

Leap-In Transposase[®] & Transposon for Improved Protein Production

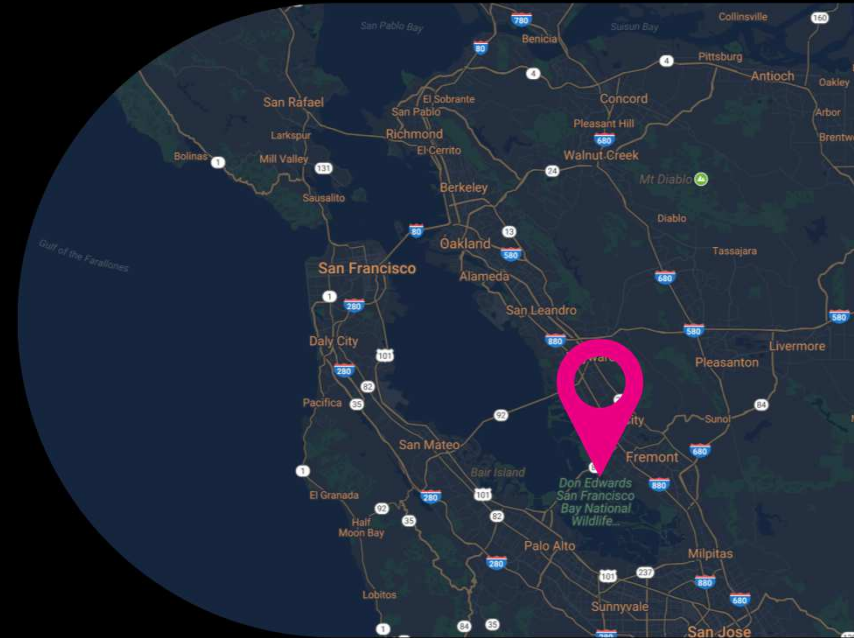
14th PEACe conference

Newport, Rhode Island
September 23, 2019

Sowmya Balasubramanian, Ph.D.

ATUM Cell Line Development

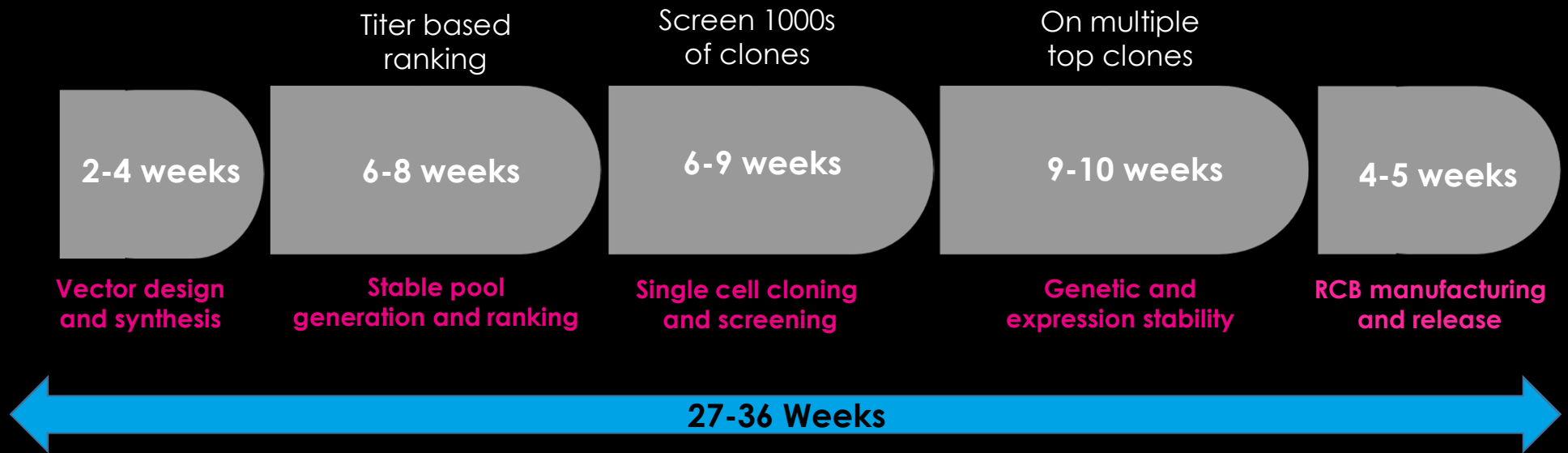
ATUM



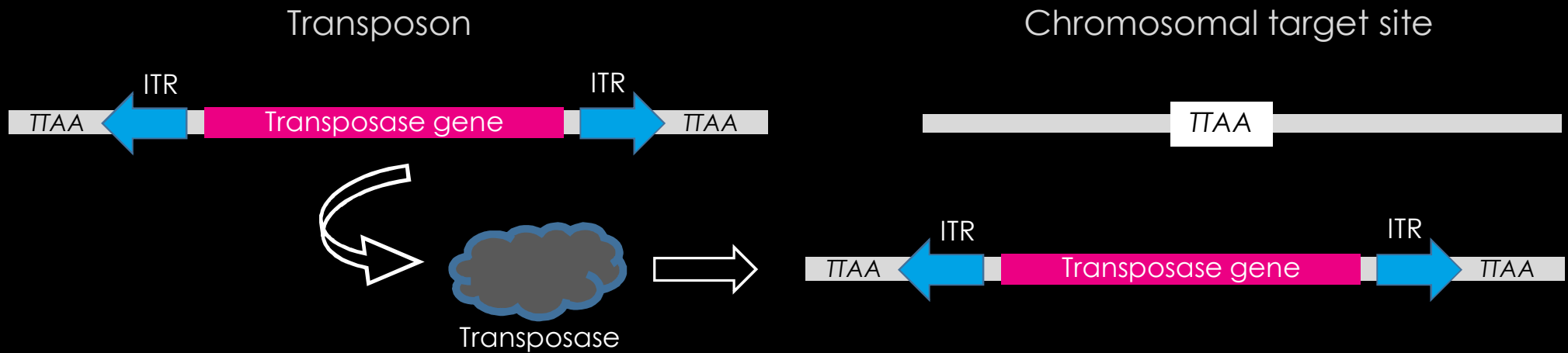
- Founded in 2003 as DNA2.0
- Organic growth, Employee owned
- ~100 employees

- >25 issued patents
- >50 peer-reviewed papers
- Services in >2,500 publications

Classic CLD workflow is slow, tedious, uncertain and labor intensive

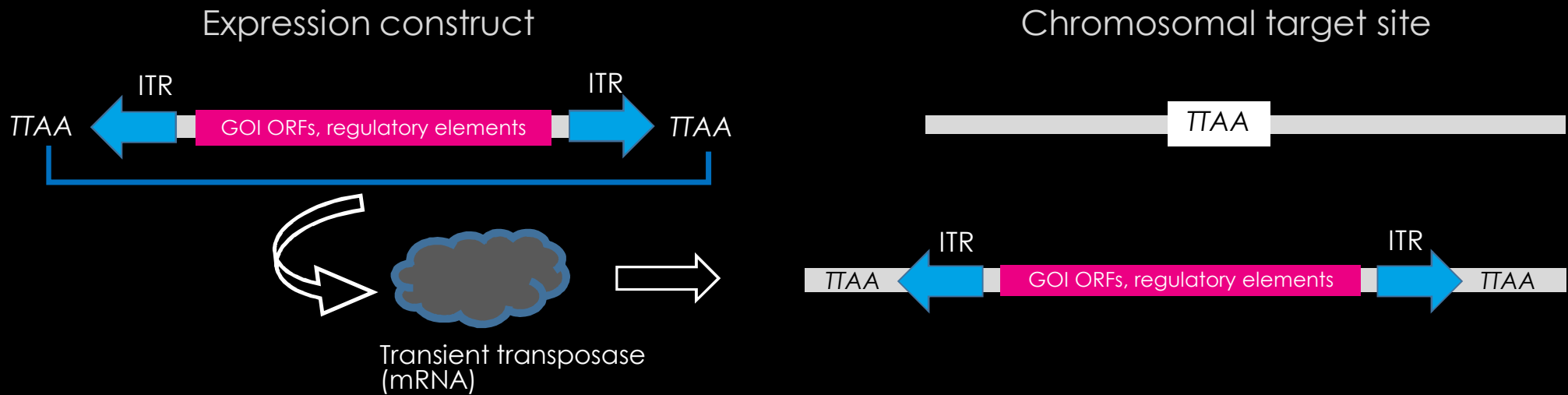


The life of a transposon-transposase pair



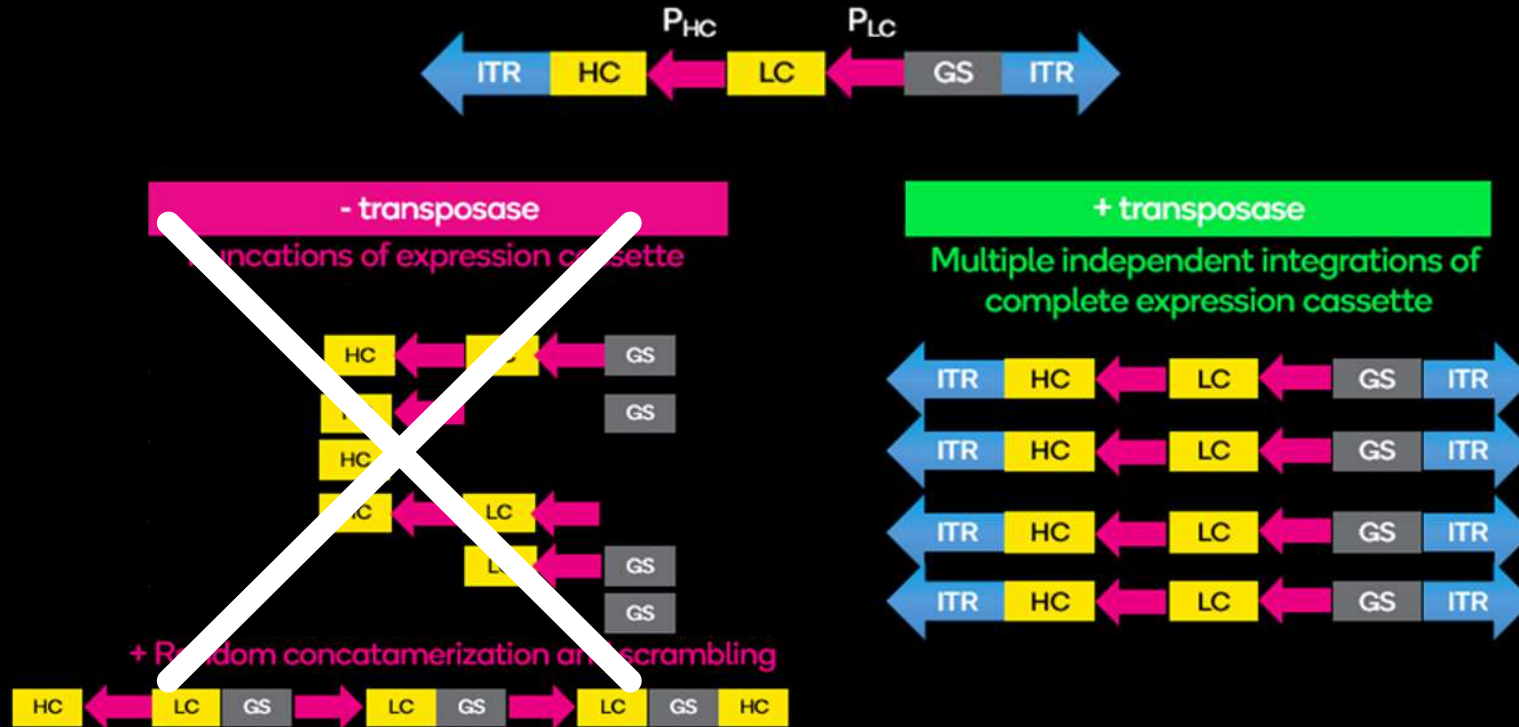
- 4 billion years of successful evolutionary history
- Cut-paste mechanism
- Single copy integration at each site
- Perfect integration of elements between ITR's

Transposase applied to stable cell line development



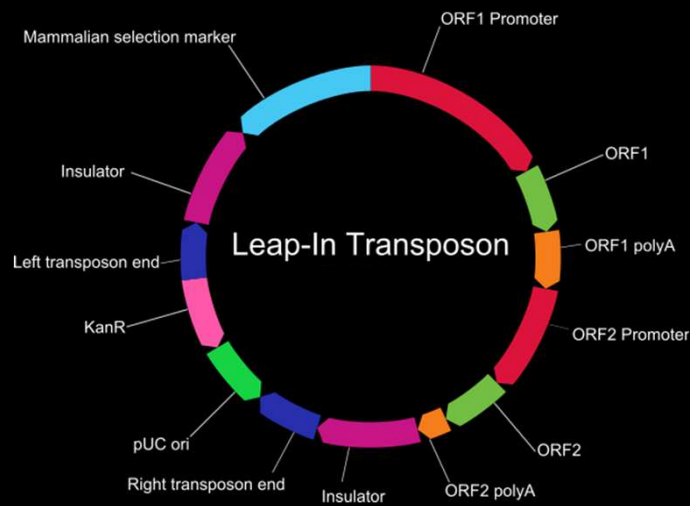
- Transient exposure to transposase = Stable insertion
- Single copy integrations at each site
- Multiple insertions (5 – 60) across the genome
- Structural integrity maintained
- No size limitation

Consistent, uniform presentation of Leap-In[®] transgenes



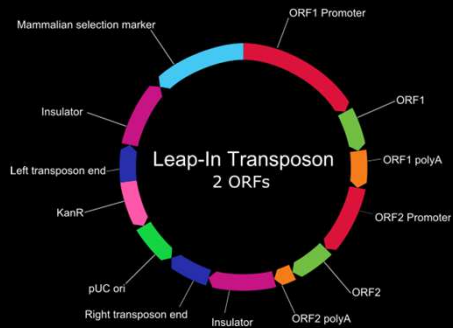
Intact constructs maintained at every integration site

VectorGPS[®] on Leap-In[®] transposon

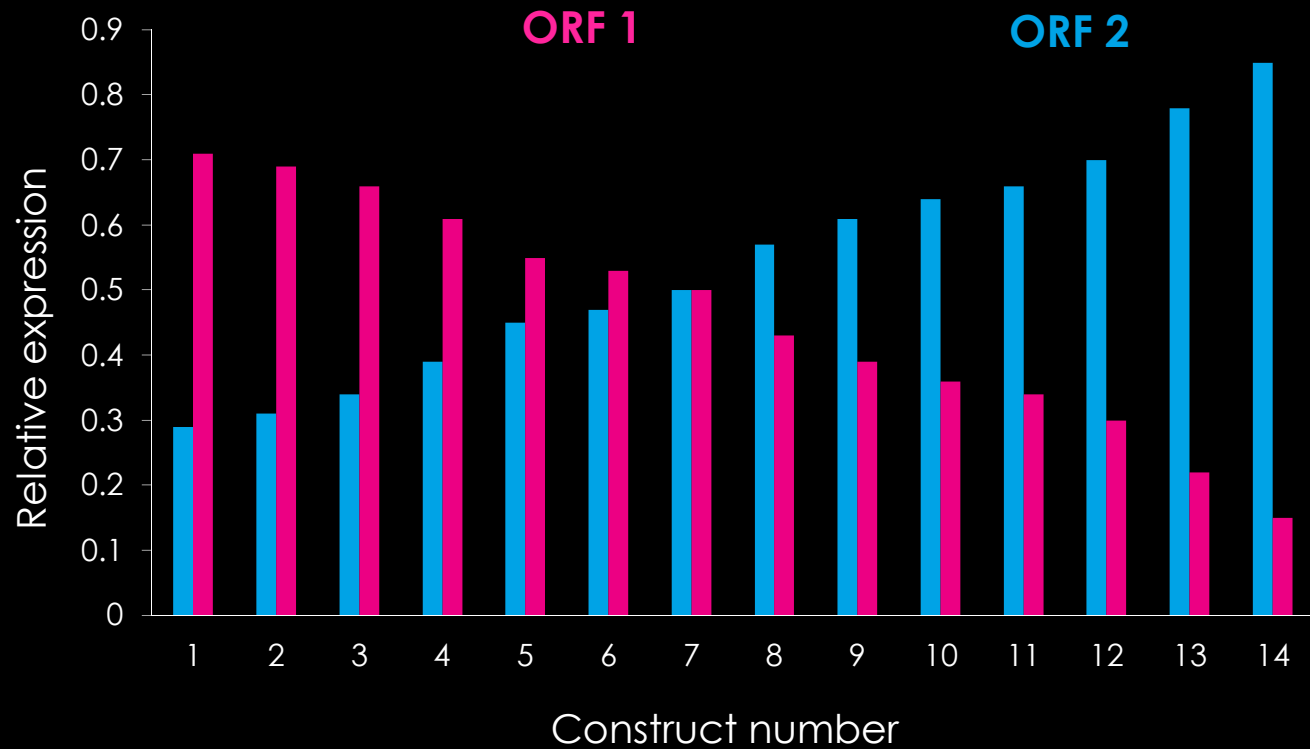


- Multiple transcriptional units/construct
- Catalog of selectable markers
- Catalog of promoters
- Catalog of insulators
- Other regulatory elements
 - Signal peptides,
 - mRNA transport sequences,
 - IRES etc.

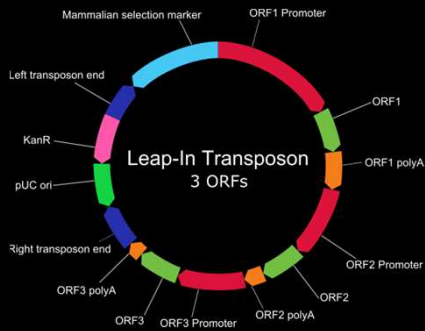
Controlling ratios with construct design – 2 ORFs



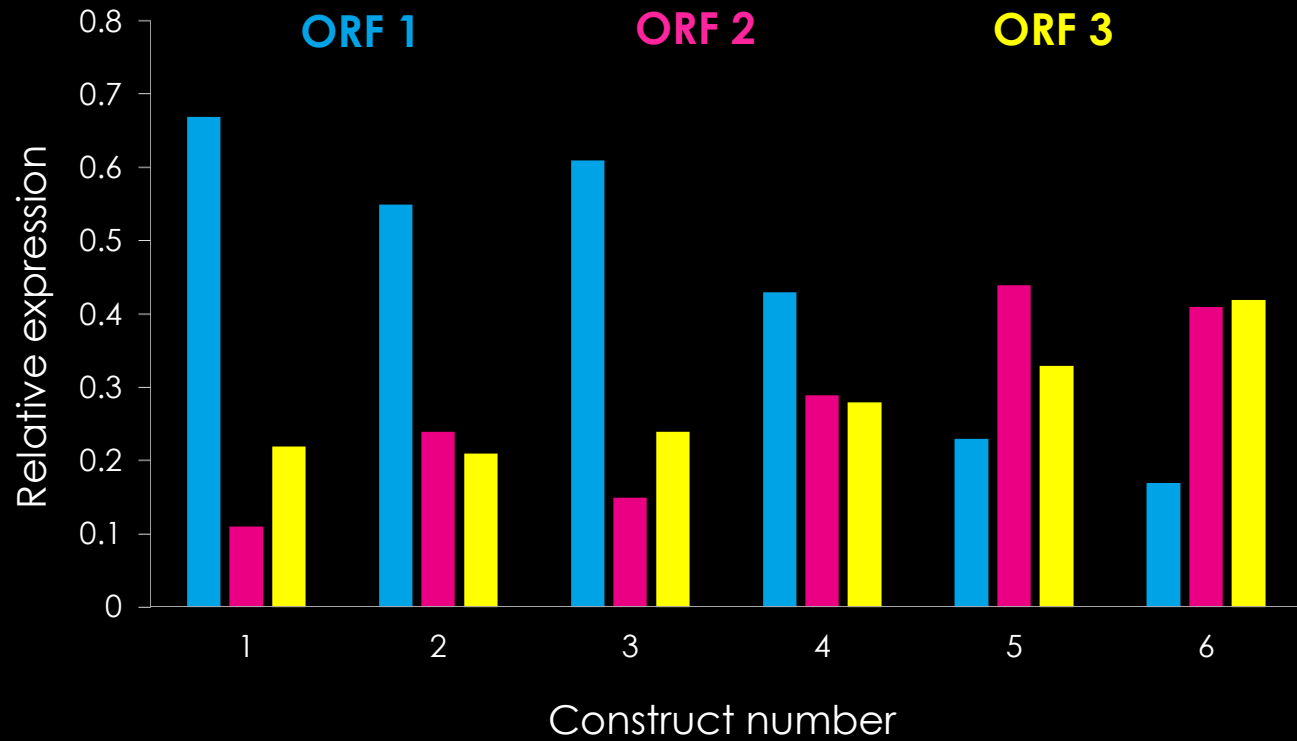
Bispecific Antibodies
chain ratio modulation



Controlling ratios with construct design – 3 ORFs

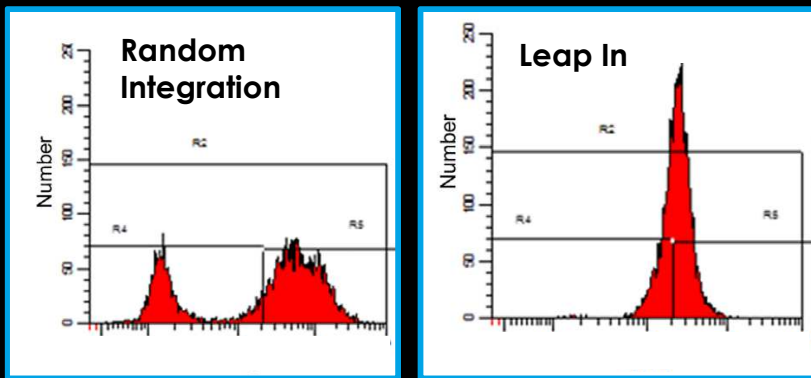


Bispecific Antibodies
chain ratio modulation



Leap-In[®] generates high expressing homogeneous pools

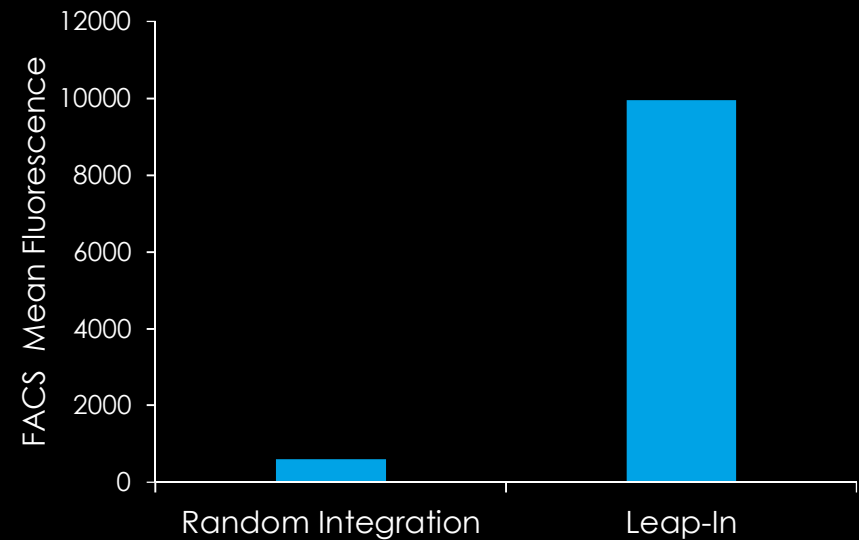
Intracellular Staining of stable pools



Balasubramanian, S, et. al., 2018, *Biotechnol. J*

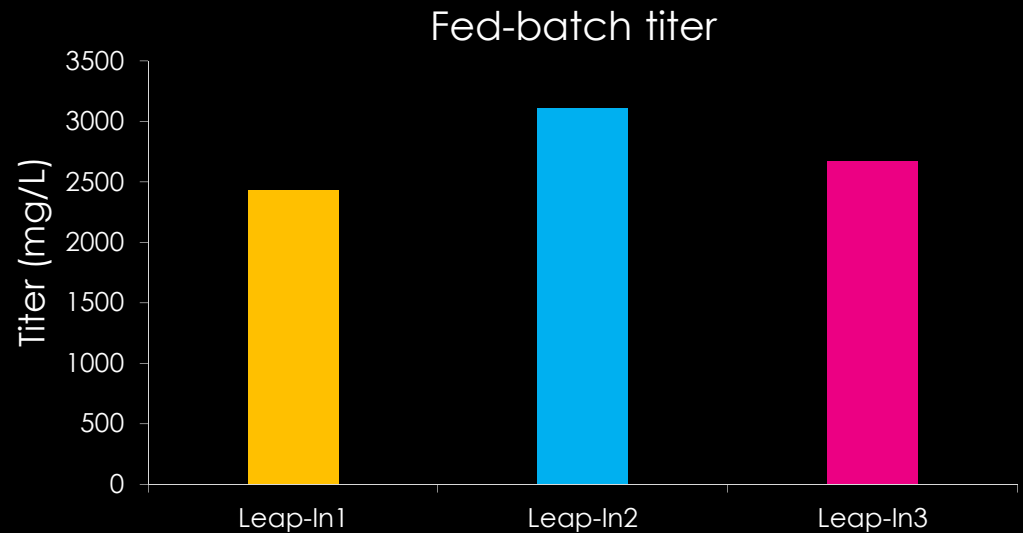
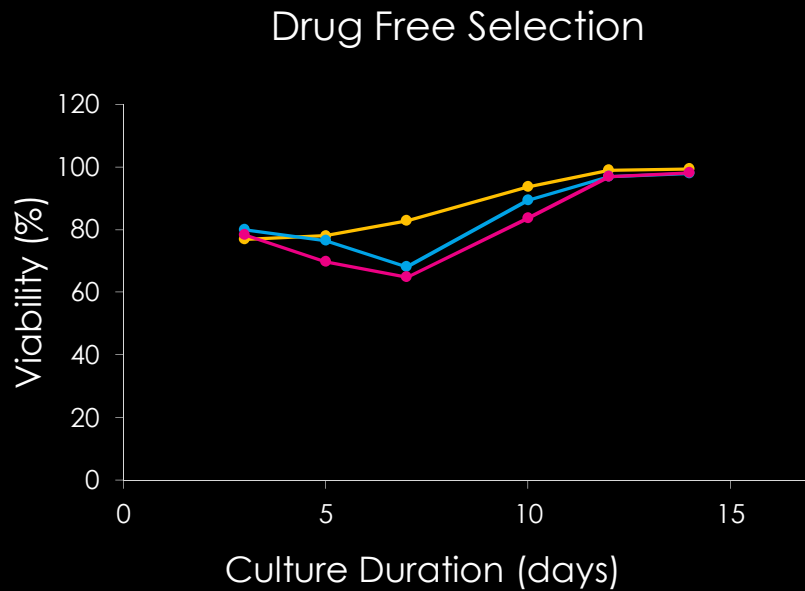
Clone-like distribution of cell pool

GFP expression in cell pools



15x higher mean fluorescence

Leap-In[®] + VectorGPS[®] = Drug free selection



- Use vector elements to modulate stringency of selection
- Drug free selection = absence of Glutamine
- Recovery in <2 weeks
- Low impact of selection dip on pool titers

horizon[™]
INSPIRED CELL SOLUTIONS

ATUM

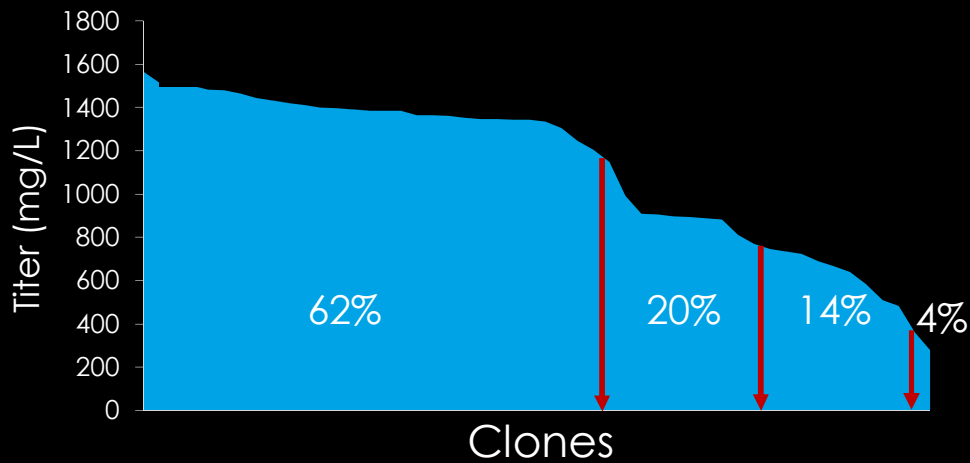
Productivity in Leap-In[®] generated stable pools

Pool Titers

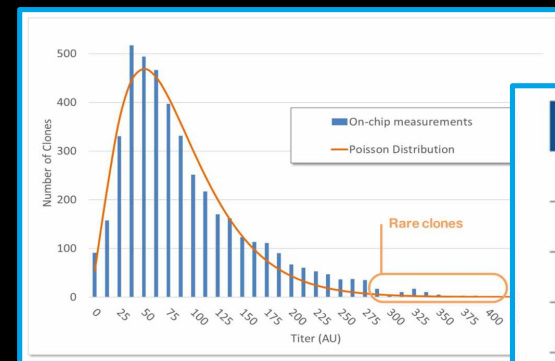
Protein	Volumetric productivity	Specific productivity
IgG1	4.2 g/L	42 pcd
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IgG1	5.9 g/L	39 pcd
IgG1	4.2 g/L	33 pcd
IgG4	5.0 g/L	43 pcd
IgG4	5.0 g/L	49 pcd

Population shift towards high producing clones

Leap-In Transposase®



Random Integration



If you sample	% chance to find it
1	0.1%
100	10%
500	39%
1000	63%
2500	92%
5000	99%

<https://www.berkeleylights.com/>

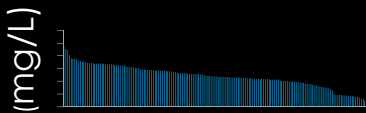
- 62% of clones in top quartile of expressers
- 82% of clones in top half of expressers
- **99% probability in under 200 clones**

- High producers rare

Leap-In[®] + VIPS[™] = reduced clone ranking effort

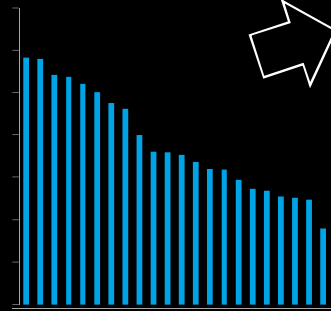


Monoclonality established



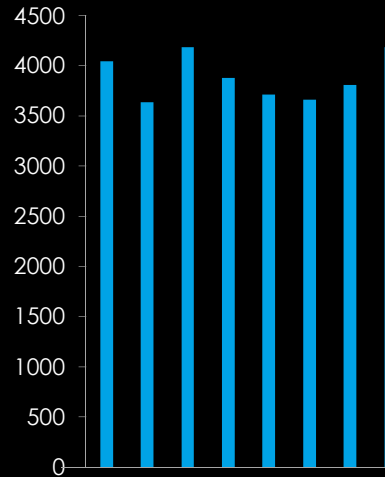
166 clones

Static 96 well plate



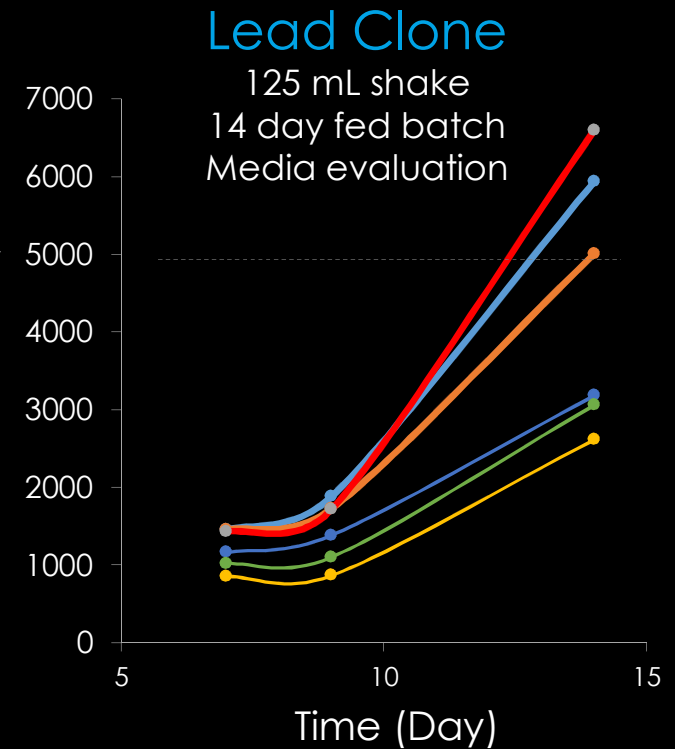
22 clones

24 deep-well scale
7 day fed batch

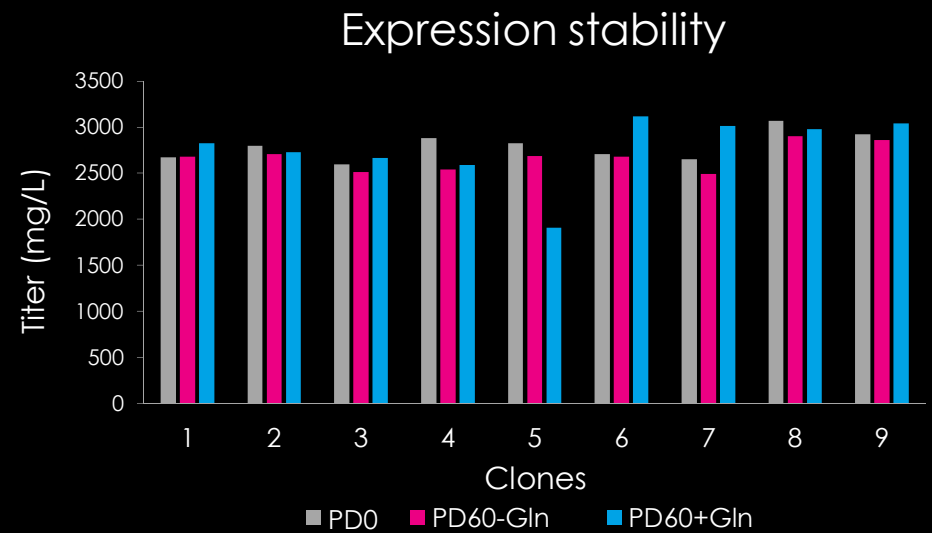
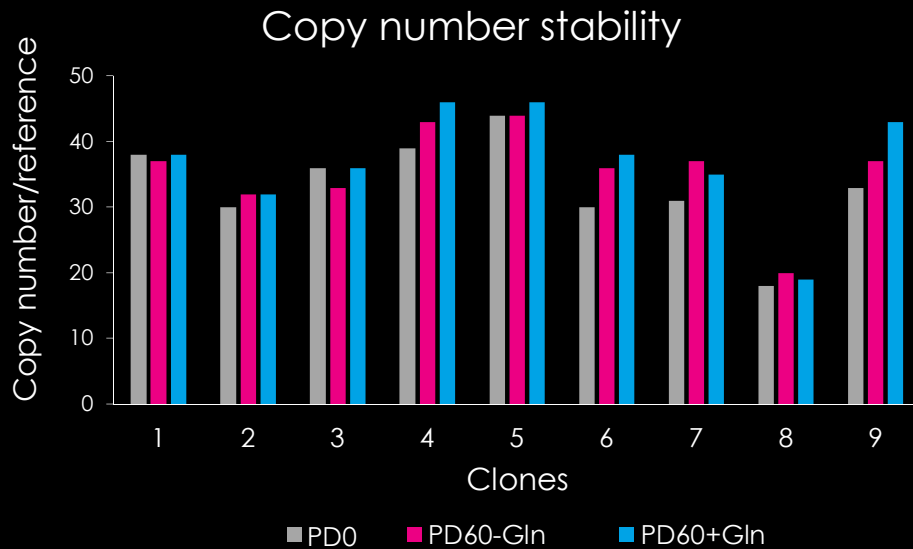


8 clones

125 mL shake
14 day fed batch



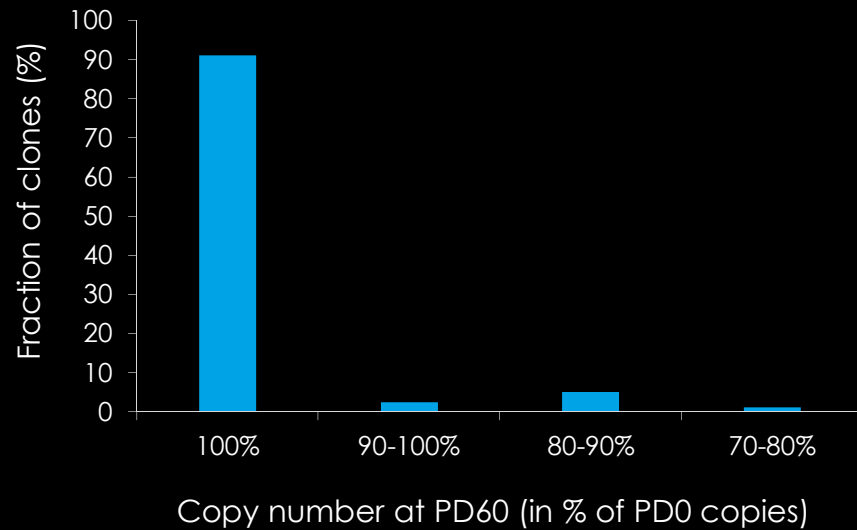
Robust expression and copy number stability



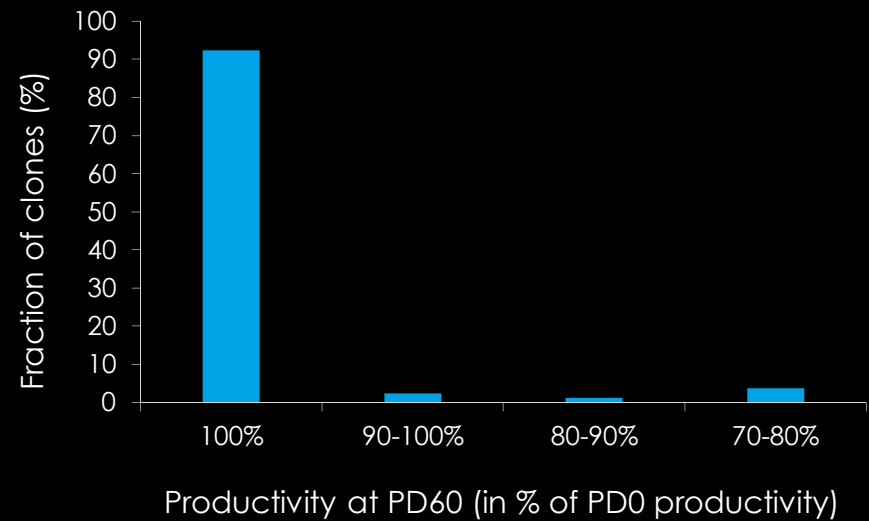
Consistent genetic stability over >60 population doublings

Genetic Stability Statistics

Copy number stability

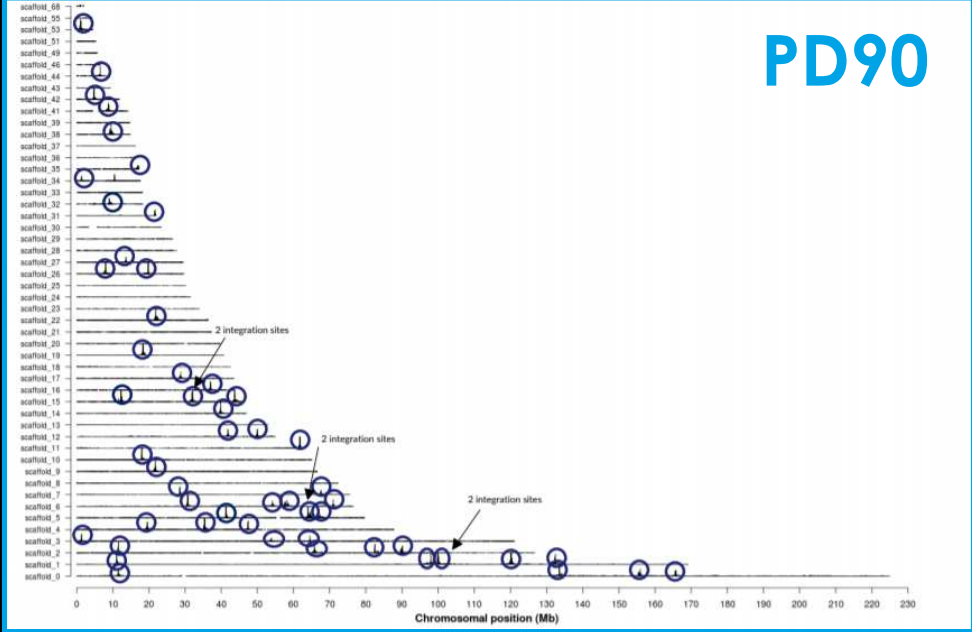
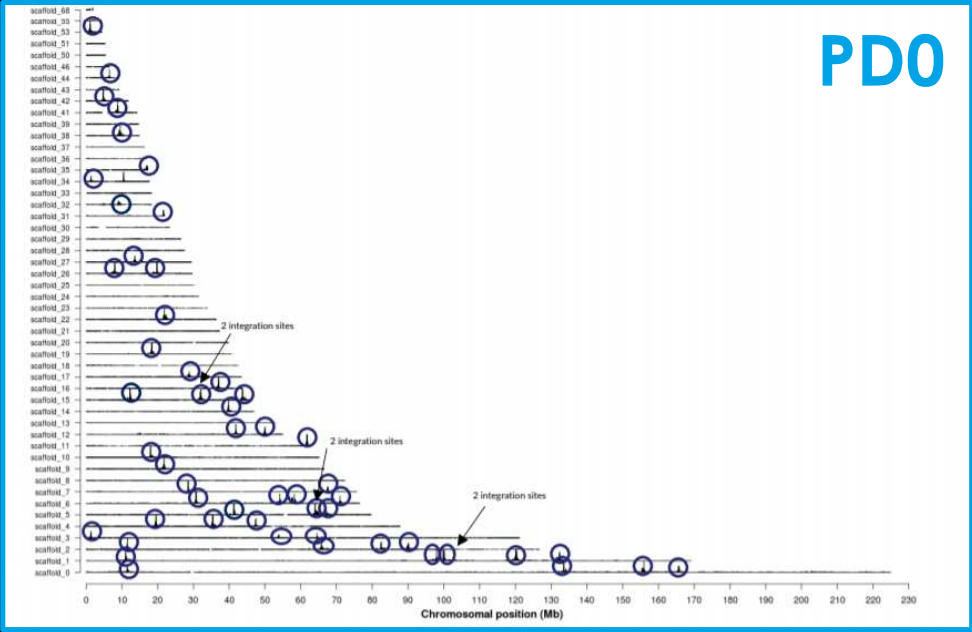


Expression stability



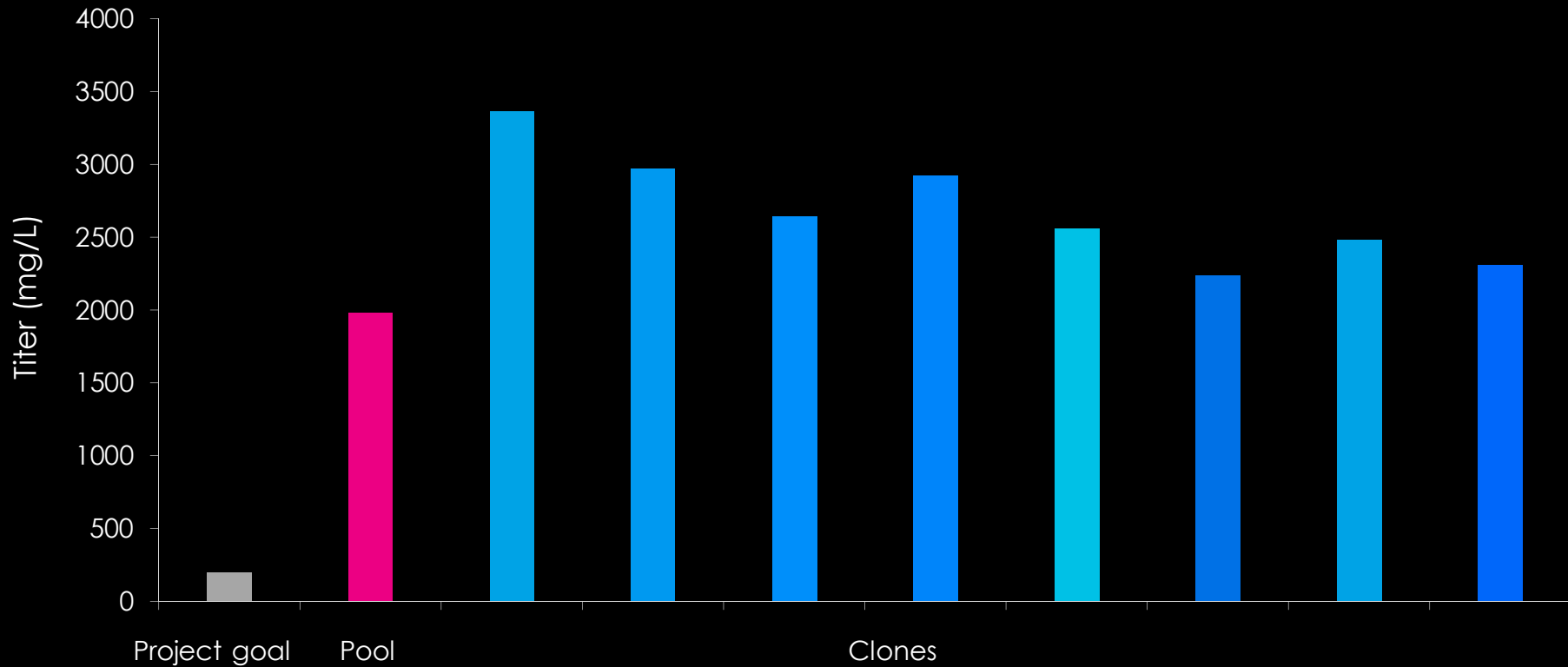
Genetic stability is not a clone ranking parameter

Structural stability of the integrated expression constructs



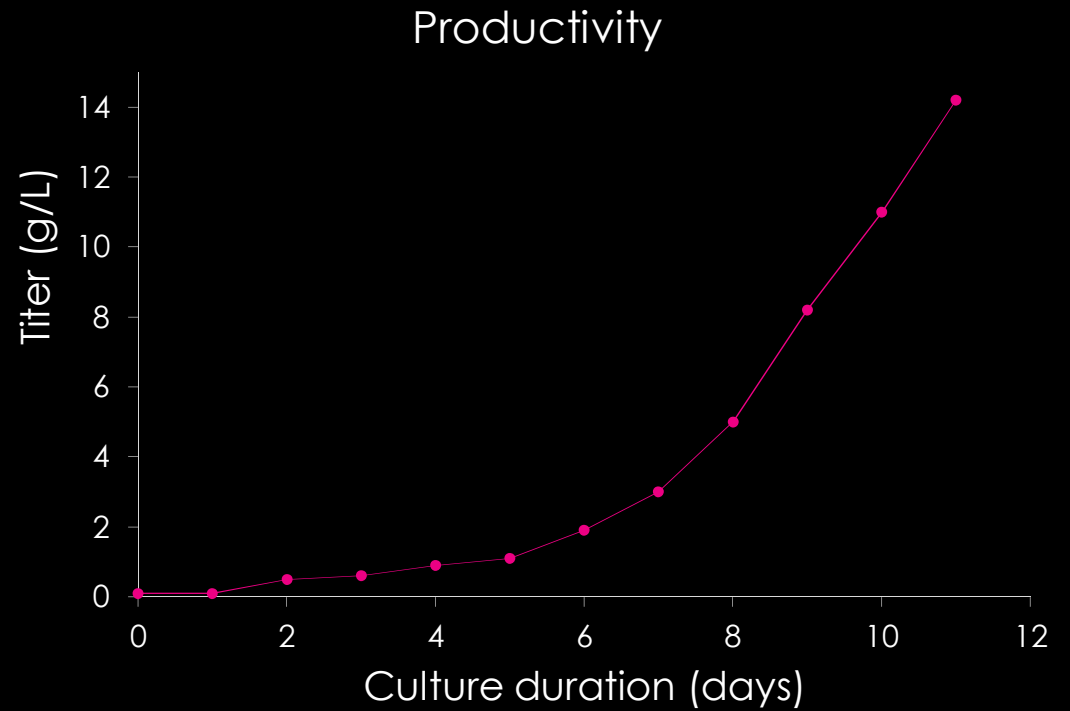
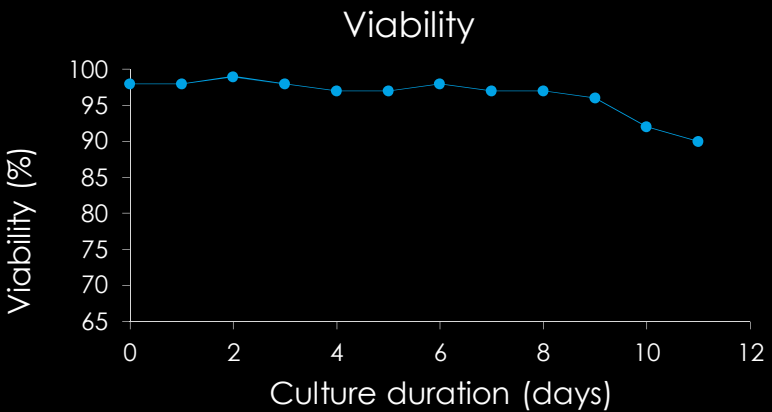
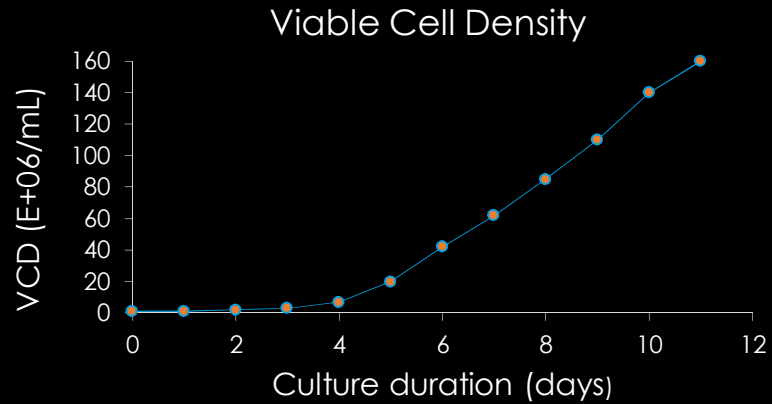
Perfect nucleotide level stability over 90 generations

Case Study: Hard to express non-CHO

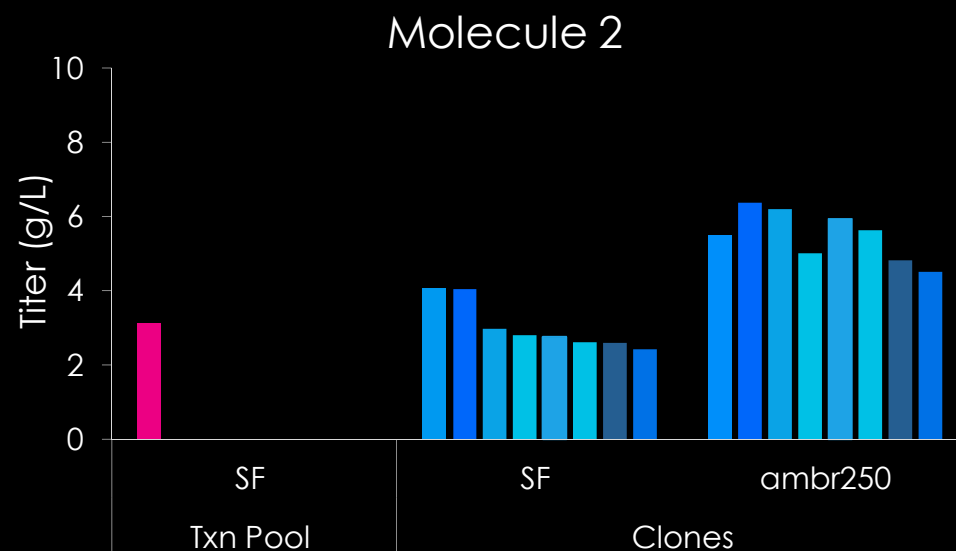
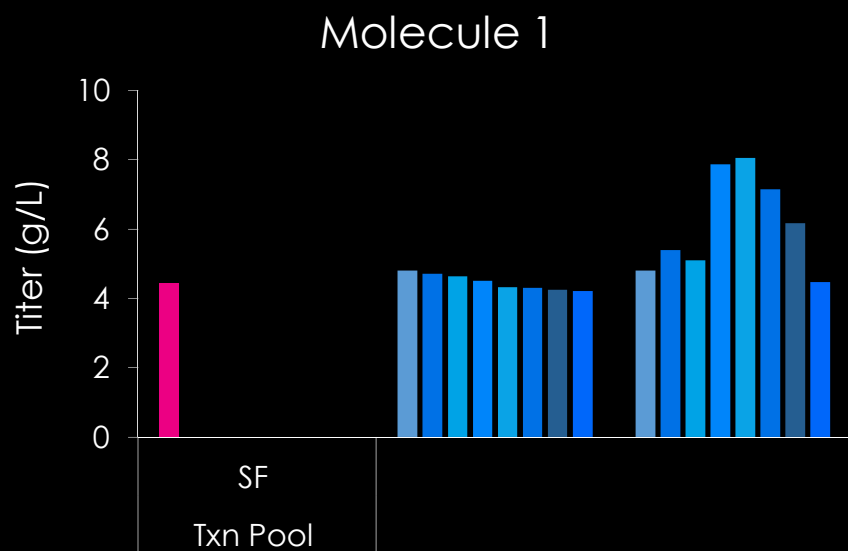


Pool titers are predictive of clone titers

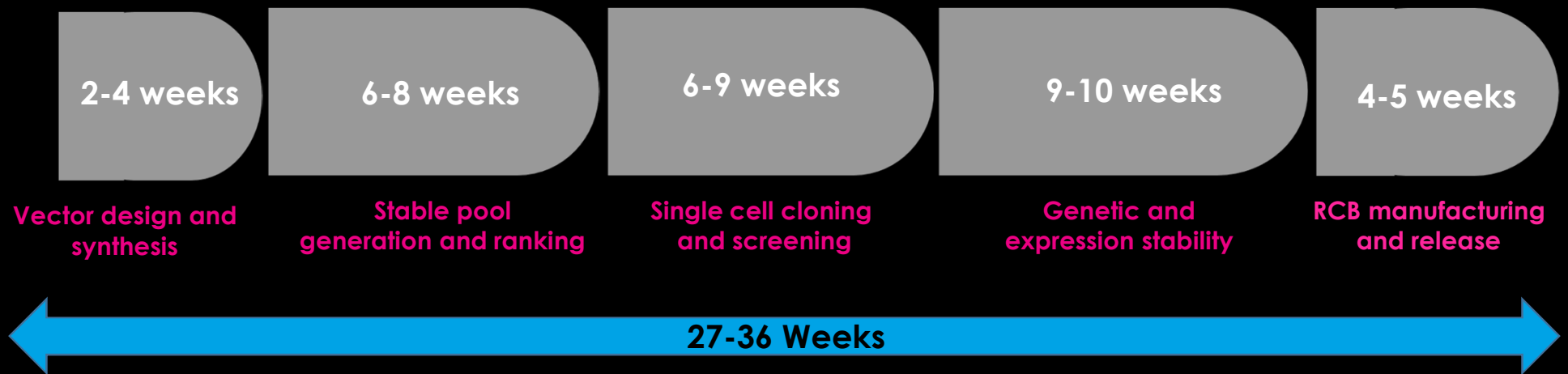
Case study: Intensified fed-batch



Case study: Stable pools predict derivative clone titers

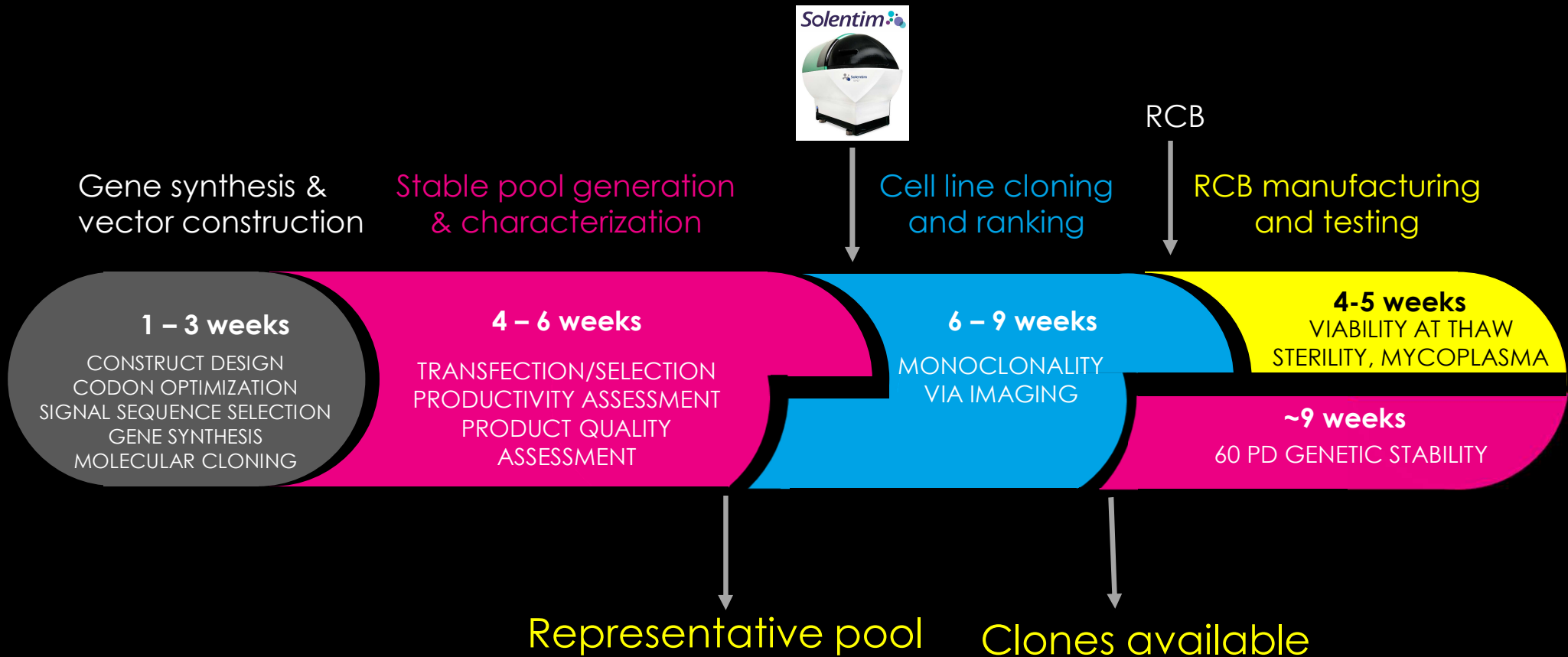


Classic CLD workflow



Classic CLD workflow is slow, tedious, uncertain and labor intensive

Leap-In CLD workflow - Transfection to RCB in 12 weeks



Summary of the Leap-In Transposase[®] Platform

Rapid Timelines

- Efficient and robust integration = Predictable selection
- From transfection to RCB in ~12 weeks
- Predictive stable pools

High Titer

- Leveraging > decade of ATUM proprietary vector elements and algorithms
- Highly uniform cell pools up to 5⁺g/L and clones in excess of 14g/L

Robust Stability

- Transposase mechanism provides very high genetic stability
- No loss in productivity or transgene copy numbers after 60+ doublings

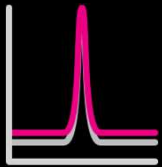
Enabling for Next Generation Biologics

- Compatible with very large inserts (e.g. >100kb)
- Able to co-express multiple genes and tune ratios
- Multiple transposases enable unique genetic engineering strategies

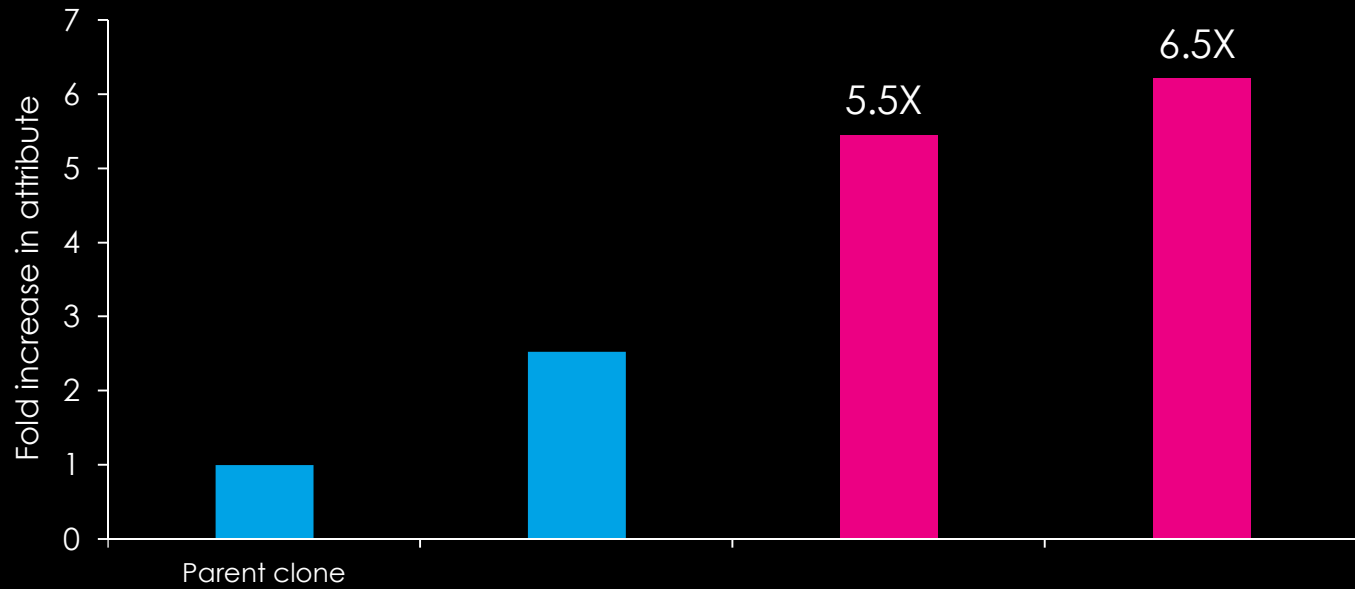
Cell engineering using Leap-In[®]



Complex large molecules
help with folding,
glycosylation and secretion



Biosimilars
specific product quality
attributes



Improving product quality through cell engineering

Cell Pools for speeding timeline

Cell pools – Risks

Low titer

Expression stability

Pool product quality \neq Clone product quality

Cell pools – Advantages

Shorter timelines

Reduced cost

Cell Line Development off critical path

Cell pool – Requirements

Clone like expression titer

Expression stability

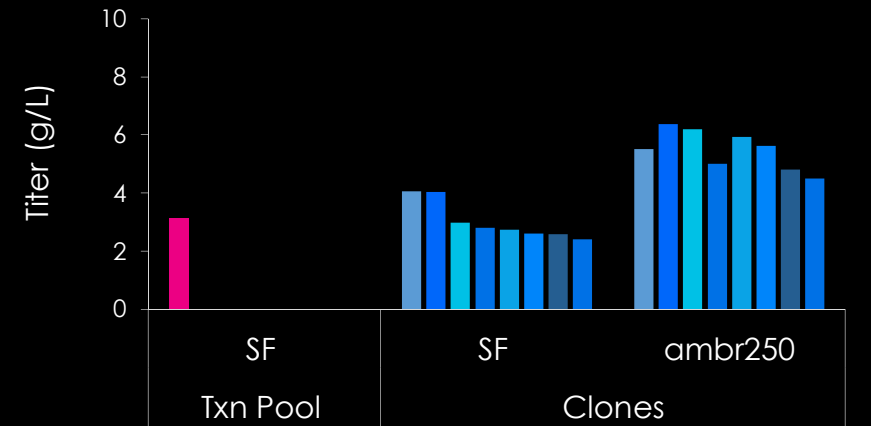
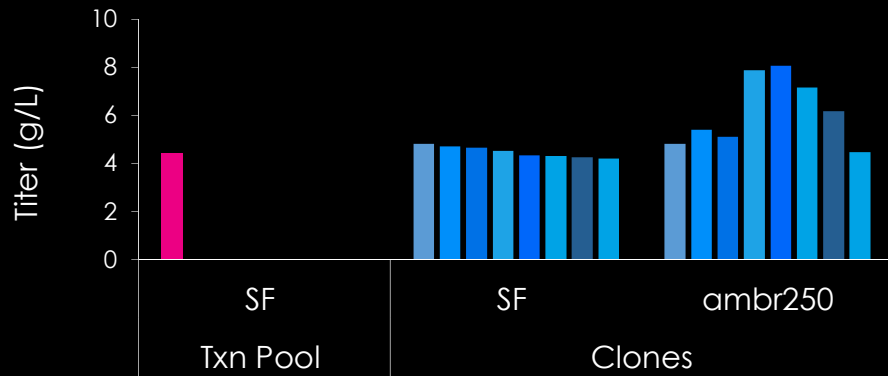
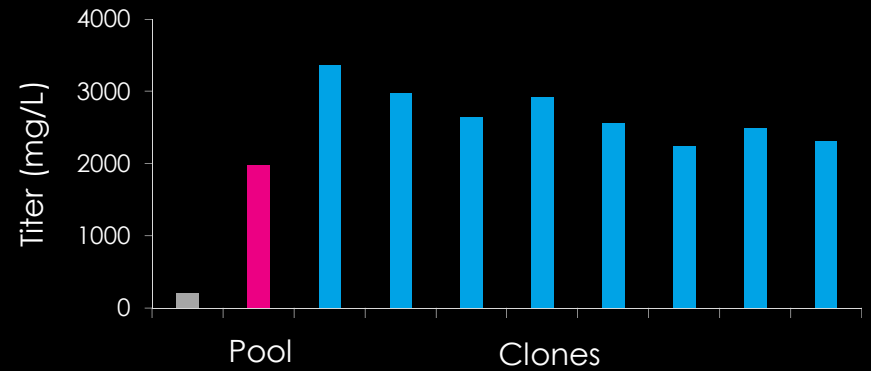
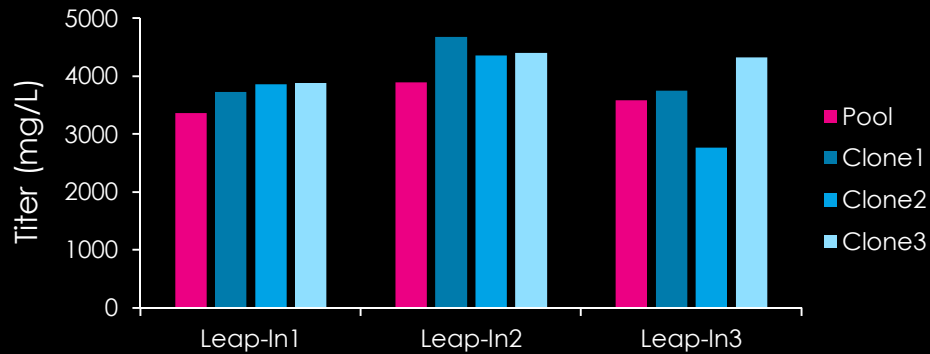
Comparable product quality to derivative clones

Leap-In[®] pools: High productivity

Pool Titers

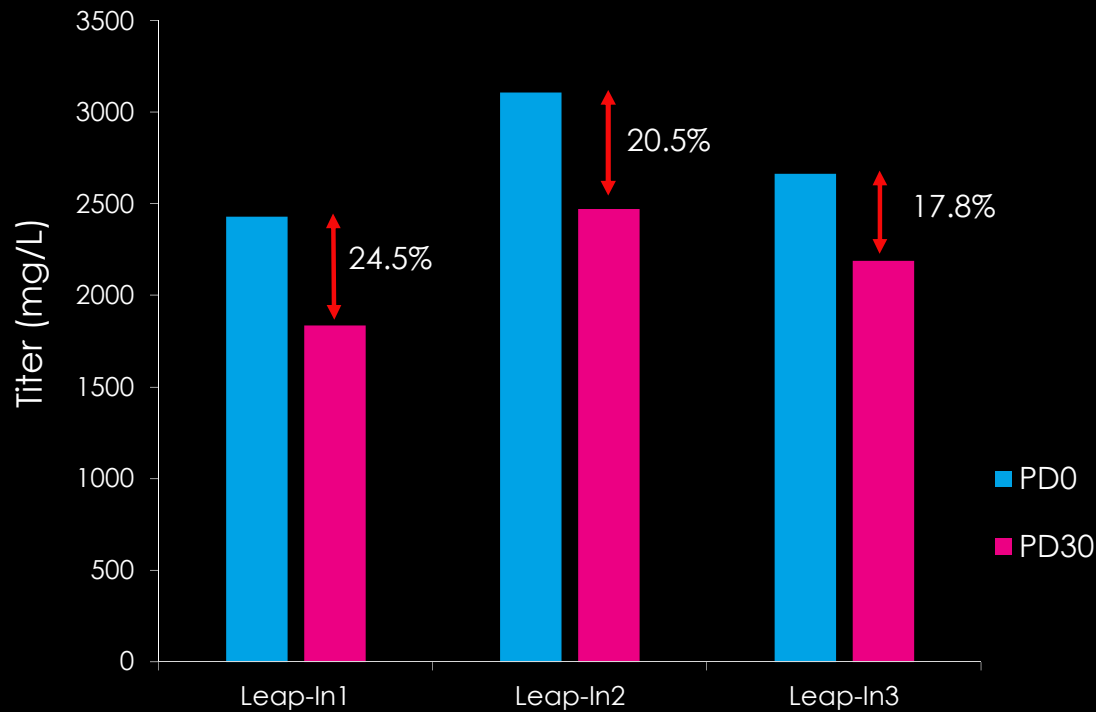
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Leap-In[®] pools: Predict derivative clone titers



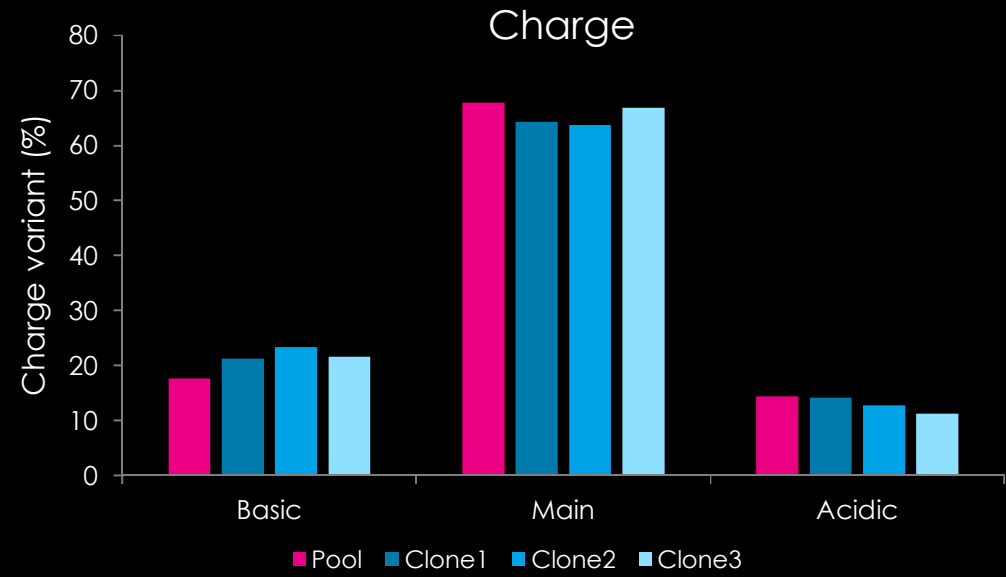
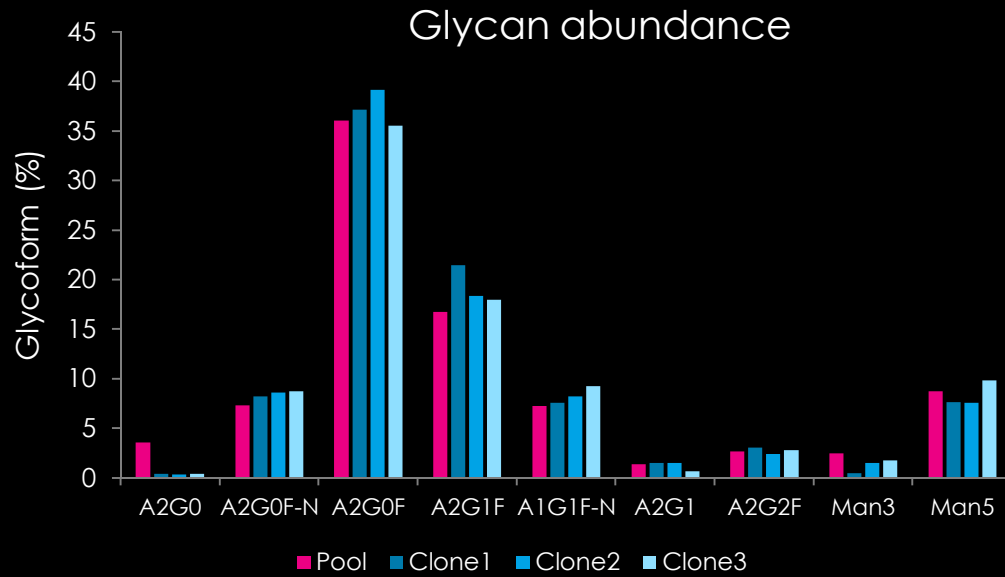
Pool titers predictive of clone titers

Leap-In[®] pools: Stable expression



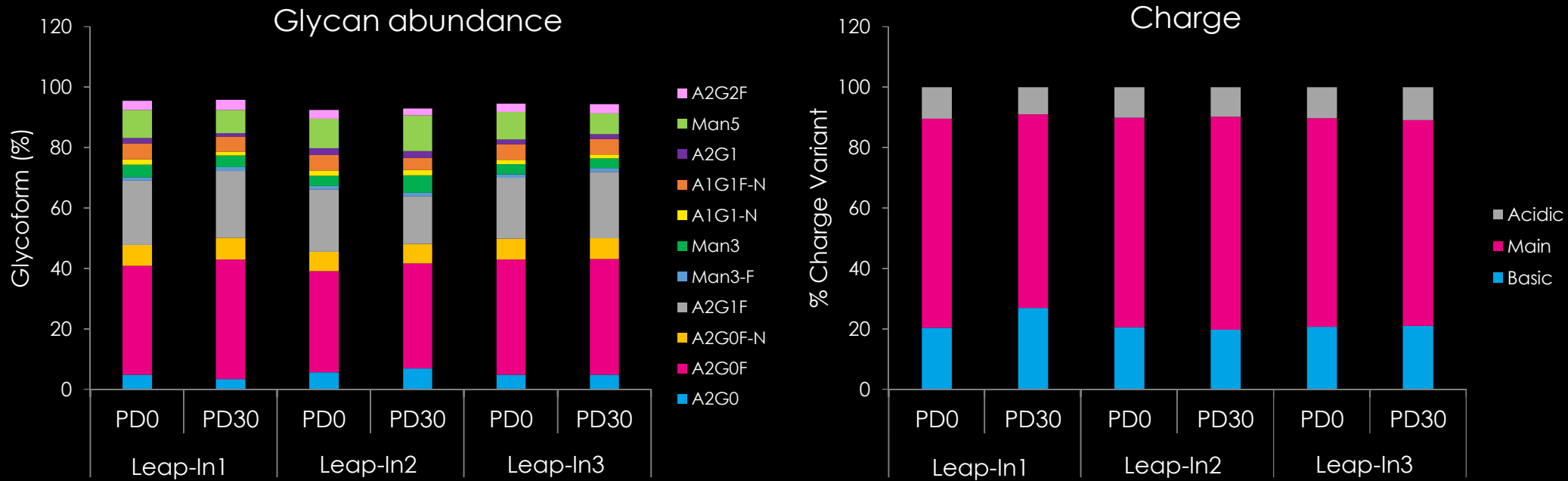
- ~80% productivity maintained
- Acceptable criteria for expression stability

Leap-In[®] pools: Predict derivative clones product quality



Comparable product quality of pools and clones

Leap-In[®] pools: : Product quality stability



Consistent product quality of stable pools

Cell Pools speeding timeline to IND

Cell pools – Risks

Low titer

Expression stability

Pool product quality \neq Clone product quality

Cell pools – Advantages

Shorter timelines

Reduced cost

Cell Line Development off critical path

Cell pools – Requirements

- ✓ **Clone like expression titer**
- ✓ **Expression stability**
- ✓ **Comparable product quality to derivative clones**

Leap-In[®] pool ranking more critical than clone ranking

Applications for cell pools

- Screen vector constructs
- Screen sequence variants
- Process development
 - Media optimizations
 - Cell engineering
- Purification method development
- Analytical and formulation development
- Generating material for IND enabling tox
- Generating Ph. I lot

Ongoing leap-In platform innovations

- The Leap-In Transposase family is expanding
- Engineered CHO host cell lines
- Optimization of alternative host cell lines
- Cell and gene therapy modalities



Thank You

CLD Partners

Solentim

VIPST™ clonality verification

Horizon Discovery

GS null CHO K1 cell line

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Technology presented is protected by issued US patents 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

