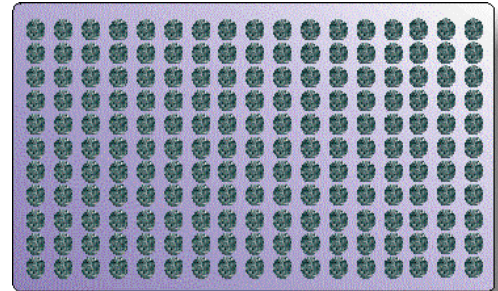


Improving a Biological Assay

Biological Assay Development

The development and validation of new biological assays is often a rate limiting step in modern pharmaceutical research. A simple way of accelerating method development is by employing the principles of Design of Experiments (DoE). A key benefit of DoE is that experiments are planned to specific objectives and endpoints so that deadlines and costs may be accurately projected in advance.



Benefits of Design of Experiments

Design of Experiments (DoE) is a method for planning and analysing experiments to give the maximum amount of information from a small number of carefully chosen experiments. The basic principle is to construct a set of experiments which cover the ranges of each factor (controlled variable) as efficiently as possible. The process begins by defining the ranges for each factor and the responses to be investigated. The next stage is to select a design which matches the experimental objective. There are three common objectives:

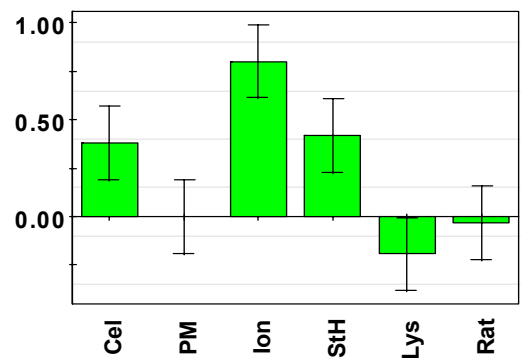
- | | |
|--------------------|---|
| Screening | Which factors are important? |
| Optimisation | What are the optimum settings of the important factors? |
| Robustness Testing | Is the method sensitive to small changes to the optimum settings? |

Reporter Gene Assay

The steps in DoE may be illustrated by an example involving the development and optimisation of a Reporter Gene Assay. The assay was developed in an inflammation study where the expression of a particular gene corresponded to a fluorescent response of a luciferase reporter gene.

The aim of the project was to increase and stabilise the signal to noise ratio of the assay.

Investigation: Reporter Gene Assay Screening (MLR)
Scaled & Centered Coefficients for S/B~



Screening Important Factors

The 6 factors varied in the experiment were:

- | | | |
|------------------|----------------------|------------------------|
| Number of cells | Amount of PMA | Amount of Ionomycin |
| Stimulation time | Lysing buffer volume | Sample:Substrate ratio |

NB PMA and Ionomycin are agents added to stimulate T-cells

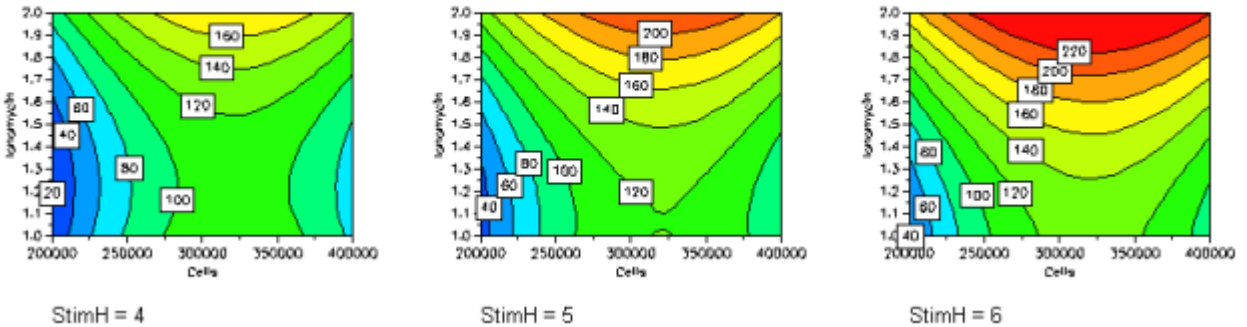
The single response was the signal to noise ratio.

64 experiments are needed to study all possible combinations of 6 factors. However, the use of DoE reduced this to just 16 experiments by focusing on the most important effects. This was achieved by using a 2^{6-2} fractional factorial design, selected in the MODDE software.

Optimisation

The screening design identified the three most important factors: the number of cells, stimulation time and the amount of Ionomycin. These were varied in a more complex (CCF) optimisation design involving 17 experiments. This design supports quadratic terms which explains the curved nature of the response contour plots shown below. These plots may be used to identify settings for the three factors at which the S/N ratio is maximised.

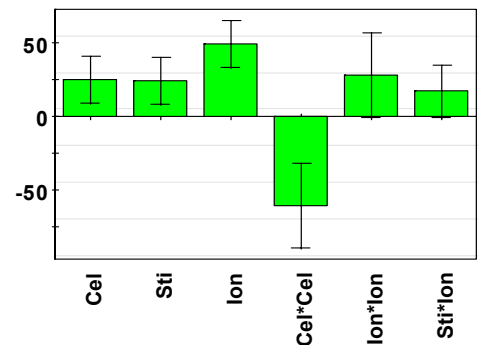
Investigation: Reporter Gene Assay RSM with CCF (MLR)
4D Contour of S/B



Robustness Testing

Having identified optimal settings for the assay, the final step is to perform a robustness test. How sensitive is the method to small changes in the key factor settings? In this case, an economical 2^{5-1} fractional factorial design was used resulting in 16 experiments. During this phase a slight sensitivity to Ionomycin concentration was discovered which resulted in the tightening of the specification for this factor.

Investigation: Reporter Gene Assay RSM with CCF (MLR)
Scaled & Centered Coefficients for S/B



We gratefully acknowledge Lena Schultz and Lisbeth Abramo of Active Biotech AB in Lund, Sweden for allowing us to use this example.

Conclusions

- The use of Design of Experiments (DoE) leads to efficient assay development.
- Screening, optimisation and robustness testing are all possible using DoE.
- MODDE software recommends an appropriate design based on the number of factors and the experimental objective.
- In DoE, the amount of work required to answer specific questions is pre-defined which expedites the setting of budgets and time lines.

MODDE 7 is our user friendly software for Design of Experiments. The unique Design Wizard and Analysis Advisor functionality guide the user to successful results.



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