Leap In Transposase Platform

How's it going?
Where we are going …

Antibody Engineering & Therapeutics

Oren Beske, Ph.D.
obeske@atum.bio

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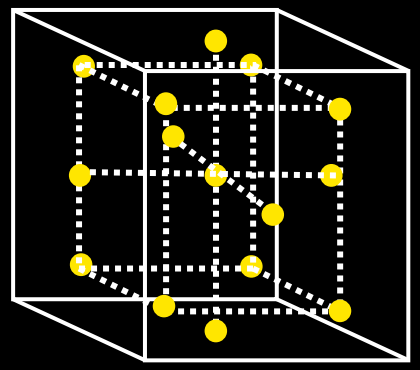
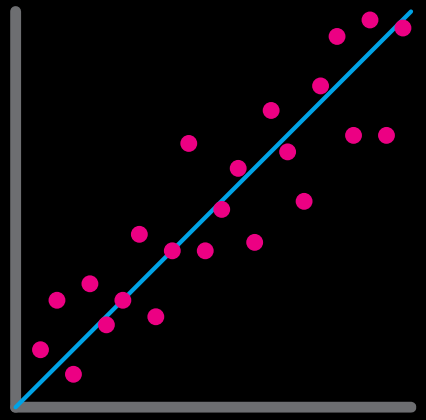
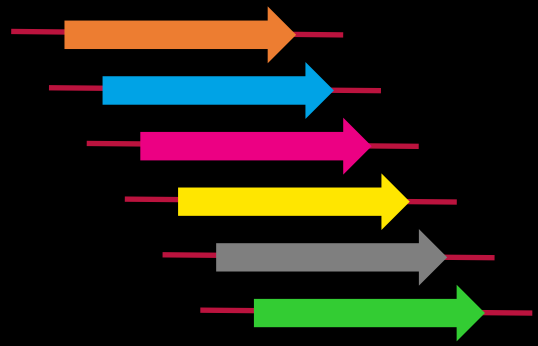
Design of Experiment



Machine Learning



GPS platform



Build

Test

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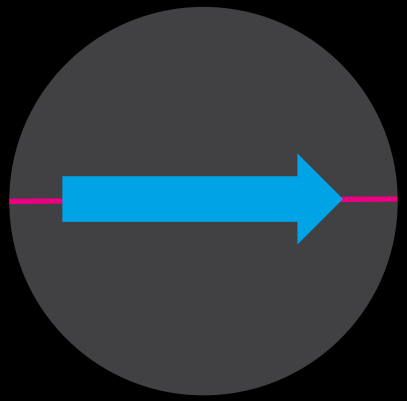
The GPS Platform



gene GPS

ORF codon optimization

vector GPS

Expression vector element optimization

protein GPS

Protein attribute optimization

Leap-In Transposase® Platform

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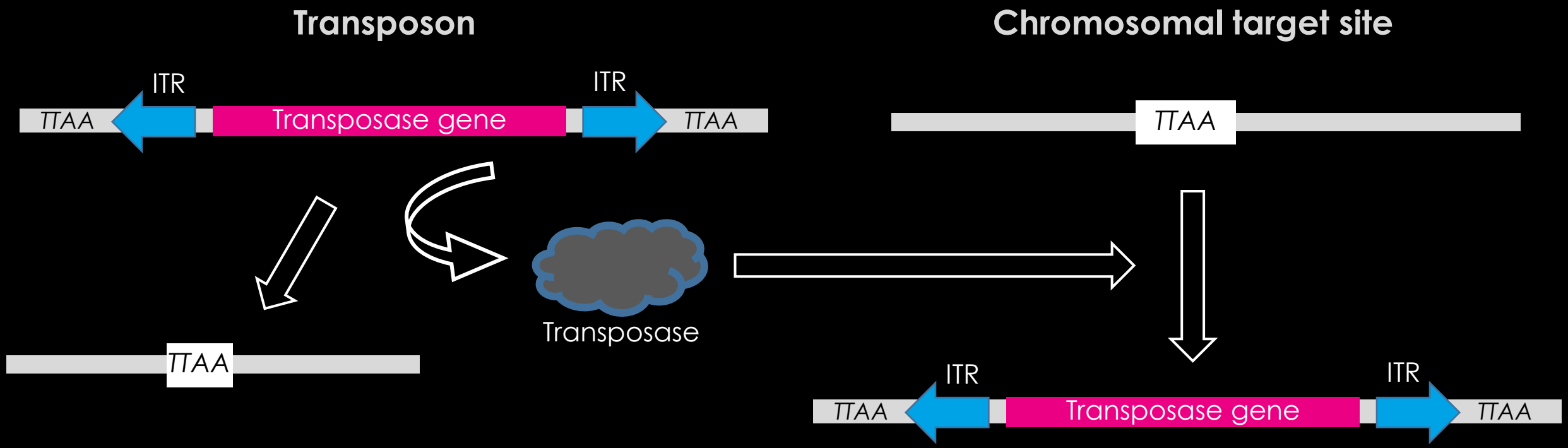
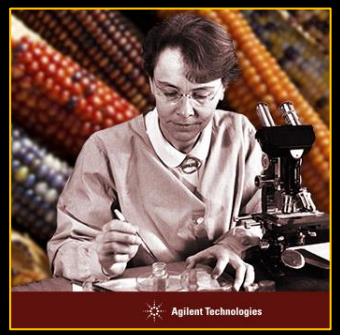
The life of a transposon-transposase pair



- Billions of years of evolutionary history
- Cut-paste mechanism
- Single copy integration at each site
- Perfect integration of elements between ITR's



1983 Nobel Prize in Physiology or Medicine

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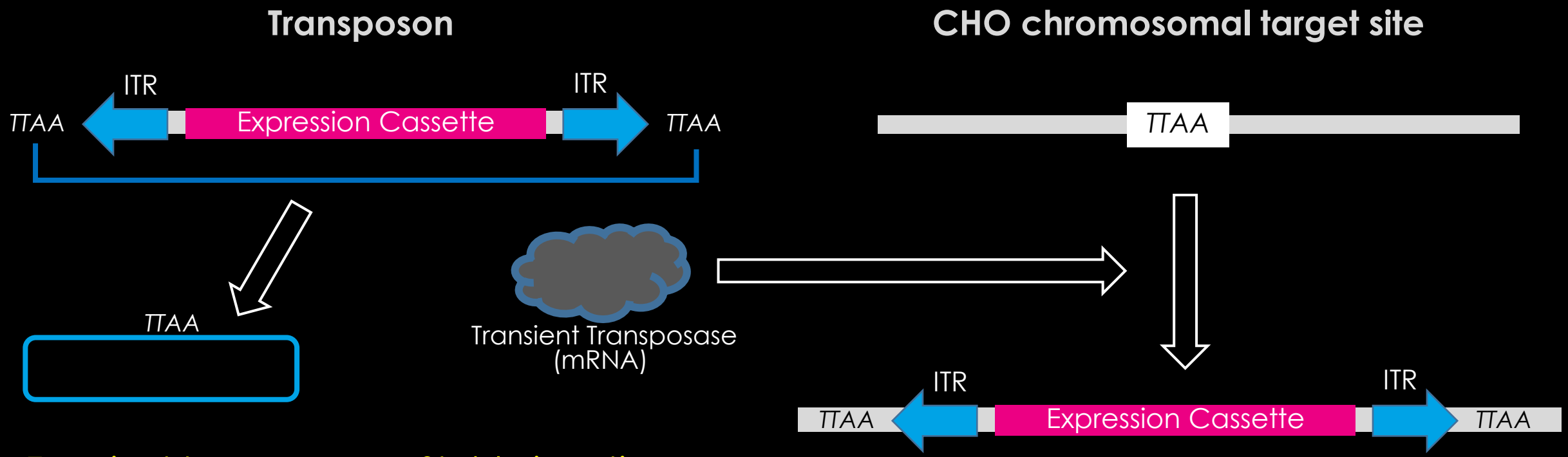
The life of a transposon-transposase pair



- Transient transposase = Stable insertion
- Single copy integrations at each site
- Multiple insertions (5 – 60+) across the genome
- Structural integrity maintained
- No size limitation

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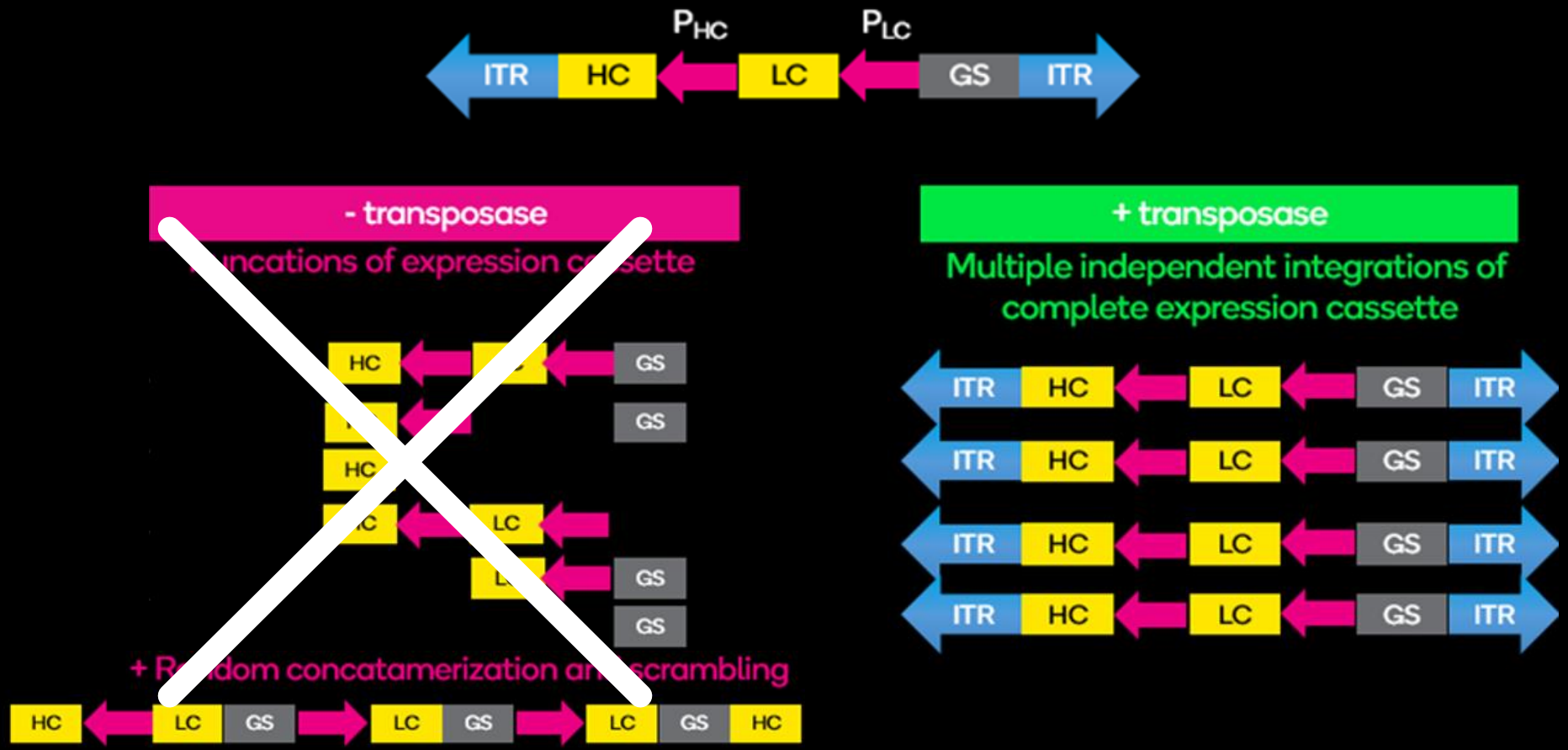
Consistent, uniform presentation of Leap-In® transgenes



- In-silico designed expression construct maintained at every integration site
- On average, functionality of each integration is comparable
  - Expression and product quality

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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

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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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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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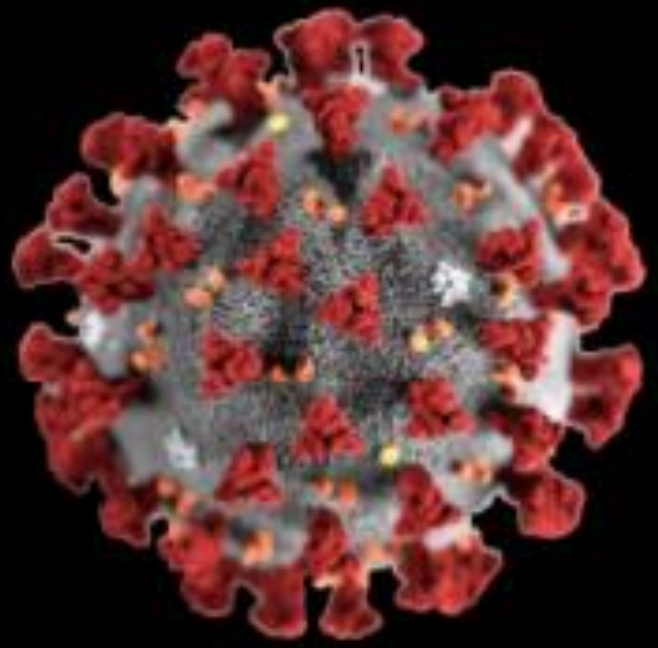
Antibodies For COVID 19 Treatment



- 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

RCB

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VIABILITY AT THAW
STERILITY, MYCOPLASMA

~9 weeks

60 PD GENETIC STABILITY

Representative pool

- High titer
- Stable
- Clinical Manufacturing?

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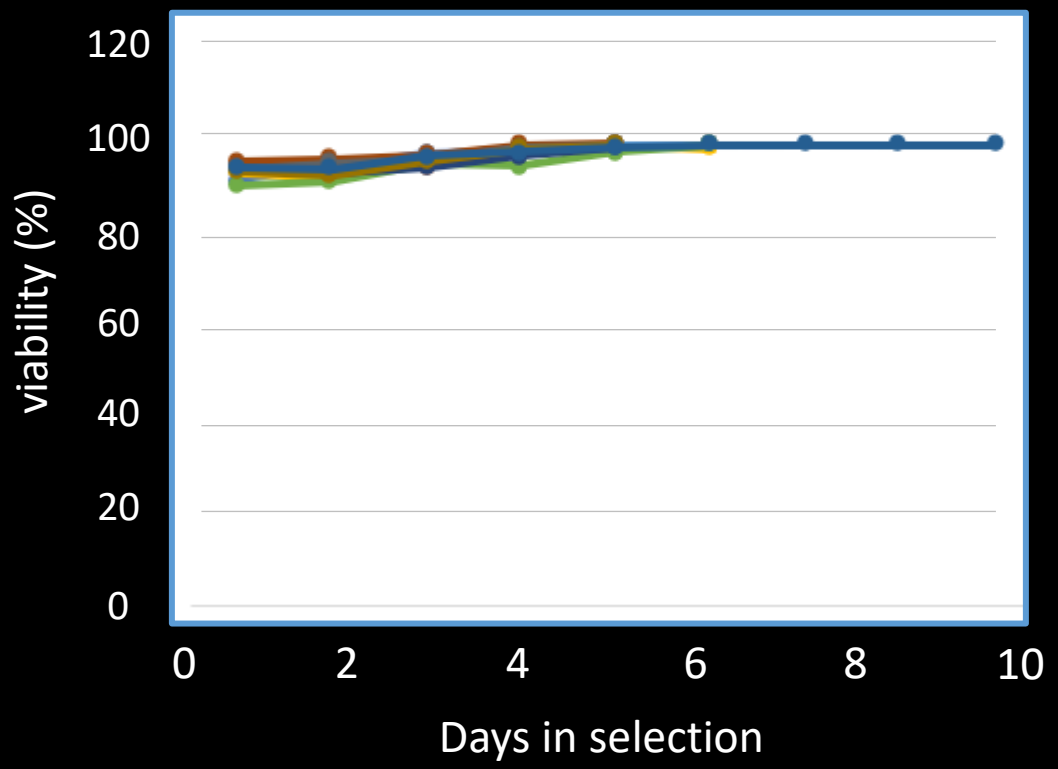
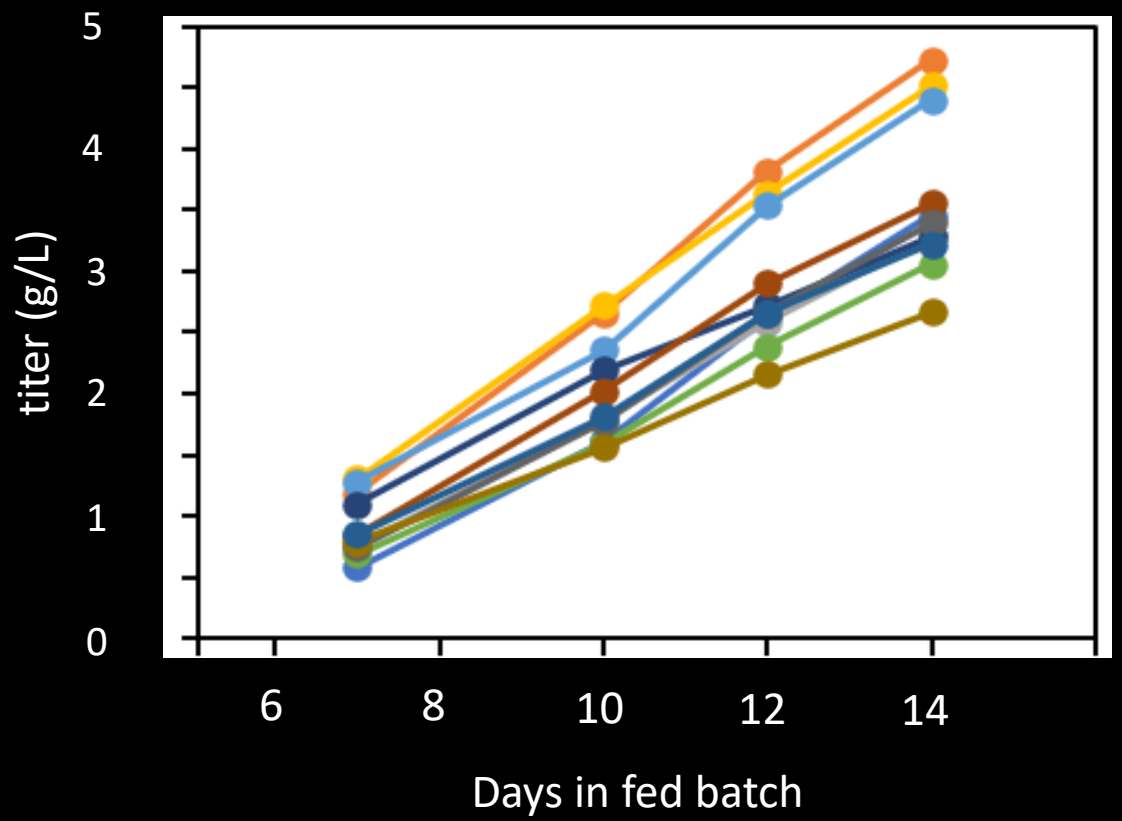
COVID 19: ATUM Accelerated Timeline: 1

Vector-2



Vector-2



Selection in ~3 days

Titer: 3.5 – 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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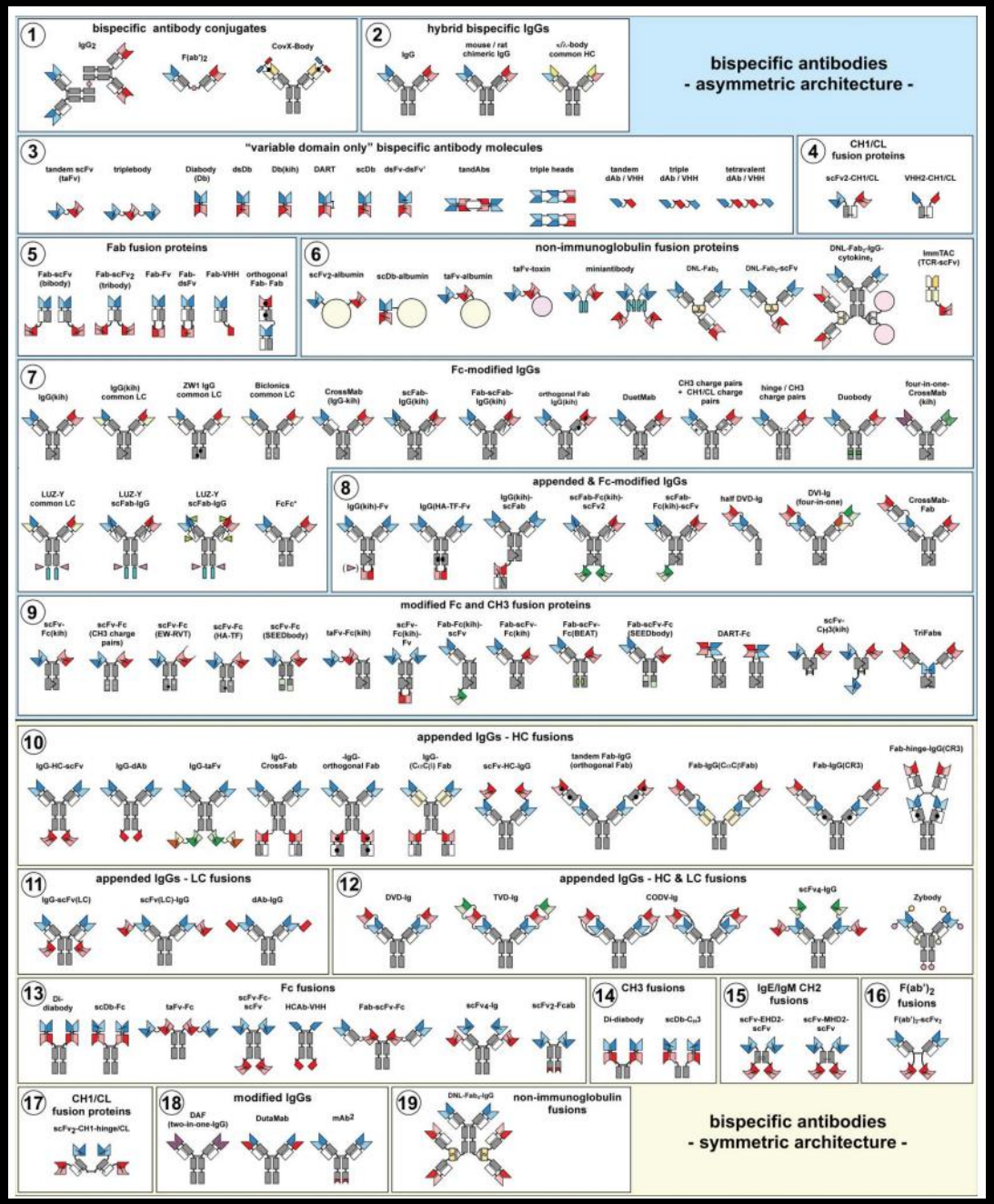

Brinkmann and Kontermann; 2017

Beyond mAb's: 2-3 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 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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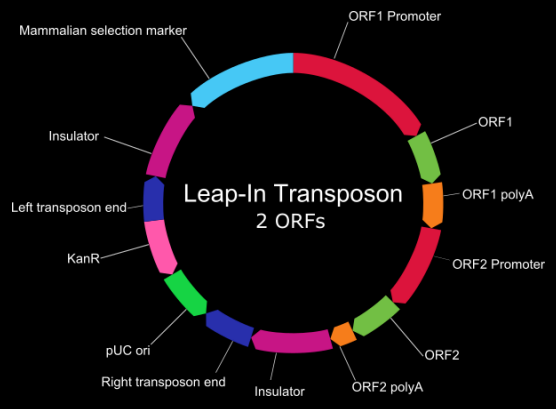
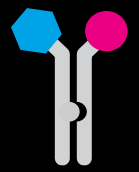
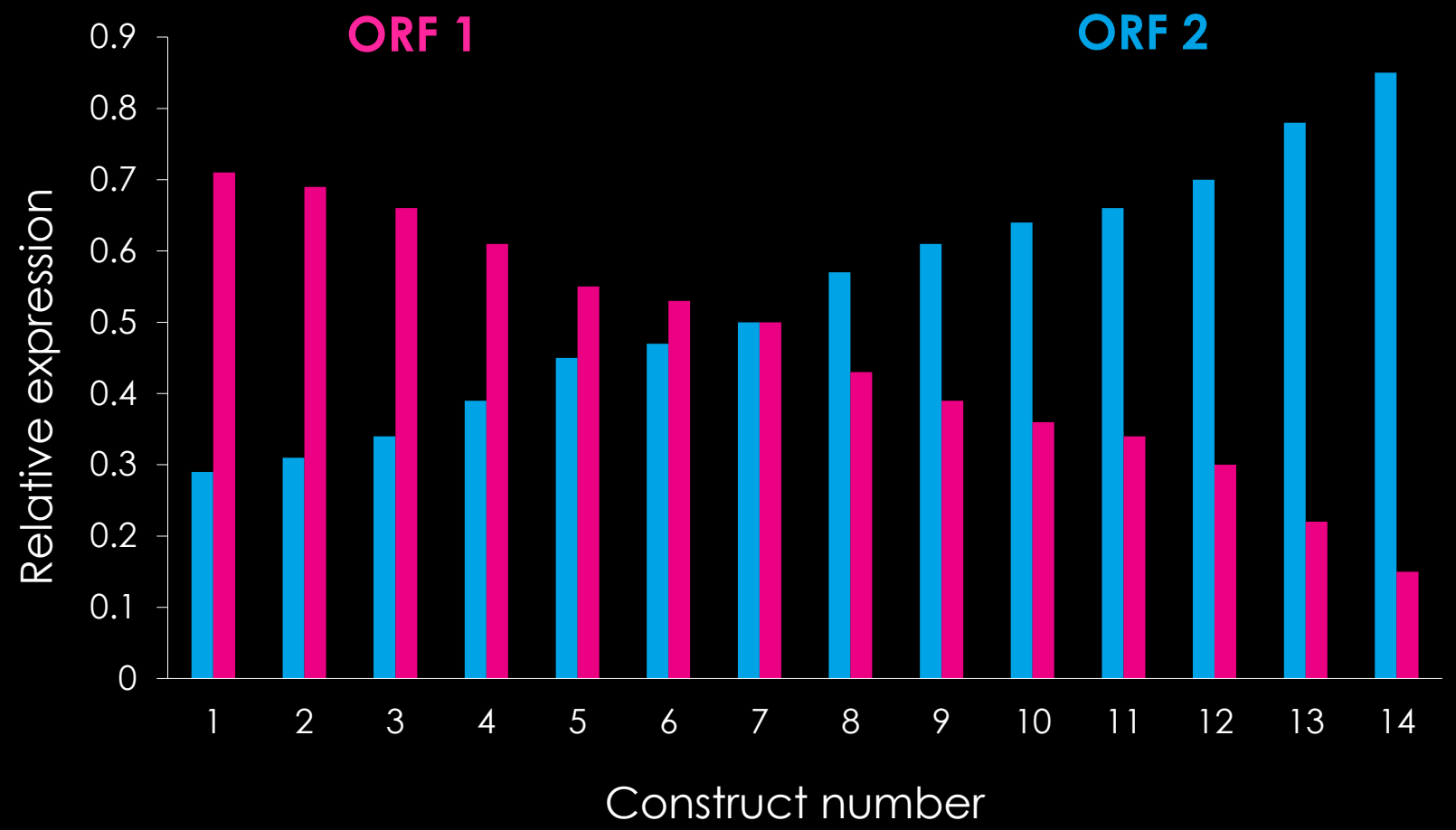
Controlling ratios with construct design: 2 ORFs

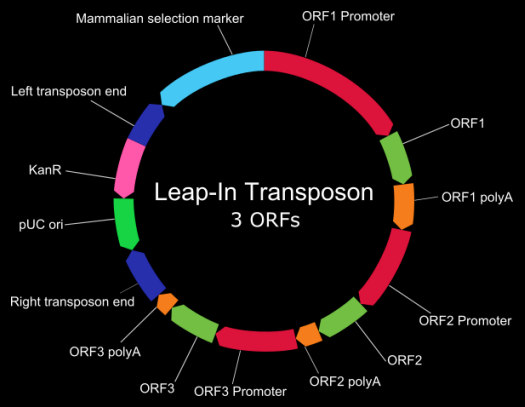




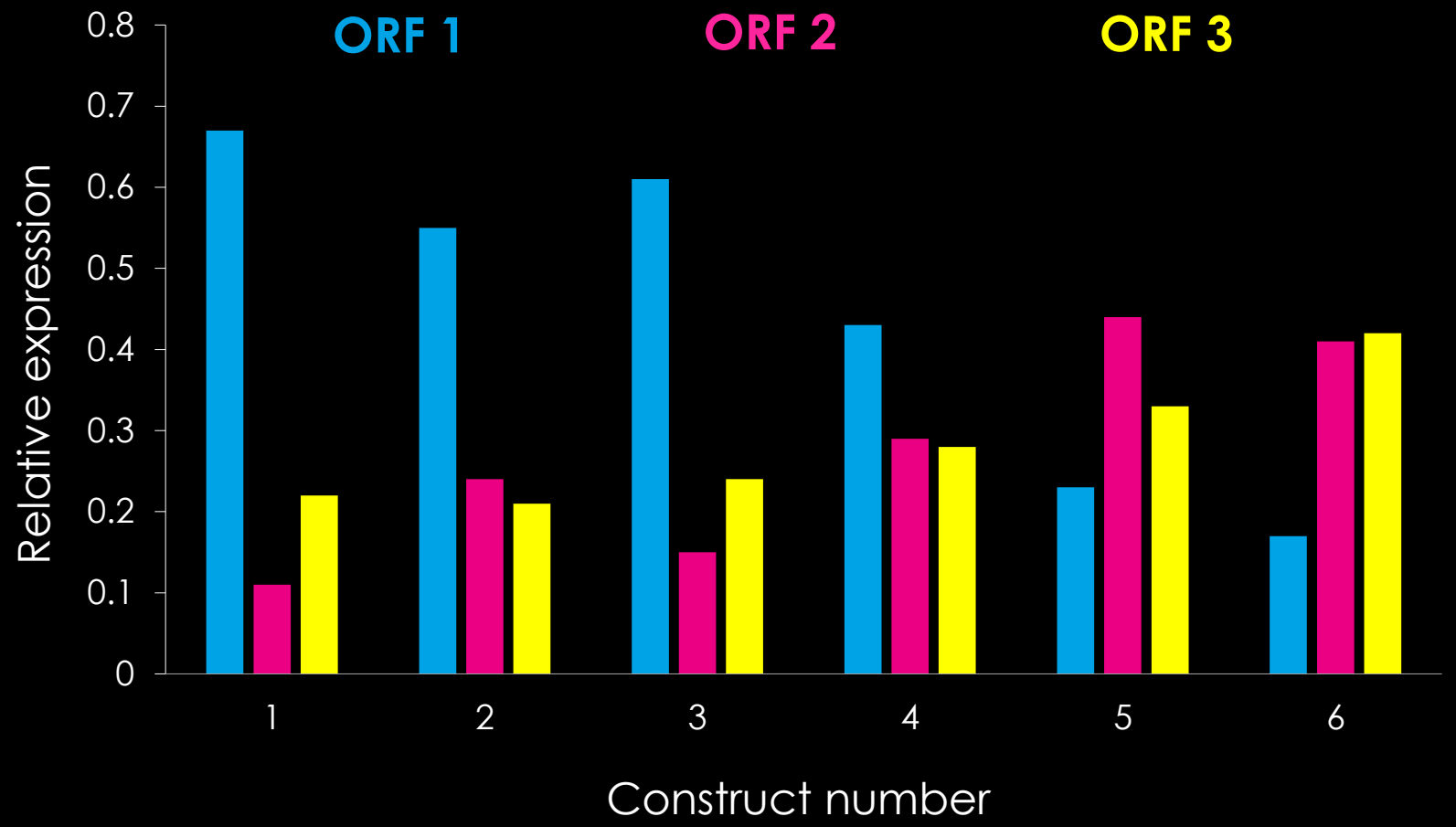
Bispecific Antibodies
chain ratio modulation



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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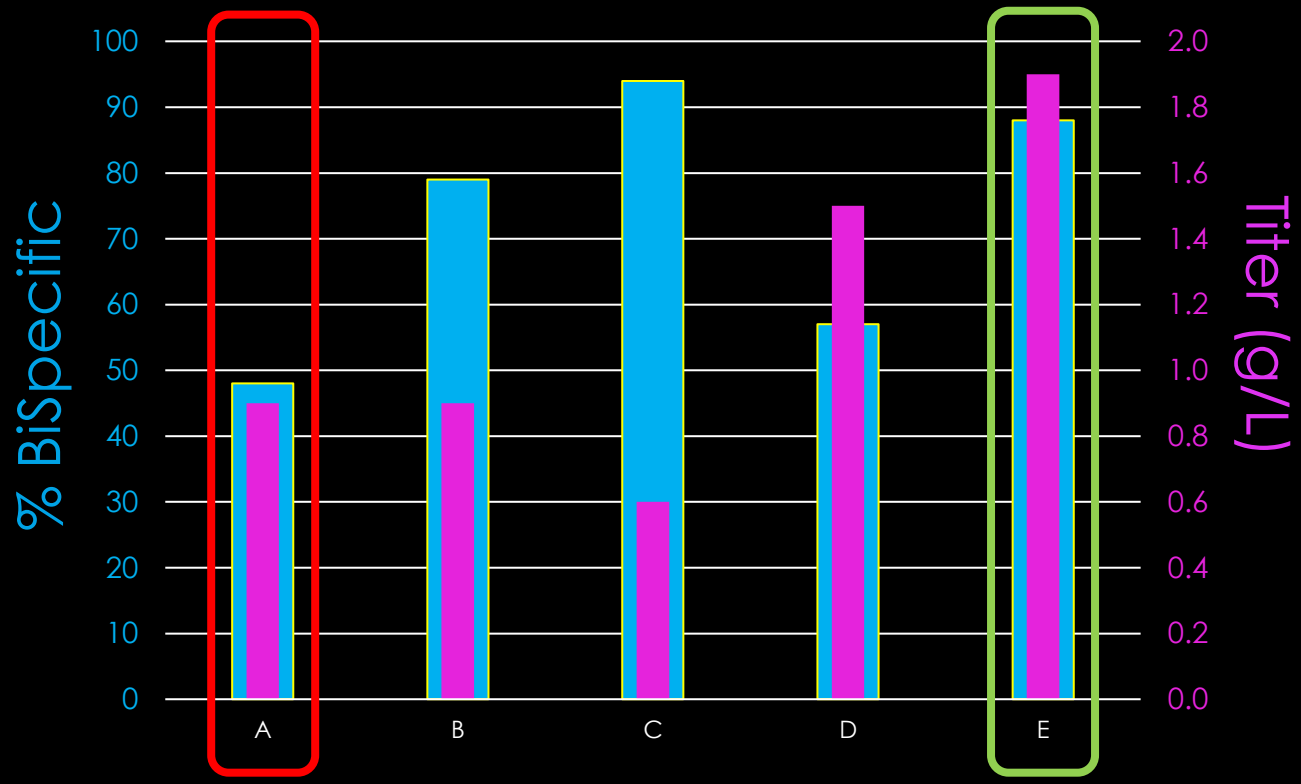
Case Study: 3-Chain Bispecific mAb

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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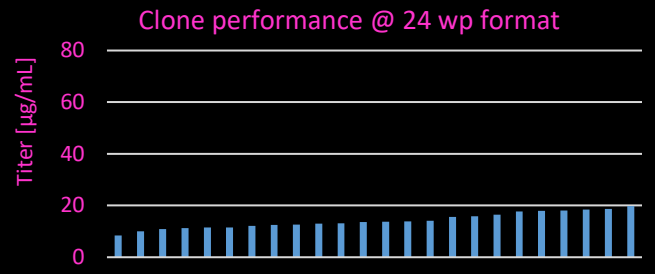
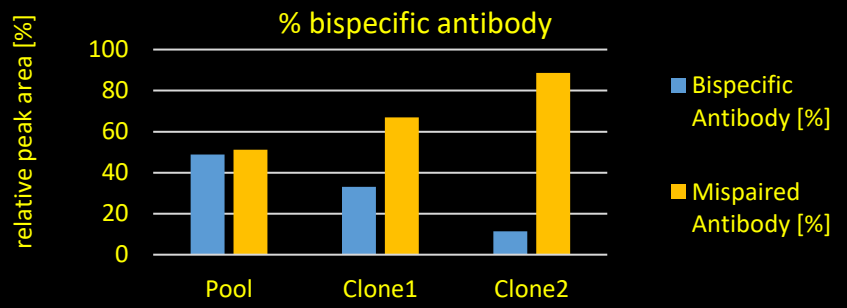
Case Study: 3-Chain BiSpecific mAb

Pool A



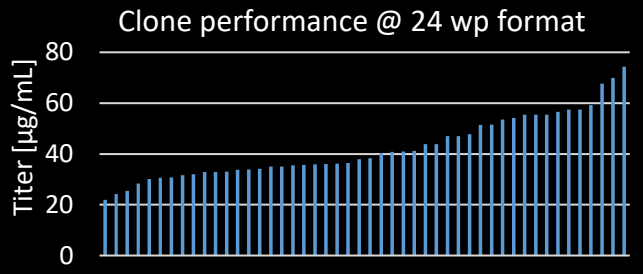


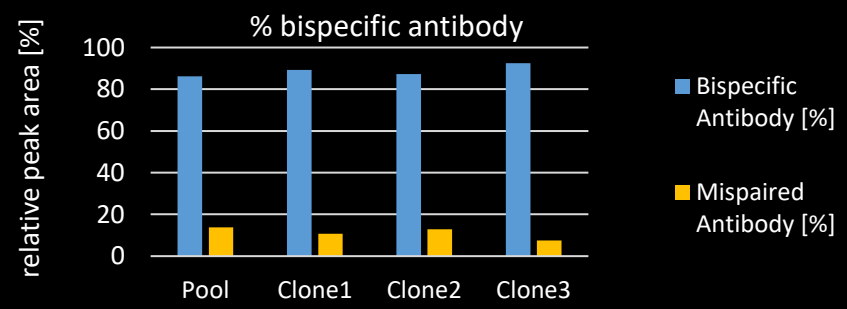
Pool and clone productivity

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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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

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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

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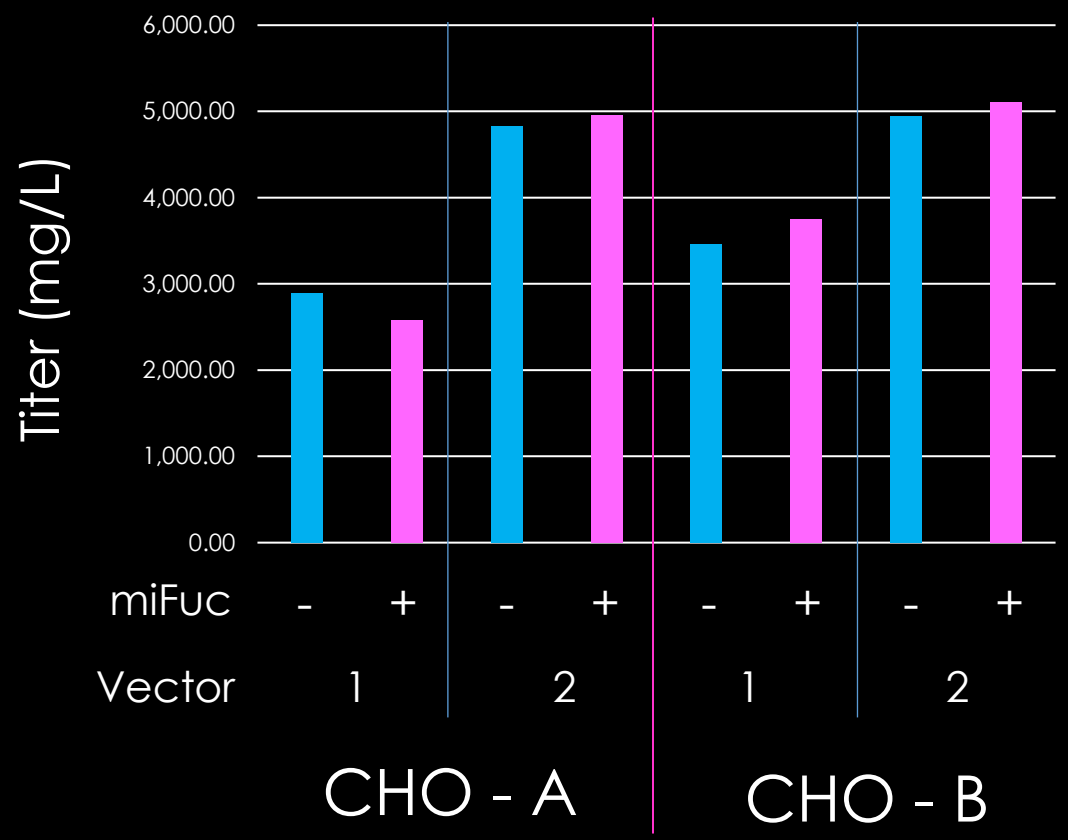
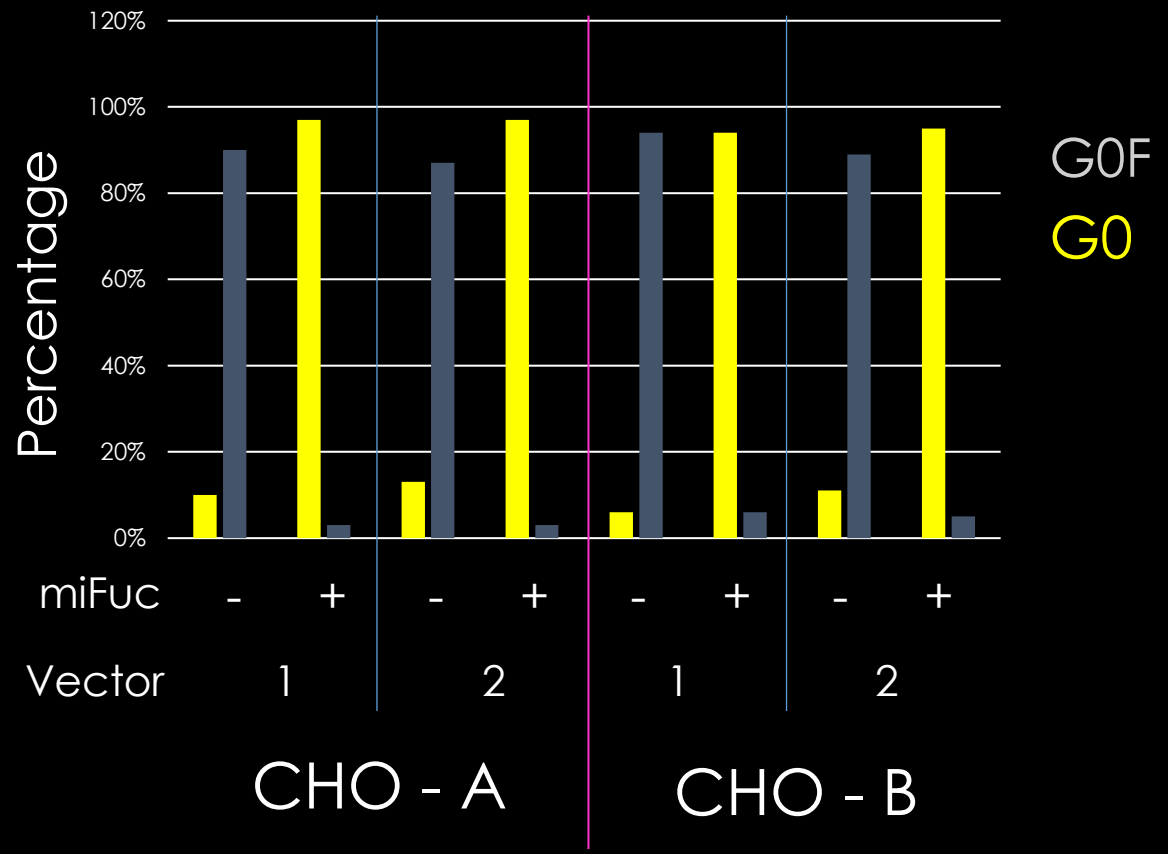
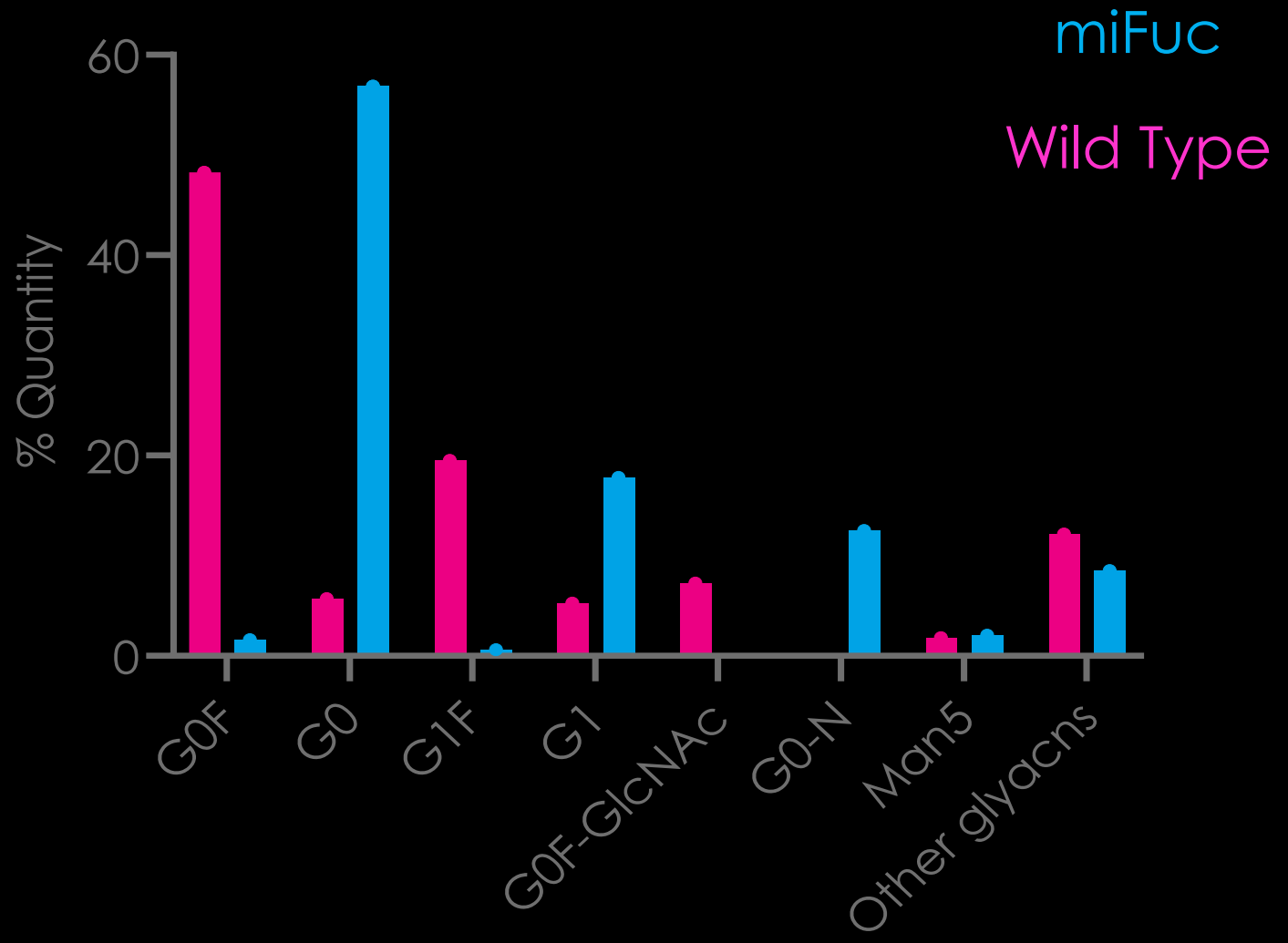
miFuc Platform: Stable Pools





miFuc Platform: Stable Pools



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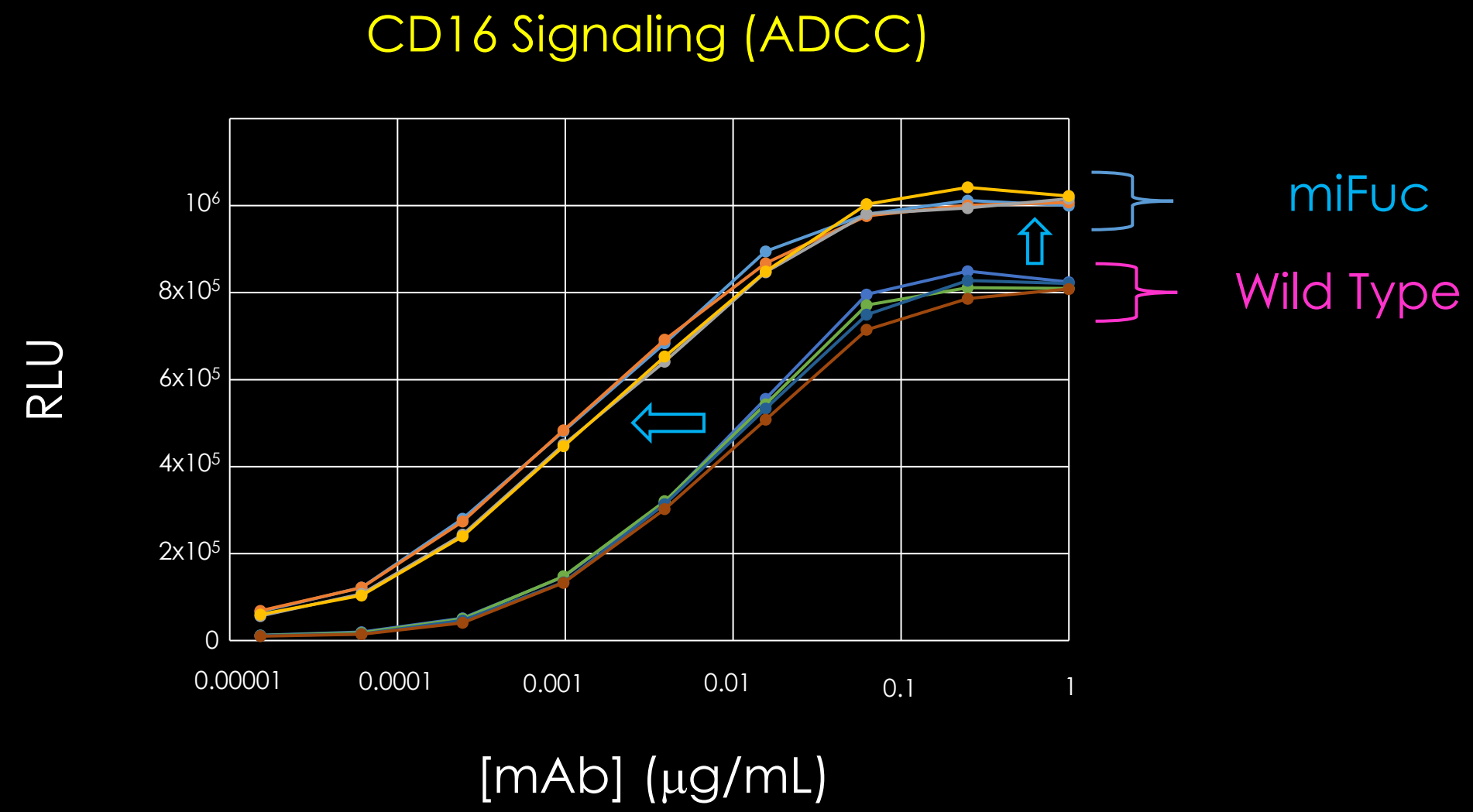
miFuc Platform: ADCC enhancement



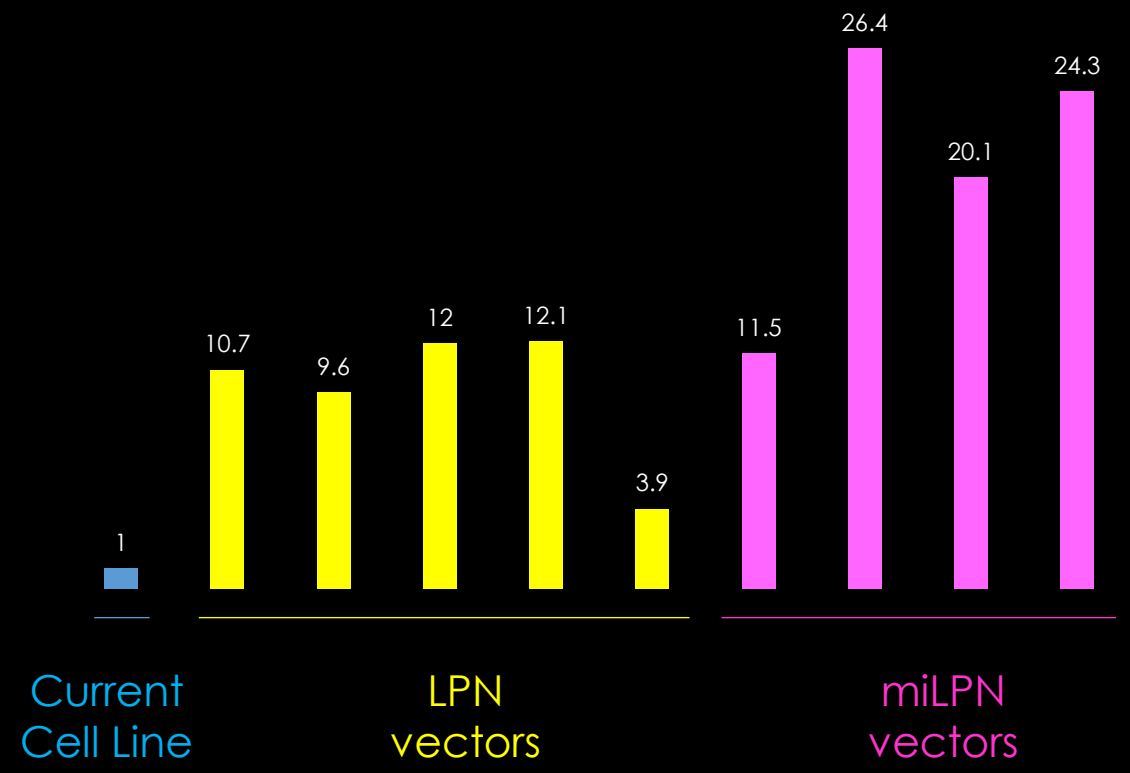
miLPN Platform: Custom Project

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

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Thank You

Oren Beske
obeske@atum.bio

Partners:

Horizon Discovery
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Our Customers