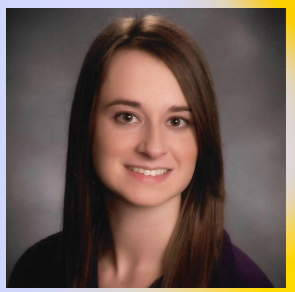
Democratizing Biomanufacturing using Perfusion Fermentation



Laura Crowell, PhD
Director R&D, Sunflower Tx

DCVMN Webinar
August 8, 2023

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Sunflower is an early-stage company delivering next-generation biomanufacturing solutions to global innovators for protein medicines, vaccines and food.

Just like anyone can grow sunflowers anywhere, our solutions are easy to use and empower anyone to efficiently make proteins anywhere.

Diverse Industries Are Producing Proteins & Need Manufacturing Solutions

Biopharma Companies

Drug Discovery
Drug Development
Process Development
Manufacturing

Research Institutions

Protein Design & Eng.
Synthetic Biology
Metabolic Engineering
Vaccine/Drug Discovery

Biotech Companies

Animal Health
Cosmetics
Foods & Nutraceuticals
Materials

Public Sector/Gov't

Vaccine/Drug Security
Biologic Defense
Pandemic Preparedness
Workforce Training

BOLD = Sunflower's Existing Traction

Conventional Biomanufacturing Capacity Requires Significant Upfront Resources & Highly Skilled Workforce

Our Goal: Make Protein Manufacturing More Accessible

Accessibility = Easy to obtain + Easy to use

- Lower Cost
- Small Footprint
- Deployable

- Simple
- Automated
- Efficient
- Flexible

Host organism and manufacturing approach impact cost, simplicity, efficiency and flexibility

Eukaryotic Microbes are Simple and Efficient Hosts for Protein Manufacturing

Eukaryotic Microbes Enable Process Simplicity

	Bacteria (*E. coli*)	Eukaryotic Microbes (*P. pastoris*)	Mammalian Cells (*CHO*)
Protein Secretion		✔	✔
Initial Purity	<<50%	>70%	<50%
No Endotoxin		✔	✔
No Adventitious Agents	✔	✔	
Process Complexity	**HIGH**	**LOW**	**MED**

Eukaryotic Microbes Enable Lower Cost

	Bacteria (*E. coli*)	Eukaryotic Microbes (*P. pastoris*)	Mammalian Cells (*CHO*)
Process Complexity	HIGH	LOW	MED
Doubling Time	<1 HR	2-3 HR	>12 HR
Facility Utilization	POOR	GOOD	POOR

Eukaryotic Microbes Enable Lower Cost

	Bacteria (*E. coli*)	Eukaryotic Microbes (*P. pastoris*)	Mammalian Cells (*CHO*)
Process Complexity	HIGH	LOW	MED
Doubling Time	<1 HR	2-3 HR	>12 HR
Facility Utilization + Raw Material Cost	**POOR** + **LOW**	**GOOD** + **LOW**	**POOR** + **HIGH**

Eukaryotic Microbes Enable Lower Cost

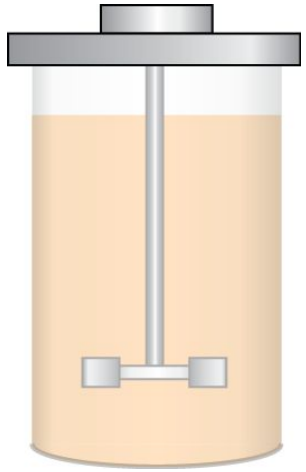
	Bacteria (*E. coli*)	Eukaryotic Microbes (*P. pastoris*)	Mammalian Cells (*CHO*)
Process Complexity	HIGH	LOW	MED
Doubling Time	<1 HR	2-3 HR	>12 HR
Facility Utilization	POOR	GOOD	POOR
+			
Raw Material Cost	LOW	LOW	HIGH
Cost of Goods Manufactured	MED	LOW	HIGH

Continuous Manufacturing Enables Efficiency, Quality and Productivity

Continuous Manufacturing Enables Efficiency

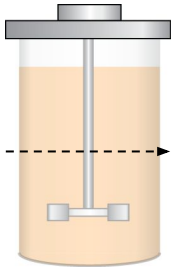
Bioreactor volume required to produce 1 kg of IgG1 in CHO in 30 days*

Fed-Batch



350 L

Continuous

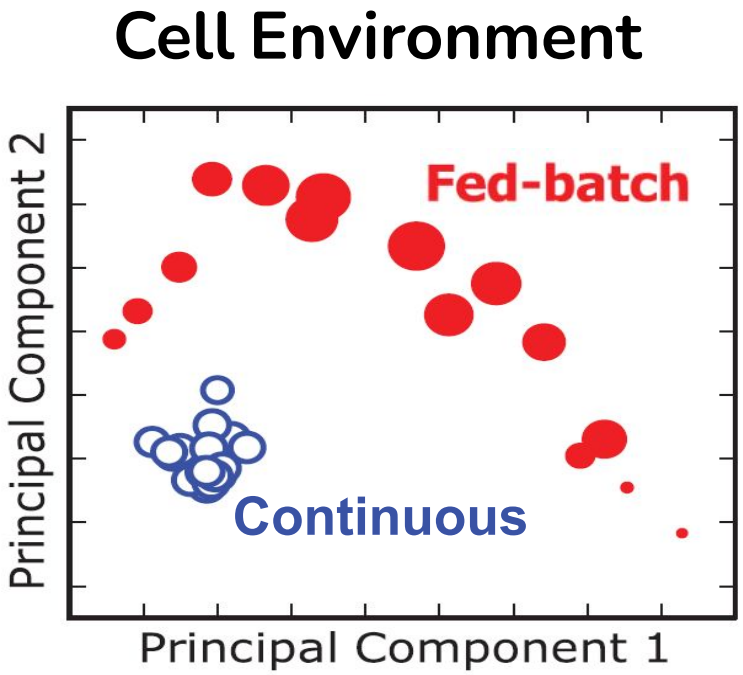


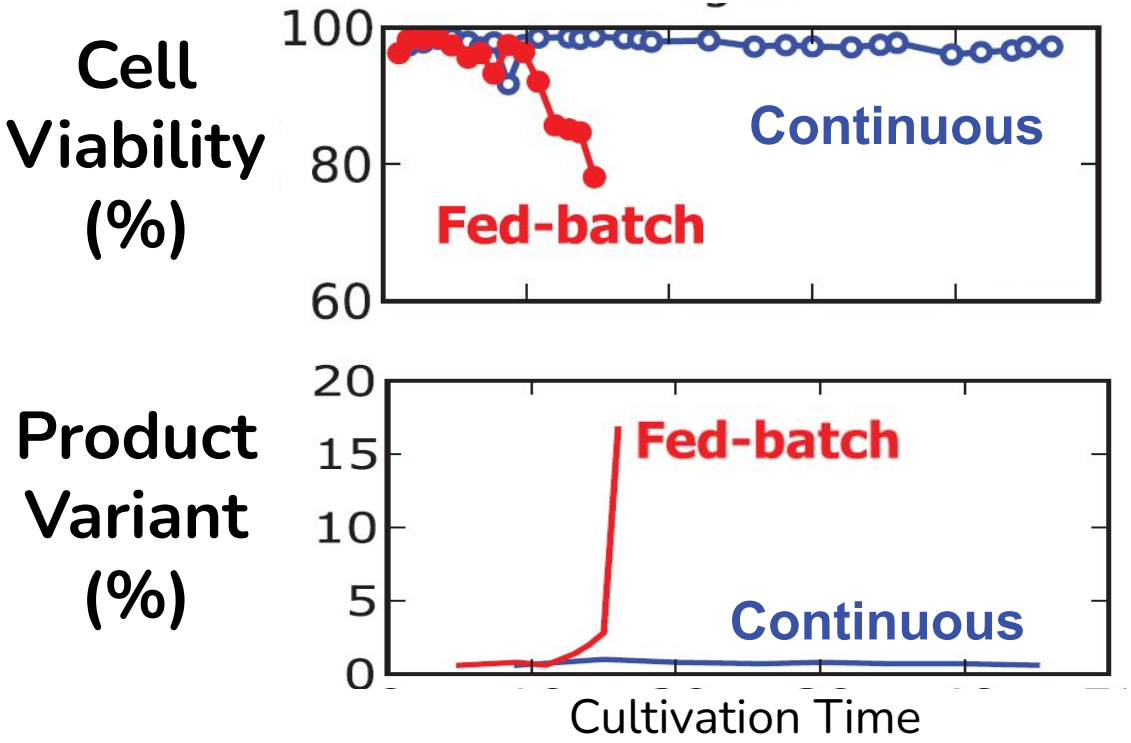
50 L

Continuous manufacturing reduces footprint by 7-fold

*Based on data from Walther et. al., Biotechnology Journal. 2018

Continuous Manufacturing Enables Quality





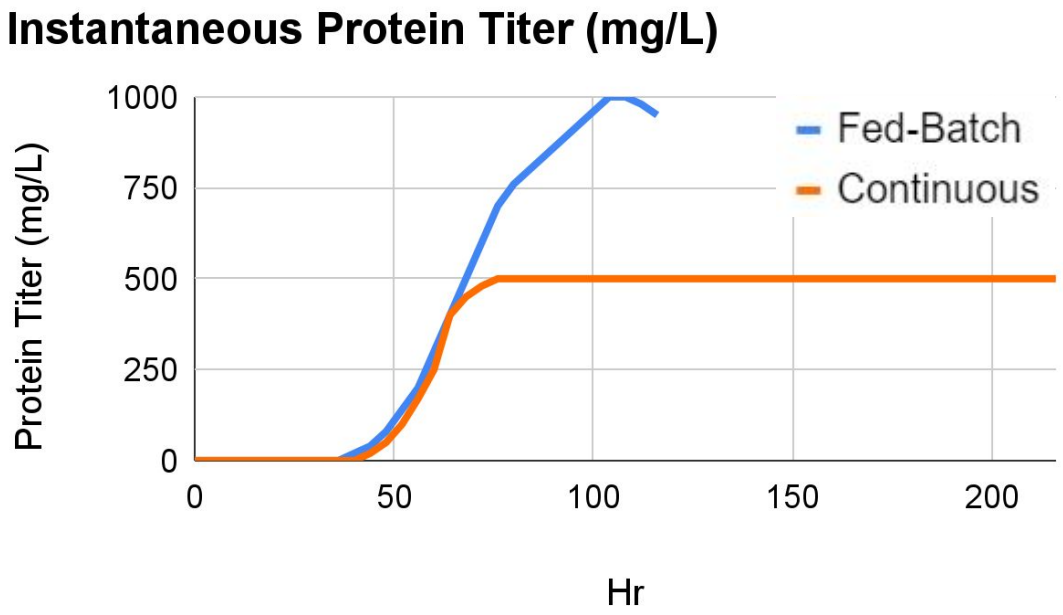
Consistent cell environment leads to higher cell viability and product quality

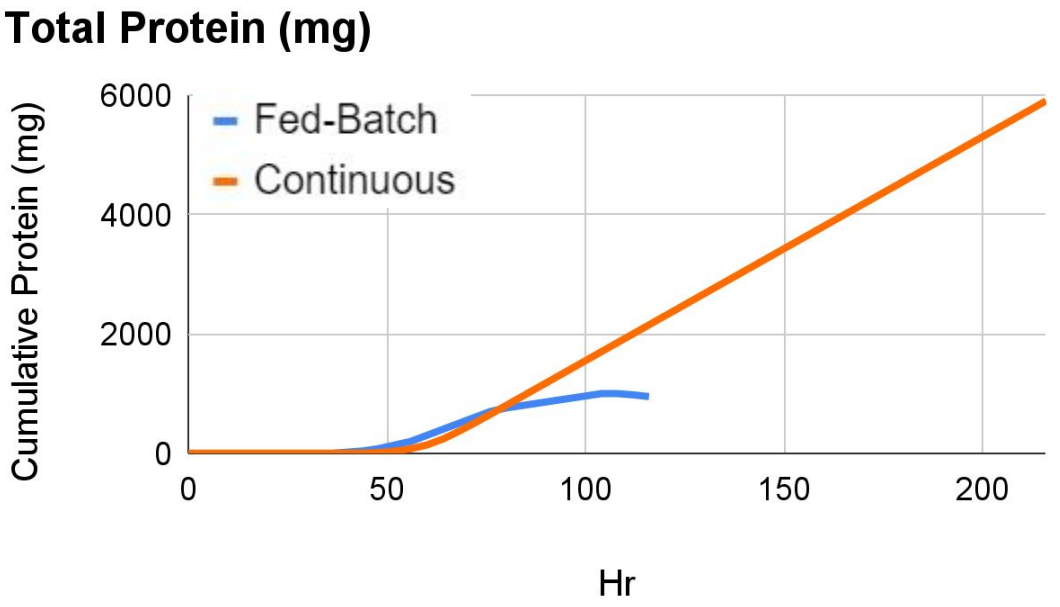
*Based on data from Walther et. al., Biotechnology Journal. 2018

Continuous Manufacturing Enables Productivity

Instantaneous protein titer is **not** a complete representation of protein produced for continuous processes

Continuous processes run longer and produce more total protein

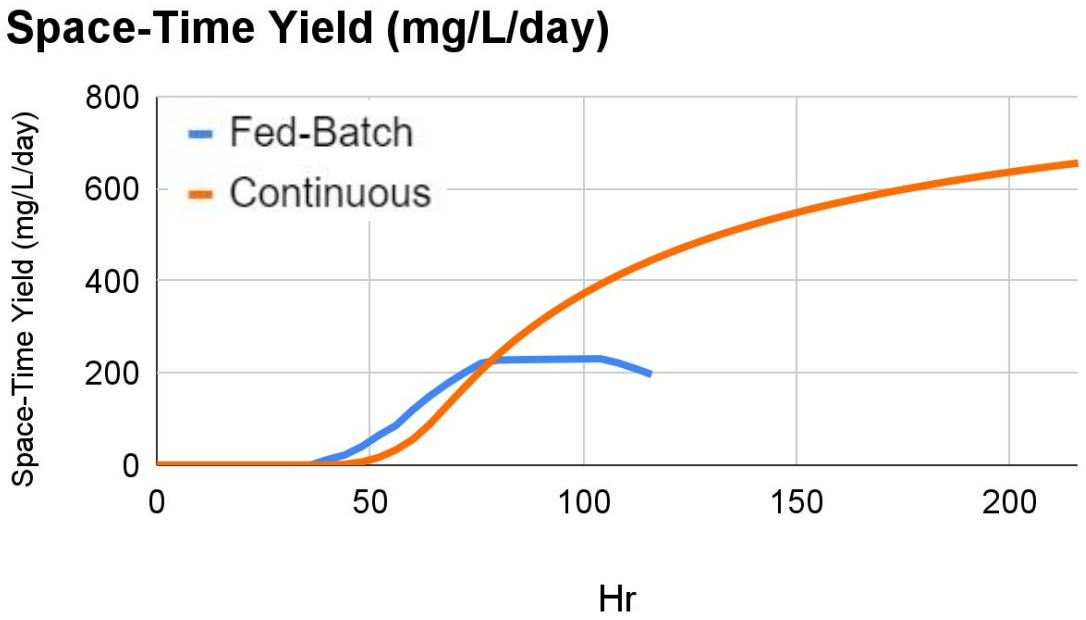




Continuous Manufacturing Enables Productivity

Space-Time Yield is a metric for normalized comparison

Space-Time Yield = **Mass of Protein** per **Bioreactor Volume** per **Cultivation Day**

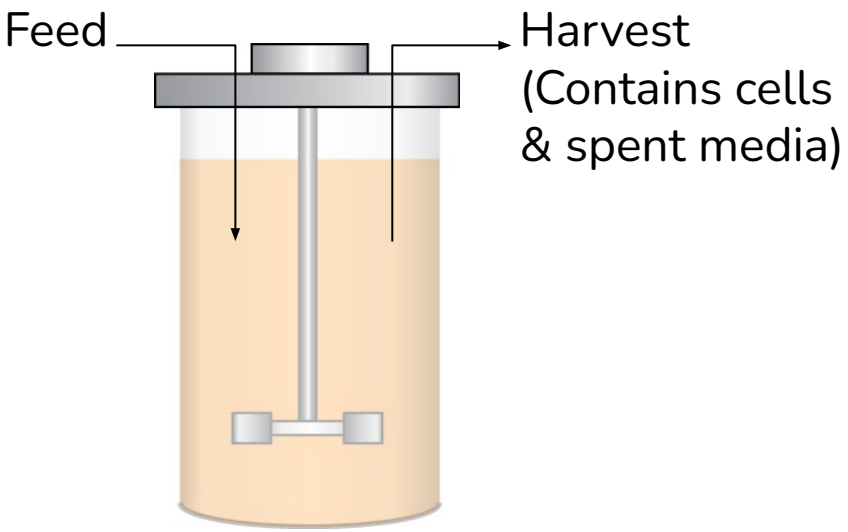


Continuous manufacturing enables significantly higher space-time yields

Perfusion Fermentation: Continuous Manufacturing for Eukaryotic Microbes

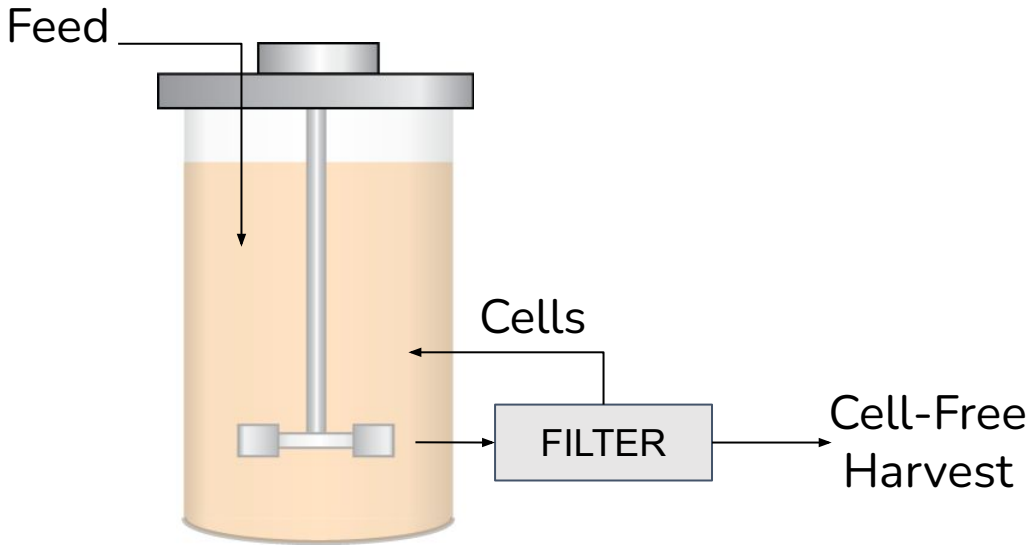
Historical Challenges To Continuous Manufacturing for Microbes

Legacy Method: Chemostat



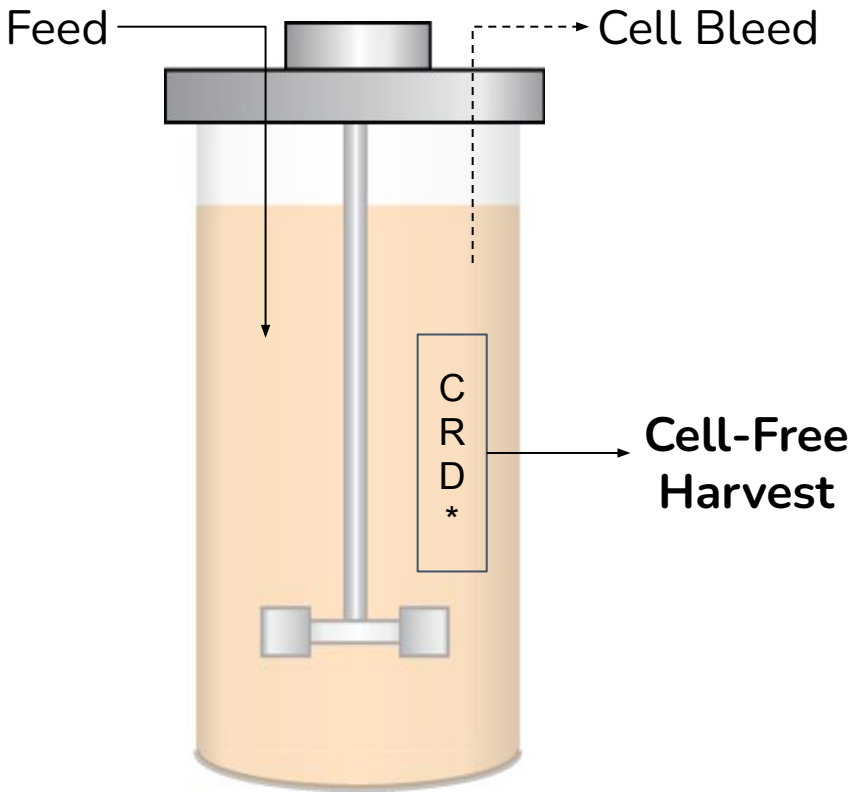
Limited achievable cell mass due to constant cell harvest

Conventional Perfusion (used with CHO)



Removes cells from the bioreactor environment leading to starvation for microbes

Sunflower's Technology Enables In-Vessel Perfusion for Eukaryotic Microbes

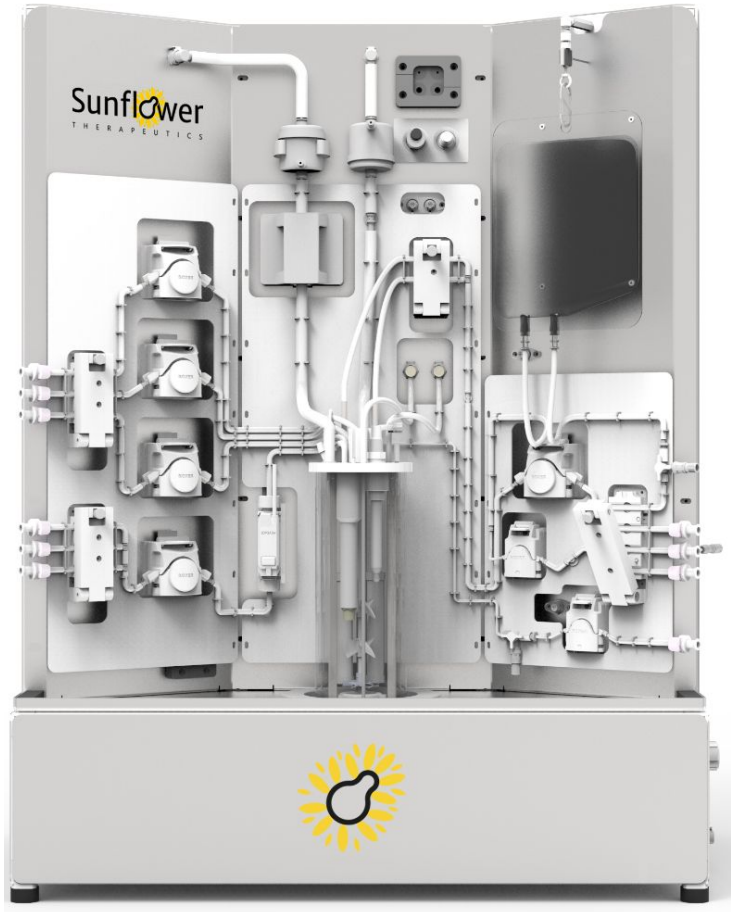


*Cell Retention Device

Benefits of In-Vessel Perfusion

- Cells never removed from bioreactor environment
- Healthier, more consistent cell culture
- Longer campaigns
- Ultra high cell mass achievable and maintainable
- Continuous harvest of secreted protein

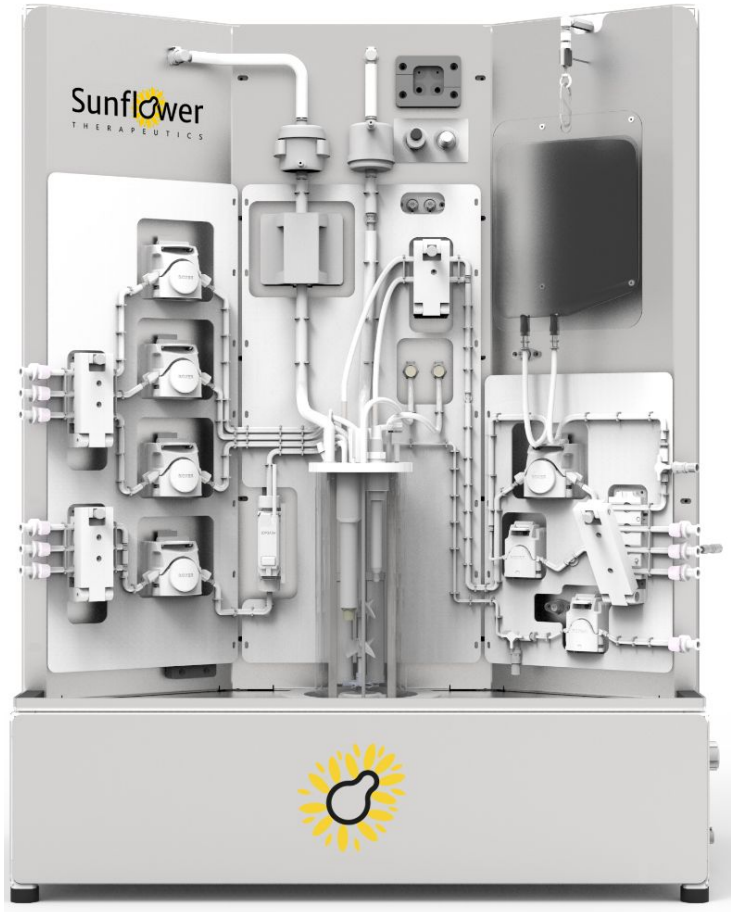
Daisy Petal™ Single-Use Bioreactor System:
Perfusion fermentation for eukaryotic microbes



EFFICIENT PRODUCTION IN A SMALL FOOTPRINT

- Bench-top sized but **produces like larger reactors** (up to grams protein in 1-2 weeks from 1L working vol.)
- Unique single-use structured consumable for **intuitive installation by non-expert users** and rapid **flexibility**
- Custom software enables **fully automated** operation
- **Immediately deployable** - just add cells & process fluids

Daisy Petal™ Single-Use Bioreactor System:
Perfusion fermentation for eukaryotic microbes



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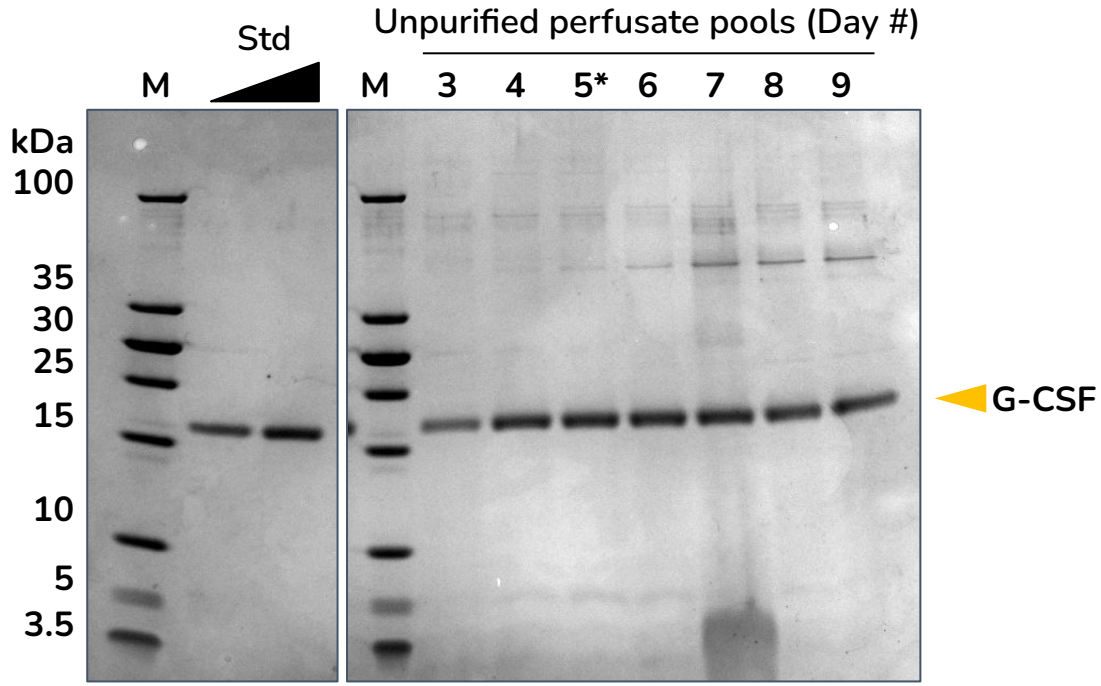
EARLY ACCESS UNITS DELIVERED SUMMER 2022

"The Daisy Petal is really simple to use. I knew exactly how to get started with the system and consumables."

- Customer in Petal Early Access Program with no previous bioprocess experience

Perfusion Fermentation Sustains Production of High-Quality Proteins



Std @ 0.05, 0.1 mg/mL
*Fed-batch processes typically end here.

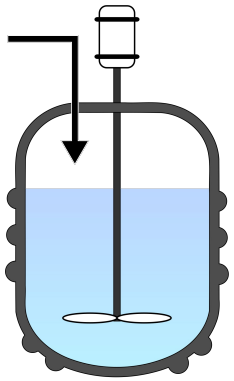
CONTINUOUS MICROBIAL PRODUCTION IS MORE EFFICIENT THAN BATCH

- **>1.2 grams** (4,200 dose equivalents) of unpurified G-CSF produced in **9 days** in 1 L working volume reactor
- Space-Time Yield: **>130 mg/L/day** (>500 mg/L fed-batch titer)
- Process ran **4 days longer** than conventional batch cultivation
- Perfusion fermentation enabled by proprietary in-vessel cell retention device

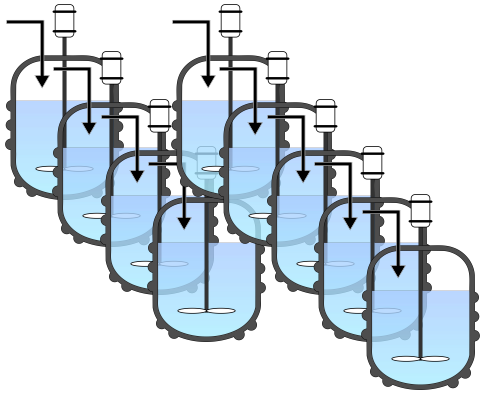
Daisy Petal™ Simplifies Fermentation Optimization

A 3 LEVEL, 2 FACTOR FULL FACTORIAL DOE REQUIRES 9 CONDITIONS

Standard Fed-Batch
9 Experiments



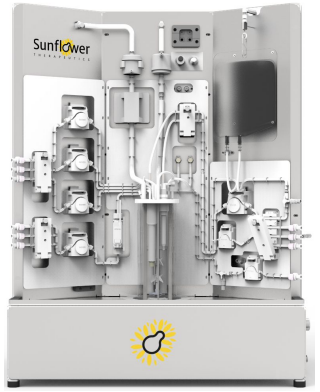
-or-



1 vessel x **36+ days**

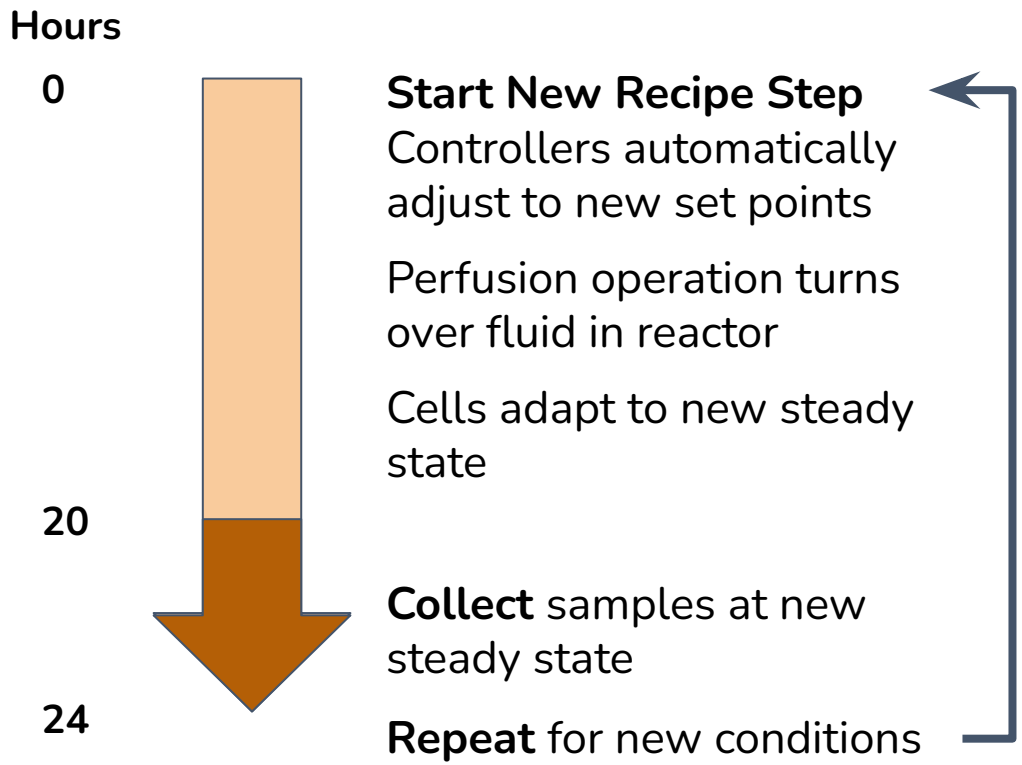
9 vessels x ~5 days

Continuous Perfusion
1 Experiment



1 vessel x 10 – 11 days

Continuous Operation Links Parameter Testing



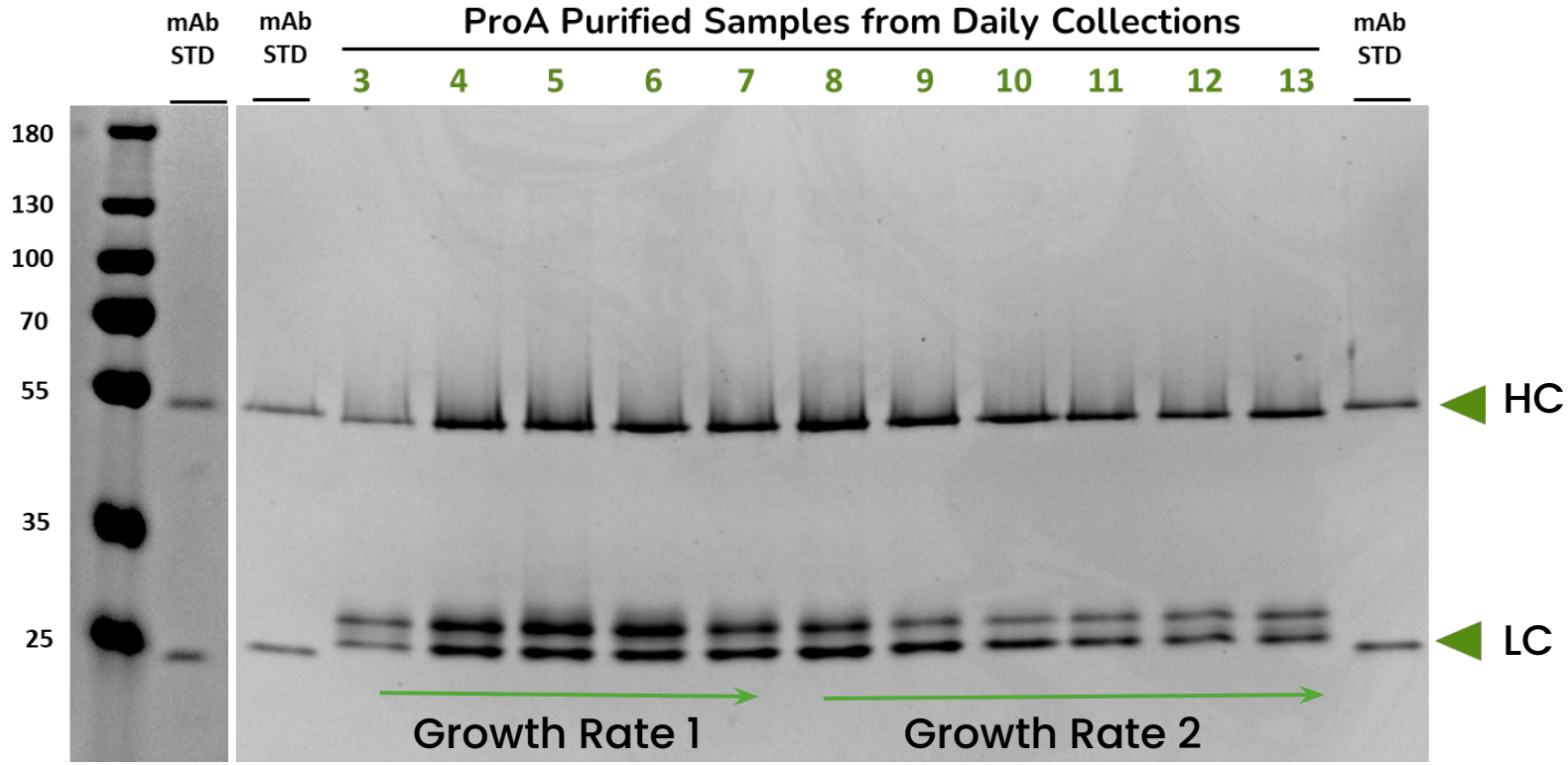
Variable Process Parameters

- pH
- Dissolved oxygen (DO)
- Temperature
- Perfusion Rate (Feeding)
- Carbon input (Feeding)
- Cell Bleed Rate

A single experiment can test multiple process conditions

Early Access Customers Demonstrate Benefits of Perfusion

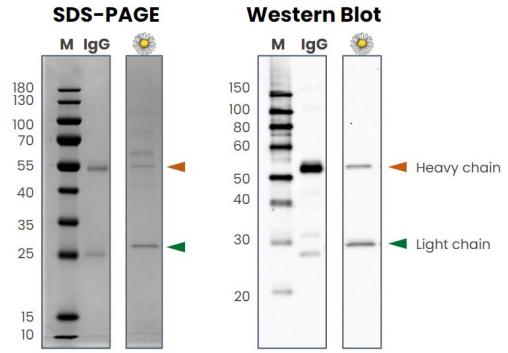
MAB PRODUCTION TESTED WITH HIGH BIOMASS (~600 WCW) AT MULTIPLE GROWTH RATES



Diverse Proteins Produced Using Daisy Petal™

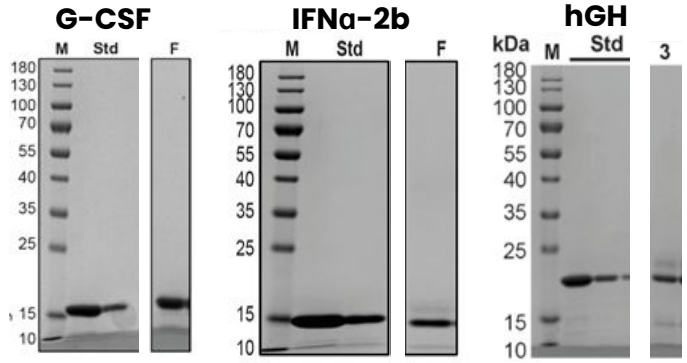
ANTIBODIES & MAB-LIKE PRODUCTS

- Monoclonal antibodies
- Single-domain antibodies (sdAb) & multimers
- Fragment antigen-binding regions (Fabs)



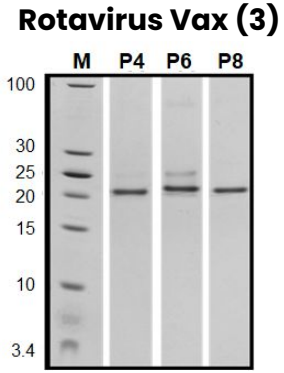
CYTOKINES & HORMONES

- G-CSF
- IFNα-2b
- hGH



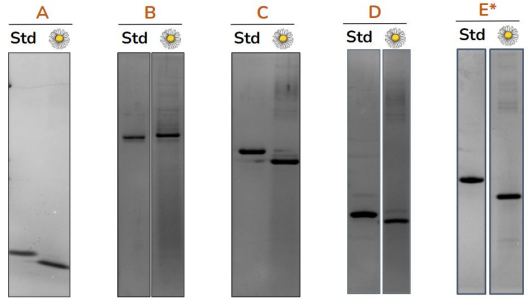
VACCINE ANTIGENS

- COVID subunit vaccine candidates
- Rotavirus subunit vaccine candidates
- Virus-like particles (VLPs)



ENZYMES & OTHER PROTEINS

- DNA / RNA editing enzymes
- Bacterial toxins



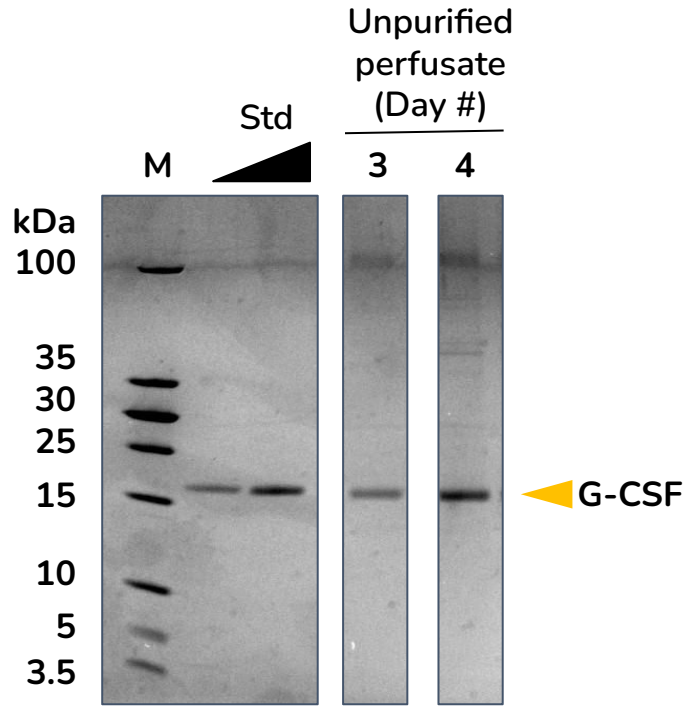
Crowell et. al. Nat. Biotechnol. 2018; Dalvie et. al. Microb. Cell Fact. 2021; Dalvie et. al. PNAS 2021; Crowell et. al. Biotechnol. Bioeng. 2021

Dahlia Petal™ is Equivalent to 200L Batch Bioreactor

15L Working Volume
Can produce **100 grams protein per week** in <20 m^2

SCALE-UP TO DAHLIA PETAL™ RESULTS IN CONSISTENT SPACE-TIME YIELDS

- G-CSF successfully produced during hardware testing
- **>5 grams** G-CSF produced in only **4 days**
- Space-Time Yield: **>70 mg/L/day***
- *Space-Time Yield from Daisy Petal™ @ 4 days: 77 mg/L/day



Std @ 0.05, 0.1 mg/mL

Benefits of Perfusion Fermentation with Sunflower Systems

Continuous - Overcomes limits of batch production for lower costs (both capital & operational)

Flexible - Accommodates a range of operations and proteins

Automated - Custom software for 'hands-free' operations

Scalable - Systems designed to transition from idea to manufacturing

Intuitive - Designed for users with diverse backgrounds in production

Deployable - Ready for installation and immediate use in many environments

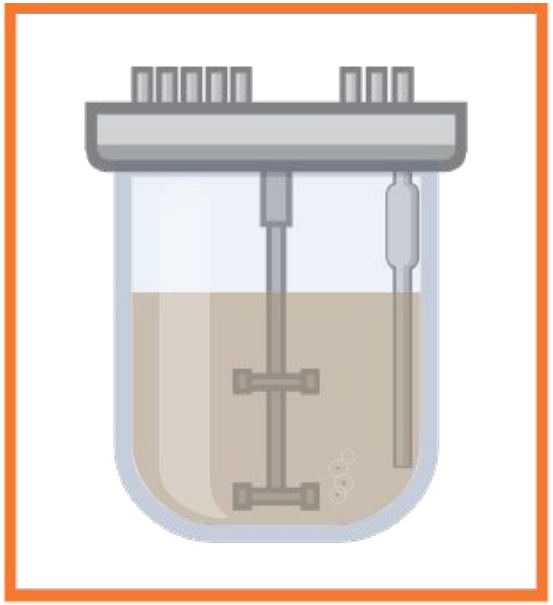
Sustainable - Reduced waste (particularly cleaning waste)

Efficient - More protein for lower cost and less space

Perfusion Fermentation Enables End-to-End Automated Biomanufacturing

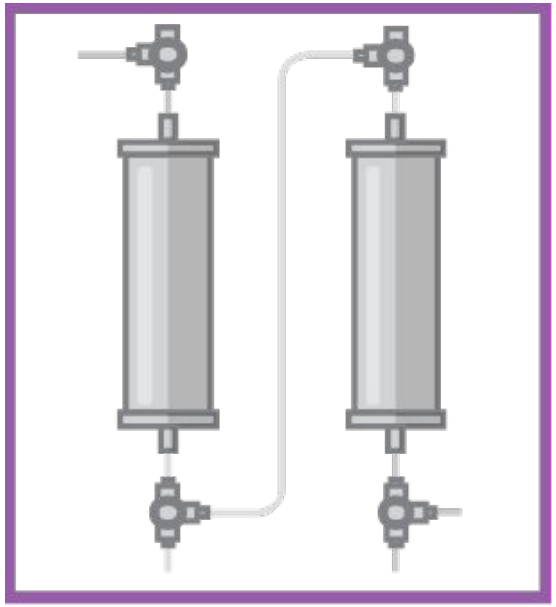
Steps Needed for End-to-End Biomanufacturing

1. Expression



Protein is produced by cells

2. Purification



Protein is separated from impurities

3. Formulation

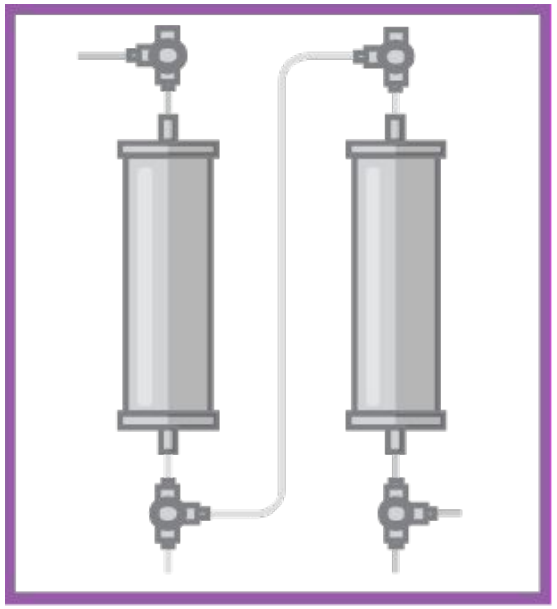
Protein is put into a buffer safe for administration

Steps Needed for End-to-End Biomanufacturing

1. Expression

Perfusion
Fermentation

2. Purification



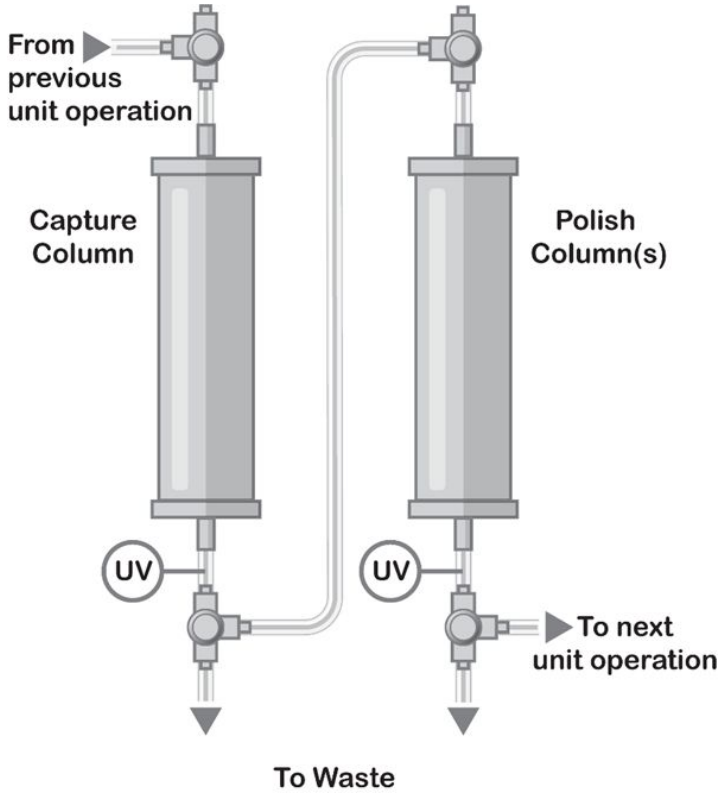
Straight-through
Chromatography

3. Formulation

Tangential Flow
Filtration

Straight-through Chromatography Enables Intensified Manufacturing

Straight-through chromatography



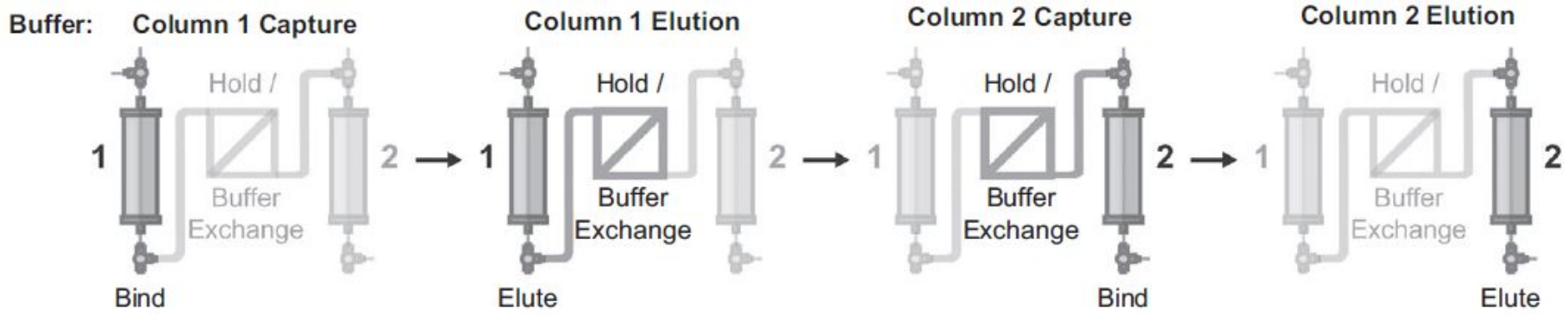
Eliminate buffer conditioning (pH / salt changes) between chromatography steps

Reduce

- # of hold tanks
- buffer usage
- processing time
- manufacturing footprint
- operator touch points

Straight-through Chromatography: 2-column example

Conventional Chromatographic Staging

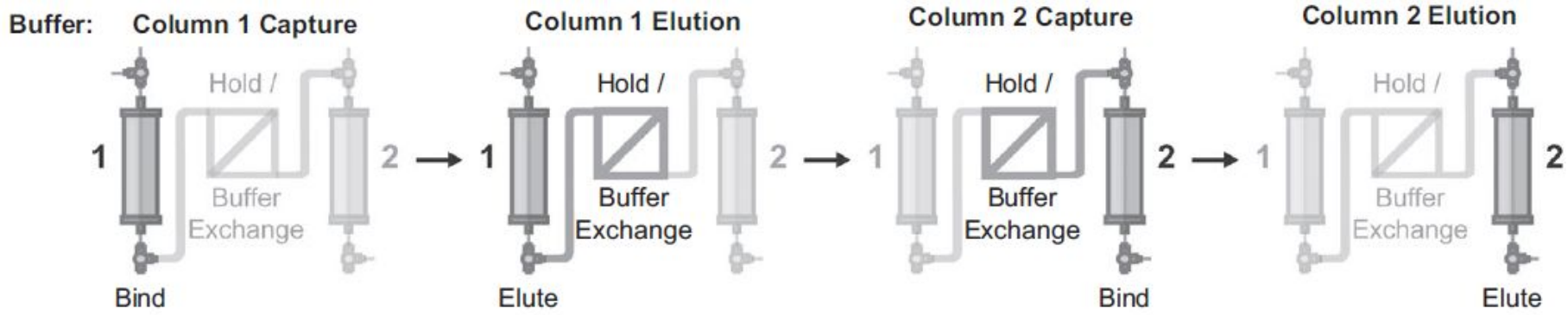


4 Buffers

1 Hold Tank and/or Buffer Exchange

Straight-through Chromatography: 2-column example

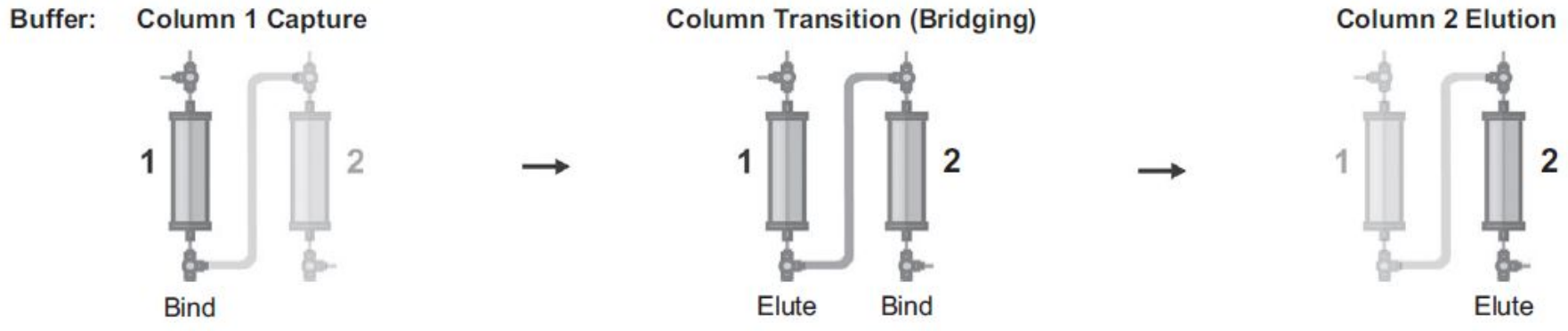
Conventional Chromatographic Staging



4 Buffers

1 Hold Tank and/or Buffer Exchange

Straight-through Integrated Staging

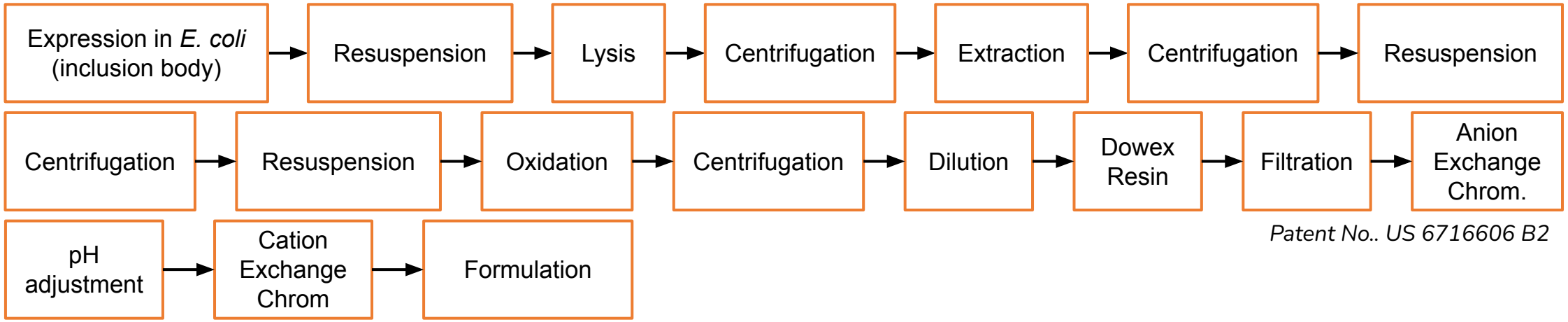


3 Buffers

NO Hold Tank and/or Buffer Exchange

Conventional Processes are Lengthy

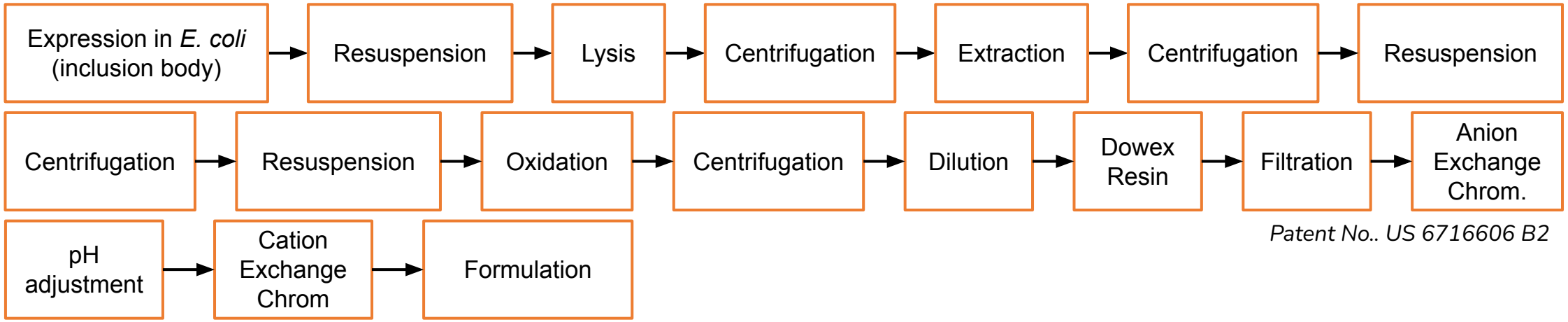
G-CSF in *E. coli* (18 Step conventional process)



Patent No.. US 6716606 B2

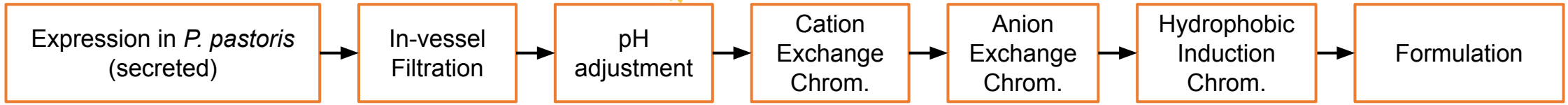
Sunflower Integrated Processes are Efficient

G-CSF in *E. coli* (18 Step conventional process)



Patent No.. US 6716606 B2

G-CSF in Sunflower Process (7 Steps)



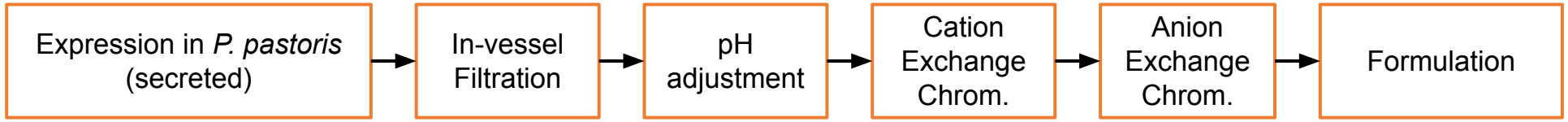
Crowell et al., Nat. Biotechnol., 2018

Perfusion fermentation and straight-through chromatography lead to a 60% reduction in the number of manufacturing steps

Sunflower Systems Use Similar Processes for Many Proteins

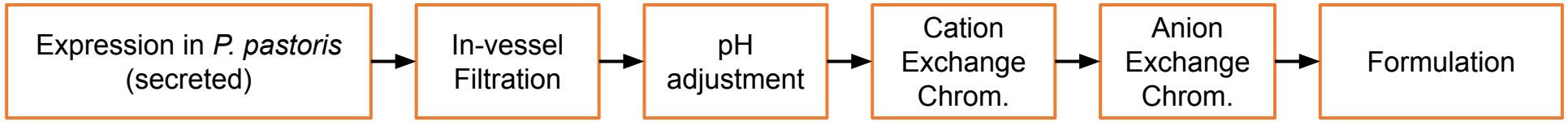
Human growth hormone

Crowell et al., Nat. Biotechnol., 2018



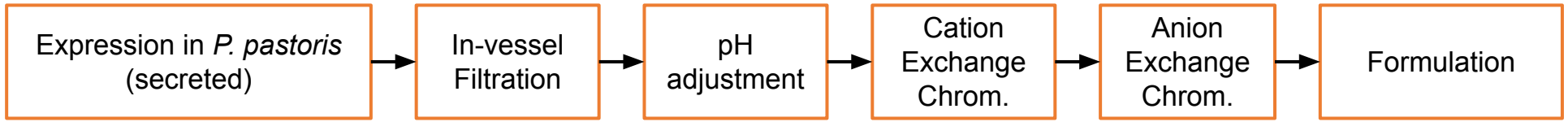
Recombinant protein vaccines

Dalvie *et al.*, *PNAS* 2021;
Dalvie *et al.*, *Microb. Cell Factories* 2021

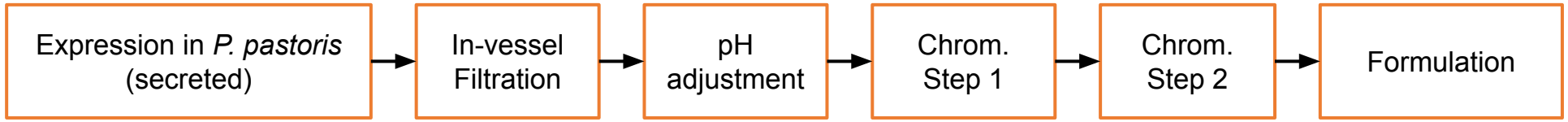


Single-domain antibody (nanobody)

Crowell *et al.*, *Biotechnol. Bioeng.* 2021

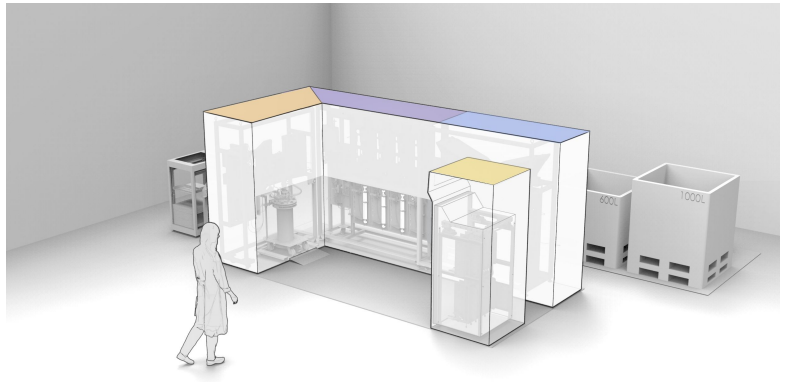


Monoclonal antibody (mAb)



Sunflower's End-to-End Manufacturing Systems:
Simple hardware to enable capacity for anyone

Immediate Process Transfer



Daisy™ System

R&D/Process Development
Automated Protein Bulk Production
(up to grams per week)

Dahlia™ System

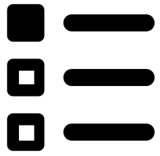
Efficient Commercial Production
Automated Protein Bulk Production
(up to 10 kg annually in <50m^2)

Sunflower End-to-End Systems: Breakthrough automated small footprint commercial protein manufacturing

INTEGRATED SYSTEM FOR CONTINUOUS PRODUCTION OF PROTEIN BULK

End-To-End: Integrated operations for expression, purification, formulation and collection

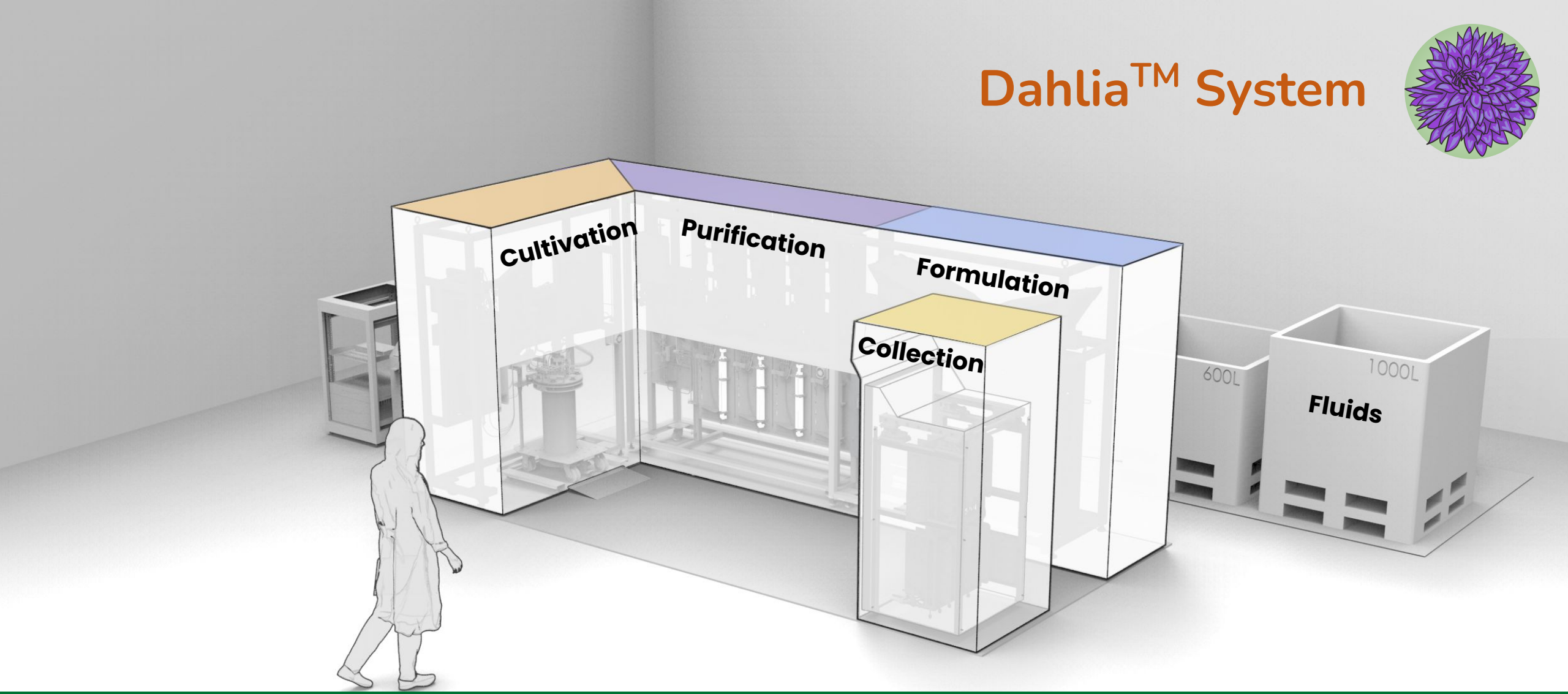
Fully Automated: Add cells & process fluids, push start, & collect protein bulk for a product in just days



Multi-Product: Closed single-use design for agility and flexibility

"Move-In" Ready: Operates in many different types of spaces and environments

Dahlia™ System

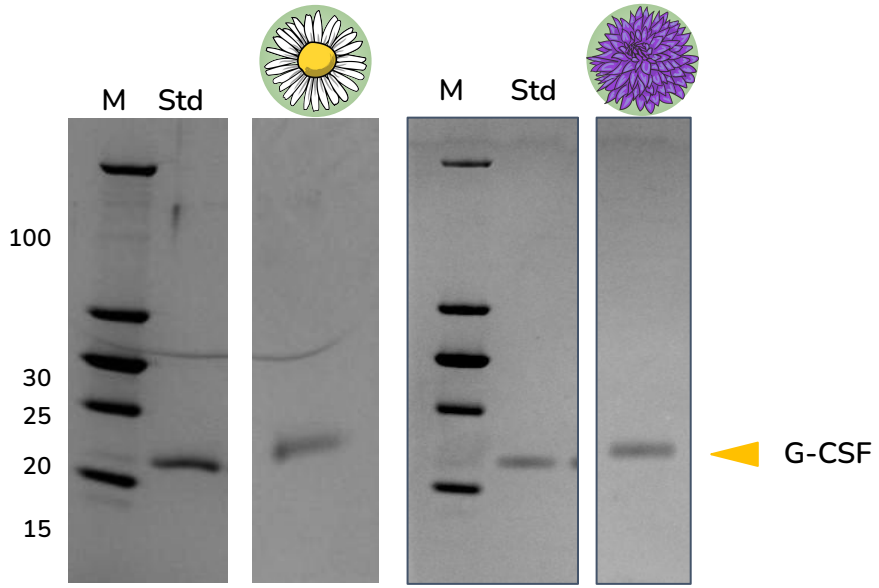


UP TO 10 KG ANNUAL PROTEIN OUTPUT IN <50m^2 WITH MULTI-PRODUCT FLEXIBILITY

High-Quality Protein Generated on Both Systems

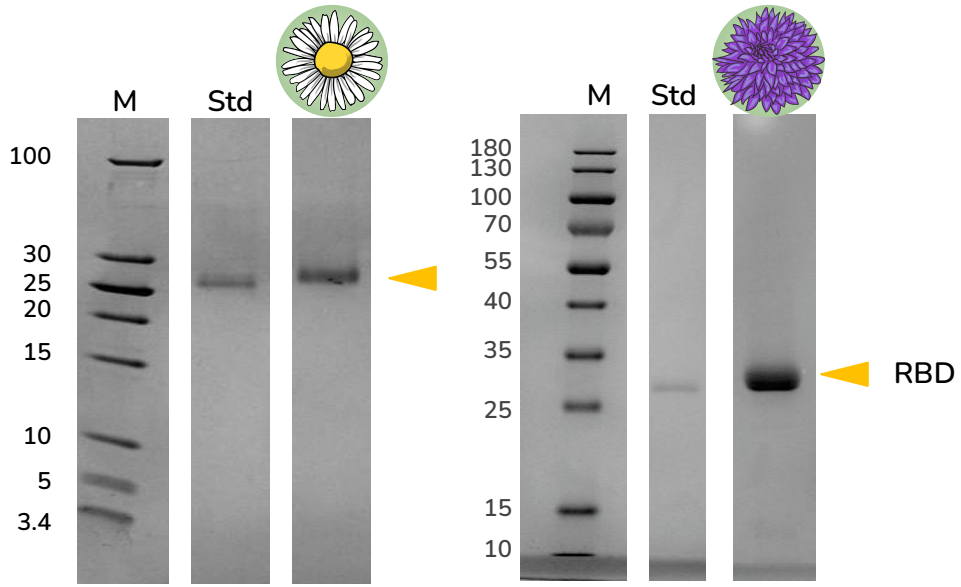
DAISY & DAHLIA SYSTEMS DEMONSTRATE MULTI-PRODUCT CAPABILITIES USING PROCESSES TRANSFERRED FROM ONE TO THE OTHER

G-CSF (filgrastim)



G-CSF Std @ 0.05 mg/mL

SARS-CoV-2 RBD (subunit vaccine)



RBD Std @ 0.05mg/mL

**Processes deployed for both molecules were not optimized for yield (Drafted for equipment demonstrations only)*

Bulk Drug Substances Produced on Systems are Similar

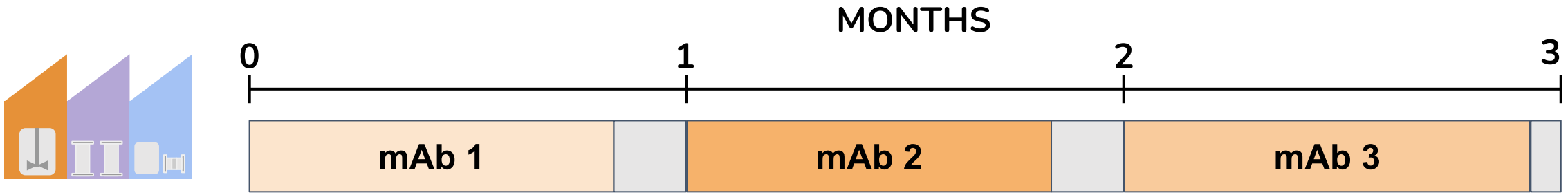
DAISY & DAHLIA SYSTEMS PRODUCED CLINICAL QUALITY BULK DRUG SUBSTANCES IN SIMPLE MANUFACTURING ENVIRONMENTS IN < 1 WEEK

Protein	System Used	Dose Equivalents	Host-cell DNA (ng DNA/mL)	Host-cell Protein (total protein)	Bioburden* (CFU/plate)
G-CSF		~100	<10	< 0.1%	0
		>1,000	<10	< 0.1%	0
COVID-19 Vax Subunit		>2,000	<10	< 0.1%	0
		>50,000	<10	< 0.1%	0

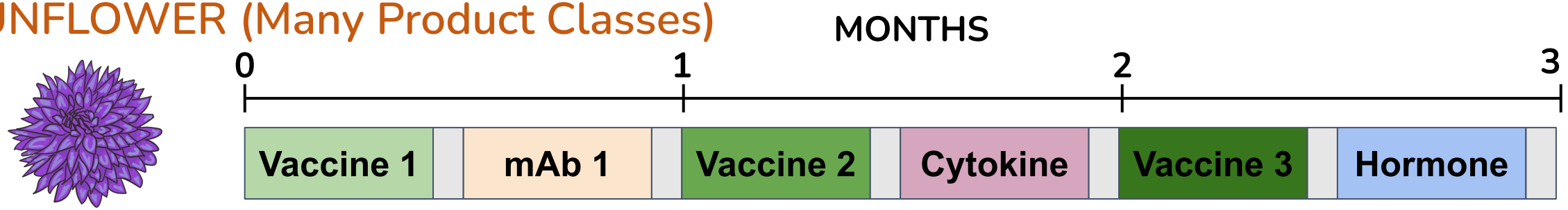
*Performed by third-party contractor according to the Compendial method USP 61 microbiological examination of Non-Sterile Products (Microbial enumeration test)

Sunflower Systems Maximize Facility Utilization

STATE-OF-THE-ART SINGLE-USE PILOT PLANT



SUNFLOWER (Many Product Classes)



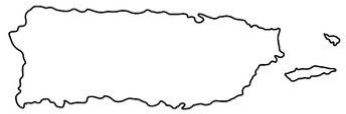
MULTI-PRODUCT CAPABILITY AND AGILITY ENABLES LOWEST MANUFACTURING COSTS

Projected Annual Product Output From Our Approach

VACCINE DOSES*
(50 ug / dose)

2.9 Million

Puerto Rico

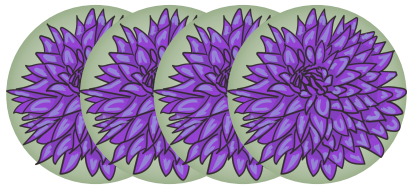


40 Million

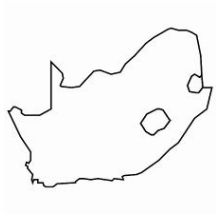
California

OR

Argentina



160 Million

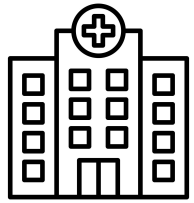


2.5x South Africa

*Medium titer process (1 g/L);
50 - 70% population vaccinated; 2 doses per person

MAB (Trastuzumab) DOSES**
(1 g / dose)

580



Hospital or Medical Center

9,000

Colombia

36,000

Southern Africa

**High titer process (4 g/L); HER2+ breast cancer patients;
18 doses per person per year; 30-50% market penetration

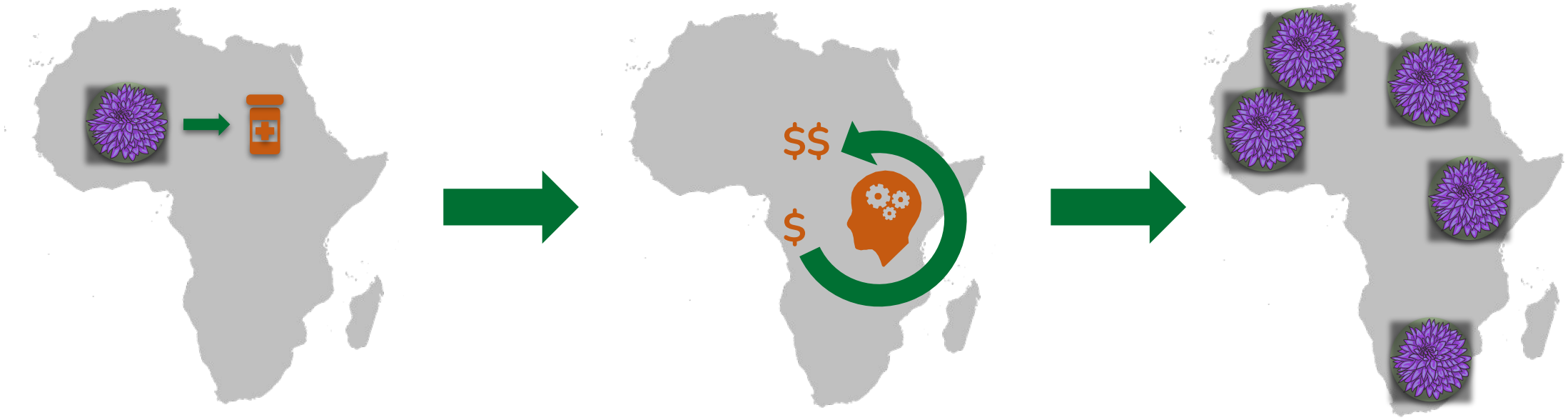
Sunflower's Vision for Democratization of Protein Supply

We believe enabling regional manufacturing capacity
for protein products can promote prosperity everywhere

ACCESSIBLE SOLUTIONS POWER
NEW PRODUCTS & BUSINESSES

NEW INNOVATIONS FUEL
REGIONAL MARKET EXPANSION

MANUFACTURING CAPACITY
GROWS TO MEET DEMAND



Sunflower Can Help You Explore the Benefits of Integrated Manufacturing using Eukaryotic Microbes

Hardware Access

- **Daisy Petal™** perfusion fermentation system commercial release in early 2024! ***Accepting Pre-Orders Now***
- Seeking **early access customers** for evaluation of **Dahlia Petal™**
- Seeking **early access customers** for evaluation of end-to-end **Dahlia™**

Contract Research Services

- Strain, media and process development in eukaryotic microbes
- Demonstrations on Daisy Petal™, Dahlia Petal™, or end-to-end Dahlia™

Acknowledgements

Sunflower Team

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Akshada Shinde
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Mary Kate Tracey
Nikhil Unde

Talk to **Kerry** and **Alex** at DCVMN Annual Meeting in Cape Town, South Africa!


SCAN ME
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