

Leap In Transposase Platform


The power of the pool



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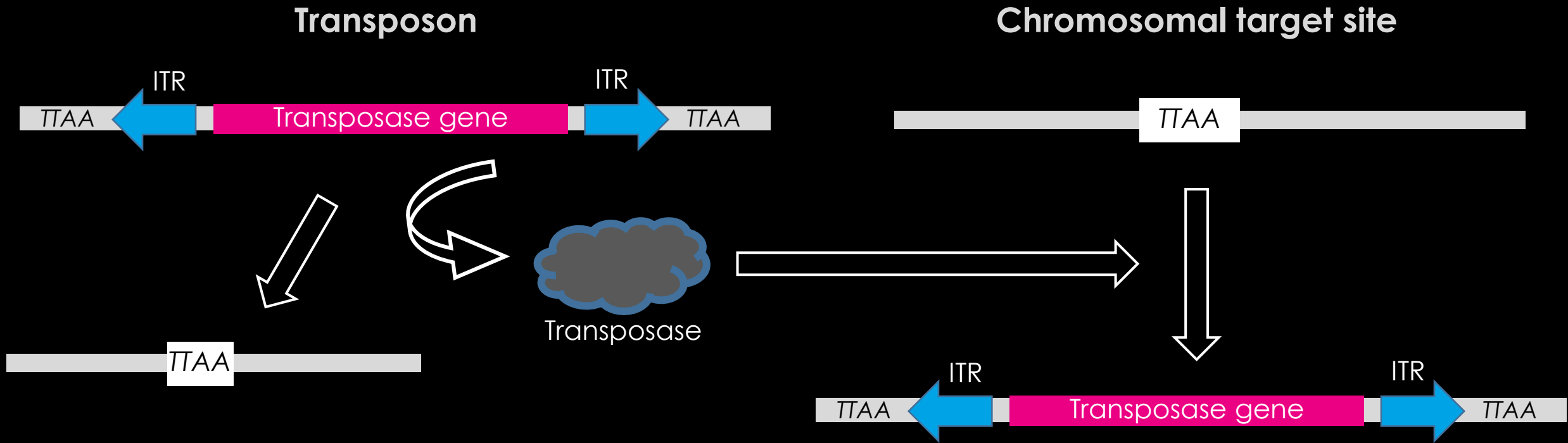


Transposase – Transposon: Mechanistic detail

Cut  Paste



The life of a transposon-transposase pair

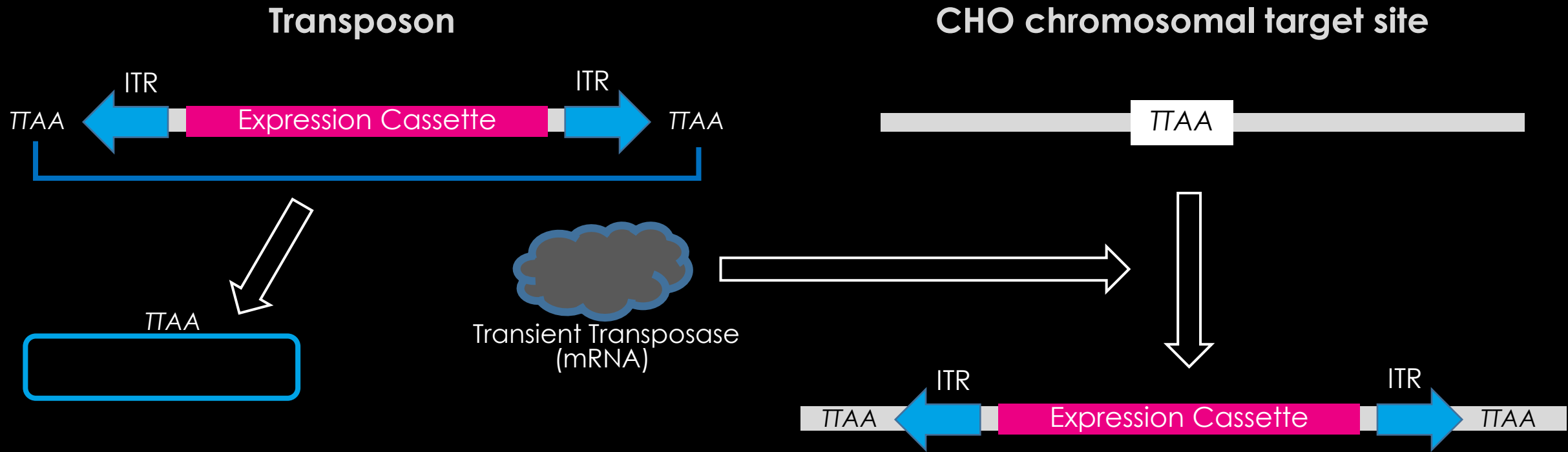


- Billions of years of evolutionary history
- Cut-paste mechanism
- Single copy integration at each site
- Perfect integration of elements between ITR's



1983 Nobel Prize in Physiology or Medicine

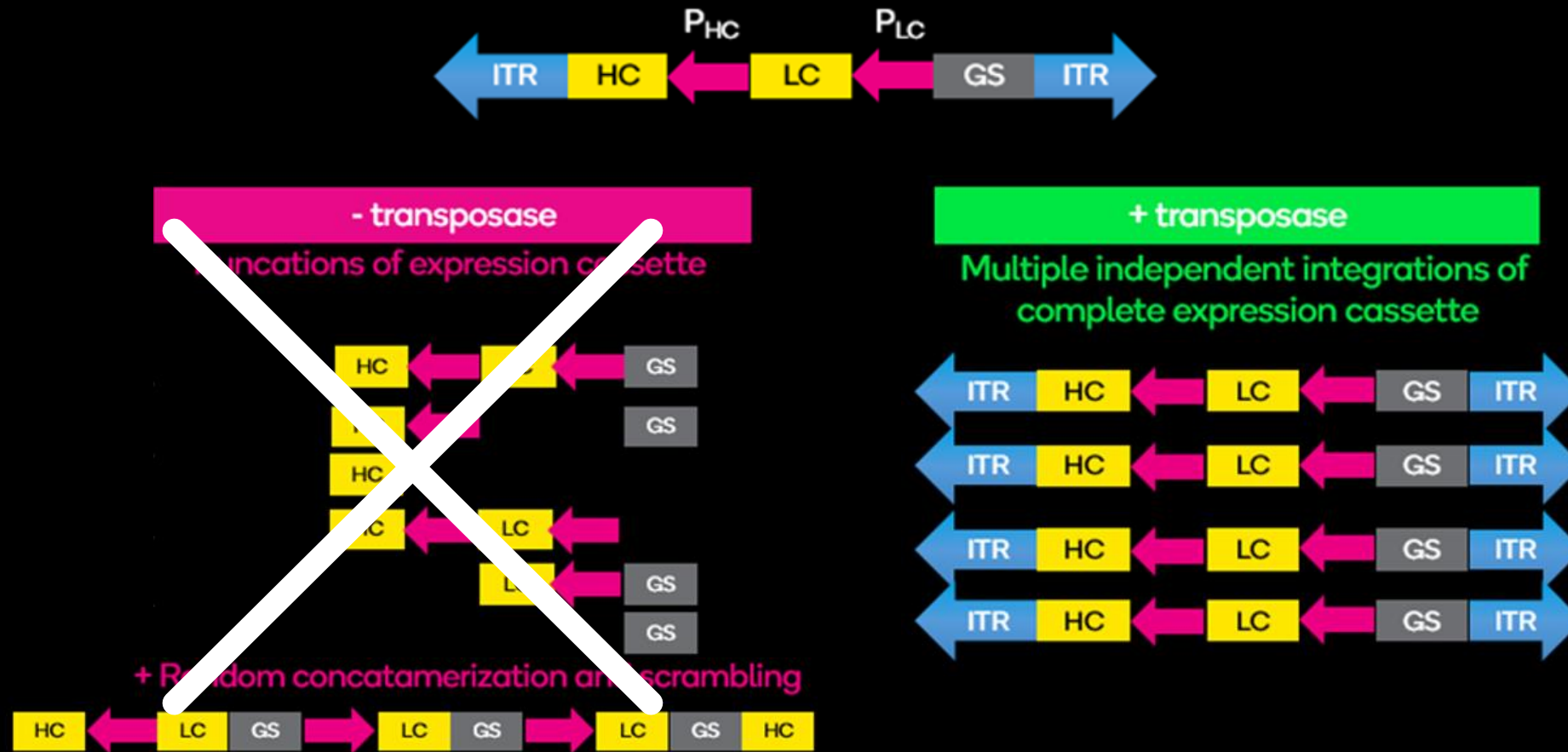
The life of a transposon-transposase pair



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



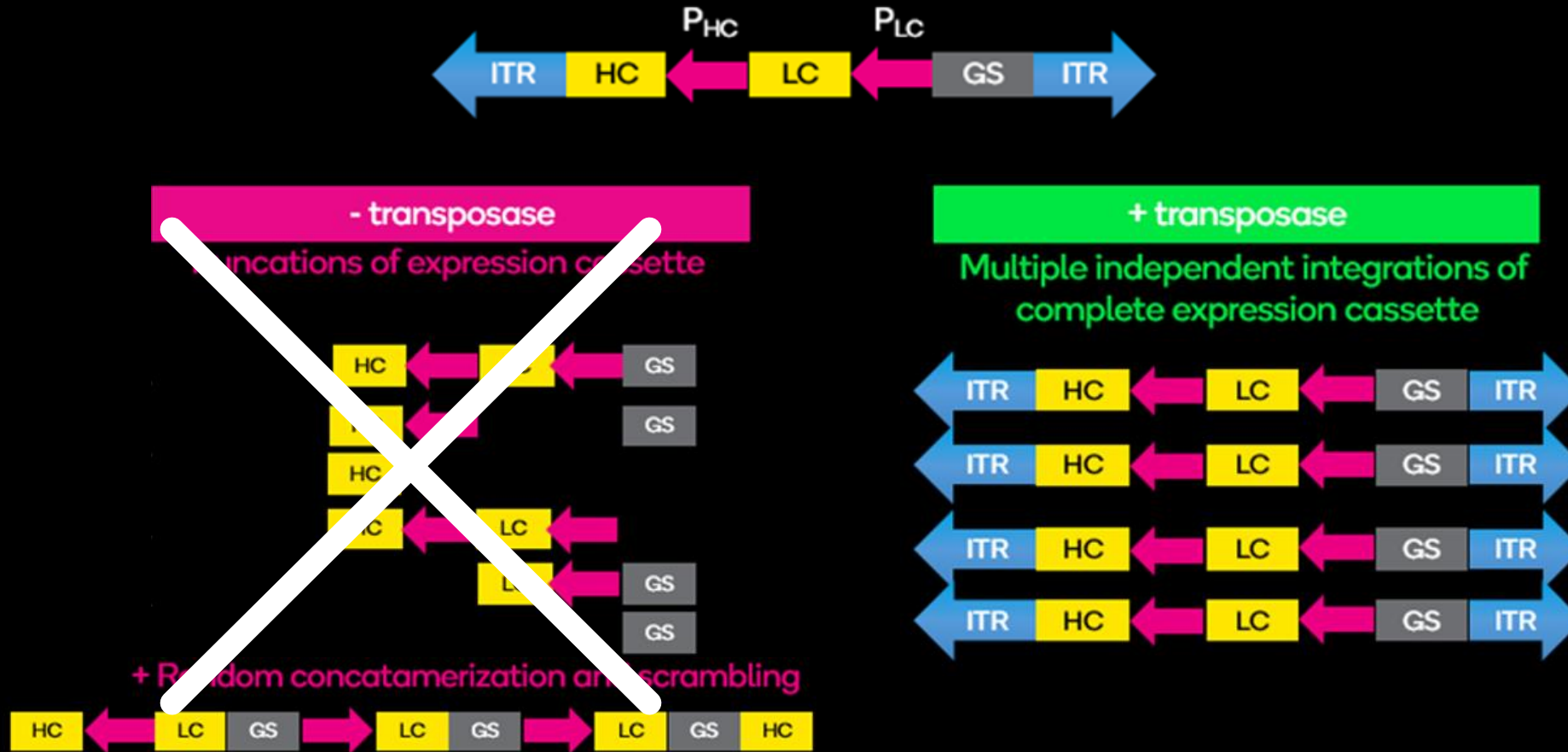
representation of Leap-In[®] transgenes



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

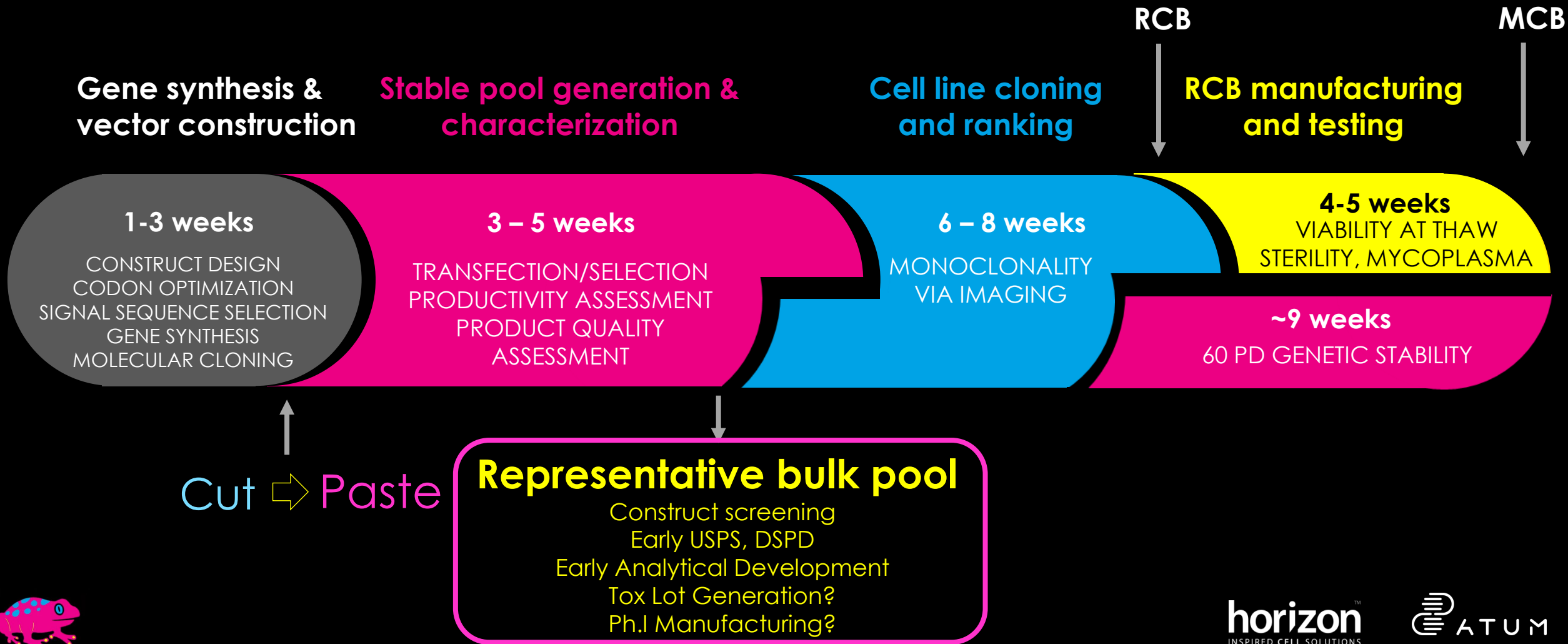


representation of Leap-In[®] transgenes



Highly uniform and predictive bulk pools

rapid and robust: sequence to MCB

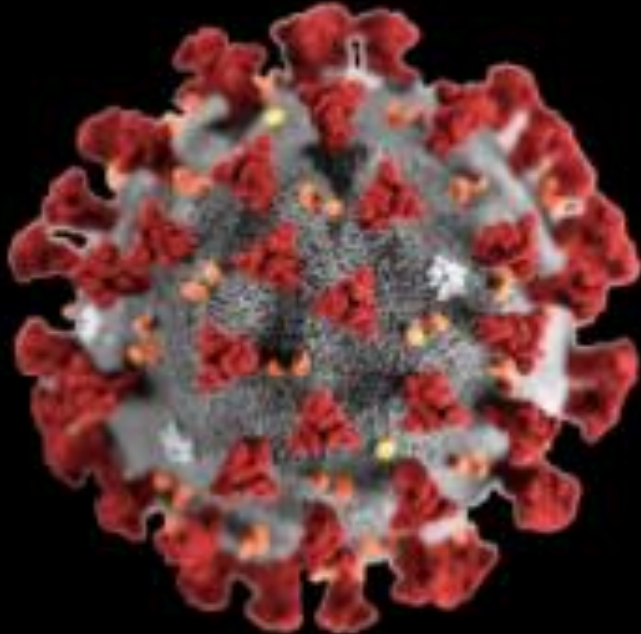


Leap-In Transposase CLD platform

- Expression construct integrity maintained
- Rapid, robust and representative bulk pool generation
- Robust, high titer and extremely stable clones



antibodies For COVID 19 treatment

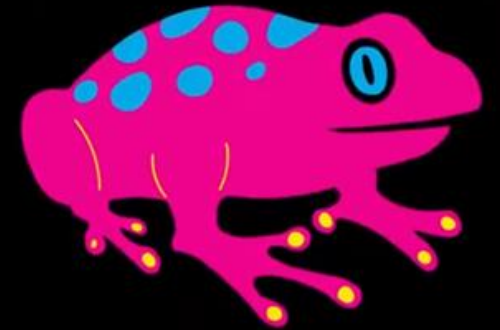


- Eleven candidate therapeutic mAb's
- Desire to initiate human trials ASAP
- Rapid progress: sequence to Ph.I
- Use bulk cell pools for GMP manufacturing

rapid cell line development: bulk pools



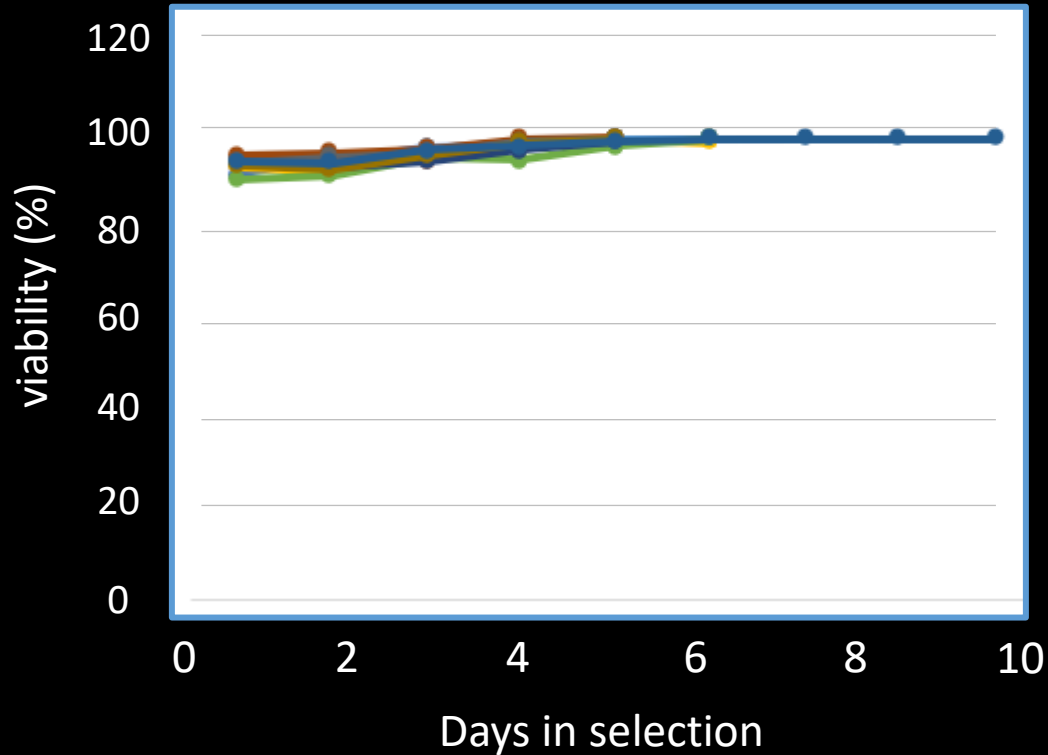
- Two vector sets for each of 11 mAb's
- Create Leap-In Transposase[®] derived pools
- Test expression in platform fed-batch format
- Freeze RCB's for transfer to CDMO



COVID 19: ATUM accelerated timeline: 1

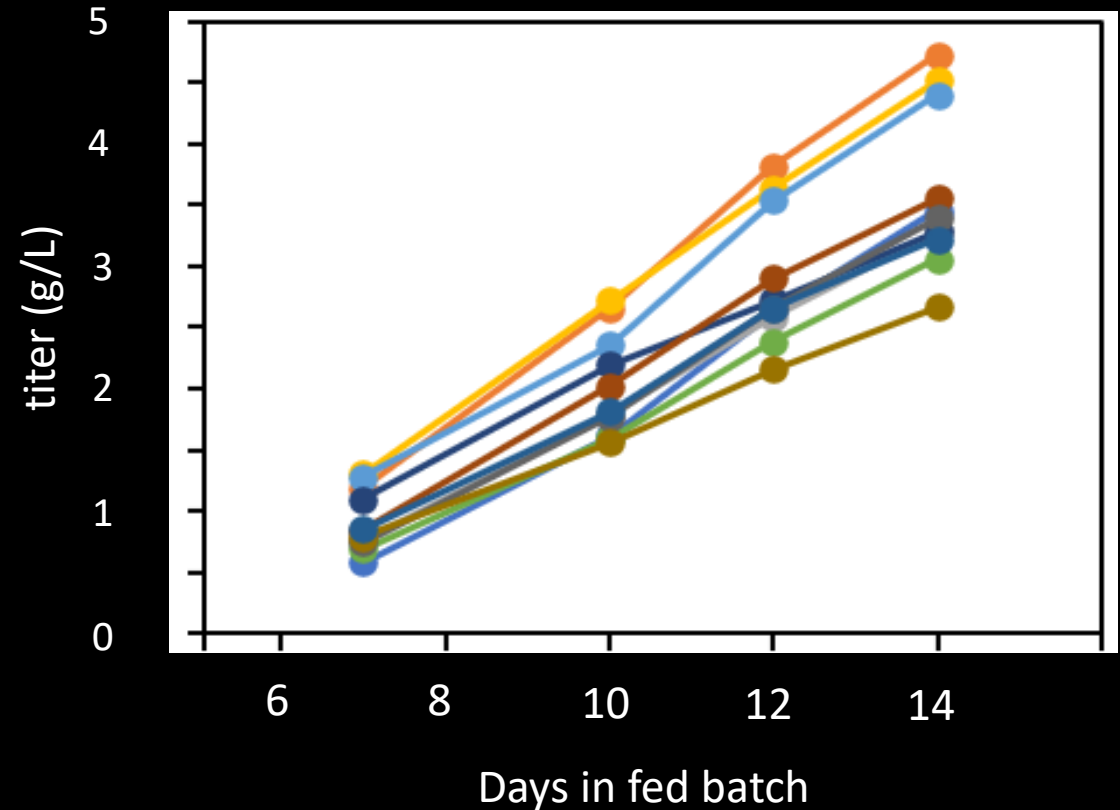


Vector-2



Selection in ~3 days

Vector-2



Titer: 2.5 – 4.8 g/L



COVID 19: ATUM Accelerated Timeline: 1



Gene synthesis

Plasmid prep

Pool Selection

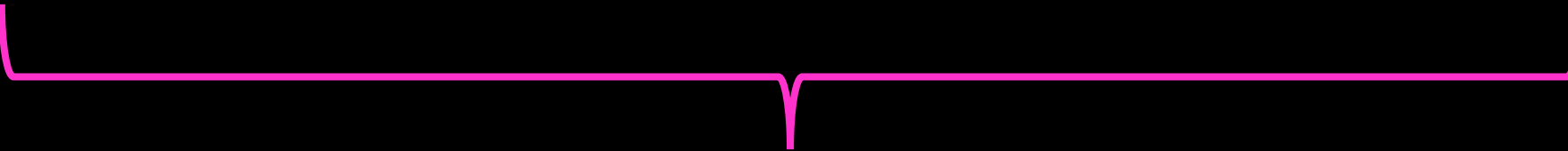
Pool Banking

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 ...”



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

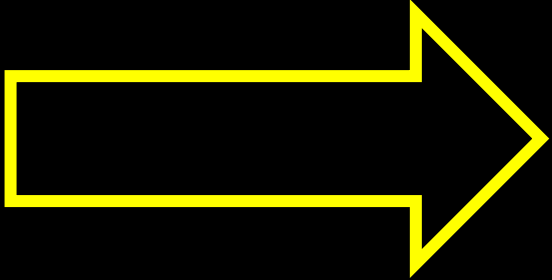
Valerie Schmieder¹, Juergen Fieder¹, Raphael Drerup², Erik Arango Gutierrez², Carina Guelch³, Jessica Stolzenberger⁴, Mihaela Stumbaum⁵, Volker Steffen Mueller⁶, Fabian Higel⁶, Martin Bergbauer⁷, Kim Bornhoeft⁸, Manuel Wittner⁹, Petra Gronemeyer¹⁰, Christian Braig¹¹, Michaela Huber¹², Anita Reisenauer-Schaupp¹³, Markus Michael Mueller¹⁴, Mark Schuette¹⁵, Sebastian Puengel¹, Benjamin Lindner¹, Moritz Schmidt¹, Patrick Schulz¹ 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



fundamental mechanism

Cut  Paste

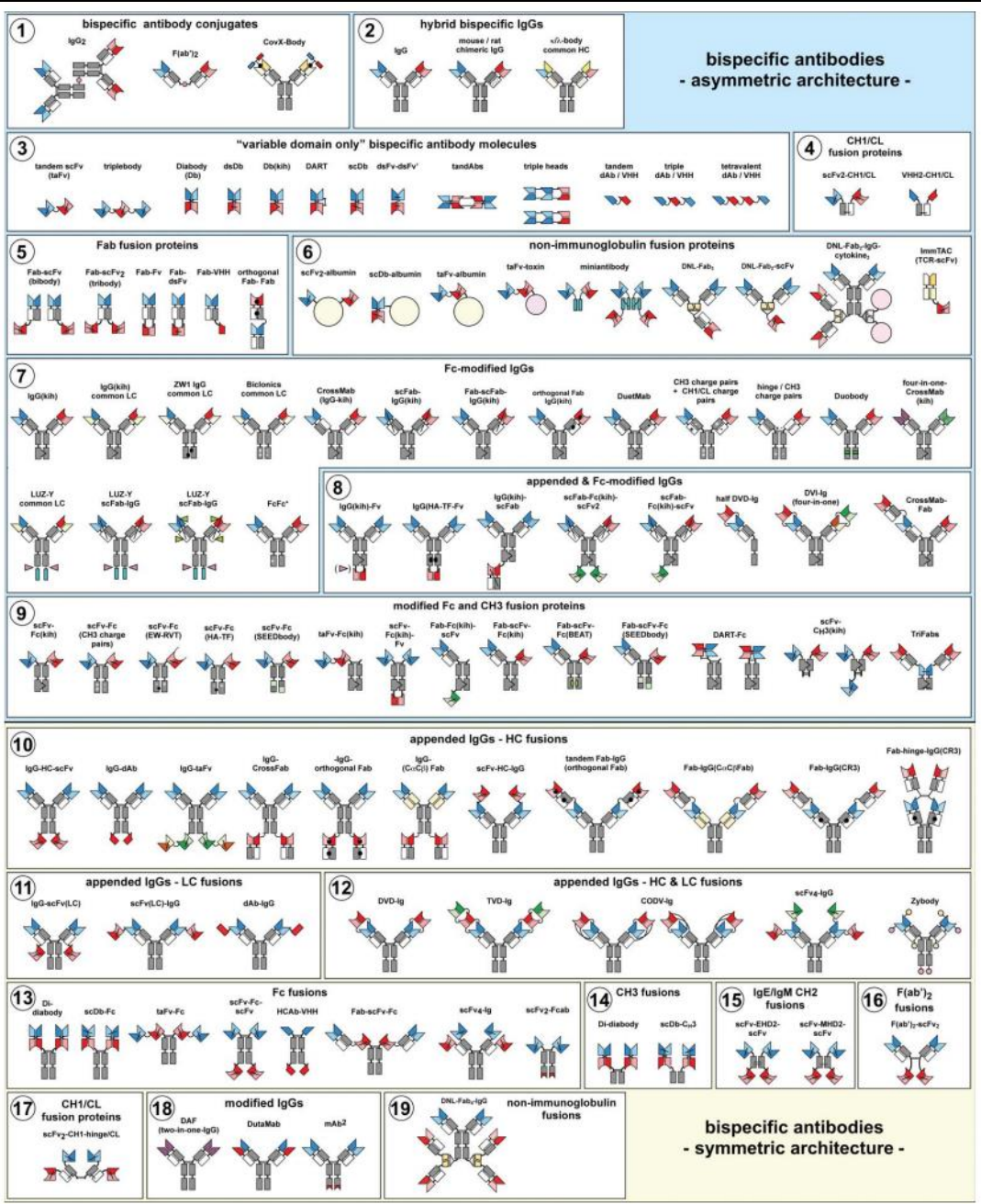
... what you paste matters ...



3 chains and more

The "zoo" of bispecifics

Chain ratio balancing is key



considerations for chain ratio balancing

Sequence

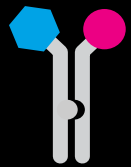
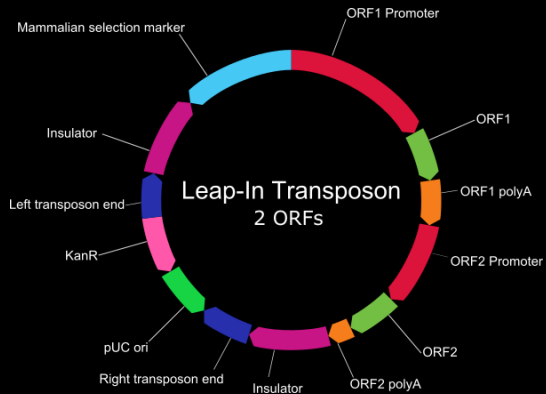
- Codon choice
- mRNA 2° structure
- Poly-A signal
- 5'/3' UTR choice
- mRNA stability
- Ribosomal entry/processivity
- Splice site donor/acceptor
- Signal sequences
- Etc.

Vector

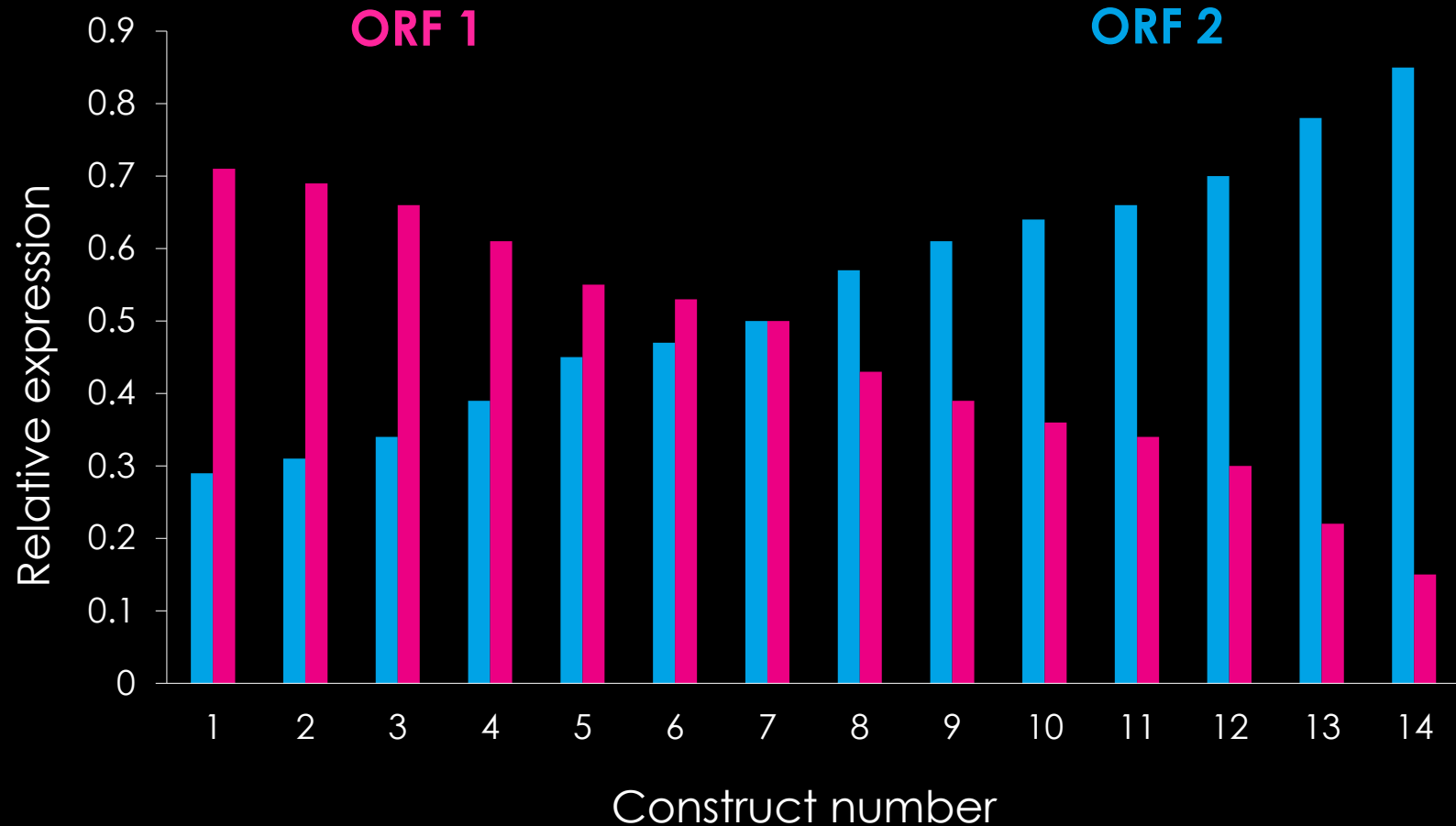
- Promoter choice
- Order of expression cassettes
- Number of expression cassettes
- Spacing of expression cassettes
- Directionality of expression cassettes
- Size of vectors
- Single vector or multiple vectors
- Choice of insulators
- Etc.



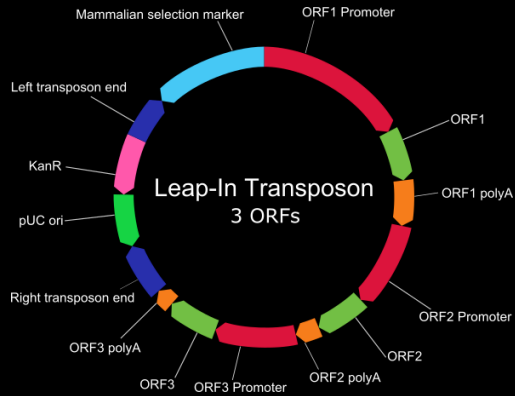
controlling ratios with construct design: 2 ORFs



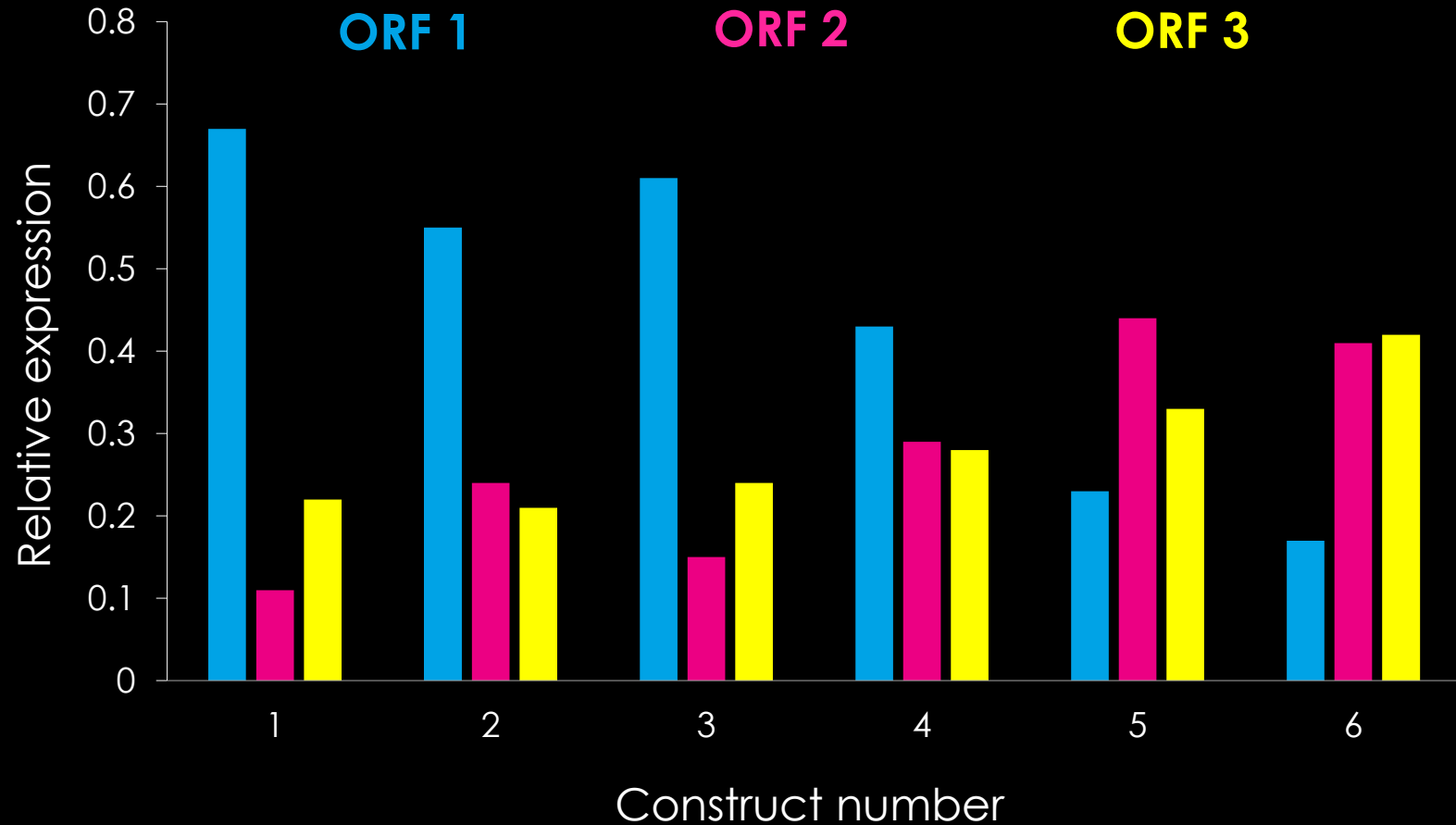
Bispecific Antibodies
chain ratio modulation



controlling ratios with construct design: 3 ORFs



Bispecific Antibodies
chain ratio modulation



case study

- 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

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

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 |



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

licensee comments

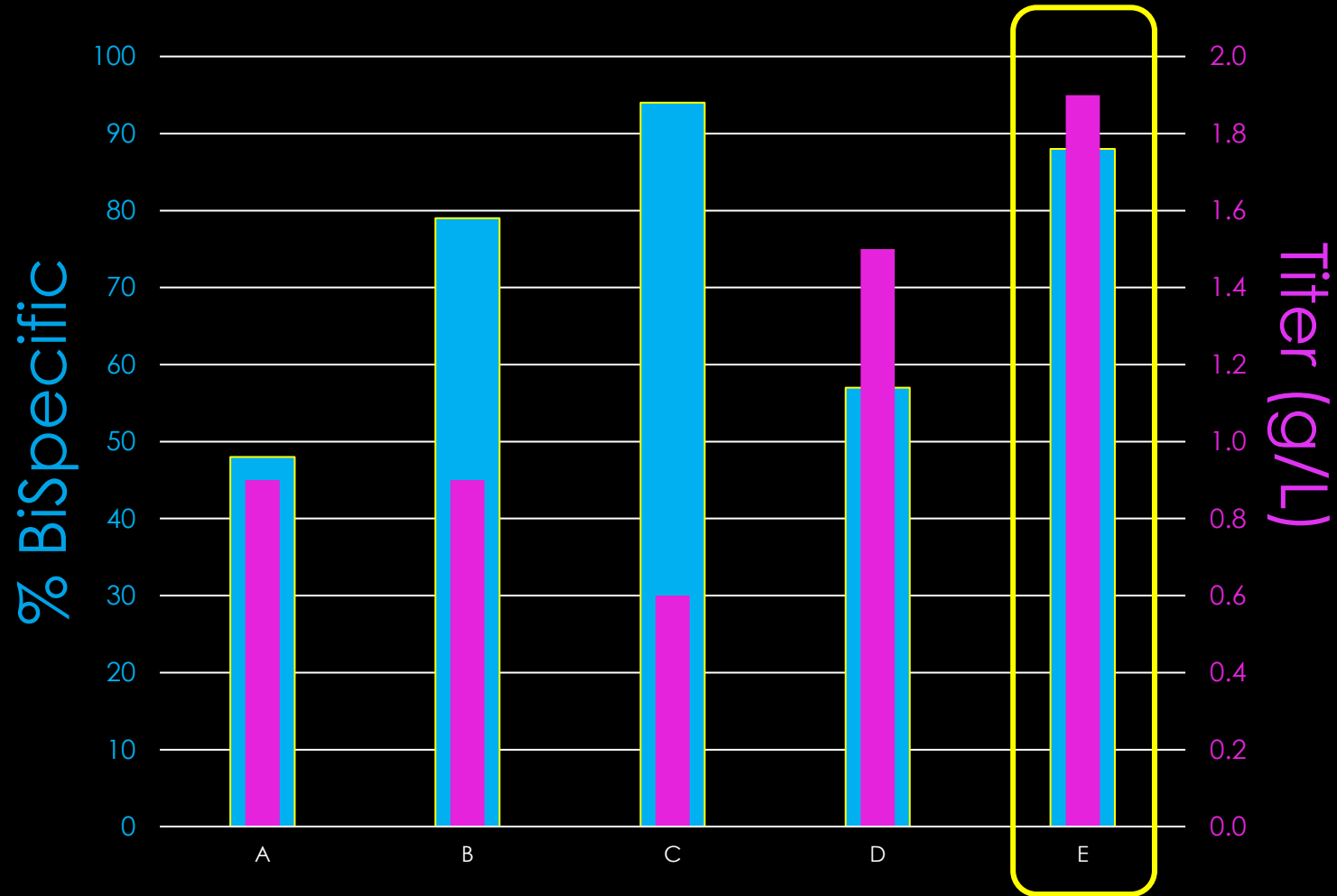
The Leap-In transposase platform enables:

- 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

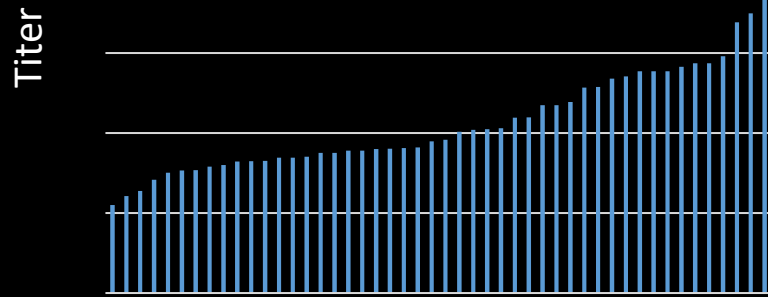
case study: 3-chain bispecific mAb - bulk pools



case study: 3-chain bispecific mAb - clones

Pool E

Clone performance @ 24 wp format

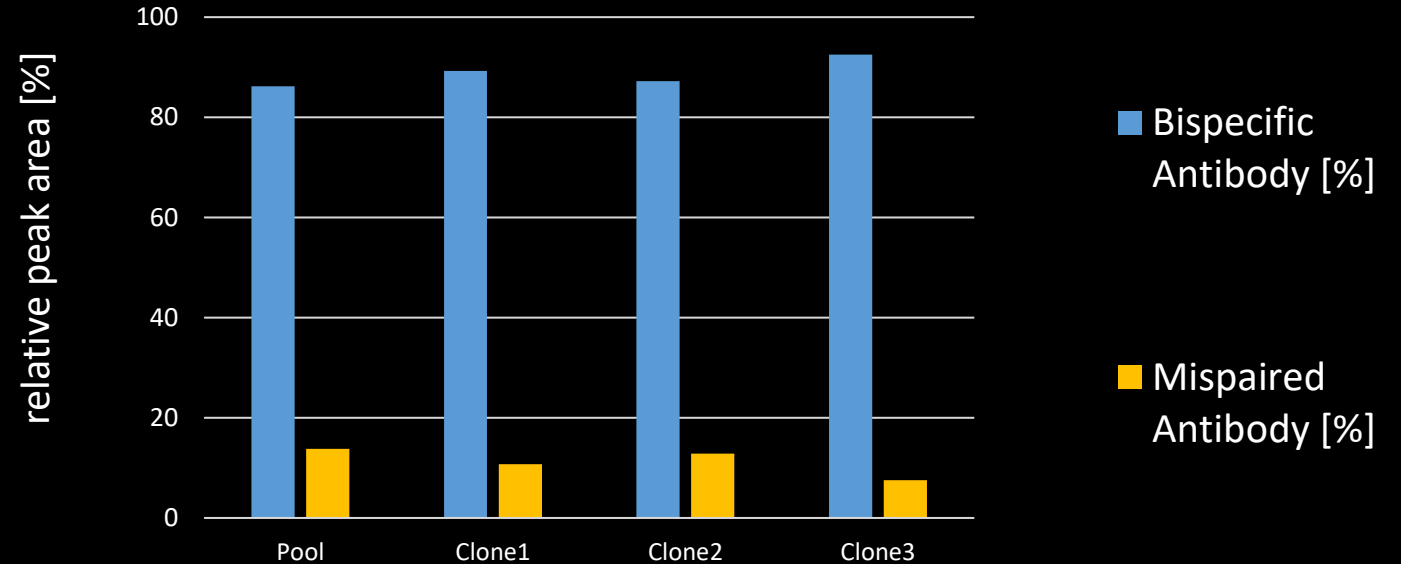


Pool and clone productivity

| Pool* | derived clones* |
|-----------|-----------------|
| 1.9 [g/L] | up to 5.5 [g/L] |

*Day 12 standard fed-batch

% bispecific antibody

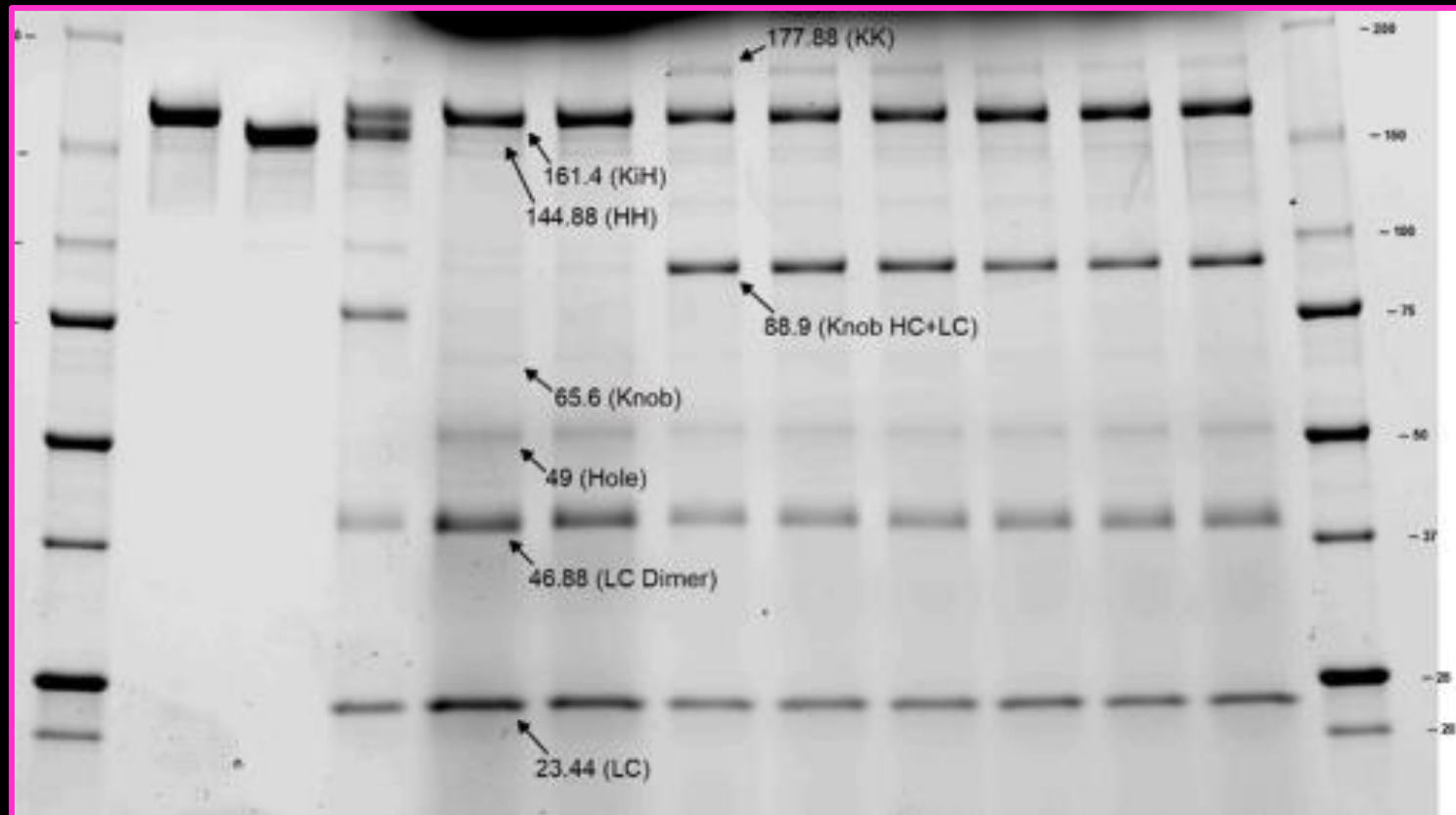


Good pools predict good clones



... and there is more ...

supernatant SDS PAGE



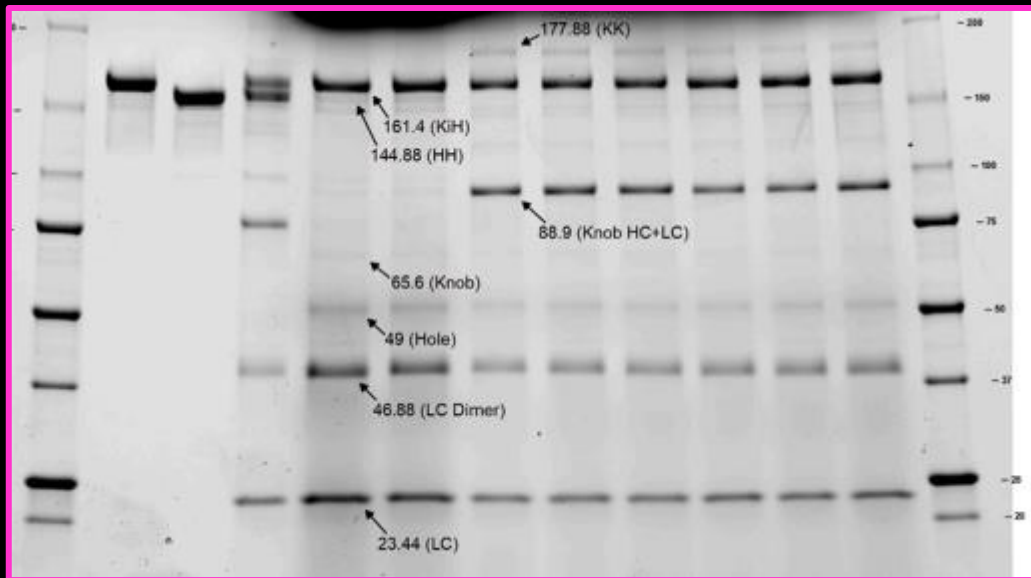
... and there is more ...

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 ... ”



... and more ...

Leap In Licensee with a 3-chain platform:

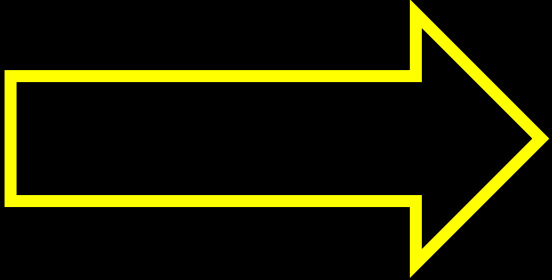
“ ... 5-7 g/L with >97% correct assembly ... ”

CLD customer with a novel 4-chain platform:

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



fundamental mechanism

Cut  Paste

... what you paste matters ...



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 \geq 13 CDMO's

IT NETWORKS



ATUM

- 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**





Thank You

Oren Beske

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Partners:

Horizon Discovery
Rentschler Biopharma
Our Customers

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

