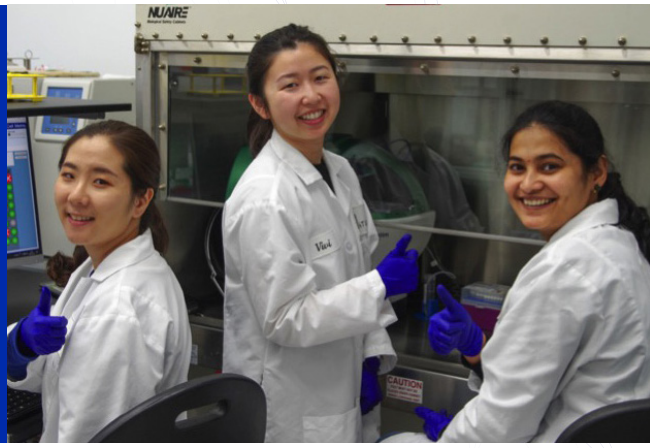
ADVANCED INSTRUMENTS

Application Note

Development of commercial manufacturing cell lines by screening less than 200 clones

using a combination of ATUM's Leap-In transposases® with the VIPS™ and Cell Metric®



Scientists are constantly striving to increase workflow efficiency, to develop the highest producing clones and to do it faster. We now demonstrate how this can be achieved in a fast and efficient way using only a handful of cloning plates.

Introduction

The typical clonal distribution, in stable pools established by random integration, dictates screening many thousands of clones to find the few gold nuggets, the high producers.In addition to being rare events, most of these clones carry rearranged, concatemerized forms of the recombinant insert leading to transgene fusions, and deletions.

Two important negative consequences of these undesired transgene structures are the unpredictable subunit ratios, potentially increasing the product related impurity levels, and, the increased probability of genetic instability.

More recently, the Leap-In transposase® expression technology developed by ATUM enables transposase-mediated integration (transposition) of structurally intact single copy inserts into multiple transcriptionally active genomic segments. The Leap-In transposase enzyme which catalyses the integration of the recombinant payload is supplied in trans as an mRNA setting a short duration for transposition. The Leap-In technology is perfectly suited to the development of clinical/commercial manufacturing cell lines.

The combination of the Leap-In technology with the VIPSTM/ Cell Metric® instrumentation drastically reduces the number of clones to rank (= actual #plates to process) per project to identify the best producers and to provide assurance of their clonality.

Materials and Methods

The Horizon Discovery BIOP3 CHOK1 GS KO host was used to produce a model antibody. The distinguishing features of the workflow are listed under the various steps.

Molecular Biology

The structural integrity of the transposase-mediated integrations allowed ATUM to develop a full suite of vectors and IP-free proprietary regulatory elements to accurately control the subunit ratios, of multi-subunit products within single expression constructs (see Figure 1).

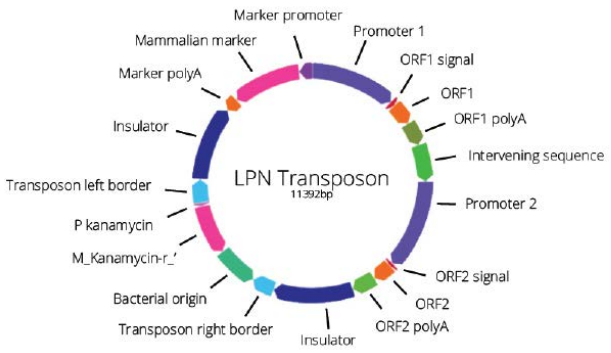


Figure 1. Leap-In Transposon

Two different proteins e.g. heavy and light chains for an antibody (see Figure 2), or three, four or more transcription units for next gen protein pharmaceuticals, can now be expressed, at consistent, optimal ratios without the need to co-transfect different plasmids, or risking ratio variations by rearranged transgenes.

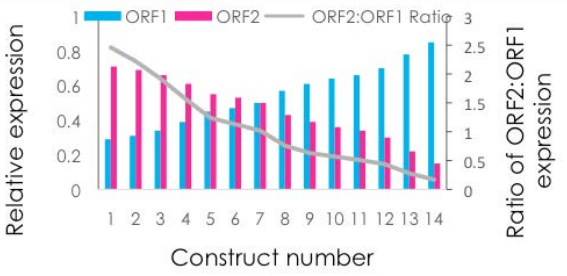


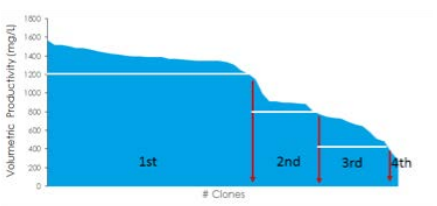
Figure 2. The consistent structural integrity, of the integrated Leap-In transposon based transgenes enable the firm control of product subunit expression ratios within one expression construct. In addition to improving product quality, this feature also reduces the number of clones to screen.

In addition, ATUM's proprietary codon optimization platform ensures maximal transcriptional and translational speed supporting high productivity.

Transfection and stable pool establishment

The host cells were co-transfected with the circular transposon-based expression construct and mRNA coding for Leap-In transposase.The transposase expression is transient as the mRNA is degraded within 48hrs in the cells. The transposase enzyme targets integration sites associated with transcriptionally active, open chromatin signatures.

The Leap-In integration mechanism ensures that a single copy transgene integrates at a given integration site. The integrated copy number within a cell is between 2-60 depending on the applied selection stringency. Following transfection, stable bulk pools are established.



Quartiles	Average % of clones
1st	42%
2nd	35%
3rd	18%
4th	5%

Figure 3. Characteristic clonal productivity distribution for Leap-In mediated stable pools. The graph on the left illustrates the clonal distribution grouped into expression level quartiles demonstrating the strong bias towards high productivity clones. The table on the right presents the average distribution values calculated from 8 stable pools expressing a variety of product types, MABs, bispecifics, fusion proteins.

The Leap-In technology has been successfully used to establish high-producing CHO stable pools using either DHFR, GS orantibiotic markers. One of the distinct characteristics, of the Leap-In mediated stable pools, is the clonal productivity distribution presented in (see Figure 3) showing a significant bias towards high producer clones. This clonal distribution facilitates the isolation of high producer clones by screening a significantly lower number of clones.

The distinctive clonal distribution leads to highly comparable product quality between stable pools and derivative single clones. This enables the performance of product quality optimization studies earlier using the stable pools with a high assurance of product comparability from the stable clones.

Single cell cloning step

The starting number of clones, to be characterized and ranked in the cell line development workflow, is further reduced by using the VIPS as it identifies the monoclonal clones right up front at the time of single cell deposition. Only a handful of 96 well cloning plates (target of 100-200 clones) need to be processed for a CLD project.

VIPS then performs the single cell cloning of the stable pool. The base media used was EX-CELL CHO Cloning Medium (Millipore-SIGMA) and the density used in the Cell Reservoir was between 9K-12K cells/ml. Cell Metric was used for daily whole well imaging from single cell to colony.

Both instruments are connected via a Sync Server so that all images from the same batch for both instruments end up in the same Clonality Report for complete documentation.

Results

Since the number of clones needed for screening is now relatively low, and single cell deposition by VIPS is a high efficiency process (see Figure 4), single cell cloning is initiated from every stable pool as soon as the pools are established.

Clonal outgrowth and stable pool ranking, by productivity and product quality, occurs simultaneously.

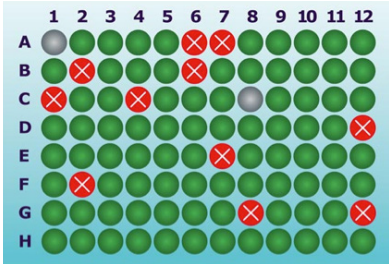


Figure 4. VIPS single cell seeding result. As an example, this plate has seeding efficiency of 87%. Green wells = single cells; red wells = >1 cell; grey = 0 cells.

Typical cloning efficiency (outgrowth rate) of HDBIOP3 derived clones is 50% resulting ~44 monoclonal isolates/ plate. After the best pool has been selected, monoclonal clones derived from that pool enter the clone ranking stage.

The Leap-In CLD workflow has 2 clone ranking stages:

Stage 1

Only a small number of clones (< 200) are needed from the VIPS 96 well single cell cloning step. The clones are transferred to 24 deep well plates and are ranked in a 7-day fed batch process.

Stage 2

The performance of the top 24 clones is evaluated in 14 day Ambr15 or shake flask/TPP cultures. Finally, the top 8 clones are taken forward into 125ml shake flasks in 14-day fed-batch.

High productivity clones are identified from the stable pools within 7-10 weeks, from single cell deposition, depending on the complexity of CQA based ranking (see Figure 5).

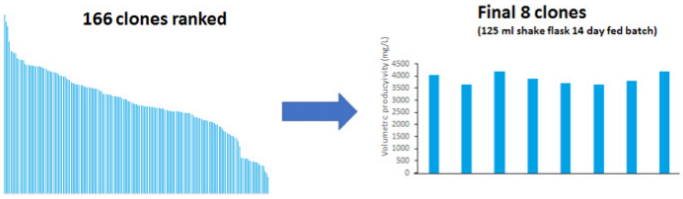


Figure 5. Example for the accelerated clone ranking workflow from Leap-In transposase mediated stable pools. The antibody expressing Leap-In mediated pool was established in Horizon Discovery's HDBIOP3 host. (Note the characteristic clonal distribution allowing to significantly reduce the number of clones to rank.) 166 clones were ranked in ATUM's typical two-stage clone ranking process. The final clones' productivity was ~4g/L.

Genetic stability is not a ranking parameter for Leap-In mediated stable clones. Up to now, all clones demonstrated exceptional stability assessed by productivity, transgene copy number, growth characteristics and transgene cDNA sequence.

Discussion

In traditional cell line development campaigns, the number of clones to be screened is in the order of many thousands.

The reduced clone screening burden (100-200 clones per project), due to the advantages of transposase-mediated stable integration, as well as the early and unequivocal monoclonality assurance with VIPS and Cell Metric instrumentation now facilitates the parallel execution of a large number of cell line development campaigns.

This requires a radical rethink for scientists who previously envisaged that to find the best producers, cell line development projects must require screening of thousands of clones.

To achieve this, they would have been considering handling tens of plates with robotics and worrying about speed per plate, or cell sorting methods to try and select "in-line" for high producers using fluorescence, or even novel high- throughput optoelectronics systems like Beacon®. Now only a handful of plates are required. The redefined workflow using the combination of Leap-in transposases with VIPS and Cell Metric is summarised below (see Figure 6).

Acknowledgements

We would like to thank Dr Ferenc Boldog, Director of Cell Line Development at ATUM, for his help in providing the figures and data.

VIPS & Cell Metric - Monoclonality established

Gene synthesis & vector construction

1-3 weeks

Construct design
Codon optimization
Signal sequence selection Gene synthesis Molecular cloning

Stable pool generation

2-3 weeks
Transfections Selection

Optional pool ranking

2-3 weeks
Focus on product quality

Stable pool available

Representative, pool derived protein (mgs)

Representative, pool derived protein (grams)

Single cell line cloning and clone ranking

6-9 weeks

Stable clone available

Clone derived protein (grams)

RCB manufacturing and release

4-5 weeks

60 PD Stablity
9-10 weeks

RCB available

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