



ONCOLOGY  
IMMUNOTHERAPY  
PATHWAYS



- CELL LINE DEVELOPMENT
- CLONE SELECTION
- PROCESS DEVELOPMENT

# Accelerating the Biotech Value Chain by Implementation of High Through-Put Technologies

Torben P. Frandsen, Ph.D.

Vice President of Process Development

# Agenda

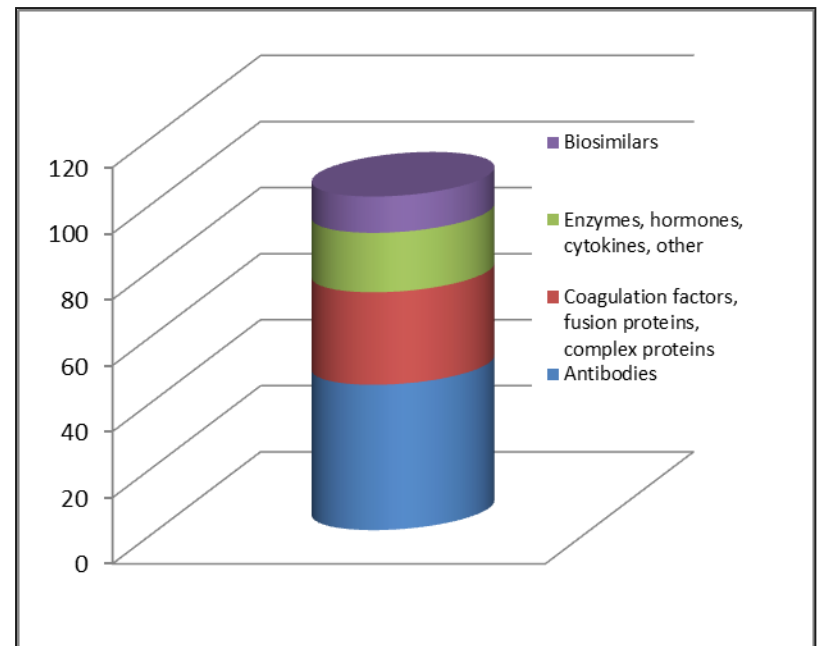
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- Introduction
  - Introduction to CMC Biologics
  - Typical timelines from DNA to IND
  - Value stream mapping and identification of bottlenecks
- The CHEF1® Technology
  - Elements of the technology and timelines
  - Pool production
- Implementation of High Through-Put Technologies
  - High through-put analytical methods
    - Octet Titer, HCP, and residual Protein-A
    - N-glycan profiling
  - AMBR 250
- Conclusions

# CMC Biologics Today

***CMC Biologics is the largest independent contract development and manufacturing organization of therapeutic proteins for your clinical and commercial production globally***

- 15 year track record of technical success in development and GMP manufacturing of therapeutic proteins
- World class GMP commercial manufacturing facilities, FDA and EMA approved
- Leader in the implementation of technologies that accelerate our customers time to market
- Devoted to our customers' satisfaction, we have an expanding portfolio of over 100 customers on five continents



# Global cGMP Manufacturing



## Copenhagen, Denmark

- ☐ Approved for commercial production by EMA and FDA
- ☐ Upstream, downstream and analytical development
- ☐ Mammalian capacity between 100L and 6000L
- ☐ Microbial capacity up to 3000L
- ☐ Bioreactor 3Pack™ Facility -- 3x2000L single-use cell culture line
- ☐ Experts in perfusion manufacturing



## Seattle (Bothell, WA)

- ☐ Approved for commercial production by EMA and FDA
- ☐ Upstream, downstream and analytical development
- ☐ Mammalian capacity between 3000 L and 12,000L using the 6pack™ technology
- ☐ Experts in bioassay and formulation technology



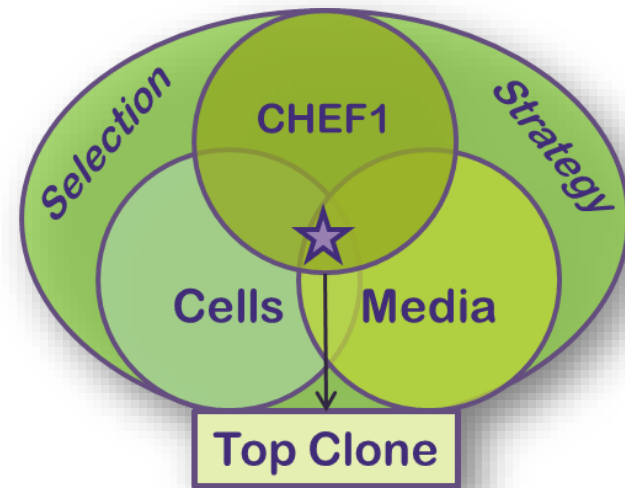
## Berkeley, CA

- ☐ Mammalian capacity between 500 L and 3000L
- ☐ Single-use cell culture line – 500L expanding to 2000L
- ☐ Expansion capacity for additional single-use bioreactor lines
- ☐ Cell banking capabilities
- ☐ Approved for early phase manufacturing

# Comprehensive Service Offering

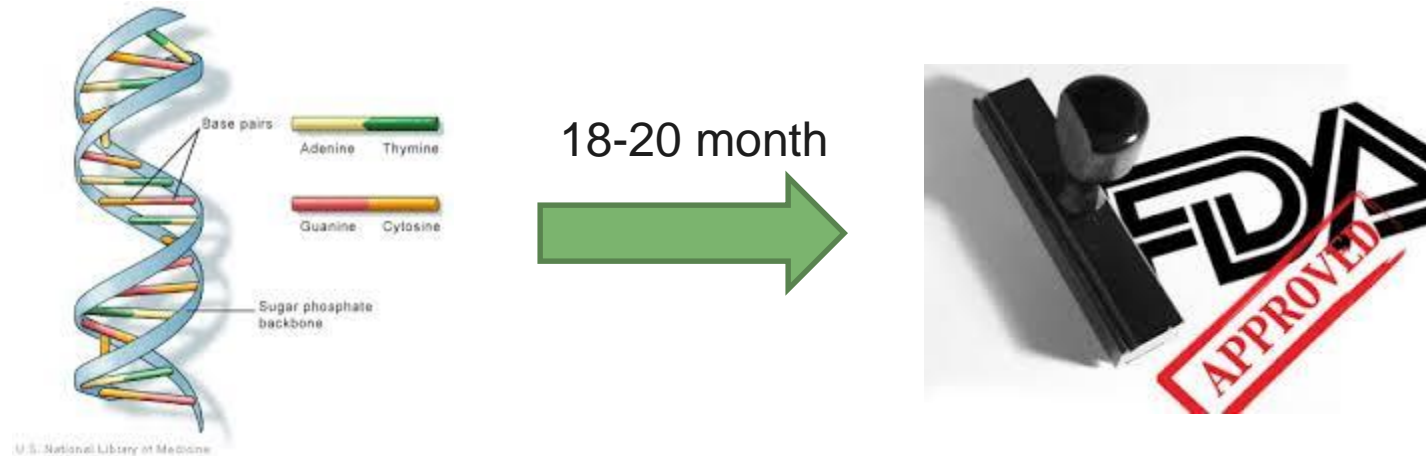
*End-to-end solutions of Integrated services taking your product from early-stage to commercial production*

- ❑ **Cell Line Development:** Creation of microbial and mammalian cell lines, CHEF1® expression technology platform
- ❑ **Process Development:** Upstream and downstream process development, process characterization and validation using QbD elements
- ❑ **Analytical Testing:** Analytical development and characterization, API and DP formulation development, stability studies, bioassays
- ❑ **Clinical Manufacturing:** Mammalian and microbial manufacturing of Tox and Phase I/II/III material according to EMA/FDA/JP/ICH guidelines
- ❑ **Commercial Manufacturing:** Mammalian and microbial manufacturing for market supply under EMA/FDA requirements
- ❑ **Quality Services:** QP release, QC testing and regulatory support
- ❑ **High Throughput Technologies:** CLD, PD, AD and Testing, Manufacturing: Enhanced Information, Accuracy, Efficiency, Reduced Timelines



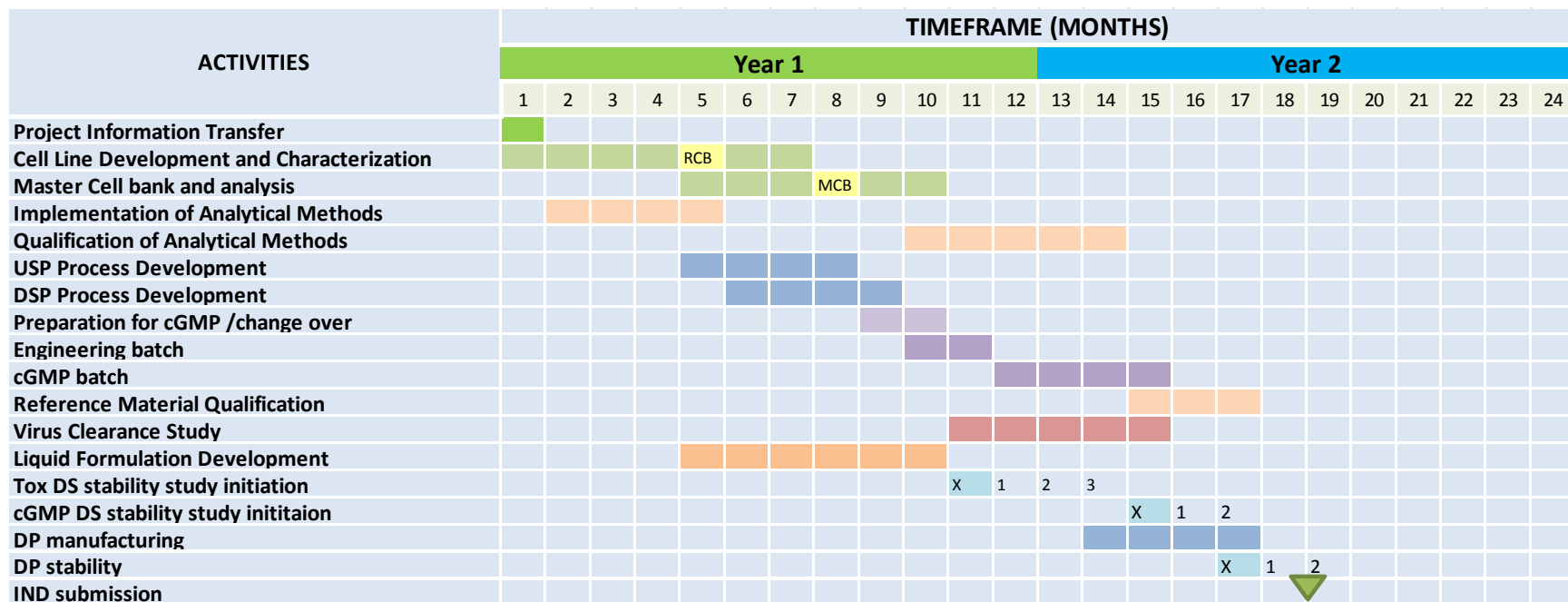


# Typical Timelines from DNA to IND



- A typical timeline from DNA to IND submission around 18-20 month
- Each program is different and the timeline and the extend of the CMC packages will depend on the demands imposed by the non-clinical and clinical development program

# Typical Timelines from DNA to IND





# High Throughput Process Development

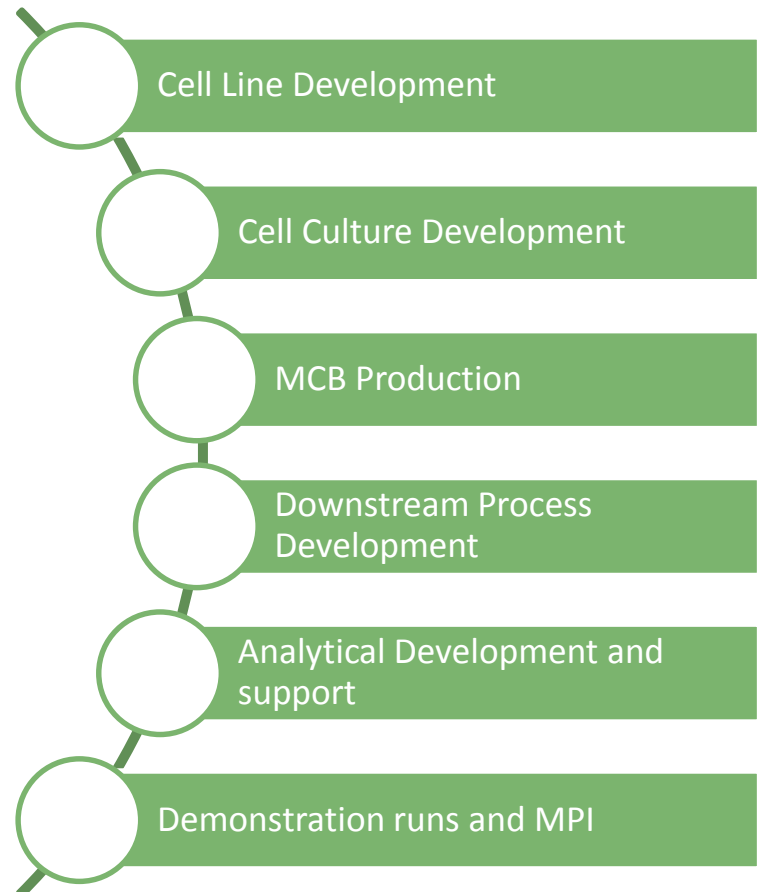
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## Scope – Speed to clinic for all molecules

- Phase I/II
- Leverage speed in CLD using CHEF1
- Equal or better productivity and quality
- Formulation development not included

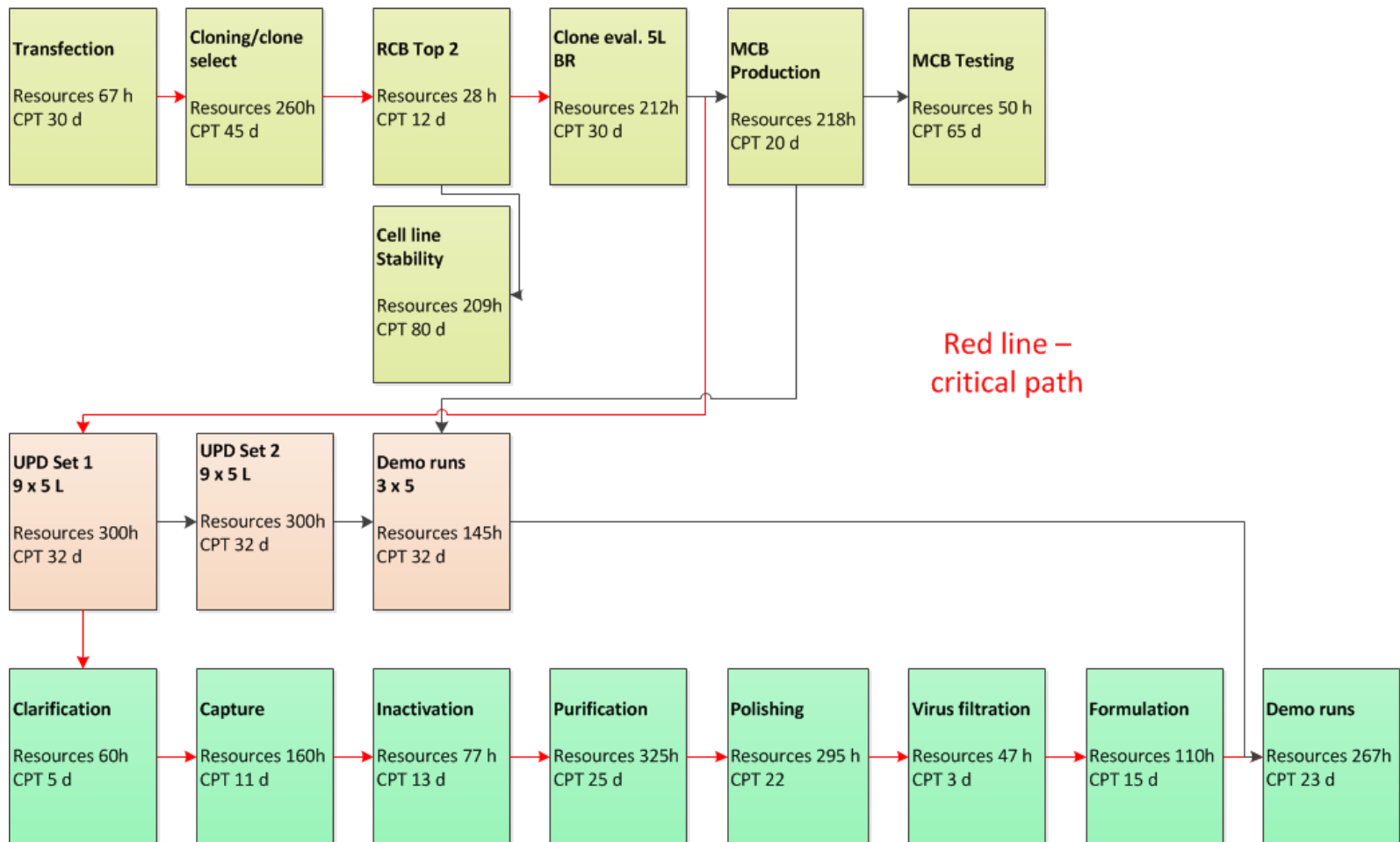
## Aim

- Time - Shorten timelines
- Cost - Reduce resources required /increase capacity
- Quality - Comply with industry best practice



# Value Stream Mapping Identifies Bottlenecks

## Current Development Process for phase I/II mAb project



# Process Development Bottlenecks

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- Analytics – 10 days from submission to results
  - Adds 90 days to timeline
- Bioreactors – limited number of reactors
  - Shake flask production models not scalable to bioreactors
- Chromatography – limited number of runs per day

# Solutions to Process Development Bottlenecks

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- Analytics – 10 days from submission to results
  - **HT analytical methods to support process development – 48 h response**
- Bioreactors – limited number of reactors
  - **High-throughput automated, disposable bioreactors - 24 reactors**
- Chromatography – limited number of runs per day
  - **Automated, small-scale chromatography using robotics - 8-16 columns in parallel**



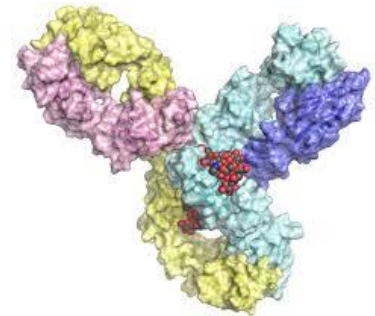
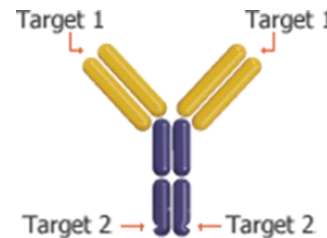
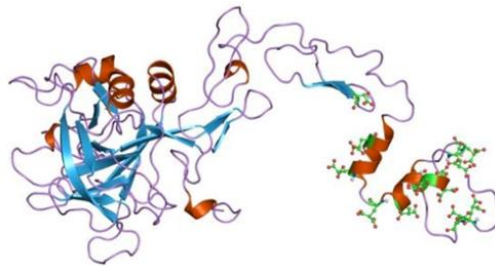
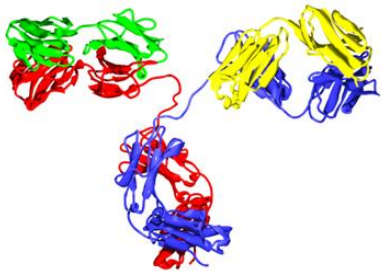
# Key Advantages of the CHEF1® Technology

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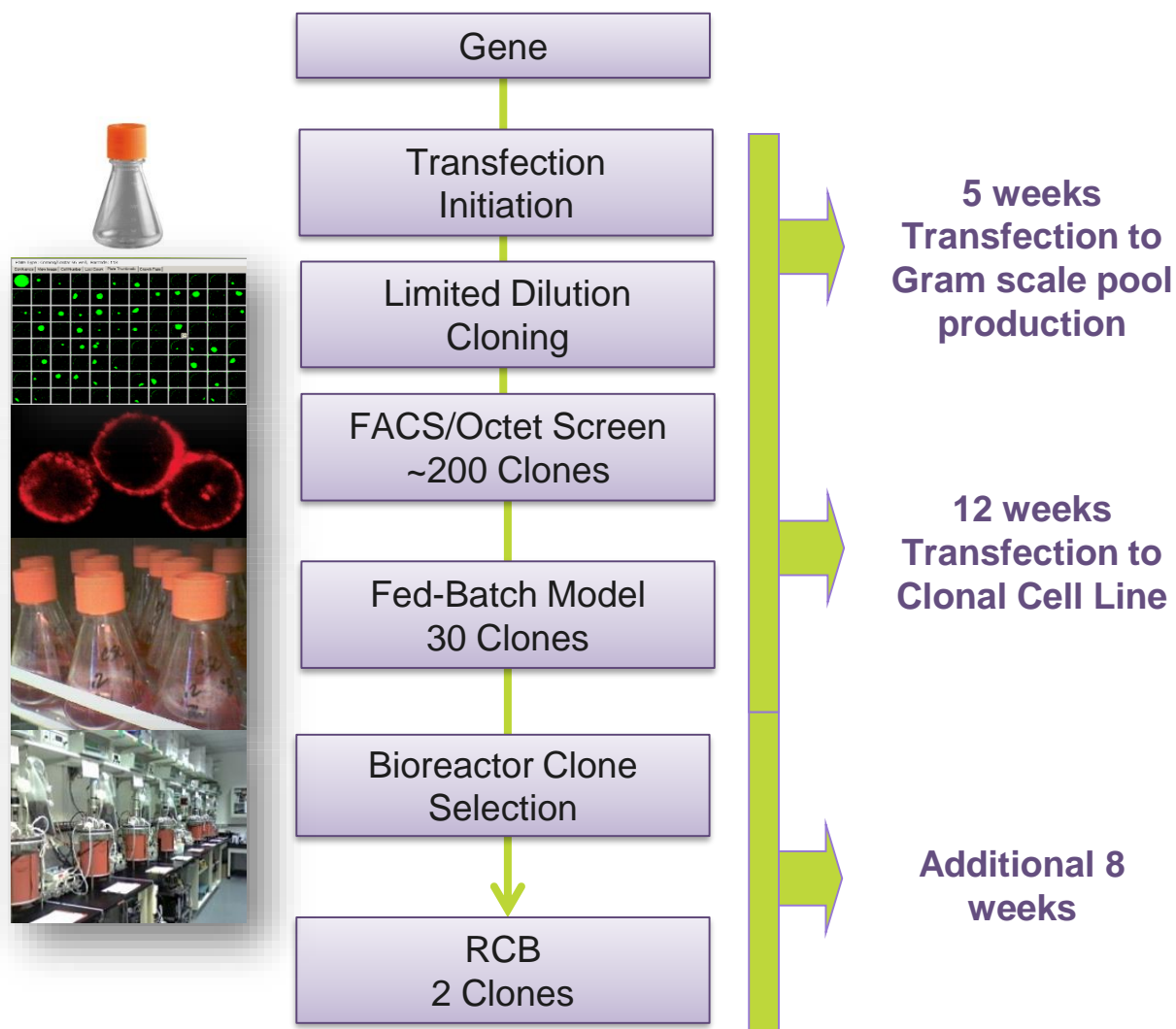
- CHEF1 Expression Plasmid Enables Rapid Cell Line Development
  - Constitutive expression (EF-1 Promoter), no MTX amplification
  - Only ~200 clones need to be screened
  - Low copy number cell lines with stable growth and titer (greater than 70 generations)
  - Competitive early development titers (>2 g/l mAb), improved with Process Development
- Chemically Defined CMC Media and Feeds Yields High Viability Growth in Production
- Serum-free, Suspension-Adapted CHO DG44 Cells Yield Consistent Growth and Productivity
  - Selected for Serum-free growth (including cloning) in CMC Media
- Expression system used for 4 commercial products and for more than 40 different projects in various stages of clinical development

# A Growing Track Record of Proteins Expressed Using CHEF1

Product class	No of products	Product Titrers
mAb (IgG1 & IgG4)	24	2 - 4 g/L
Biosimilars	5	1 – 3 g/L
FC-fusion	1	Up to 1 g/L
Complex glycoprotein	5	50 mg/L – 250 mg/L
Antibody Formats	5	300 mg/L – 2 g/L
Enzyme	1	1 g/L in pools
	41	

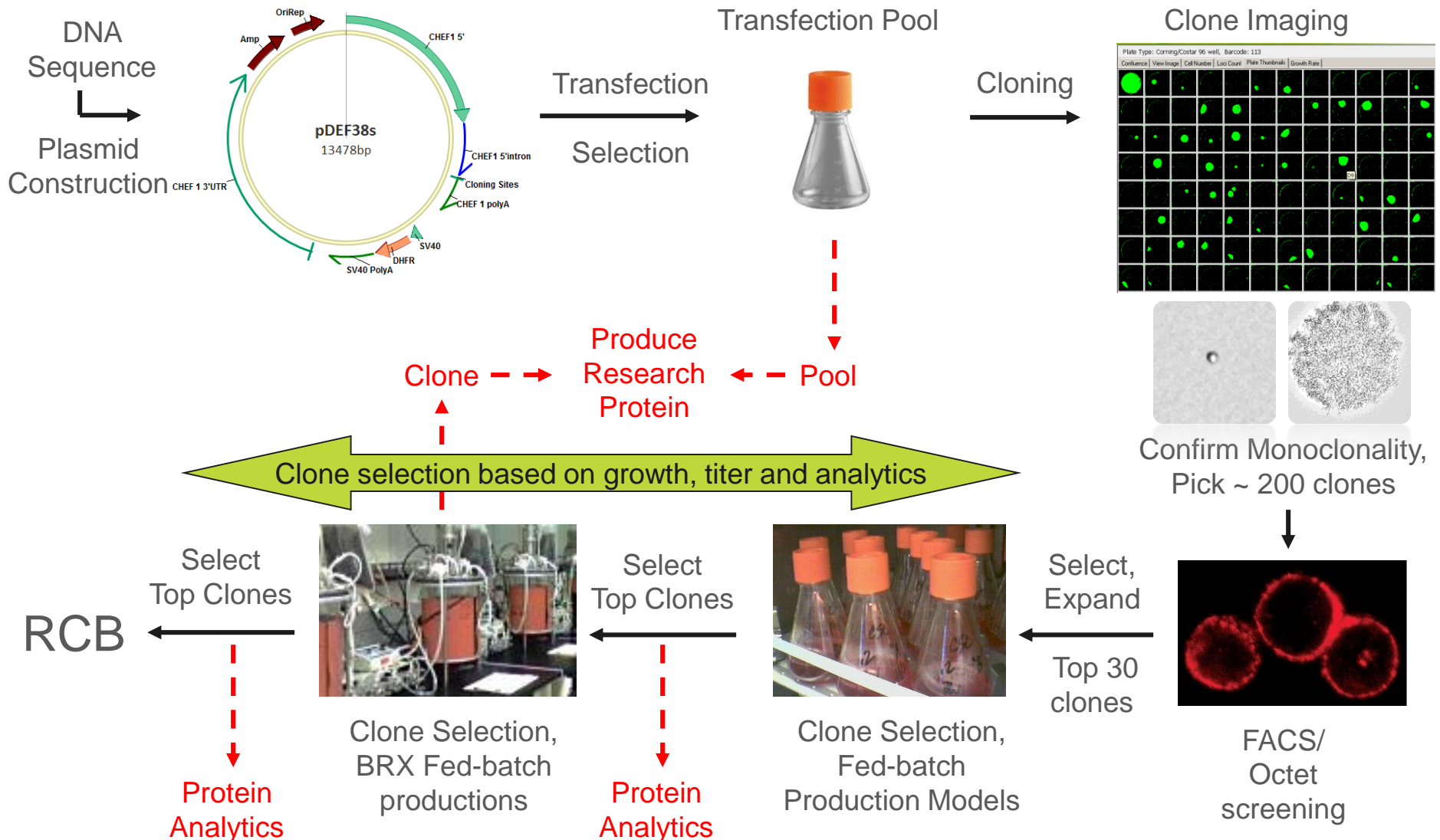


# CHEF1 Development Timeline





# CHEF1 Cell Line Development



# Selection of Best Antibody Candidate (IgG4)

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- Started with 12 IgG4 variants  
(6 HC's in combination with 2 LC's)
- **6 variants expressed from stable pools to deliver >30 mg of each for mice studies**
- **4 variants expressed from stable pools to deliver >1.5 g for monkey studies**
- 4 variants initiated in cell line development (staggered with 3 weeks in-between)

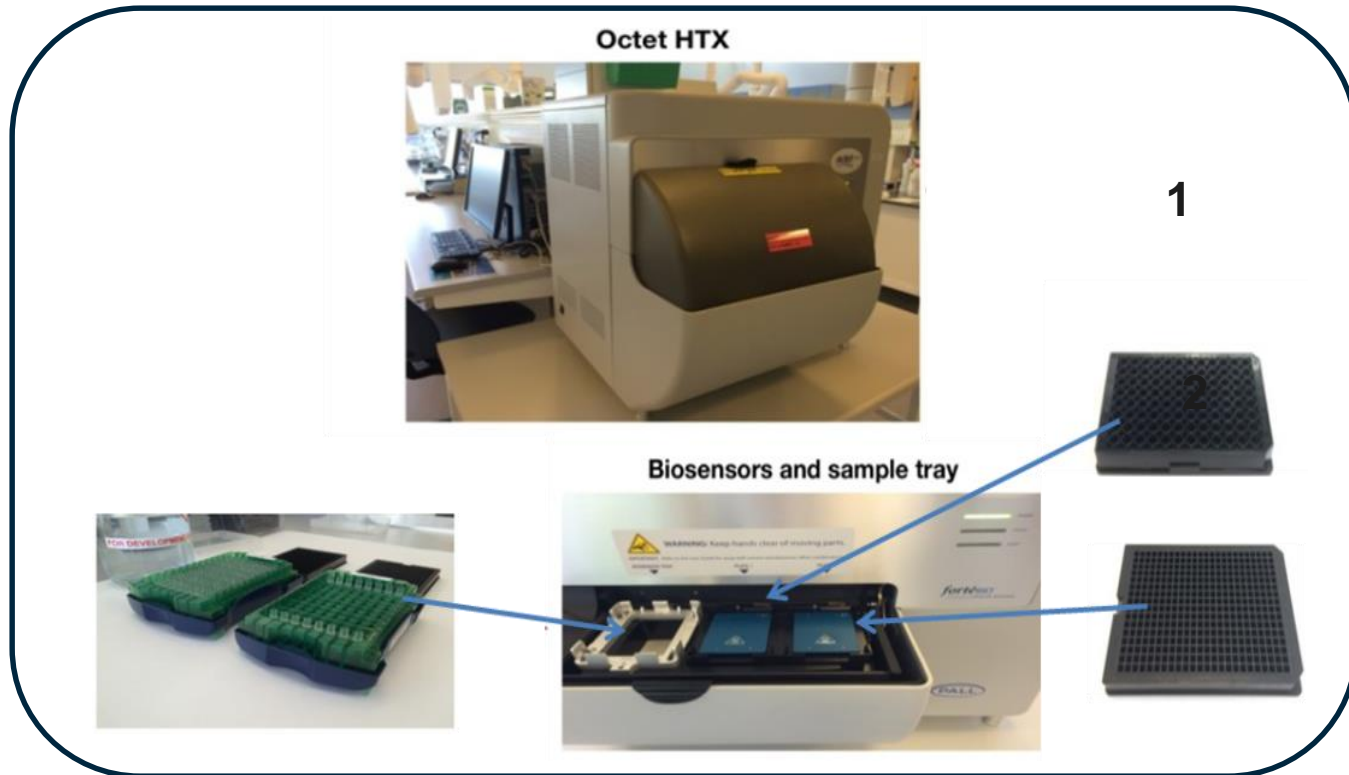


IgG4 variants  
de-selected by  
customer

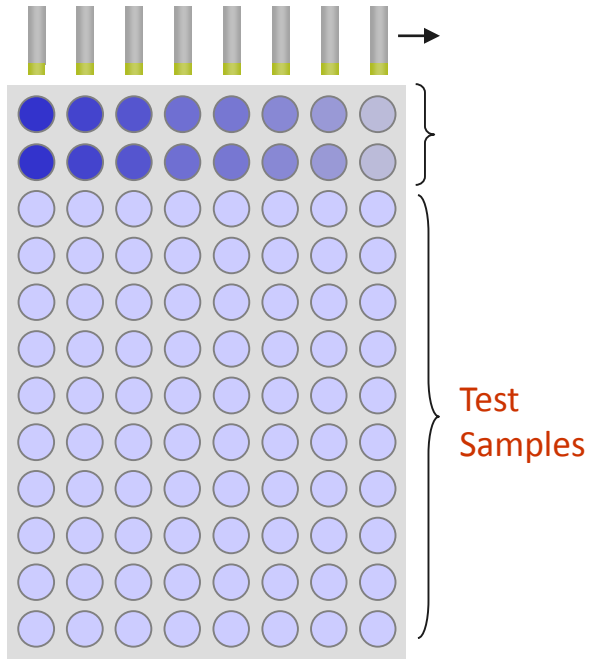


# Octet HTX - Plates

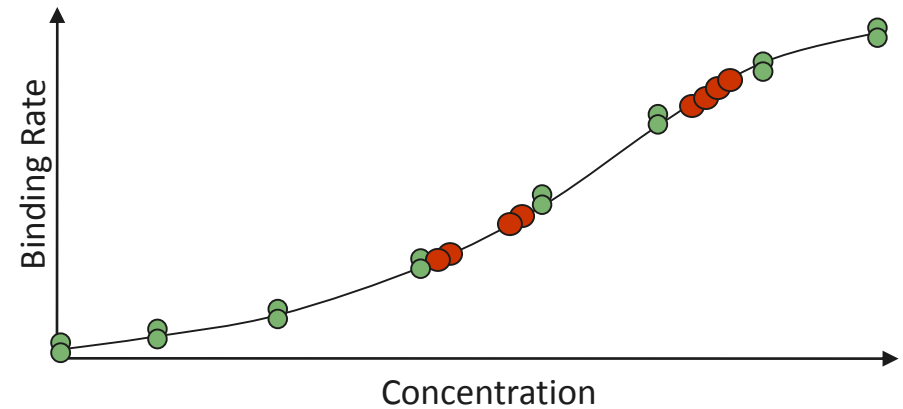
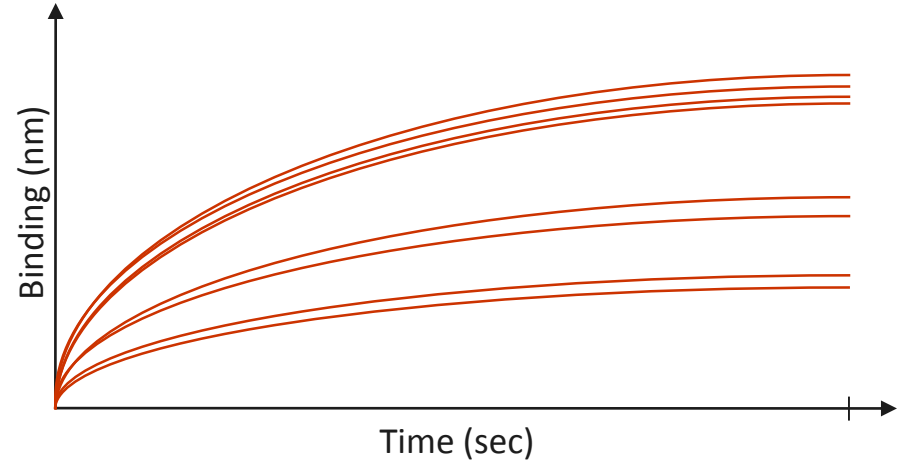
- Bio-layer interferometry
- Optical analytical technique that measures interference patterns between waves of light
- Operated with 96/384 well plates



# Octet Workflow for Quantification

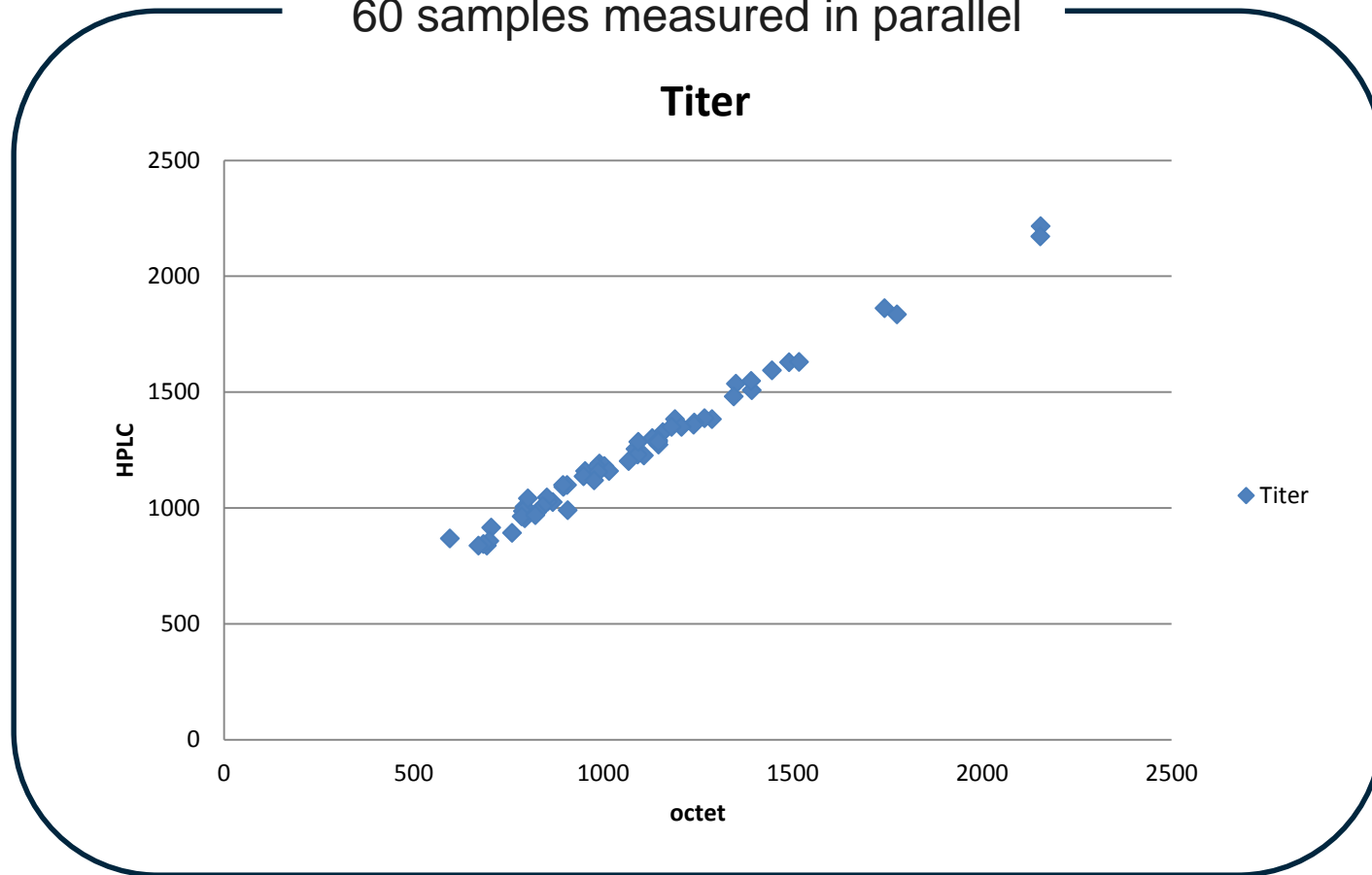


- The binding rates of test samples are measured and interpolated from the standard curve to determine concentration
- 96 samples analyzed in 15 minutes



# Titer – HPLC vs. Octet C62401

60 samples measured in parallel

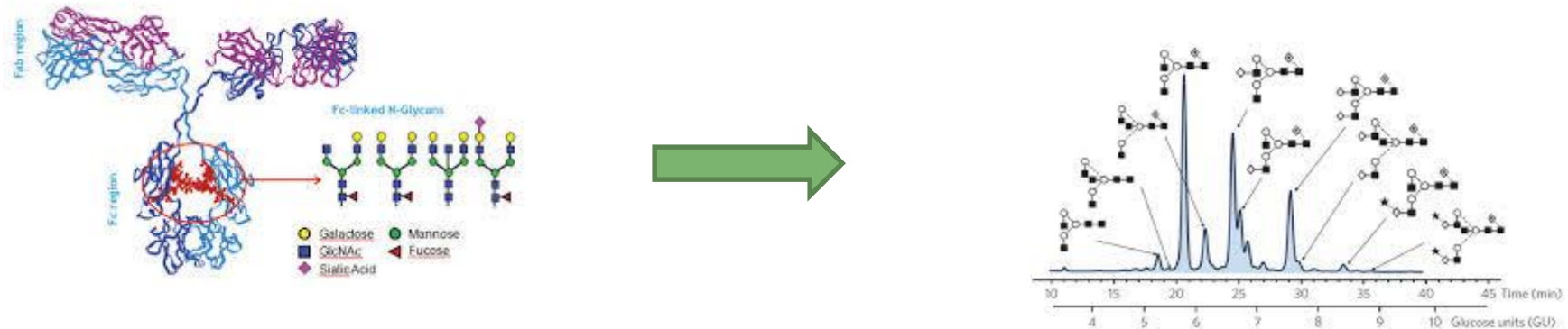


# Implementation of HT Octet Methods

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- The use of Octet have been successfully implemented for the following methods
  - Titer determination (Protein A)
  - Titer determination (Protein L)
  - Titer determination (Penta-His)
  - Residual Protein A
  - CHO HCP

# Implementation of HT N-glycan Profiling



- Implementation of microtiter plates (24 well format)
- Reductions in preparation, digestion and labelling time



# Implementation of HT N-glycan Profiling

	Platform	Waters	Prozyme (Glykoprep)	Prozyme (In solution)
<b>Label</b>	2AA	Rapi-fluor	Instant PC	Instant PC
<b>Total Prep Time</b>	overnight+ 9 hrs	2 hours	4 hours	2 hours
<b>Cleanup</b>	plate + vac	micro plate + vac	centrifuge	plate + vac
<b>Digest</b>	overnight	10 min	1 hr	10 min
<b>Label time</b>	1 hr	5 min	5 min	1 min
<b>Price</b>		\$6000 / plate	\$6000 / plate	\$6000 / plate
<b>Drawbacks</b>	<ul style="list-style-type: none"> <li>Lengthy</li> <li>Requires vacuum</li> <li>Lower fluorescence response</li> </ul>	<ul style="list-style-type: none"> <li>Vacuum cleanup was problematic</li> <li>Small quantities of protein can be tougher to handle</li> <li>Dye is unstable (<i>i.e.</i> must be used soon after being reconstituted)</li> </ul>	<ul style="list-style-type: none"> <li>Lots of centrifuging</li> <li>Poorer recovery?</li> </ul>	<ul style="list-style-type: none"> <li>Need no salt/detergent present</li> <li>Need to buy vacuum system</li> </ul>

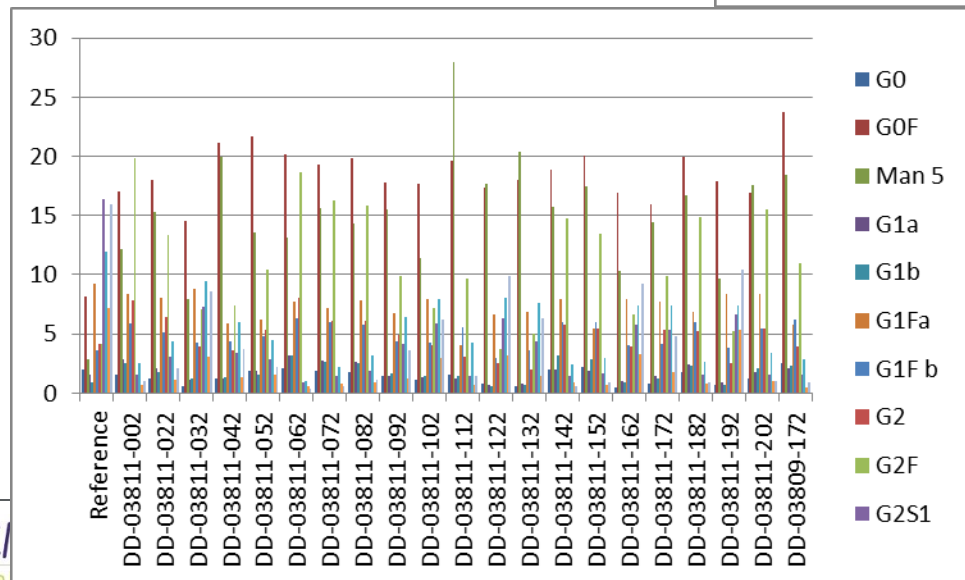
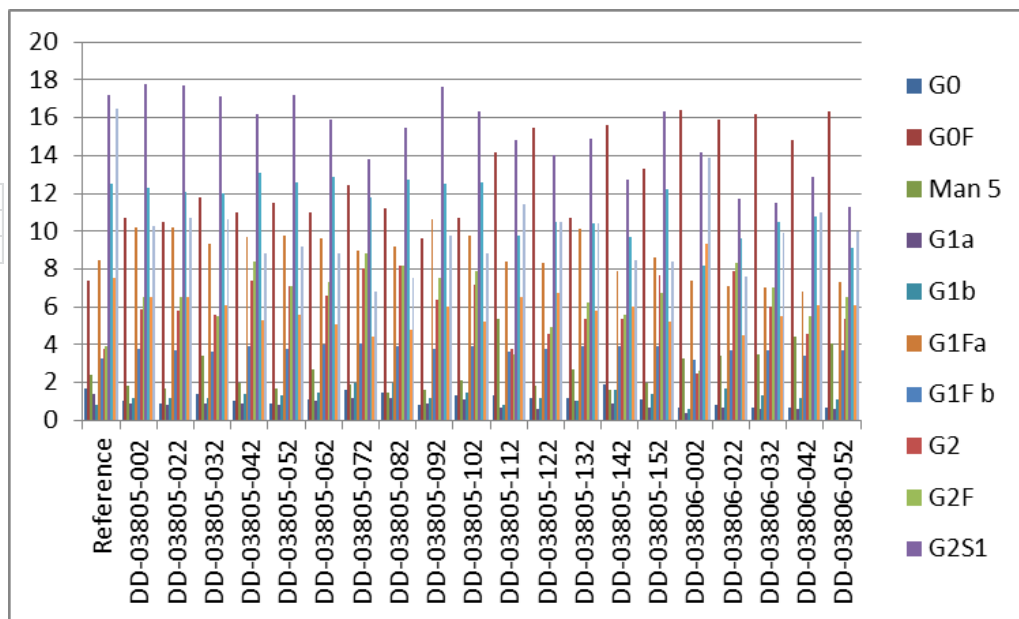
- Evaluation of 3 different methods compared to standard 2AA/HPLC
- Substantial reductions in overall preparation time
- Overall increase in sample through-put

# N-Glycan Case Study. Analysis of 500 Samples

	G0	G0F	Man 5	G1a	G1b	G1Fa
Reference	1.7	7.4	2.4	1.4	0.8	8.5
DD-03805-002	1	10.7	1.8	0.9	1.2	10.2

	G1F b	G2	G2F	G2S1	G2FS1	G2S2	G2FS2
Reference	3.3	3.8	3.9	17.2	12.5	7.5	16.5
DD-03805-002	3.8	5.9	6.5	17.8	12.3	6.5	10.3



	G0	G0F	Man 5	G1a	G1b	G1Fa
Reference	2	8.2	2.8	1.5	0.9	9.2
DD-03811-002	1.5	17	12.2	2.8	2.5	8.4

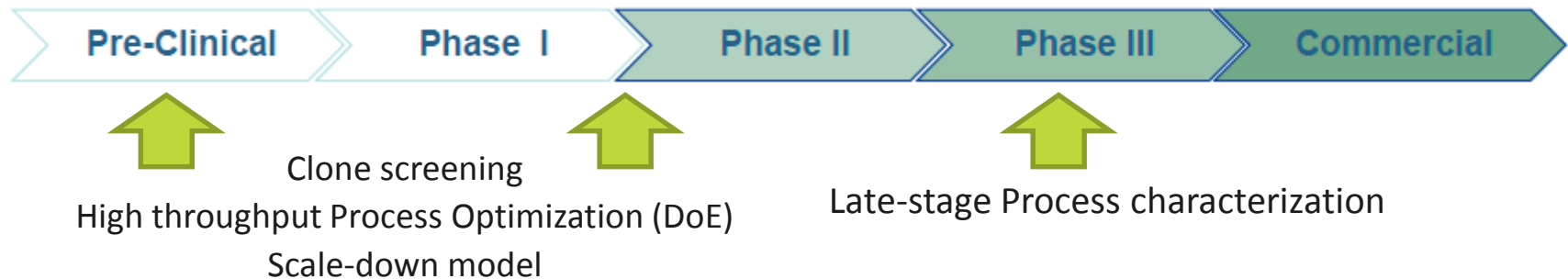
  

	G1F b	G2	G2F	G2S1	G2FS1	G2S2	G2FS2
Reference	3.6	4.1	4.1	16.4	11.9	7.2	16
DD-03811-002	5.9	7.8	19.8	1.6	2.5	0.7	1



# AMBR250 – the system

AMBR250 - 24 independently controlled 250 ml bioreactors  
Implemented in CMC's Upstream PD (CPH, Aug-2016; SEA, Nov-2016)



**Advantages:** Mini Single-use bioreactors significantly reduce material, time, and labor demands while providing more experiments than previously possible.

**Small, but accurate bioreactor model** => lower cost

**Automation** => Accurate experiments, lower labor cost

**24 bioreactors** => Massive parallel processes, resulting in faster optimization/evaluation of design space



# AMBR250

AMBR250 bioreactors



AMBR250 system



- Cell Counts are performed automatically



# Implementation experiments

- Process template programming ✓
- Determination of AMBR250-specific key parameters (agitation settings, relevant gas/CO<sub>2</sub> flows, pH probe drift) ✓
- Scalability to 5L, e.g. run DoE runs in both (Data collection ongoing ✓)
- Scalability to 2000L; e.g. satellite 5L's and 250 ml's



# Implementation: Scalability to 5L - run DoE runs in both

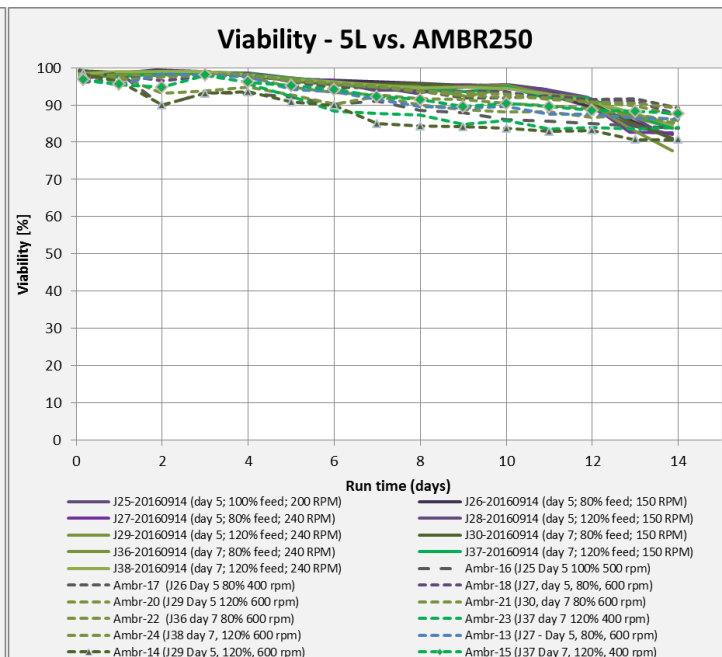
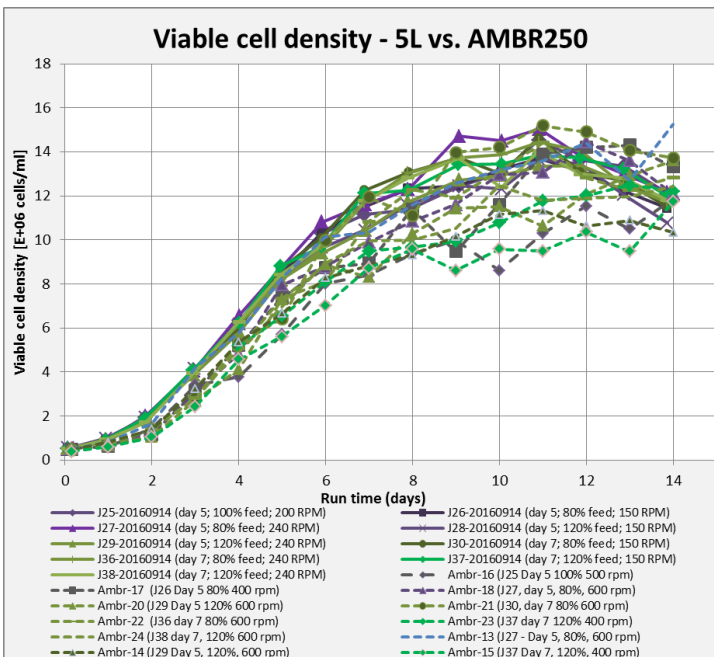
	5L vessel#	AMBR vessel#	Day of temp. shift	Feeding (% of platform)	5L vessel#	AMBR vessel#
Run#					Agitation (RPM)	Agitation (RPM)
1	J25	16	5	100	200	500
2	J26	17	5	80	150	400
3	J27	18	5	80	240	600
4	J28	19	5	120	150	400
5	J29	20	5	120	240	600
6	J30	21	7	80	150	400
7	J36	22	7	80	240	600
8	J37	23	7	120	150	400
9	J38	24	7	120	240	600



250 ml AMBR



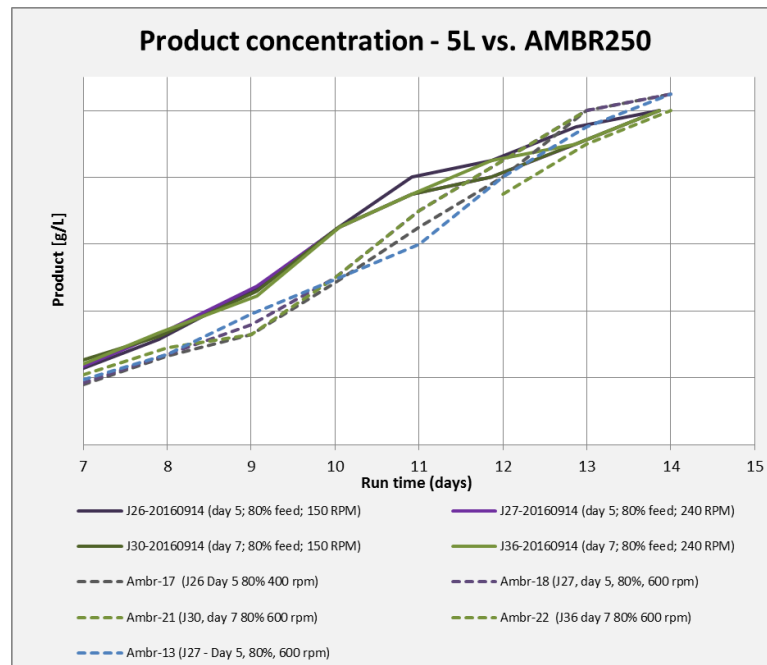
5L Glass vessel





# Implementation: Scalability to 5L, e.g. run DoE runs in both

	5L vessel#	AMBR vessel#	Day of temp. shift	Feeding (% of platform)	5L vessel#	AMBR vessel#
Run#					Agitation (RPM)	Agitation (RPM)
1	J25	16	5	100	200	500
2	J26	17	5	80	150	400
3	J27	18	5	80	240	600
4	J28	19	5	120	150	400
5	J29	20	5	120	240	600
6	J30	21	7	80	150	400
7	J36	22	7	80	240	600
8	J37	23	7	120	150	400
9	J38	24	7	120	240	600



# Conclusions

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- Identification of key bottlenecks affecting the process development value chain
- We have successfully impleted some of these technologies
- Substantial capacity improvements within current laboratory footprint
- Full implementation will lead to substantial timeline reductions
- Other types of projects, like e.g. development of biosimilars will also benefit from implementation of HT analytics and AMBR250
- We also plan to use e.g. AMBR250 in future process characterization projects





**COPENHAGEN**

Vandtaamsvej 83B  
DK-2860 Soeborg  
Copenhagen  
Denmark  
Phone: +45 7020 9470  
Fax: +45 7020 9476

**SEATTLE**

22021 20th Avenue SE  
Bothell, WA 98021  
USA  
Phone: +1 425 485 1900  
Fax: +1 425 486 0300

**BERKELEY**

890 Heinz Avenue  
Berkeley, CA 94710  
USA  
Phone: +1 425 485 1900  
Fax: +1 425 486 0300

[cmcbiologics.com](http://cmcbiologics.com)  
[contact@cmcbio.com](mailto:contact@cmcbio.com)