



## Developing single use technology (SUT) cell expansion process to improve cell culture processes

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# Outline



- Development strategy of single use technology (SUT) cell expansion process
- Example of developing a SUT cell expansion process
  - DOE analysis
  - $K_La$  and mixing study
- Considerations of implementing SUT cell expansion process

# SUT Benefit Summary



**Reduces  
safety and  
ergonomic  
risks when  
handling  
glass  
spinners**



**Eliminates the  
environmental  
monitoring,  
process warm  
rooms**



**Eliminates  
intermediate  
transfer  
vessels**

# Cost Analysis: Glass spinner versus SUT seed train processes

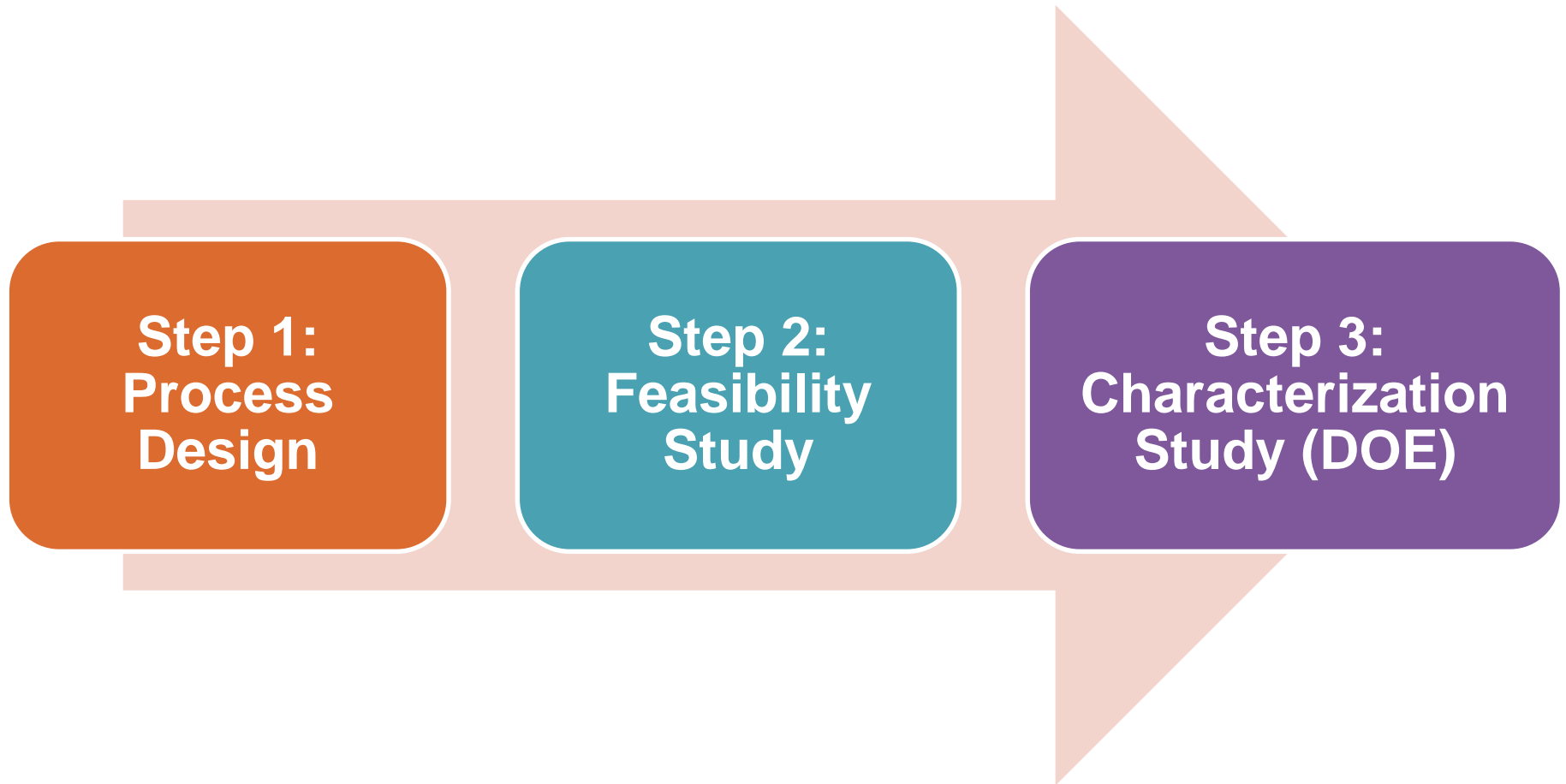
	Glass Spinner Seed Train	SUT Seed Train
CAPEX	No additional investment required	Shaker Incubators, rockers, pumps and sterile tubing welders
OPEX	<u>Material:</u> <ul style="list-style-type: none"> <li>•Medium and consumable</li> </ul> <u>Labor:</u> <ul style="list-style-type: none"> <li>•Spinner preparation</li> <li>•Up keeping autoclave and parts washers</li> <li>•Environmental Monitoring (EM)</li> </ul>	<u>Material:</u> <ul style="list-style-type: none"> <li>•Medium is comparable to glass spinner process</li> <li>•Consumable- single use shake flasks, process bags and connectors</li> </ul> <u>Labor:</u> <ul style="list-style-type: none"> <li>•No vessel preparation</li> <li>•No Autoclave and parts washers required in the process</li> <li>•Minimal EM</li> </ul>

3-4 year to break even



# Strategy

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# Step 1: Process Design- *think manufacturability*

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- Platforms Consideration (shake flasks, rocker platform)\*
  - Vessel sizes\*
  - Working volumes\*
  - Operational conditions
- Process Scale-up Strategy
  - Cell bank size (cell number per thaw)
  - Stages/ Dilution ratio/ Scale up criteria of each stage



Leverage design from publications and/or internal experiences

# Step 1: Process Design

## Shake Flask + Rocker Platform

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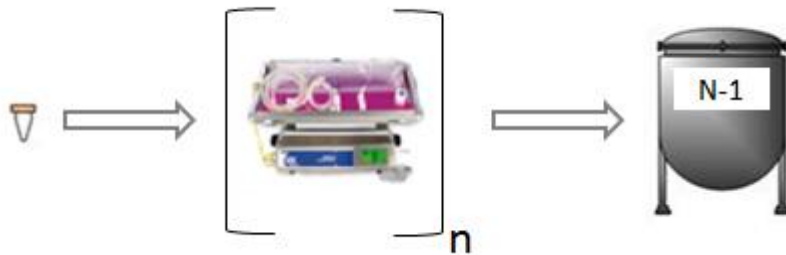
# Could we thaw into bags?

- **Problem Statements:**

- Proposed SUT seed train still requires one stage of open process, which requires daily sampling and scale up
- Shaker incubator has humidity control which could be potential contamination source

- **Approach:**

- Direct thaw into bags or incubator without humidity control



Or





Diagram illustrating a bioreactor setup for cell thawing, highlighting several critical factors for successful cell recovery:

- Low cell density thaw:** Indicated by a callout pointing to the top of the vessel, suggesting a low concentration of cells during the thawing process.
- Evaporation:** A callout points to the headspace of the vessel, indicating the risk of media loss due to evaporation during the thawing process.
- Suboptimal condition for pH and DO sensor reads:** A callout points to the liquid surface, indicating that the presence of cells or media components can interfere with accurate pH and Dissolved Oxygen (DO) measurements.
- Cell clumps and attachments:** A callout points to the bottom of the vessel, where cells are shown clumping and attaching to the stirrer or bottom surface, potentially leading to cell death or inaccurate sampling.
- Sample volume and technique:** A callout points to the liquid surface, emphasizing the importance of using the correct sampling technique and volume to avoid contamination or inaccurate readings.

# Low working volume: Mixing challenge

Eibl et al., Adv Biochem Engin/Biotechnol (2009) 115:55-87

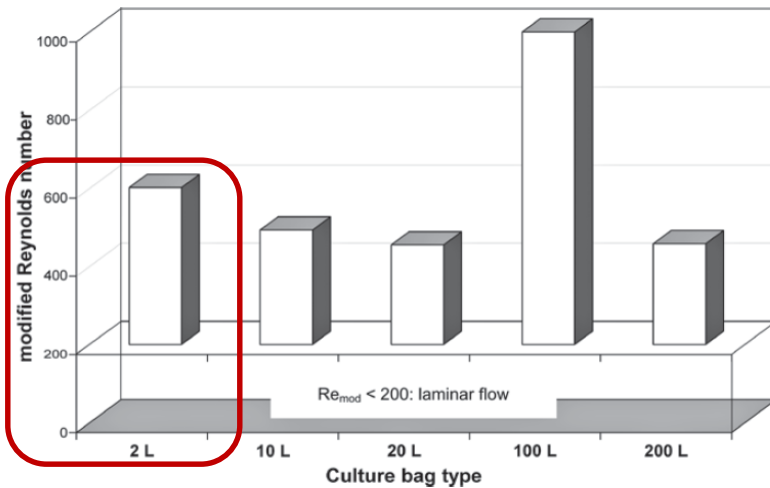
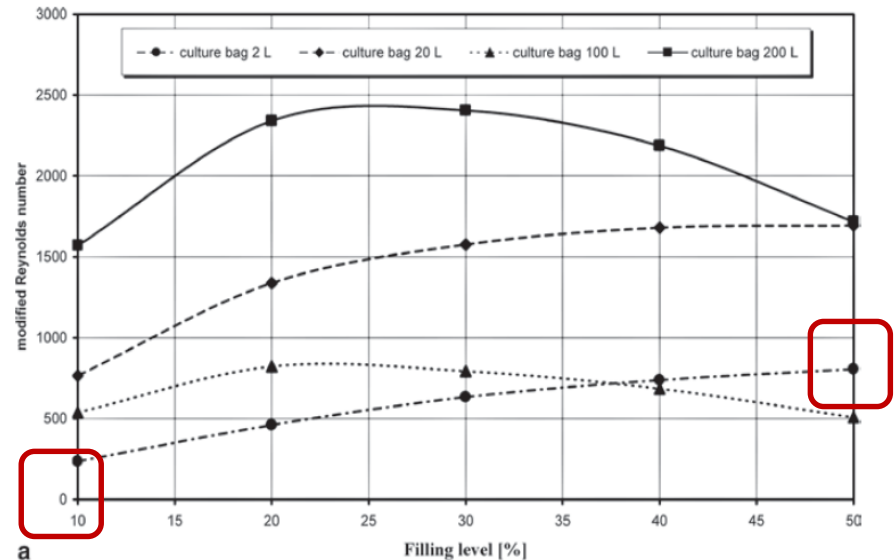


Fig. 6 Transition areas from laminar flow to turbulent flow for different culture bags (filling level 10–50%)

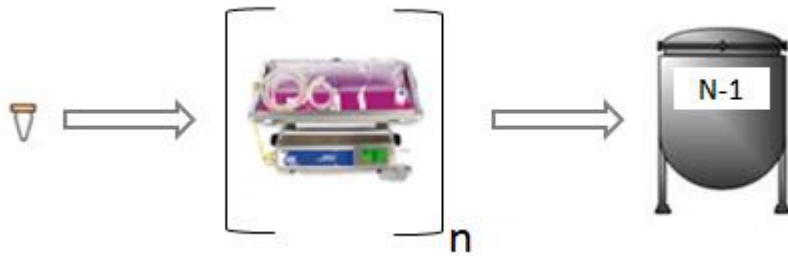


Rocking action 1) creates large turbulent surface for oxygen transfer and 2) sweeps up cells and prevents settling.

Post thaw working volume in a 2L bag would be within laminar/transition flow range. When scale up to 1L ww in the same 2L bag, it falls into transition flow or starts to become turbulent flow.

# Focus on eliminating the humidity control

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Or



~~With  
Humidity  
Control~~

## Step 2: Feasibility Study

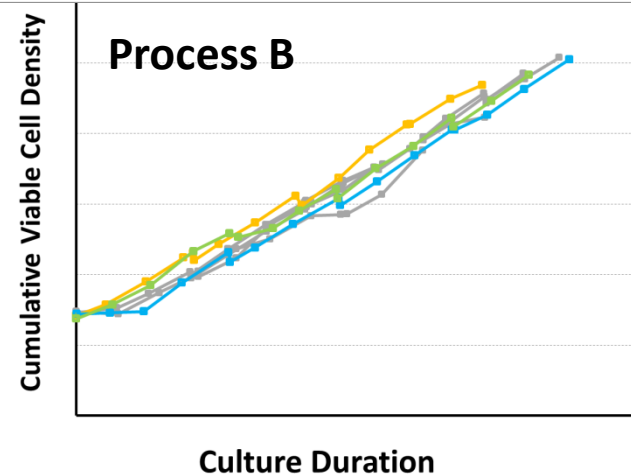
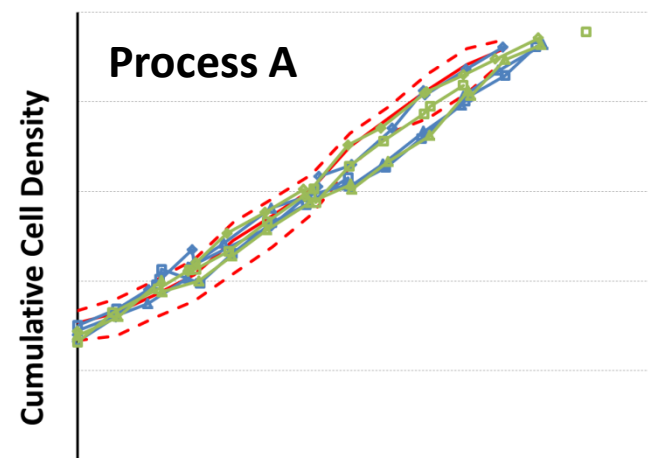
### Shake Flask w/o Humidity Control + Rocker Platform

- **Two Feasibility Examples:**

- Process A: Same dilution factors as glass spinner process
- Process A and B: Same final cell density and volume as glass spinner process

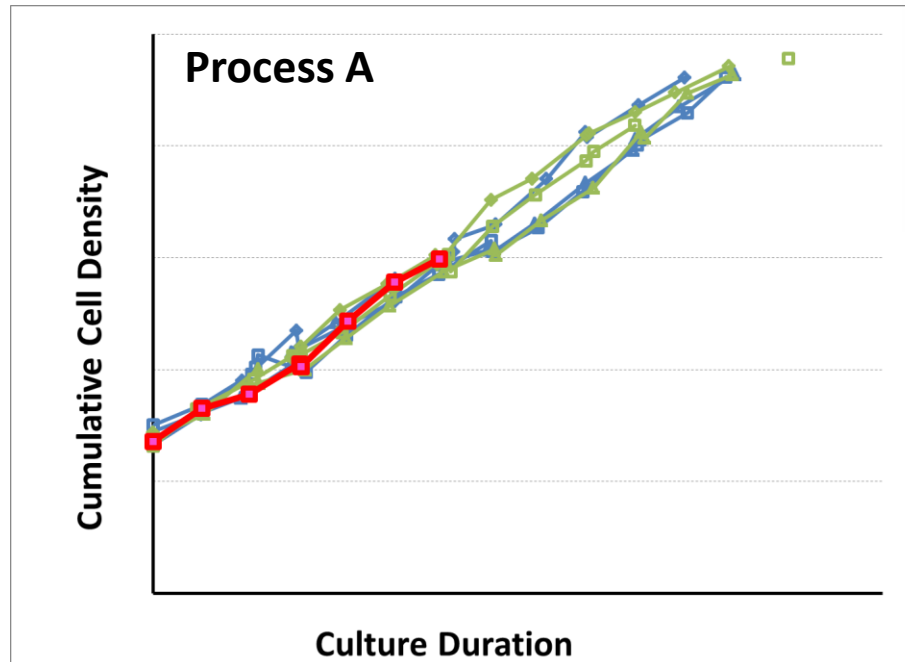
- **Results:**

- SUT seed train comparable to glass spinner seed train



# Further Equipment Consideration: What if CO<sub>2</sub> shaker incubator is not available?

Closed Cap Shake Flask +  
Rocker Platform



# Step 3: Characterization Study

## ● Objective:

- To establish Setpoints and Acceptable Ranges (ARs) for selected Process Control Elements (PCEs)

## ● Approach:

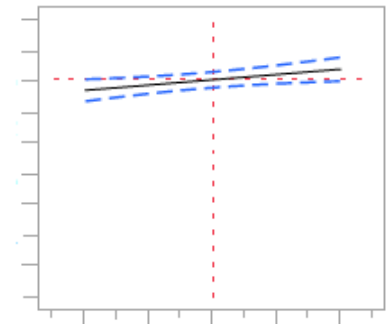
- Design of Experiment (DOE) study
- 2 Levels, 8 Factors split plot design
- 22 Runs, including 2 center points
- Run center points and extreme conditions at beginning
- Establish Models for each stage

Whole Plot	P1 Temp	P1 Agitation Rate	P2 Temp	P2 Rocking Speed and Angle	P3 Temp	P4 Rocking Speed and Angle	P4 Temp	P4 Rocking Speed and Angle
1	0	0	0	00	0	0	0	0
1	0	0	0	00	0	0	0	0
2	+	+	+	--	+	--	+	++
2	+	+	+	--	+	--	+	++
3	+	+	+	--	+	--	+	++
3	+	+	+	--	+	--	+	++
4	+	+	+	--	+	--	+	++
4	+	+	+	--	+	--	+	++
5	+	+	+	--	+	--	+	++
5	+	+	+	--	+	--	+	++
6	+	+	+	--	+	--	+	++
6	+	+	+	--	+	--	+	++
7	+	+	+	--	+	--	+	++
7	+	+	+	--	+	--	+	++
8	-	-	-	--	+	++	+	++
8	-	-	-	--	+	++	+	++
9								
9								
10								
10								
11								
11								

2 Level, 8 Factors  
Split Plot Design

### Prediction Profiler

Cell Viability (%)



P1 Parameter 1

# DOE Analysis

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Use cell density curve to estimate the duration of each stage

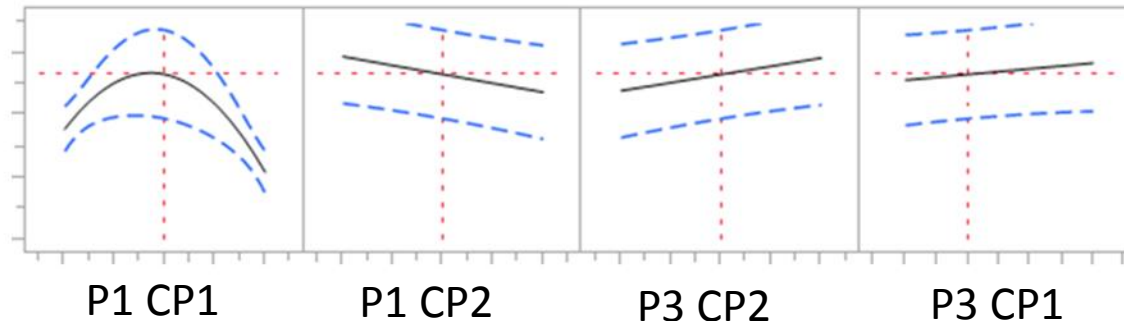
Use the estimated duration and cell density data to build models

Cell density, viability, and duration of each stage

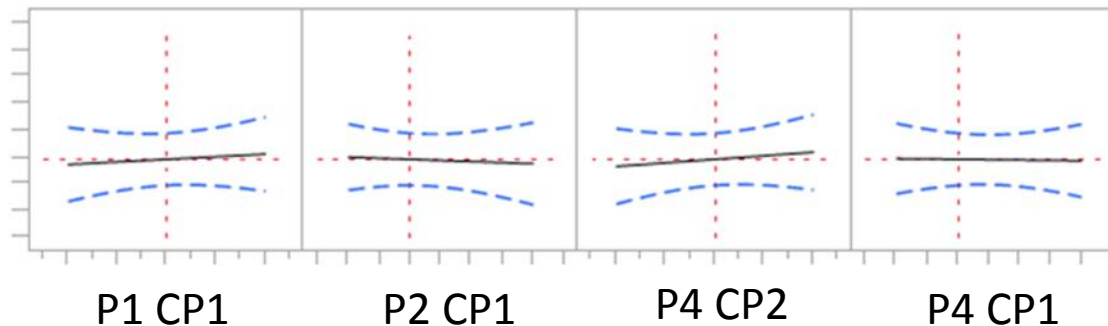
Use models to confirm process operational ranges could be set at DOE study range

# Step 3: Characterization Study: Example DOE Results

**Prediction Profiler** Stage Duration



**Prediction Profiler** Cell Density

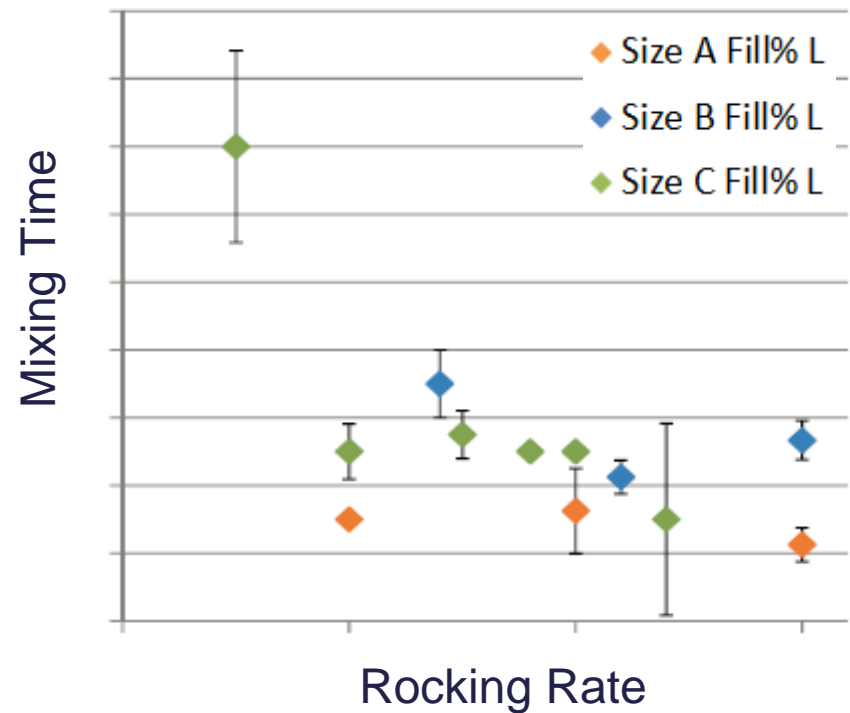
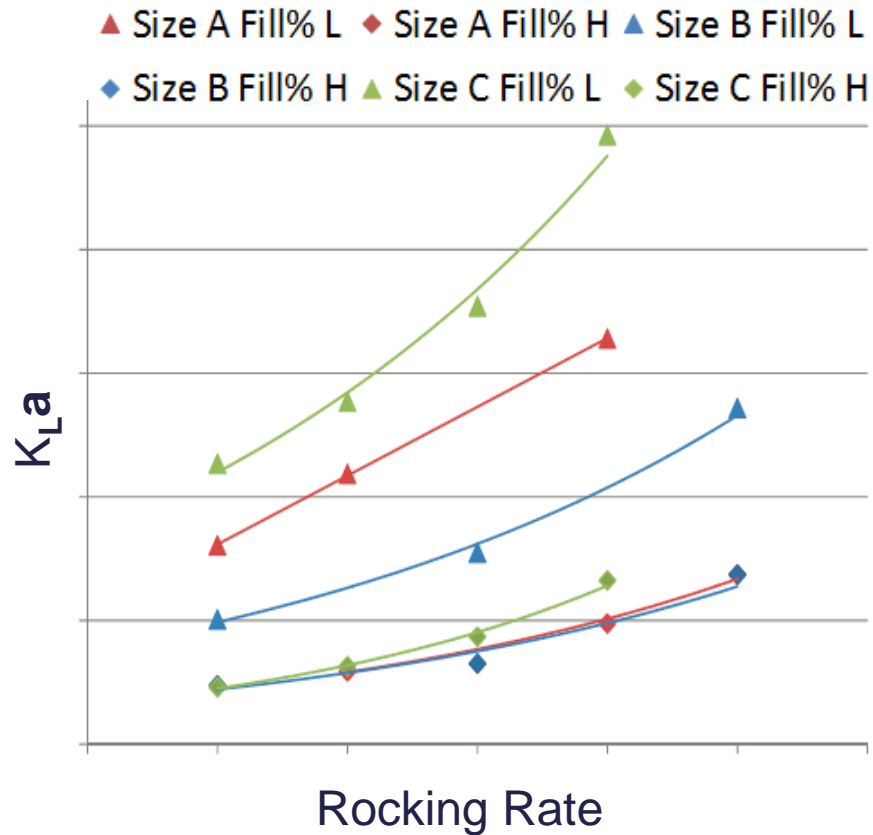


CP: control parameter



# Step 3: Characterization Study: $K_L a$ / Mixing Time Study

**Example:** Rocking Angle: 8

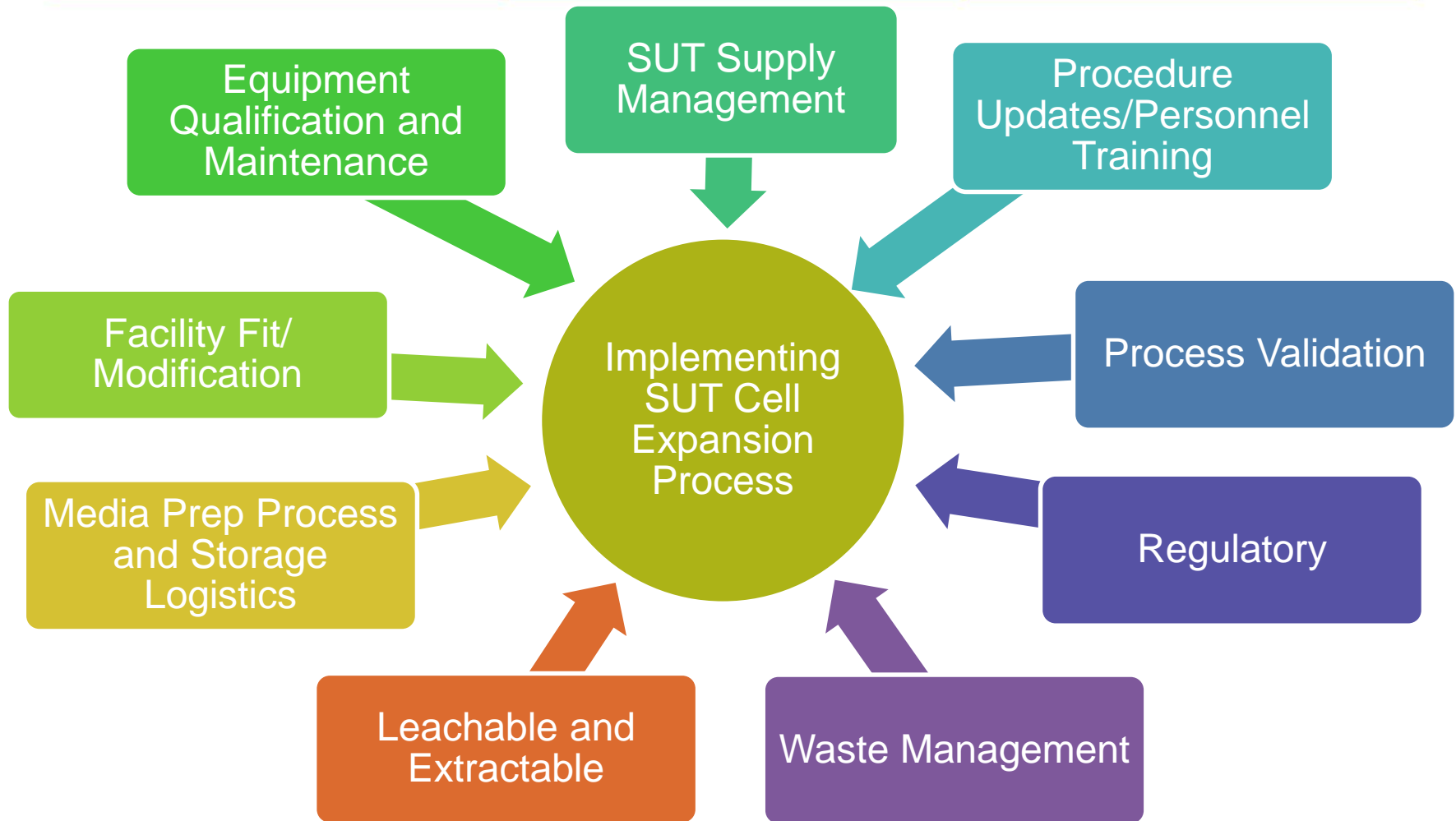


# Process Development Summary

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- **Manufacturability** must be considered in the process design and proposal steps should involve **multi-functional** teams' effort
- Comparable performance between SUT and glass spinner processes is achievable with the benefits to eliminate
  - Glassware handling (cleaning, assembly, transferring)
  - Environmental monitoring in open processes
- Departments that could benefit from this process including Equipment and Media Preparation, Cell Culture, and Quality Control

# Considerations of implementing SUT cell expansion process



# Considerations of implementing SUT cell expansion process

## Leachable and Extractable:

- Assess risk of impact the safety and efficacy of the final drug product
- Performed internally or by vendor
- CQA
- Seed Train CPP/KPP

# Considerations of implementing SUT cell expansion process

## Media Prep Process & Storage Logistics:

- Streamline Media Prep via SUT
  - Sterile connection comparability between small scale and media prep processes
- Bottles vs Bags



# Considerations of implementing SUT cell expansion process

## Facility Fit/Modification:

- Utilities (process gas sources)
- Storage (SUT supplies and media inventory)



# Considerations of implementing SUT cell expansion process

## Equipment Qualification & Maintenance:

- Establish User Requirement etc.
- Internal/External Service
- PM Frequency
- Qualification Criteria (Multi/Single Process)



# Considerations of implementing SUT cell expansion process

## SUT Supplies Management:

- Off-the-shelf vs. customized
- Shelf life
- Lead Time
- Alternative supplies
  - Comparability (bag geometry, sensors, connectors)





# Considerations of implementing SUT cell expansion process

## Process Updates & Personnel Training

- Regulatory Filing
- Process Control Strategy (PCS)
- SOP/Batch Records
- On-the-Job Training



# Considerations of implementing SUT cell expansion process

## Process Validation

- Development Runs and Engineering Runs
- Protocol
  - determine process acceptable ranges based c development and/or engineering run results
- Define Scope (*Where* and *When* is the Change)
  - Combine or separate from Media SUT process PV runs
  - Thaw to N-1 stage
  - Number of runs
  - Lot-to-lot variations
  - Scheduling
    - Room for expansion
    - Family approach
- Establish Inventory Supply Chain



# Considerations of implementing SUT cell expansion process

## Regulatory

- Assess Regulatory impact
- Filing requirement different with different authorities
- Data package & Response Time



# Considerations of implementing SUT cell expansion process

## Waste Management

- Recyclable OR biohazard waste consumables
  - Media bottles or bags
  - SUT Shake flasks
  - Process bags
  - Package materials



Extractable

waste management

# Summary Conclusion

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- Development strategy of single use technology (SUT) cell expansion process
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  - Kla and mixing study
- Considerations of implementing SUT cell expansion process

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Thank you