Leap-in Transposases® - Changing the Paradigm of Cell Line Development

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SCAN ME

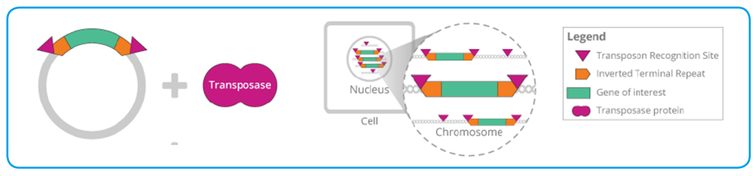
Abstract

The generation of robust and stable cell lines for the commercial production of protein therapeutics is critical. Current methodologies to introduce recombinant genes into production strains relies on random integration, a method limited by poor integration rates, concatemer formation, transgene rearrangements and instability. To address these limitations and others, ATUM has developed the Leap-In Transposase® platform. This flexible and robust platform enables the precise and stable integration of genes of interest. This remains true with large and complex constructs with multiple open reading frames (ORF's) each under discrete expression control.
Taken together, the Leap-In platform enables the robust generation of stable high expressing cell lines for routine and complex molecules such as bispecifics and multispecifics.

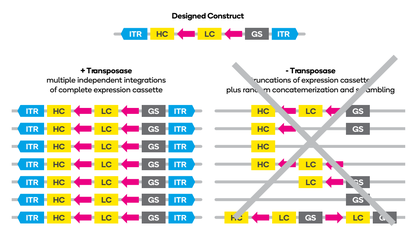
Leap-In transposase® benefits

1	2	3
Optimized expression constructs	**Robust and valuable stable pools**	**Stable integration combined with structural integrity**
Maximize expression levels	Highly uniform clonal distribution	Precise integration= structural integrity
Adjust and tune expression levels	Significantly reduced screening required	Extremely stable integration and transgene expression
Express complex multi-ORF proteins	Pools predictive of clones	Control integration copy number

Leap-In transposase mediated integration



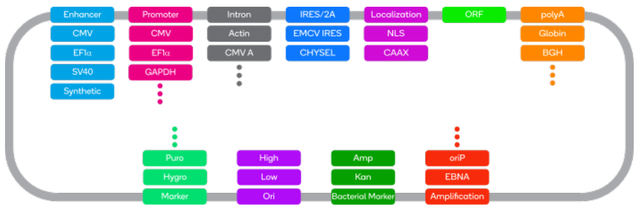
- Single copy integrations at each site
- Maintains integrity of the expression cassette
- Multiple insertions across the genome
- Robust activity in CHO and non-CHO host cells



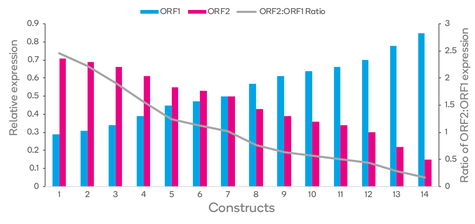
1 Optimized expression constructs

The Leap-In platform consists of:

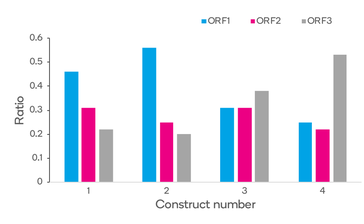
- Instant access to gene design and synthesis capacity
- Proprietary codon optimization technology
- Modular vector design optimized for flexibility and yield
- Engineered Leap-In transposase provided as mRNA
- Optimized protocols



Adjustment of expression ratios for multi-ORF vectors

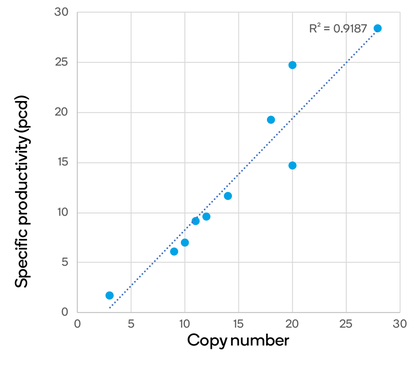


14 separate constructs demonstrating the ability to control ORF expression ratios.



Tuning of expression ratios of 3 ORFs.

Strong correlation: copy number - productivity

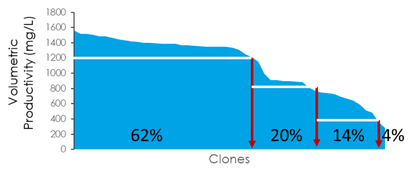


The correlation between copy number and specific productivity indicates that each integrated copy is intact and functional.

2 High productivity stable pools

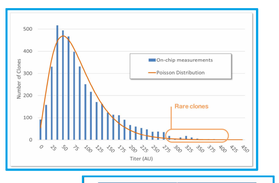
Leap-In Transposase®

Clonal distribution in Leap-In mediated stable pools



- 62% of clones in top quartile of expressers
- 82% of clones in top half of expressers
- 99% probability of finding a high producer from <200 clones

Random Integration



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

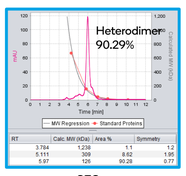
https://www.berkeleylights.com/

- High producers rare

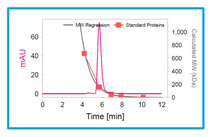
Protein	Volumetric productivity	Specific productivity
IgG1	5.9 g/L	39 pcd
IgG4	5.0 g/L	43 pcd
IgG4	5.0 g/L	49 pcd
IgG1	4.3 g/L	22 pcd
IgG1	4.2 g/L	42 pcd
IgG1	4.2 g/L	33 pcd
IgG1	4.0 g/L	44 pcd
IgG1	3.6 g/L	29 pcd
IgG1	3.3 g/L	29 pcd
IgG1	2.8 g/L	30 pcd

Ratio balancing enables high titers, improvements in product quality and manufacturability

Advanced Analytics for bispecifics



SEC



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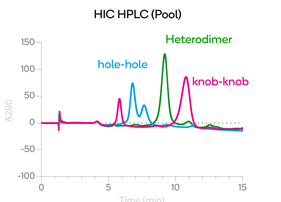
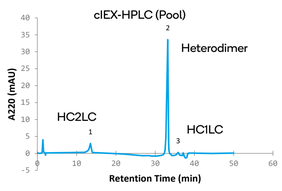
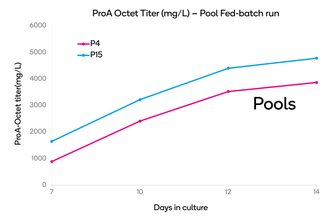
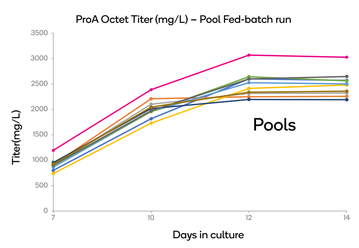


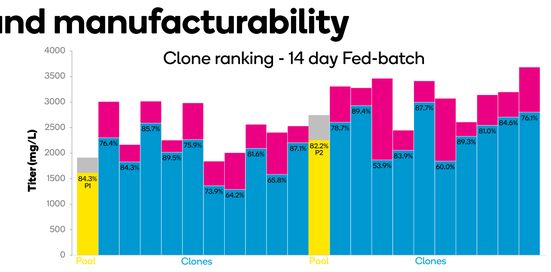


4 chain bsIgG1

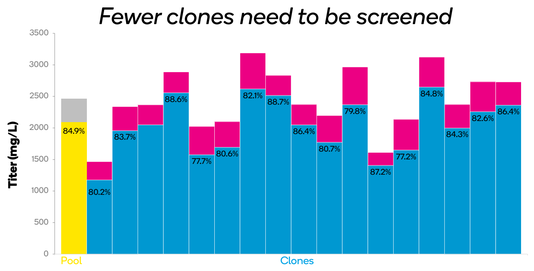
3 chain bsIgG4







Pools = Clones
Fewer clones need to be screened



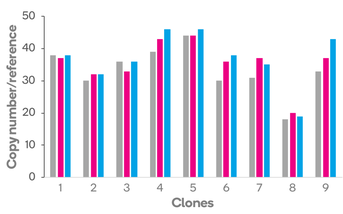
Better separation of heterodimers from homodimers with HIC HPLC and cIEX HPLC

Screening, characterizing and ranking fewer clones eliminates the need to invest in expensive, high-throughput instrumentation and allows more cell line development projects to be executed with limited resources.

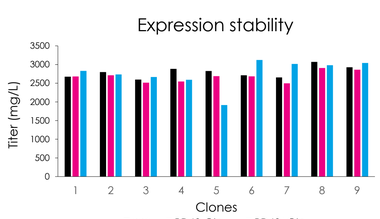
ATUM's miCHO™ GS KO cell line used to stably express 4 chain and 3 chain bispecifics.
Solentim: *Integration with VIPS™ (Verified In-Situ Plate Seeding) technology.*
Leap-In platform and VectorGPS® is broadly applicable and is host agnostic.

3 Robust expression and copy number stability

Copy number stability

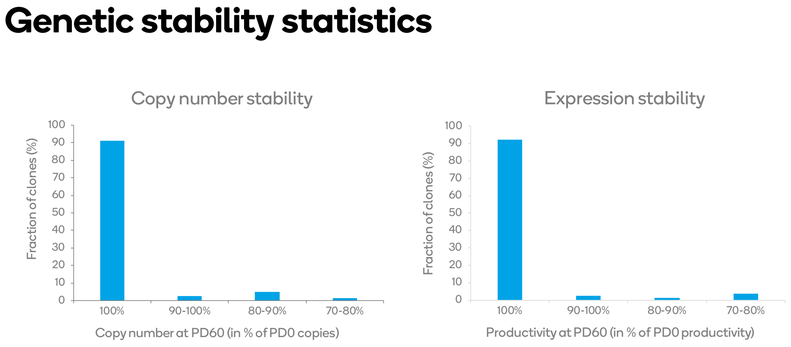


Expression stability



Consistent genetic stability over >60 population doublings

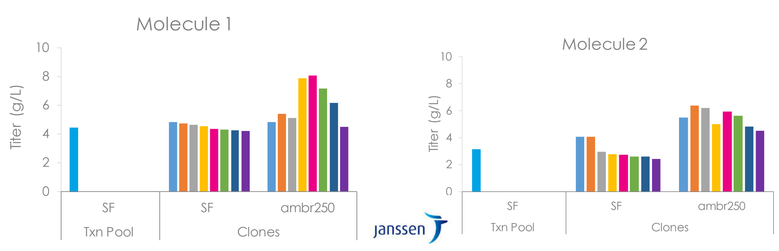
Genetic stability statistics



>90% of clones retain 100% of expression and gene copy number

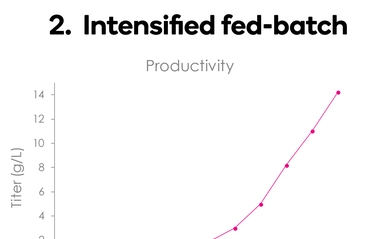
Case studies

1. Stable pools predict derivative clone titers



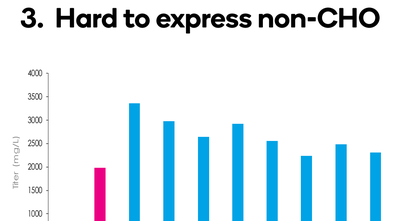
Selected pools reproducibly lead to robust, high expressing clones

2. Intensified fed-batch



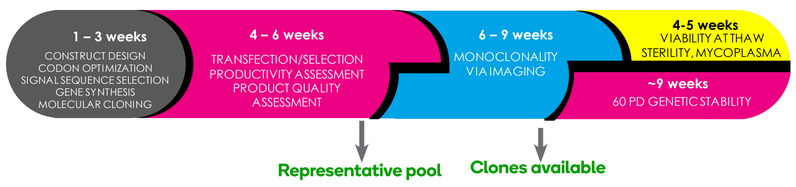
Novel process yielded high cell density and titer in excess of 14 g/L

3. Hard to express non-CHO



Robust and high expression results from a non-CHO host cell line

Summary



Rapid Timelines - Transfection to RCB in ~12 weeks

- Efficient and robust integration = Predictable selection

High Titer

- Highly uniform cell pools up to 5+ g/L and clones up to 10+ g/L

Robust Stability

- No loss in productivity or transgene copy numbers after 90+ doublings

Enabling for Next Generation Biologics

- Compatible with very large inserts (e.g. >100kb)
- Multiple transposases enable unique genetic engineering strategies
- Improved product quality and manufacturability

Regulatory validation as of May 2022

- 22 approved IND filings in three jurisdictions
- Licensed by >50% of top 20 Pharma
- >120 projects delivered

Resources

Website - https://www.atum.bio/pipeline/cld

Publications:

Accelerating and de-risking CMC development with transposon-derived manufacturing cell lines; Biotechnol Bioeng 2021 Jun;118(6):2301-2311. doi: 10.1002/bit.27742. Epub 2021 Apr 2; Rajendran S, Balasubramanian S, Webster L, Lee M, Vavilala D, Kulikov N, Choi J, Tang C, Hunter M, Wang R, Kaur H, Karunakaran S, Sitaraman V, Minshull J, Boldog F.