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DISCOVERY SUMMIT
EXPLORING DATA
INSPIRING INNOVATION

Variance components analysis – Nested design

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Abstract

With cost and timeline pressures for process development there is a drive to use high throughput methods. Recent commercial availability of small scale down system hardware when coupled with design of experiments software enables the potential discovery of the few critical process parameters from the many, in the short timelines required. For process evaluation, early phase development of fermentation recombinant protein processes often uses generic assay methods, but the high throughput methods are not necessarily optimised for accuracy and precision. Thus the overall process analysis is the sum of process variability and that of the measurement system. The estimate of experimental error (noise) will determine the size of response difference (signal) that can be readily detected in the experimental design. JMP software contains process quality analysis tools such as gauge analysis and measurement system analysis that can be used to identify the sources of variation. For expression of an intracellular model protein in *E coli* the work presented here will show how JMP can carry out variance component analysis on a nested design of the overall process analysis. This information can then be used in the subsequent JMP design of experiments evaluation.

Introduction

As a Contract Development Manufacturing Organisation (CDMO) our focus is on supporting our customers with the development and manufacture of recombinant proteins, viral vaccines and gene therapies. Process Development (PD) establishes processes suitable for manufacture and QC. In upstream process development (USP) microbial strain construction and cell line development lead to cell lines making the desired product – typically recombinant proteins. Fermentation / cell culture of these strains / cell lines can investigate the process parameters that influence product formation (either product formation inside the cell or product secreted into the medium). Subsequent primary separations will separate the product from the cell biomass and filter this through a 0.2 µm filtration membrane, prior to purification.

Fujifilm platform pAVEway™ and customer processes can be optimised / characterised using a scale down model in the Sartorius ambr™250 parallel bioreactor system (Figure 1).

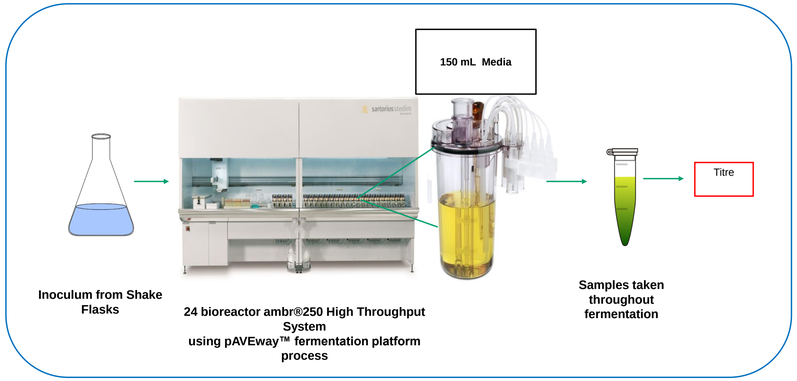

Figure 1: 24 bioreactor ambr®250 High Throughput System

In order to automate the product titre analysis, the samples from the ambr™250 bioreactors are diluted, then a protein preparation prepared using 2 automated robot (Tecan) stages before loading onto the LabChip and analysing the results. A schematic process flow is shown in Figure 2.

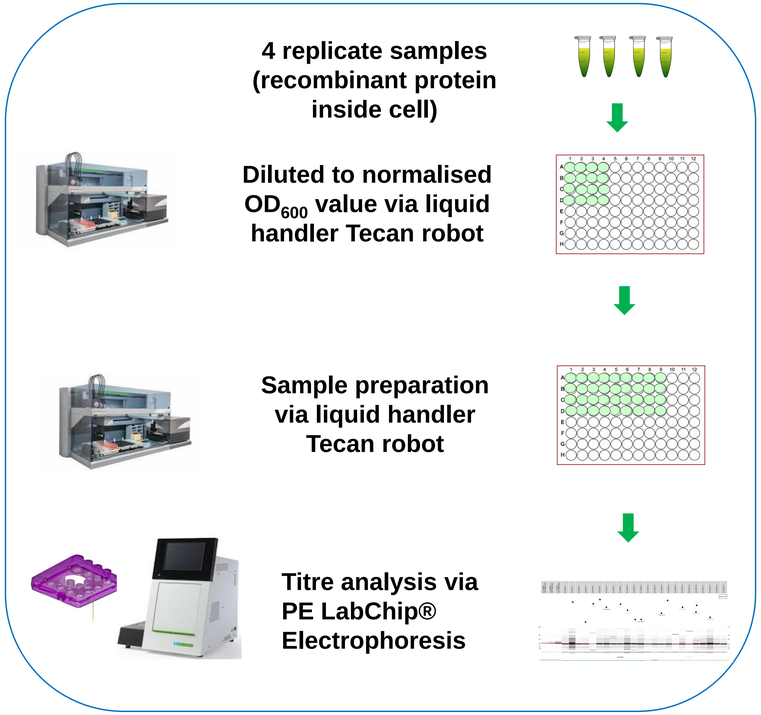

Figure 2: Process flow for sample preparation and analysis

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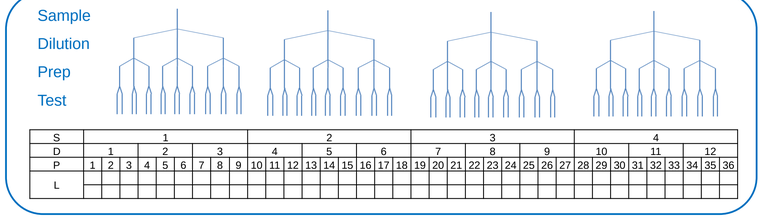
Objective

Determine the relative contributions of assay steps to the overall assay variation.

Methods

Replicate centre point samples were processed identically through the dilution, protein preparation, and loading onto the LabChip. Product band intensities were quantified using the LabChip software to generate a relative quantity value for each protein sample / replicate load. The experimental design used was a nested design (Figure 3). The Design can be generated in a JMP data table (Full factorial variant or enter manually) where the labels (sample (S), dilution (D), preparation (P), and LabChip (L) results) in Figure 3 are transposed into columns.



S	1									2									3									4								
D	1			2			3			4			5			6			7			8			9			10			11			12		
P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
L																																				

Figure 3: Nested design

Results

The relative quantity values for each of the sample replicates is pasted into the JMP Data Table. Using the JMP Analyse / Distribution menu in JMP a histogram plot of the results can be generated (Figure 4).

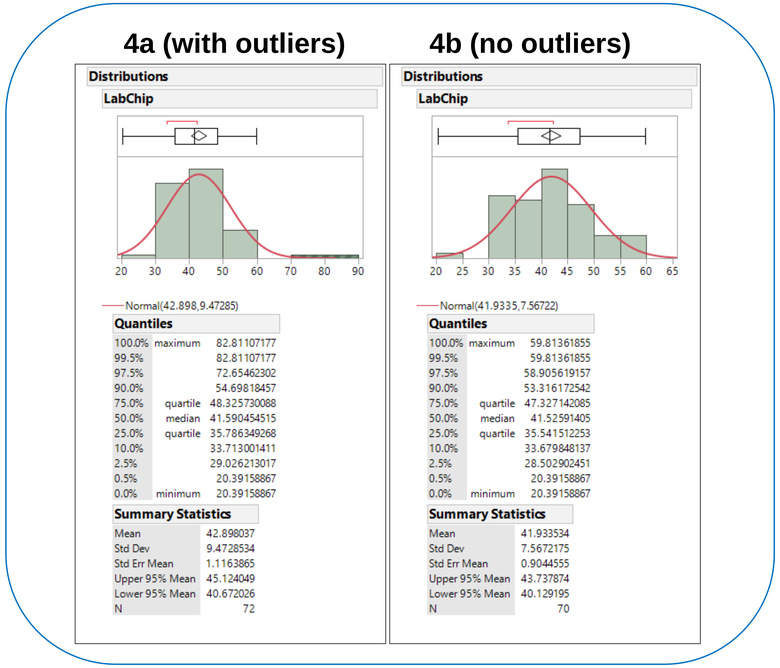


Figure 4: Histogram Charts with Fitted Normal plot

From the JMP/Analyse/Quality and Process/Variability & Attribute gauge chart menu a variability chart for the LabChip results can customised (Figure 5).

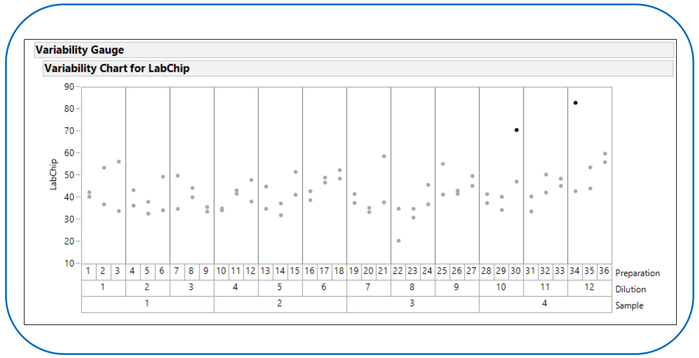


Figure 5: JMP Variability Chart for LabChip

From the histogram plot (Figure 4) and variability chart (Figure 5) there appear to be 2 outlier values. In JMP these values can be selected to hide (and exclude from further analysis). In Figure 5 we can see the 2 LabChip readouts for each sample replicate along with the range and mean (can customise using the red triangle as required).

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Results

Variance Components

Component	Var Component	% of Total	20 40 60 80	Sqrt(Var Comp)
Sample	2.960725	5.4		1.7207
Dilution[Sample]	6.350444	11.5		2.5200
Preparation[Sample,Dilution]	7.433079	13.5		2.7264
Within	38.366828	69.6		6.1941
Total	55.111077	100.0		7.4237

Figure 6: The Variance Components Table

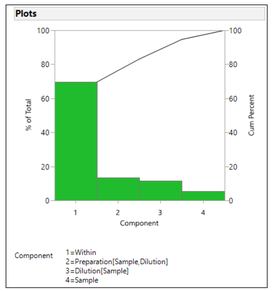

Figure 7. Variance components Pareto Chart

From the Variability Chart, the variance components option can be selected and this can be made into a new data table as shown in Figure 6. Hide and excluding the total row and then selecting the JMP Analyse/Quality and process/Pareto option will enable a Pareto Chart of the factors to be shown (customise using the red triangle) in Figure 7. The corresponding experimental design analysis (alternative approach) is shown in Figure 8.

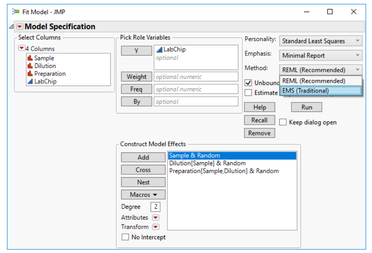

Figure 8: Experimental design analysis

Conclusions

- JMP allows a relatively easy approach to data visualisation of variability and generation of variance component tables.
- JMP has the advantage in that if the balanced design becomes unbalanced following removal of outliers, the variance components can be automatically analysed using REML / Bayesian methods. A similar analysis of the original data (outliers included) using the expected mean squares method also indicated the same significant factor.
- The main component of assay variation is the LabChip stage of the assay. Further efforts on reducing assay variation for this step are underway.
- Replication of this step may helpful if variation still an issue.

ACKNOWLEDGEMENTS

Amy Woodhall – did the wet work in the lab
Ambr™250 with help from Mary Greenwell & Julia Kraemer
Tecan & LabChip with help from Deidre Boland
Phil Greaves – generated the design and did the statistics (initially the hard way)
Paul Nelson (PRISM)
Who introduced the concept (if not worked example) of nested designs many years ago (SB)
Phil Kay (JMP)
Who suggested the JMP Quality and Process route to get the Variance components

REFERENCES

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Box, Hunter, & Hunter (Wiley, 2005) Statistics for Experimenters. pp350 Table 9.8
Montgommery (Wiley, 1997) Design and analysis of experiments. pp510 Table 12-3
https://www.prismtc.co.uk/resources/blogs-and-articles/how-to-nested-analysis
Drain (CRC Press, 1998) Statistical Methods for Industrial Process Control. Chap 3, pp191 - 227
Examples in C:\Program Files\SAS\JMP\14\Samples\Data\Variability Data
2 factors nested.jmp
3 factors nested.jmp
https://www.fujifilmdiosynth.com/resource/ambr250 - Dewar & Slingsby White paper