Shortening the Biologics Clinical Timeline with a Novel Method for Generating Stable, High Producing Cell Pools and Clones

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Abstract

Reducing drug development timelines is an industry-wide goal to bring medicines to patients in need more quickly. This was exemplified in the COVID-19 pandemic where reducing development timelines had a direct impact on the number of lives lost to the disease. The use of drug substance produced using cell pools, as opposed to clones, has the potential to shorten development timelines. Toward this goal, we have developed a novel technology, GPEx® Lightning, that allows for rapid, reproducible, targeted recombination of transgenes into more than 200 Dock sites in the CHO genome. This allows for rapid production of high expressing stable cell pools and clones that reach titers of 4 to 12 g/L in generic fed-batch production. These pools and clones are highly stable in both titer and glycosylation, showing strong similarity in glycosylation profiles.

Introduction

Pool Cell Lines for Development

Biologic pharmaceutical drug developers have been contemplating using uncloned stable mammalian cell pools for GLP toxicology production, GMP production, and Phase 1 clinical products for a number of years (Rajendra, 2017; Stuible, 2018). The advantages of stable mammalian cell pools include faster timelines and reduced costs to initiate clinical trials; however, until COVID-19 very few programs used this approach. In addition to traditional biologic therapeutics, other biologic products requiring cGMP production (e.g. molecules that are used in diagnostic assays, medical devices, or cell or gene therapy production) may also benefit from this type of approach. The main concerns around cell pool production have been both product and process consistency. As compared to clonal cell lines, there are limited published data showing that cell pools can reliably provide the product and process consistency necessary for clinical production. To our knowledge, there are few successful examples of this approach, with the documented ones published only recently (Agostinetto, 2022; Zhang, 2021; Xu et al, 2022). If cell pools are going to be used as outlined above, the characteristics of the product from a cell pool should mimic a clonal production cell line. The characteristics requiring consistency are high titer, production behavior, production stability, scalability, and protein quality. In addition, the process to produce pools should be fast and work well for a variety of molecule types. The technology described here meets these criteria to accelerate biologic product development.

Targeted Integration Cell Line Development Overview

The generation of high expressing, stable cell lines producing proteins with the desired product quality is the goal of any cell line development program. Typically, these requirements need to be achieved on a shortened timeline with the goal of getting to First in Human as quickly as possible. Toward this goal, many novel cell line engineering technologies have been developed for integration of transgenes into the CHO genome. These technologies can be broadly grouped into three main categories: random integration (Grandjean, 2011), semi-targeted integration (Bleck, 2005; Rajendran, 2021), and targeted recombination (Feary, 2020; Ng, 2021). Here, we explore the advantages of combining the latter two technologies by placing a relatively high number (>200) of Dock sites (aka landing pads) throughout the genome, followed by targeted recombination of an expression construct (Boat) into these dock sites.

GPEx® and GPEx® Lightning Technology

GPEx® technology (Figure 1A) is a versatile system designed to insert genes of interest into a wide variety of mammalian host cells (Bleck, 2005). It is based on the use of replication incompetent retroviral vectors (retrovectors) to actively insert the desired genes into or around the transcription start point of genes (Wu, 2003; Mitchell,2004). This preference for transcriptionally "active" regions of the genome allows for higher, more consistent levels of expression per copy of the gene inserted as compared to other methods of gene insertion. These integrated genes are maintained in an extremely stable manner through subsequent cell divisions as if they were small endogenous cellular genes (Bleck, 2012).

GPEx® Lightning technology leverages the GPEx® technology to insert Docks throughout the CHO genome. This parental Dock cell line is then co-transfected with a Boat plasmid containing the gene(s) of interest (GOI), and the recombinase plasmid. This leads to the targeted integration of multiple copies of the GOI into Dock sites throughout the genome. To select for cells containing a high number of targeted recombination events, we utilized a unique glutamine synthetase (GS) selection method in a GS knock-out cell line. This allows for enrichment of high producing cells without the use of selective chemicals (i.e. methionine sulphoximine, methotrexate). Here we demonstrate that GPEx® Lightning technology produces stable pools with high titer and consistent product quality which is maintained between pools and clones. This enables the use of cell pool-produced drug substance for toxicology, and potentially for clinical studies, to reduce product development timelines and costs.

Methods

Dock and Boat Construct Design

The Dock sites contain in order: a modified Long Terminal Repeat (mLTR) which possesses very weak promoter activity, an enhanced packaging region required for retroviral packaging, the Phi-C31 recombinase *att* achment sequence from the Phi-C31 *P* hage genome (attP), and another mLTR (Figure 2).

The single gene Boat expression construct contains (in order) the Phi-C31 recombinase *att* achment sequence from the *Streptomyces b* acterial genome (attB), the Glutamine Synthetase (GS) gene, a strong mammalian promoter from the simian cytomegalovirus immediate-early gene, the gene of interest (GOI), an mRNA export element and a polyadenylation signal (Figure 2). The two gene Boat expression construct adds a human cytomegalovirus immediate-early gene promoter, intron, GOI, and polyadenylation sequence at the end of the single gene Boat expression construct. The Boat constructs are designed to utilize a promoter trap approach such that random integration would not result in significant GS expression, since the Boat lacks its own promoter to drive GS expression. However, upon proper targeted integration into the Dock, the GS gene is situated downstream of the mLTR which possesses weak promoter activity. Multiple other methods are used to reduce GS transcription and translation, allowing for survival of clones with high recombined

Boat copy numbers while clones containing few or no copies of integrated Boat are outcompeted by the high producing cell lines.

Retrovector Production and Transduction to Create the Dock Parental Cell Line

GPEx technology leverages an HEK 293 cell line that constitutively expresses MMLV gag, pro, and pol proteins (Bleck, 2005). The dock construct described above was co-transfected with an envelope expression plasmid. Retrovector was subsequently concentrated by ultracentrifugation and used for transduction of the CHOZN® (MilliporeSigma) Chinese Hamster Ovary parental cell line. Sequential rounds of transduction were performed, and cells were routinely maintained in media supplemented with 6 mM glutamine.

Production of GPEx® Lightning Technology Pools

Coding sequences for commercially available biotherapeutics for monoclonal IgG1-kappa antibodies and an Fc fusion protein were cloned into either a single gene or two gene Boat expression construct. 3 million GPEx® Lightning Parental Dock cells were transfected with 2 micrograms (total) of Boat plasmid and recombinase plasmid DNA using ExpiFectamine^{TM} CHO (ThermoFisher Scientific). Cells were allowed to recover in Ex-Cell® Advanced CHO Fed-Batch media (MilliporeSigma) supplemented with 6 mM glutamine and 1% ClonaCell-CHO ACF Supplement (Stem Cell Technologies) until viability returned to greater than 95% before glutamine was withdrawn. Viability was monitored until the resulting selected cell pools returned to greater than 95% viability and doubling times reached 18-24 hours, typically 16 to 22 days after transfection.

Single Cell Cloning

The Berkeley Lights Beacon(r) instrument was used for single cell cloning (Kravitz, 2019). The individual clones from the GPEx(r) Lightning Dock pool were ranked and selected based on desirable doubling time (17.5-23.5 hours). All clones were exported onto 96 well plates, expanded, frozen, and subjected to quantitative Polymerase Chain Reaction (QPCR) to quantify the number of docks per cell. All Dock clones were tested by PCR for retroviral components to ensure no viral genes had been incorporated into the genome, and thus future cell lines made from the Parental Dock line could not produce replication competent retrovirus.

Clonal lines from pools expressing protein products were ranked and exported based on the Beacon Spotlight assay which measures relative titer through the Fc portion of the molecules. All clones were exported onto 96 well plates, expanded, and frozen.

Quantification of Recombination:

Genomic DNA from 3 million cells was isolated using a Qiagen DNEasy kit. QPCR using SYBR(r) green dye (Thermo Fisher Scientific) was employed to estimate the number of specific recombination events into the Dock sites. AttR formation is the result of site-specific recombination between attP in the genome (Dock) and attB on the transgene construct (Boat). QPCR was performed to quantify attR using a forward primer in the attP sequence in the Dock and a reverse primer in the attB sequence in the Boat. Amplification using this primer pair only detects the transgene construct when it is recombined into a Dock but does not detect empty Dock, random integration, integration into a pseudo-attP site in the genome (Thyagarajan, 2005), or free plasmid. Cycle threshold (Ct) values from the attR primer set were subtracted from the Ct value from an internal reference gene primer set to determine the delta-Ct values. Those values were used to estimate copy number using the equation: copy number =2ˆ(delta-Ct). The amplification efficiency of both primer sets was determined to be 90-100% via a template DNA sequential dilution assay.

Fed-Batch Production

Generic Fed-Batch Strategy 1 (GFB1)

Cells were inoculated at 600,000 cells per mL in Ex-Cell(r) Advanced CHO Fed-Batch media (MilliporeSigma) and incubated in a humidified (70-80%) shaking incubator with 5% CO_2 and temperature of 37degC (shift to 34degC on day 4). Cultures were fed every other day starting on day 2 with 6.25% (V:V) of a feed blend containing 66% Ex-Cell(r) Advanced CHO Feed 1 and 33% Cellvento(r) 4Feed (MilliporeSigma). Glucose was monitored daily and supplemented if below 5 g/L. Cultures were terminated when viabilities were [?] 70% or at the end of day 20. Cell concentration and viability were measured by a Roche Cedex HiRes(r) instrument and cell metabolites, glucose, and IgG titer were measured by a Roche Cedex Bio HT(r) instrument.

Generic Fed-Batch Strategy 2 (GFB2)

Methodology was as described in GFB1 except media and feeds were replaced with ActiPro^{TM} media and 5% CellBoost^{TM} 7a and 0.5% CellBoost^{TM}7b feeds (Cytiva).

Production in Ambr(r) 250 Bioreactors

All methodologies were the same as GFB2 except Ambr(r) 250 (Sartorius) vessels were inoculated at 6.5 x 10^5 cells/mL, pH set point was 7.0 with deadband of -0.2 and +0.5. Baseline agitation was set to 20 watts/m^3 and increased as needed to reach the dissolve oxygen set point of 30%. Actual rates ranged from 340-500 RPM.

Stability Studies

Pools or clones were continually passaged in Ex-Cell(r) Advanced CHO Fed-Batch media (MilliporeSigma) or ActiPro^{TM} (Cytiva) media and frozen weekly. All generations were simultaneously thawed and evaluated for production.

Glycosylation Analysis

Sample Preparation

Protein-A purified protein was denatured in 1% RapiGest^{TM} (Waters) solution, followed by the simultaneous release of N-glycans using Rapid^{TM}PNGaseF (New England BioLabs) and derivatization with RapiFluor-MS (Waters). The GlycoWorks SPE Reagents-Automation kit (Waters) and GlycoWorks HILIC µElution plate (Waters) were used to purify and concentrate the released and fluorescently labeled N-glycans.

Chromatography Conditions

These N-glycans were separated and analyzed using a Waters Acquity UPLC (Ultra Performance Liquid Chromatography) H-Class equipped with fluorescence detector, an Acquity® Glycan BEH130 Amide, 2.1x150mm (Waters) column, and acetonitrile (mobile phase A) and 50mM ammonium formate (mobile phase B). A gradient of 25% to 46% B 0.4 mL/min in 35.0 min, then a gradient shift to 100% B 0.2 mL/min to 36.5 min, followed by an isocratic step at 100% B 0.2 mL/min from 36.5 to 39.5 min, then a gradient shift to 25% B 0.2 mL/min to 43.1min, and then equilibration at 25% B 0.4 mL/min from 47.6 min to 55.0 min. Column temperature was 60°C and a 10 µL injection volume was used.

Glycan Detection and Identification

N-glycans were fluorescently detected (λ_{ex} = 265nm, λ_{em} = 425nm) at a sample rate of 10 Hz and mass confirmed using a Waters Xevo® G2-XS Q-ToF mass spectrometer. RapiFluor-MS^{TM} Calibration Ladder was injected (1 µL) at the beginning and the end of the sequence to determine the glycan units (GU) for the

samples tested and assist in assignment of identity. The calculated glycan units for each observed species in conjunction with mass confirmation were compared to the Waters RFMS Glycan Library for released N-glycan identification.

Results

Creation of the Parental Dock Cell Line

Docks were placed in transcriptionally active sites throughout the genome of CHOZN® GS-/- cells with sequential rounds of transduction using GPEx® retrovector technology (Figure 1). The Dock cell pool contained approximately 75 Dock copies per cell on average. To test the integration efficiency and expression of the pool, we co-transfected with Phi-C31 Recombinase and Boat construct expressing an Fc fusion protein (Figure 2). After transfection, pools were allowed to recover for 2-4 days prior to selection by removal of glutamine from the media.

The system utilizes multiple methods to reduce GS expression resulting in survival of only high copy number clones regardless of the initial copy number of the pool (see methods). For example, a pool containing 31 inserted copies per cell on average prior to selection via glutamine withdrawal resulted in 63 copies after selection while another pool that contained 64 copies per cell average before selection reached 94 copies after selection. Even though the second pool already contained 64 copies per cell on average, selection still resulted in a substantial increase in copy number.

After pools recovered from selection, QPCR using the attR primers, which only detect Boat that has integrated into the Dock site, revealed that these pools contained approximately 30 copies of recombined Boat per cell. In generic fed-batch production, these pools produce up to 5 g/L indicating the system was working as designed.

To isolate a high Dock copy number clone that had good growth characteristics, the Dock pool was subjected to single cell cloning using the Berkeley Lights Beacon® instrument. Multiple clones with the highest number of Dock copies were co-transfected with plasmid encoding the Phi-C31 recombinase and the same test Boat construct expressing an Fc fusion protein. After recovering from selection, the cell pool generated from the top clone contained about 40 integrated Boat copies on average. This clone was found to have suitable reproducibility of insertion, averaging 40 to 130 copies per pool. Robust growth characteristics and high protein expression were observed; therefore, this clone was used as the parental Dock cell line for all further process optimization studies and projects.

Creation of Cell Pools Expressing Proteins of Interest

To test the insertion efficiency, expression, and consistency of the technology, we also utilized a Boat expression construct that contains two cloning sites for the independent expression of both heavy chain and light chain sequences in the same Boat as opposed to the single chain construct described above. The GPEx® Lightning Parental Dock line was co-transfected with Phi-C31 recombinase plasmid and each Boat plasmid construct in triplicate. To select high expressing cells, glutamine was removed from the media and viability was monitored during selection (Figure 3A). Each triplicate transfection showed very consistent recovery from selection, and selection dynamics were very similar between molecules. Pools consistently achieved about 50 integrated Boat copies per cell on average (Figure 3B).

Fed Batch Production from Cell Pools

Pools were subjected to a fed-batch production using a generic, unoptimized feeding strategy (GFB1) for each of the three replicate pools. All three replicate pools of mAb1 reached approximately 20 x 10^6 cells per mL, maintained high viability for the full 20 days of production, and produced very consistent titers of about

6.5 g/L (Figure 4A). The same feed strategy was used for production of the Fc fusion protein (test molecule) and three other mAb molecules. Average viable cell densities for the three replicates of each molecule reached 18-26 million cells per mL and viability remained high throughout production (Figure 4B). Average final titers ranged from 3.5 to 5.5 g/L across these molecules. These data indicate that the technology consistently produces robust and high titer producing pools.

Upstream process development was performed on mAb1 pool population #3. Media/feed scouting experiments identified the GFB2 strategy as superior to GFB1 (Figure 5). The Ambr® 250 bioreactor system was used for further control of the GFB2 process. Using the optimized strategy, the pool reached 28 million cells per mL viable cell density, maintained high viability throughout production, and attained a final titer of approximately 12 g/L.

Stability of Cell Pools

To determine the stability of pools expressing the Fc fusion protein, three unique pools made at three different generations from the GPEx® Lightning Dock Pool (not clone) were subjected to stability analysis. For this study, two different media and feed strategies were employed. GFB1 typically provides robust titer and culture longevity for most products. GFB2 is more variable but often results in higher cell density and significantly higher titers than GFB1. Final titers and average number of integrated transgenes for all generations in each of the two medias remained stable for more than 40 generations (Figure 6A).

The genetic and production stability of pools expressing mAb1 and mAb2 made using the Parental Dock clone were also assessed using GFB1 (Figure 6B). These pools also showed high production and genetic stability across more than 40 generations. Together, these data indicate robust stability of transgene copy number and protein expression. Stability and consistency of expression are critical requirements for the potential use of these stable cell pools in drug substance manufacturing.

Expression and Stability of Clones

To determine the expression and stability of clones, the highest titer pool expressing the Fc Fusion molecules (pool population 4, Figure 6) was cloned using the Berkeley Lights Beacon® instrument. Clones were ranked and exported based on relative expression using the Spotlight™ Assay. 26 clones were expanded and their attR copy numbers ranged from 37 to approximately 200. At high copy numbers such as this, intrinsic variability in QPCR assays make it difficult to accurately measure precise copy number (D'haene, 2010). Since QPCR is better able to quantify lower copy numbers, we used primers that amplify attP, which is only present in empty dock sites. Highlighting the efficiency of the process, the attP amplicon could not be detected in one of the clones indicating that this clone had all dock sites filled.

Next, we performed a small-scale 16-day production run on these 26 clones using the GFB1 strategy. Titers ranged from 2.5 to 5 g/L in this production run. We found that Boat copy number correlated well with final titer (Figure 7A). To determine the stability of the three highest titer clones, a study was performed, this time for over 60 generations. As with cell pools, the clones showed the same robust stability of transgene copy number (not shown) and product expression (Figure 7B).

Protein Quality Characterization of Pools and Clones

To examine the consistency of the protein product quality, a generational stability study was conducted using two pools made at different times, but expressing the same Fc-fusion protein, which contains five potential N-glycan attachment sites (Figure 8A and Table 1). While there are minor differences between the quantity of each particular N-glycan between the two pools, different generations within each pool are essentially identical, demonstrating consistent quality over the generations studied.

Comparisons of product quality between pools and their clones were performed for 3 different antibody products. To create sample diversity for the evaluation, we chose clones that produced significantly more product than the pool (mAb05), less product than the pool (mAb06), and about the same amount of product as the pool (mAb07). No matter how the clones produced relative to the pool, no significant differences in glycosylation pattern or levels were observed (Figure 8B and Table 2). In each case the pools and their corresponding clone(s) are superimposable. Table 2 shows more detail and concordance for each species between the pool and the clone(s). Of particular interest is the similarity in high mannose and total fucosylated species.

Discussion

Here we described the development and testing of the GPEx® Lightning technology. Combining GPEx®, GS selection, and targeted recombination technologies leverages the advantages of both semi-targeted integration and site-specific recombination for cell line development. This technology consistently results in stable cell pools containing approximately 50+ copies of recombined Boat gene construct per cell on average. Clones isolated from these pools can contain 150 to 200+ copies of Boat expression construct. This represents a total of up to 1.4 million base pairs of DNA stably inserted into the CHO genome at multiple chromosome sites from a single transfection.

Generic fed-batch production of the resulting pools and clones showed that Boat gene copy number correlated with final titer. Titers from pool populations typically reach 3-7 grams per liter in non-optimized conditions and up to 12 g/L with minimal optimization. Clones typically reach 4-12 g/L and up to 15 g/L. Both pools and clones reach high cell density with prolonged cell viability. Recent efforts focusing on process intensification (i.e. higher inoculation density, feed rates, and temperature) have achieved similar titers in shorter production runs (not shown). Additionally, these pools and clones show very high genetic and production stability over greater than 40 generations. The N-glycan distribution across 40+ generations also remains remarkably consistent, especially the amount of high-mannose and total fucosylated species. Clones show N-glycan profiles consistent with the pools from which they were isolated mitigating the risk of clones having divergent protein quality.

This technology also allows for shortened development timelines. Once the Boat gene construct is cloned and purified, transfection and selection typically take less than 25 days to produce pool research cell banks. These banks can then be scaled for large scale production. When combined with the Berkeley Lights Beacon instrument for cloning, clones can be produced, expanded, banked, and characterized in an additional 12 weeks.

Many pharmaceutical/biotech companies and CDMOs have moved away from random integration approaches to cell line development in favor of semi-targeted or targeted approaches to improve process consistency, increase yield, and reduce timelines (Rajendra, 2017; Rajendran, 2021; Feary, 2020; Ng, 2021). To our knowledge this is the first example of a technology that combines the two latter approaches, allowing for a very high number of transgenes to be inserted at consistent locations in the genome. The speed of development, high and stable expression with consistent glycosylation, and ability to scale to bioreactors make GPEx Lightning pool-derived material suitable for early phase clinical studies. The use of pool-derived material should also allow for cGMP production of diagnostic assay components, molecules that are part of medical devices, or process reagents needed to produce cell/gene therapy products to be produced not only in a more cost-effective manner but also with greatly reduced timelines. As we have seen with the COVID pandemic, reducing timelines and costs through approaches like this can have significant impact for drug development, patients, and society.

Figure Legends

Figure 1. GPEx® Technology Overview

A. The GPEx® cell line development process utilizes retrovector technology to insert the product expressing gene of interest (GOI) into transcriptionally active sites in the host genome and can be used for most mammalian cell lines. B. GPEx® Lightning combines the benefits of GPEx® with GS selection and targeted recombination technology. GPEx® technology was leveraged to place >200 Dock sites, which contain recombinase attachment sequences (att), into transcriptionally active sites throughout the genome, creating a parental GPEx Lightning Dock cell line. Transfection of this parental line is performed with a Boat construct containing att, GS, and the GOI, plus a separate recombinase construct (not shown). After transfection, selection results in the rapid and efficient insertion of up to approximately 200 copies of the GOI into transcriptionally active sites in the genome.

Figure 2. GPEx® Lightning Boat Insertion Process

Dock sites containing the attP site flanked by modified retroviral long terminal repeats (mLTR) sequences were placed in transcriptionally active sites throughout the CHO genome using GPEx® technology. See Figure 1 and main text for detailed description of Dock site placement and GPEx® Lightning Parental Dock cell line development. The parental Dock cell line is co-transfected with a Boat construct (containing attB, GS, and the GOI) and a construct expressing recombinase. Recombinase protein mediates the insertion of the entire Boat construct into the attP site in the Dock; this process occurs simultaneously across many dock sites. Following integration, the upstream att site, now called attR, is comprised of the upstream portion of attP (light blue) and the downstream portion of attB (light purple). Conversely, the downstream att site, attL, is comprised of the upstream portion of attB (light purple) and the downstream portion of attP (light blue). The location of forward (green) and reverse (red) primers used in the attR QPCR assay to quantify integration are indicated.

Figure 3. Pool Selection

A. Percent cell viability over time for pools expressing four different monoclonal antibodies (mAb) or an Fc fusion protein. B. Approximate number of integrated Boat copies per cell in each pool. Error bars represent standard deviation of triplicate transfections.

Figure 4. Pool Generic Fed-Batch Production

Results of fed-batch production from pools using the GFB1 strategy. Viable cell density, viability, and titer for A) three replicate pools expressing mAb1, B) average of replicates for pools expressing mAb2 (n=2), mAb3 (n=3), mAb4 (n=3), and an Fc Fusion protein (n=1). Average standard deviation for each molecule across the time course for each of the 4 mAbs were less than 10%, 3%, and 13% for VCD, viability, and titer, respectively (not displayed).

Figure 5. Pool Ambr® 250 Bioreactor Production

Viable cell density, viability, and titer for mAb1 pp3 production using GFB2 and the optimal GFB2-based production strategy identified in an Ambr® 250 process optimization campaign. Results from the GFB1 strategy (from Figure 4) are shown for comparison.

Figure 6. Pool Stability

A. Final titer and integrated Boat copy number from a stability study of 40+ generations of 3 independent pools expressing an Fc fusion protein. Each pool stability study was performed in 2 different media. Error bars intentionally omitted for clarity. B. Final titer and integrated Boat copy number from a 40+ generation

stability study on 2 pools expressing mAb 1 and mAb2. Production was performed using the GFB1 strategy in duplicate. Error bars indicate standard deviation.

Figure 7. Clone Generic Fed-Batch Production and Stability

A. Linear regression of final titer from a 16-day productivity using GFB1 plotted against percent Dock fill for 26 clones isolated from Fc Fusion pp2. B. Final titer from a 40+ generation stability study on the top 3 clones from Fc Fusion pp2. Productivity was performed using the GFB1 strategy. Error bars indicate standard deviation.

Figure 8. Released N-glycan Profile Stability and Pool to Clone comparison

A. Overlay of UPLC traces of fluorescently labeled and released N-glycans from two different Fc-fusion pools. Terminal samples from productivity of the indicated generations (Gen) were analyzed. B. Overlay of UPLC traces of fluorescently labeled and released N-glycans from three different monoclonal antibodies. Terminal samples from productivity of a pool and a clone derived from that pool were analyzed.

Table 1. Released N-glycan Profile Stability

A comparison of individual N-glycan species released from Figure 9. Numbers shown are percentages.

Table 2. N-glycan Species from Pools and Clones.

A comparison of individual N-glycan species released from Figure 10. Numbers shown are percentages. ND = not detected or below 0.4% area reporting threshold.

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