Leap In Transposase Platform

The power of the pool

EUROPEAN BISPECIFIC & MULTISPECIFIC ANTIBODY CONGRESS

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

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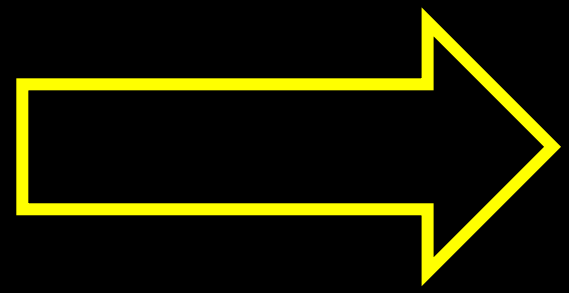
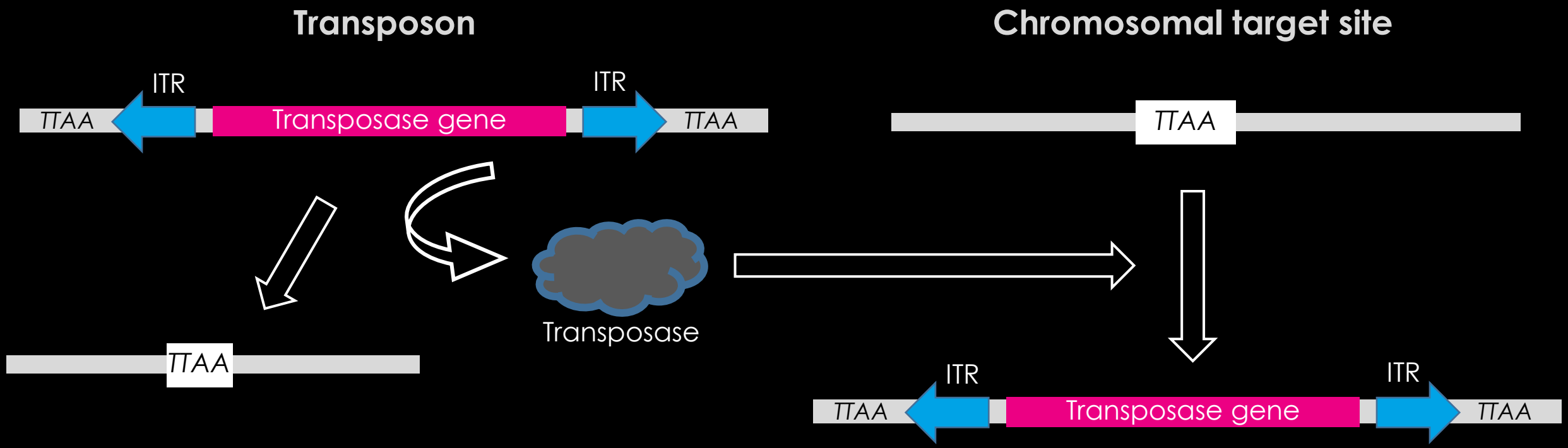
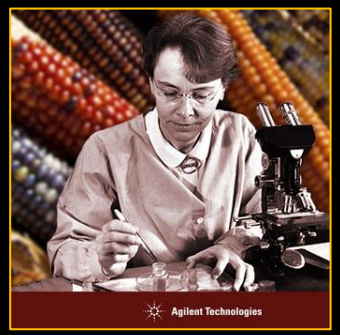
Transposase – Transposon: Mechanistic detail

Cut  Paste

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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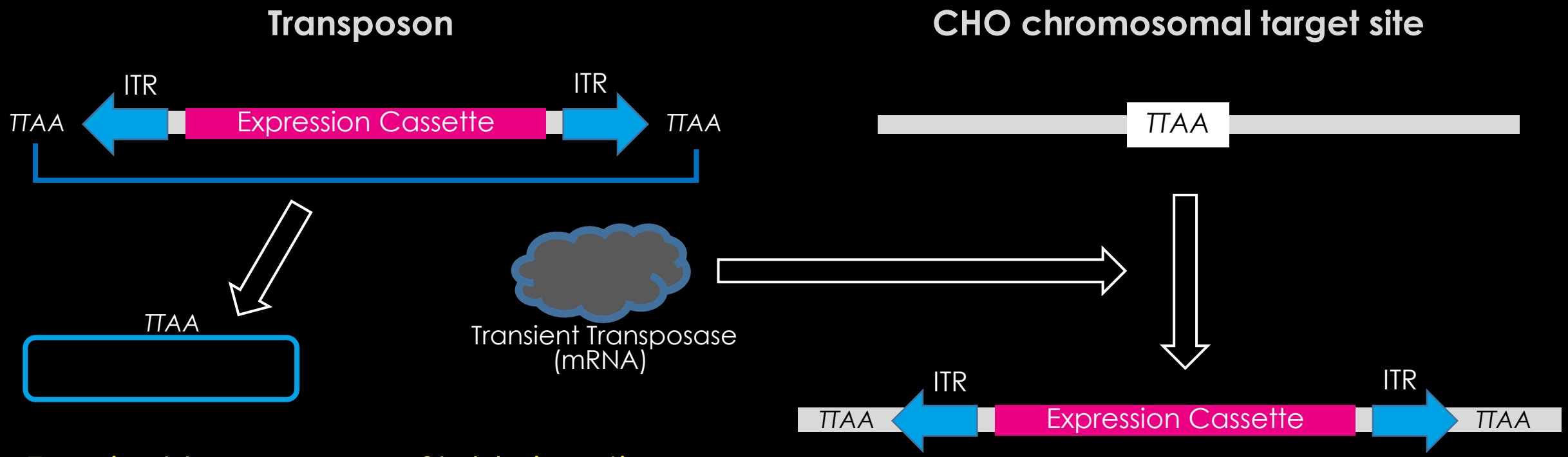
The life of a transposon-transposase pair



- 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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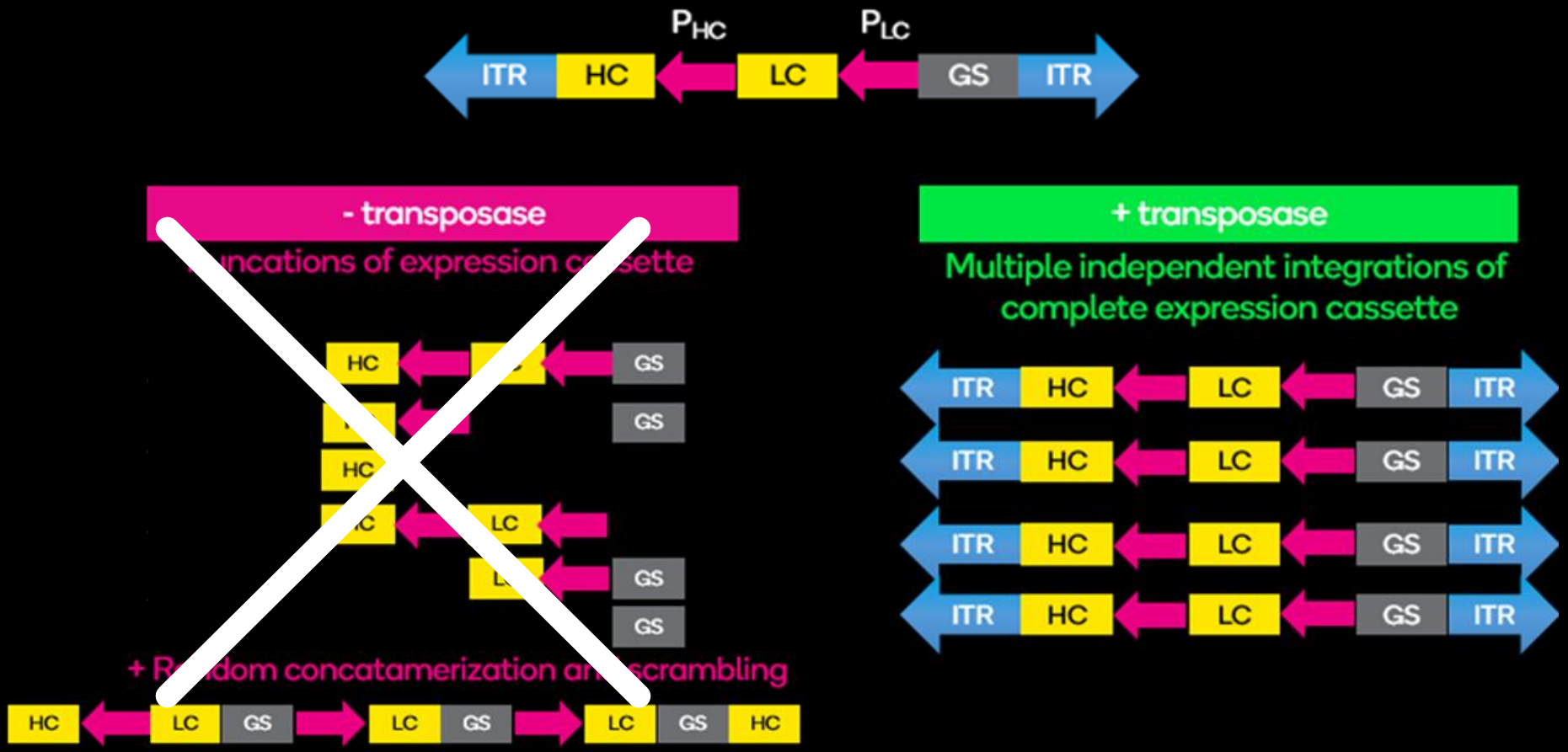
representation of Leap-In® transgenes



- 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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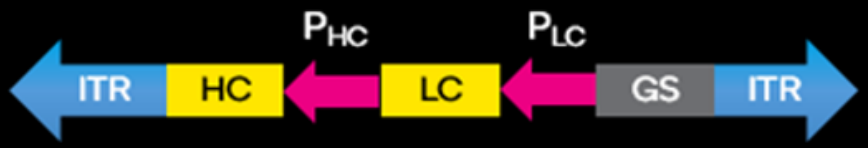
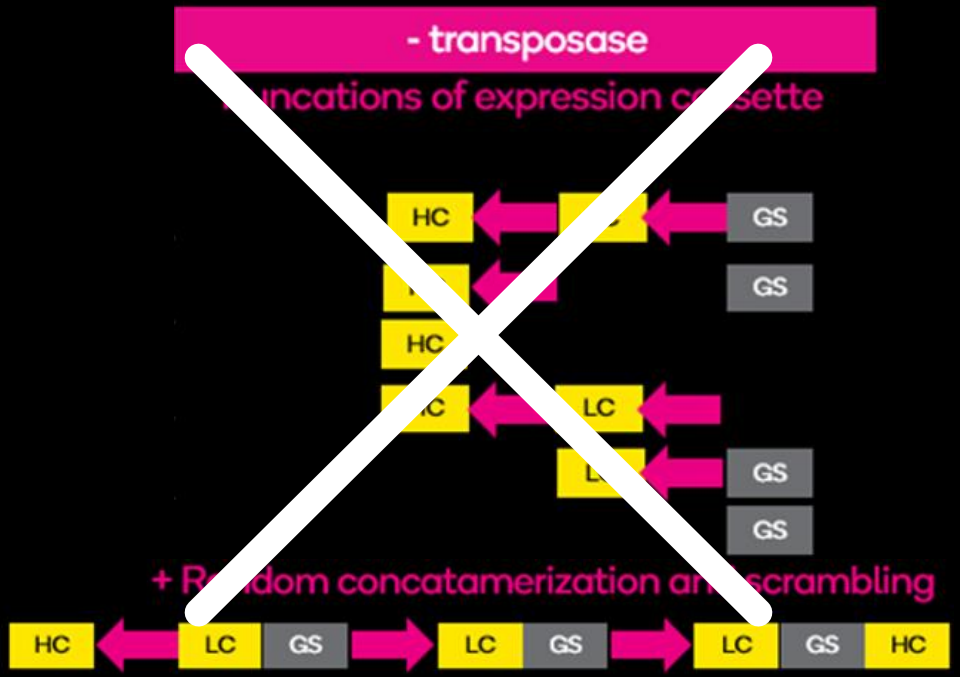
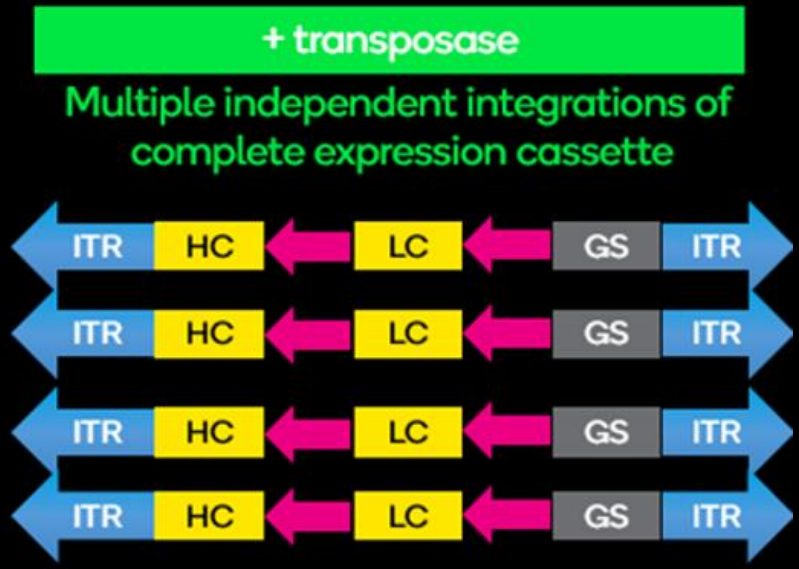
representation of Leap-In® transgenes

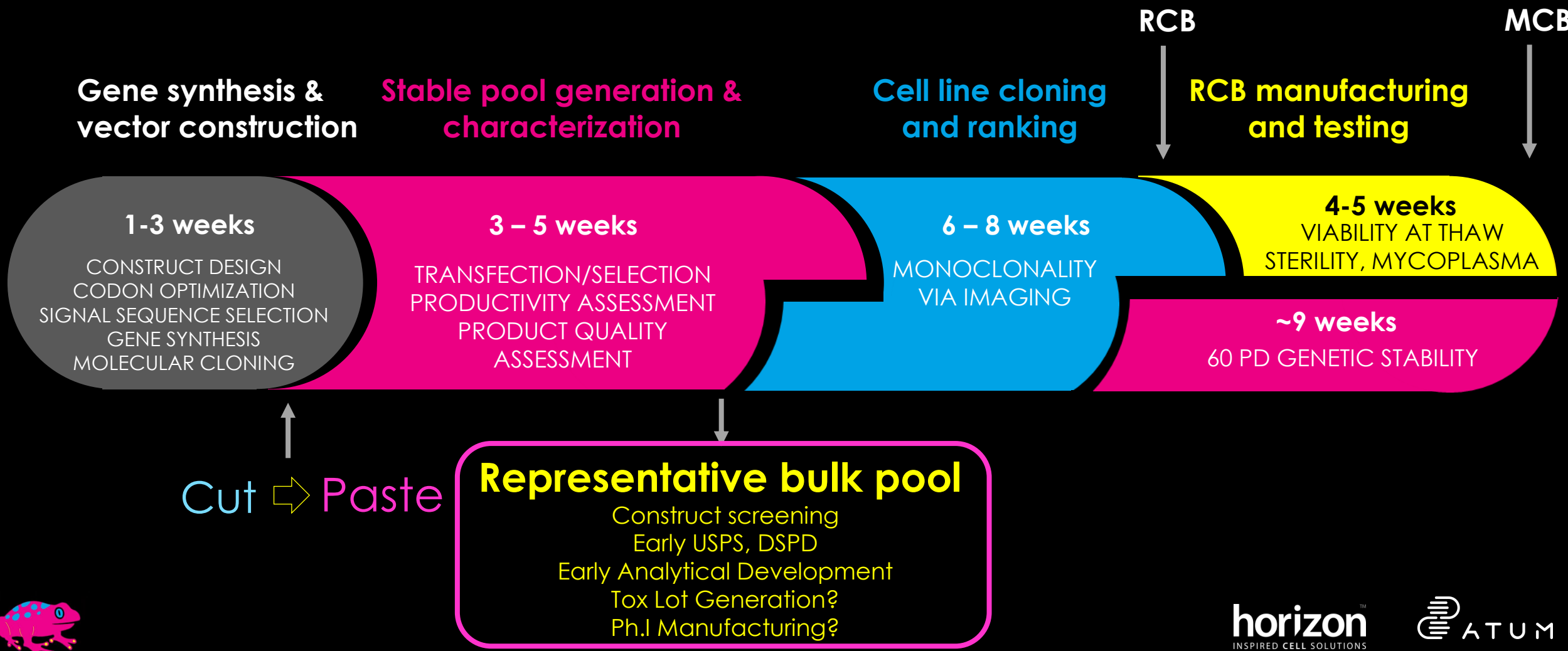






Highly uniform and predictive bulk pools

rapid and robust: sequence to MCB



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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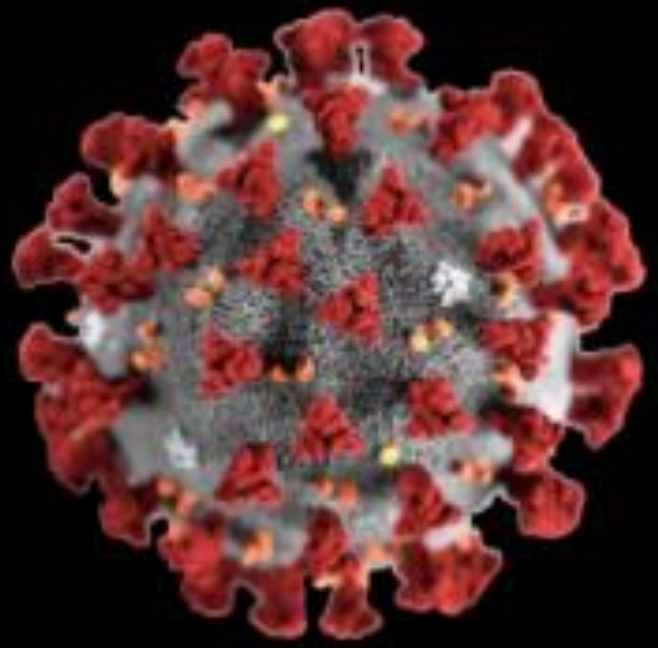
antibodies For COVID 19 treatment

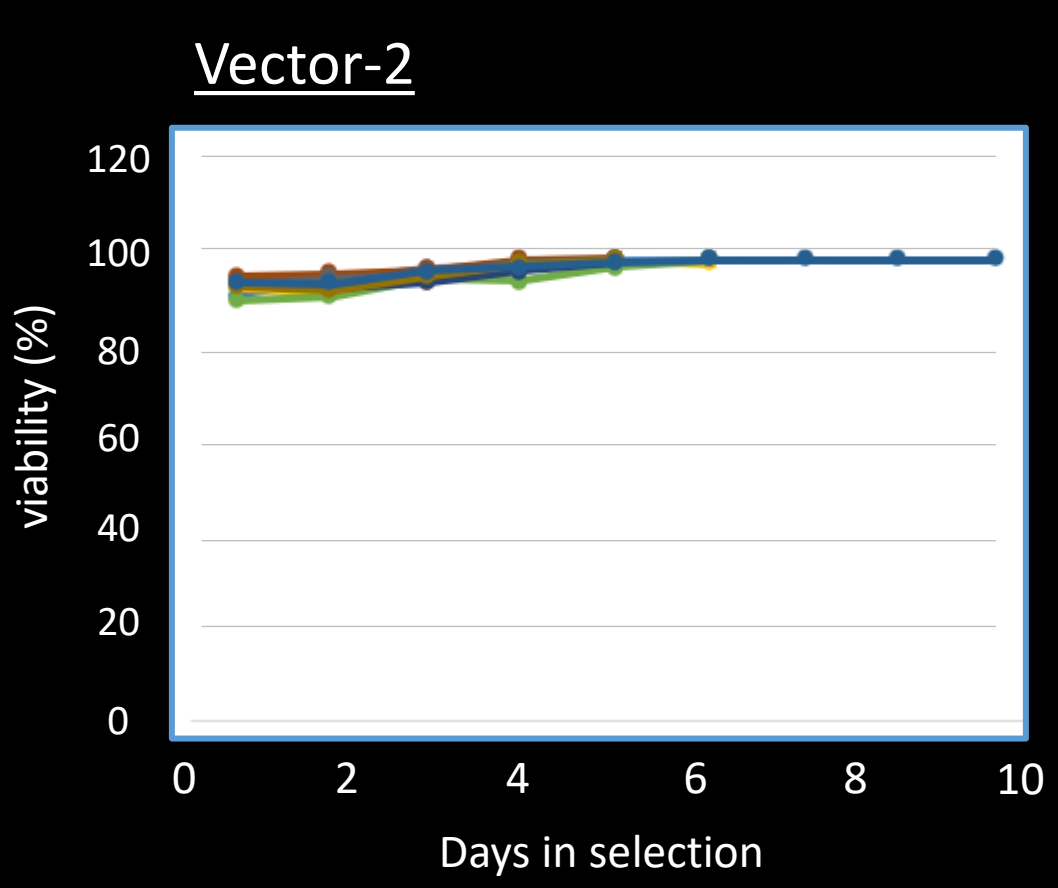


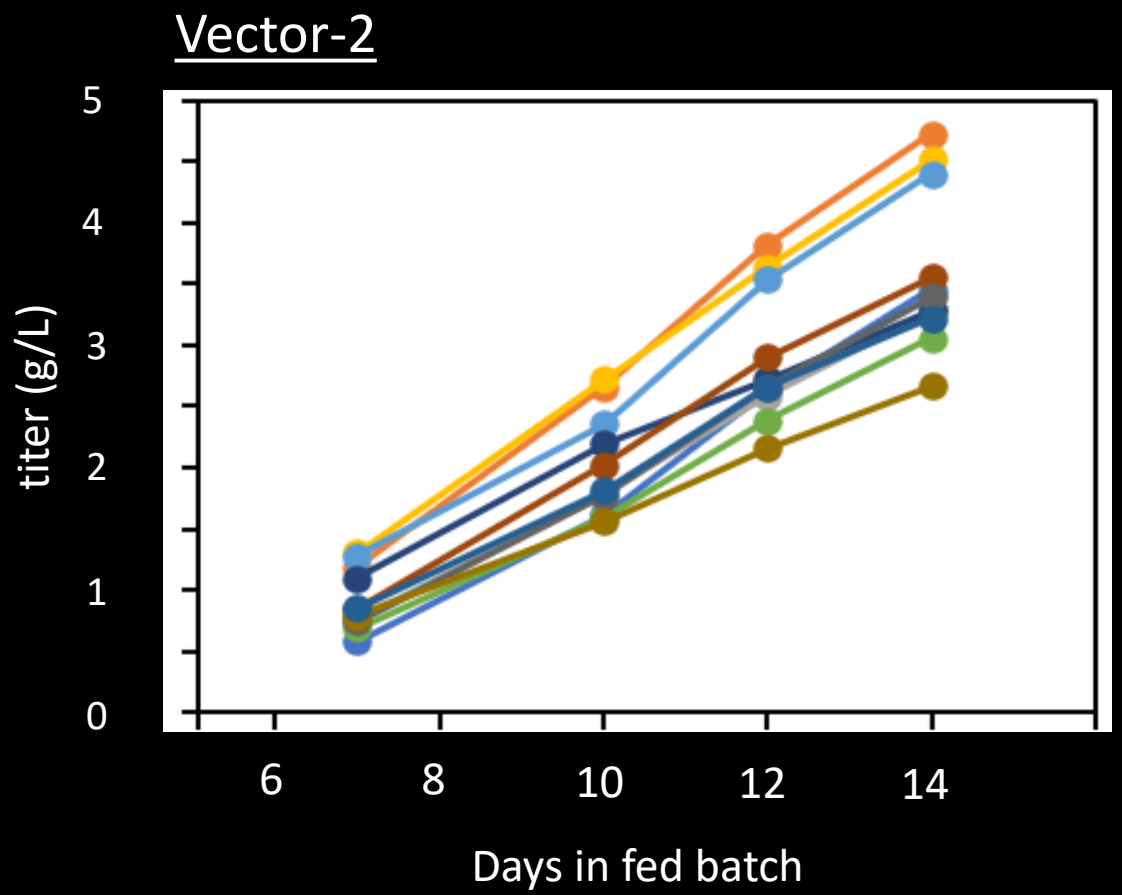
- Eleven candidate therapeutic mAb’s
- Desire to initiate human trials ASAP
- Rapid progress: sequence to Ph.I
- Use bulk cell pools for GMP manufacturing

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





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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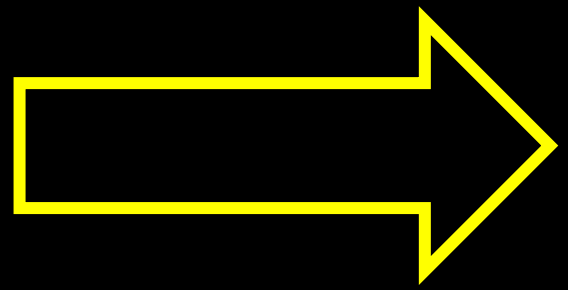
fundamental mechanism

Cut  Paste

… what you paste matters …

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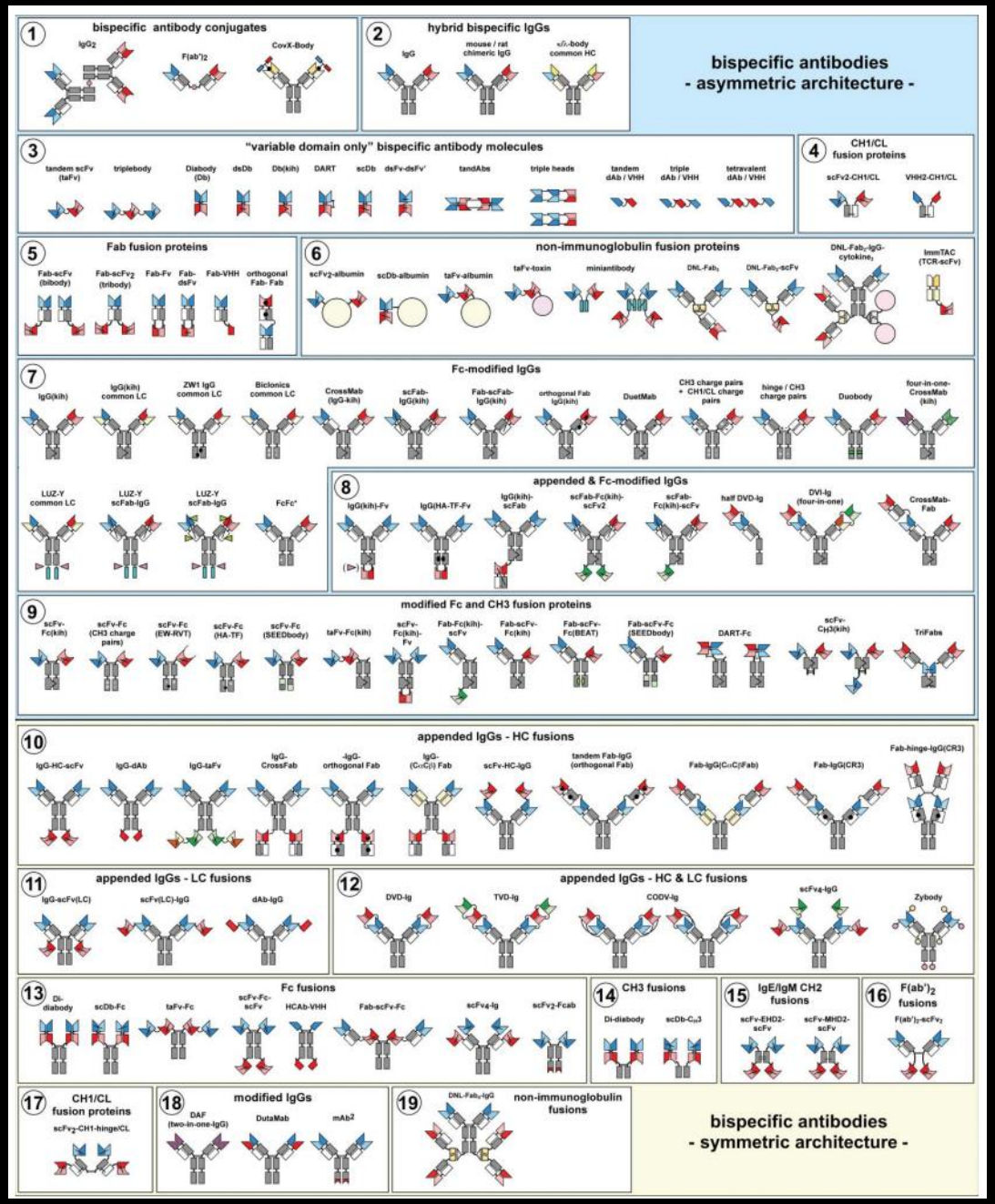

Brinkmann and Kontermann; 2017

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 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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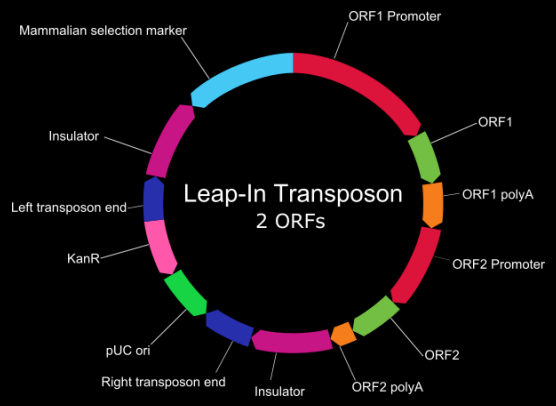
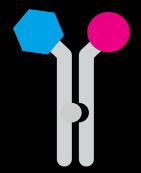
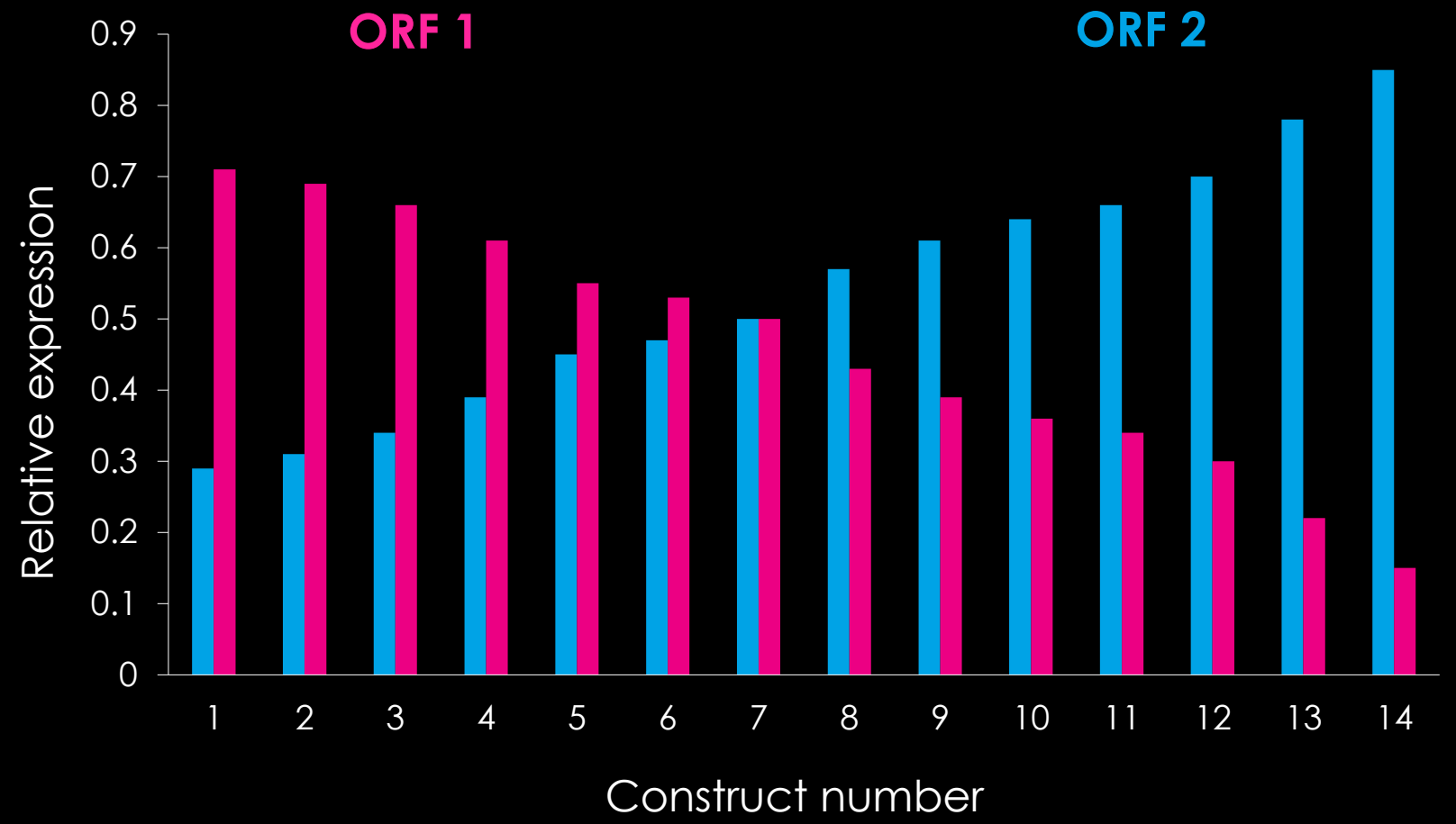
controlling ratios with construct design: 2 ORFs

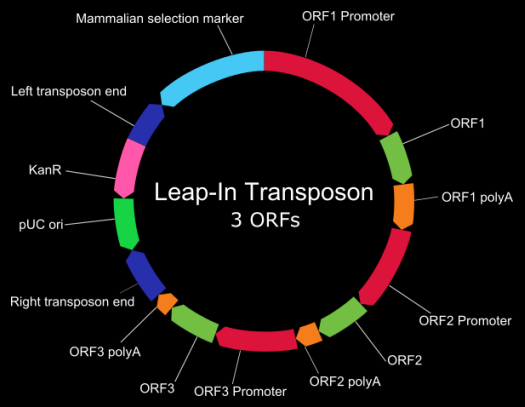




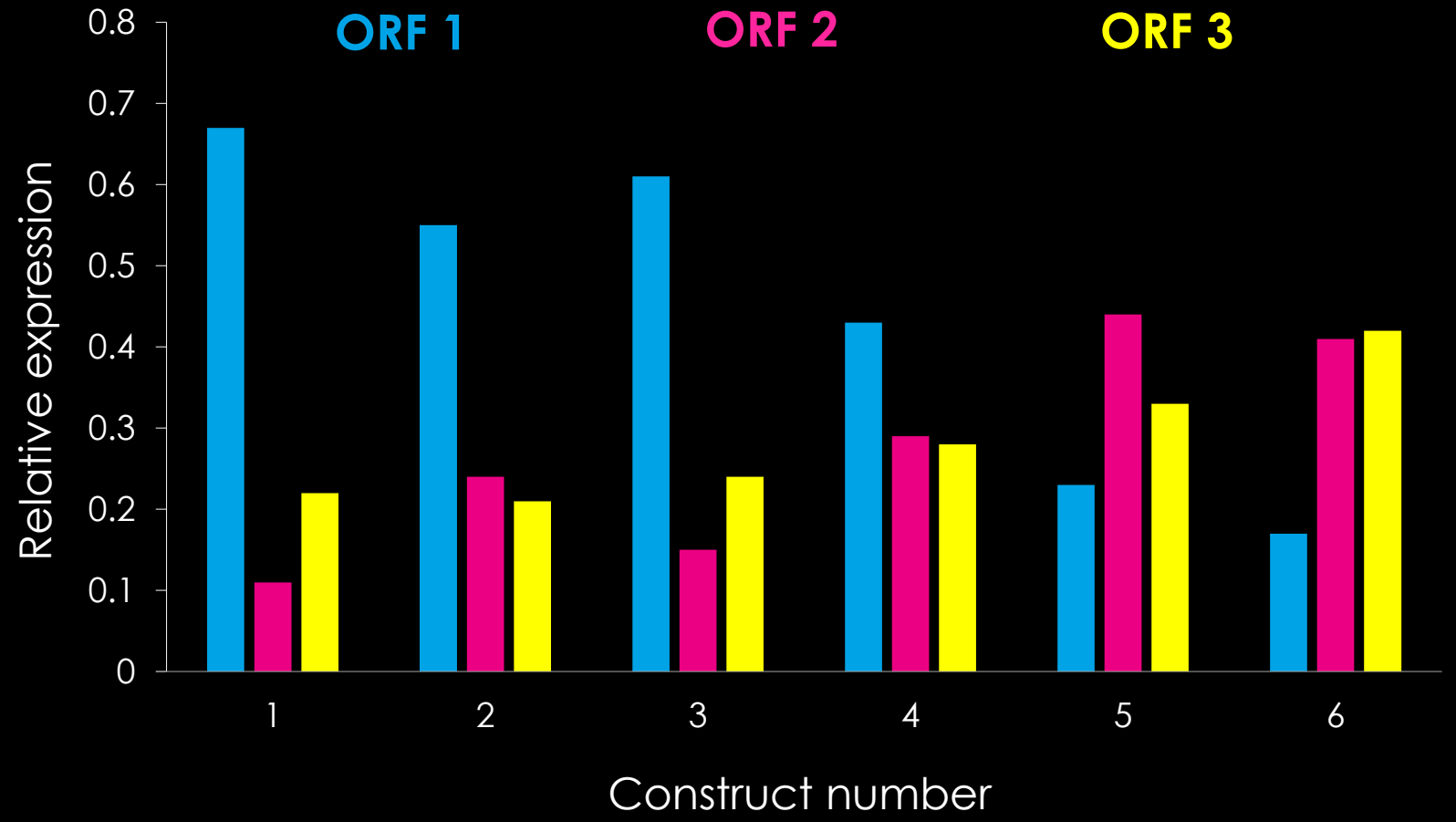
Bispecific Antibodies
chain ratio modulation



controlling ratios with construct design: 3 ORFs



Bispecific Antibodies
chain ratio modulation



case study

- Medium sized biotech
- Leap In Transposase licensee
- Two and four chain bispecifics
- Strong internal biologics capabilities

(… heavily redacted data set …)

case study-1: two chain bispecific

Random Integration Pool

Fed-batch Expression d14: Normalized = 1

61.4% Proper Assembly

Leap In Transposase Pool

Fed-batch Expression d16: Normalized =1.9

90% Proper Assembly

Leap In Transposase Clones

Clone	Titer (Normalized)	% Assembly
A	2.5	98.4
B	2.5	98.3
C	2.6	98.2
D	2.5	98.0
E	2.4	94.9

Good pools predict good clones

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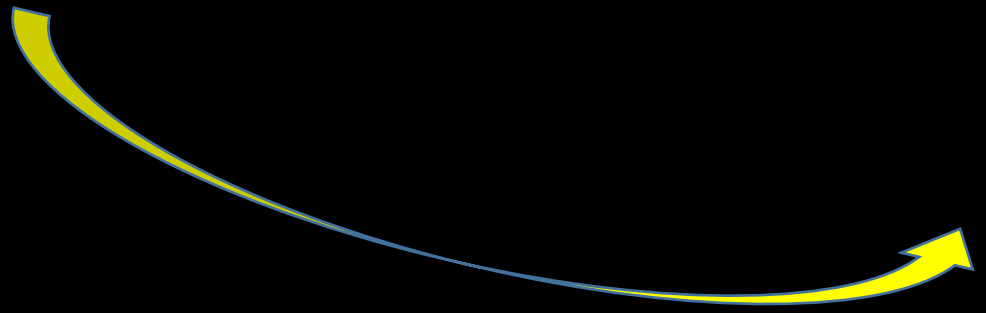
case study-2: four chain bispecific

Random Integration Pool

Fed-batch Expression d14: Normalized = 1

10.6% Proper Assembly



Random Integration Clones

Clone	Titer (Normalized)	% Assembly
A	0.82	87.5
B	0.71	86.0
C	0.44	72.1
D	0.55	77.1
E	0.52	60.5

Random integration clones lost expression and few clones with reasonable product quality

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case study-2: four chain bispecific

Random Integration Pool

Fed-batch Expression d14: Normalized = 1

10.6% Proper Assembly

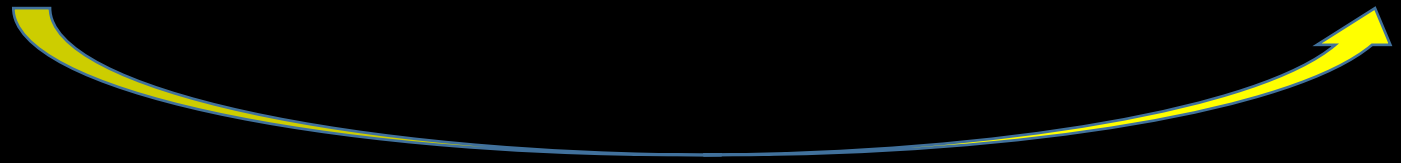
Leap In Transposase Pool

Fed-batch Expression d16: Normalized = 1.1

85.6% Proper Assembly

Leap In Transposase Clones

Clone	Titer (Normalized)	% Assembly
A	1.0	92.9
B	1.1	93.5
C	0.96	91.8
D	0.95	92.8
E	1.2	88.8



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case study-2: four chain bispecific

Random Integration Pool

Fed-batch Expression d14: Normalized = 1

10.6% Proper Assembly

Leap In Transposase Pool

Fed-batch Expression d16: Normalized = 1.1

85.6% Proper Assembly

Leap In Transposase Clones

Clone	Titer (Normalized)	% Assembly
A	1.0	92.9
B	1.1	93.5
C	0.96	91.8
D	0.95	92.8
E	1.2	88.8

Leap In derived clones retained expression and all top clones had excellent product quality

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licensee comments

The Leap-In transposase platform enables:

- Higher titers and better protein quality in stable pools.
- More homogenous cell populations in stable pools.
- Low clonal variability = fewer clones need to be screened.
- Timelines can be shortened by ~8 weeks.

case study-3: 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

Rentschler Biopharma

(… heavily redacted data set …)

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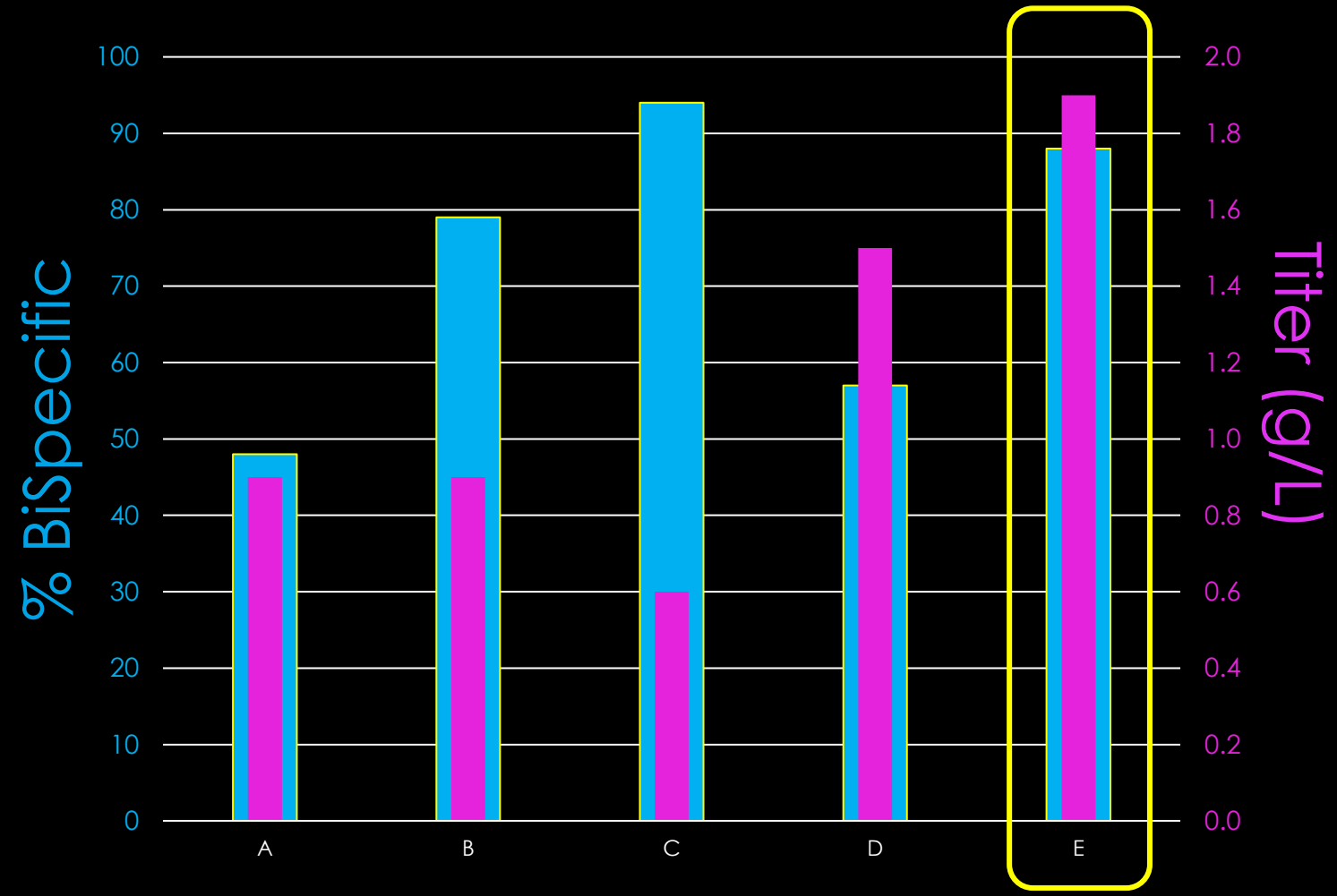
case study: 3-chain bispecific mAb - bulk pools



Rentschler Biopharma

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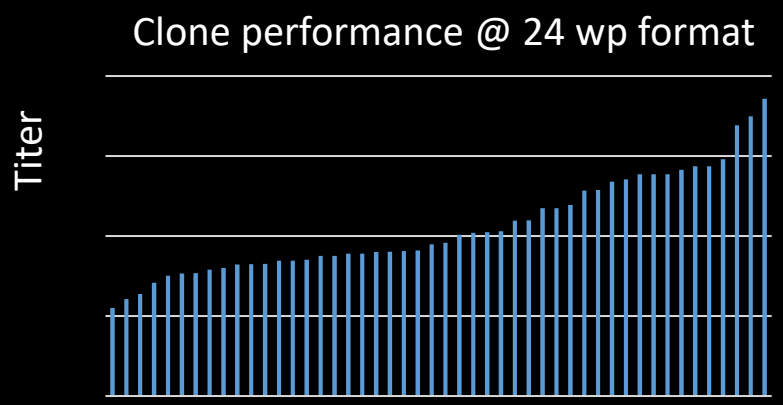
case study: 3-chain bispecific mAb - clones

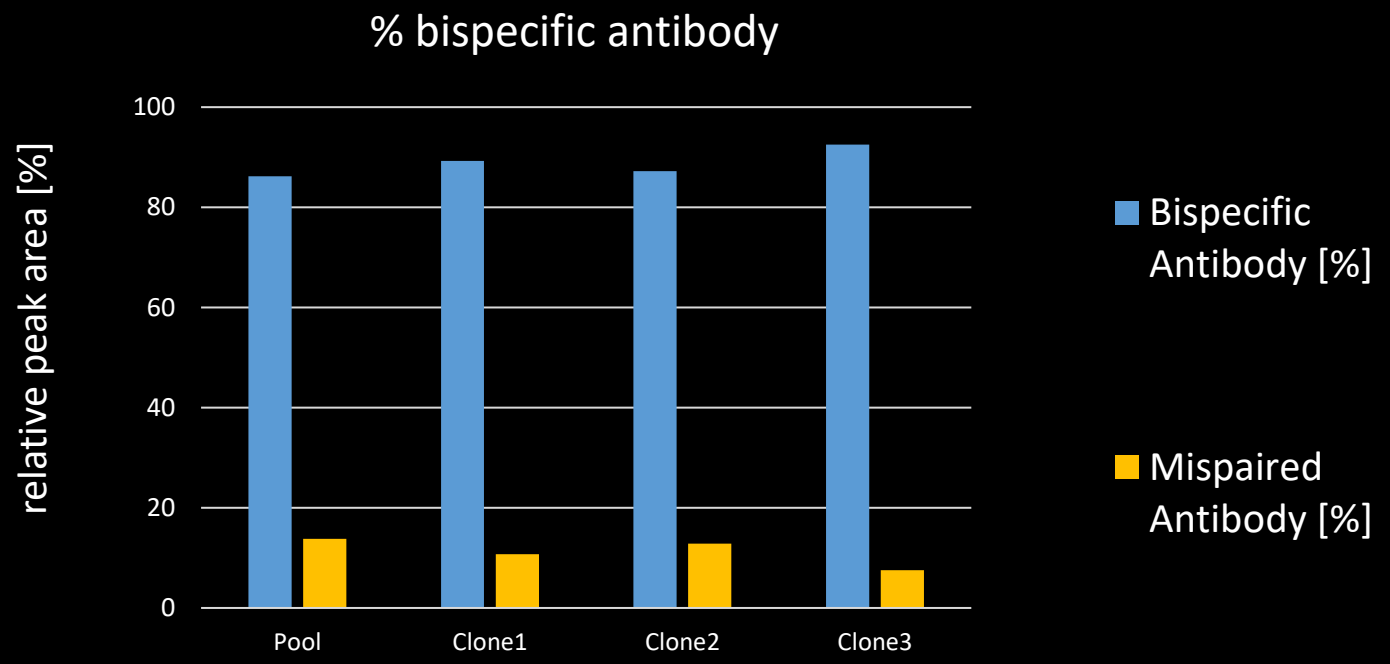
Pool E



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

Rentschler Biopharma

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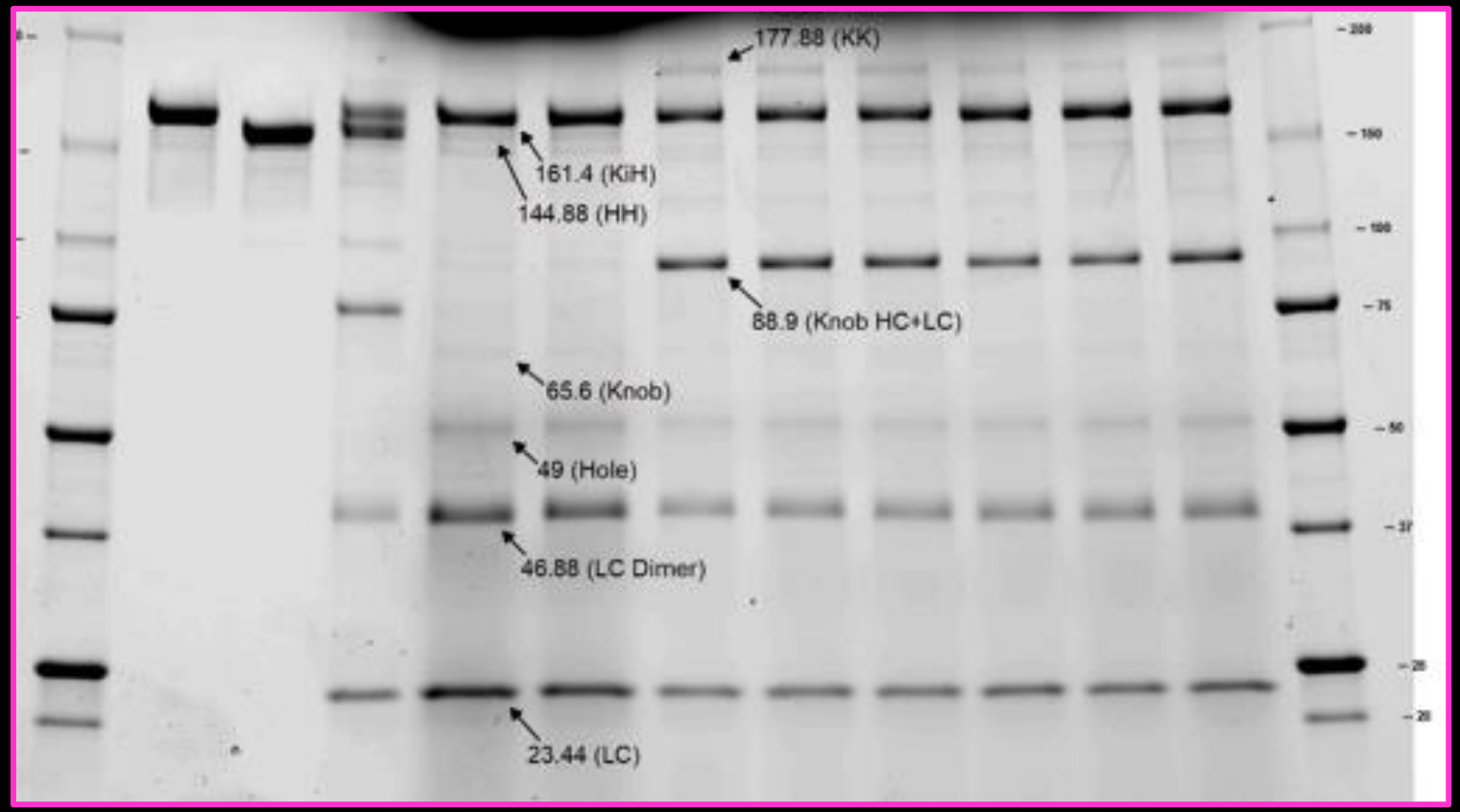
… and there is more …

supernatant SDS PAGE

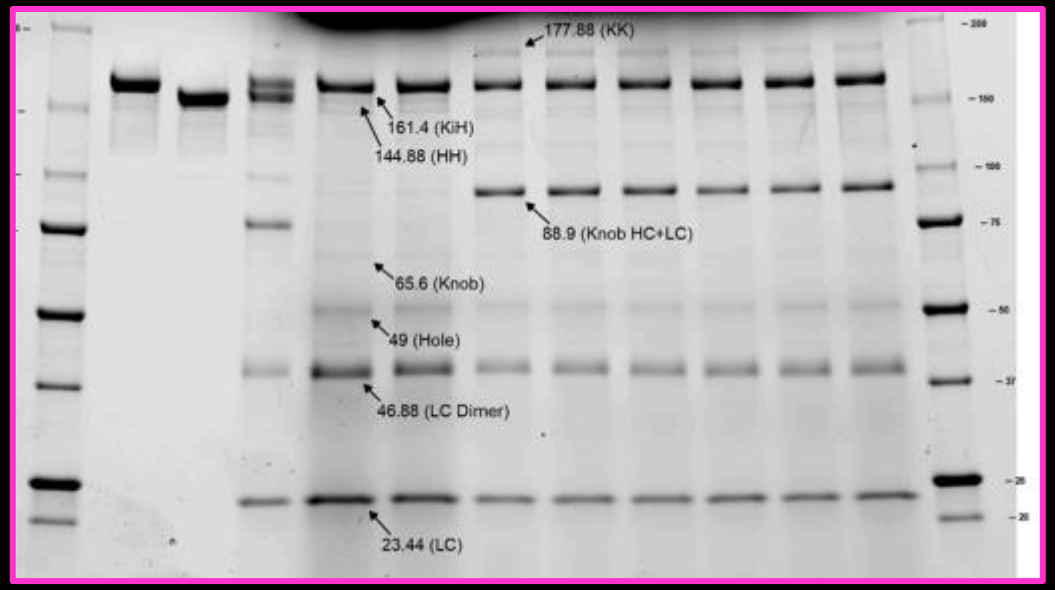


… and there is more …

An innovative platform to improve asymmetric bispecific antibody assembly, purity, and expression level in stable pool and cell line development☆☆,☆

Yanling Wang [a,*,1], Haoran Qiu [a,1], Jeremy Minshull [b], Wilburt Tam [a], Xichan Hu [a], Carl Mieczkowski [a], Weibin Zheng [a], Chun Chu [a], Wenqiang Liu [a], Ferenc Boldog [b], Claes Gustafsson [b], Jean-Michel Gries [a], Wenfeng Xu [a]

[a] *Hengenix Biotech Inc., 430 N McCarthy Blvd, Milpitas, CA, USA*
[b] *ATUM, 37950 Central Court, Newark, CA, USA*



" … efficiently generate clones with titer above 6 g/L within 3 months from vector to top 10 clones … "

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… and more …

Leap In Licensee with a 3-chain platform:

“ … 5-7 g/L with >97% correct assembly … ”

CLD customer with a novel 4-chain platform:

“ … 6-11 g/L with >85% correct assembly * … ”

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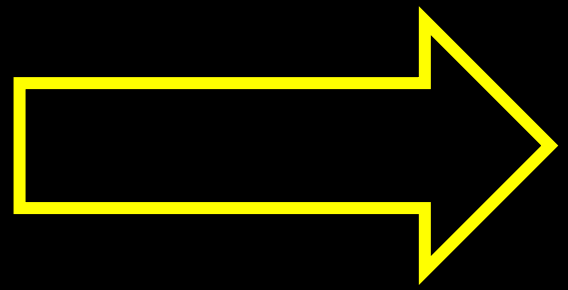
fundamental mechanism

Cut  Paste

… what you paste matters …

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robust market adoption

- Launched ~4.5 years ago
- Offered as a service by ATUM: >175 projects delivered
- >40 active licensees: 5 of top 7 pharma
- >20 regulatory filings in ~3 years: Including Ph.II/III
- Tech transfer to ≥ 13 CDMO's

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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
obeske@atum.bio

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Our Customers