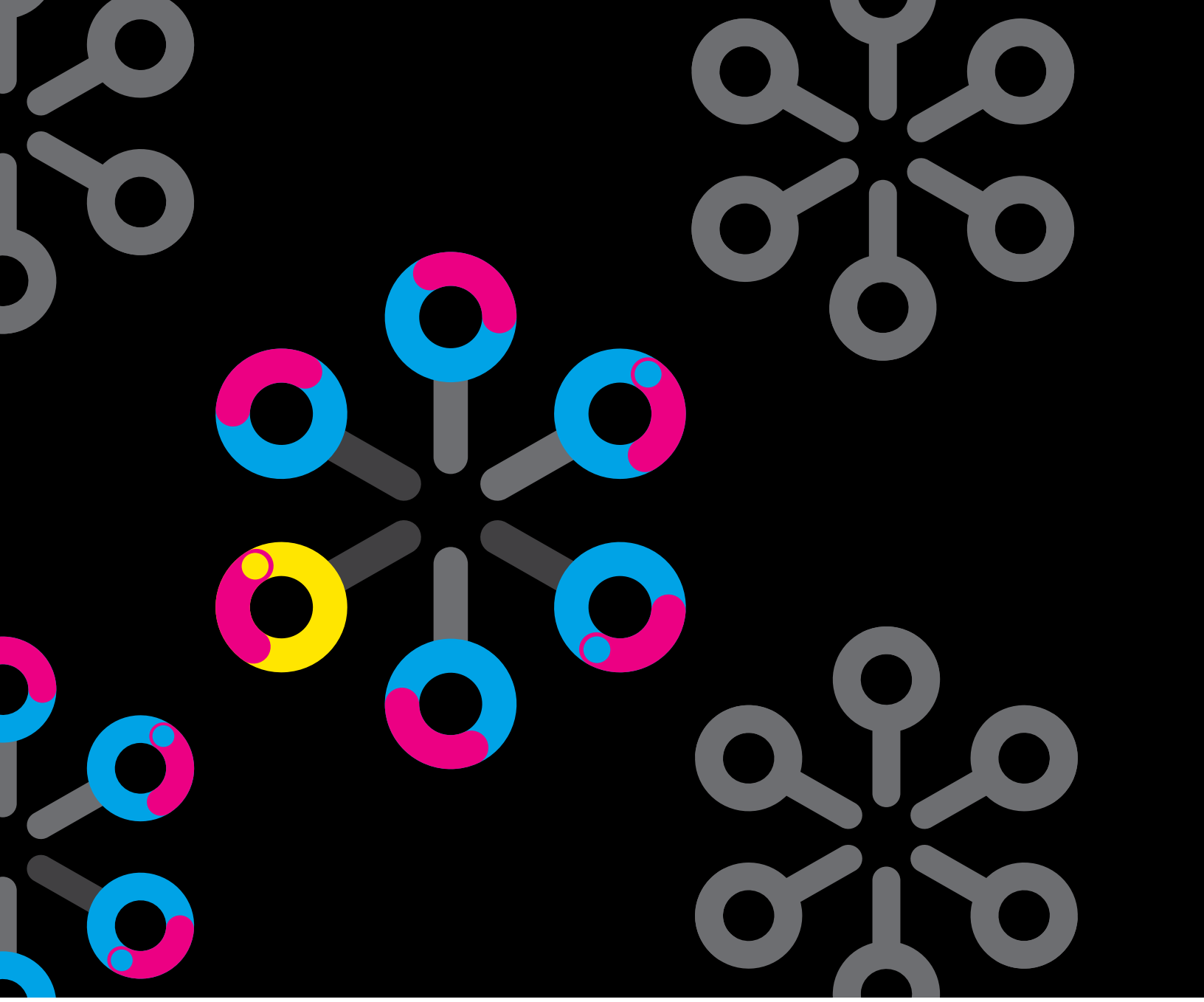


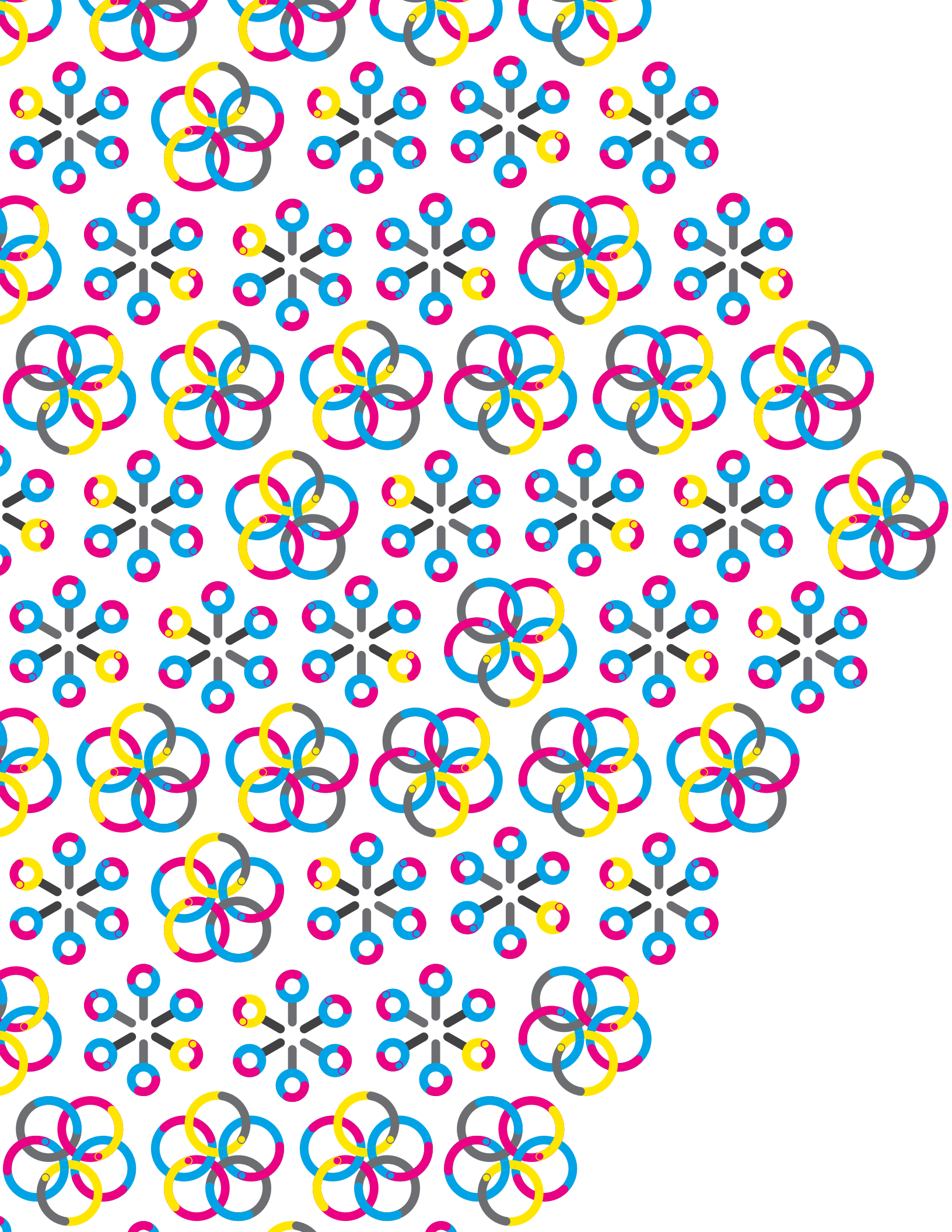
cell line development

Develop stable cell lines faster

High productivity

Achieve your goals efficiently

ATUM

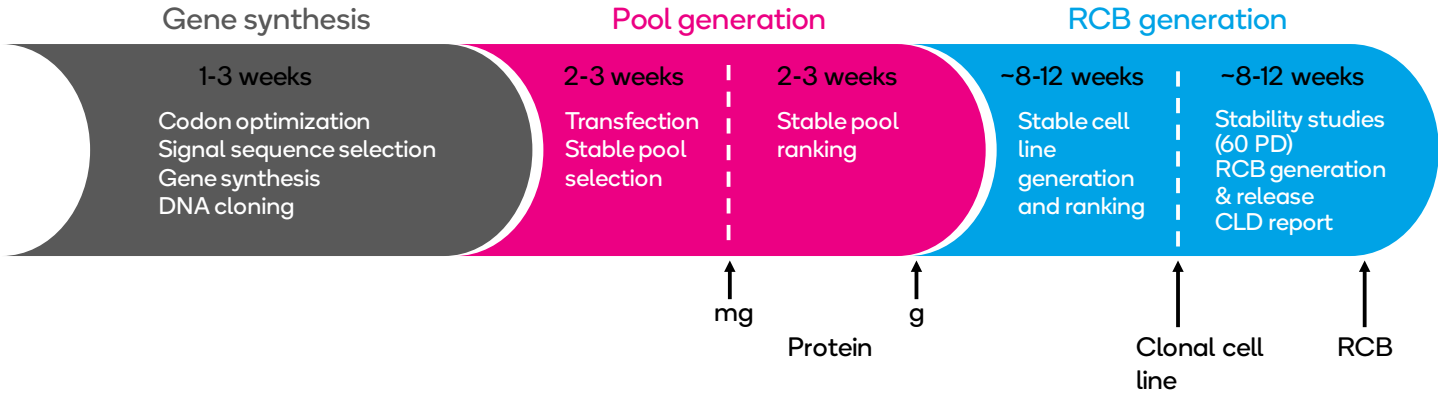


cell line development

ATUM's Cell Line Development Services are based on our many integrated capabilities all housed under one roof at our facility in Newark, CA. To efficiently build your cell line, we combine our codon optimization algorithms, state-of-the-art gene synthesis, secretion signal toolbox and flexible expression vector configurations with the Leap-In® transposase system and high productivity cell lines.

Stable Expression on Transient Timelines

Generate High Expressing Stable Pools in 14 days



- Stable pool-derived protein is available as early as 2 weeks post-transfection.
- Clonal cell line (without stability information) is available as early as 12 weeks post-transfection.
- RCBs (Research Cell Banks) released as early as 20 weeks post-transfection.
- Process Development (PD) can start as early as 4 weeks post-transfection saving valuable development time.
- Faster to IND / Clinical trials.

Production Hosts

ATUM uses two well characterized mammalian cell lines for bioproduction:

- HD-BIOP3 GS null CHOK1 cell line from Horizon Discovery
- DG44 from the lab of Dr. Lawrence Chasin (Columbia Univ), adapted to serum-free suspension

Key Benefits:
- Lineage traceability
- Host bank characterized (tested for mycoplasma, sterility, adventitious agents by PCR)
- Commercially available chemically defined media formulations.

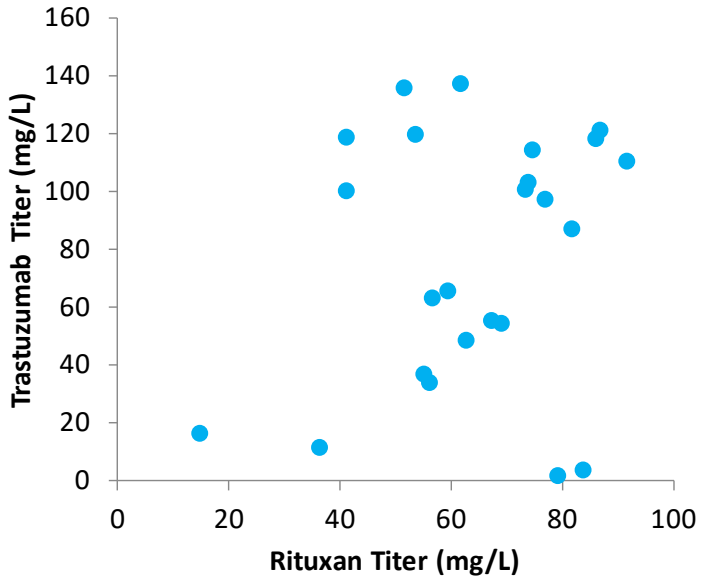
ATUM

cell line development process

step 1

Coding sequence optimization

Expression of proteins in mammalian cells may be limited by codon usage and secretion signals. If expression of your gene appears limited, we recommend testing a re-coded version of the open reading frame, and/or a different signal sequence.



Expression of two different antibodies (trastuzumab and rituxan shown) was tested using 24 different heavy and light chain signal sequence combinations. Each blue circle represents a combination of two signal sequences, one for each antibody chain. Signals that work well for one antibody may be mediocre for another.

We recommend starting cell line development with a transient expression pilot study in cases where expression of the sequence is known to be unsatisfactory.

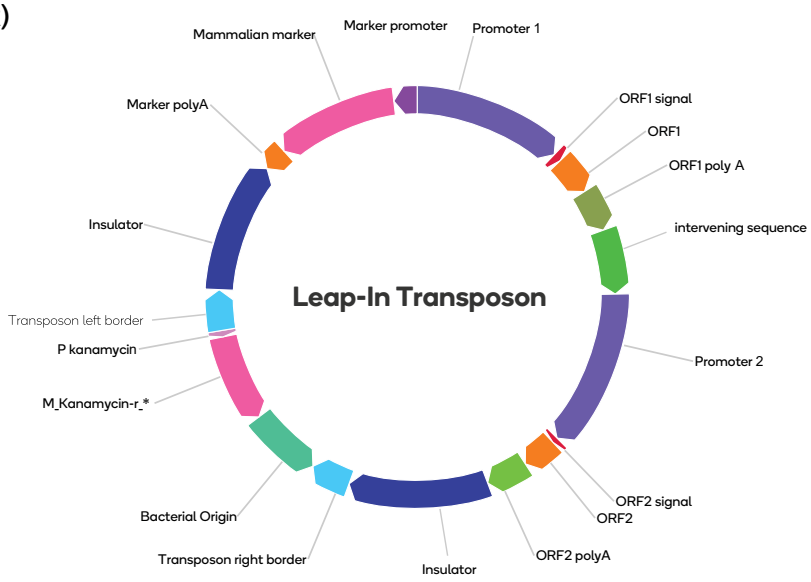
- Test the effect of ORF re-coding
- Identify more effective secretion signals

step 2

Stable vector selection

ATUM uses pD3600 vectors with glutamine synthase (GS), dihydrofolate reductase (DHFR) or puromycin selectable markers. A series of selectable marker expression levels are available. Lower levels of selectable marker expression produce a more stringent selection, which in turn results in higher expression of client proteins.

ATUM may clone client sequences into several different vectors to obtain maximal expression levels. Our stable expression vectors have combinations of promoters for expressing two, three or four open reading frames at controlled ratios. In some cases it may not be known what the desired expression ratios should be. ATUM can then create several different versions, and use pool productivity to guide us to the optimal configuration.



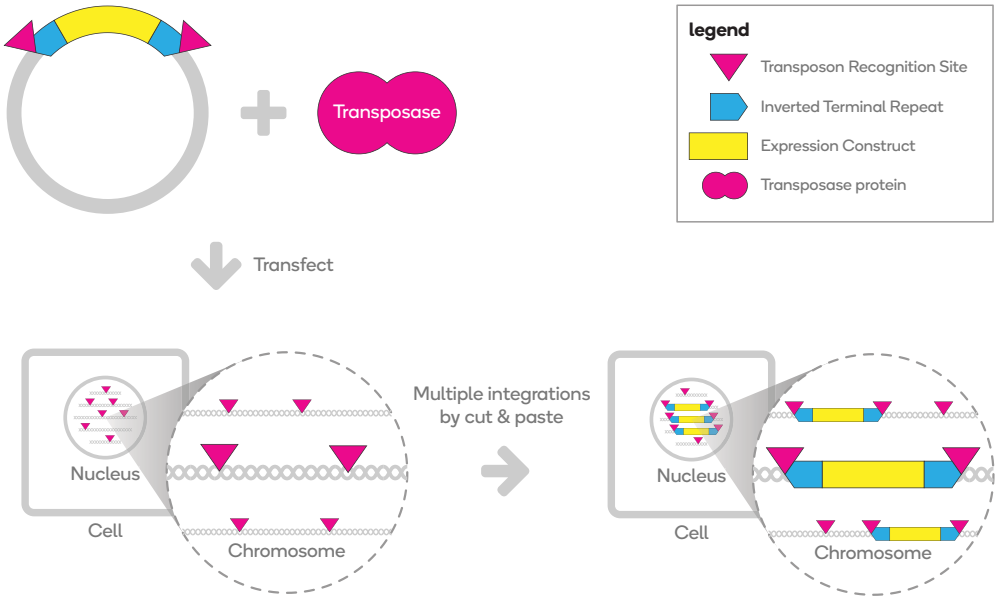
The pD3600 vectors are constructed in a modular way that allows easy modification of component elements. Versions are available that allow expression of up to four open reading frames in addition to the selectable marker.

ATUM vectors are transposons, which accelerates the generation of highly productive stable pools and lines.

step 3

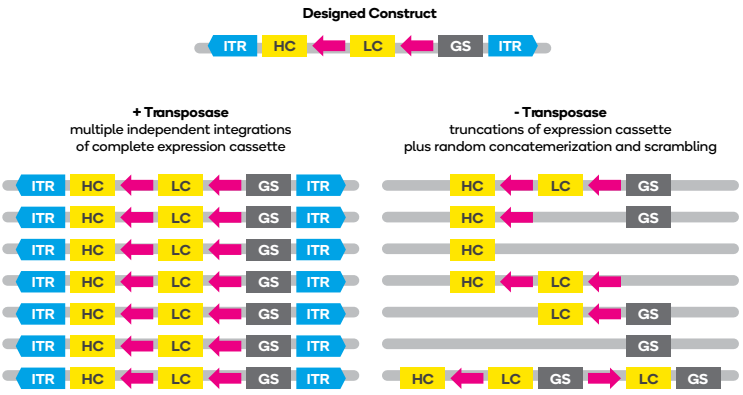
Stable pool generation

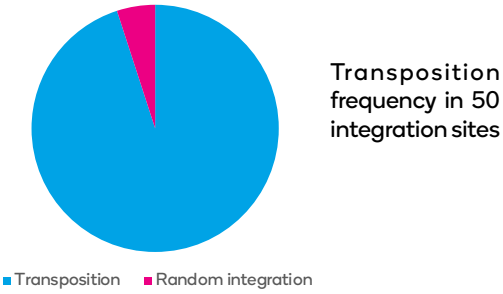
Plasmid DNA is co-transfected with transposase mRNA. Transposons preferentially integrate in transcriptionally active chromatin. ATUM's transposons are flanked by insulators to reduce silencing after genomic integration. Multiple copies of a transposon can be independently integrated into the genome of a single cell, increasing expression levels.



Transposases maintain transposon structural integrity

Transposases integrate the entire sequence between two transposon ends into a host genome, so ALL chains of an antibody or bispecific are integrated, along with the selectable marker.





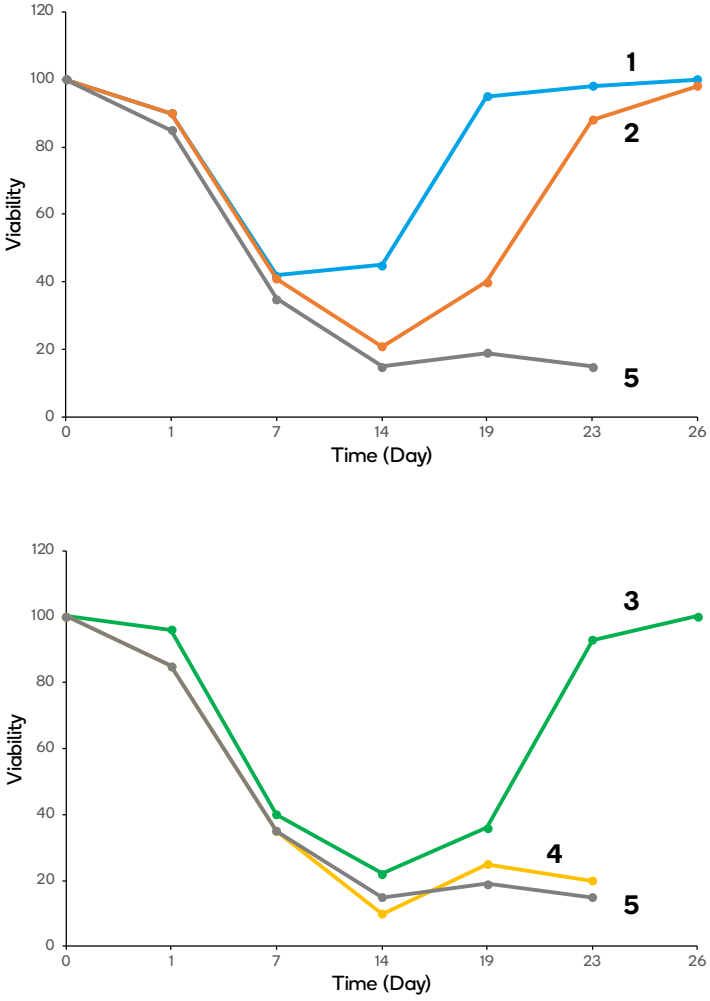
Transposition frequency in 50 integration sites

Targeted locus amplification (TLA) results performed by Cergentis demonstrated that 96% of the stable integrations were Leap-In transposase mediated transpositions and resulted in single copy, structurally intact transgenes.

Structural integrity of the complete expression cassette is maintained in the presence of transposase as shown in the left panel. Random fragmentation and integration in the absence of transposase (right panel), compromises the structural integrity of the expression cassette which may be truncated, scrambled and concatemerized. Concatemers may be unstable, and increase the risk of repeat-induced silencing.

Transposases generate stable pools faster

Transposases are much more efficient at integrating DNA into a host genome than random fragmentation and non-homologous integration. Transposon integration sites are also biased toward transcriptionally active parts of the genome. This means that a population of cells transfected with a transposon system will result in a very high fraction of cells that have an integrant, and a good proportion of these cells will be viable under selection conditions. This leads to faster pool recovery.



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

Transposase speeds recovery. CHO K1 GS KO cell line was co-transfected with ATUM's transposons and wild type or hyperactive transposase as shown in table. Upper graph: Under typical selection conditions (strong GS promoter, 50 µM MSX), cell pools created using transposase (curve 1) recover viability >7 days faster than cell pools created by random integration (curve 2). Lower graph: Under drug-free selection conditions (attenuated GS promoter), cell pools created using transposase (curve 3) recover viability in ~3 weeks, whereas cell pools created by random integration (curve 4) fail to recover.

Examples of stable pool productivity

Cells are grown under selective conditions to produce stable pools. These are ranked by productivity and product quality. At this point material can be generated from the stable pools and single clone isolation is initiated.

HC	GS promoter	volumetric productivity	specific productivity
IgG1-Hs	ht	4.2 g/l	42 pcd
IgG1-Hs	ht	3.6 g/l	29 pcd
IgG1-Hs	ht	3.3 g/l	29 pcd
IgG1-Hs	ht	2.8 g/l	30 pcd
IgG1-Hs	hxt	4.2 g/l	33 pcd
IgG4-Hs	hxt	5.0 g/l	43 pcd
IgG4-Hs	hxt	5.0 g/l	49 pcd

HD-BIOP3 GS null CHOK1 cell line from Horizon Discovery was co-transfected with ATUM's transposase and transposons with different vector and antibody combinations. Stable pools were established and productivity measured in non-optimized small scale shake flask or deep-well cultures.

step 4

Isolation of monoclonal lines

Single cells are isolated from selected pools using a FACS-based cell printer. Monoclonality is also demonstrated and documented using orthogonal imaging technologies. Clones are ranked by productivity and product quality. Product from the best pools or clones can be provided to client for additional function-specific analyses.

Clonal cell lines established using Leap-In transposase exhibit genetic stability over >60 population doublings.

Stable pools can be used to speed up discovery research including developmental candidate selection and lead optimization.

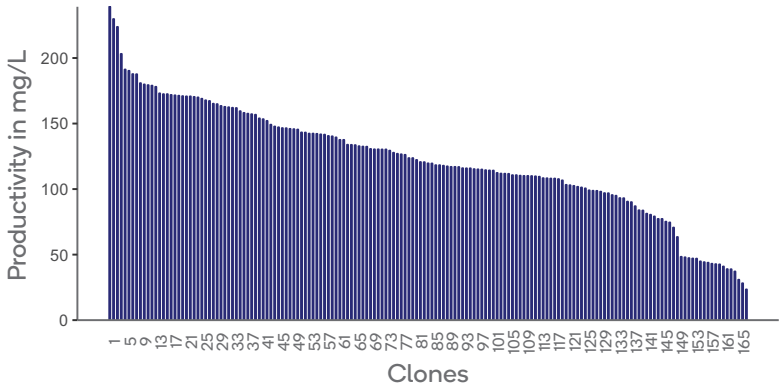
Product quality attributes from a stable clone and derivative clones is highly comparable, thereby shortening CMC development timelines - initiate tox study ~4 months earlier.

case study:

High Productivity Stable Clones in ~12 weeks

Establish Stable Pools

Plating and Ranking of 165 clones



8 clones selected for productivity assessment

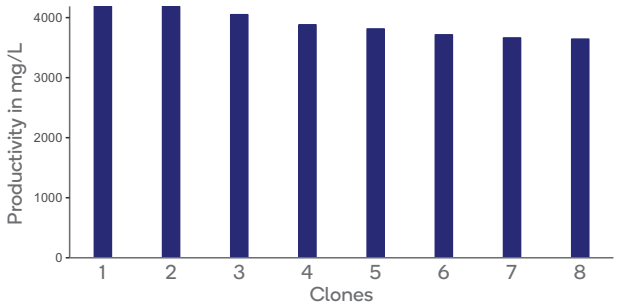


Figure illustrates clonal distribution in stable pools generated by Leap-In hyperactive transposase mRNA. A transposon based antibody construct was co-transfected with transposase mRNA in HD-BIOP3 GS null CHOK1 cells from Horizon Discovery. Stable pools were established in glutamine-free conditions and the absence of MSX. 165 clones were isolated by limited dilution cloning in 96-well plates. Productivity of individual clones grown for 20 days in a 96-well plate with feeding was measured by Octet®.

The top 24 clones of 165 were further ranked based on productivity in a 7 day 24 deep well plate fed batch culture and the 8 most productive clones were selected. Productivity was measured in a non-optimized 14 day fed batch 125 ml shake flask culture. **The specific productivity of clonal isolates was >40 pcd (picogram/cell /day).**

Screening of only a small number of clones generates highly productive candidates

Product quality attributes between a stable clone and three derivative clones is comparable

Pool and selected clones from above were tested for product quality attributes:

SEC

	HD-BIOP3			
	Clone 1	Clone 3	Clone 5	Pool
SEC main peak (%)	100	100	100	100
Reduced gel	comparable			
Non-reduced gel	comparable			

Glycan species

	HD-BIOP3			
	Clone 1	Clone 3	Clone 5	Pool
G0	8.17	5.45	8.39	7.4
G0F	49.73	49.89	45.13	45.8
G1Fa	28.79	30.1	31.34	30.1
G1Fb	4.83	5.51	5.05	5.45
G2F	8.47	9.21	10.18	11.3

Charge variants

	Charge %		
	Basic	Main	Acidic
Pool	18.58	46.43	34.98
Clone 1	18.47	44.35	37.18
Clone 2	20.84	46.48	32.68
Clone 3	20.05	49.97	29.99

step 5

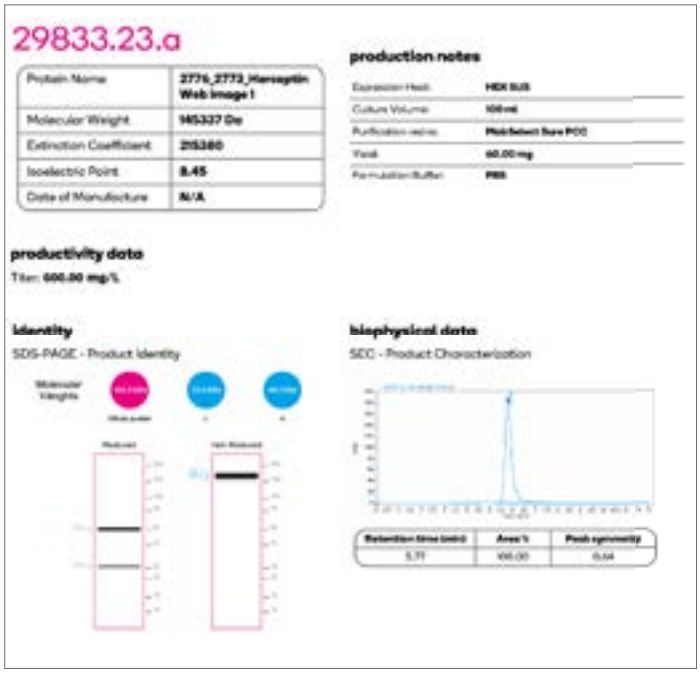
Clone ranking and Research Cell Bank (RCB) generation

Clones are ranked by productivity and critical quality attribute (CQA) driven product quality data.

Standard clone ranking analysis

- **Productivity**
  Octet and ELISA
- **Binding affinity**
  Octet
- **Molecular weight**
  Reduced and non-reduced gels
  Mass spectroscopy
- **Macromolecular structures / aggregation**
  SEC-HPLC
- **Total glycan analysis**
  Mass spectroscopy
- **Thermal stability**
  Tm analysis

Sample report:



Product from the best pools can be provided to client for additional function-specific analyses.

Genetic stability is assessed by demonstrating consistent productivity and growth rate over 60 generations and by genomic analysis (Southern blotting, copy number and sequencing).

Research cell banks (~30 vials) are established from the top performing 1-3 clones. Cell banks are released after testing for sterility, mycoplasma, transgene integrity by Southern blot and cDNA sequence analysis.

Workflow processes are tracked using a proprietary informatics package.

Cell line development report is provided.

Regulatory filing assistance is available upon request.

leap-in transposase benefits

Leap-In Benefits	Advantages for Cell Line Development
Leap-In transposase co-transfected as mRNA	Transposase mRNA short half-life results in stable transgene copy; no chromosomal integration
>90% integration efficiency	Fast recovery of high productivity stable pools
DNA ligase is the only host derived factor required for transposition	Broad host cell range from yeast to mammalian cells
Multiple single copy integrations in the target genome	No repeat induced silencing; increased genetic stability and increased production
Entire region between the flanking ITRs integrates	No re-arrangement of designed expression constructs for complex biologics
No payload limit	Multi ORF (multichain) products can be expressed from one expression construct
Integration directed to transcriptionally active chromatin	High productivity and genetically stable recombinant pools and clones; small number of clones need to be screened and ranked
Productivity and product quality are highly comparable between pools and clones	Representative stable pool derived product facilitates Process Development (PD) and Tox process development shortening the overall timeline
Accurately tunable selection stringency	Selection conditions leading to the highest productivity without compromising product quality are identified at stable pool stage
Two independent transposon/transposase pairs	Efficient production of complex biologics; engineering performance of the production host

Contact us for information on licensing options & cell line development services

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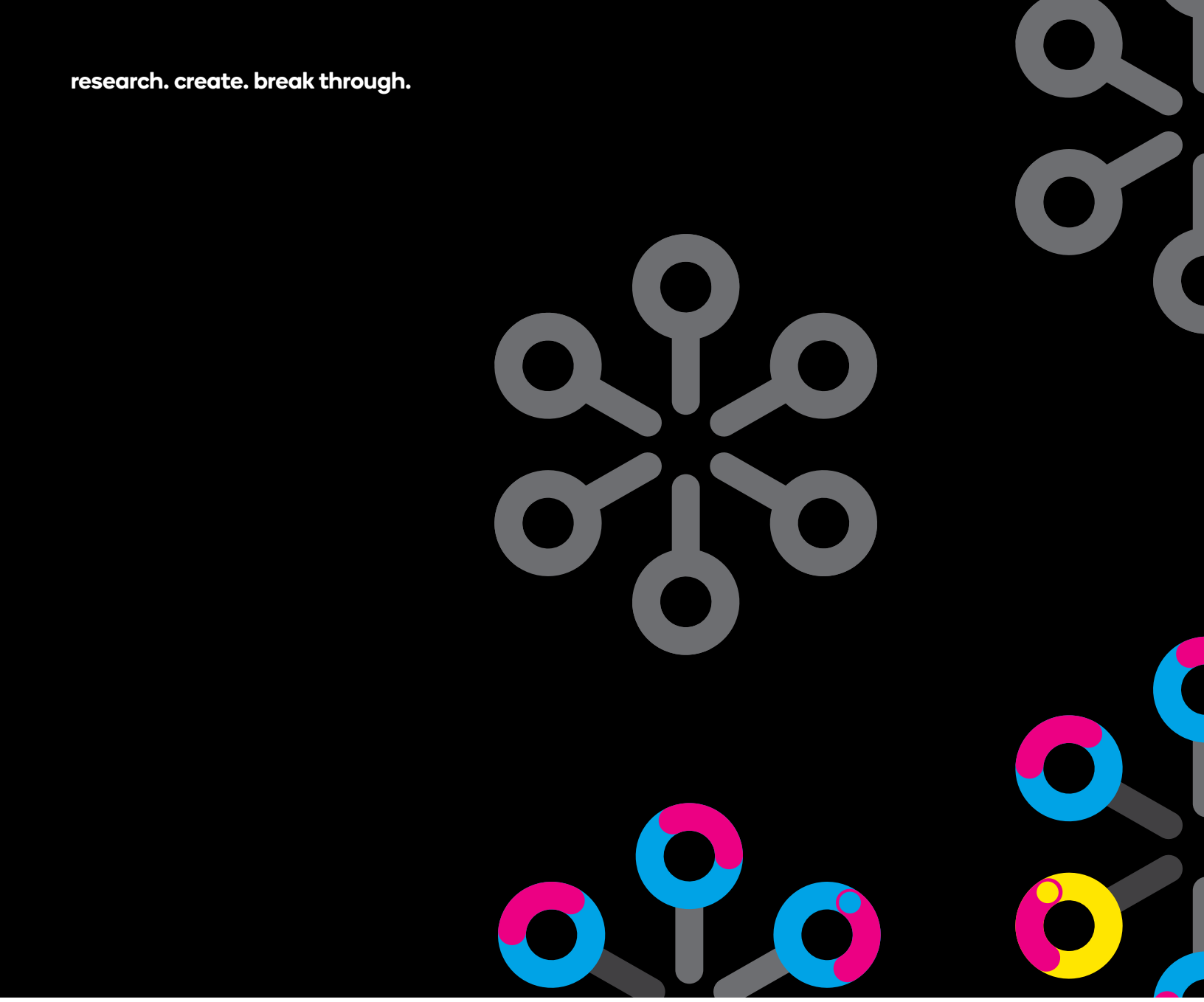
research. create. break through.



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