Leap In Transposase Platform

From shiny and new to tried and true

Oren Beske, Ph.D.
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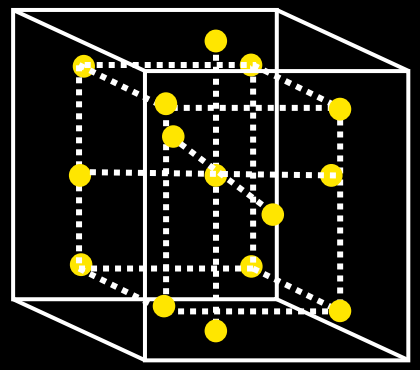
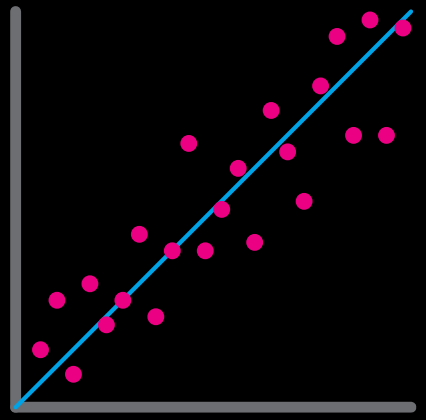
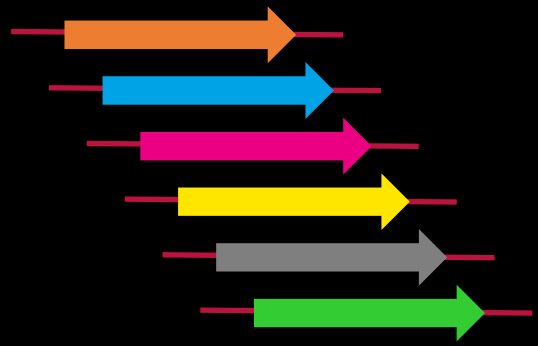
Design of Experiment



Machine Learning



GPS platform



Build

Test

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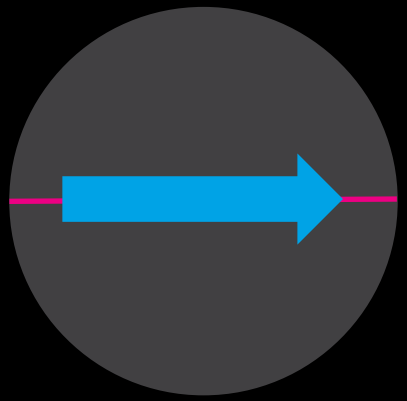
The GPS Platform



gene GPS

ORF codon optimization

vector GPS

Expression vector element optimization

protein GPS

Protein attribute optimization

Leap-In Transposase® Platform

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Leap-In Transposase CLD Platform

- Expression construct integrity maintained
  - No concatemers, scrambling, deletions, etc.
  - Design in silico = structure in chromosome
- Rapid and robust pool generation
  - High titer – predictive of clones (5^+ g/L – 10^+ g/L)
  - Product quality – predictive of clones (glycans, charge, etc.)
- Extremely stable clones
  - >90% of clones retain 100% of titer & copy number

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Transfection to RCB in ~10-12 weeks

RCB

Gene synthesis & vector construction

Stable pool generation & characterization

Cell line cloning and ranking

RCB manufacturing and testing

2-3 weeks

CONSTRUCT DESIGN
CODON OPTIMIZATION
SIGNAL SEQUENCE SELECTION
GENE SYNTHESIS
MOLECULAR CLONING

3 – 5 weeks

TRANSFECTION/SELECTION
PRODUCTIVITY ASSESSMENT
PRODUCT QUALITY ASSESSMENT

6 – 8 weeks

MONOCLONALITY VIA IMAGING

4-5 weeks

VIABILITY AT THAW
STERILITY, MYCOPLASMA

~9 weeks

60 PD GENETIC STABILITY

Representative pool

Early USPS, DSPD
Early Analytical Development
Tox Lot Generation

Clones available

Finalize Processes
Verify CPQA's

horizon INSPIRED CELL SOLUTIONS

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Robust Market Adoption

- Offered as a service by ATUM: >70 projects delivered
- >30 active licensees: 11 of top 20 pharma
- 10 IND's filed in less than two years:
  - Seven IND's filed and accepted in US
  - One IND filed and accepted in China
  - Two IND's filed and accepted in EU

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Moving Beyond the Routine

- COVID-19 Response
- Chain ratio balancing for titer and product quality
- Reduction of target gene expression

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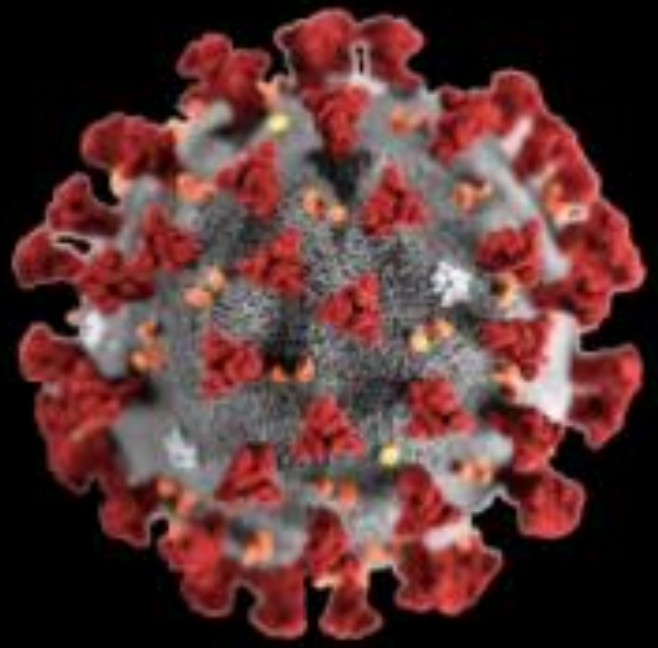
Antibodies For COVID 19 Treatment



- 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: Pools

- Two vector sets for each of 11 mAb's
- Create Leap-In Transposase® derived pools
- Freeze RCB's for transfer to CDMO
- Test expression in 10mL tube spin format

Transfection to RCB in ~10-12 weeks

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60 PD GENETIC STABILITY

How fast can we go?

Representative pool

- High titer
- Stable
- Low risk

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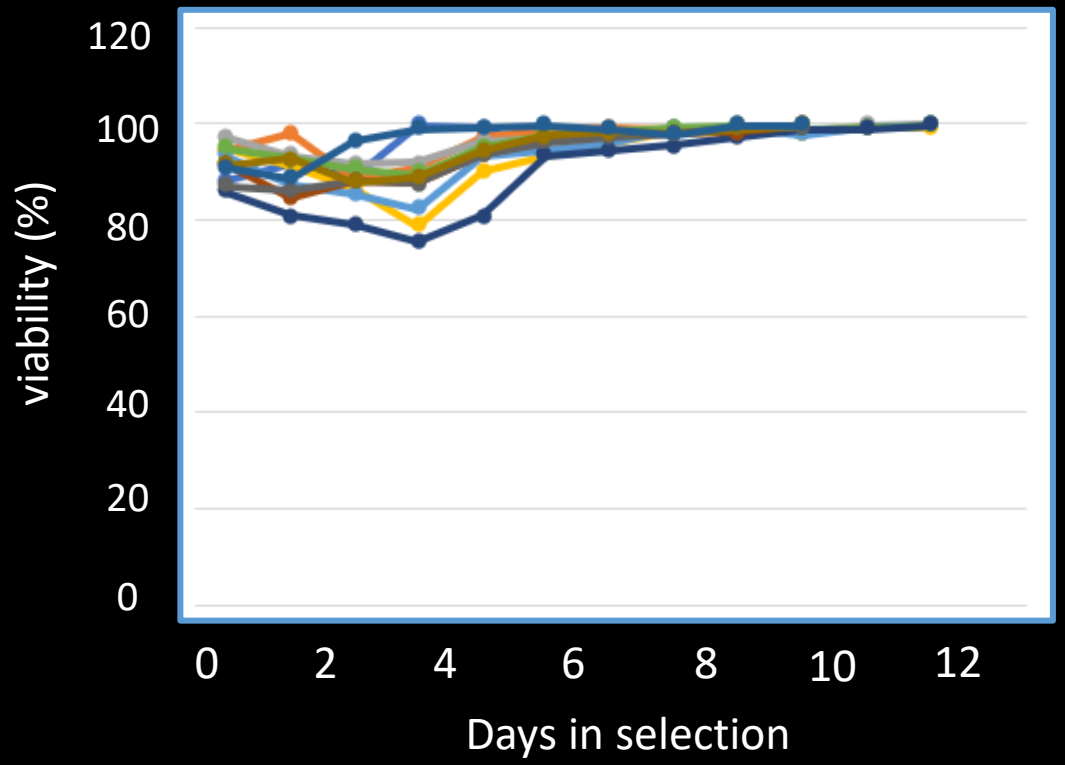
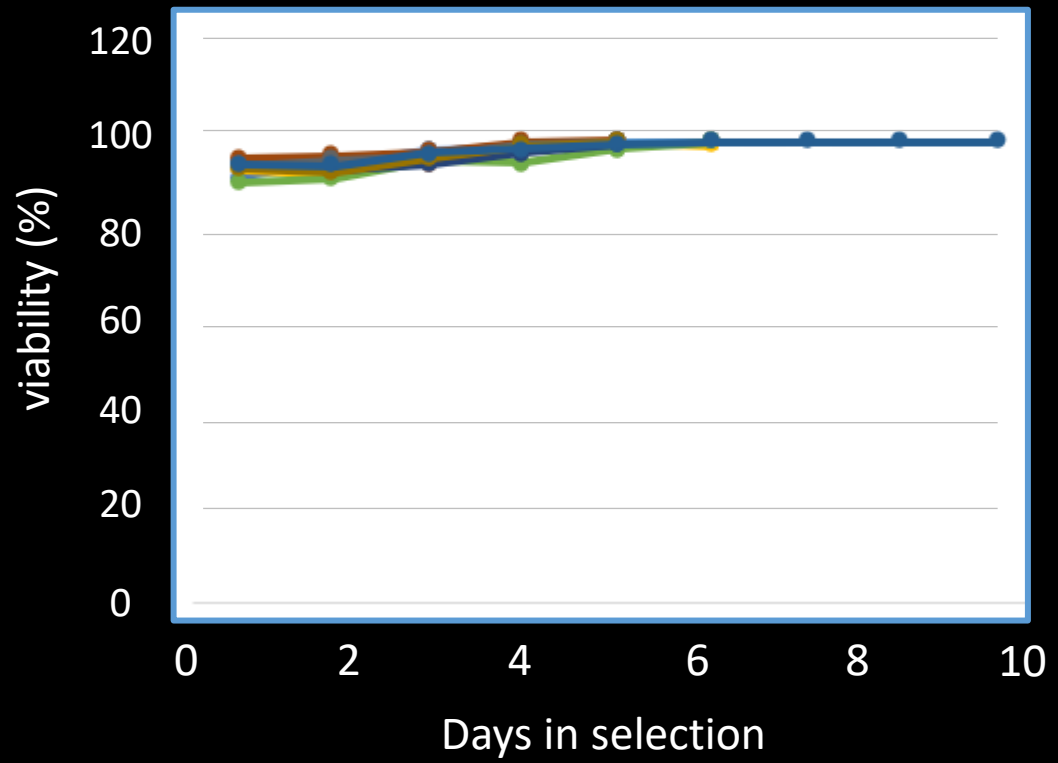
COVID 19: ATUM Accelerated Timeline: 1

Vector-1



Selection in ~4-6 days

Vector-2



Selection in ~3 days

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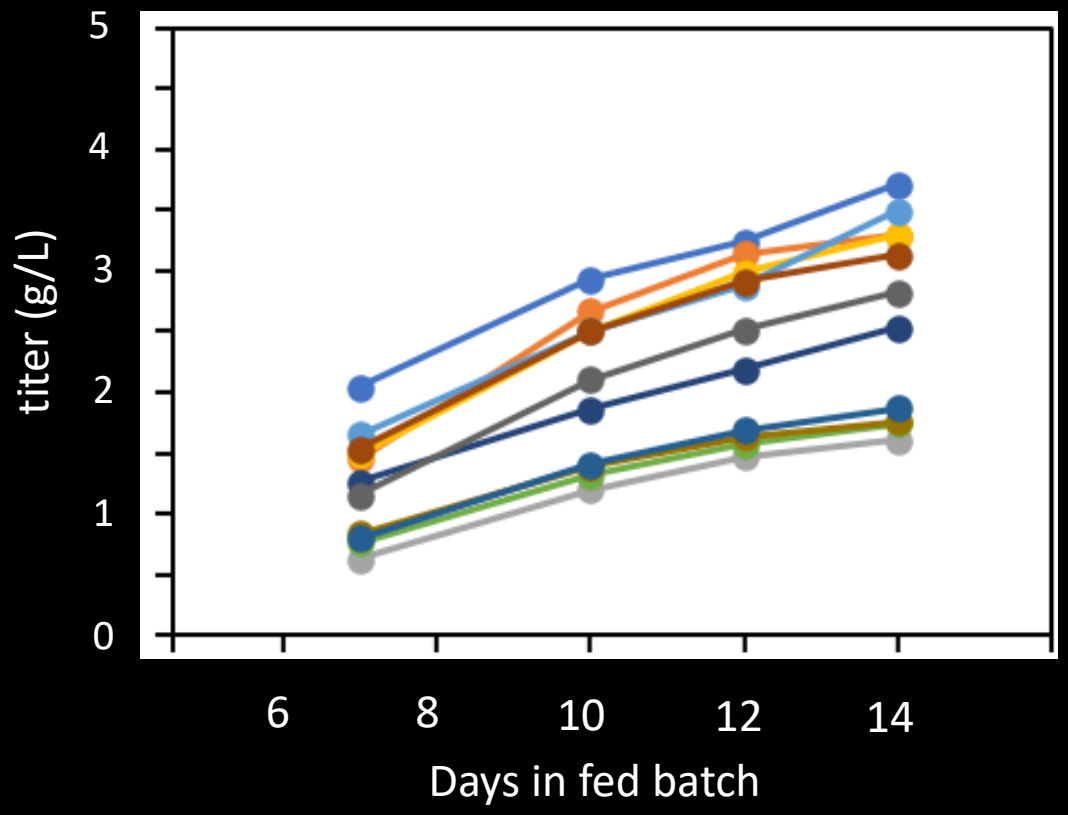
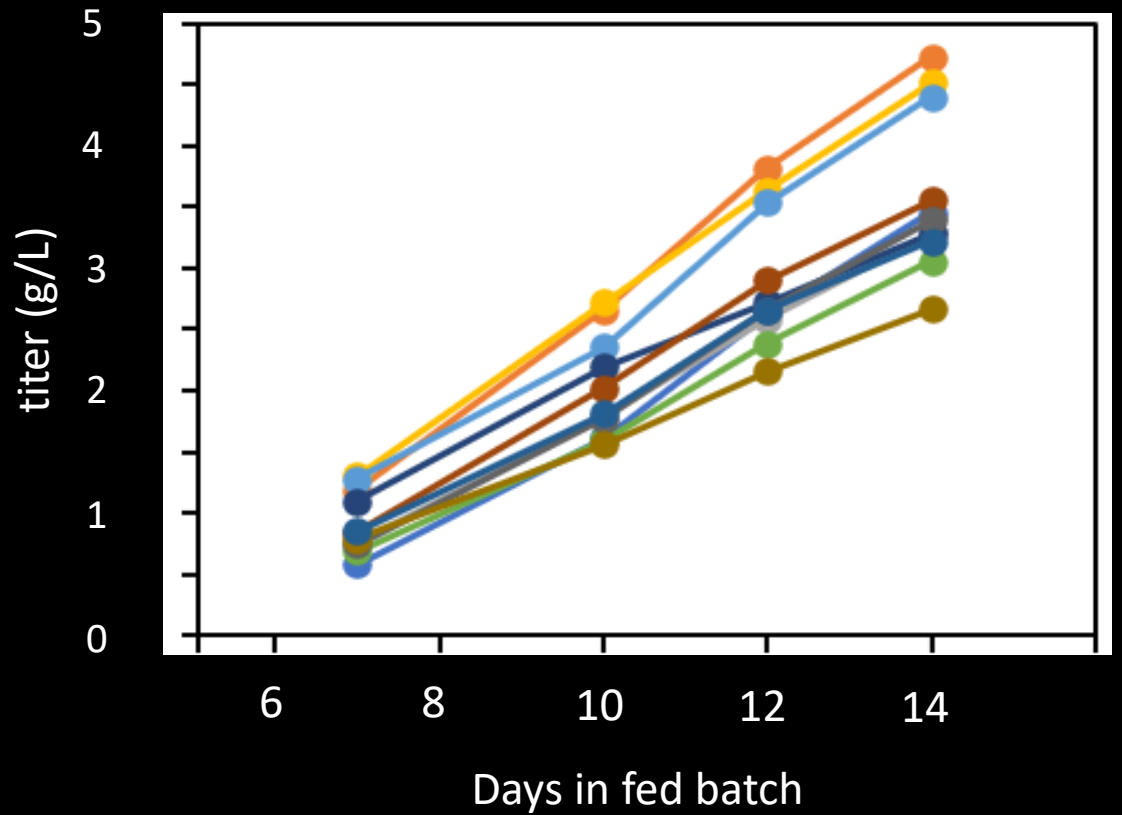
COVID 19: ATUM Accelerated Timeline: 1

Vector-1



Vector-2



Titer: 1.4 – 3.5 g/L

Titer: 3.5 – 4.8 g/L

Multiple pools banked and transferred to CDMO

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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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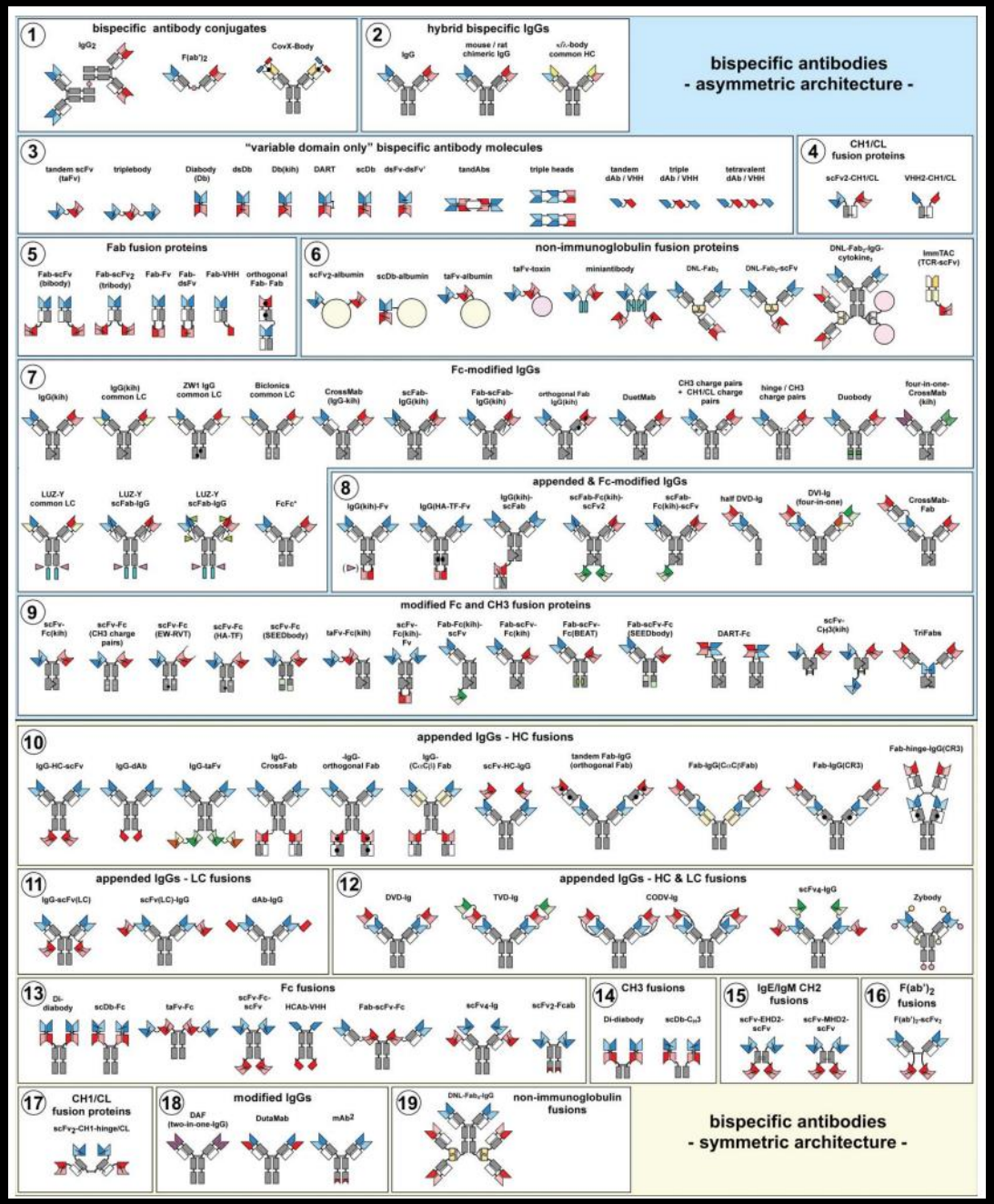

Brinkmann and Kontermann; 2017

Beyond mAb's: 3 Chains and More

The "zoo" of bispecifics

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Considerations for chain ratio balancing

Sequence

- Codon choice
- mRNA 2° 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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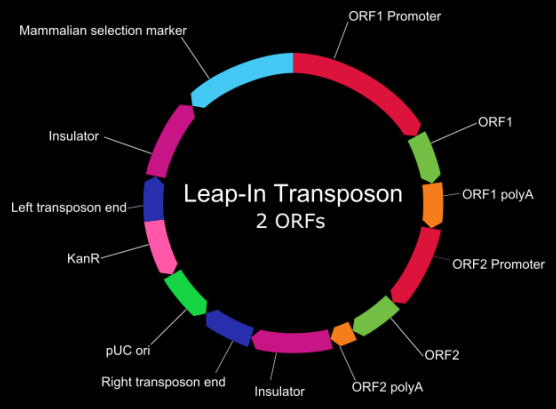
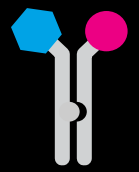
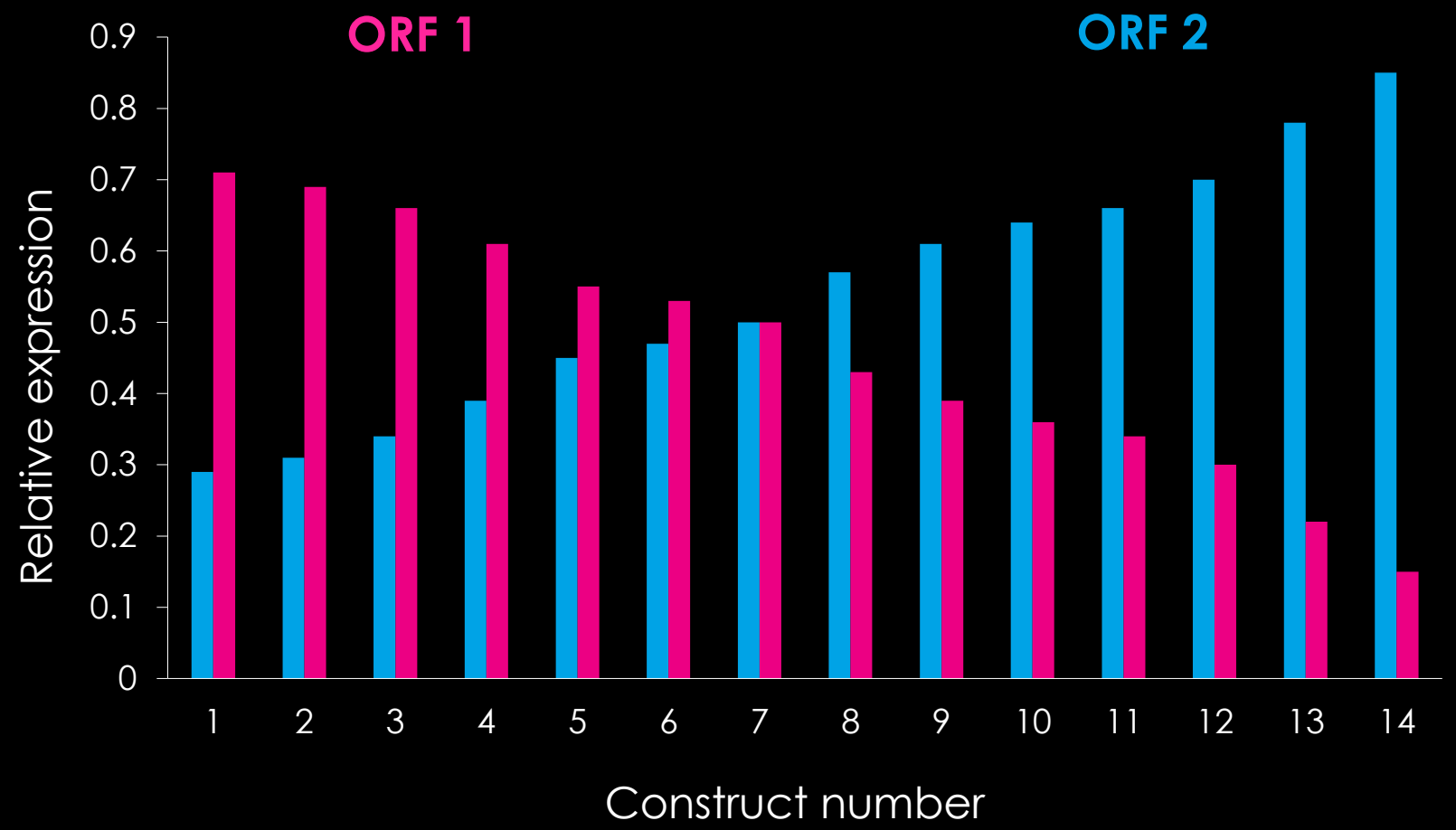
Controlling ratios with construct design: 2 ORFs

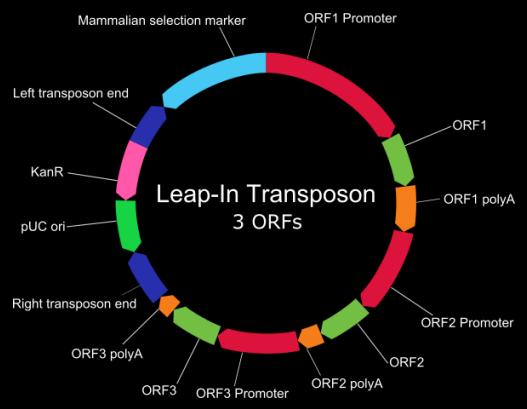




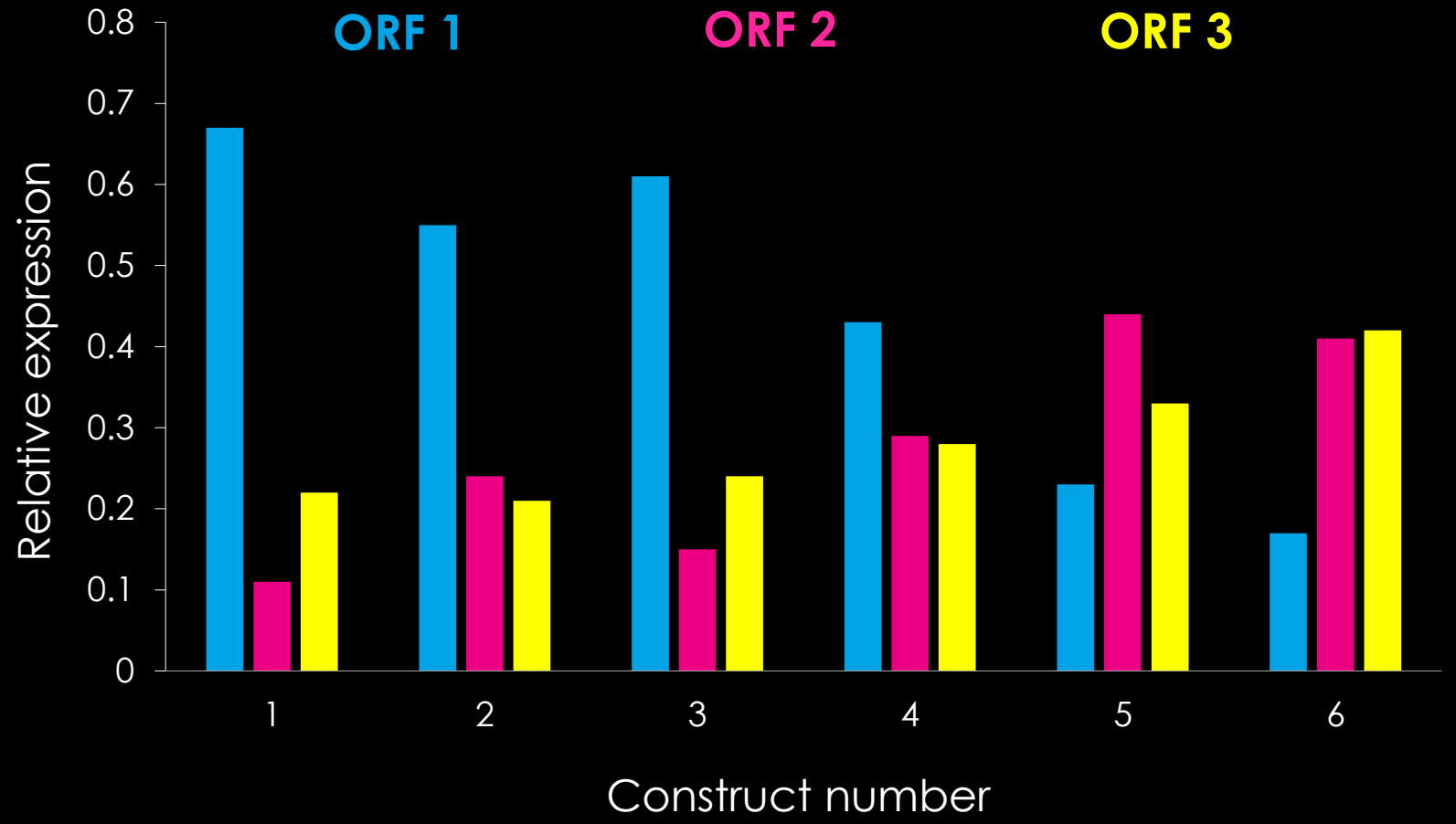
Bispecific Antibodies
chain ratio modulation



Controlling ratios with construct design: 3 ORFs



Bispecific Antibodies
chain ratio modulation



Case Study: 3-Chain Bispecific mAb

- 14 vector configurations
  - Varying expression levels
  - Varying expression ratios
- Leap In Transposase based pool selection
- Analytical assessment
  - Total titer
  - Chain expression: Relative and Amount
  - % Bispecific

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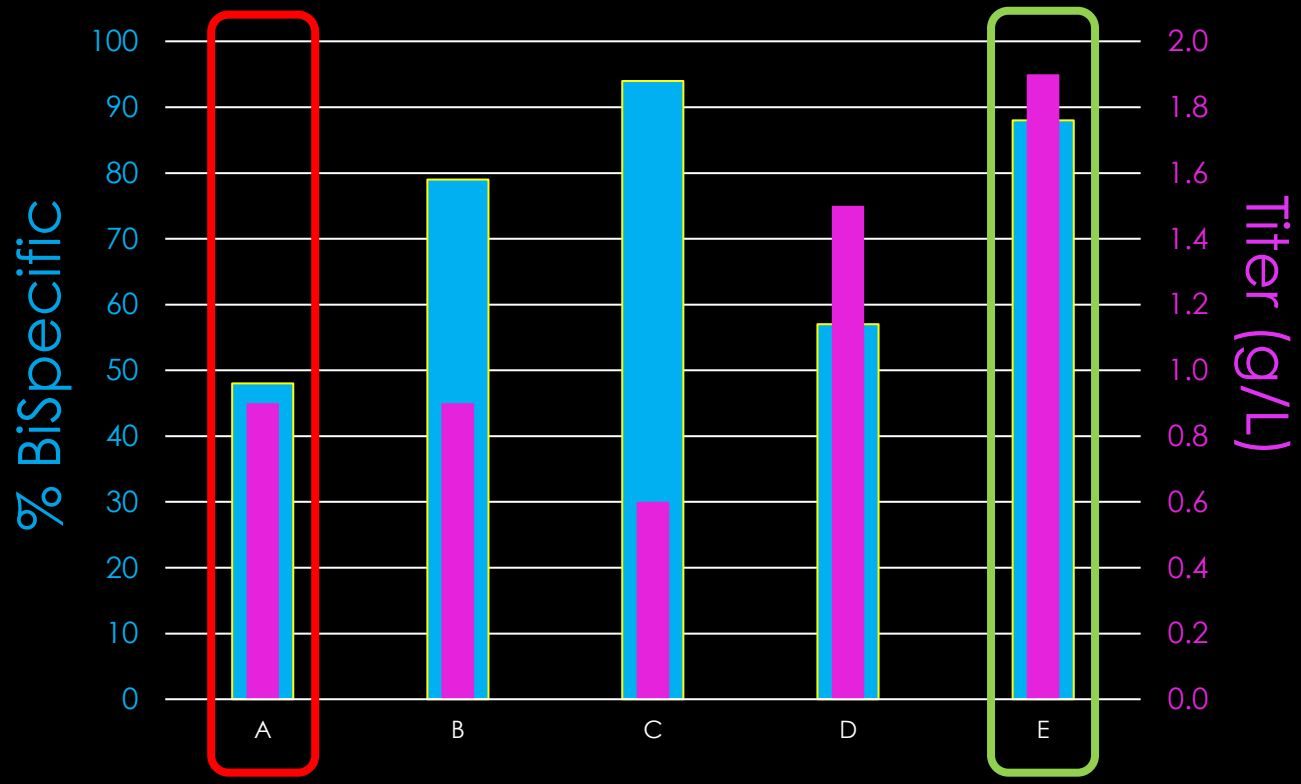
Case Study: 3-Chain Bispecific mAb

Vector*	Expression Level [relative]		Expression Level
	LC	Sum of HC1+HC2 (normalized)	
A	comparable	1	med-low
B	comparable	1	low
C	significantly higher	1	low
D	moderately higher	1	high
E	comparable	1	high

* Subset of 14 vectors screened



Screening vectors at pool stage enables ID of high value pools

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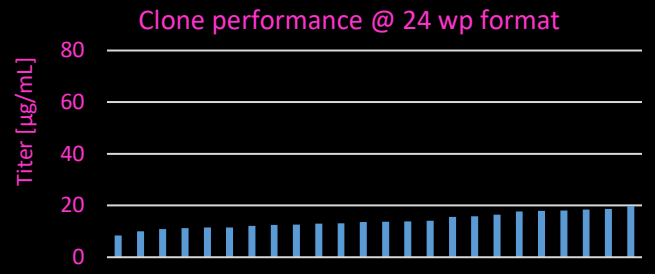
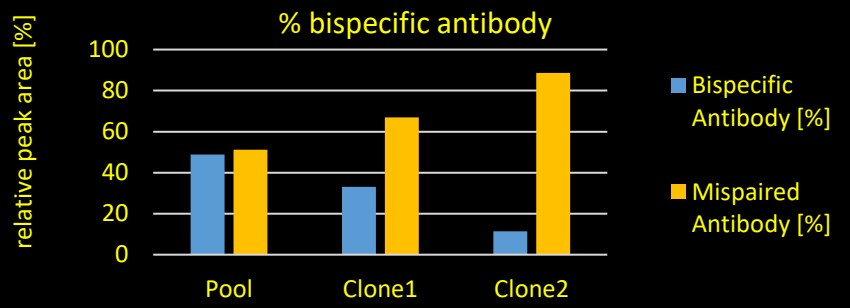
Case Study: 3-Chain BiSpecific mAb

Pool A



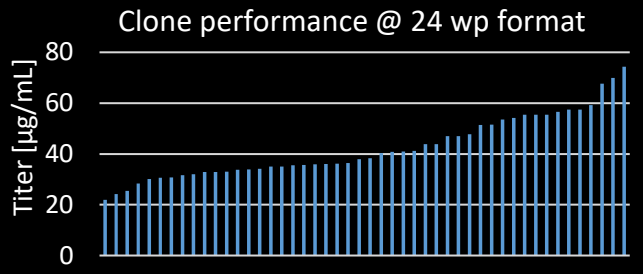


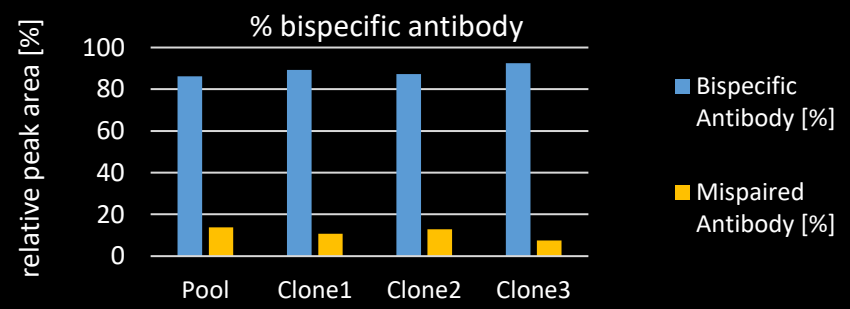
Pool and clone productivity

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

**Day 12 standard fed-batch*

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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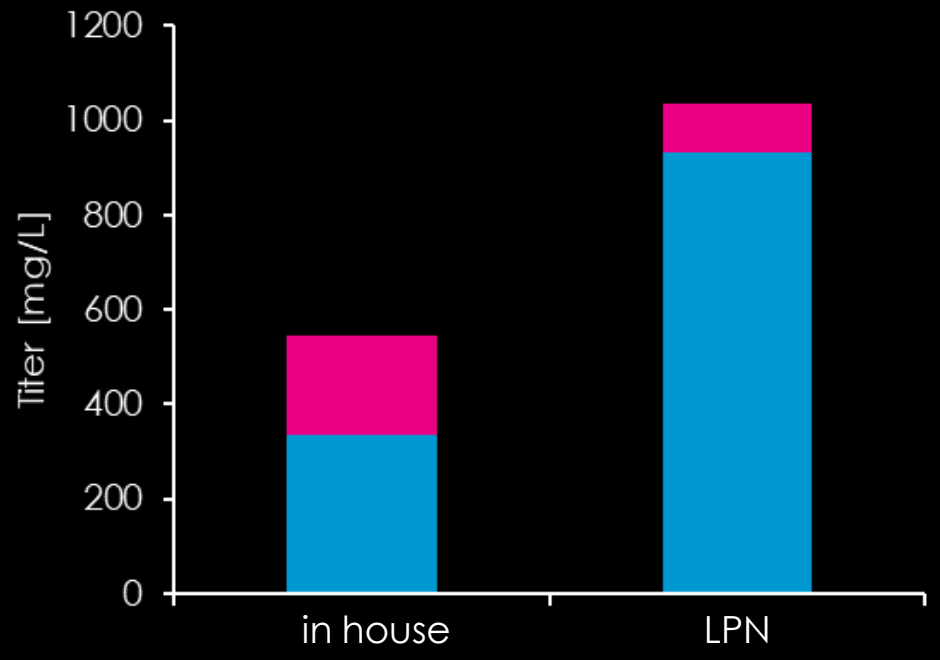
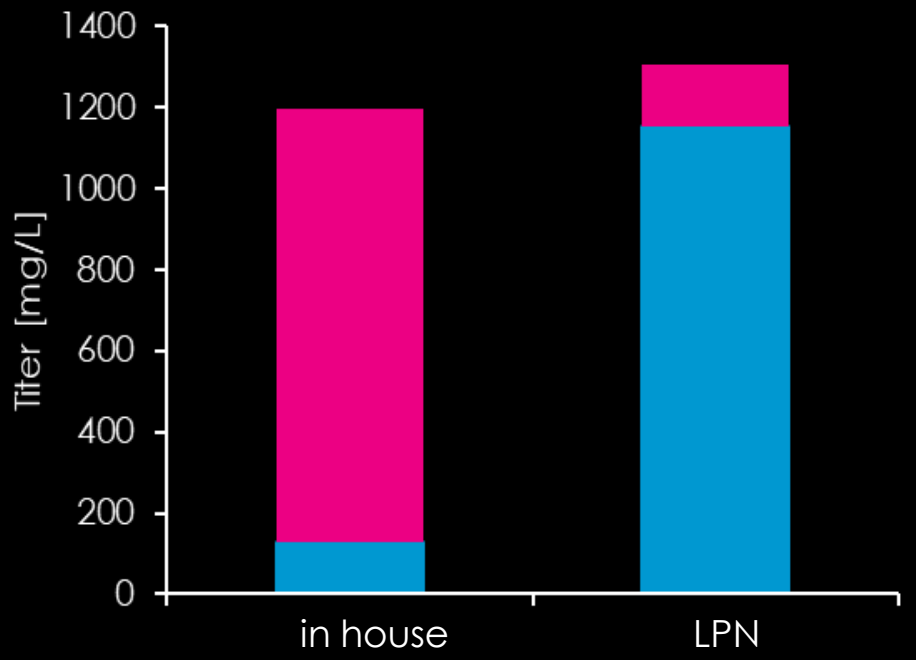
Controlling ratios with construct design: 3 ORFs

Desired product



Assembly variant



Leap-In enabled chain balancing = significantly improved product assembly

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The miLPN platform

Use Leap In Transposase platform to reduce gene expression

miCHO-GS

- K1 derived
- GS deficient
- GMP Cell Bank

miFuc

- Vector based
- Host cell agnostic
- Modify existing expression cell line

miLPN

- Custom projects

ATUM

The miLPN platform

Use Leap In Transposase platform to reduce gene expression

miCHO-GS	miFuc	miLPN
• K1 derived • GS deficient • GMP Cell Bank	• Vector based • Host cell agnostic • Modify existing expression cell line	• Custom projects

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miFuc Platform: Overview

Transient:

Modified HEK host
Modified CHO host

Stable:

Vector based approach
Unmodified cell host
Engineer existing cell line

Proof of concept stage: seeking early access partners

ATUM

miFuc Platform: Overview

Transient:

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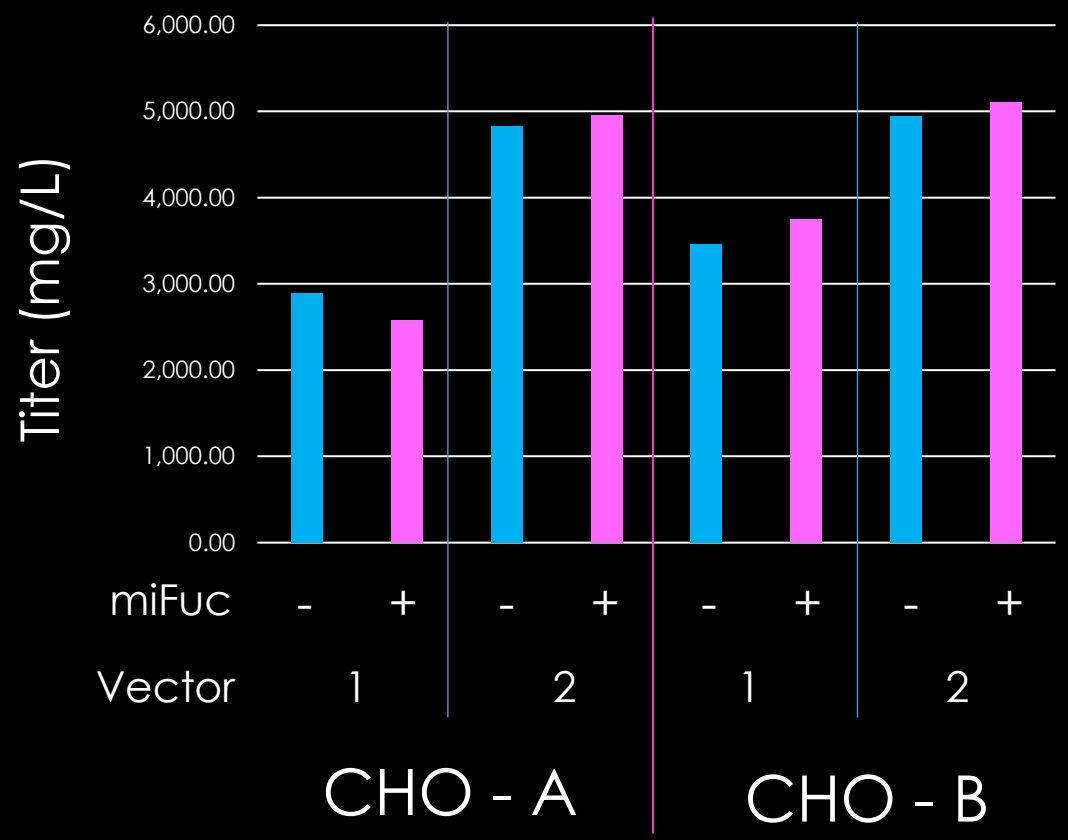
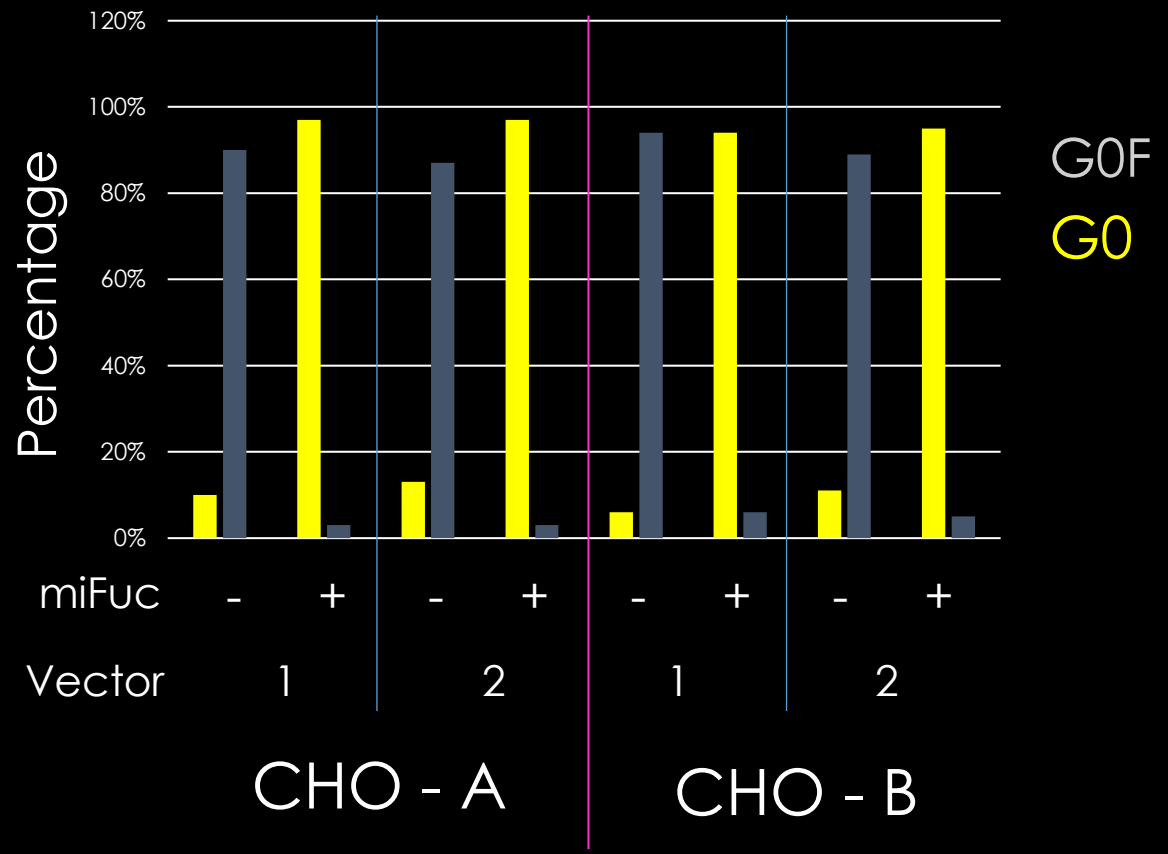
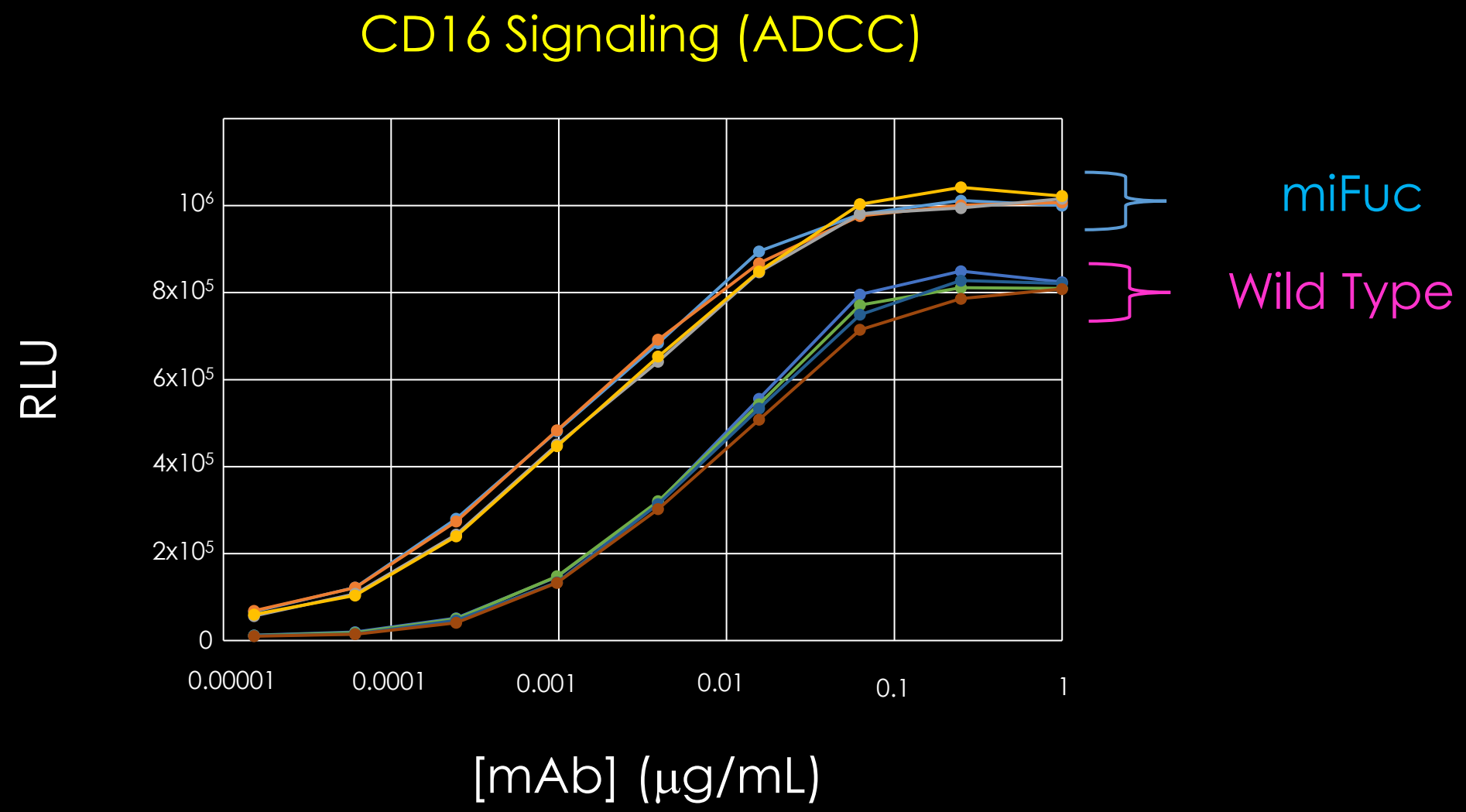
miFuc Platform: Stable Pools

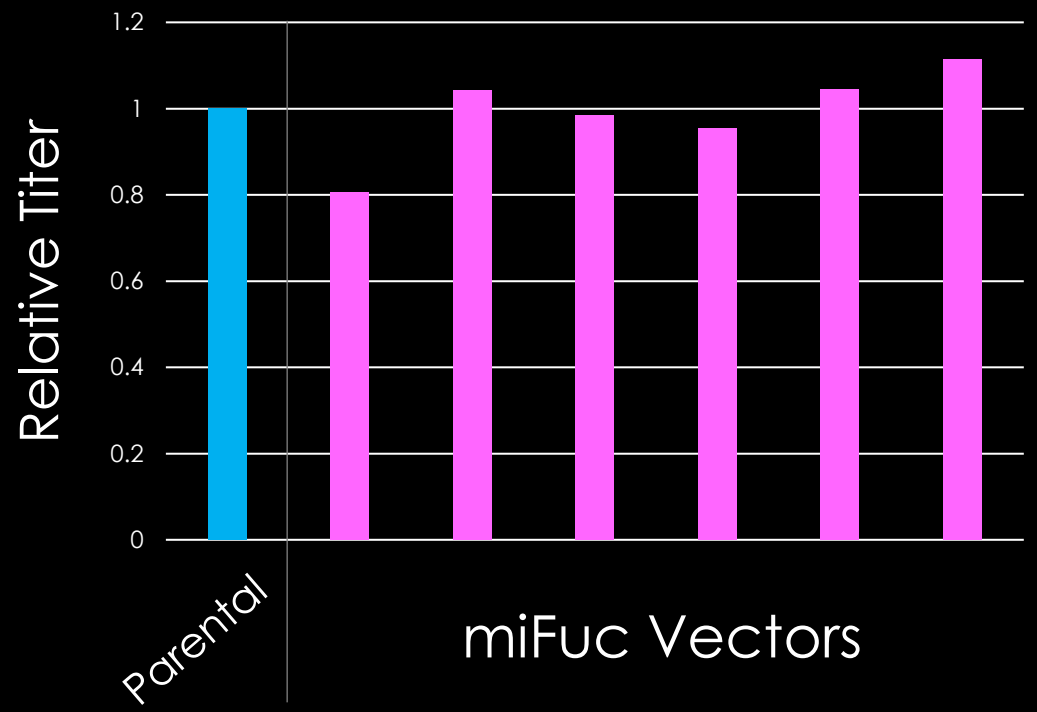


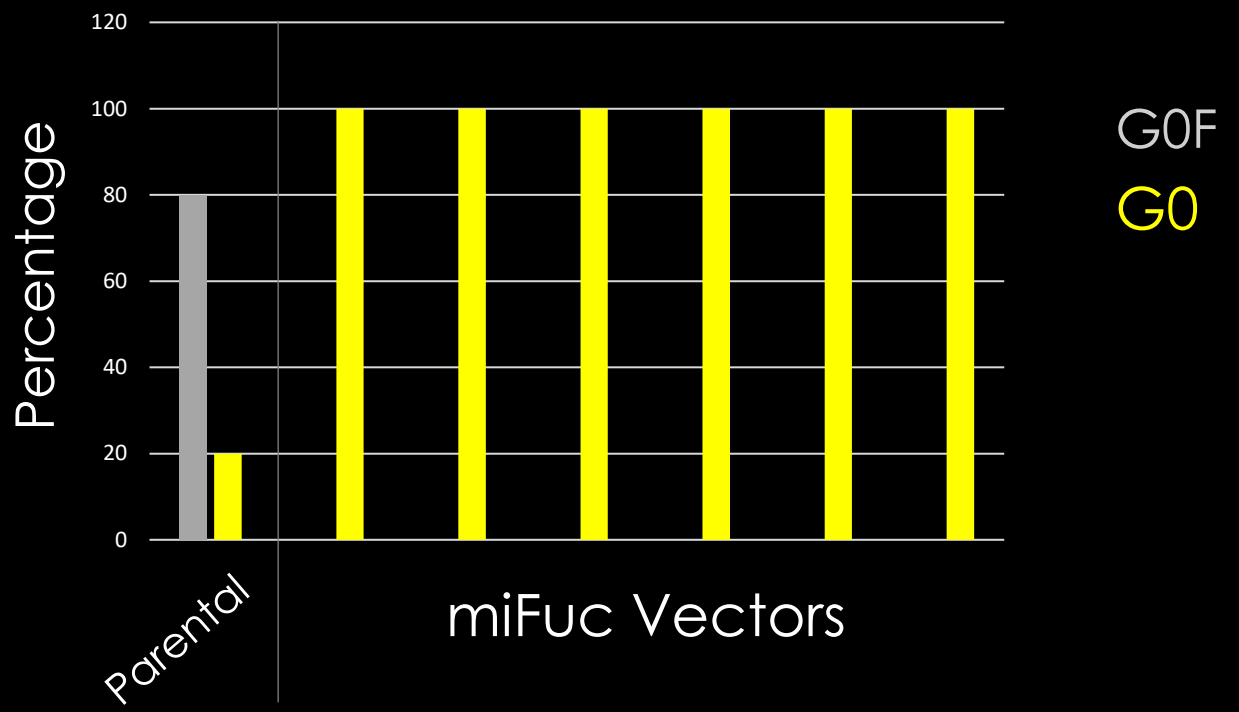


miFuc Platform: ADCC enhancement



miFuc Platform: Existing cell line





miFuc technology works on existing mAb expression line

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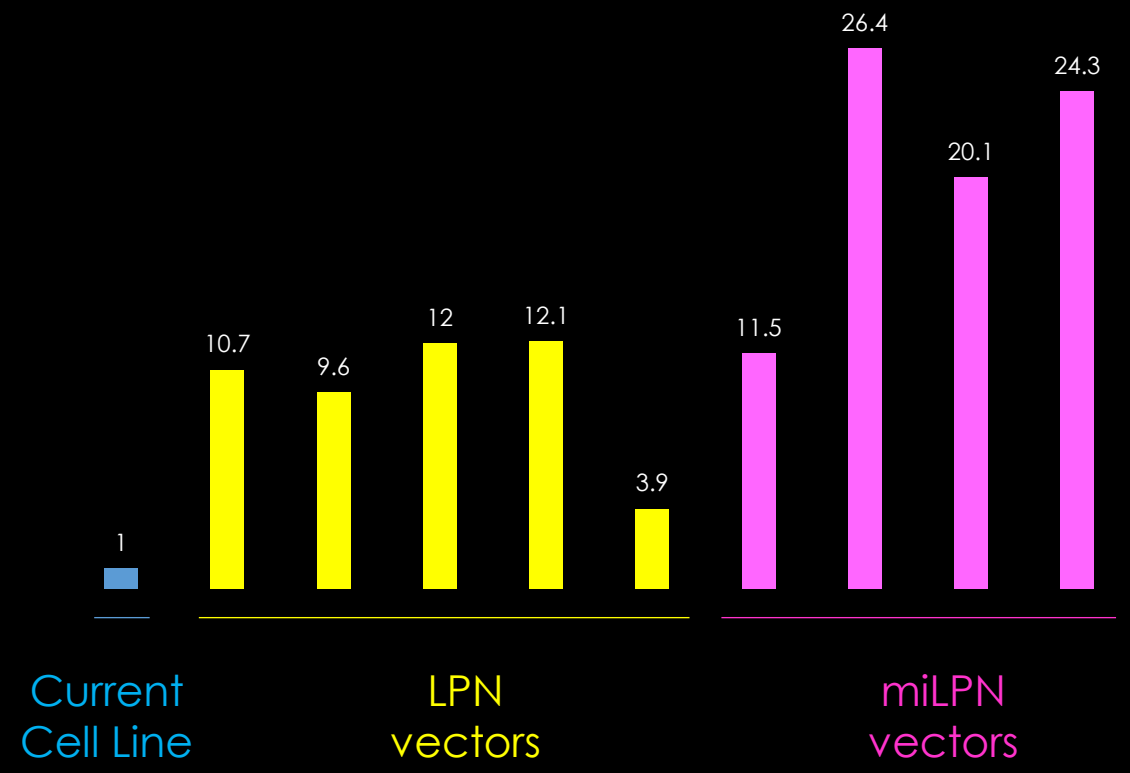
miLPN Platform: Custom Project

Overview

Cytokine therapeutic
Low expressor

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

Use miLPN technology to reduce endogenous receptor expression on host cell



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Leap In Transposase Platform

- From shiny and new to tried and true
  - Robust market adoption
  - Ten IND's in 2 years, >30 licensees, >70 projects
- Rapid COVID 19 response
  - Bulk selected pools for IND filing
- Chain expression ratio balancing
  - Increased titer and product quality
- miLPN technology to reduce gene expression
  - miCHO-GS, miFuc, miLPN receptor knock down

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- Gene synthesis, vectors
  - Large, complex, routine
  - 1000's to chose from
- Protein production
  - 96-well to multi-gram
  - mAbs to others
  - Mammalian, e. coli, other
- Protein analytics
  - MS, HPLC, other
  - Developability
- Cell based assays
  - FACS, signaling, other
  - Primary immune cells
- Protein Engineering

ATUM

Thank You

Oren Beske
obeske@atum.bio

Partners:

Horizon Discovery
Rentschler Biopharma
Our Customers

Lucia Kirchgeorf - Rentschler: 9:30A Salo Clave

Lydia Caro - Ichnos: 3:45P Salo Clave

Technology presented is protected by issued US patents 10435696, 10344285, 10287590, 10253321, 10233454, 10041077, 9771402, 9580697, 9574209, 9534234, 9493521, 9428767, 9290552, 9206433, 9102944, 8975042, 8825411, 8635029, 8412461, 8401798, 8323930, 8158391, 8126653, 8005620, 7805252, 7561973, 7561972 and pending applications