ATUM

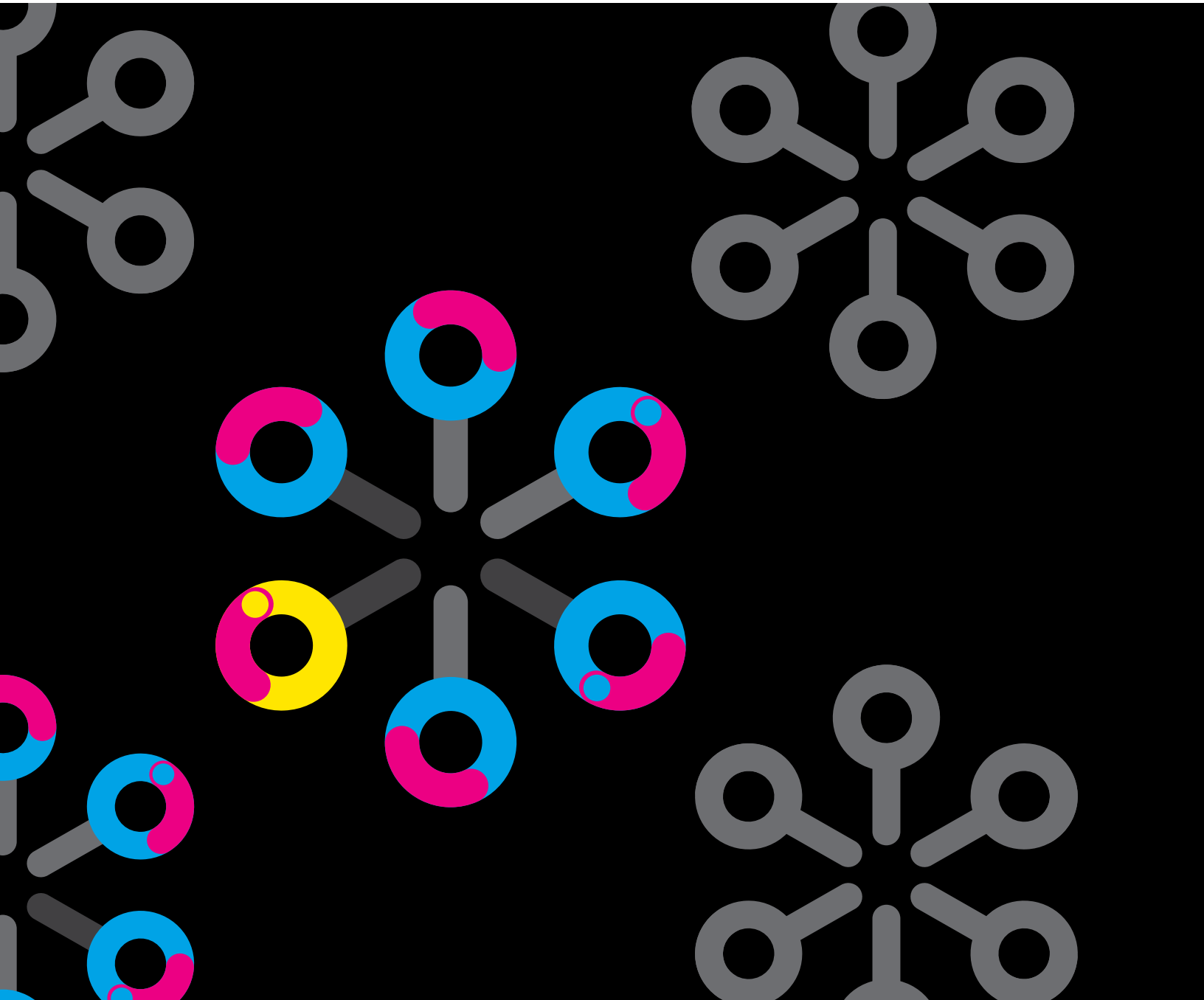
cell line development

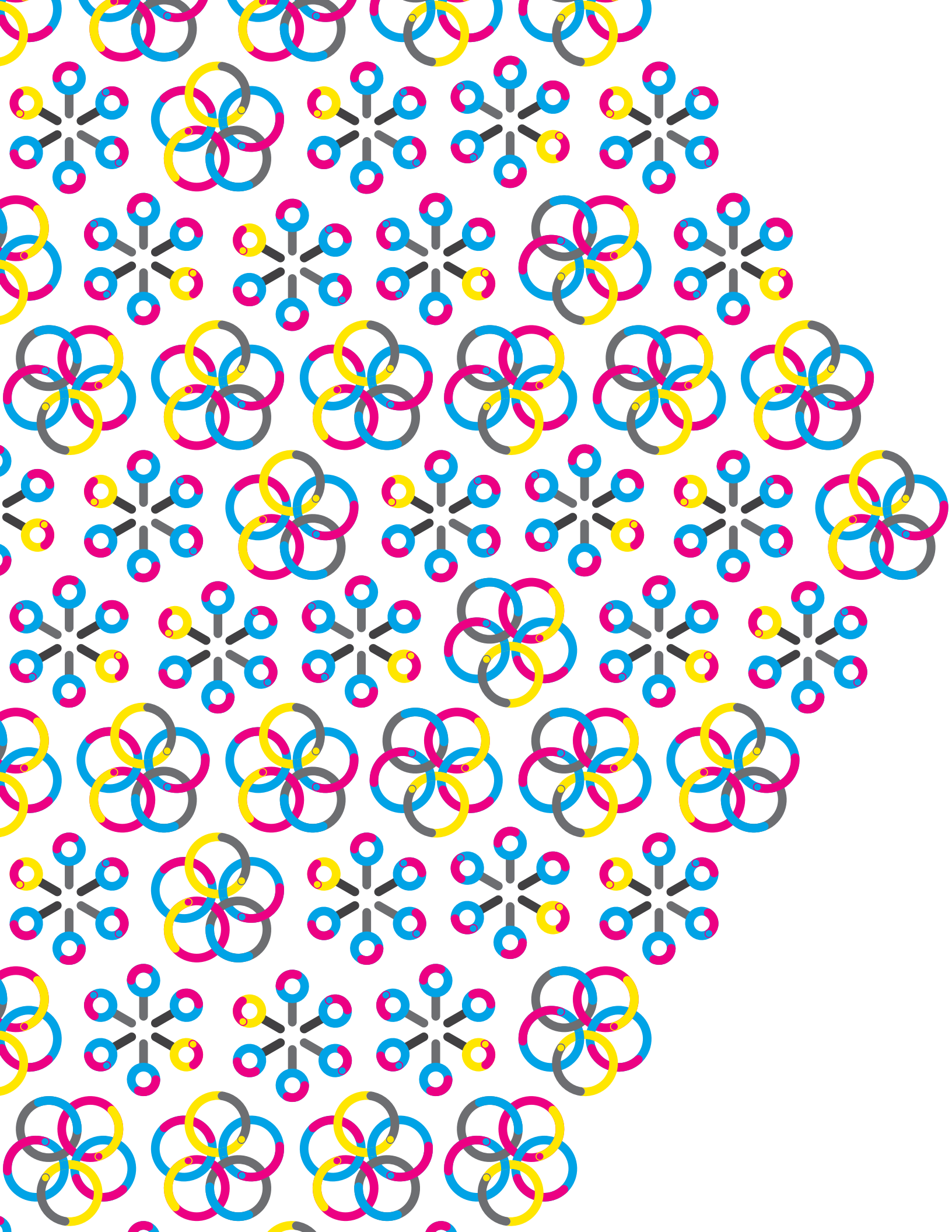
Leap-In Transposase® mediated cell line development results in:

Shorter timelines

High productivity

Genetic stability



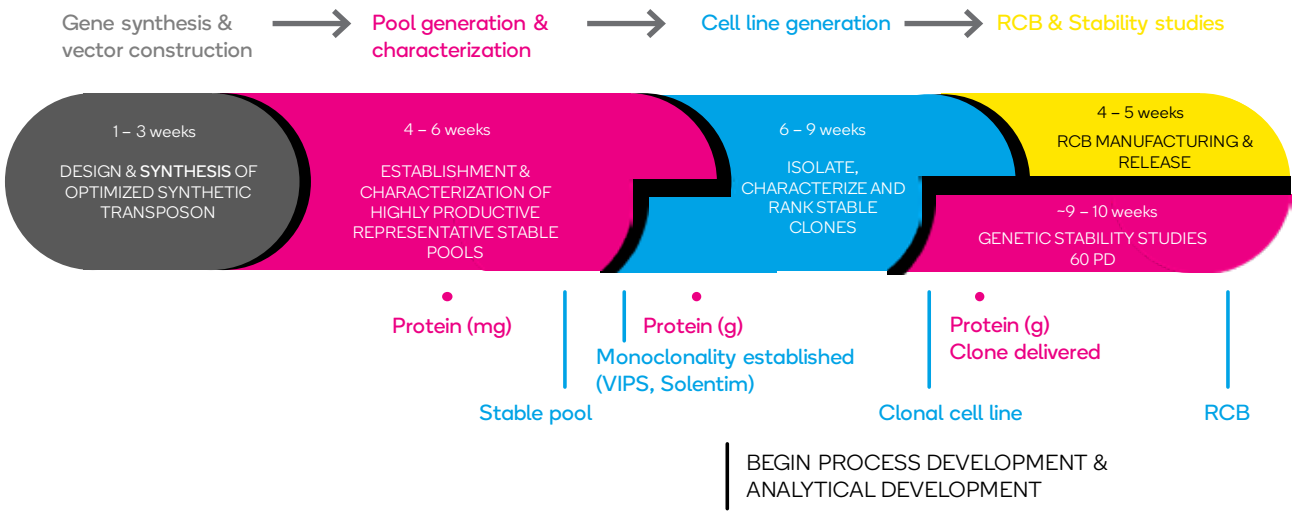


Cell Line Development

ATUM's Leap-In transposases® and synthetic transposons enable a cost-effective, easily implemented cell line development workflow. The technology delivers high productivity pools and cell lines, resulting in consistent product quality. The Leap-In technology underpins ATUM's cell line development services and is also licensable for your in-house use.

Stable Expression on Transient Timelines

Generate High Expressing Stable Pools in 14 days



Yield

With Leap-In technology, generate highly productive stable pools. Pool titers are very predictive of the titers of final clonal lines.

Stability

Exceptional genetic stability leads to stable productivity of Leap-In-generated clonal cell lines. De-risk your downstream process development, scale-up and manufacturing.

Speed

Product quality and productivity are highly comparable between stable pools and derivative clones. Initiate analytical development, process development, and GLP toxicology studies early, shaving months off the CMC timeline.

Efficiency

Cell line development using Leap-In technology is less labor intensive and does not require automation. Many cell line development projects may be conducted simultaneously by a small team.

ATUM

Cell Line Development Workflow

1 Production Hosts

ATUM currently uses two well characterized mammalian cell lines for bioproduction. No raw materials of animal origin are used during our CLD process.

- HD-BIOP3 GS null CHOK1 cell line from Horizon Discovery
- DG44 from the lab of Dr. Lawrence Chasin (Columbia University)

Key Benefits:

- Lineage traceability and documented provenance
- Host bank characterized (tested for mycoplasma, sterility, adventitious agents by PCR)
- Adapted to commercially available chemically-defined serum-free media formulations.

Timeline Post-Transfection

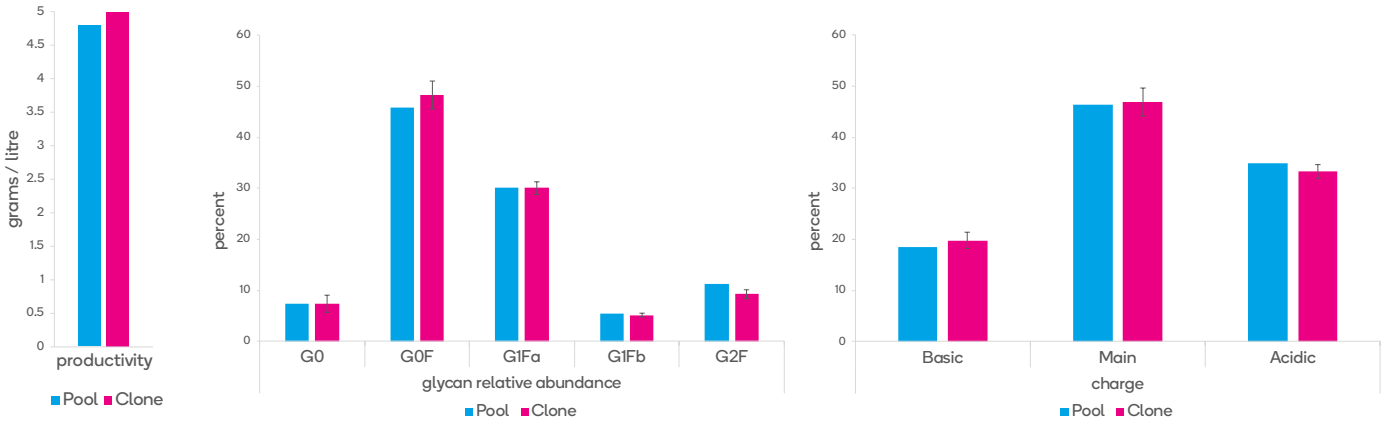
- Stable pool derived protein is available in 2 weeks
- High yielding pools and clones ~5-7 g/l for a typical IgG
- Clonal cell line (without stability information) is available in 12 weeks
- Research Cell Banks (RCBs) released in 20 weeks
- Process Development (PD) can start in 4 weeks saving valuable development time
- Faster to IND/Clinical trials

2 Stable Pool Selection

Leap-In transposase® is very efficient and will integrate into >90% of all transfected cells. Most cells that are transfected with Leap-In transposons and transposase will integrate multiple (3-70) independent, non-concatemerized copies of the transposon into their genomes. This results in rapid recovery of stable pools consisting of many very similar high producing cells. The productivity and product quality of clones derived from these pools are similar to the pools themselves. Process and analytical development can therefore start as early as a few weeks post-transfection. Material for toxicology studies can be prepared from stable pools to further shorten overall CMC timelines.

Productivity

Product Quality



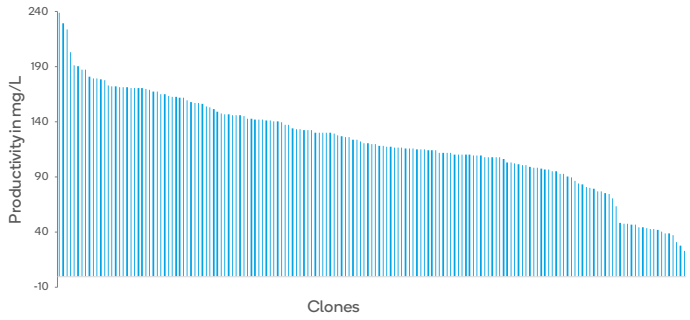
Product quality (glycan relative abundance %, charge %) and productivity are comparable between stable pool and derivative clones.

3

Stable Clone Isolation

In stable pools generated by Leap-In technology, the clonal distributions within the pools are strongly biased towards high productivity. This reduces the number of clones that need to be tested to at most a few hundred.

166 clones
Static 96-well



Rapid screening of stable clones with high titers

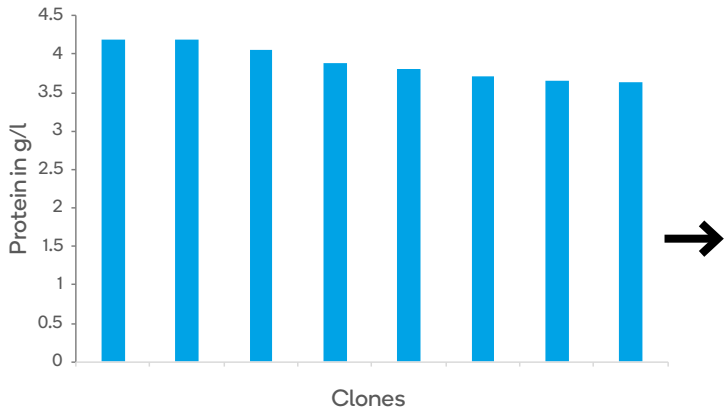
Host: Horizon Discovery's HD -BIOP3 CHOK1 GS⁻ cells

Molecule: Human IgG1 antibody

Monoclonality established using Solentim's VIPS™ instrument

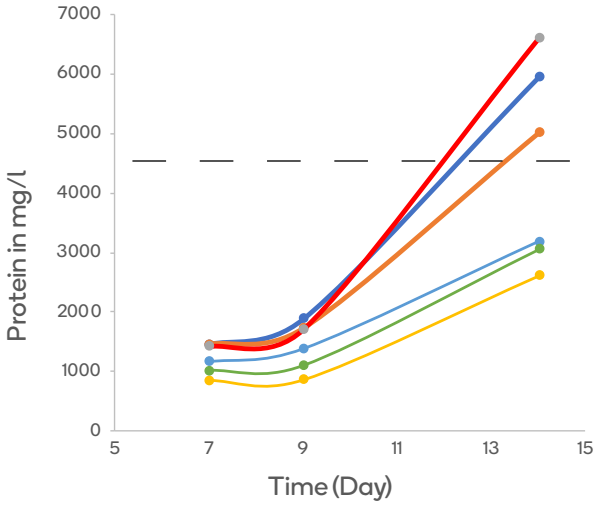
8 clones

Highest ranked clones, 125 ml shake, 14 day fed batch



Lead Clone

125 ml shake, 14 day fed batch, Media evaluation



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 managed simultaneously.

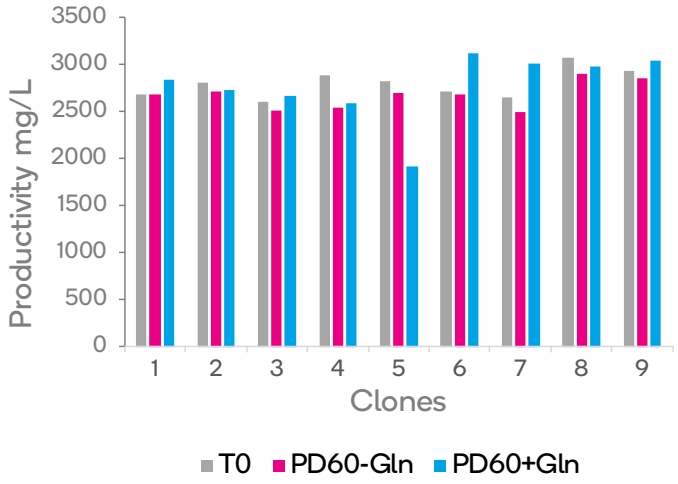
4

Stability Testing

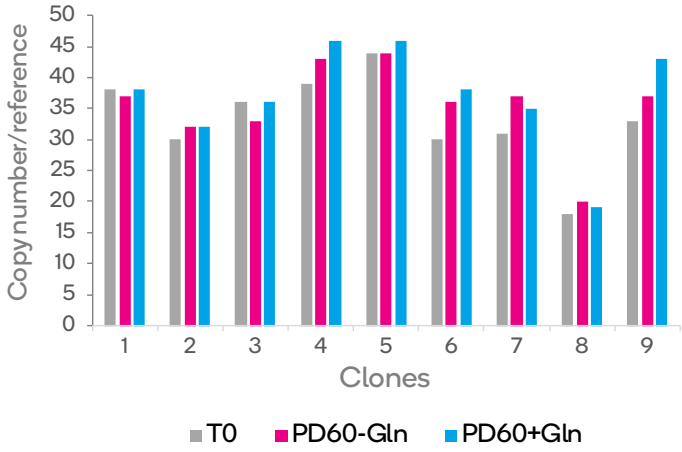
Leap-In transposase mRNA is co-transfected with the synthetic transposon into the cell. The mRNA is translated and the Leap-In transposase protein acts to integrate the transposon into the host genome. Once the transposase mRNA has been degraded through the normal cellular RNA turnover pathways, the cell is unable to generate transposase to mobilize the transposon, so the integration structures are stably maintained. Because each integration contains a single copy of the transposon, there is no instability resulting from concatemer recombination or repeat-induced silencing.

Clonal cell lines are grown for 60 population doublings, then assayed for genetic stability by ddPCR, and productivity stability. A large majority of clones created using Leap-In technologies exhibit no significant decreases in their volumetric productivity and integrated transposon copy number.

Productivity Maintained



Copy Number Maintained



Graphs show the maintenance of productivity and transposon copy number in clonal cell lines after 60 population doublings (PD). A subset of data taken from 47 clones created using Leap-In technologies is shown. All clones retained at least 70% of their initial productivity and transposon copy number.

Productivity and copy number is maintained after 60 population doublings.

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.

Examples of Stable Pool Productivity for Different Molecule Types

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

5 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.

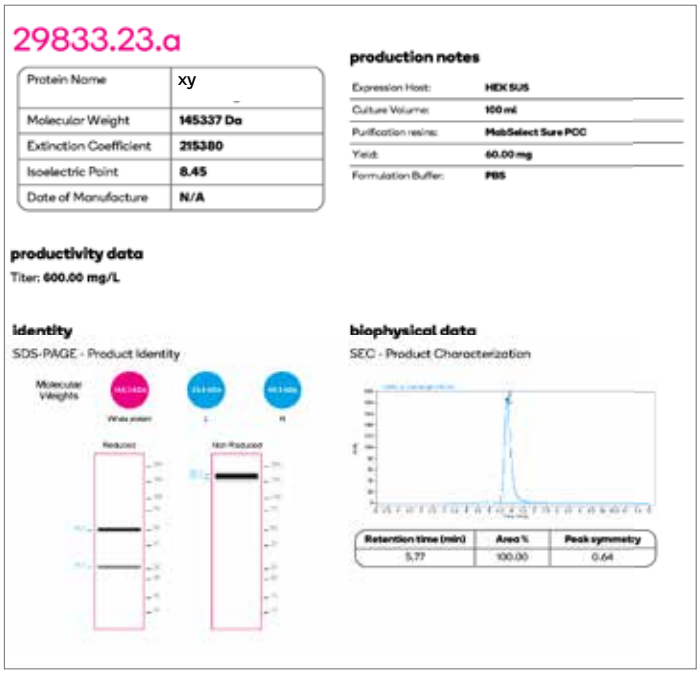
6 Clone Ranking and Research Cell Bank (RCB) generation

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:



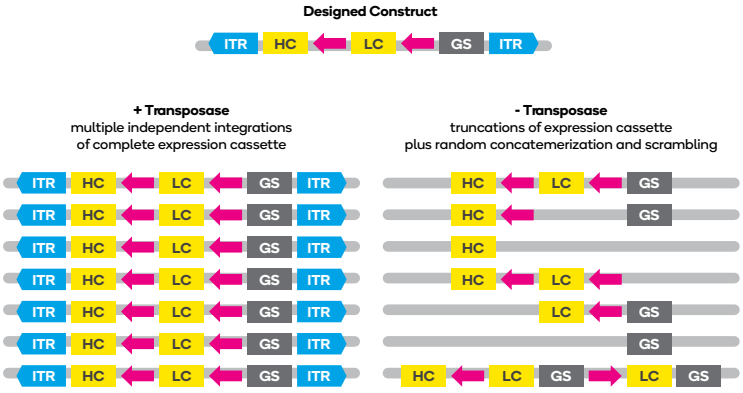
- Product from the best pools can be provided to client for additional function-specific analyses.
- Genetic stability is assessed by demonstrating consistent productivity and growth rate over 60 generations and by genomic analysis (Southern blotting, copy number and sequencing).
- Research cell banks (~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.

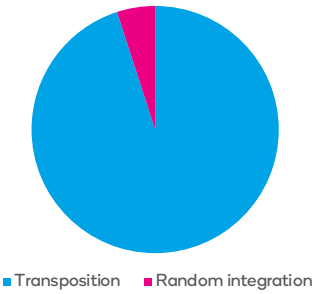
Leap-in Transposase®

Leap-In transposases integrate single copies of the entire synthetic transposon into multiple transcriptionally active genomic loci. Expression constructs integrated by Leap-In transposases do not exhibit the rearrangements and concatemerization that are common, problematic hallmarks of random non-homologous recombination driven integrations. Maintaining structural integrity of the sequence integrated into the host genome ensures that regulatory elements remain associated with the appropriate open reading frames, and desired balances between multiple open reading frames are maintained.

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.



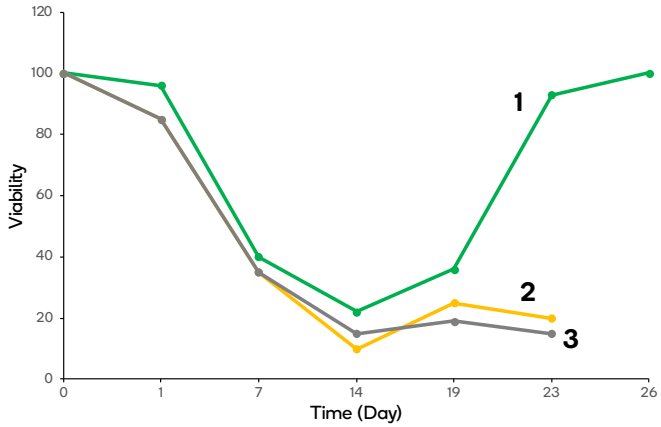


Targeted locus amplification (TLA) results performed by Cergentis demonstrated that 96% of the stable integrations were Leap-In transposase mediated transpositions and resulted in single copy, structurally intact transgenes.

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.



Transposase speeds recovery. CHO K1 GS KO cell line was co-transfected with ATUM's transposons and hyperactive transposase. Under drug-free selection conditions (attenuated GS promoter), cell pools created using transposase (curve 1) recover viability in ~3 weeks, whereas cell pools created by random integration (curve 2) fail to recover, curve 3 is control.

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
Multiple 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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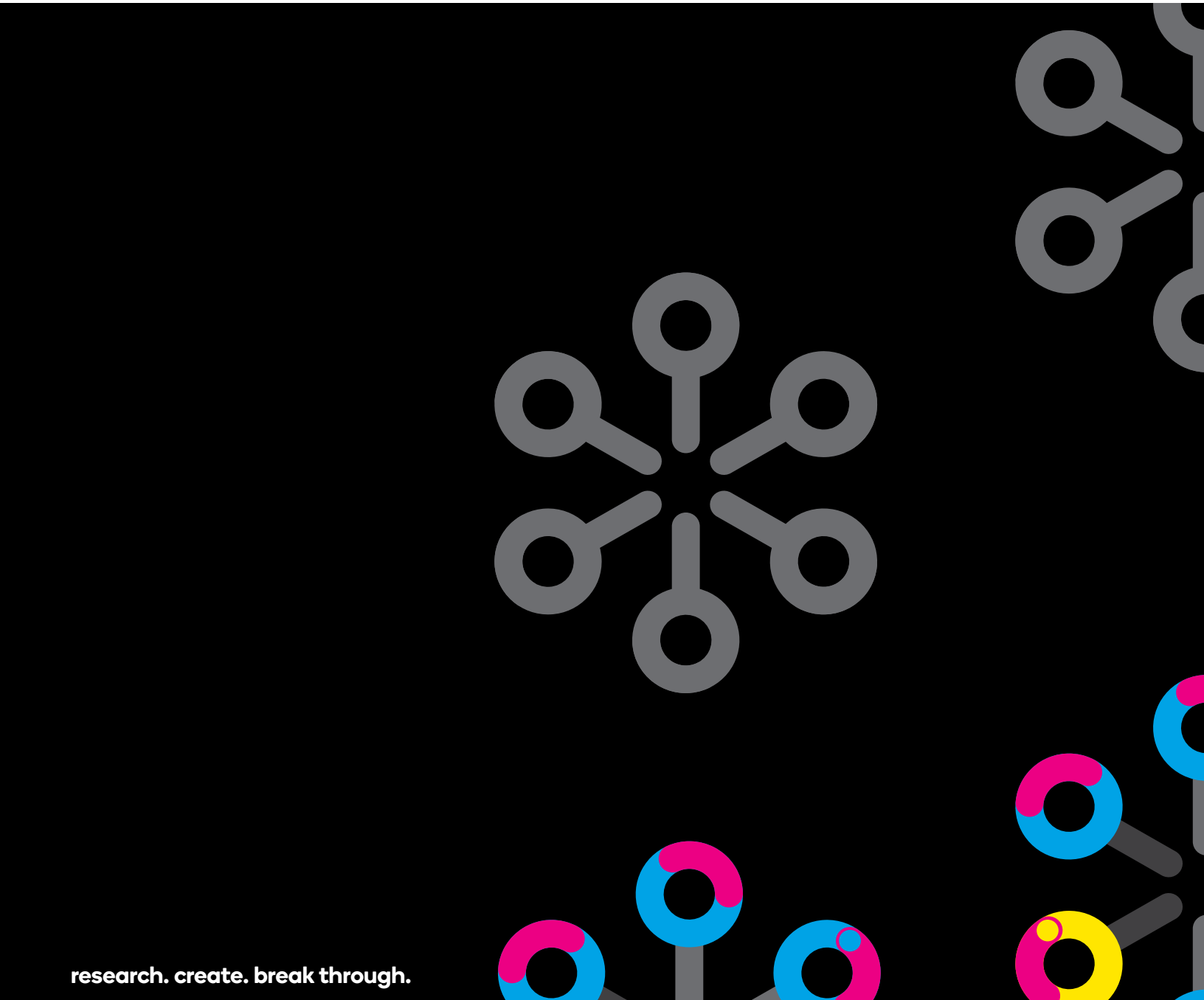

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