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NOTE

Cell Culture and Tissue Engineering

SPEED-MODE cell line development (CLD): Reducing Chinese hamster ovary (CHO) CLD timelines via earlier suspension adaptation and maximizing time spent in the exponential growth phase

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Abstract

Chinese hamster ovary (CHO) cells are the preferred system for expression of therapeutic proteins and the majority of all biotherapeutics are being expressed by these cell lines. CHO expression systems are readily scalable, resistant to human adventitious agents, and have desirable post-translational modifications, such as glycosylation. Regardless, drug development as a whole is a very costly, complicated, and time-consuming process. Therefore, any improvements that result in reducing timelines are valuable and can provide patients with life-saving drugs earlier. Here we report an effective method (termed SPEED-MODE, herein) to speed up the Cell line Development (CLD) process in a targeted integration (TI) CHO CLD system. Our findings show that (1) earlier single cell cloning (SCC) of transfection pools, (2) speeding up initial titer screening turnaround time, (3) starting suspension adaptation of cultures sooner, and (4) maximizing the time CHO cultures spend in the exponential growth phase can reduce CLD timelines from ~4 to ~3 months. Interestingly, SPEED-MODE timelines closely match the theoretical minimum timeline for CHO CLD assuming that CHO cell division is the rate limiting factor. Clones obtained from SPEED-MODE CLD yielded comparable titer and product quality to those obtained via a standard CLD process. Hence, SPEED-MODE CLD is advantageous for manufacturing biotherapeutics in an industrial setting as it can significantly reduce CLD timelines without compromising titer or product quality.

KEYWORDS
cell line development (CLD), exponential growth phase, suspension adaptation, targeted integration (TI)

1 | INTRODUCTION

At Genentech, we aim to deliver new medicines to patients with unmet medical needs fast and at the lowest cost possible. Although there are many challenges associated with developing life-saving drugs, reducing timelines at every stage of the process (from research to manufacturing) has a significant impact on reducing cost and faster delivery of drugs to patients. Chinese hamster ovary (CHO) cells are the preferred protein expression platforms across the industry with almost 80% of all biotherapeutics being expressed using this

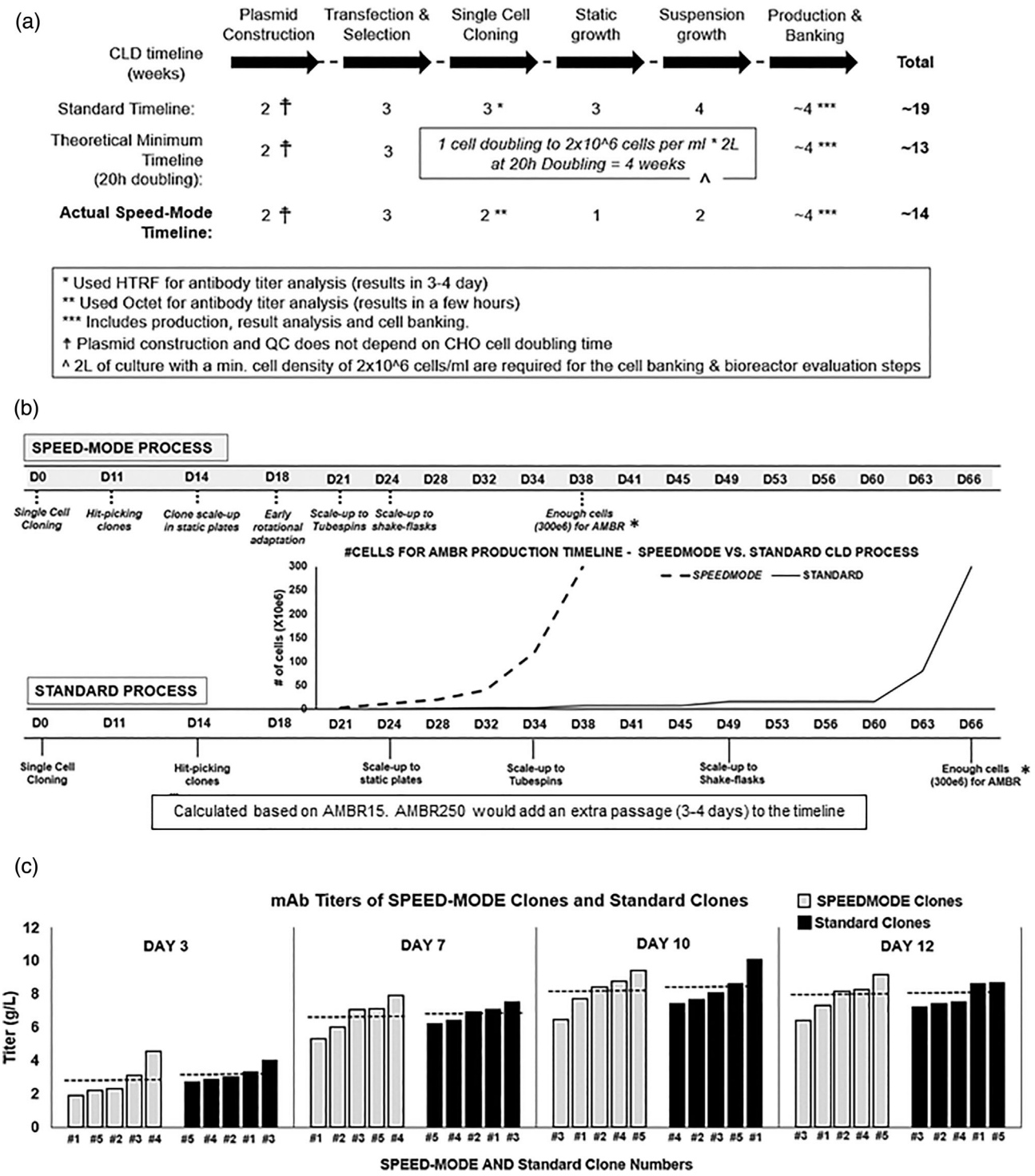

FIGURE 1 SPEED-MODE CLD generates clones with comparable titers to the standard process. (a) Schematic depiction of standard, SPEED-MODE, and theoretical CLD timelines. (b) Detail schematic of CLD steps and important milestones achieved for standard and SPEED-MODE CLD approaches. (c) Titer measurements (g/L) for days 3, 7, 10, and 12 cultures for standard and SPEED-MODE clones after a 12-day fed-batch production.

mammalian expression system. Among these biotherapeutic molecules, mAbs dominate the market and encompass more than 50% of the total sales value.[1,2] Besides IgG1, many other new molecular formats and modalities such as bispecific antibodies, antibody-drug conjugates (ADCs), and antigen binding fragments (Fabs), seem to gain momentum across the industry.[3] With more than 200 antibody-based molecules being approved around the globe as of June of 2022, CHO mammalian expression remains the ideal system for expressing many different therapeutic modalities.[2,3] However, manufacturing biotherapeutics is very costly and time consuming with various challenges that

could impact the timeline of biologics entering clinical trials,[4] hence, reducing timelines and being first to market is critical for faster delivery of life-saving medicines to patients and gaining market advantage.[5,6]

Chemistry Manufacturing and Controls (CMC) activities often begin with cell line development (CLD) and proceeds all the way to production and release of drug substance and drug product for Phase I clinical trials, a process which can take upwards of 1–2 years for a mAb product.[7] Shortening these timelines without compromising product quality or titer is an effective way to deliver drugs faster and perhaps, at a reduced cost. The CLD process takes several months to complete and it starts with transfection of expression vectors followed by single cell cloning, extensive clone screening to identify the lead clones, and cell banking.[8] In the targeted integration (TI) CLD process, vector(s) expressing transgene(s) of interest are integrated into a predetermined and transcriptionally-active genomic hotspot and this provides many advantages compared to random integration (RI) CLD processes. These include generation of highly stable clones, abolishing the need for screening large numbers of clones, and hence reducing the overall CLD timelines and labor compared to the RI process.[9,10] However, further improvements to strike a balance between speed, process robustness, reliability, and product quality is always desired. Normally, the standard TI CLD for a mAb takes approximately 18–20 weeks. This includes plasmid construction, transfection and stable pool generation followed by single cell cloning, clone screening, and identification of the lead clone.[11] To shorten CLD timelines, we first estimated the theoretical fastest timeline possible based on the doubling time (20 h on average) of our CHO host cells which is estimated to be about 12.5–13 weeks beginning from plasmid construction to production and cell banking, approximately 4–6 weeks shorter than the standard TI CLD timelines (Figure 1a).

Based on this, we identified specific rate-limiting steps in the standard TI CLD that imposed unnecessary delays to the process. We focused on two main areas: (A) automated single cell cloning (SCC) and clone selection, which involved SCC, hitpicking and static culture growth, and (B) suspension adaptation and scale up, which involved adapting the cells to grow in suspension and culture scale up followed by a production screen and assays to identify the top clones. The automation portion of TI CLD followed by static scale-up took about 6 weeks with the rate-limiting step being the turnaround time for the initial Homogeneous Time Resolved Fluorescence (HTRF) titer assay post clone hitpicking on week 2. The turnaround time (from sample submission to getting results back) for the HTRF assay was approximately 3–4 days, which on average delayed the clone expansion time frame by an additional 3–4 days. Therefore, the top clones were not scaled up to the next vessel (a 24-well static plate) until week 3. We hypothesized that such delays may result in cells transitioning from the exponential growth phase into stationary phase, which can slow down cell growth during the scale up step. To mitigate this, we decided to perform SCC on the transfection pool as soon as possible and use an OCTET system for titer analysis with a turnaround time of only a few hours. It was estimated that with this approach we could potentially expand the top clones from 96-well plates after one passage, ensuring that the cells were maintained in an exponential growth phase.

Suspension adaptation and scale-up portion of the CLD process on average took about 8 weeks (Figure 1a). To shorten this, we decided to start suspension adaptation earlier and maintain and expand CHO cultures at the exponential growth phase throughout the CLD process. Early suspension adaptation involved switching from 24-well static plates directly to 24 deep-well blocks instead of using an intermediary 6-well static plate. We hypothesized that by starting suspension adaptation earlier and performing culture scale-up at an exponential growth phase without discarding any cells, we should be able to achieve the culture volumes required for production and cell banking significantly faster than the standard CLD process.

2 | MATERIALS AND METHODS

2.1 | Cell culture conditions and TI CLD

CHO cells were routinely cultured in the in-house (DMEM/F12 based) serum free media at a seeding density of 0.4 million cells/mL in shake-flasks at 37°C, 5% CO_2, a shaking speed of 150 rpm and split every 3–4 days as previously described.[9] Transfection & Stable Pool Generation was performed using: Front and Back expression vectors each having one copy of mAb-X heavy chain (HC) and two copies of mAb-X light chain (LC) with each HC or LC subunit under the control of cytomegalovirus (CMV) promoter. Front and back vector configurations were: HC-LC-LC (2 copies of heavy chain and 4 copies of light chains total). These vectors were then transfected into the CHO TI host via recombinase mediated cassette exchange (RMCE) as described in Reference 12. Transfections were performed using Maxcyte STX Electroporation (Maxcyte, Gaithersburg, MD) and stable pools were generated by puromycin selection.

2.2 | SCC and scale-up

SCC was performed by plating the cells at 1 cell/well in 384-well plates using CYTENA (Cytena SCP). For the SPEED-MODE process, clones were hitpicked in to 96-well plates on Day 11 based on confluence imaging (>25% confluency) and the top 12 clones per pool were immediately scaled to 24-well static plates on Day 14 based on in-house OCTET (Sartorius Octet HTX) titer analysis and image review.[13]

2.3 | Suspension adaptation and fed-batch production

The selected clones from static plates were scaled to 24-deep well blocks on Day 17 for early rotational adaptation in the SPEED-MODE process while in the standard process clones were expanded to 24-well and then 6-well static plates. Next, clones were scaled up

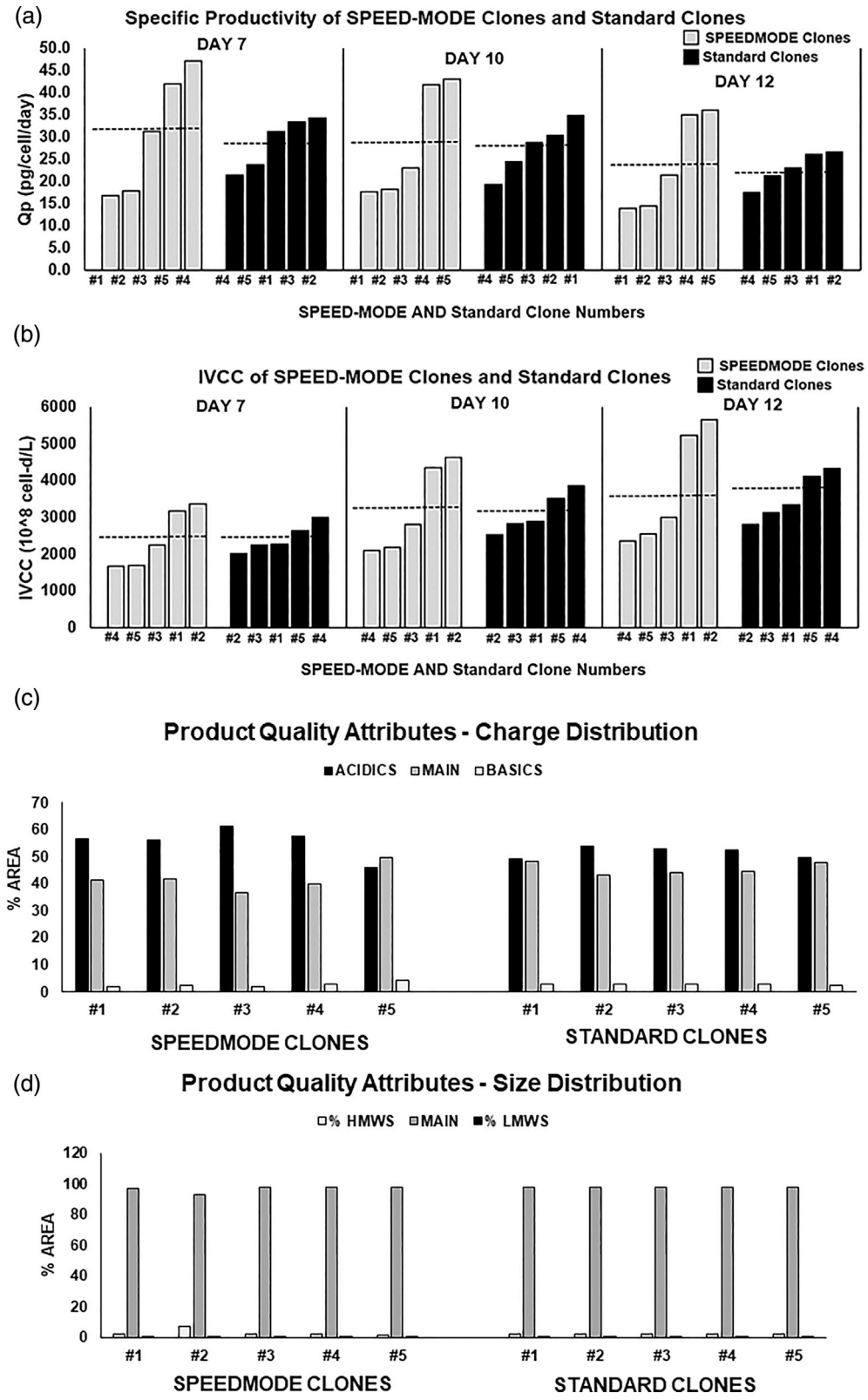

FIGURE 2 (a) Specific productivity, (b) growth, (c) charge distribution, and (d) size distribution for SPEED-MODE and standard CLD clones.

to tube-spins and shake flasks without discarding any cells for the SPEED-MODE process and the top 5 clones were selected for production screen based on initial titer and copy number analysis. During the 12-day production process in AMBR15 bioreactors (Sartorius Ambr® 15 Cell Culture),[9,12] SPEED-MODE clones and clones generated by the standard CLD process were evaluated side-by-side for cell growth, viability, specific productivity, titers, and product quality. The AMBR15 production run was performed as described.[14] Product quality attributes such as protein charge and aggregation were measured using in-house developed assays such as imaged capillary

isoelectric focusing (icIEF) and size exclusion chromatography (SEC), respectively.[15]

3 | RESULTS & DISCUSSION

Genentech's TI CHO cells utilize integration of two separate expression plasmids into a predetermined and transcriptionally-active hotspot within the CHO genome where the promoter and cDNA of the selection marker are split between the two plasmids.[9,12] This ensures accurate recombination of expression vectors into the TI site, permits optimization of gene dosage/position for each subunit, and enables systematic investigation of each subunits' impact on protein folding, secretion, and ultimately titer.[9,10,12] Generally, the TI CLD timelines from plasmid construction to cell banking takes approximately 18–20 weeks. However, based on an estimated theoretical cell doubling time of 20 hours per cell division for CHO cells, this process should only take about 12 to 13 weeks starting from plasmid construction to cell banking (Figure 1a). Several rate-limiting steps in the CLD process were identified and streamlined. These included: (1) performing SCC immediately after pool recovery, which reduced the timeline by 0.5 weeks (Figure 1a), (2) replacing HTRF assays with OCTET for initial titer analysis to identify top clones, which shortened the process by 1–1.5 weeks (Figure 1a), (3) switching to 24-deep well shaking blocks enabled faster suspension adaptation of CHO culture, shortening the culture expansion timelines from hitpicking to tube-spin by half for the SPEED-MODE (10 days) versus standard (20 days) CLD process (Figure 1b), and finally (4) maintaining the cultures in an exponential growth phase allowed expanding the cultures from tube-spin to the required volumes (target of 300 million cells) to perform production assay in approximately 2.5 weeks for SPEED-MODE compared to 4.5 weeks for the standard CLD process (Figure 1b). The latter was made possible by timely expansion of all the viable cells in the suspension culture to keep the cells in exponential growth phase and avoiding discarding cells unnecessarily. Indeed, incorporating all of these process optimizations demonstrated that CLD timelines could be reduced significantly. SPEED-MODE CLD could be completed in 14 weeks as opposed to the standard CLD approach, which on average takes ~19 weeks, translating to a time savings of 4–5 weeks (Figure 1a,b).

To confirm whether the process optimizations that were implemented during the SPEED-MODE approach impacted the final clone productivity and product quality, the top 5 clones generated by either standard or SPEED-MODE CLD processes were evaluated side by side in an Ambr® 15 Cell Culture production run. SPEED-MODE clones had, on average, comparable titers to the standard clones throughout the course of production, with titers ranging from 6 to 9 g/L for SPEED-MODE clones and 7–8.5 g/L for standard clones on Day 12 of production (Figure 1c). Similarly, the specific productivities (Figure 2a) and growth rates (Figure 2b) were on average comparable between SPEED-MODE and standard clones, with the SPEED-MODE clones displaying somewhat of a wider range for specific productivity and growth among the clones (Figure 2a,b). Furthermore, the product quality attributes such as charge distribution (Figure 2c, and Table 1), size distribution (Figure 2d) and glycosylation profiles (Table 2) were on average comparable between the SPEED-MODE and standard clones. The variations in the levels of charge, aggregation, or glycan species observed between different clones are within the expected range (which is about 10%–15%). These observed differences between different clones can be attributed to assay error range and/or normal culture variations, irrespective of CLD process (SPEED-MODE or Standard). This demonstrates that the faster timelines of the SPEED-MODE process does not compromise key cell culture performance indicators and product quality attributes.

TABLE 1 Charge distribution of SPEED-MODE and standard clones.

	Acidics	Main	Basics
SPEED-MODE CLONE #1	56.77	41.29	1.94
SPEED-MODE CLONE #2	56.1	41.58	2.32
SPEED-MODE CLONE #3	61.06	36.92	2.02
SPEED-MODE CLONE #4	57.44	39.91	2.65
SPEED-MODE CLONE #5	46.04	49.82	4.14
Average for SPEED-MODE clones	55.48	41.90	2.61
STDEV for SPEED-MODE clones	5.6	4.8	0.9
STD CLONE #1	49.05	48.13	2.83
STD CLONE #2	53.65	43.41	2.93
STD CLONE #3	52.91	44.26	2.83
STD CLONE #4	52.45	44.78	2.77
STD CLONE #5	49.77	48	2.22
Average for Standard clones	51.57	45.72	2.72
STDEV for Standard clones	2.0	2.2	0.3

The SPEED-MODE process also has an impact on reducing overall CLD costs compared to the standard process. One of the key areas where we observed a significant impact on cost reduction is in the automation portion of the process. Performing SCC immediately after pool recovery reduced the time the pools were in culture, saving media, resources and labor required for maintenance of these cultures. Next, by early hit-picking and using the OCTET assay, we saved on the materials and resources costs associated with performing the HTRF assay. Additionally, since the OCTET assay has a faster turnaround time, it enabled a speedy transition to scale up clones for suspension growth, further saving time and costs associated with clone maintenance. Finally, clone expansion post suspension adaptation was also significantly shorter compared to the standard process, thus saving costs associated with media, cell culture materials and labor. Altogether, our study highlights that SPEED-MODE approach saves CLD time, resources, and albeit associated costs compared to the standard CLD process.

TABLE 2 Glycan profile of SPEED-MODE and standard clones.

	% AFUCOSYLATION	% MAN5	%G0 F	% G1 F	% G2 F	% Major glycan species
SPEED-MODE CLONE #1	4.8	2.0	62.3	22.2	2.9	94.3
SPEED-MODE CLONE #2	4.7	2.2	64.1	22.0	2.8	95.8
SPEED-MODE CLONE #3	5.3	1.9	60.3	24.3	2.7	94.5
SPEED-MODE CLONE #4	5.5	2.2	62.0	20.9	2.4	93.0
SPEED-MODE CLONE #5	9.0	3.9	56.9	23.5	2.8	96.1
Average for SPEED-MODE Clones	*5.9*	*2.4*	*61.1*	*22.6*	*2.7*	
STDEV for SPEED-MODE Clones	1.8	0.9	2.7	1.3	0.2	
STD CLONE#1	5.2	1.8	60.4	24.5	3.2	95.0
STD CLONE#2	4.9	2.1	56.2	27.6	3.7	94.5
STD CLONE#3	5.1	2.3	55.6	27.8	3.6	94.4
STD CLONE#4	4.2	1.6	63.2	22.8	2.5	94.3
STD CLONE#5	4.4	1.6	66.3	21.0	2.1	95.5
Average for Standard Clones	4.8	1.9	60.4	24.7	3.0	
STDEV for Standard Clones	0.4	0.3	4.6	3.0	0.7	

4 | CONCLUSION

Various strategies have been proposed to accelerate the drug development process when the need for speed is important, for example during pandemics, and reducing CLD timelines constitutes the first and most critical step in such strategies.[6] In this context, use of Genentech's TI CHO host has specifically been advantageous because TI CLD is less labor intensive, faster, and enables seamless isolation of stable high titer clones compared to random integration (RI) CLD processes.[9,16,17] Such advantages are indeed critical for expression of complex or difficult to express molecules, however, improving CLD timelines via the SPEED-MODE process furthers the benefits of TI CLD approach. Our data confirms that TI CLD timelines can indeed be shortened by as much as 4–5 weeks via the SPEED-MODE process, which simply entails: (1) performing SCC immediately after pool recovery, (2) quick turnaround for initial titer screening results, (3) earlier start of suspension adaptation, and (4) maintaining cultures in exponential growth phase post suspension adaptation. Considering that CLD constitutes a significant portion (on average 30%–50%) of DNA to IND timelines, shortening CLD timelines by over 1 month can substantially reduce DNA to IND timelines and help with providing life-saving medicine to patients faster.

AUTHOR CONTRIBUTIONS

Kavya Ganapathy: Investigation; writing – original draft; methodology; validation; visualization; data curation. **Cynthia Lam:** Investigation; methodology; validation; visualization; writing – review and editing. **Joni Tsukuda:** Investigation; methodology; validation; visualization; writing – review and editing; data curation. **Alyssa Sargon:** Investigation; methodology; validation; visualization; data curation. **Adrian Nava:** Investigation; methodology; validation; visualization; writing – review and editing; data curation. **Peter Harms:** Investigation; methodology; validation; visualization; writing – review and editing; data curation. **Amy Shen:** Writing – review and editing; visualization; validation; methodology; supervision. **Gavin Barnard:** Conceptualization; investigation; methodology; validation; visualization; writing – review and editing; supervision. **Shahram Misaghi:** Conceptualization; investigation; writing – original draft; methodology; validation; visualization; writing – review and editing; supervision; data curation.

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CONFLICT OF INTEREST STATEMENT

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PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/btpr.3479.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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