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Ferenc Boldog
ATUM (formerly DNA2.0), USA, fboldog@atum.bio

Jeremy Minshull
ATUM (formerly DNA2.0), USA

Mark Welch
ATUM (formerly DNA2.0), USA

Kate Caves
ATUM (formerly DNA2.0), USA

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Novel Transposases for Cell-Line Development & Engineering

Ferenc Boldog*, Sowmya Rajendran, Maggie Lee, Molly Hunter, Candice Talsalt, Lynn Webster, Vivi Truong, Elizabeth Hart, Nicolay Kulikov, Thomas Purcell, Mark Welch, Miles Scotcher**, Claes Gustafsson*** and Jeremy Minshull
ATUM (formerly DNA2.0), Newark, CA USA. *fboldog@atum.bio, **miles@atum.bio, ***claes@atum.bio

Abstract

The generation of stable cell lines is critical for the commercial production of protein therapeutics. Current methodologies to introduce engineered recombinant genes into production strains relies on homologous recombination, a method limited by poor integration rates (<<1%), concatemer formation and common transgene rearrangements. To address this limitation, ATUM has identified and engineered two orthologous transposases, designated Leap-In 1 (from frog) and Leap-In 2 (from insect), that stably integrate synthetic transposons into the host genome with close to 100% integration frequency and an absolute minimum of concatemers or other genetic instabilities. Each synthetic transposon can encode up to 4 unique open reading frames (ORFs) flanked by inverted terminal repeats recognized by the cognate transposase. Each ORF is under discrete expression control thereby enabling tuning of expression ratios by adjustment of each ORF independently. Leap-In transposases speed cell recovery times, enabling the generation of high expressing stable pools (up to 5g/L) in as little as 2 weeks post transfection.

Leap-In transposase benefits

1 Application-optimized expression constructs	2 Shortened stable pool recovery times	3 Stable integration combined with structural integrity
Maximize expression levels using ATUM's proprietary gene sequence and vector optimization tools.	Clonal productivity distribution is characteristically **higher** and **more uniform** in stable pools	Transposases integrate the entire, intact transposon, thereby maintaining its **structural integrity**, and **expression balance**.
Adjust and tune expression levels of your gene(s) by testing combinations of vector elements.	**Fewer clones need to be screened** to enable identification of highly productive clonal cell lines.	Clonal cells lines created using Leap-In transposases exhibit **genetic stability and stable productivity** over 60 population doublings.
Express **multichain proteins** (such as bispecific antibodies) or **multiple genes within a pathway**.	**Initiate process development earlier** with more representative pool derived reagents.	**Control integration copy number** by manipulating dose of transposase and synthetic transposon. **Foot-print free excision** of transposon from host genome.

Leap-In transposase process

Transposase → Nucleus, Cell, Chromosome

Legend: Transposon Recognition Site; Inverted Terminal Repeat; Gene of interest; Transposase protein

- Transposon and transposase (as mRNA) co-transfected into target cell
- Broad host cell range
- ATUM uses 2 in-house cell lines, both with full traceability
  - HD-BIOP3 GS null (Horizon Discovery)
  - DG44 DHFR null (Prof. Lawrence Chasin)
- Single copies of entire transposon are independently integrated by a "cut and paste" mechanism into multiple sites across the host genome
- 48 hours post transfection, no mRNA or transposase are detectable

Stable pools → Stable pool ranking → Clonal cell line generation → Stability testing → Research Cell Bank

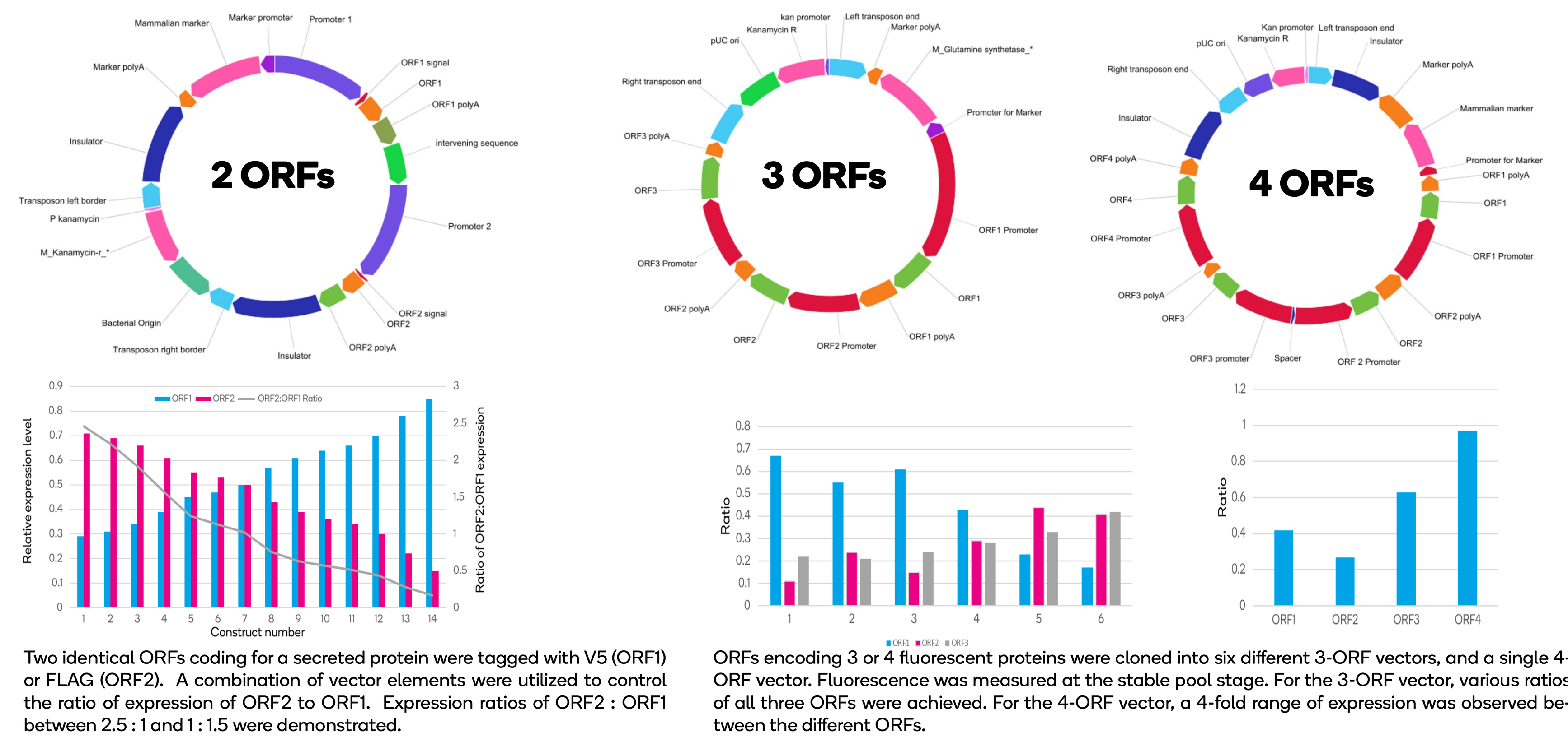
1 Application - optimized expression constructs

- Up to 4 open reading frames under independent expression control.
- No size limit for synthetic transposon.
- Multiple, parallel selection stringencies.
- High productivities in stable pools up to 5g/l and clonal up to 6.5g/l.

protein	selection stringency	transposon copy/cell	specific productivity (pcd)
300 kDa glycoprotein-Fc fusion	low	5 to 7	~4
bispecific antibody	medium	~16	7 to 12
antibody	medium	20 to 25	35 to 50
antibody	high	35 to 45	50 to 70

HD-BIOP3 GS null CHO-K1 cell line from Horizon Discovery was co-transfected with ATUM's transposase and transposons with different vector and antibody combinations to reach the highest specific productivity without compromising product quality. Stable pools were established and productivity measured at day 14 in non-optimized small scale shake flask or deep-well cultures.

Adjustment of expression ratios for 2-, 3- and 4-ORF vectors



Two identical ORFs coding for a secreted protein were tagged with V5 (ORF1) or FLAG (ORF2). A combination of vector elements were utilized to control the ratio of expression of ORF2 to ORF1. Expression ratios of ORF2 : ORF1 between 2.5 : 1 and 1 : 1.5 were demonstrated.

ORFs encoding 3 or 4 fluorescent proteins were cloned into six different 3-ORF vectors, and a single 4-ORF vector. Fluorescence was measured at the stable pool stage. For the 3-ORF vector, various ratios of all three ORFs were achieved. For the 4-ORF vector, a 4-fold range of expression was observed between the different ORFs.

- Consistent ranking of highly productive candidates = only a small number of clones need to be screened.
- Product quality attributes are consistent from pools to clones, enabling early initiation of process development and GLP-toxicology studies.

2 Shortened timelines

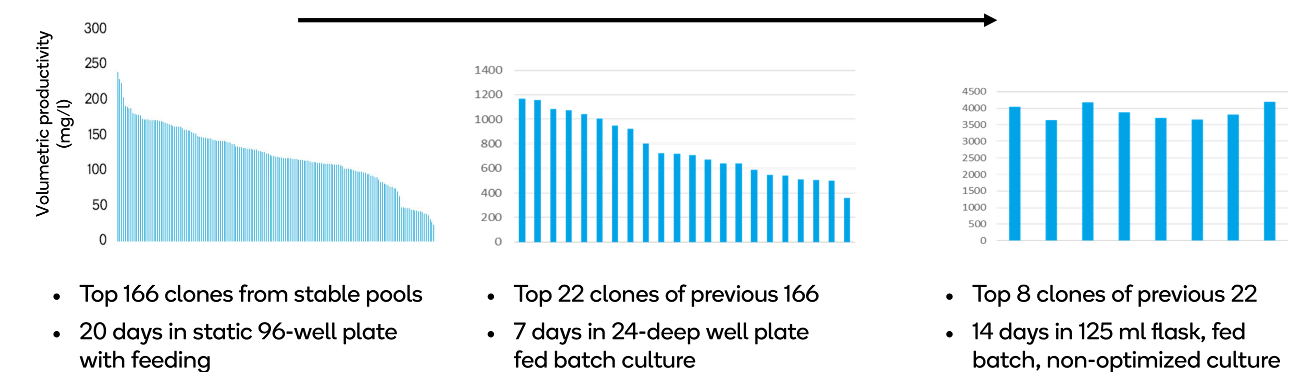
Transposases speed cell pool recovery post-transfection.

Stable pool	GS promoter	Transposase	MSX (µM)
	Variable Parameters		
1	mPGK	YES (wild type)	50
2	mPGK	NO	50
3	Attenuated-1	YES (wild type)	0
4	Attenuated-1	NO	0
5	negative control		

Upper graph - Under typical selection conditions (strong GS promoter, 50 µM MSX), cell pools created using tranposases recover viability >7 days faster than cell pools created by random integration.

Lower graph - Under drug-free selection conditions (attenuated GS promoter), cell pools created using transposases recover viability in ~ 3 weeks, whereas cell pools created by random integration fail to recover.

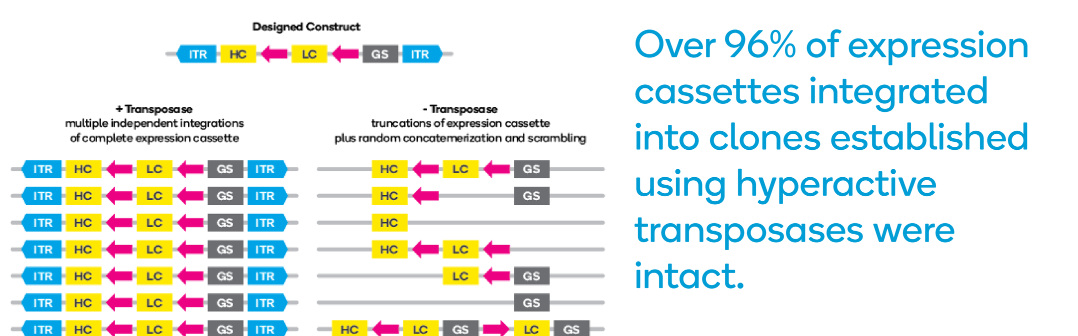
Screening and selection of clones expressing transposon-based antibody construct



- Top 166 clones from stable pools
- 20 days in static 96-well plate with feeding
- Top 22 clones of previous 166
- 7 days in 24-deep well plate fed batch culture
- Top 8 clones of previous 22
- 14 days in 125 ml flask, fed batch, non-optimized culture

3 Stable integration combined with structural integrity

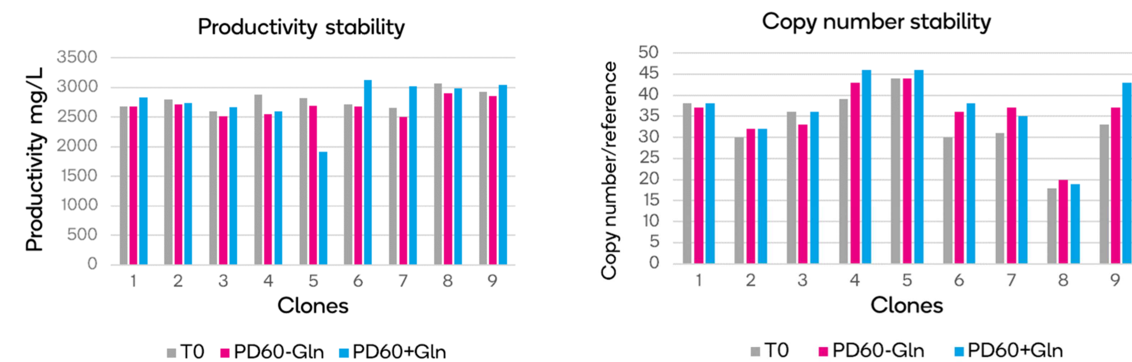
Transposase	Transposition based integration	Random integration	TG/TG fusion
+	24/25	1/25	2
+	23/24	1/24	1
-	No site identified	No site identified	23



Over 96% of expression cassettes integrated into clones established using hyperactive transposases were intact.

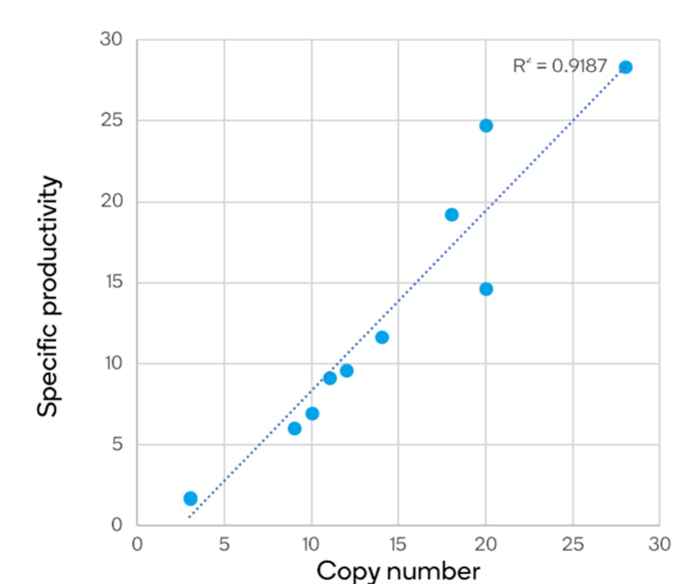
An antibody expression construct was transfected into HD-BIOP3 GS null CHOK1 cells (Horizon Discovery) in the presence (+ transposase) or absence (- transposase) of Leap-In transposase. Targeted Locus Amplification was used to analyze the structural integrity of expression cassette integrations.

Clonal cell lines generated using Leap-In transposases exhibit genetic stability and stable productivity over 60 population doublings.



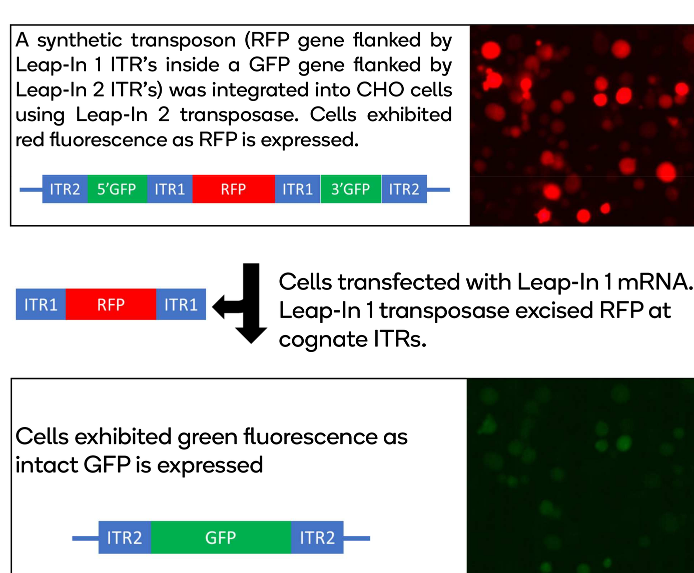
Nine CHO GS KO clones, derived from the transposase-mediated integration of an antibody expression cassette, were grown in 24-deep well plate for 60 generations (population doublings (PD)) in the presence (-Gln) and absence (+Gln) of selection. Productivity and integrated copy number of clones after 60 PD were compared to T0. No significant change in productivity or copy number under the with glutamine (+Gln) and minus glutamine (-Gln) growth conditions were observed compared to T0.

1:1 correlation between copy number and specific productivity in transposase mediated DG44 stable pools.



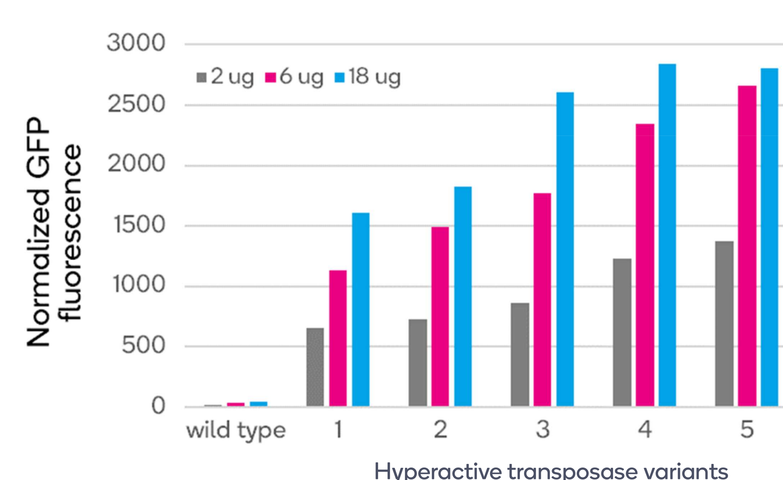
Stable pools derived from the transposase-mediated integration of an antibody expression cassette in DG44 cells, were grown under fed-batch conditions. Productivity and copy number were measured. The correlation between copy number and specific productivity indicates that **each integrated copy is intact and functional.**

Foot-print free excision of transposon from host genome.



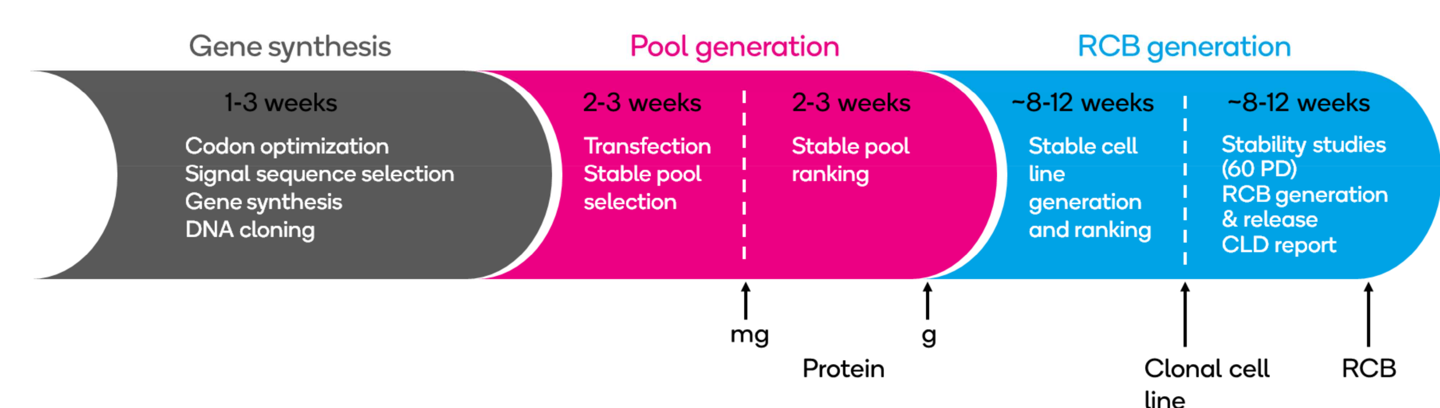
Studies are currently ongoing to demonstrate that integration of single copies of transposons is possible.

Excision efficiency of wild type and engineered hyperactive Leap-In® 1 transposases



Hyperactive transposase variants designed using ProteinGPS® demonstrate foot-print free excision. Variants were ranked by excision efficiency measured as per figure on left. Different concentrations of hyperactive transposase mRNA demonstrated a dose response. Hyperactive transposase variants tested show an approximately 80-fold higher activity compared to wild type in this assay.

Typical timeline of cell line development at ATUM



- Stable pool-derived protein is available as early as 2 weeks post transfection.
- Clonal cell line without stability information, available as early as 12 weeks post -transfection.
- RCBs (Research Cell Banks) released as early as 20 weeks post-transfection.