

Leap In Transposase Platform

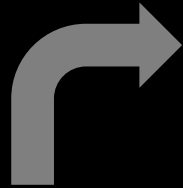
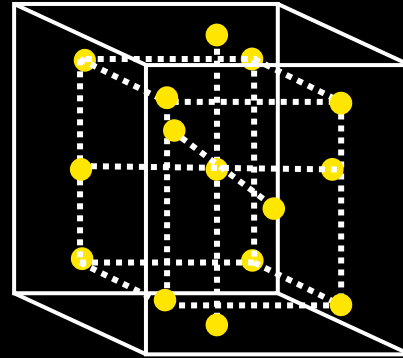
From shiny and new to tried and true



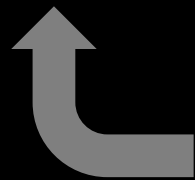
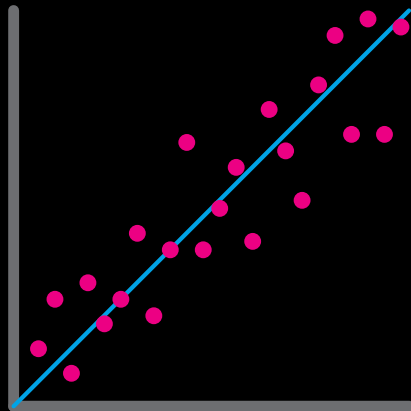
Oren Beske, Ph.D.
obeske@atum.bio



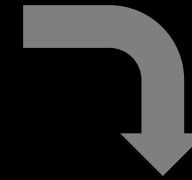
Design of Experiment



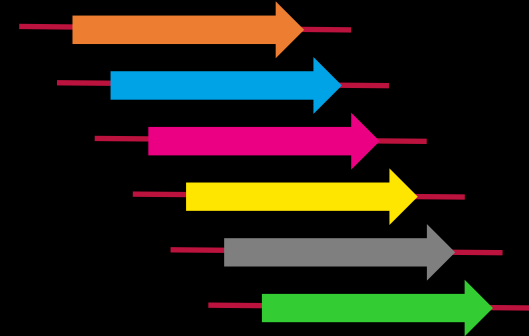
Machine Learning



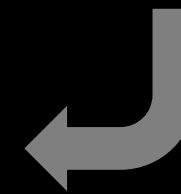
GPS platform



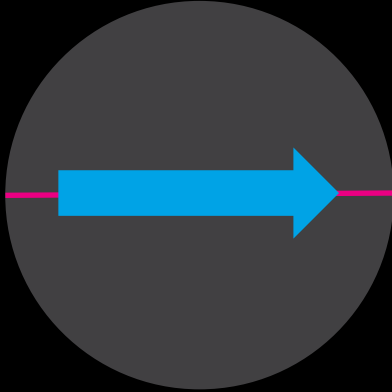
Build



Test



The GPS Platform



gene GPS

ORF codon optimization



vector GPS

Expression vector element
optimization



protein GPS

Protein attribute
optimization



Leap-In Transposase® Platform

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



Transfection to RCB in ~10-12 weeks



Gene synthesis & vector construction

Stable pool generation & characterization

Cell line cloning and ranking

RCB

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



Robust Market Adoption

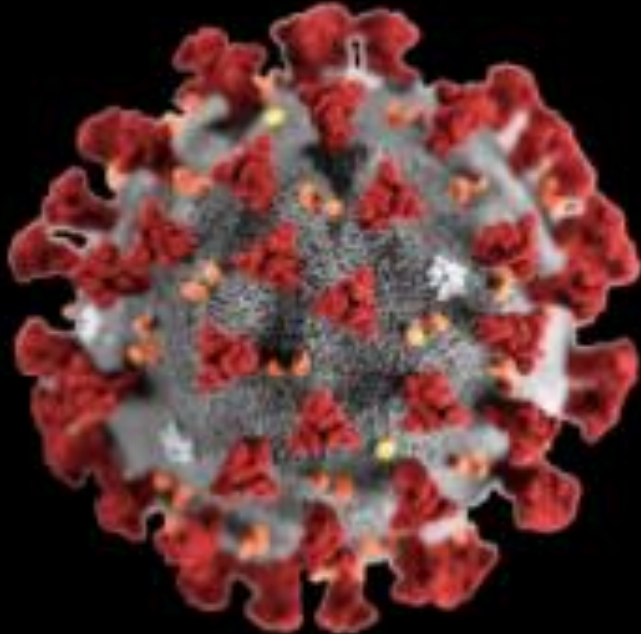
- Offered as a service by ATUM: >70 projects delivered
- >30 active licensees: 11 of top 20 pharma
- 10 IND's filed in less than two years:
 - Seven IND's filed and accepted in US
 - One IND filed and accepted in China
 - Two IND's filed and accepted in EU



Moving Beyond the Routine

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

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

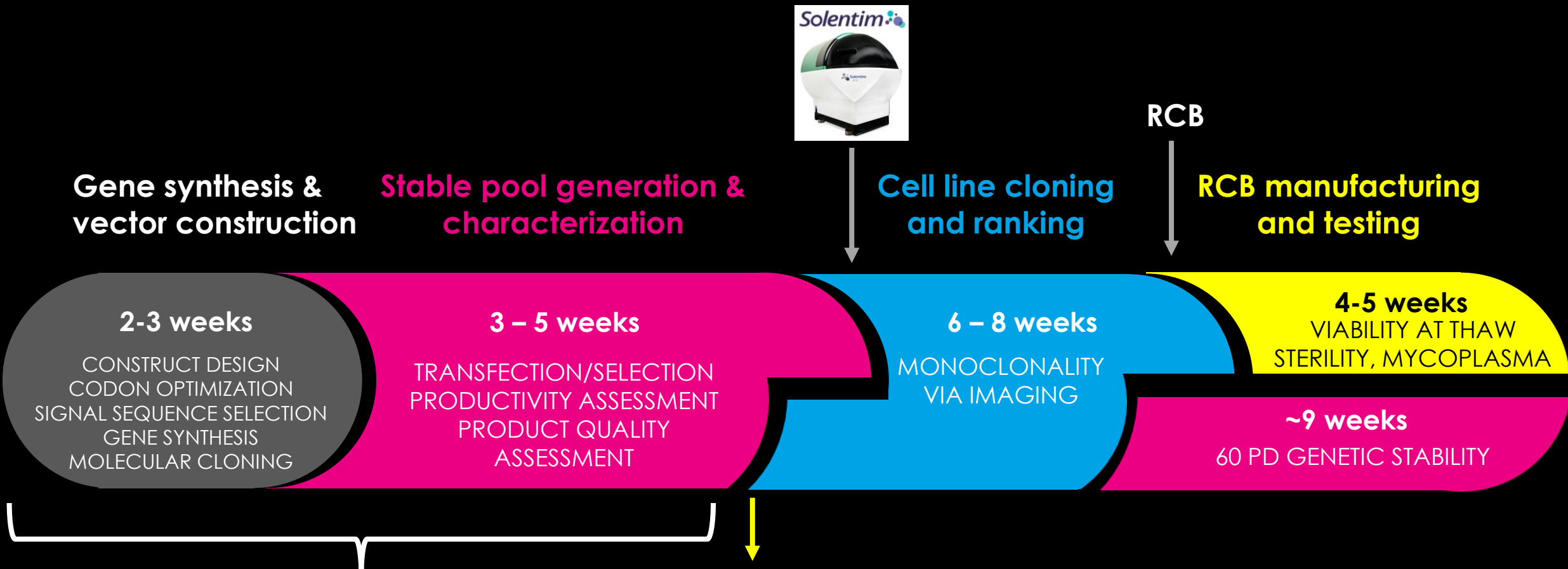
Rapid Cell Line Development: Pools



- 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



Representative pool

How fast can we go?

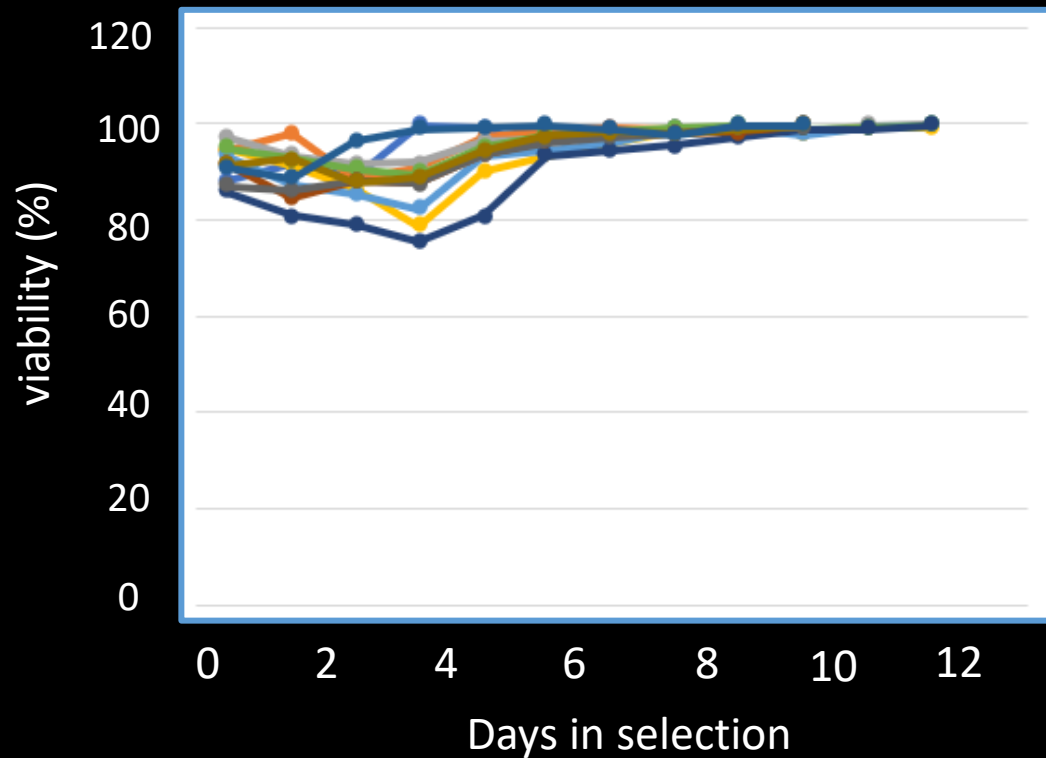
- High titer
- Stable
- Low risk



COVID 19: ATUM Accelerated Timeline: 1

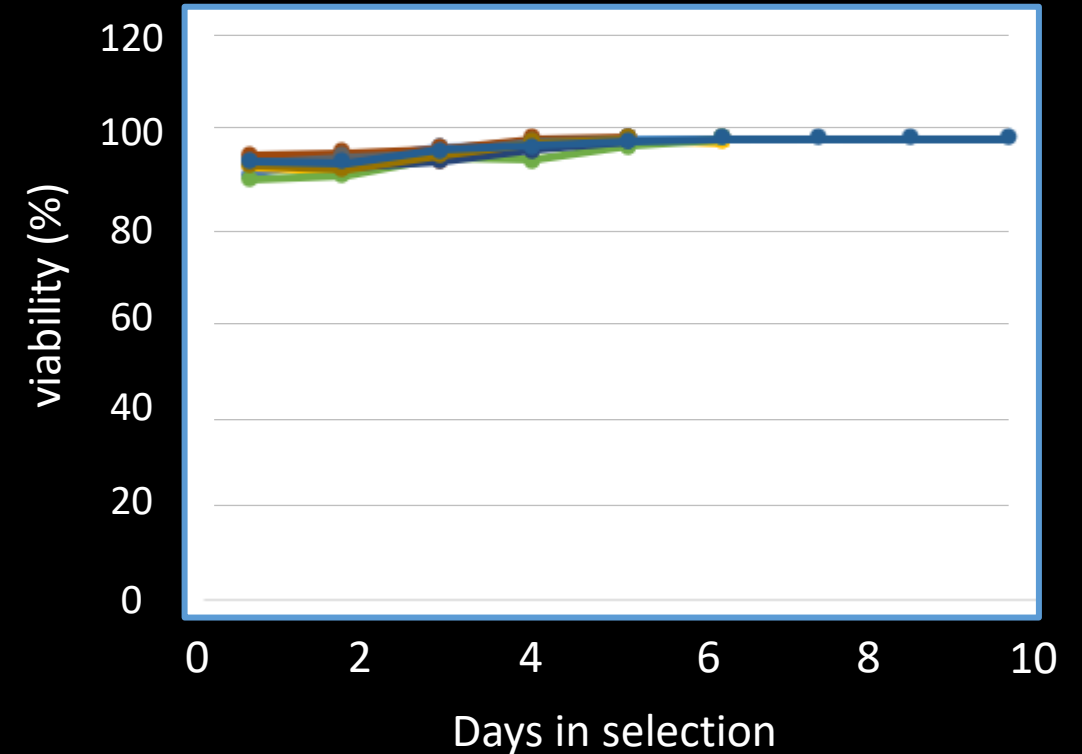


Vector-1



Selection in ~4-6 days

Vector-2



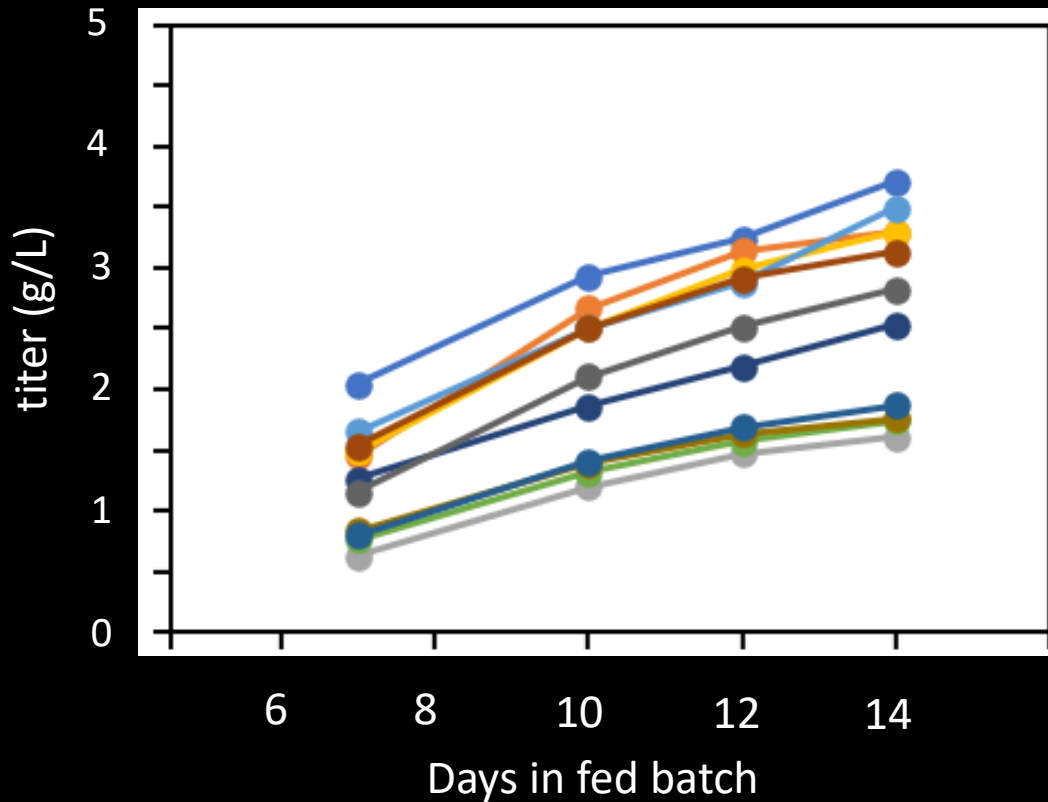
Selection in ~3 days



COVID 19: ATUM Accelerated Timeline: 1

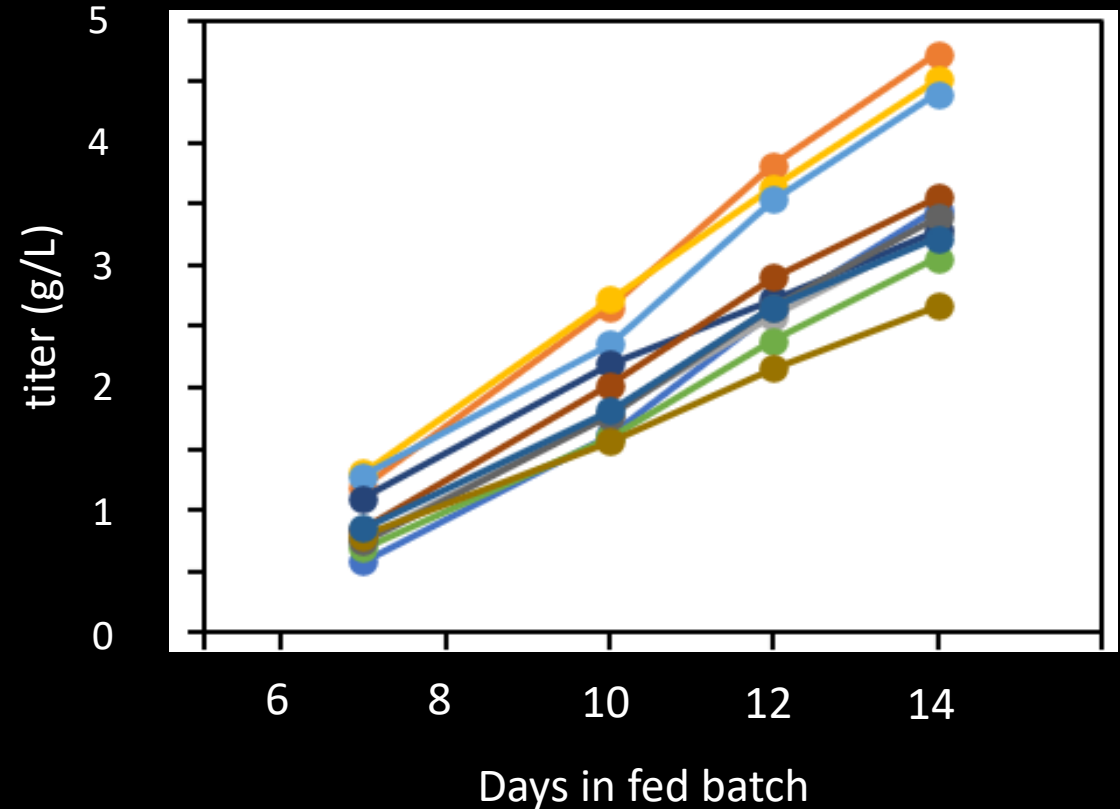


Vector-1



Titer: 1.4 – 3.5 g/L

Vector-2



Titer: 3.5 – 4.8 g/L

Multiple pools banked and transferred to CDMO



COVID 19: ATUM Accelerated Timeline: 1



Gene synthesis

Plasmid prep

Pool Selection

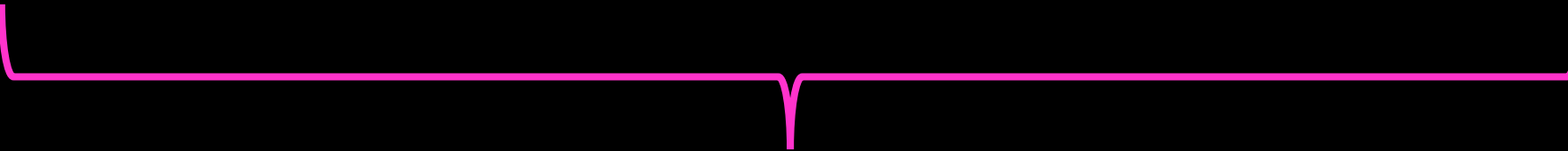
Pool Banking

Bank Testing



Fed Batch

Ship to CDMO



38 days!

IND filed

CDMO: Intensified fed batch process

>12 g/L



COVID 19: ATUM Accelerated Timeline: 2



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

Rita Agostinetto¹, Jessica Dawson², Angela Lim², Mirva Hejjaoui-simoneau³, Cyril Boucher³, Bernhard Valldorf⁴, Adin Ross-gillespie³, Joseph Jardine⁵, Devin Sok⁵, Dennis Burton⁵, Thomas Hassell⁶, Hervé Broly⁷, Wolf Palinsky³, Philippe Dupraz³, Mark Feinberg⁶, and Antu Dey⁸

¹Merck Serono SpA

²EMD Serono Biotech Center Inc

³Ares Trading SA

⁴Merck KGaA

⁵The Scripps Research Institute

⁶International Aids Vaccine Initiative

⁷Merck Serono SA-Corsier-sur-Vevey

⁸Greenlight Biosciences Inc

Pools 6.0 g/L



200L Preclinical Safety



2000L Phase I

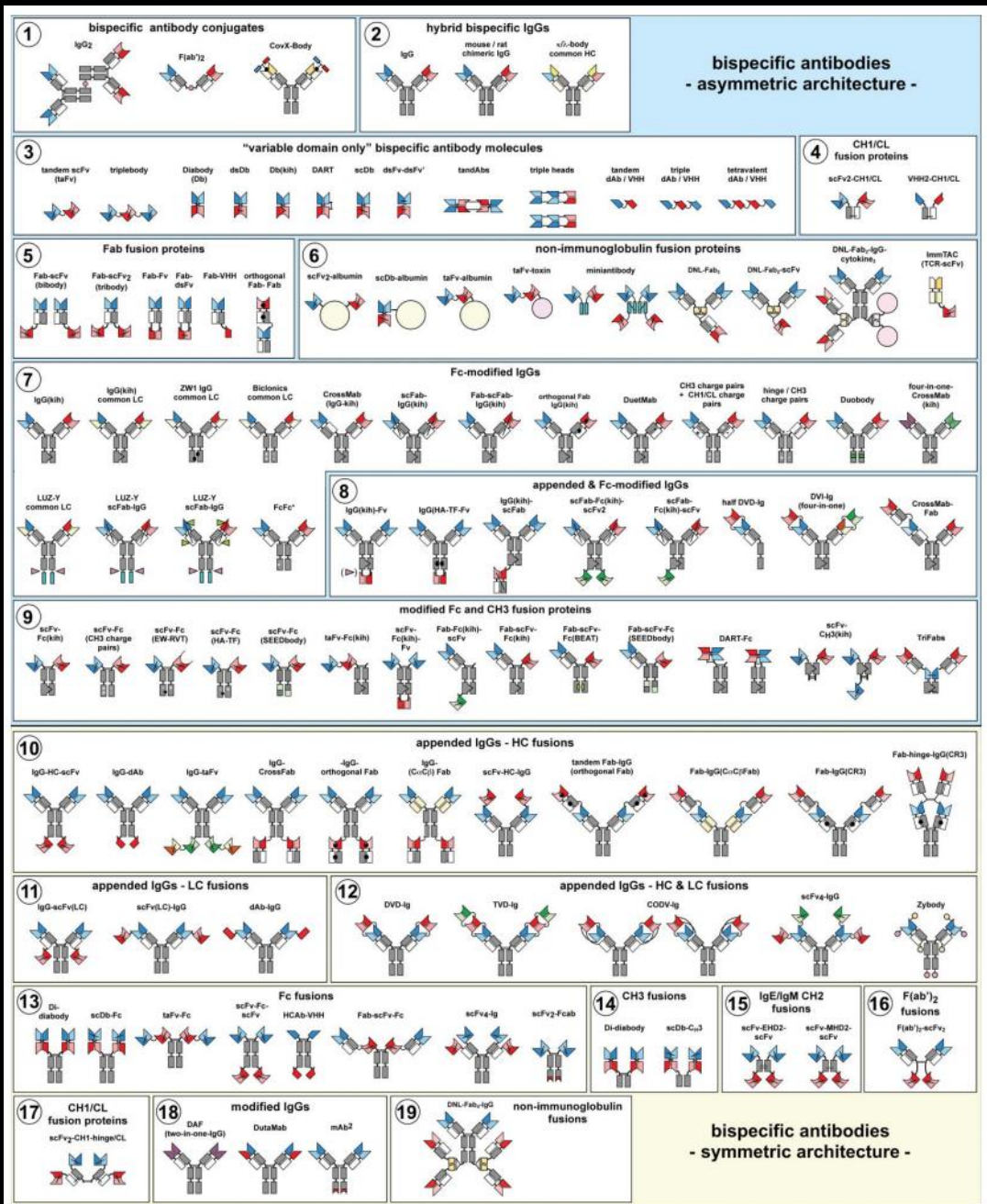
Preprint on Authorea.com

“.. Enabled manufacturing of early clinical trial material within 4.5 months ...”



Beyond mAb's: 3 Chains and More

The "zoo" of bispecifics



Considerations for chain ratio balancing

Sequence

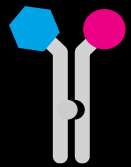
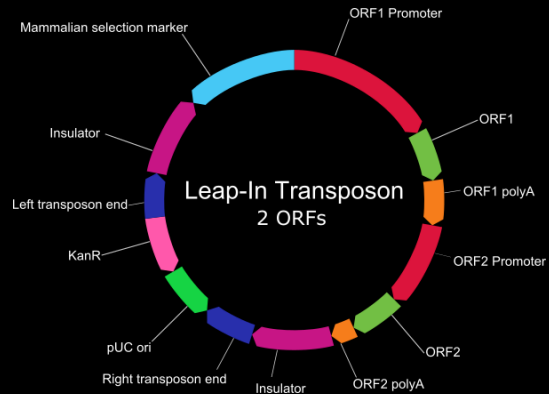
- Codon choice
- mRNA 2° structure
- Poly-A signal
- 5'/3' UTR choice
- mRNA stability
- Ribosomal entry/processivity
- Splice site donor/acceptor
- Signal sequences
- Etc.

Vector

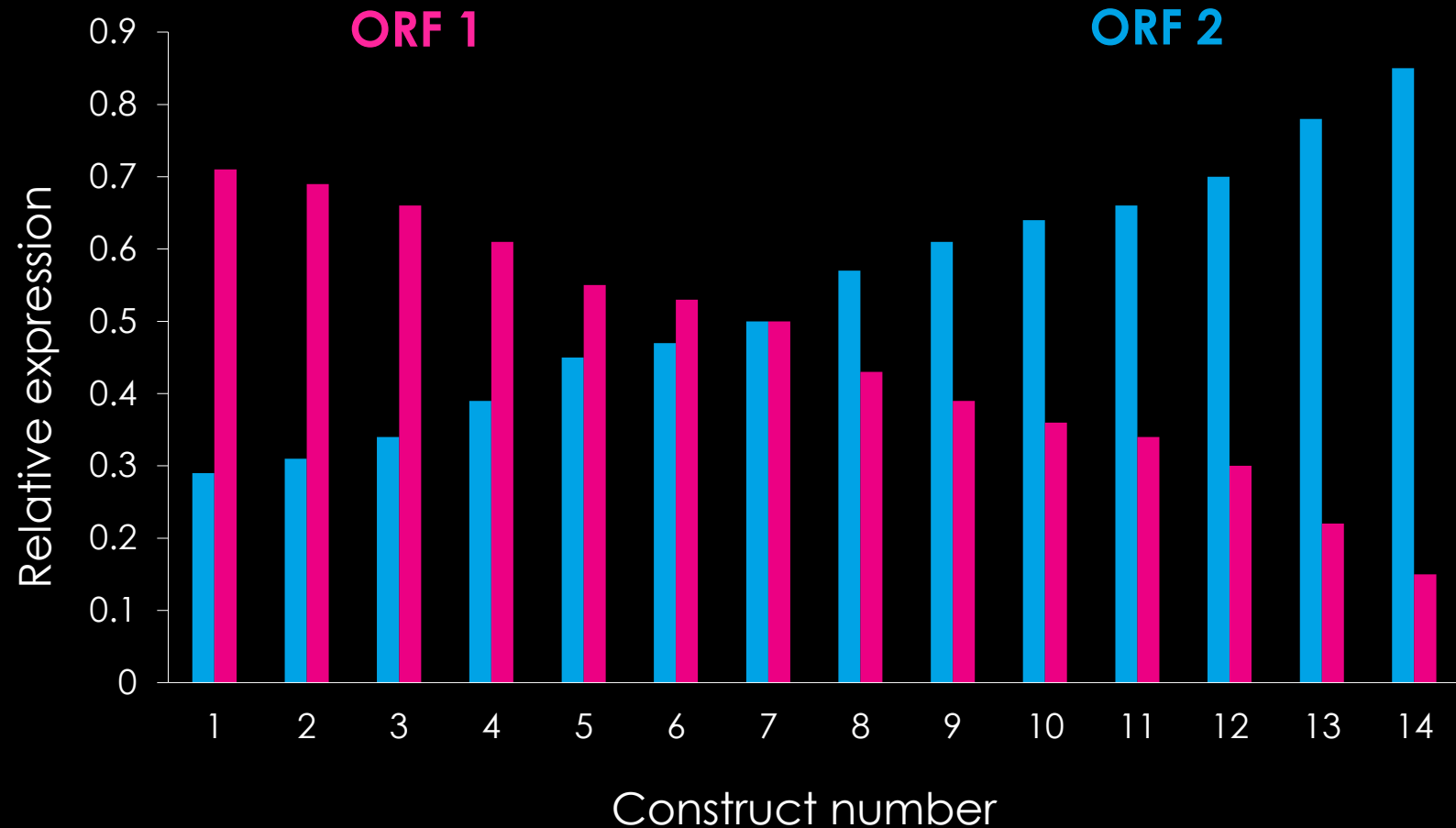
- Promoter choice
- Order of expression cassettes
- Number of expression cassettes
- Spacing of expression cassettes
- Directionality of expression cassettes
- Size of vectors
- Single vector or multiple vectors
- Choice of insulators
- Etc.



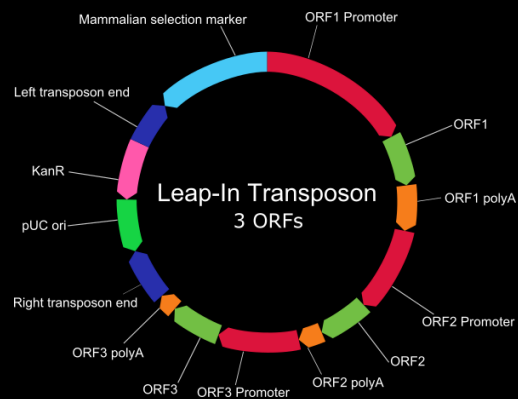
Controlling ratios with construct design: 2 ORFs



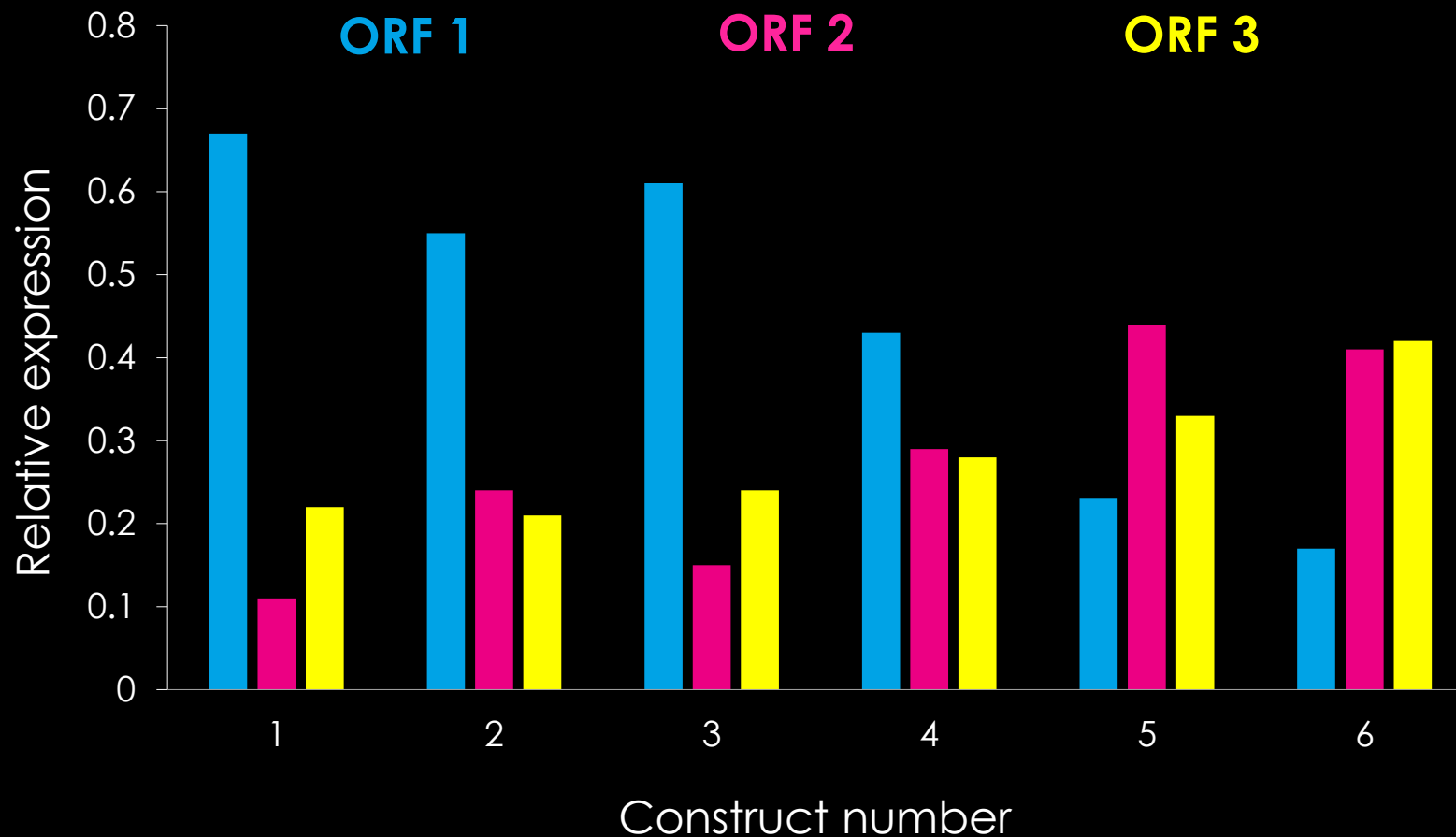
Bispecific Antibodies
chain ratio modulation



Controlling ratios with construct design: 3 ORFs



Bispecific Antibodies
chain ratio modulation



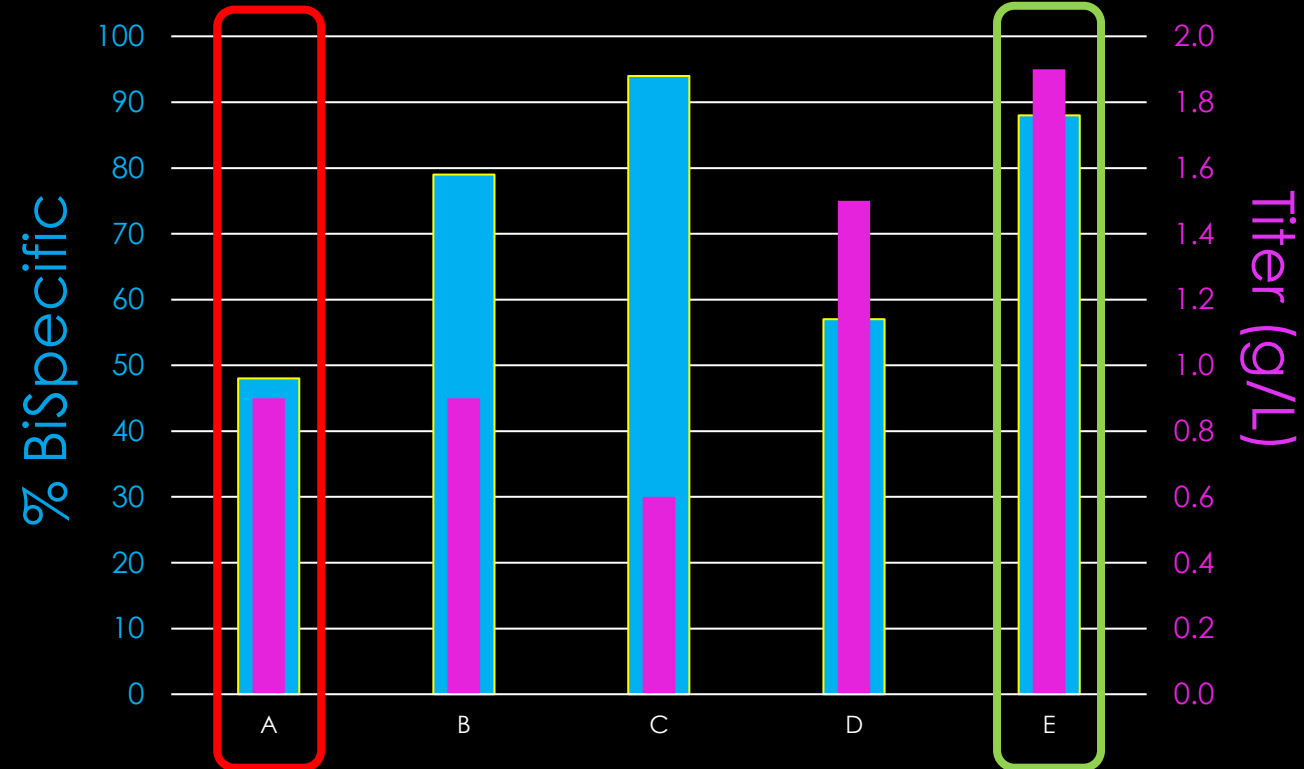
Case Study: 3-Chain Bispecific mAb

- 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

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

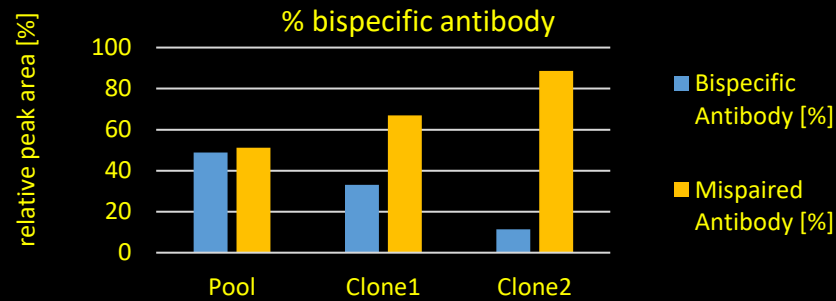
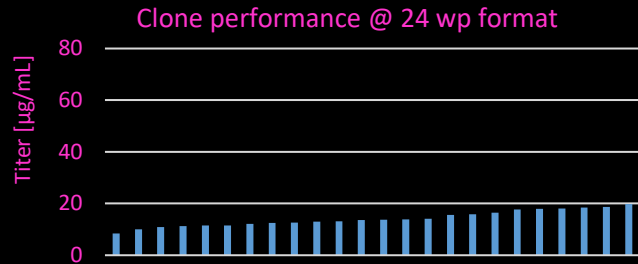


Screening vectors at pool stage enables ID of high value pools



Case Study: 3-Chain BiSpecific mAb

Pool A

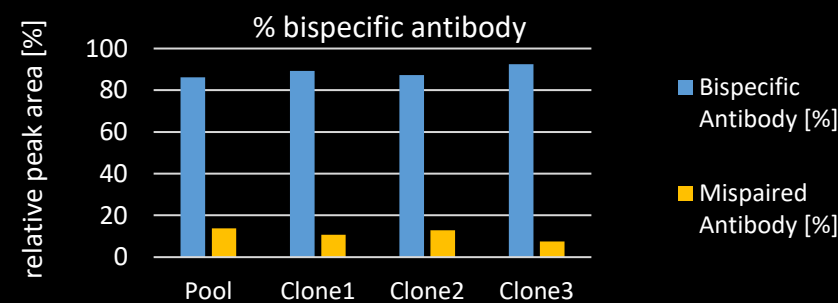
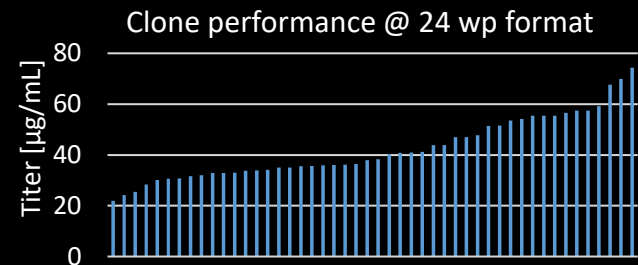


Pool and clone productivity

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

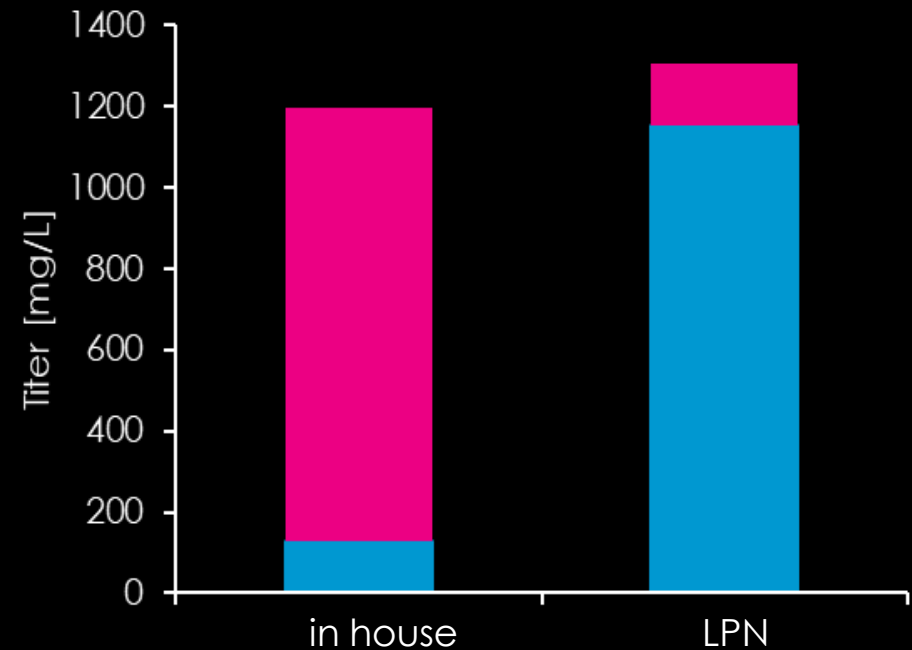
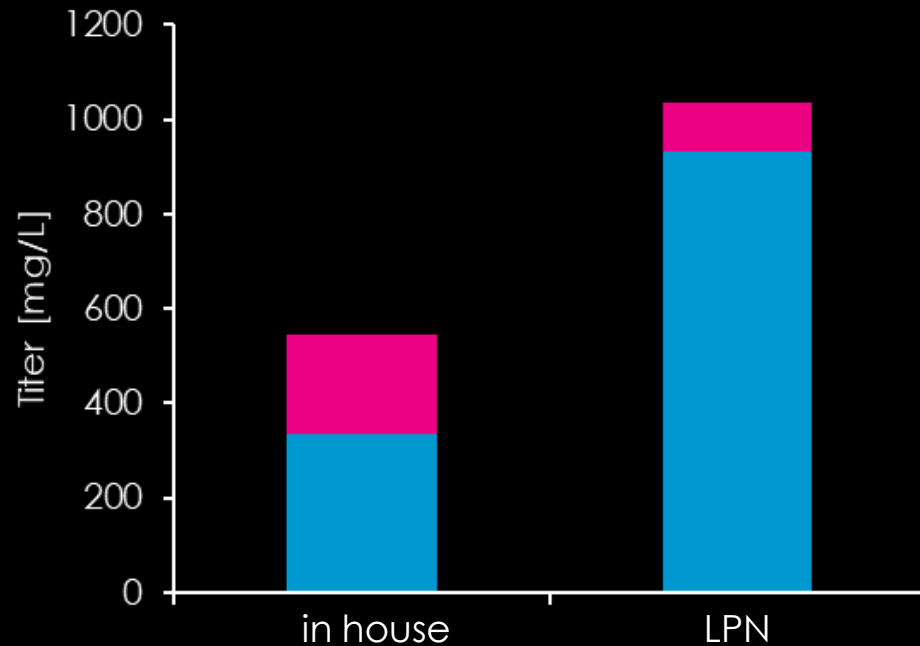
Good pools predict good clones



Controlling ratios with construct design: 3 ORFs

Desired product

Assembly variant



Leap-In enabled chain balancing = significantly improved product assembly



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



The miLPN platform

Use Leap In Transposase platform to reduce gene expression

miCHO-GS

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miFuc

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miLPN

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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



miFuc Platform: Overview

Transient:

Modified HEK host
Modified CHO host

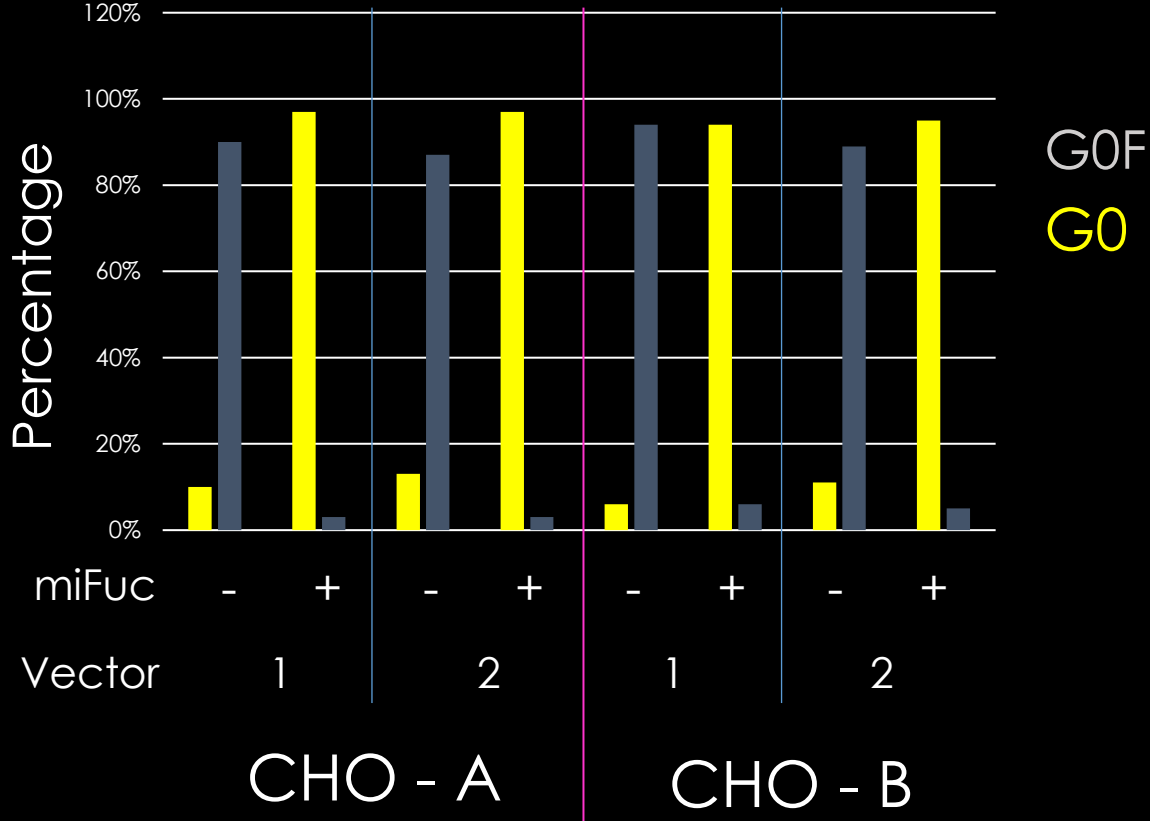
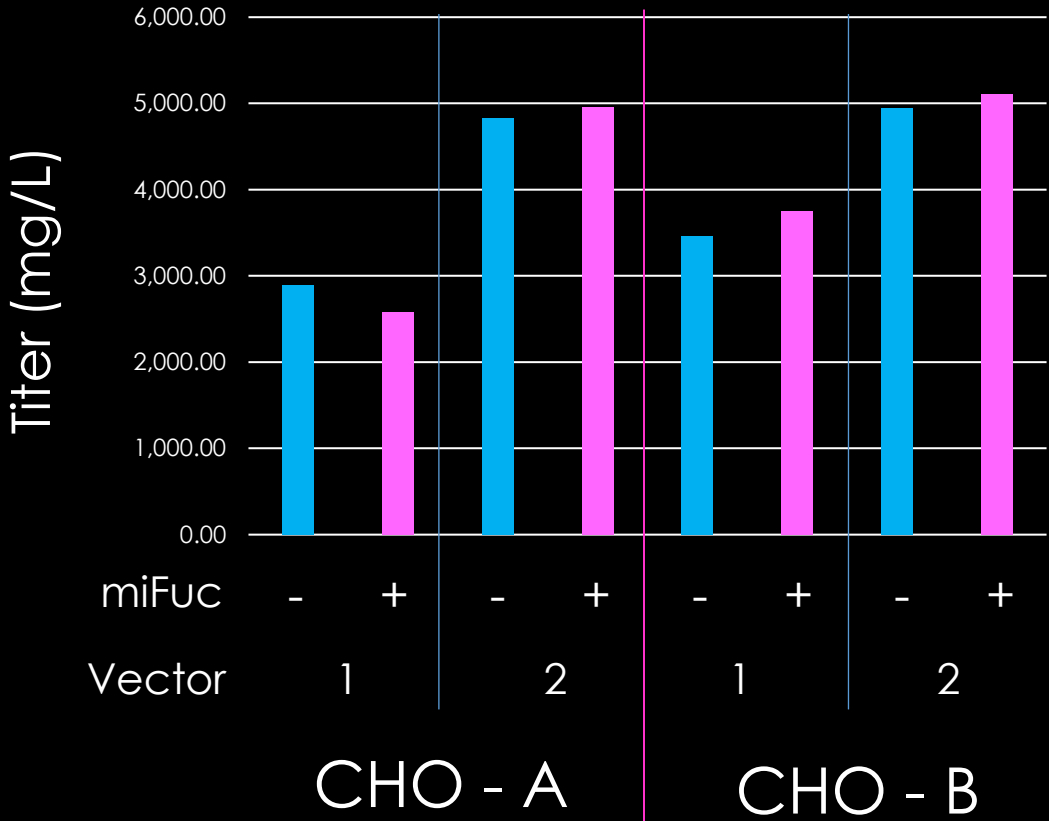
Stable:

Vector based approach
Unmodified cell host
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Proof of concept stage: seeking early access partners

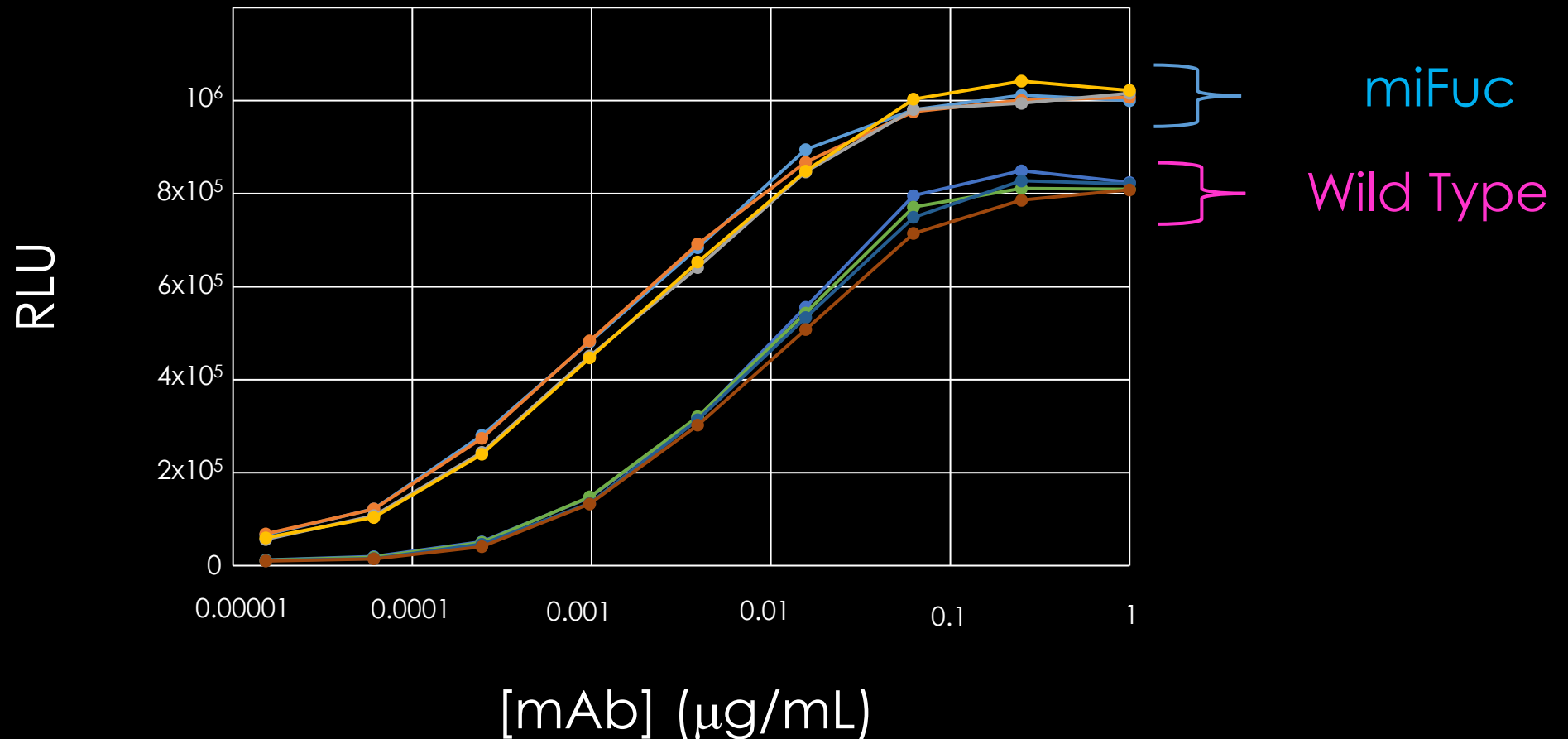


miFuc Platform: Stable Pools

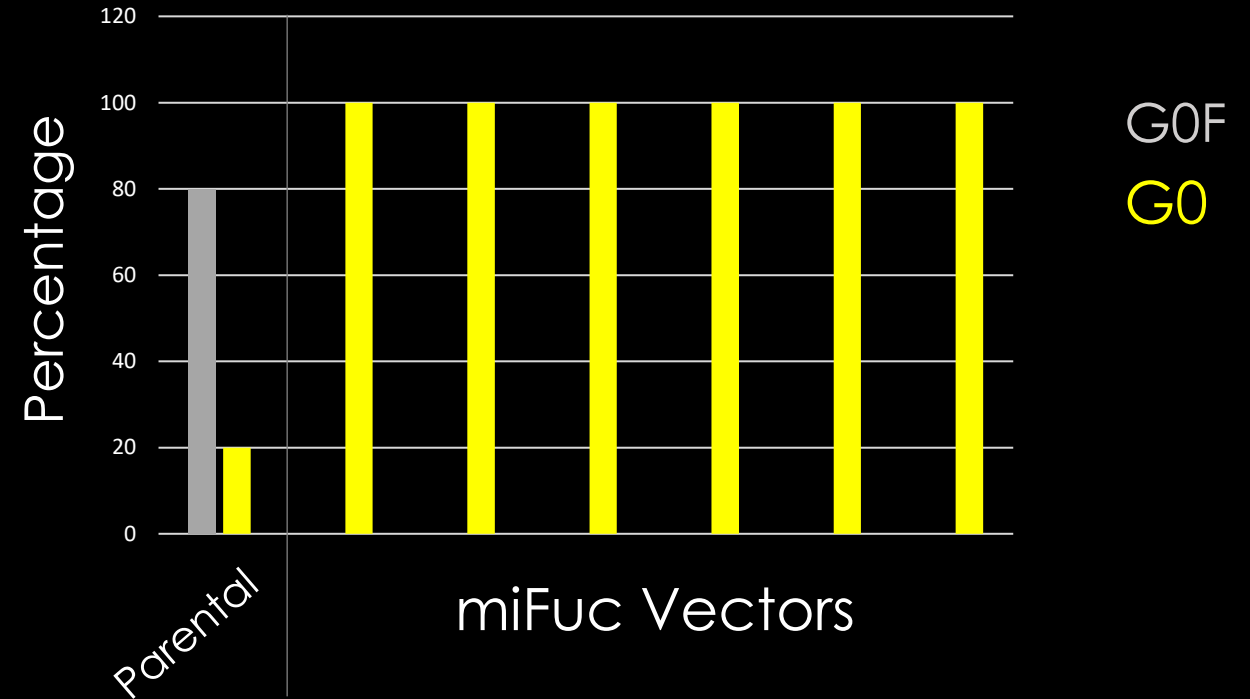
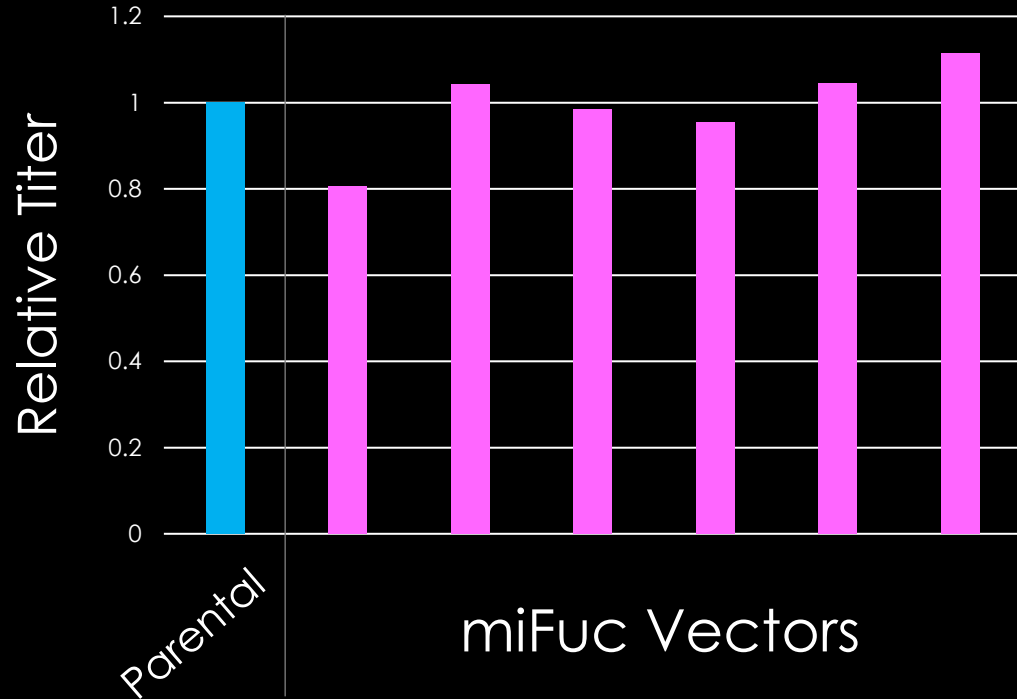


miFuc Platform: ADCC enhancement

CD16 Signaling (ADCC)



miFuc Platform: Existing cell line



miFuc technology works on existing mAb expression line



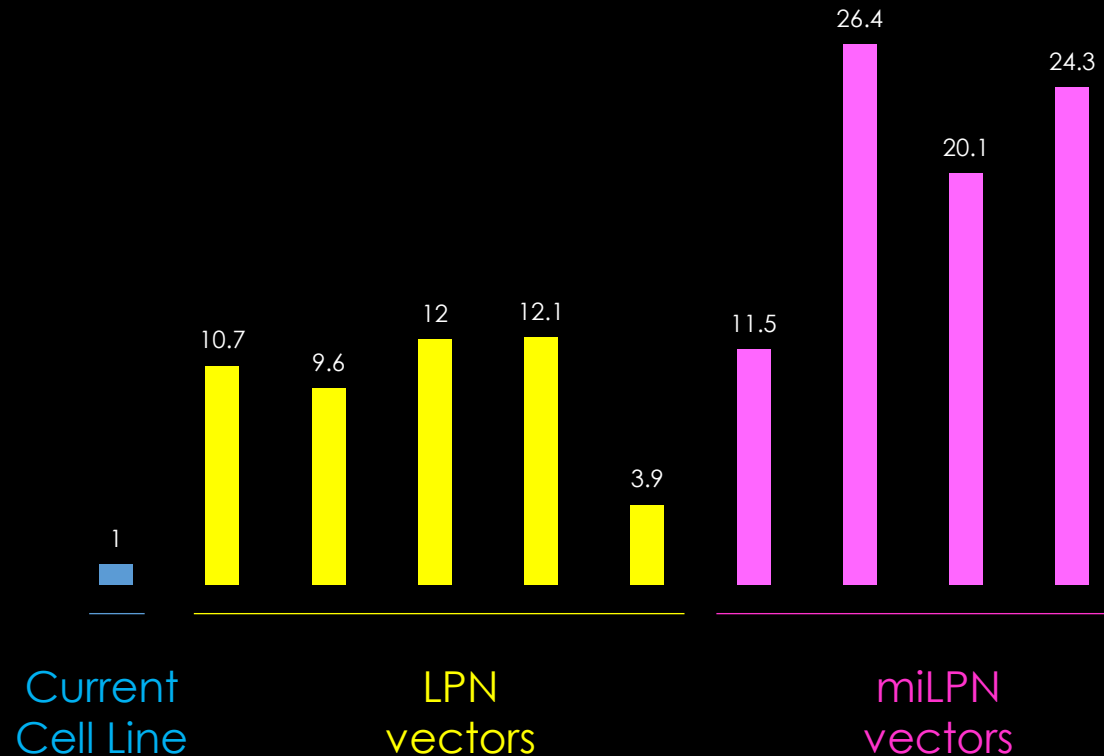
miLPN Platform: Custom Project

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



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



ATUM

- 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





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

