A SMART Platform for Scalable Biotherapeutic Development: cDNA to 200L Single-use Stirred-tank Bioreactor

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Introduction

Establishing a platform for developing and manufacturing therapeutic proteins will eliminate the bottleneck between cell-line development and tech transfer. The Celltheon SMART™ Technology Platform in combination with Pall Biotech's manufacturing equipment accelerates time from cDNA to pilot scale with a pre-defined fed-batch and downstream processing platform for monoclonal antibodies and difficult to express therapeutic proteins. In this case study, Celltheon demonstrates a workflow established to develop an RCB followed by scale-up and reproducible product quality from bench to pilot scale in the 200L Allegro STR single-use bioreactor.

CURRENT INDUSTRY NEEDS	CELLTHEON SMART™ PLATFORM
COST OF MANUFACTURING	LOWER COST OF GOODS
FASTER TIME TO MARKET	cDNA to RCB IN 16 WEEKS
HIGH TITERS	STABLE POOLS: 1.5g/L; SINGLE CLONES: 4-5g/L
PROCESS SCALABILITY	TECH TRANSFER PLATFORM TO 200L
CRITICAL QUALITY ATTRIBUTES	CQAs MATCHING BETWEEN 3L AND 200L STR
CELL LINE STABILITY	>90% OF CLONES STABLE FOR >120 GENERATIONS

Materials and Methods

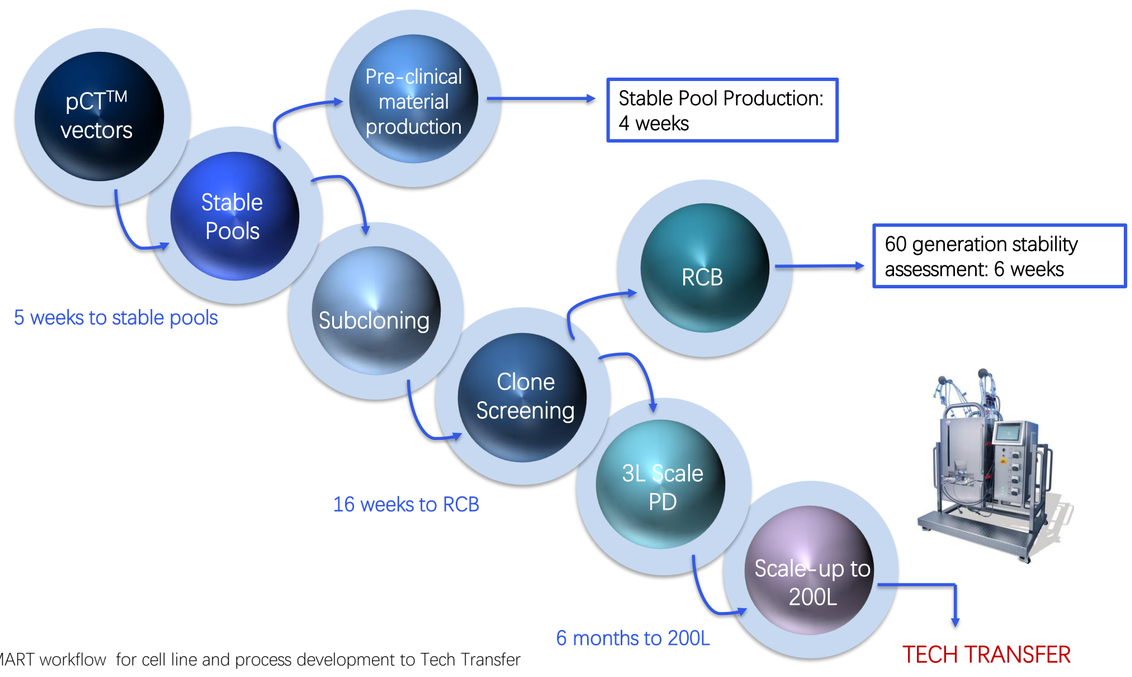
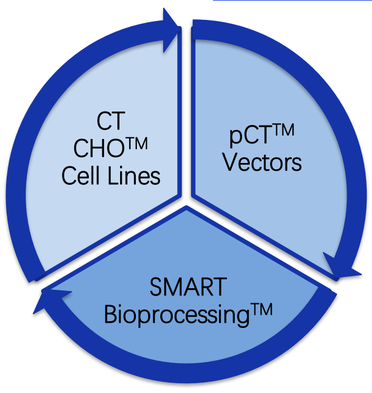


Fig 1. SMART workflow for cell line and process development to Tech Transfer

Celltheon SMART™ Technology Platform includes:



- CHO-K1 derived cells, fully documented host cell line
- No clumping, 18 h doubling time, low lactate and ammonia
- 97% monomer protein produced
- Patented RNA and DNA elements (*CT Stabilizer™ Element*)
- Cell line stability: 120+ generations
- Clone Titers: >4-5g/L
- Pre-defined commercial media/ feed platform
- Optimized process parameters identified at 3L scale
- Integrated scale up equipment: Allegro STR 200L, STAXmAx

Celltheon SMART™ Technology Output

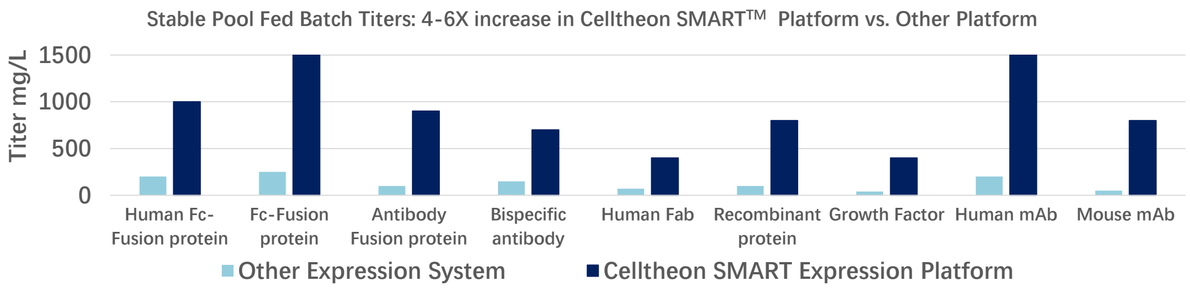


Fig 2. CT-CHO™ cells have been optimized for high expression of hard to express proteins via directed evolution technologies. In addition, Celltheon's novel genetic elements (such as the CT Stabilizer™ Element) vastly improve expression with no additional PD.

Impact of Celltheon Genetic Elements on clone distribution, Qp and clone stability

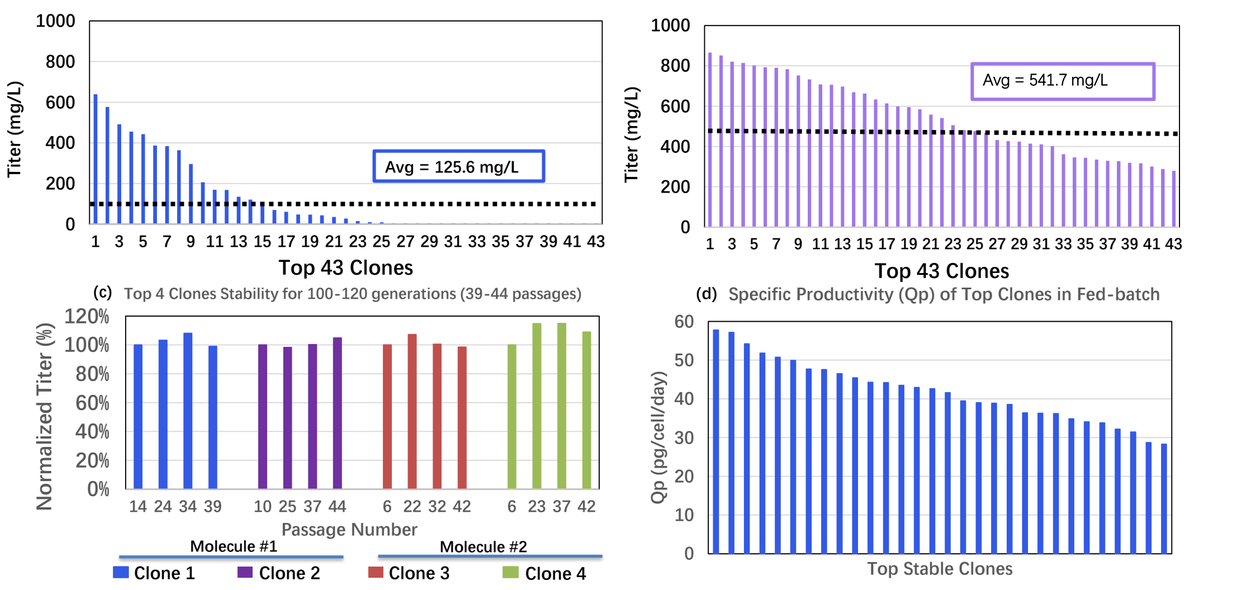


Fig 3 a, b, c & d.: To further compare the impact of Celltheon's novel genetic element (CT Stabilizer™ Element) one stable pool from each platform expressing the same protein was subcloned and top clones were selected from each for assessment in batch culture (a) (-) CT Stabilizer™ Element and (b) (+) CT Stabilizer™ Element. Clones obtained from the pool with (+) CT Stabilizer™ Element were homogenously high-expressing and had 4x higher titer on average. (c) The top 2 clones from two different molecules expressed in the Celltheon SMART™ Expression platform were further assessed for cell line stability from 10-120 generations (passages 6 – 44), All 4 clones were highly stable with <3% RSD. (d) The top 30 clones from (+) CT Stabilizer™ Element were further assayed using the platform fed-batch process, the Qp of the clones ranged from 28-58 pg./cell/day.

Result: Celltheon SMART™ Cell Line Development

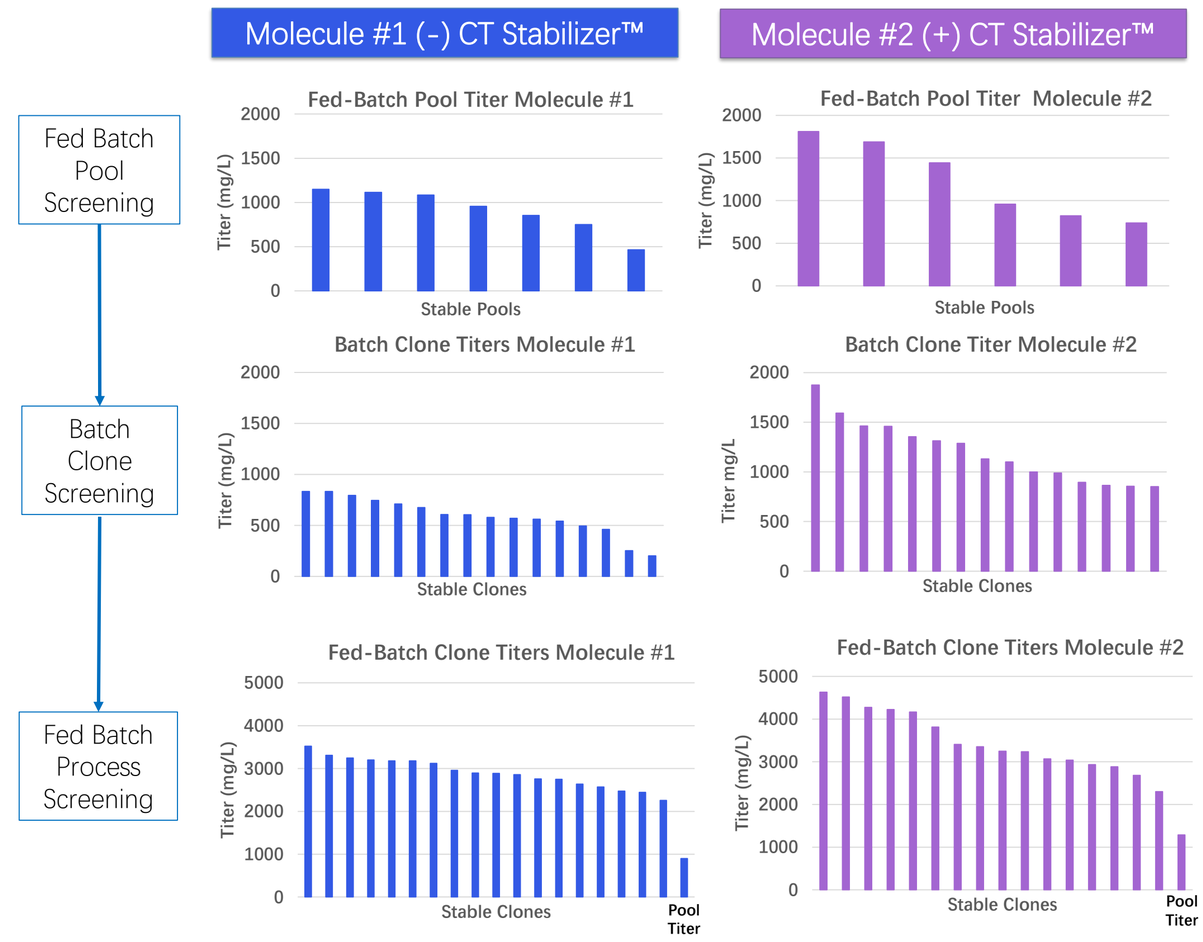


Fig 4: CT-CHO™ cells were transfected with 2 different mAbs that are known to yield similar titers. Full cell line development was performed, comparing the effect of a novel regulatory element (CT Stabilizer™) throughout development stages. At every stage, ~2-fold higher distribution in expression is observed using the novel CT Stabilizer™ element.

Scalability to Allegro 200L STR

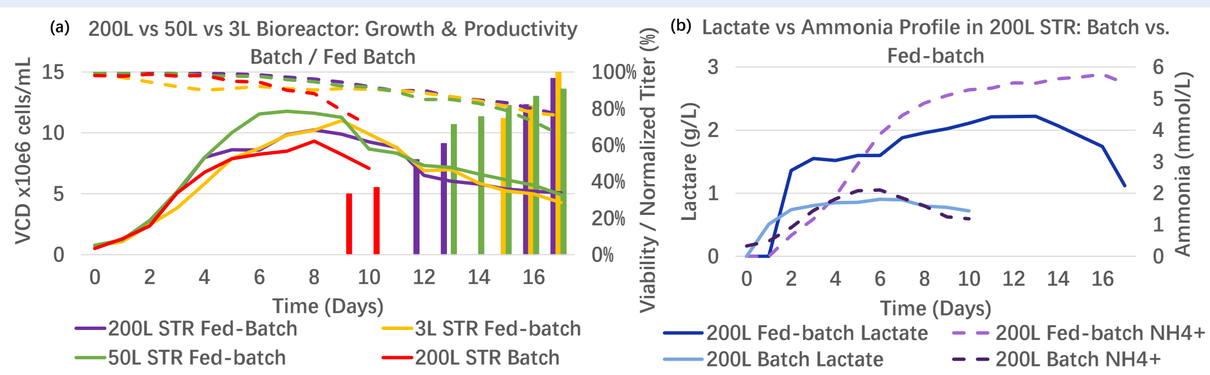


Fig. 5 a & b: Critical process parameters were established at 3L scale; the process was scaled up in a 50L STR and in the Allegro STR 200 in Batch and Fed-Batch. (a) Productivity and growth profiles were comparable at 3L, 50L and 200L pilot scale in fed-batch. (b) The established fed-batch process results in low ammonia (>6mM) and lactate production (>2.2g/L,) which is consumed toward the end of the culture.

Scaling up the 3L and 50L bioreactor process to Pall's Allegro STR 200 resulted in highly similar cell growth, titer and product quality profiles. The process was scaled up by P/V and process parameters were maintained between all three scales. The baffles and square design of the Allegro STR Single-Use Bioreactors enable higher kLa and P/V in order to reproduce optimized scale-down models.[4]

Product quality: 3L vs 200L STR

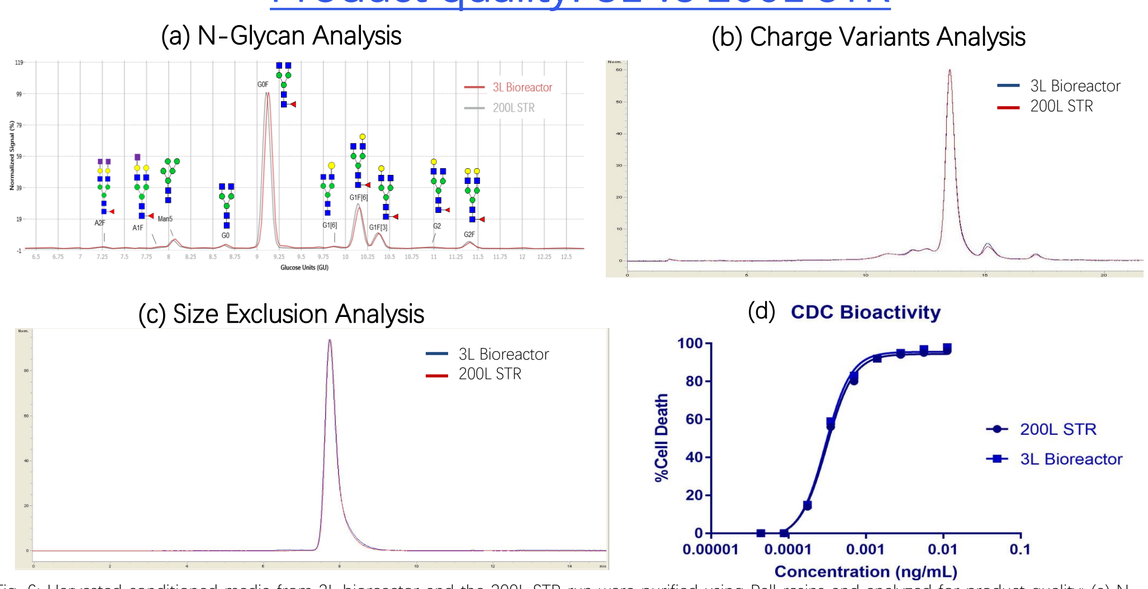


Fig. 6: Harvested conditioned media from 3L bioreactor and the 200L STR run were purified using Pall resins and analyzed for product quality: (a) N-Glycan assay (b) Charge variant Cation Exchange-HPLC (c) Size Exclusion- HPLC to evaluate aggregation (d) Cell based CDC assay to assess the functionality of the purified molecule. Result: Purified protein has same characteristics at 3L and 200L scale

Conclusion

- ✓ The Celltheon SMART™ Platform yields homogenously, high-expressing clones (**4.5g/L**) with high specific productivity (**30-60pg/cell/day**) and >95% of clones are stable for **100-120 generations.**
- ✓ Pall's Allegro STR Single-Use Bioreactors feature a novel, square design with baffles resulting in higher kLa and P/V, eliminating limitations in scale-up from bench to production scale.
- ✓ Described here is a SMART scalable platform that shows a process with **reproducible product quality, productivity and cell growth** profiles between 3L bench to 200L pilot scale STR.

- Integrated, scalable fed-batch process reduces timelines to **6 months – ready for tech transfer**
- Allegro STR SUBs: scalable production equipment eliminate limitations in production
- The Celltheon SMART™ Platform has been adopted and **licensed for several programs**

References

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