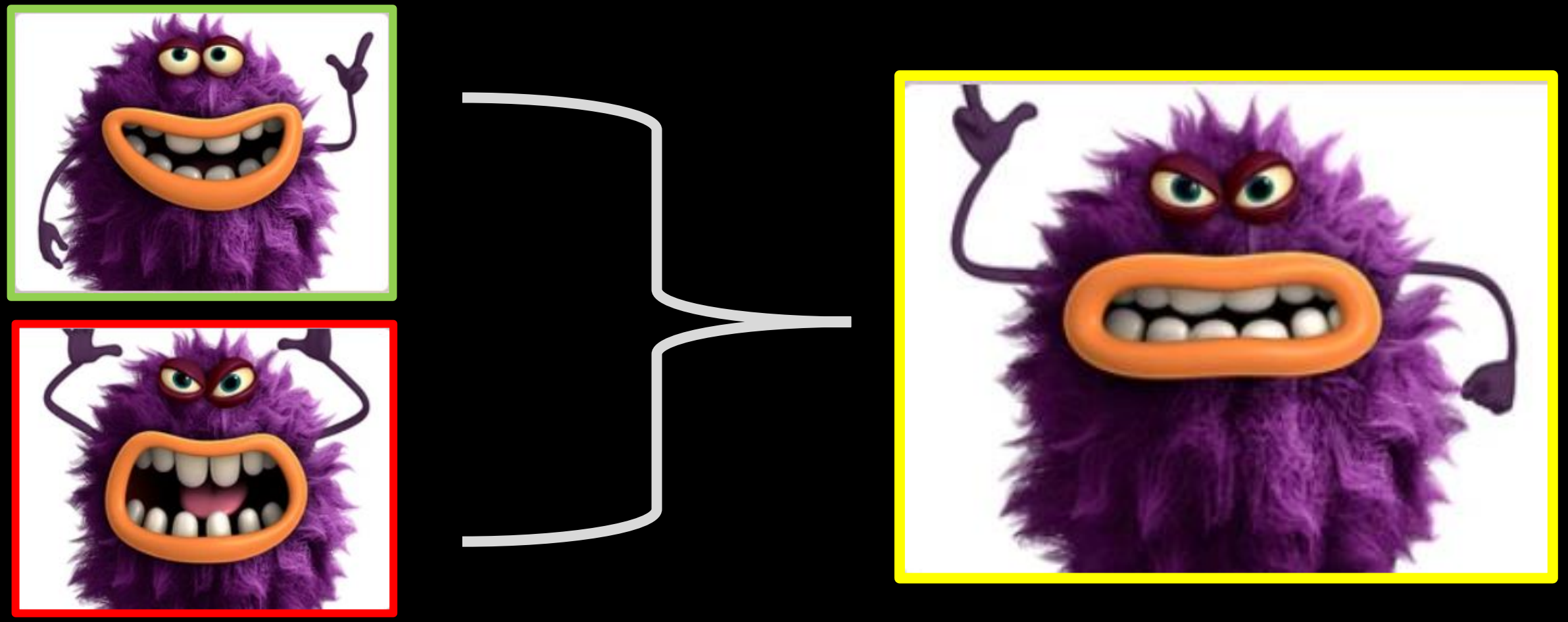
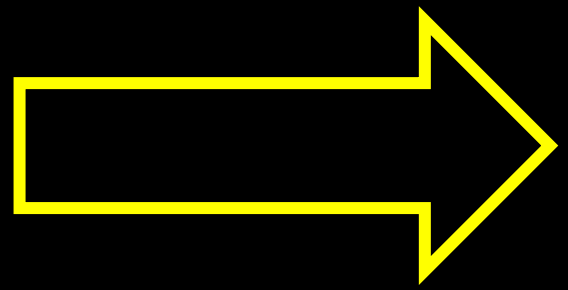
REGULATORY RISK



fundamental mechanism

Cut  Paste

… what you paste matters …

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Leap In Transposase platform

- From shiny and new to tried and true
  - Robust market adoption
  - 20 regulatory filings in ~3 yrs, ~40 licensees, >100 projects
- miLPN technology to reduce gene expression
  - miCHO-GS, miUC, miLPN receptor knock down
- Chain expression ratio balancing
  - Increased titer and product quality
- Bulk selected pools for clinical material
  - COVID-19 rapid response

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- Gene synthesis, vectors
  - Large, complex, routine
  - Host optimized
- Protein production
  - 96-well to 100's of grams
  - mAbs to others
  - Mammalian, e. coli, other
- Protein analytics
  - MS, HPLC, CE, other
  - Developability
- Cell based assays
  - FACS, signaling, other
  - Primary immune cells
- **Protein Engineering**

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Thank You

Oren Beske
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Our Customers