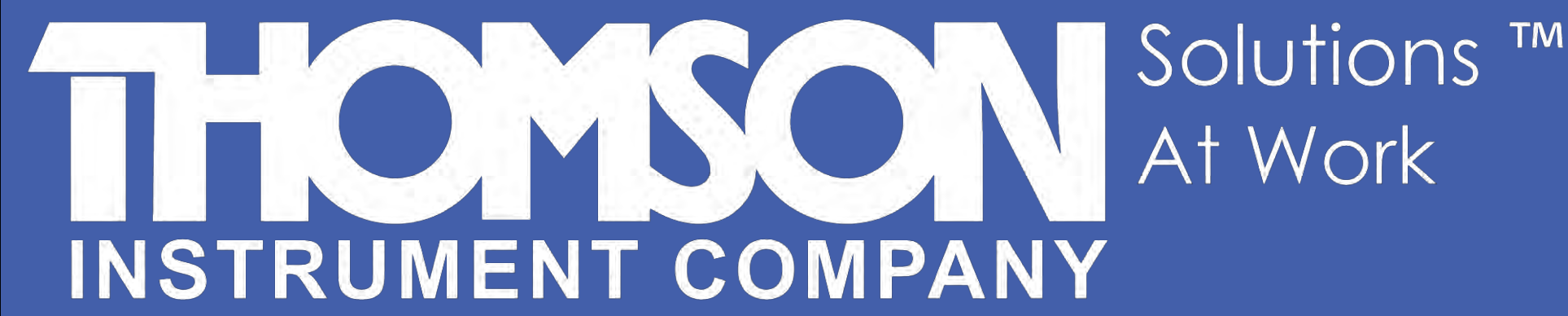


Bridging the Gap of Screening and Scale Up of Insect, CHO, Hybridoma, and HEK293 Cell Lines

Single Use Optimum Growth™ Flasks 125mL-5L Flasks with Transfer Caps, and Ports

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ABSTRACT

Optimum Growth™ Flasks (patented) give excellent growth with space saving capability. By using Optimum Growth™ Flasks users are able to grow 25mL-3L of Cell Culture versus the competitors max capacity of 1L. The Optimum Growth™ Flasks have replaced expensive, disposable, Fernbach flasks and also small Wave® Bags (5L & 10L). The Optimum Growth™ Flasks also give high viability cultures with a great use of space as shown by our data in Insect, CHO, Hybridoma, and HEK293 Cell Lines. Transfer Caps (patented) allow for the flasks to be used as seed culture for Cell Bags and Bioreactors. We will show data from GPCR Proteins, Soluble Proteins, and Antibodies.

INTRODUCTION

Thomson has recently introduced 125mL, 250mL, 500mL and 1.6L Optimum Growth™ Flasks to compliment the industry standard 5L Optimum Growth™ Flask. These smaller flasks allow enhanced growth with the same footprint as conventional flasks, but with up to 300% higher working volume for maximum shaker spacer utilization. With the addition of the smaller flask sizes, Thomson Optimum Growth™ Flasks are scalable from 125mL to 5L. Transfer Caps (patented) allow easy, contamination free inoculation of larger vessels directly from Optimum Growth™ Flasks.

2 Membrane Proteins Expressed in Thomson 250mL Optimum Growth™ and 500mL Corning® Flasks

Materials and Methods

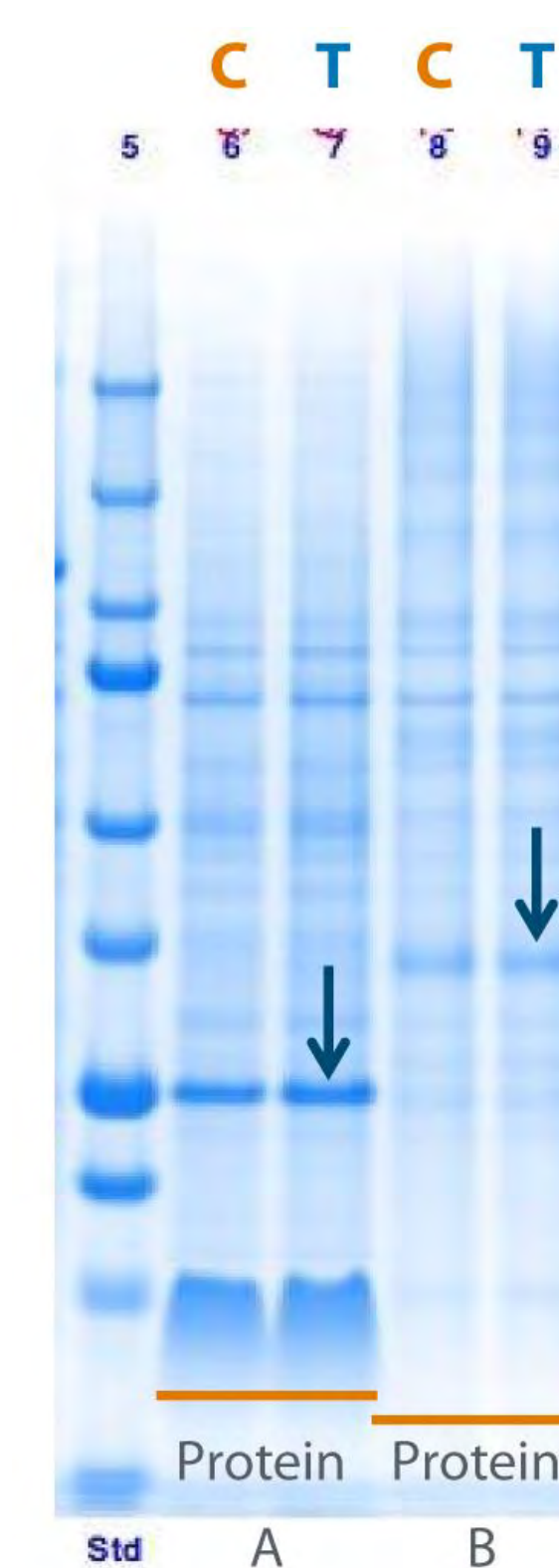
Genentech expressed two membrane proteins using 200mL of working volume in 500mL Corning® Flasks and 150mL of working volume in Thomson 250mL Optimum Growth™ Flasks. Protein A is a 34kd moderately expressing membrane protein. Protein B is a 45kd low expressing membrane protein. 4mL from each flask was purified over Ni-NTA and 12µL of each purified fraction was resolved on a Coomassie stained gel.

Results

Gel Analysis of Protein A & B Following Ni-NTA Purification

C= Corning® 500mL Flask

T= Thomson 250mL Optimum Growth™ Flask



Conclusions

The Thomson 250mL Optimum Growth™ Flasks produced as much comparable protein from 150mL of working volume as 200mL of working volume in the Corning® 500mL flasks with an improved working volume to footprint ratio.

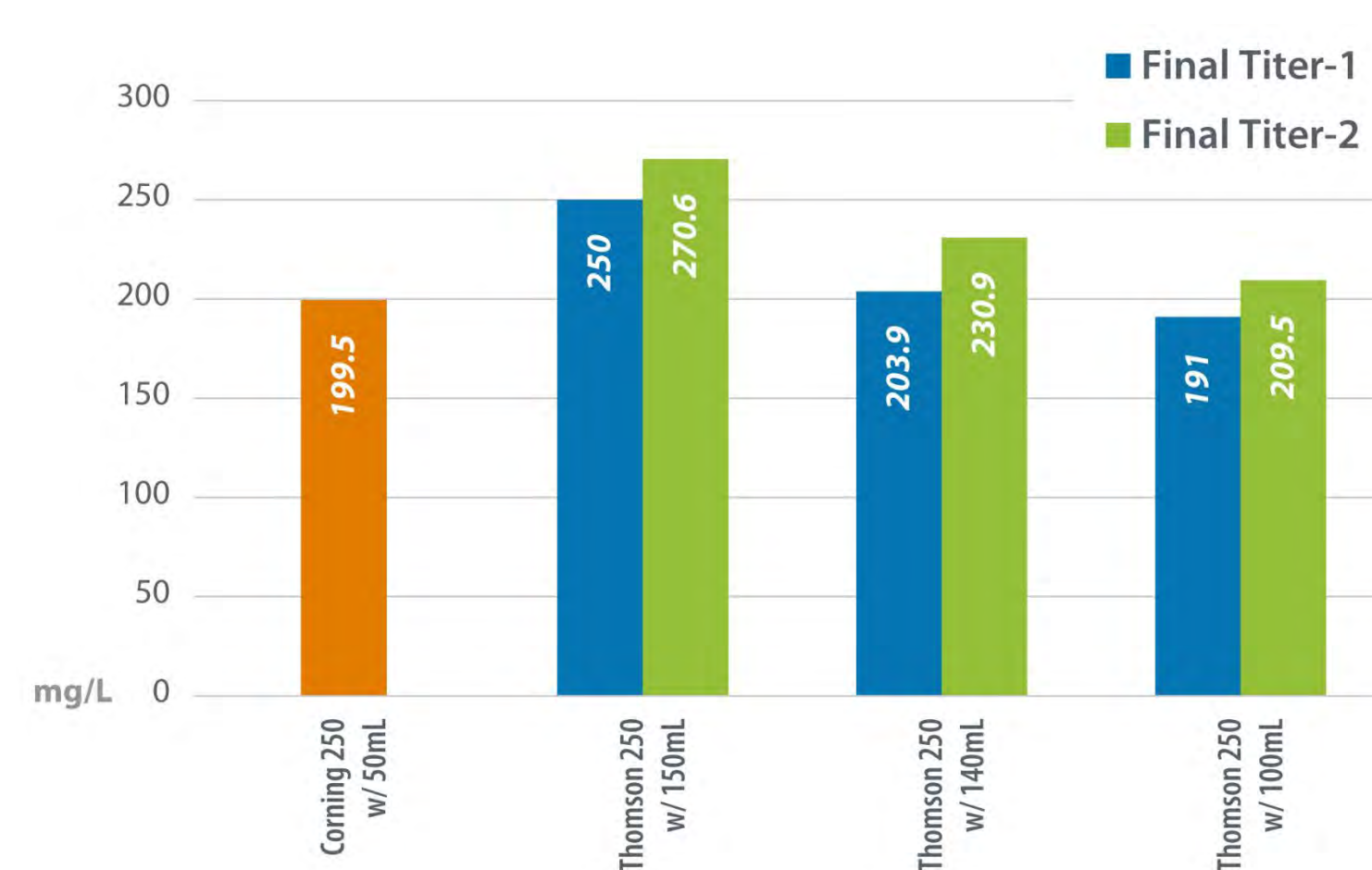
HEK 293 Transient Expressing Cells in Thomson 250mL Optimum Growth™ and 250mL Corning® Flasks

Materials and Methods

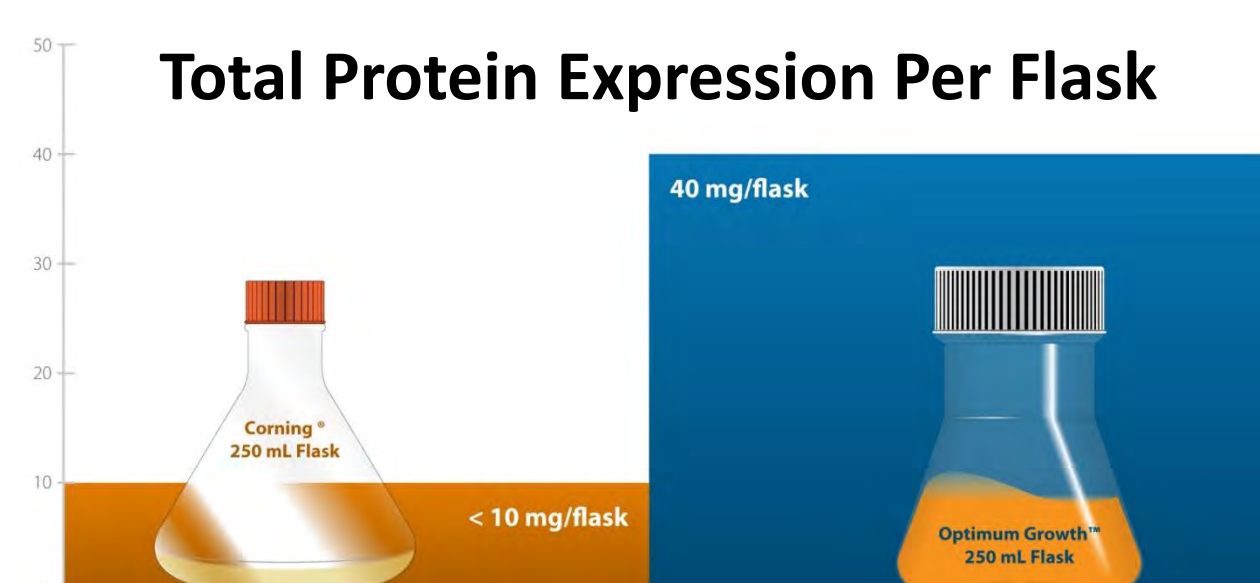
Amgen expressed protein in transiently transfected HEK 293 cells in Corning® 250mL flasks and Thomson 250mL Optimum Growth™ Flasks using Gibco® /Life Technologies™ media. The cells were transfected using PEI Max in F17 media. 50mL of working volume in a Corning® 250mL flask was compared to 100mL, 140mL, and 150mL of working volume in Thomson 250 mL Optimum Growth™ Flasks for titer, VCD, and viability. All flasks were shaken at 150RPM in a 1" orbit and fed on day 3 with glucose.

Results

HEK 293 Day 6 Titer – 150 RPM

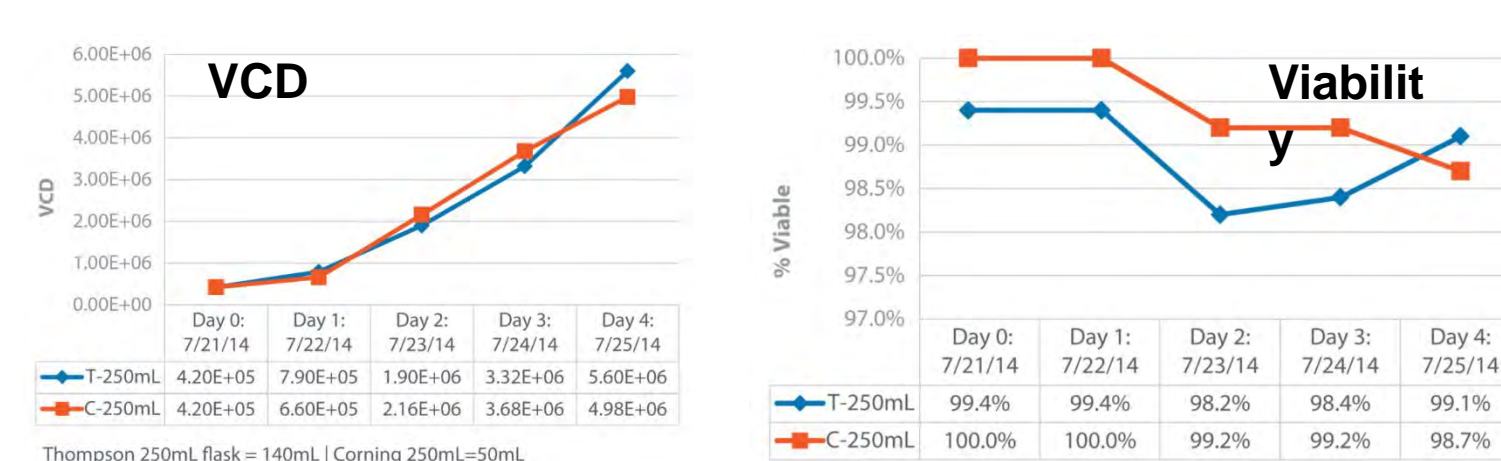


The Thomson Optimum Growth™ Flask had the highest final titer and was 25% higher than the titer of the 250mL Corning® flask.



Less than 10mg of protein was expressed in the 250mL Corning® flask and 40mg of protein was expressed in the Thomson 250mL Optimum Growth™ Flask

250mL Optimum Growth™ Flask-HEK 293 Growth Test



The VCD and cell viability of the Thomson 250mL Optimum Growth™ Flask with 140mL of working volume were comparable to the Corning® 250mL flask with 50mL of working volume.

Conclusions

The combination of the 25% increase in titer and 300% increase in working volume of the Thomson 250mL Optimum Growth™ Flask compared to the Corning® 250mL flask produced 400% more protein in the same amount of shaker space.

HEK 293 Transient Expressing Cells in Thomson 1.6L Optimum Growth™ and 3L Corning® Flasks

Materials and Methods

Amgen expressed protein in transiently transfected HEK 293 cells in Corning 3L flasks and Thomson 1.6L Optimum Growth™ Flasks using Gibco® / Life Technologies™ media. The cells were transfected using PEI Max in F17 media. 900mL of working volume in Corning® 3L flasks was compared to 900mL of working volume in Thomson 1.6L Optimum Growth™ Flasks for titer, VCD, and viability. The cells were fed with glucose on day 3.

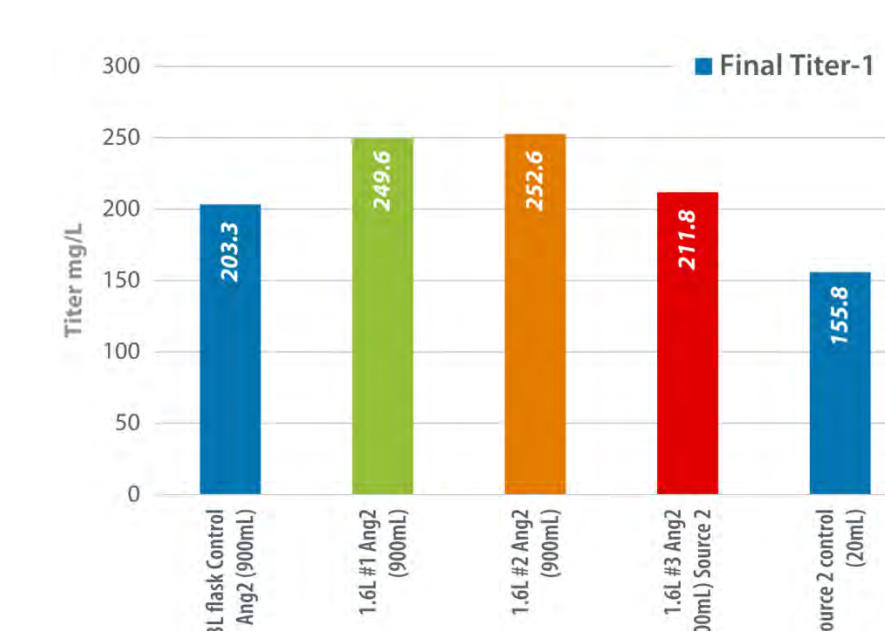
Results

18 Thomson 1.6L with 18L vs. 6 Flasks 3L Fernbach Flasks with 6L, Same Footprint



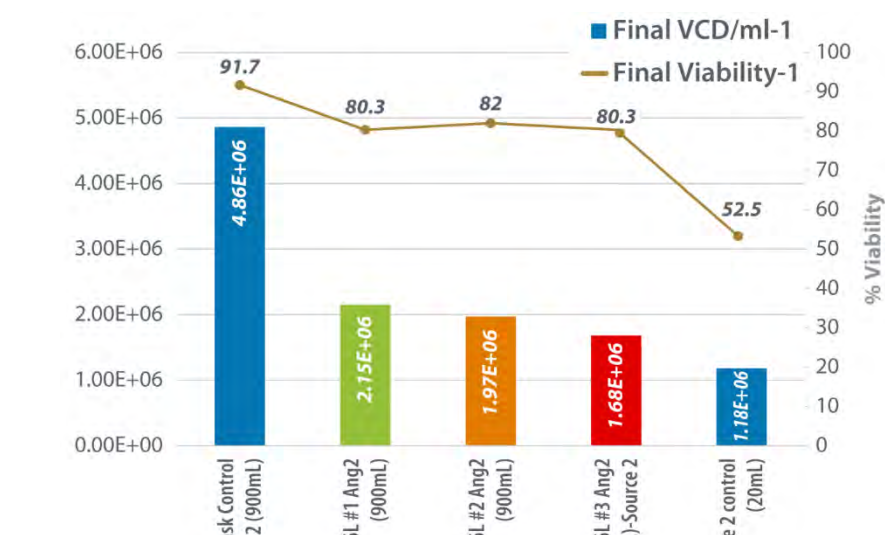
1.6L Optimum Growth™ Flask -HEK 293 Final Titer

*HEK 293 cells for the 1.6L #1 and #2 flask came from source flask #1. Cells from 1.6L #3 came from source flask #2.



The Thomson 1.6L Optimum Growth™ Flasks from source flask #1 had a 25% higher titer than the 3L Corning® control flask.

1.6L Optimum Growth™ Flask-HEK 293 Final Growth



The final cell viabilities of the three Thomson 1.6L Optimum Growth™ Flasks were 80.3%, 82%, and 80.3%. The final cell viability for the Corning® 3L flask was 91.7%.

Conclusions

The Thomson 1.6L Optimum Growth™ Flask provided 25% higher titers than the Corning® 3L control flask and maintained good cell viability. The small footprint of the 1.6L Optimum Growth™ Flask allows 18 flasks with a total of 16.2L of working volume to fit in an INFORS HT Multitron shaker compared to 6L of working volume in 6 Corning® 3L Flasks.

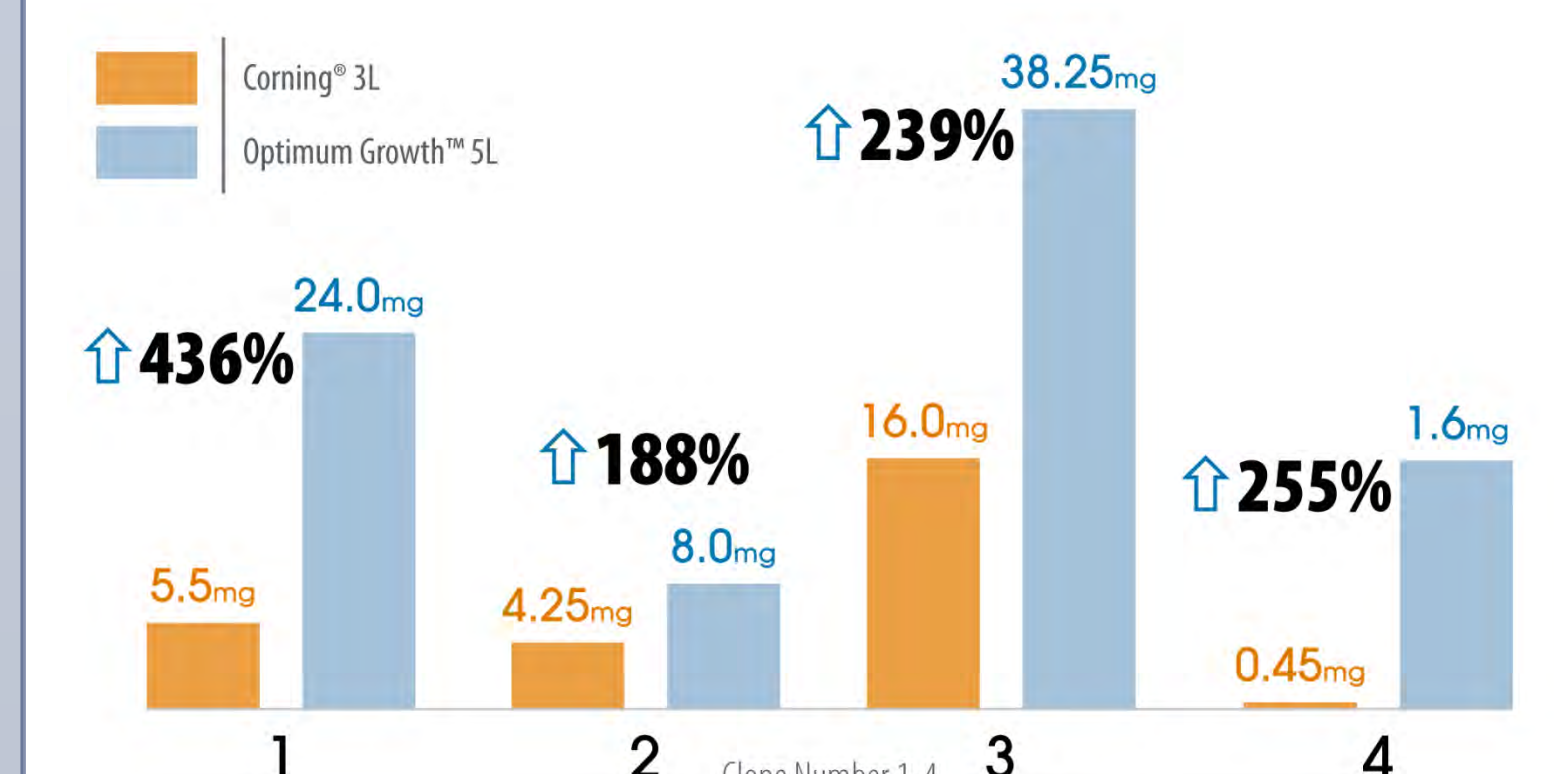
Insect Cells in Thomson 5L Optimum Growth™ and 3L Corning® Flasks

Materials and Methods

New York Structural Genomics compared protein expression in insect cells between two Corning® 3L Flasks with 2L of media in each flask totaling 4L and 3L of media in one Thomson Optimum Growth™ 5L flask. High Five cells at 1x10⁶/mL were infected with 500µL of P3 virus per liter of culture. The culture conditions were 100 RPM shaking speed, 50mm throw, and Express Hi5® media from Invitrogen™. Secreted targets were harvested at 96 hours post infection.

Results

214% Yield Increase From Insect Cells Protein Production/Flask



The average increase in protein production was 214% across 8 clones. Data from Clones 5-8 not shown.

Conclusions

- One Thomson 5L Optimum Growth™ Flask with 3L of working volume produced 214% more protein across eight clones than 4L of working volume in two 3L Corning® flasks. Using Thomson 5L Optimum Growth™ Flasks reduced the shaker space required by 50% while increasing titers.

Recommended Fill Volumes & Shake Speeds

CHO Stable Cells, CHO Transient, HEK 293 Transient

| Flask Size | Best Fill Volume | *RPM in 1"/2" |
|------------|------------------|---------------|
| 125mL | 63mL | 150/110 |
| 250mL | 150mL | 150/110 |
| 500mL | 250mL | 150/110 |
| 1.6mL | 900mL | 150/110 |
| 5L | 2.0L - 3.0L | 120/90 |

Hybridoma Cells

| Flask Size | Best Fill Volume | *RPM in 1"/2" |
|------------|------------------|---------------|
| 125mL | 36mL | 70/50 |
| 250mL | 75mL | 70/50 |
| 500mL | 150mL | 70/50 |
| 1.6mL | 480mL | 70/50 |
| 5L | 1.5L | 80/60 |

Insect Cells

| Flask Size | Best Fill Volume | *RPM in 1"/2" |
|------------|------------------|---------------|
| 125mL | 63mL-75mL | 150/110 |
| 250mL | 150mL | 150/110 |
| 500mL | 250mL | 150/110 |
| 1.6mL | 900mL | 150/110 |
| 5L | 2.0L - 3.0L | 135/90 |

Microbes/E.coli

| Flask Size | Best Fill Volume | *RPM in 1"/2" |
|------------|------------------|---------------|
| 125mL | 63mL | 250/110 |
| 250mL | 125mL | 250/110 |
| 500mL | 250mL | 250/110 |
| 1.6mL | 900mL | 250/110 |
| 5L | 2.0L - 3.0L | 250/150 |

* 1" orbit = 25mm and 2" orbit = 50mm

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