Cell Line Development for Multi-chain Biologics:

Leveraging the Leap In Transposase Platform

The 13th Annual BIOPROCESSING SUMMIT
IN-PERSON • VIRTUAL
FLEXIBLE REGISTRATION

THE POWER OF THE POOL

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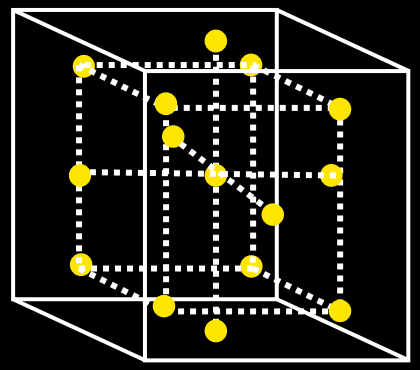
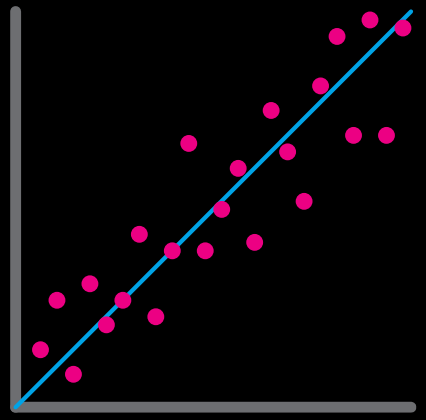
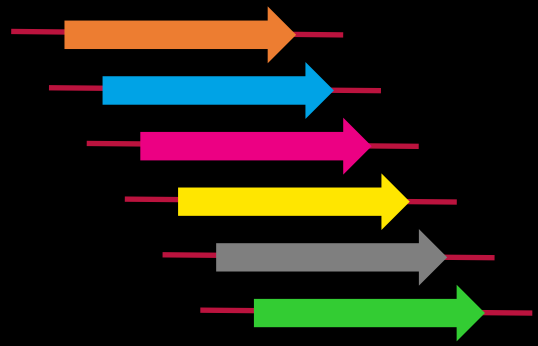
Design of Experiment



Machine Learning



GPS platform



Build

Test

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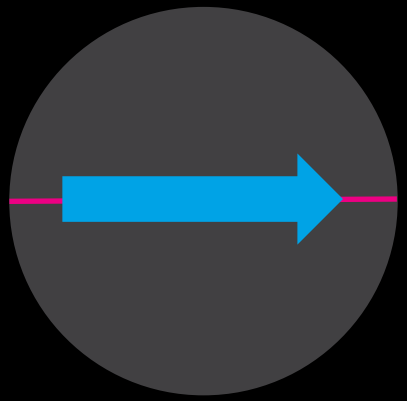
The GPS Platform



gene GPS

ORF codon optimization

vector GPS

Expression vector element optimization

protein GPS

Protein attribute optimization

Leap-In Transposase® Platform

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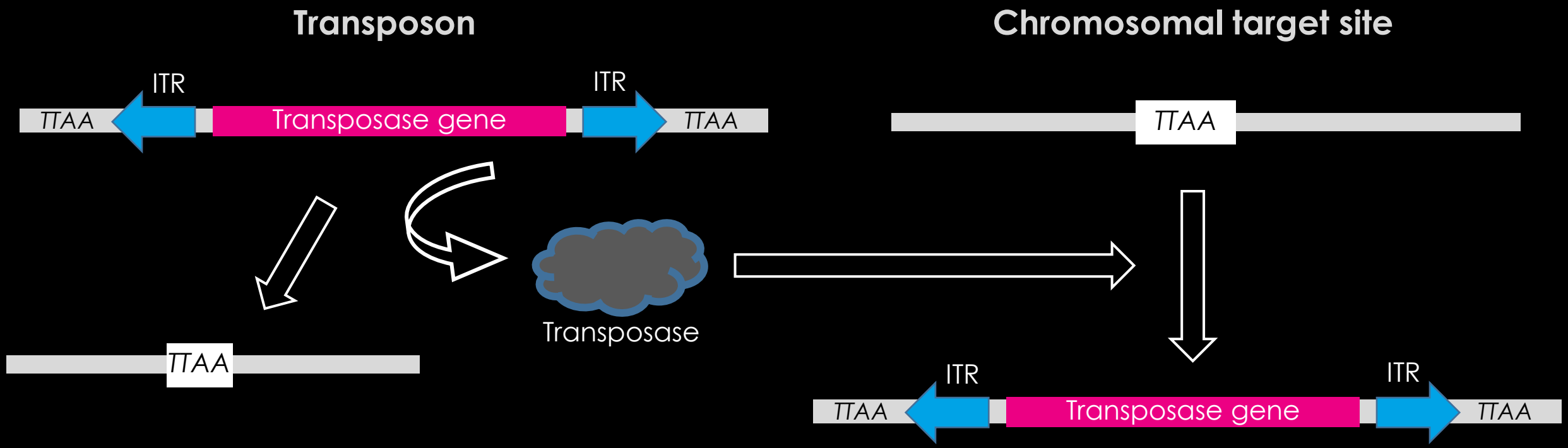
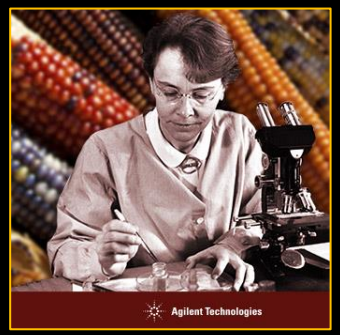
The life of a transposon-transposase pair



- 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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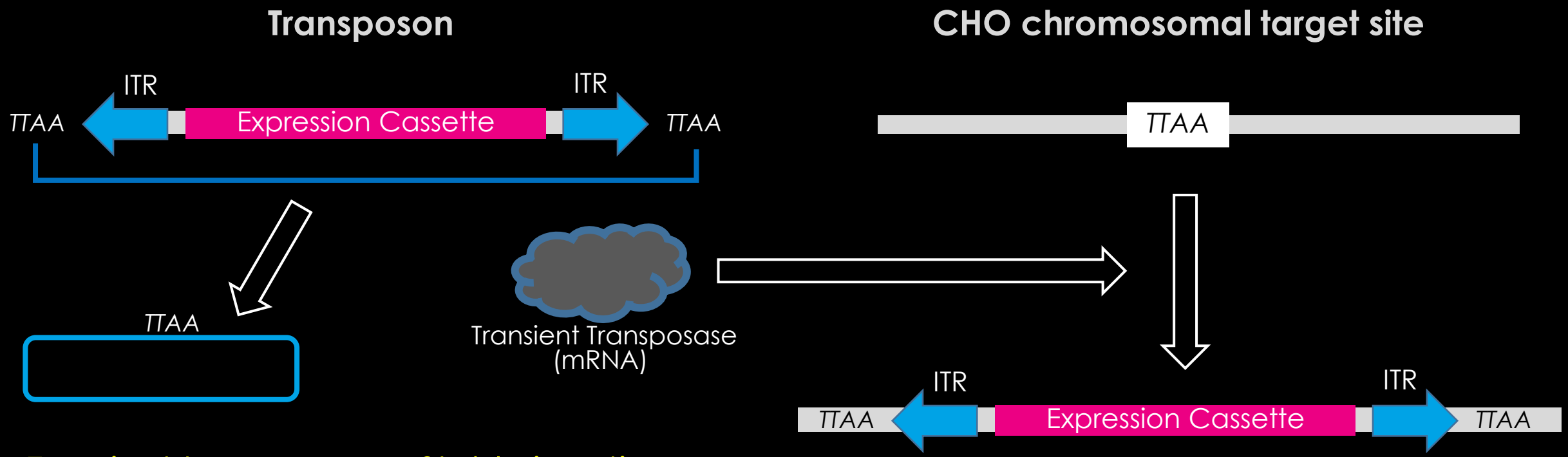
The life of a transposon-transposase pair



- 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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Transfection to RCB in ~12 weeks

RCB

Gene synthesis & vector construction

Stable pool generation & characterization

Cell line cloning and ranking

RCB manufacturing and testing

1 – 2 weeks

CONSTRUCT DESIGN
CODON OPTIMIZATION
SIGNAL SEQUENCE SELECTION
GENE SYNTHESIS
MOLECULAR CLONING

4 – 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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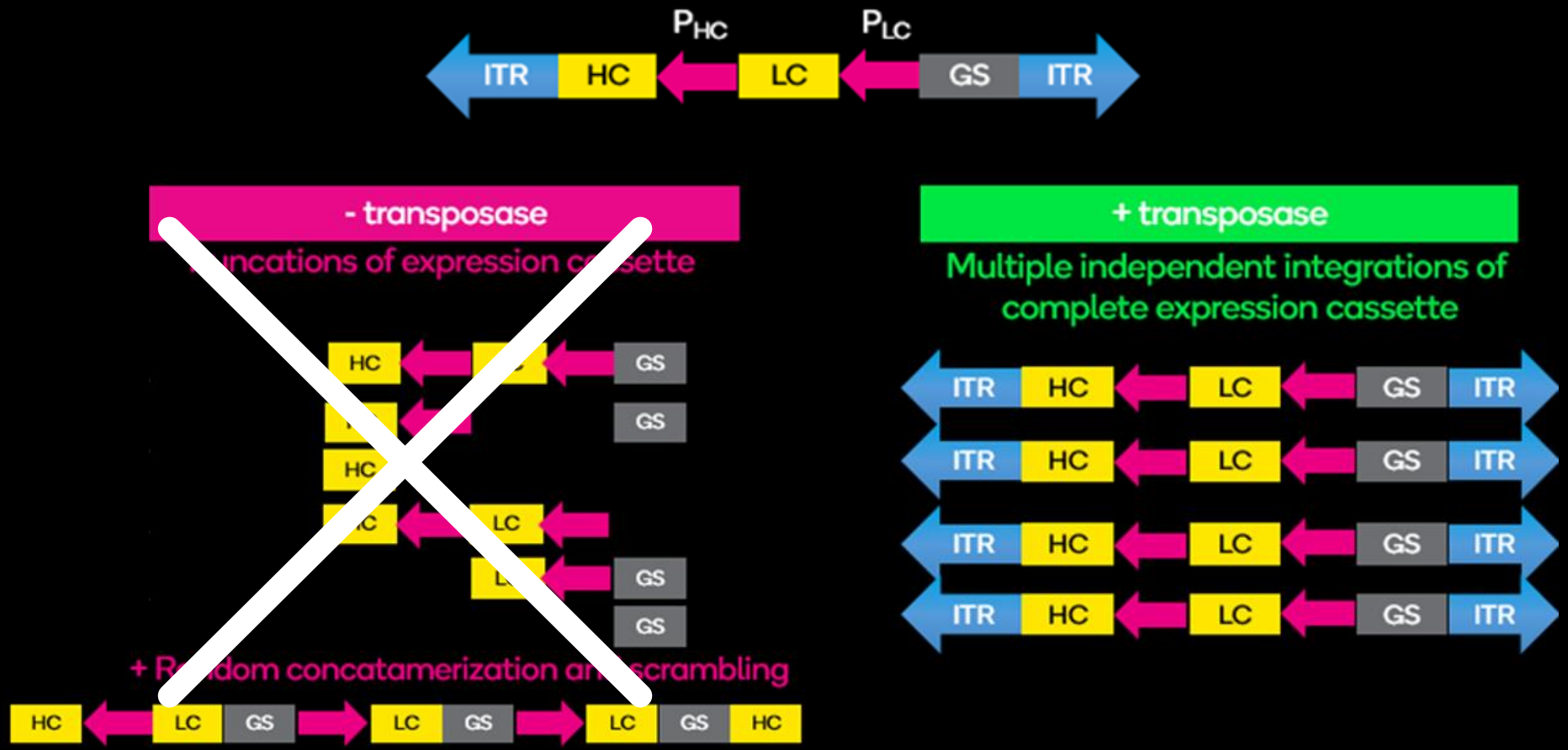
Consistent, uniform presentation of Leap-In® transgenes



- 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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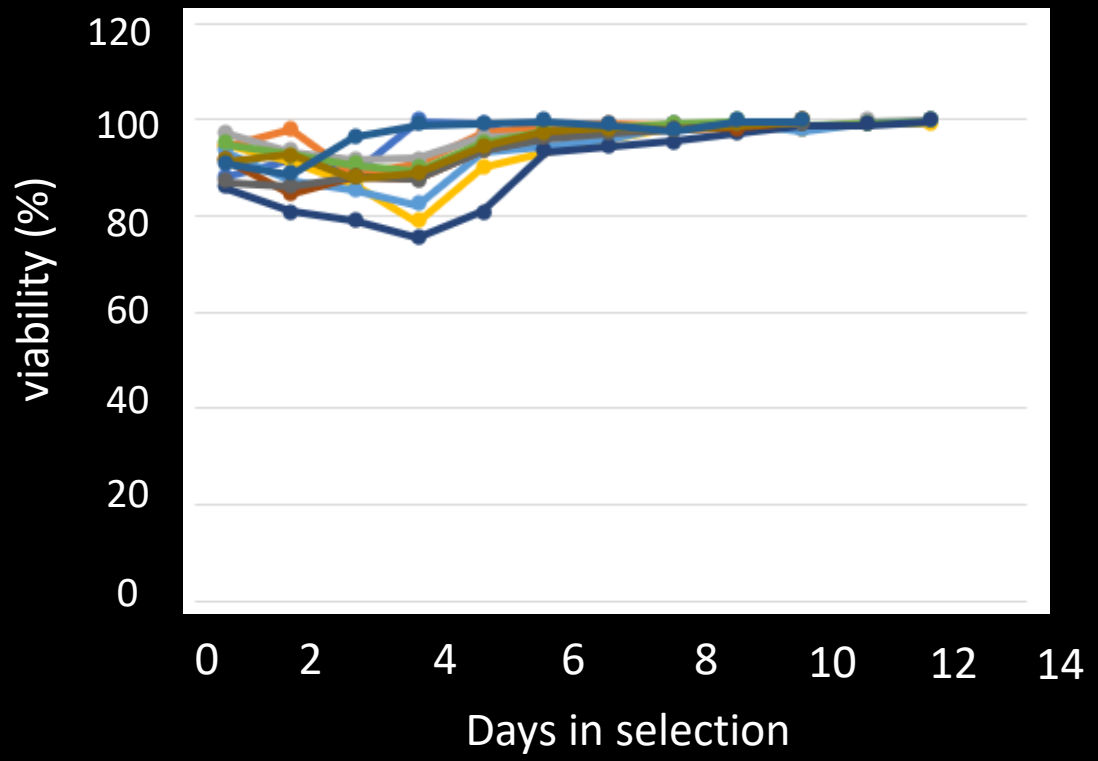
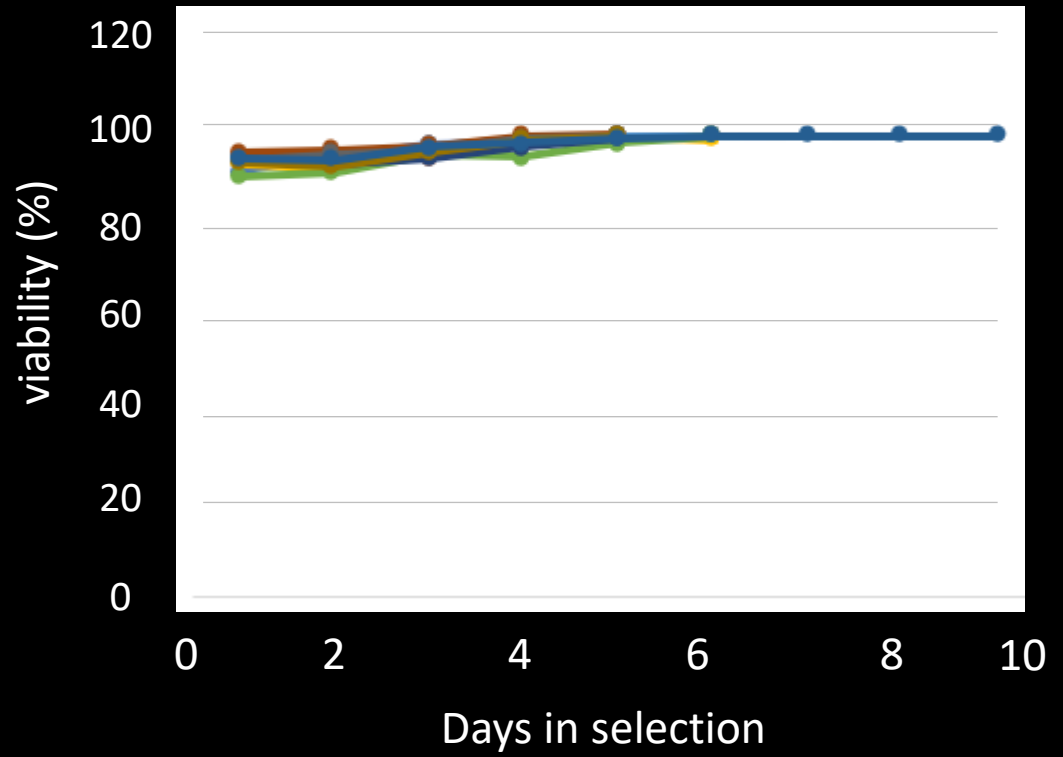
Rapid Stable CHO Pool Selection

Vector-1



Selection in ~7-8 days

Vector-2



Selection in ~3-4 days

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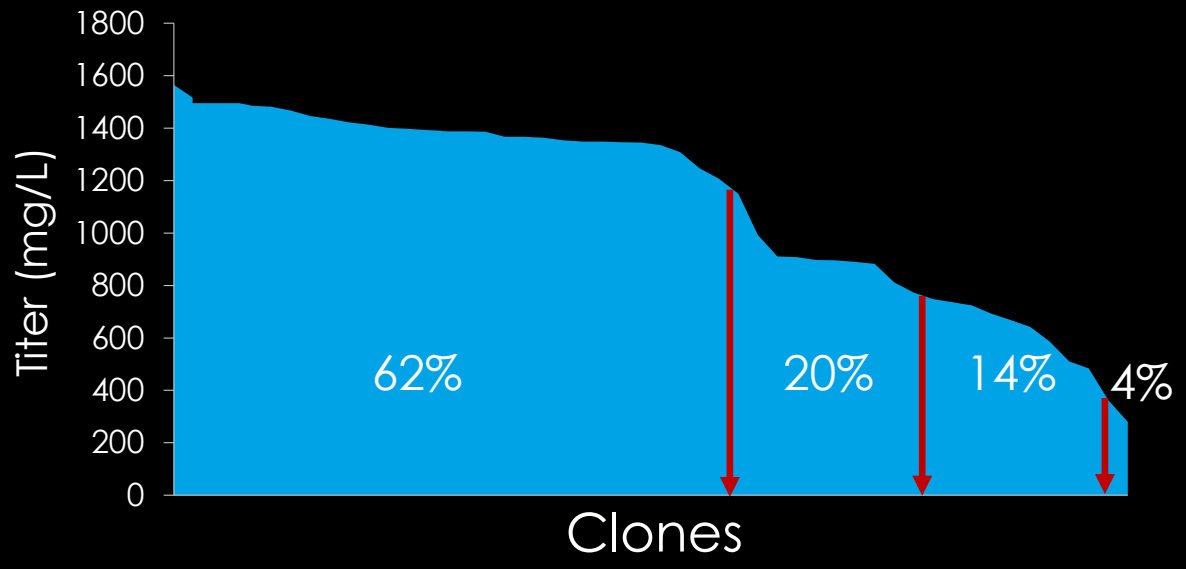
CHO pools uniformity: Titer

Leap-In Transposase®



- 62% of clones in top quartile of expressers
- 82% of clones in top half of expressers
- **99% probability in under 200 clones**

Random Integration

https://www.berkeleylights.com/

- High producers rare

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Robust High Titer Stable CHO Pools

Protein	Volumetric productivity
IgG1	7.7 g/L
IgG1	5.9 g/L
IgG1	5.3 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	~7 g/L
3 ORF Bispecific	3 g/L

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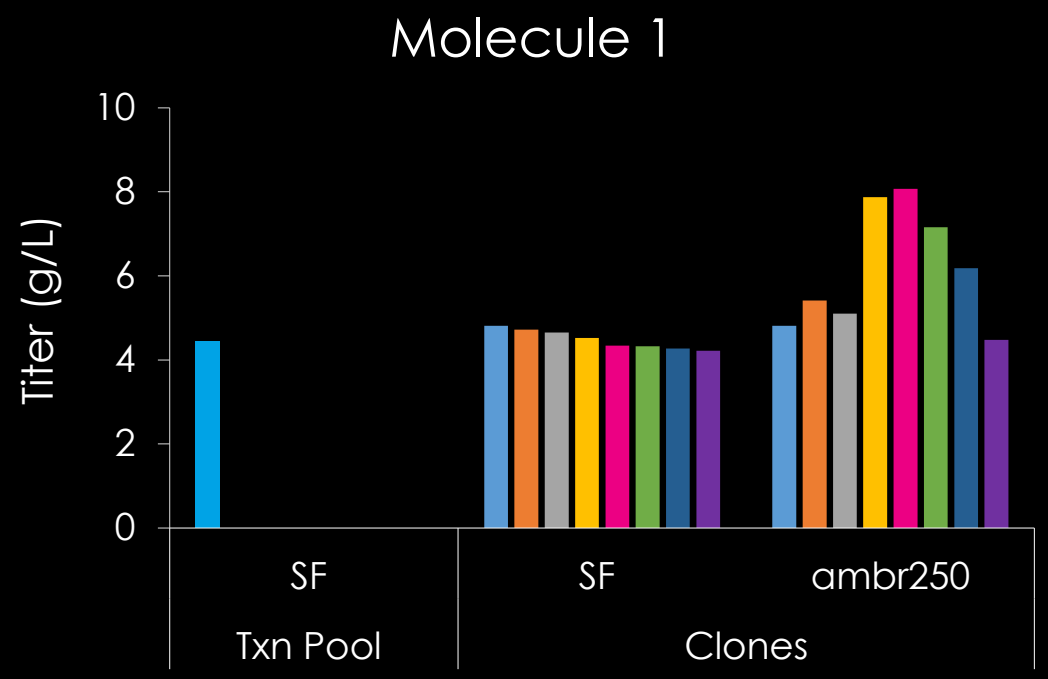
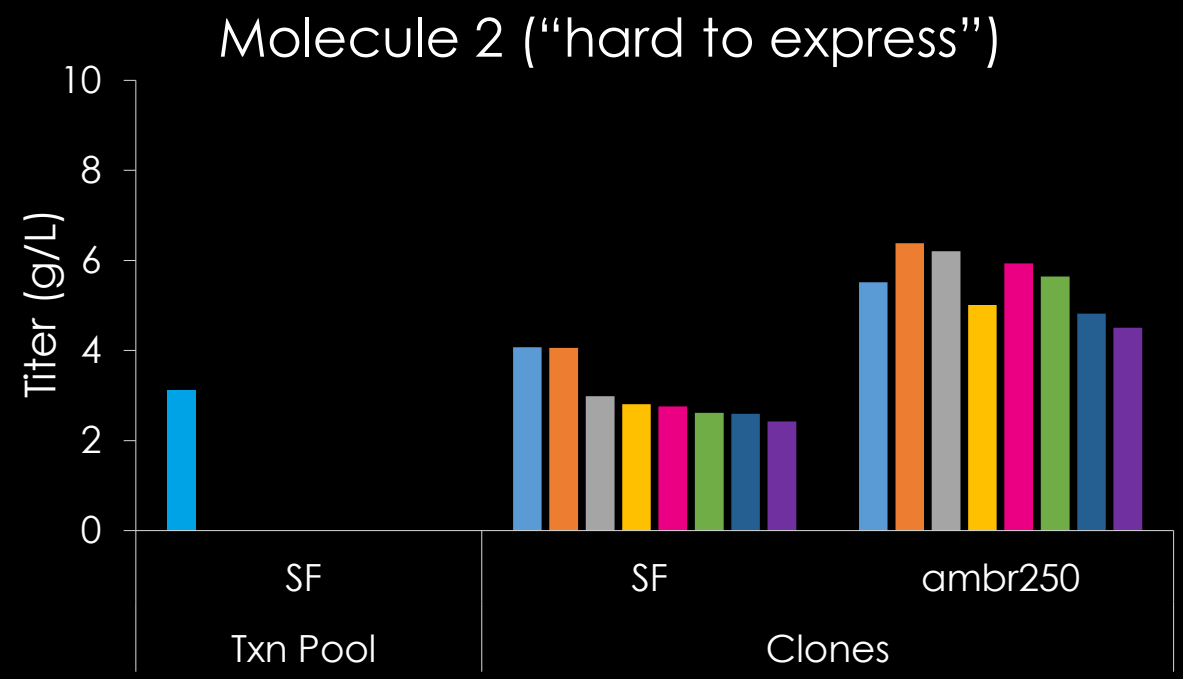
Pools predict clones: Titer





janssen
PHARMACEUTICAL COMPANIES
OF Johnson & Johnson

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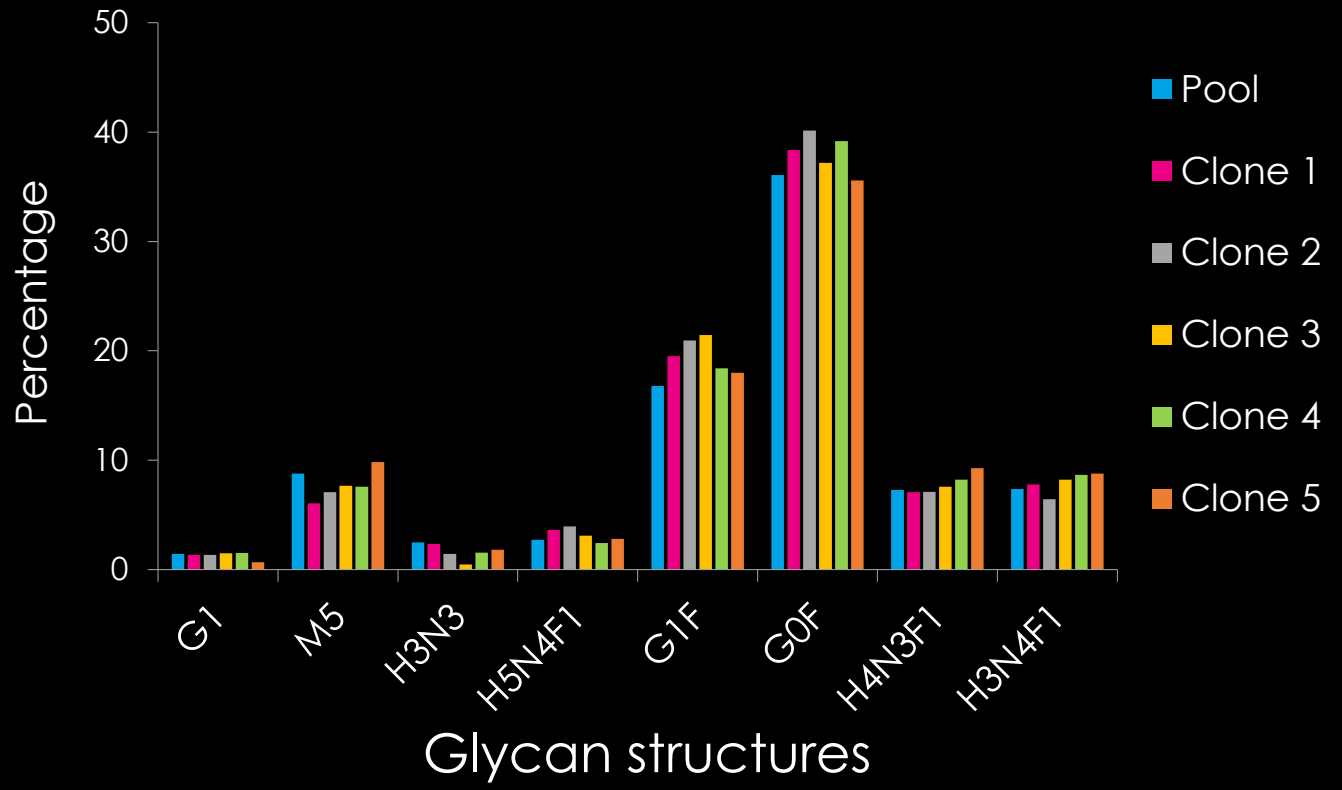
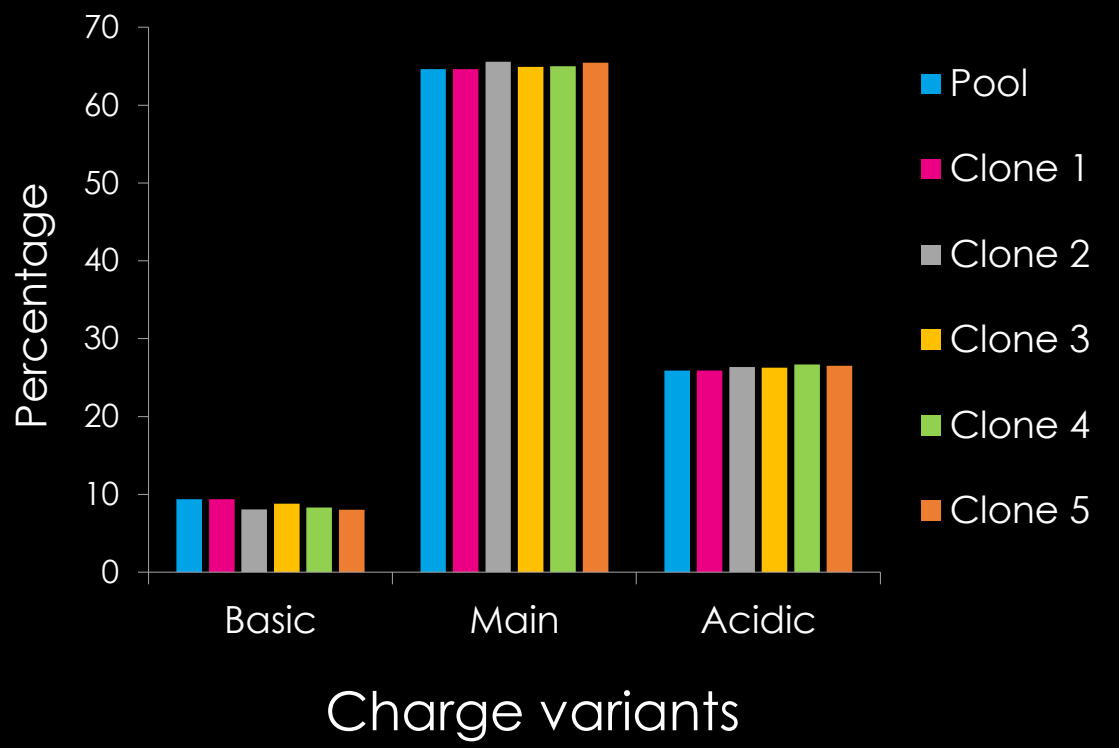
Pools predict clones: Product quality

Glycans: Pool vs Clones



Charge variants: Pool vs Clones



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COVID 19: ATUM Accelerated Timeline

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

38 days!

IND accepted

CDMO: Intensified fed batch process

>12 g/L

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Leap-In transgenes structural feature summary

- Expression construct integrity maintained
- Each integration is equally functional
- Rapid, robust and predictive pool generation

THE POWER OF THE POOL

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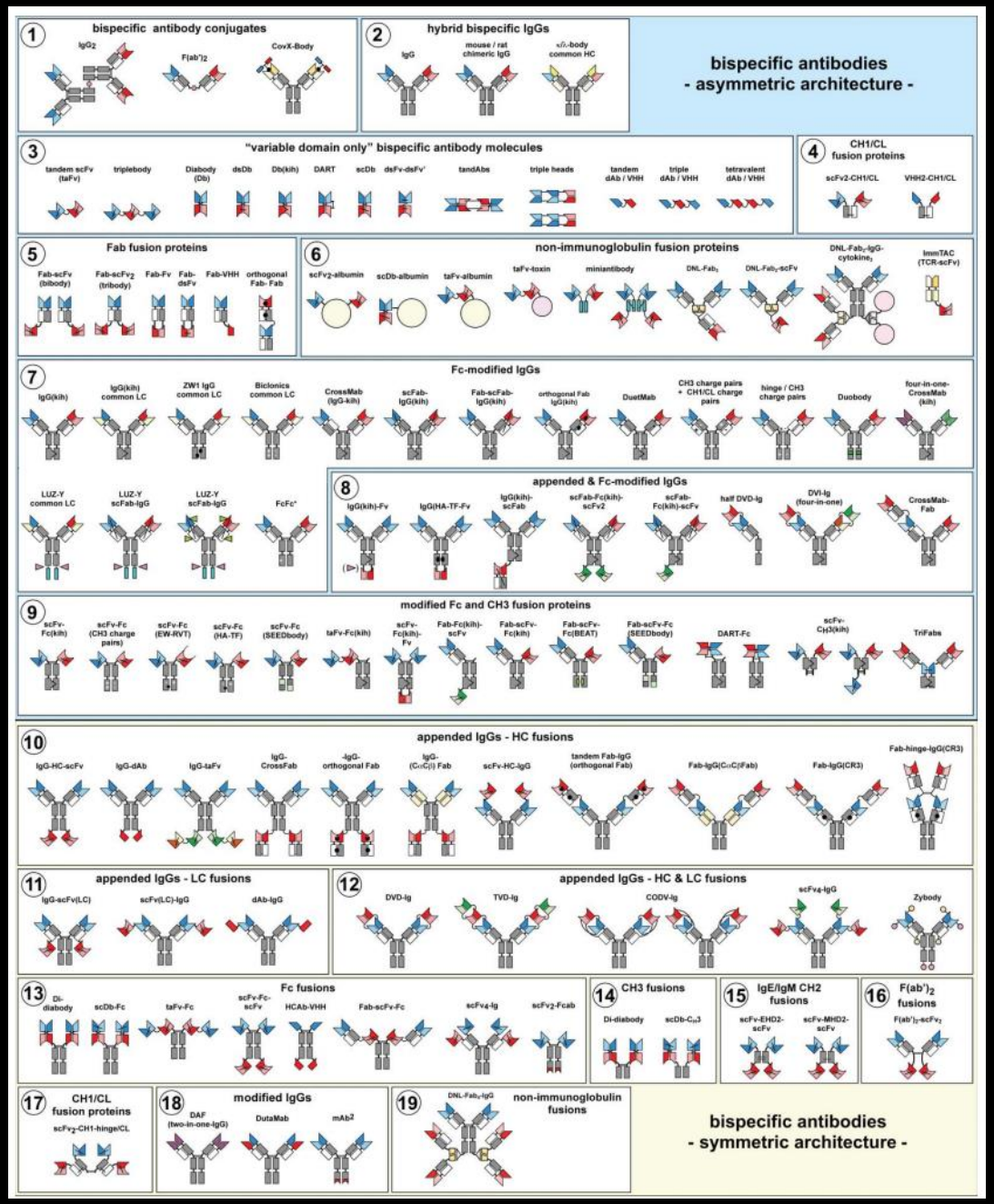

Brinkmann and Kontermann; 2017

Beyond mAb's: 3 Chains and More

The "zoo" of bispecifics

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Considerations for chain ratio balancing

Sequence

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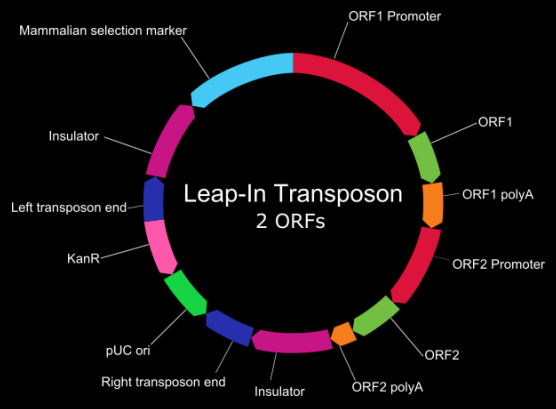
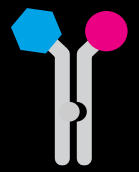
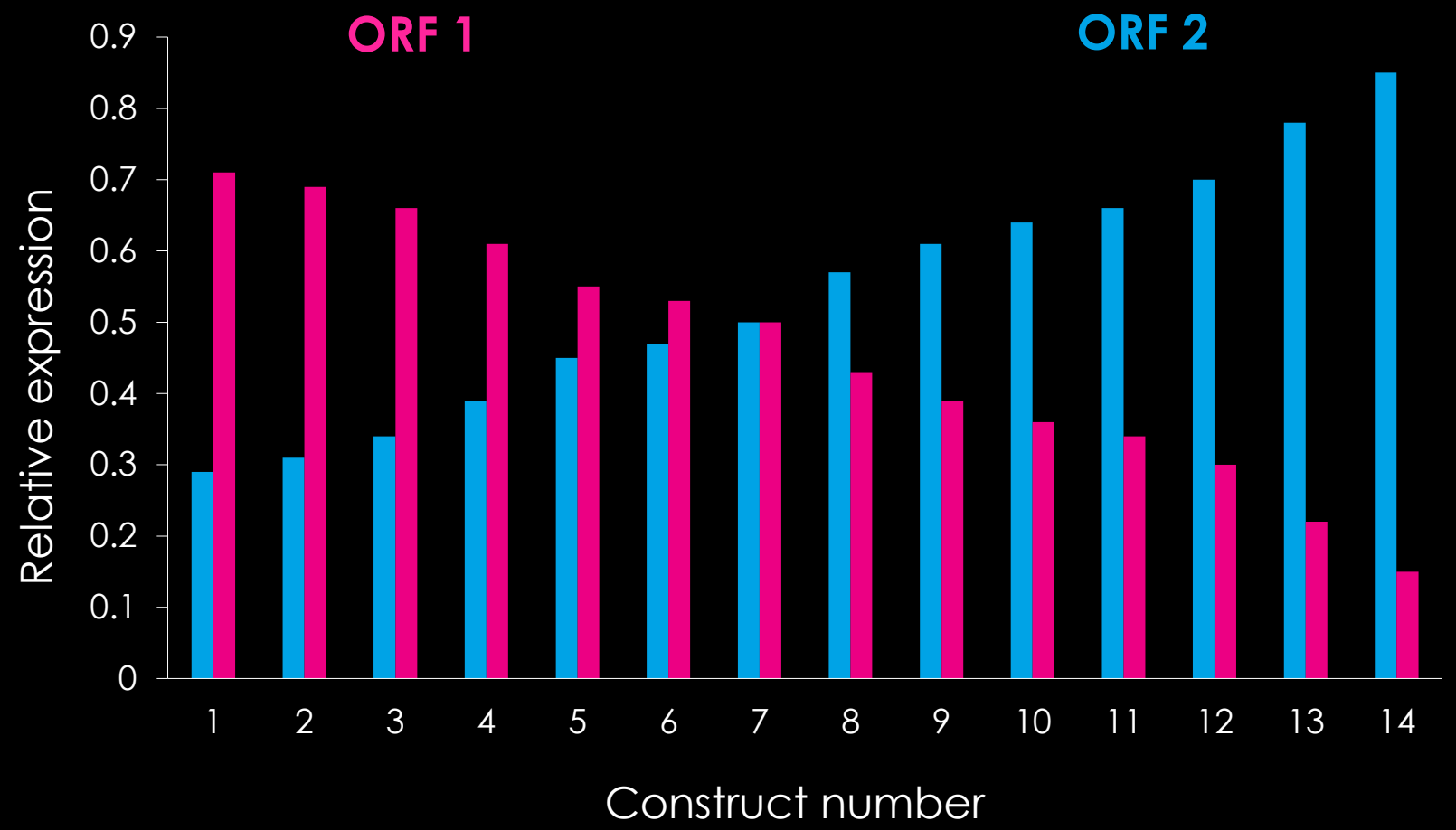
Controlling ratios with construct design: 2 ORFs

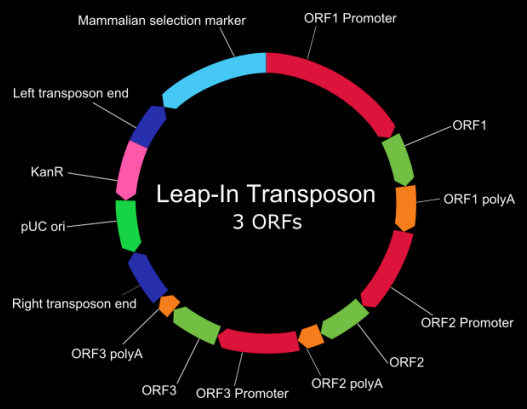




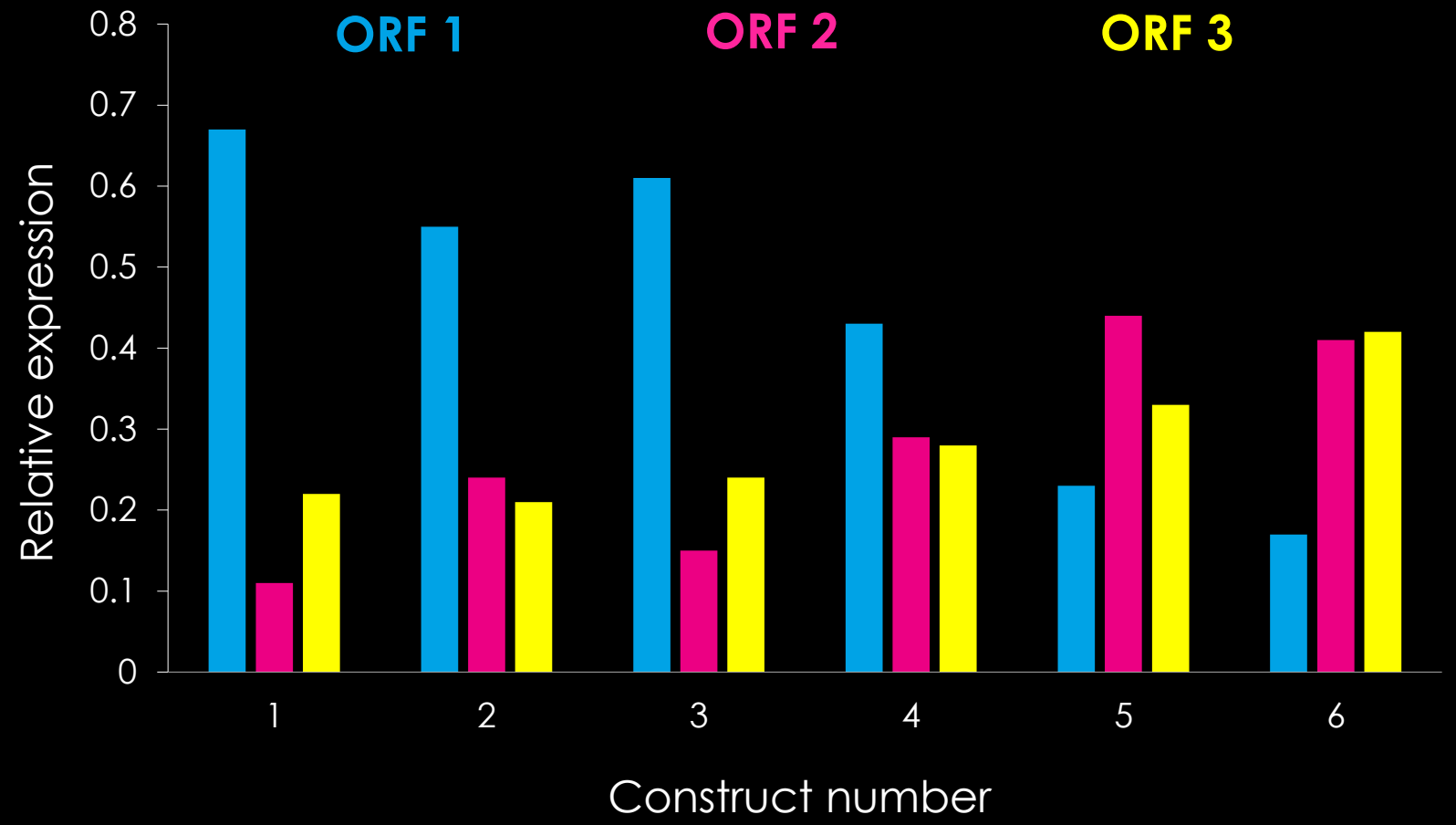
Bispecific Antibodies
chain ratio modulation



Controlling ratios with construct design: 3 ORFs

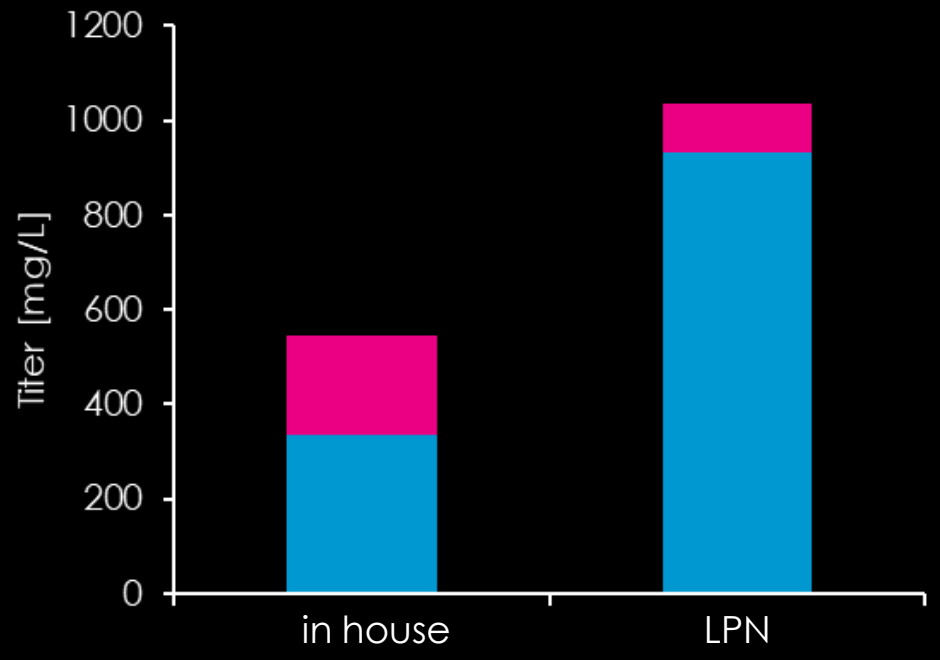


Bispecific Antibodies
chain ratio modulation

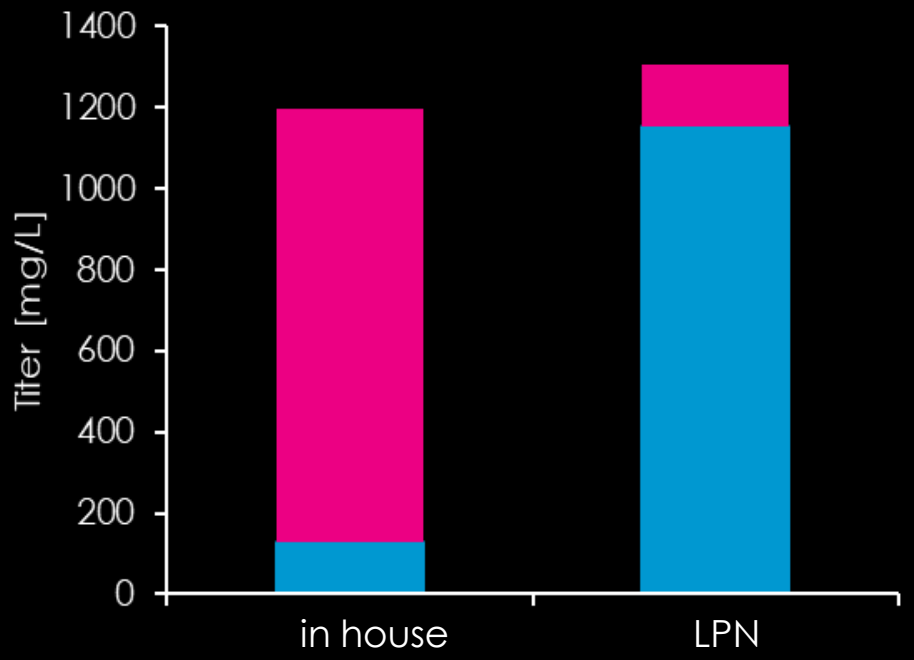


Controlling ratios with construct design: 3 ORFs

Desired product



Assembly variant



Leap-In enabled chain balancing = significantly improved product assembly

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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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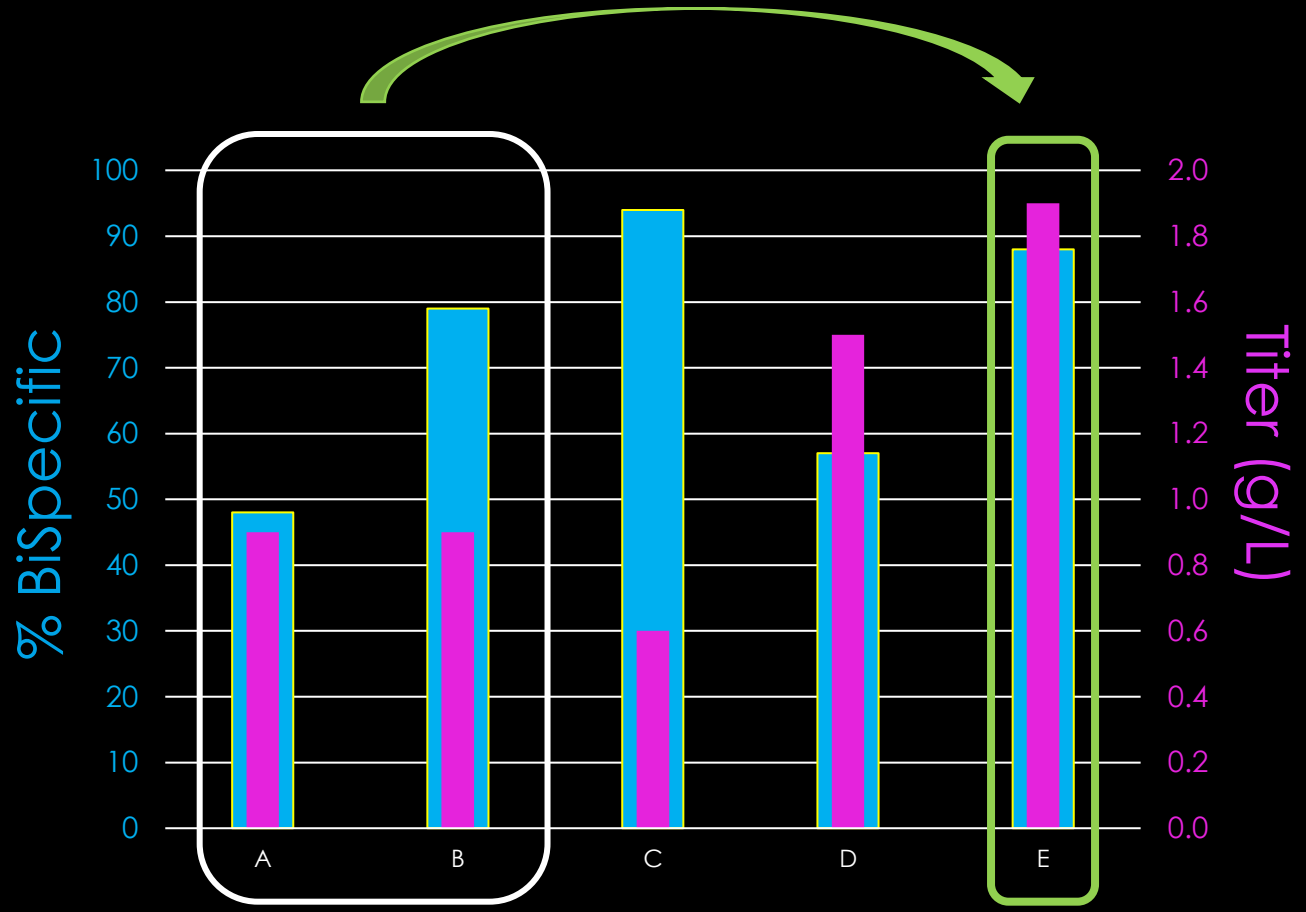
Case Study: 3-Chain Bispecific mAb

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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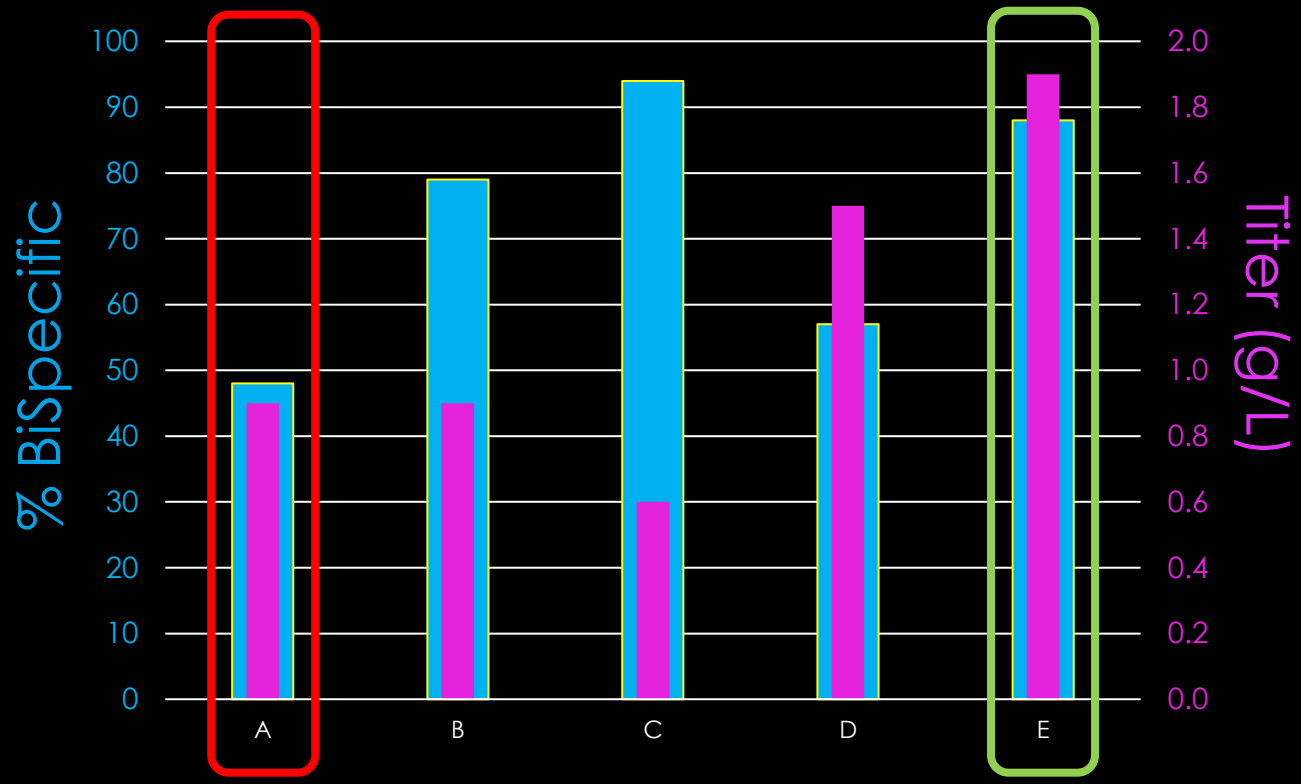
Case Study: 3-Chain Bispecific mAb

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

* Subset of 14 vectors screened



Screening vectors at pool stage enables ID of high value pools

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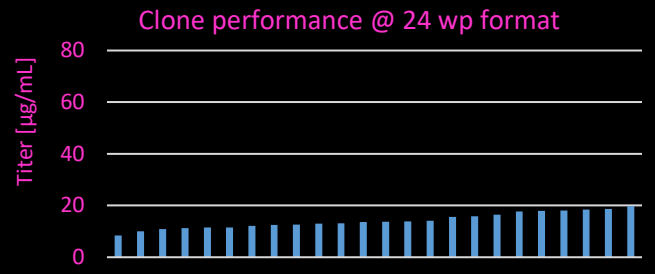
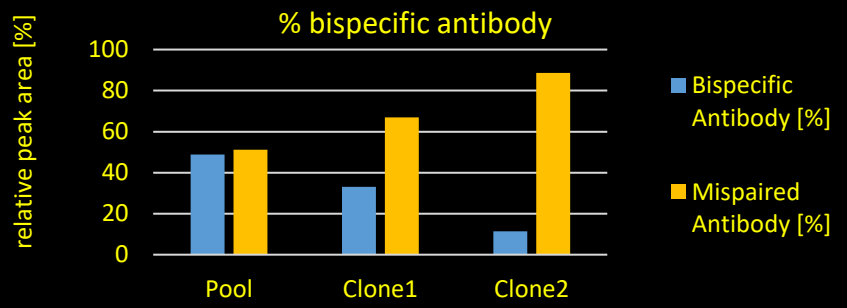
Case Study: 3-Chain BiSpecific mAb

Pool A



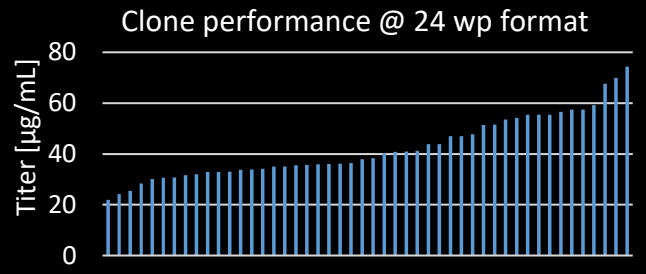


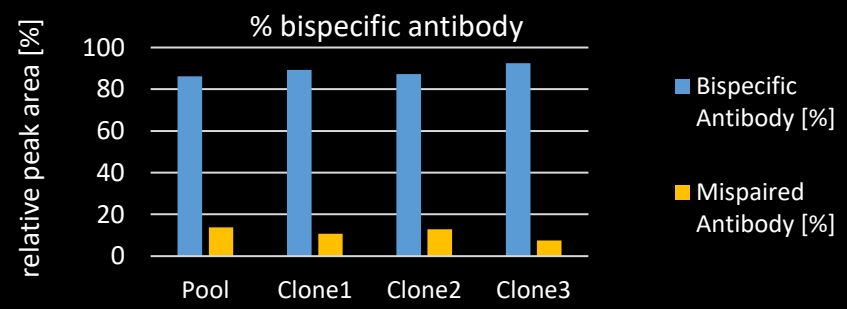
Pool and clone productivity

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 Power of the Pool

- Pools predict clones
  - Titer, glycan, charge variants, assembly
- Screen vector configurations at pool stage
- Uniquely enabling for complex molecules
- Enables rapid progress towards IND
  - Early transfer to CDMO
  - Process development in parallel with clone selection
  - Pools for tox lot and possibly Ph.I

ATUM

Thank You

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