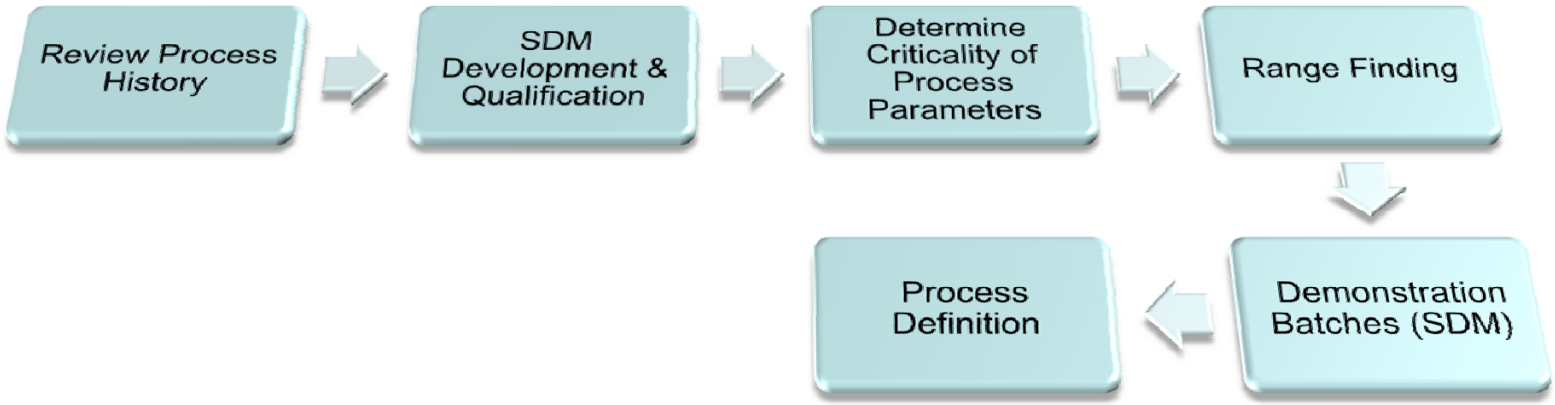
Eden Approach To Bioprocess Characterisation

Philip Mellors
USP Development & Transfer Team Leader

Process Characterisation

- **What is Process Characterisation?**

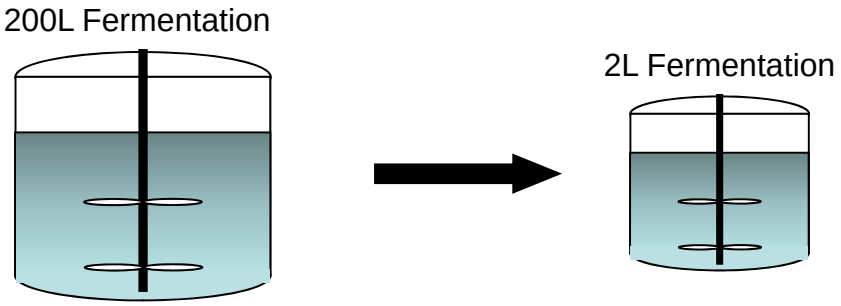
“ A late stage study that evaluates the process to increase process knowledge and examines proposed operational ranges and their individual and/or combined impact on target protein quality and process performance”.



Scale Down Model Development & Qualification

- Impractical to execute process characterisation studies at production scale.
- Process characterisation studies are performed using a Scale Down Model (SDM).
- Scale Down Model for given unit operation must be **representative** and **predictive** of that unit operation when performed at full scale.

SDM Development & Qualification Strategy



- **Overview of Activities**
  - Develop suitable SDMs for US and DS process steps.
    - For mammalian fermentations, relative gas flow rates (v/v/min) and impeller tip speed are maintained across scales.
    - For chromatography, bed-height, linear flow rates, load ratios, CVs of buffer for wash & elution are maintained across scales.
  - Qualification Activities:
    - Perform 3 batches of SDM
    - Process parameters run at base line conditions (i.e. at production set point).
    - Pre-defined acceptance criteria for process outputs (e.g. growth kinetics, yield, purity etc.).

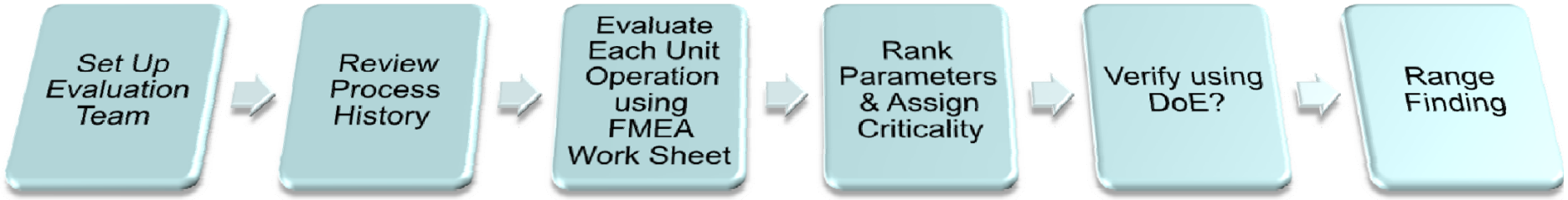
Determine Criticality of Process Parameters

- Definitions:
  - Critical Process Parameter (CPP):
    - "*Process parameter that should be maintained within a narrow range so not affect critical quality attributes (CQA)".*
  - Key Operational Parameter (KOP):
    - "*Process parameter that should be maintained within a narrow range and is essential for process performance".*
  - Non Key Operational Parameter (NKOP):
    - "*Process parameter that is easily controlled or has a wide acceptable limit. May have an impact on CQAs or process performance*".

FMEA

- Failure Modes & Effects Analysis (FMEA) is a risk assessment tool.
- Methodology for identifying and evaluating potential modes of product or process failure.
- Can be used to help determine the criticality of process parameters.
- Based on ranking 1 to 10 (low to high) 3 criteria, Severity, Occurrence and Detectability.
- **Severity**: impact on CQAs & process performance if the parameter varies from the operational range.
- **Occurrence**: probability that the parameter may vary from the operational range.
- **Detectability:** probability of detecting that a parameter has varied from operational range.
- The product of the 3 results (S x O x D= RPN) provide the Risk Priority Number (1-1000).

FMEA Work Flow

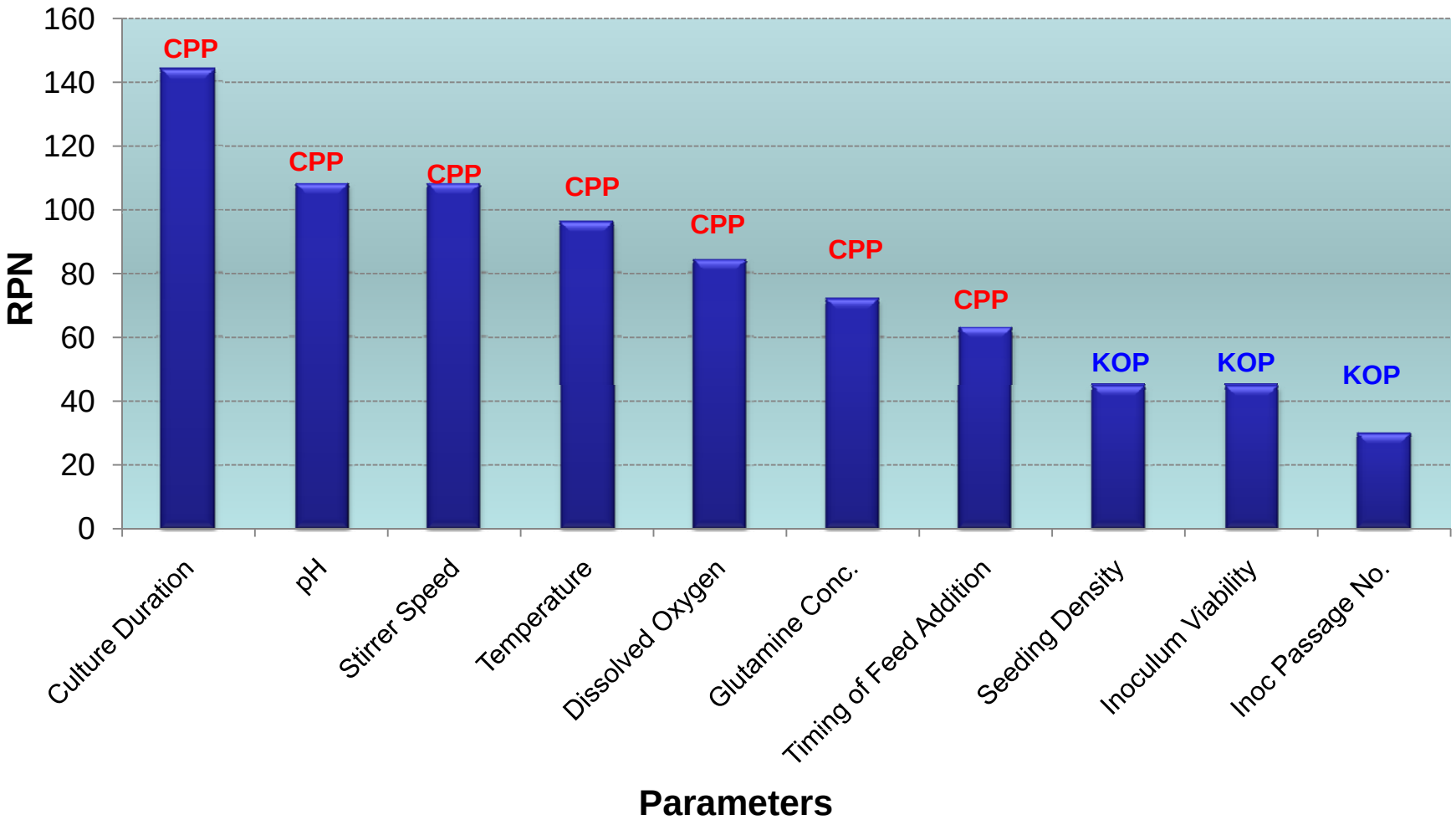


- When is FMEA as a stand alone tool acceptable to determine the criticality of process parameters?
  - i) after extensive development preferably using DoE to map the design space.
  - ii) multiple batches at full scale.

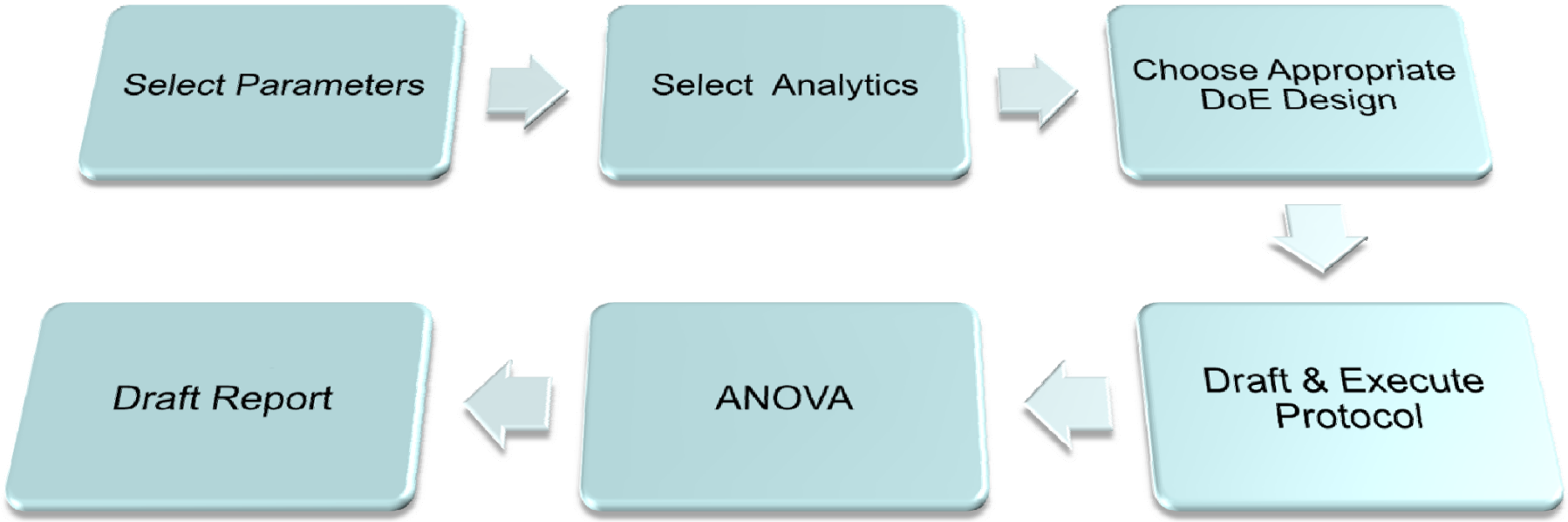
Case Study Part 1 FMEA

Operational Parameter	Failure Modes	Causes	Effects	S	O	D	RPN
Seeding Density	<1 x 10^5 cells/mL	• Operator error	• Reduced Product Yield	5	3	3	45
Inoculum Viability	< 80%	• Operator error	• Reduced Product Yield	5	3	3	45
Inoculum Passage #	>50	• Operator error	• Reduced Product Yield	5	2	3	30
Glutamine Conc. In Base Media	< 2mM or > 8mM	• Operator error • Faulty Balance	• Reduced Product Yield • Reduced Product Quality	8	3	3	72
Timing of Feed Addition	> Day 3	• Operator error	• Reduced Product Yield • Reduced Product Quality	7	3	3	63
Dissolved Oxygen	<10%	• Incorrect Calibration • Faulty Probe	•Reduced Product Yield • Reduced Product Quality	7	4	3	84
Stirrer Speed	<70rpm or >90rpm	• Operator error	•Reduced Product Yield • Reduced Product Quality	9	4	3	108
Temperature	> 39ºC	• Incorrect Calibration • Faulty Probe • Faulty Heat Exchanger	•Reduced Product Yield • Reduced Product Quality	6	4	4	96
pH	<6.8 or >7.4	• Incorrect Calibration • Faulty Probe	• Reduced Product Yield • Reduced Product Quality	9	4	3	108
Culture Duration	TBD	• Operator error	•Reduced Product Yield • Reduced Product Quality	9	4	4	144

FMEA Pareto Chart



Determine Criticality of Process Parameters Using DoE



Case Study Part 2 DoE Verification

- Examined 7 parameters (glutamine Concentration, timing of feed addition, DO, stirrer speed, temperature, pH and culture duration)
- DoE Design:
  - Fractional Factorial.
  - 2 Levels.
  - 15 Experiments.
  - 3 Centre Points.
- Analytics:
  - Max Product Titre, Cumulative Cell Time (CTT), Max Viable Cell Density (Max VCD), Max Ammonia conc., Max Lactate conc, Max CO_2 and Product Quality .

Summary of Effects

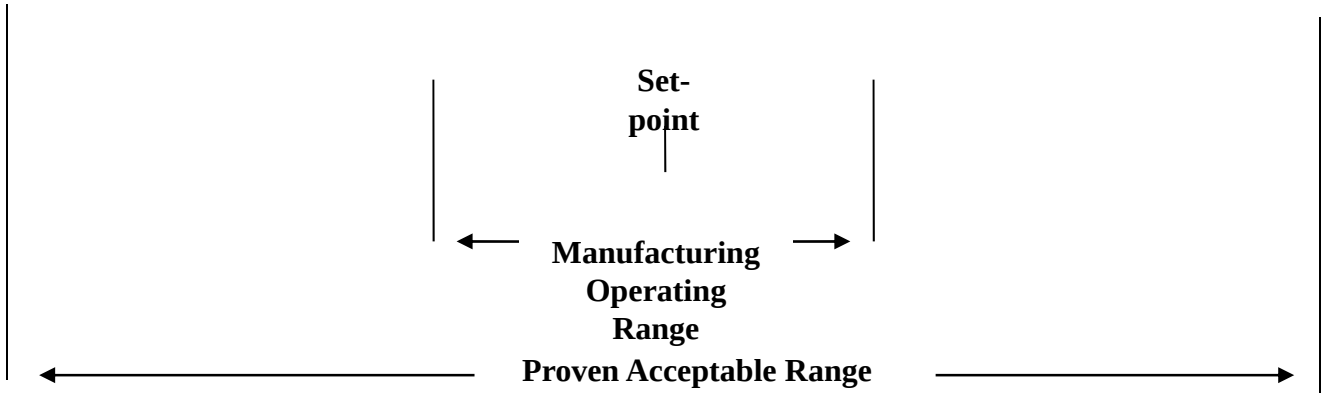
Parameter	Max Titre	CCT	Max NH_3	Max VCD	Max Lactate	Max CO_2	Product Quality	Criticality
Glutamine Conc.	(-)		+	(-)				KOP
Timing of Feed	(-)	(-)		(-)				KOP
DO	(+)	(+)		(+)	-			KOP
Stirrer Speed	+	+		+	-	-	+	CPP
Temp	+	+	(+)	+	(+)			KOP
pH	-	-	(+)	-	(+)		-	CPP
Culture Duration			+		(+)		-	CPP

- Increasing input results in decreasing output >95% Confidence.
+ Increasing input results in increasing output > 95% Confidence.
(-) Increasing input results in decreasing output >90% Confidence.
(+) Increasing input results in increasing output >90% Confidence.

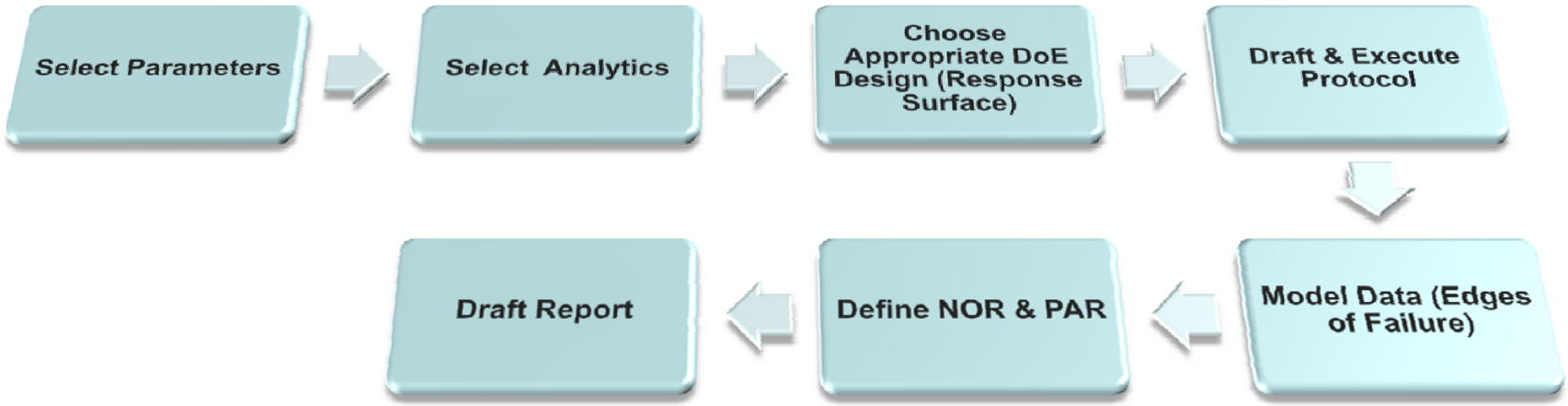
Range Finding

- Proven Acceptable Range:
  - *"The range at which a process parameter can be controlled at, to deliver a product of acceptable quality."*

- Range Finding:
  - *"To explore the operational range for individual CPP (& some KOPs) to determine the Proven Acceptable Range (PAR) and propose Manufacturing Operating Range."*



Range Finding Using DoE



Case Study Part 3 DoE Range Finding

- Examined 3 parameters (pH, culture duration and stirrer speed).
- DoE Design:
  - Box Behnken Design (Response Surface Design).
  - 3 Levels.
  - 12 experiments.
  - 3 Centre Points.
- Analytics:
  - Max Product Titre, Cumulative Cell Time (CTT), Max Viable Cell Density (Max VCD), Max Ammonia conc., Max Lactate conc, Max CO_2 and Product Quality .

DoE Design

Run #	Pattern	pH	Culture Duration	Stirrer Speed
1	- -0	6.8	10	85
2	+ - 0	7.5	10	85
3	- + 0	6.8	14	85
4	+ + 0	7.5	14	85
5	- 0 -	6.8	12	70
6	+ 0 -	7.5	12	70
7	- 0 +	6.8	12	100
8	+ 0 +	7.5	12	100
9	0 - -	7.15	10	70
10	0 + -	7.15	14	70
11	0 - +	7.15	10	100
12	0 + +	7.15	14	100
13	0 0 0	7.15	12	85
14	0 0 0	7.15	12	85
15	0 0 0	7.15	12	85

Summary of Effects

Parameter	Max Titre	CCT	Max NH_3	Max VCD	Max Lactate	Max CO_2	Product Quality
Stirrer Speed	+	+		+	-	-	+
pH	-	-	+	-	(+)		-
Culture Duration		+	+		(+)		-
(Stirrer Speed)^2							
(pH)^2	-	-	+	-	+		+
(Culture Duration)^2	(+)						+
No interations							

Ranges

Parameter	Proposed Operating Range	Predicted PAR
pH	7.1 ± 0.1	6.8-7.3
Culture Duration	12 days	11-13 days
Stirrer Speed	90 ± 10 rpm	80-110rpm

Follow On

- If through the predictive modelling edges failure are outside the original design (e.g. pH <6.8 or >7.5) then verification studies are required to confirm PAR.
- For identified CPPs preventative measures should be incorporated into the batch manufacturing records to mitigate against parameter deviation (e.g. dual sign off, additional in process analysis, use of redundant probes etc).
- If any of the original set points are close to the edges of failure, the process may have to be modified. To ensure the modified process is robust and capable of delivering product of the required quality, small scale demonstration batches are usually performed.

Acknowledgements

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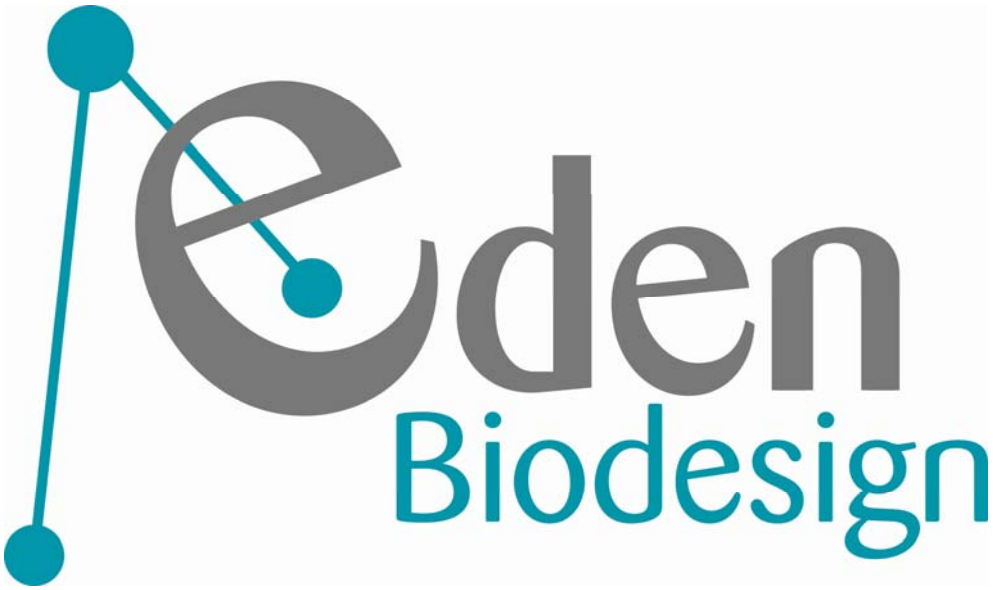
Any questions are welcome and encouraged, they can be asked by e-mailing:

eden.biodesign@propelmg.com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