Dr. Johannes Solzin

Analytical Development Biologicals,

Potency Assay Skill Center

3 in 1: Increasing Efficiency of Potency Assay Optimization via DoE

CASSS, April 16th, 2018

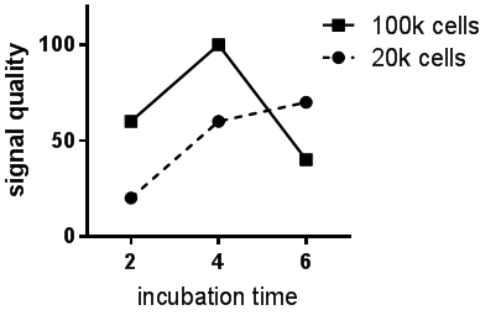
Boehringer Ingelheim

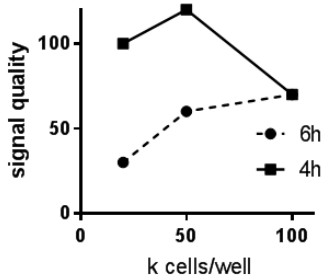
Overview

- Priniciple of DoE vs. One Factor at a Time
- „Classic“ DoE vs. „how we set up“ DoE
- Which response to assess the quality of a bioassay?
- Case studies: Examples of outcomes of DoEs
- Using DoE to assess robustness
- Capacities: Comparison of DoE approaches (and OFaT)

Theory of OFaT vs. DoE

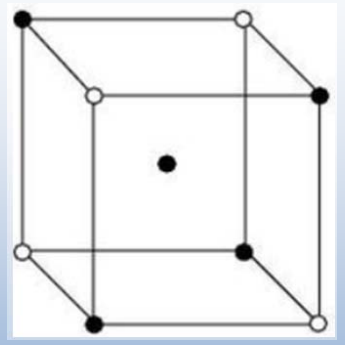
- **One Factor at a Time (OFaT):**





- not possible to detect two-factor interactions
- hard to find maximum with reasonable number of experiments

- **DoE:**
  – changing all factors at the same time
  ➔ easy to find maximum and/or two-factor interactions (quite often observed in biological systems)



How we set up DoEs

How we set up DoE

„Classic “ Approach:

Typically 3 DoEs for assay development:

	Design space	Goal
1. screening (all potential factors; only linear model with low resolution)	wide	→*identify relevant factors*
2. optimization (only relevant factors; RSM with higher resolution)	medium	→*two-factor-interactions and quadratic dependencies to find optimum*
3. robustness (only relevant factors; only linear model with low resolution)	small	→ *no changes of responses over design space*

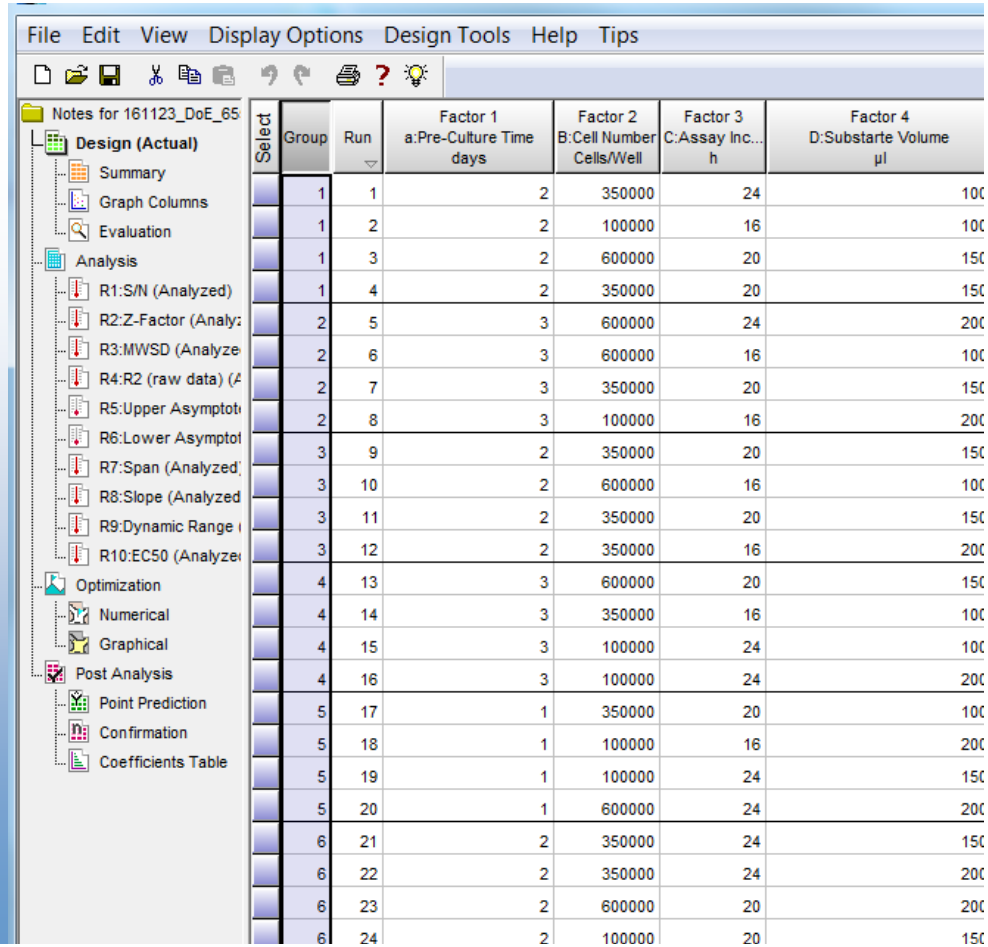
Don´t tell your technicians…

How we set up DoEs

3 in 1 approach (i.e. screening, optimization and robustness in 1 DoE)**:**

- constraining the Design Space via:
  - hands-on experience during „proof of concept“ (e.g. incubation time proliferation assay)
  - theoretical assessment of Design Space (e.g. substrate volume, cell-number)

- plan a **3 in 1 DoE** i.e. with all potentially relevant factors (including *hard-to-change* factors)



Group	Run	Factor 1 a:Pre-Culture Time days	Factor 2 B:Cell Number Cells/Well	Factor 3 C:Assay Inc... h	Factor 4 D:Substarte Volume µl
1	1	2	350000	24	100
1	2	2	100000	16	100
1	3	2	600000	20	150
1	4	2	350000	20	150
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2	7	3	350000	20	150
2	8	3	100000	16	200
3	9	2	350000	20	150
3	10	2	600000	16	100
3	11	2	350000	20	150
3	12	2	350000	16	200
4	13	3	600000	20	150
4	14	3	350000	16	100
4	15	3	100000	24	100
4	16	3	100000	24	200
5	17	1	350000	20	100
5	18	1	100000	16	200
5	19	1	100000	24	150
5	20	1	600000	24	200
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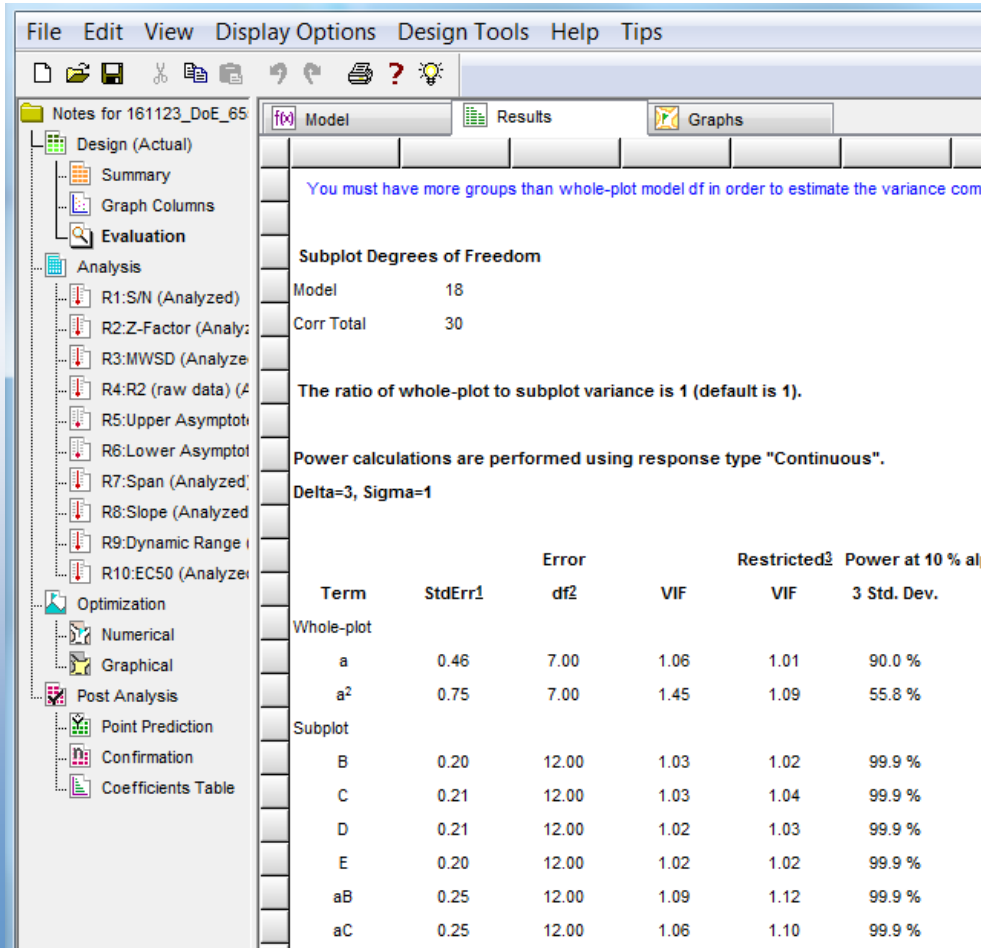
Advantages vs. „Classic“ Approach:

- only single *RSM* DoE
  → *far less experiments*

- more replicates per factor in comparison to „classical optimization DoE“
  →*higher statistical power* (unless not all factors turn out as relevant)

- hard-to-change factors allow identification of edge-of-failure at 1st experiment



How we set up DoEs

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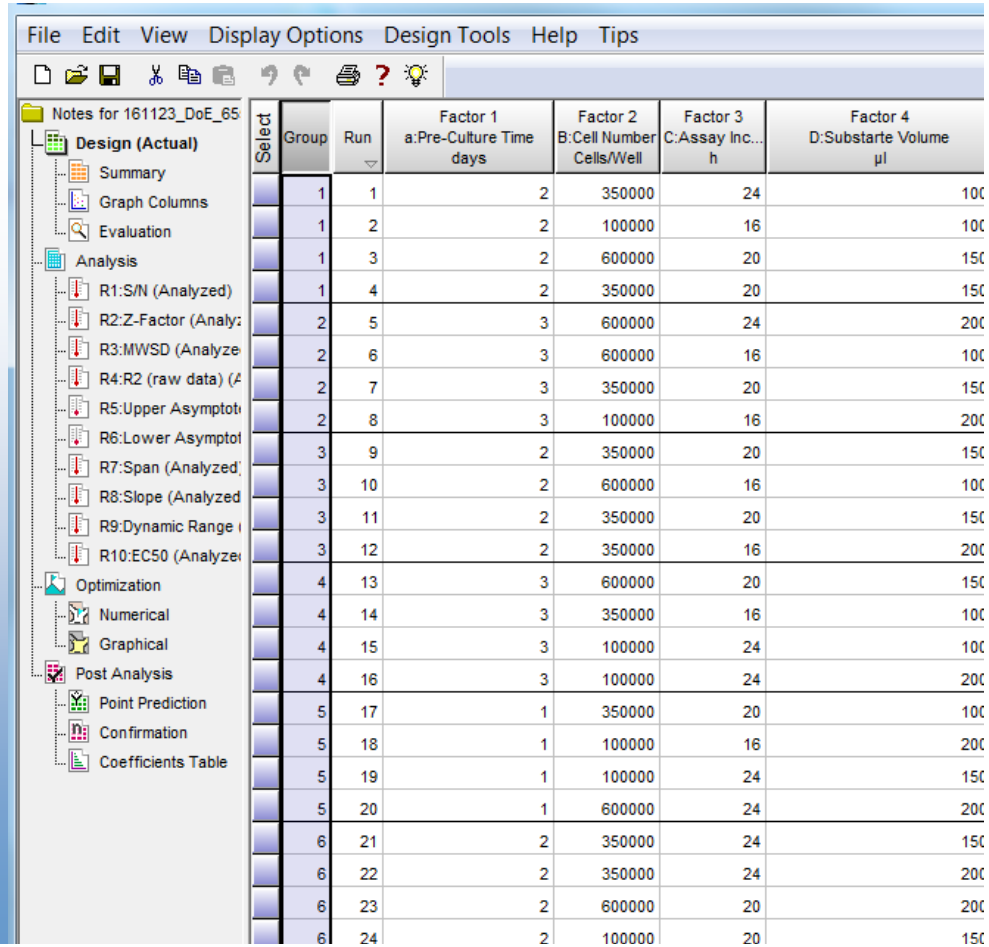

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	2	7	3	350000	20	150
	2	8	3	100000	16	200
	3	9	2	350000	20	150
	3	10	2	600000	16	100
	3	11	2	350000	20	150
	3	12	2	350000	16	200
	4	13	3	600000	20	150
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	4	15	3	100000	24	100
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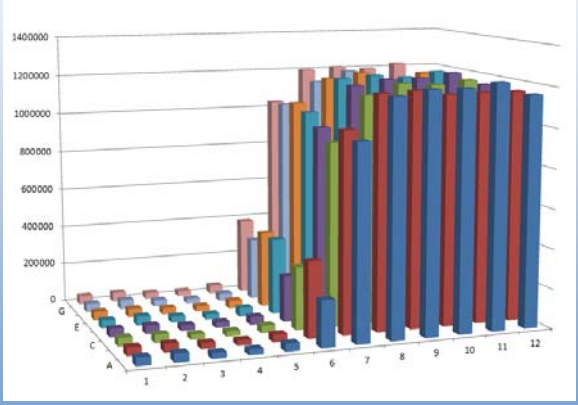
How we set up DoEs

Performance of experiments:

- 1 plate per run with octaplicates to assess scattering of data
- serial diltion e.g. >>1:2 steps
- swiss clock like technician
- knowing his limits in statistics

(e.g. no rearrangement of data; no changes in design space)

Plate layout:



File Edit View Display Options Design Tools Help Tips

Notes for 161123_DoE_65
Design (Actual)
Summary
Graph Columns
Evaluation
Analysis
R1:S/N (Analyzed)
R2:Z-Factor (Analyz
R3:MWSD (Analyze
R4:R2 (raw data) (A
R5:Upper Asymptot
R6:Lower Asymptot
R7:Span (Analyzed)
R8:Slope (Analyzed
R9:Dynamic Range
R10:EC50 (Analyze
Optimization
Numerical
Graphical
Post Analysis
Point Prediction
Confirmation
Coefficients Table

Select	Group	Run	Factor 1 a:Pre-Culture Time days	Factor 2 B:Cell Number Cells/Well	Factor 3 C:Assay Inc... h	Factor 4 D:Substarte Volume µl
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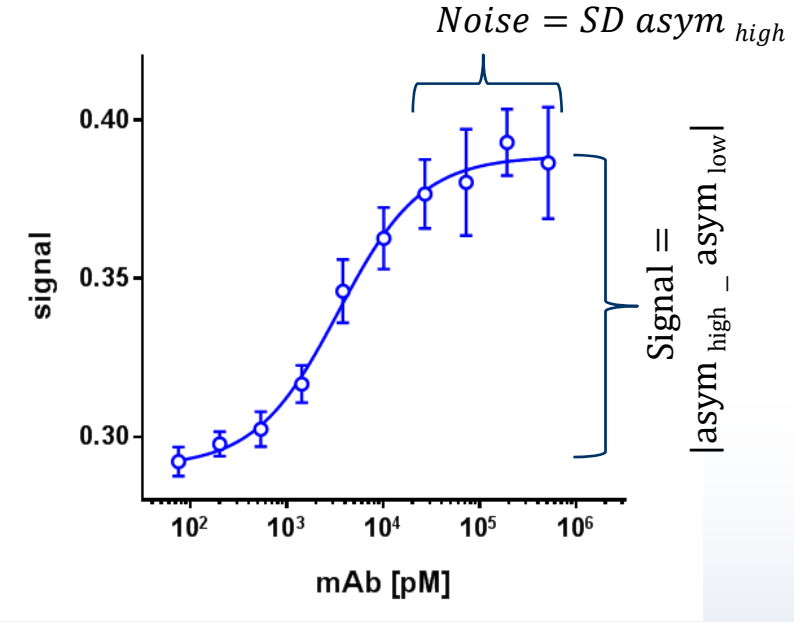
Choosing the right parameter

Choosing the right parameter

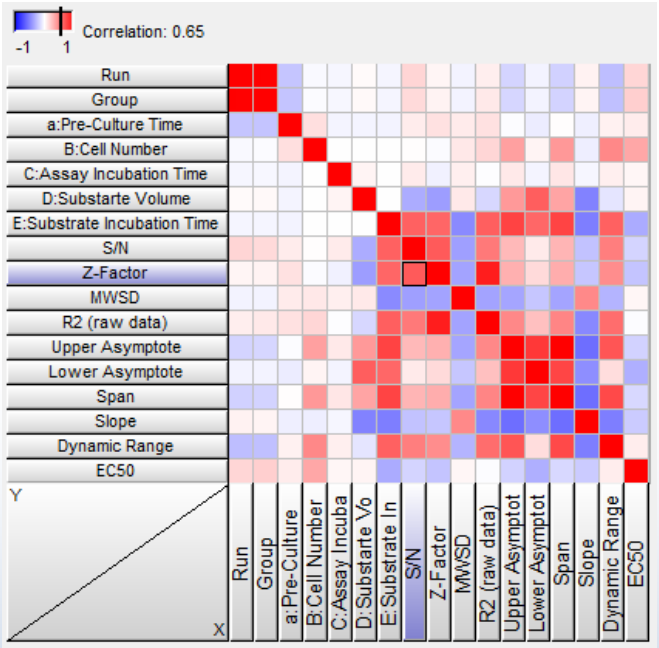
Which factor to assess quality of bioassay?

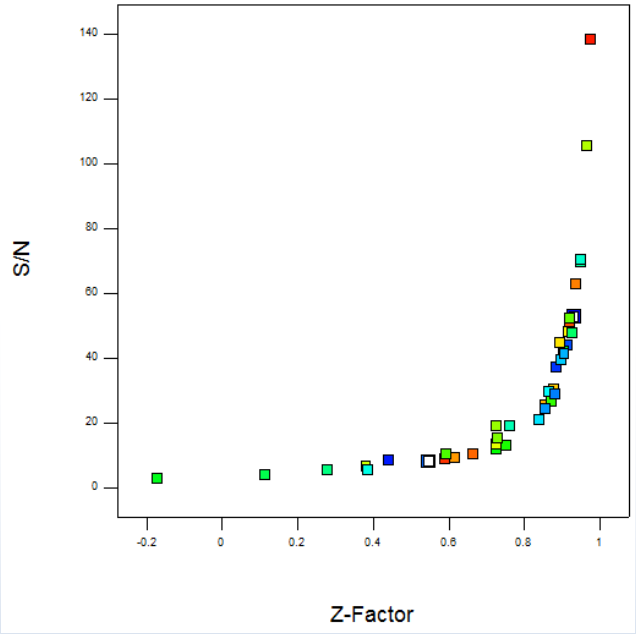
- dynamic range (DR) = $\left(\frac{asym_{high}}{asym_{low}}\right)$
- coefficient of correlation (R2): $\sum_{i=1}^{n}(y_i - \bar{y})^2$
- mean-weighted-standard-deviation (MWSD): $\sqrt{\frac{\sum(SD^2)}{n}}$
- signal-to-noise (S/N): $\frac{SD\ asym_{high}}{|asym_{high} \_ asym_{low}|}$
- Z-factor * = $1 - \frac{(3\mathrm{SD\ asym}_{high} + 3\mathrm{SD\ asym}_{low})}{|\mathrm{asym}_{high} \_ \mathrm{asym}_{low}|}$



* J. Zhang et al., (1999), Journal of Biomolecular Screening

Choosing the right parameter



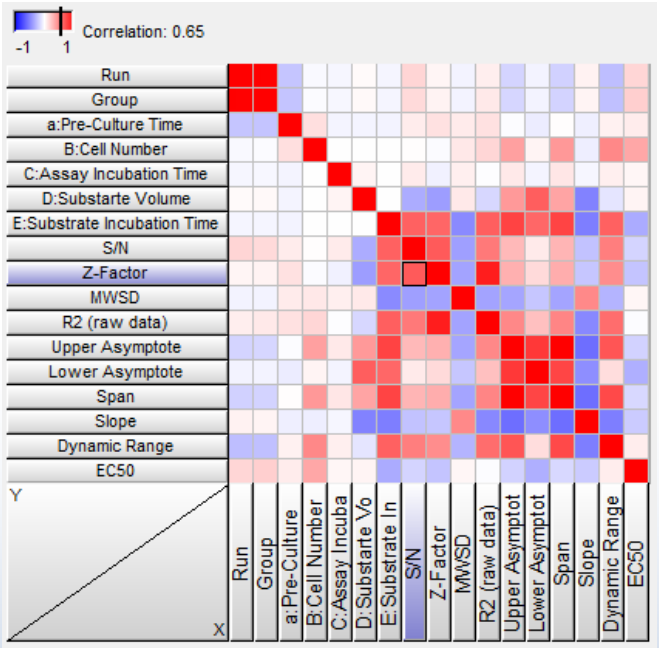


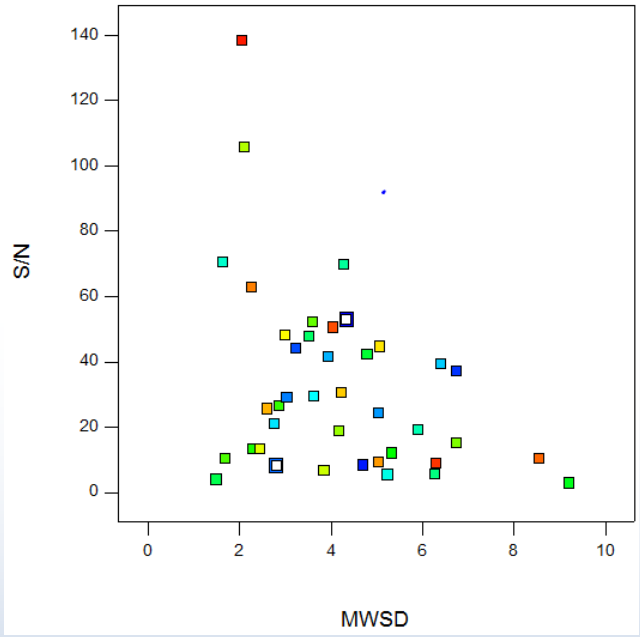
Conclusion:

- Z-factor strongly correlates with S/N

→ Focus on S/N as changes in Z-factor are small due to normalization

Choosing the right parameter



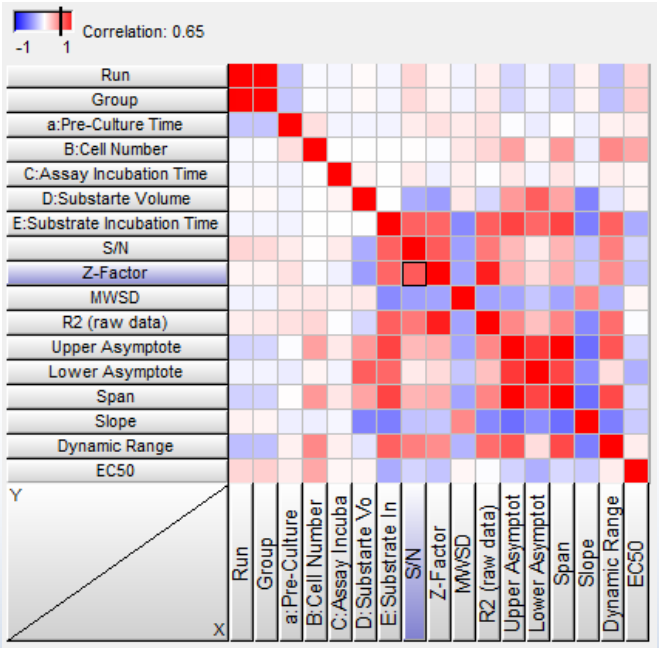


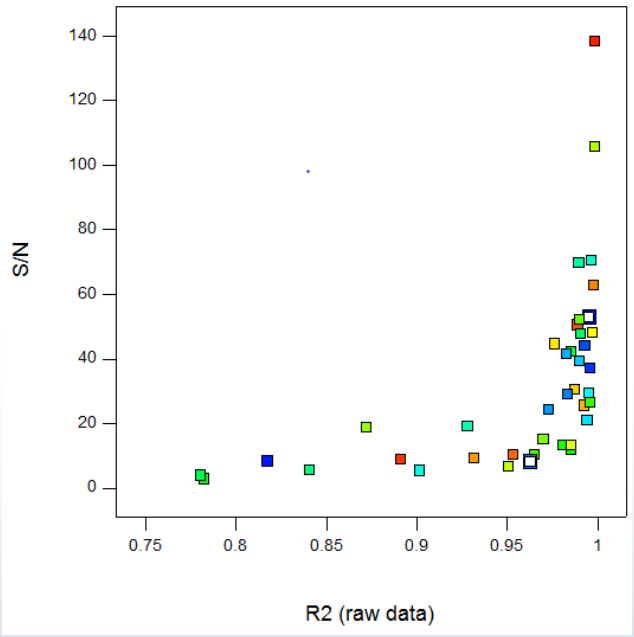
Conclusion:

- MWSD does not correlate with S/N

→ MWSD interesting to asses model quality

Choosing the right parameter



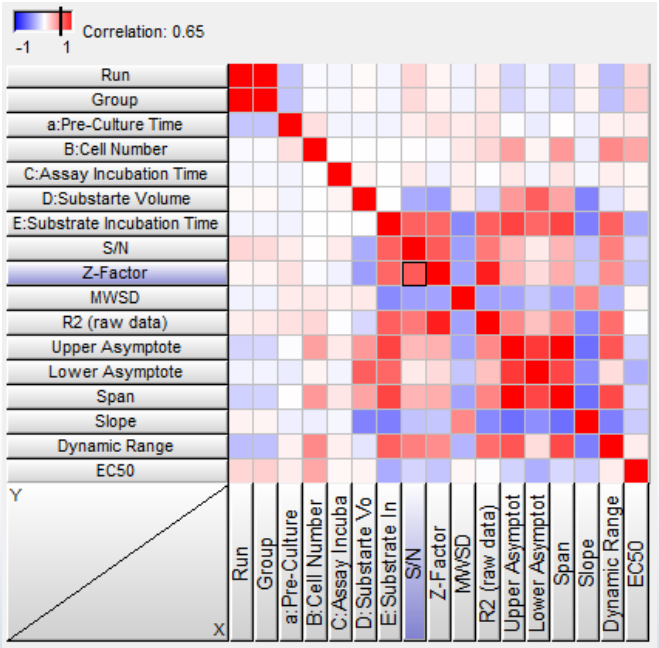


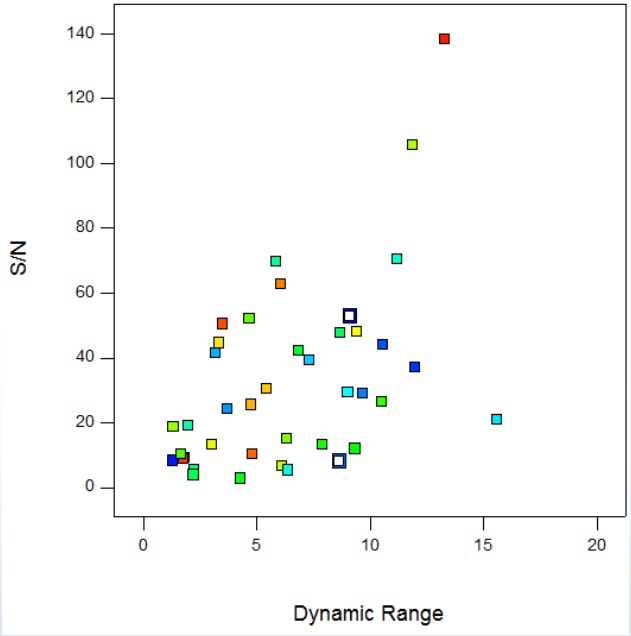
Conclusion:

- R2 correlates well but it rather describes fit-quality than noise

→ have a glance at both models

Choosing the right parameter





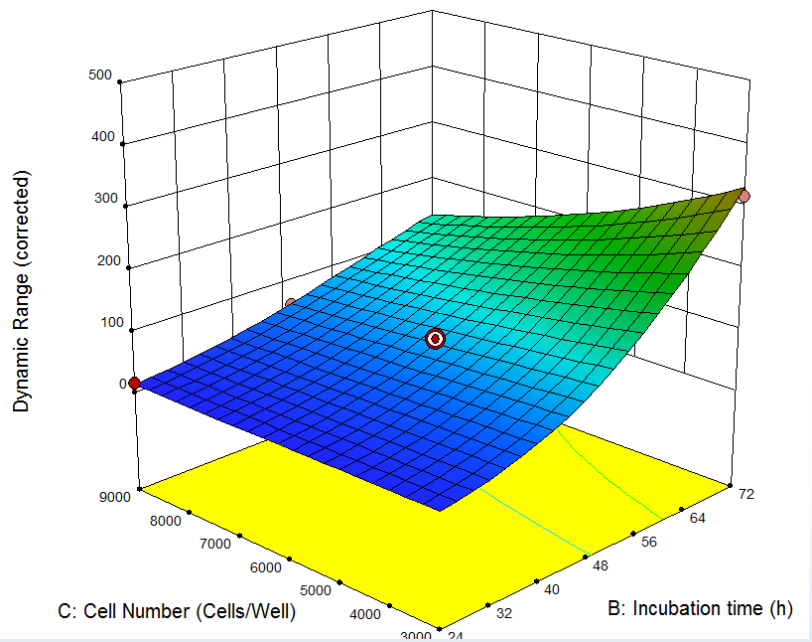
Conclusion:

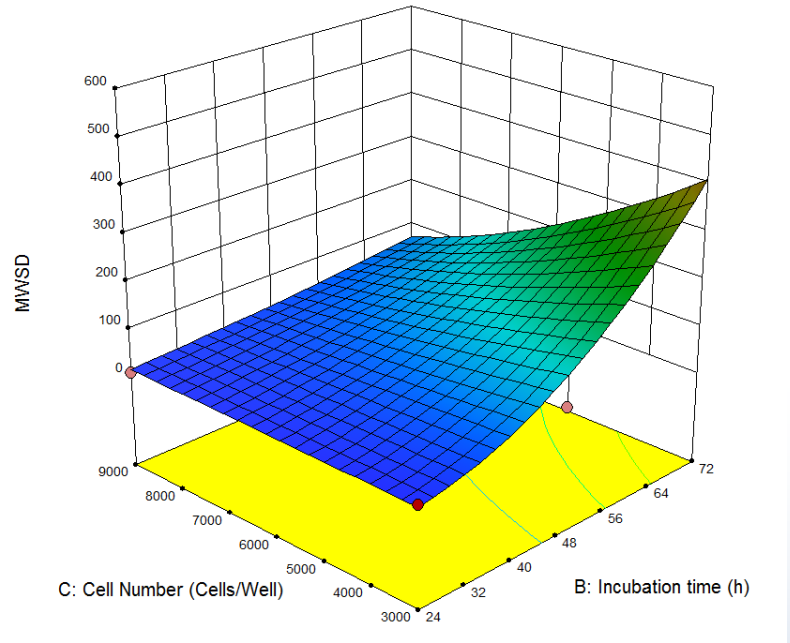
- S/N only weakly correlates with Dynamic Range

→ assess both parameters

→ careful using Dynamic Range exclusively…

Choosing the right parameter



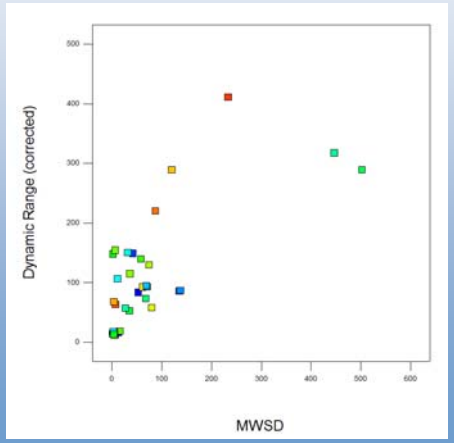


Conclusion:

- both parameters correlate ….. but opposite outcome!

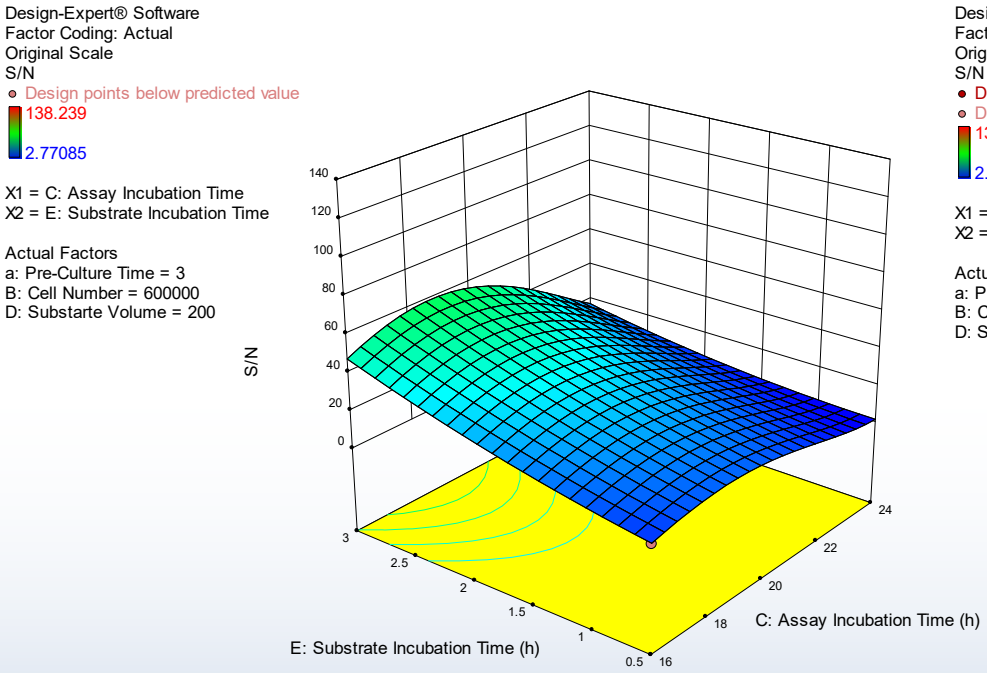
→ careful using Dynamic Range exclusively

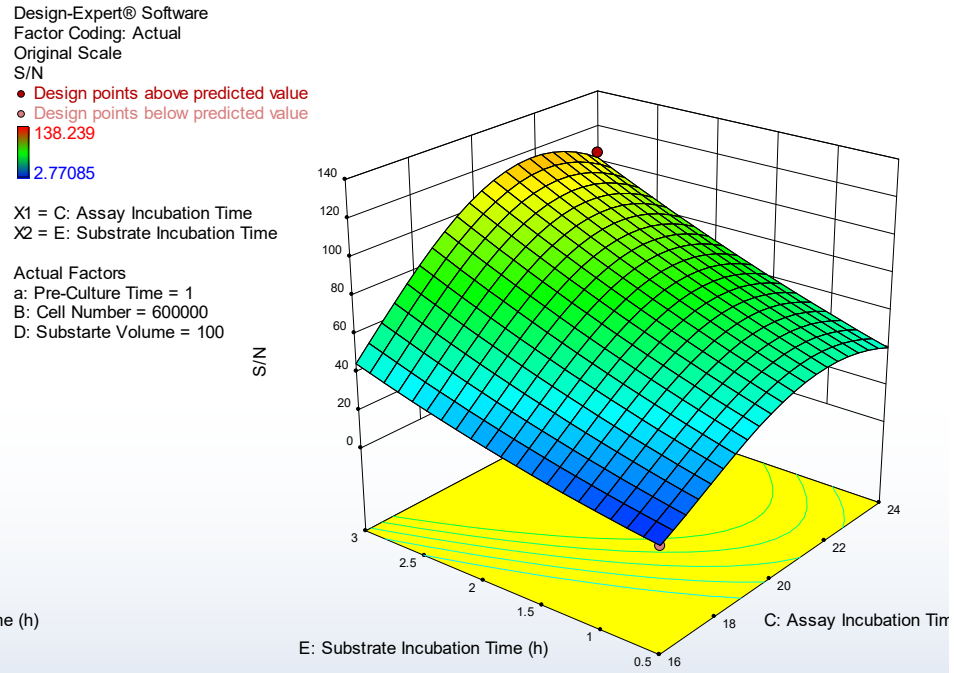
→ ***reasonable to evaluate different responses***



Case studies

Case study #1: DoE of Reporter-Gene Assay





R-Squared	0.87	
Adj R-Squared	0.81	
	Coefficient	Standard
Source	Estimate	Error
Intercept	5.97	0.40
Whole-plot Terms:		
a-Pre-Culture Time	0.064	0.30
a^2	1.08	0.48
Subplot Terms:		
B-Cell Number	-0.016	0.18
C-Assay Incubation Time	0.14	0.19
D-Substarte Volume	-0.89	0.19
E-Substrate Incubation Time	1.89	0.19
aB	-0.64	0.22
aC	-0.81	0.22
CD	-0.75	0.22
C^2	-2.01	0.33

Conclusion:

- good model
- identification of maximum and of two-factor interactions

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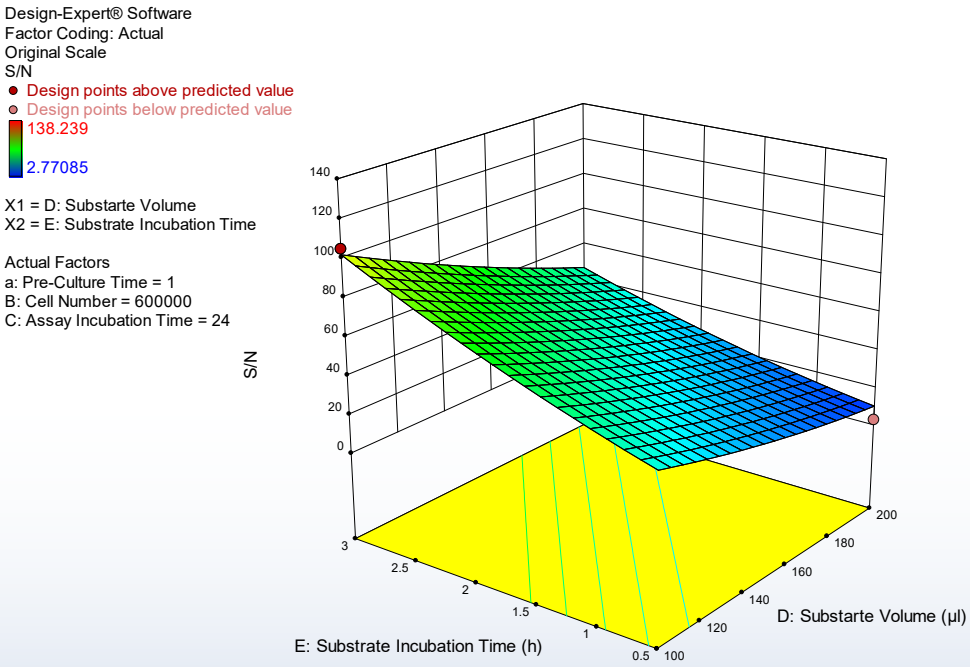

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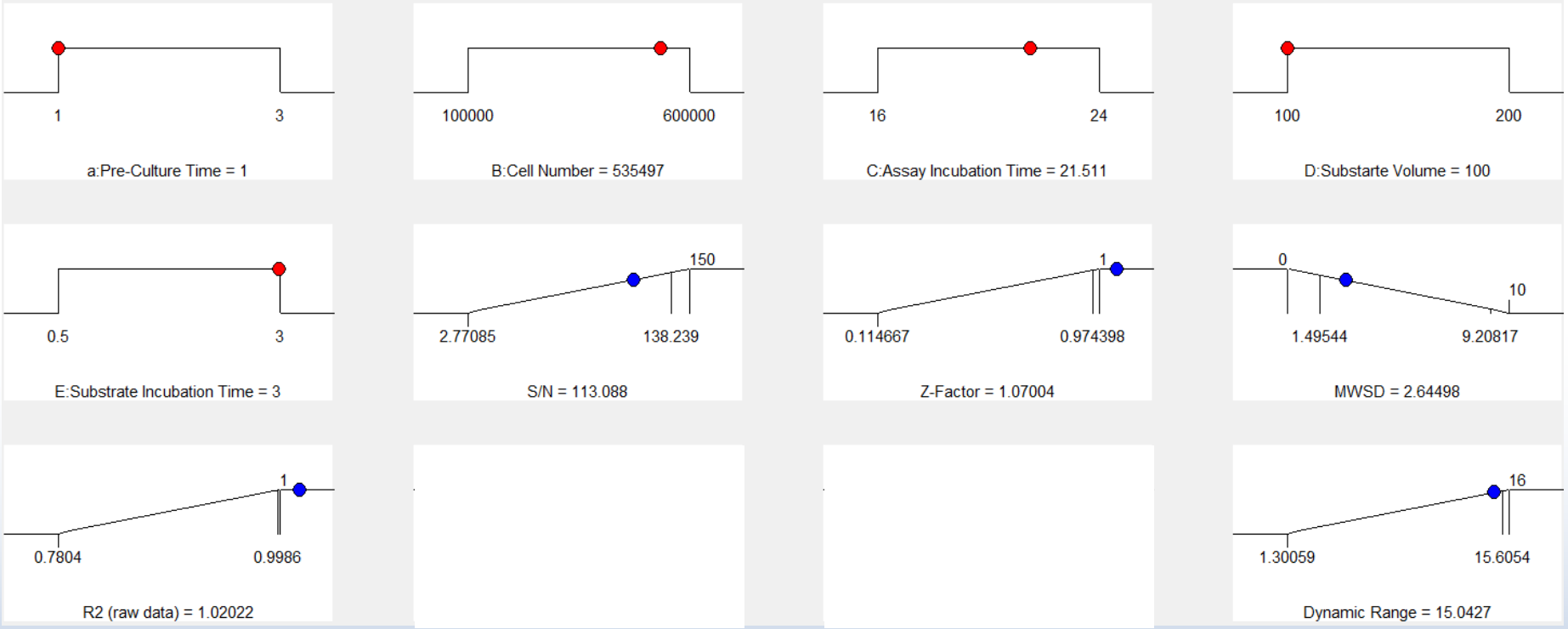
Conclusion:

- good model
- identification of maximum and of two-factor interactions
- lower substrate volume contributes to better S/N-ratio (even supported by model from MWSD)...

→ ***2 in 1 works…***

→ ***….and how about OFaT?!***

Case study: DoE of Reporter-Gene Assay



Conclusion:

- „numerical optimization“ allows to determine settings of all tested factors at which best assay performance is achived

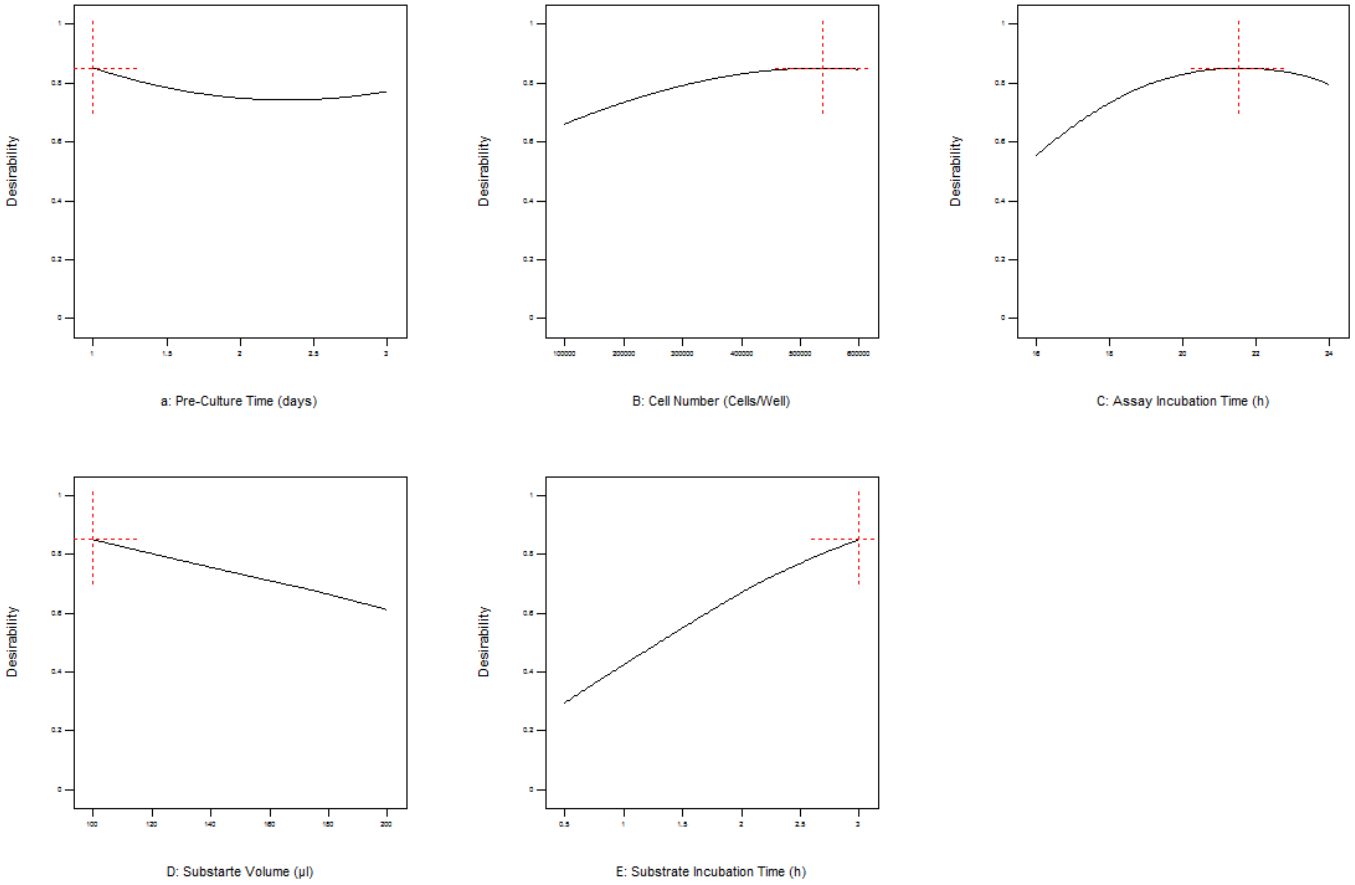
Assessment of Robustness via DoE model

Assessment of Robustness via DoE model

Background:

- often assessed via Potency …"very indirect" approach
- rather assay quality important….

Acceptance Criteria	**Significance**
easy	low
difficult (only „soft" AC)	high



Conclusion:

Desirability plots show edge of failures and ranges of factors which do not effect assay

Approach is efficient and reasonable way to determine robustness at least for early phases

→ ***even 3 in 1 works***

Case study #2: Apoptosis-Assay

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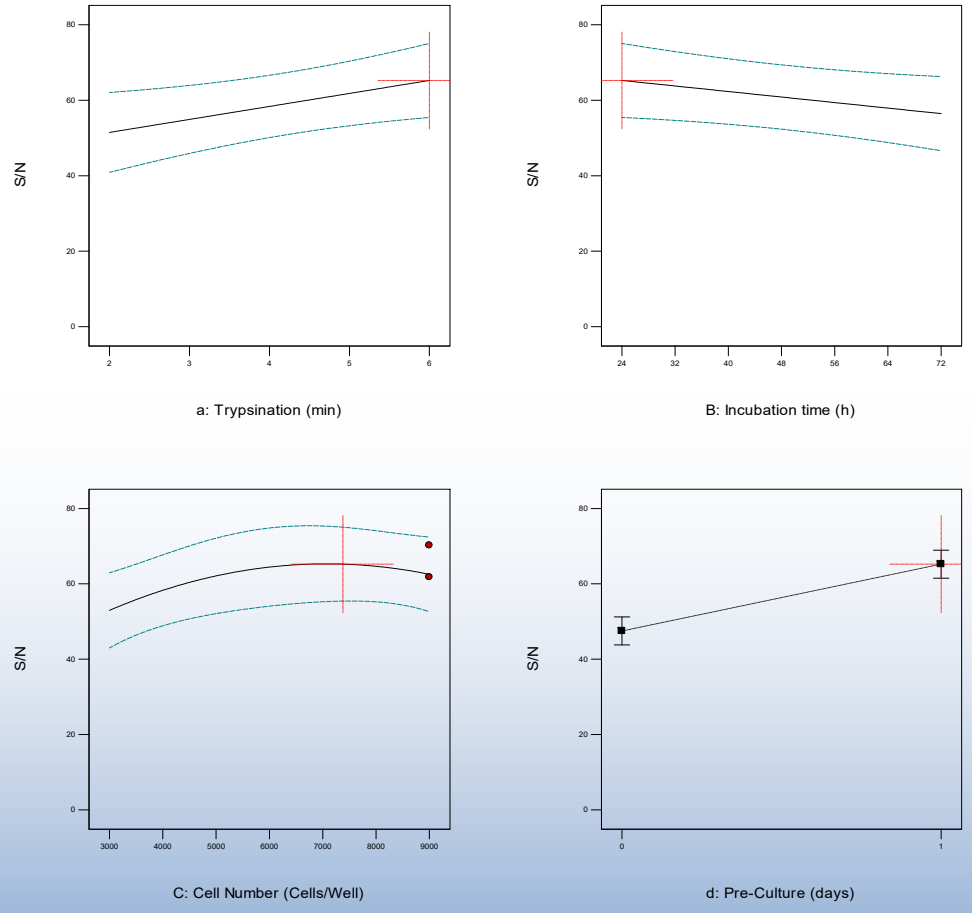
Design-Expert® Software
Factor Coding: Actual
S/N

Actual Factors
a: Trypsination = 6
B: Incubation time = 24
C: Cell Number = 7378.38
d: Pre-Culture = 1



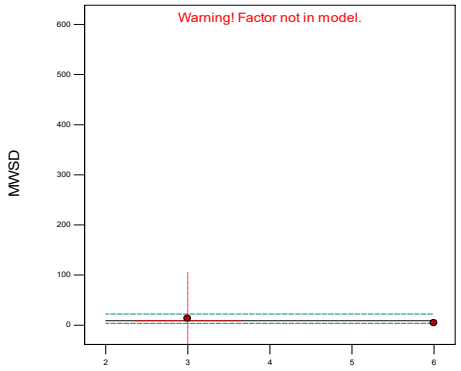
Conclusion:

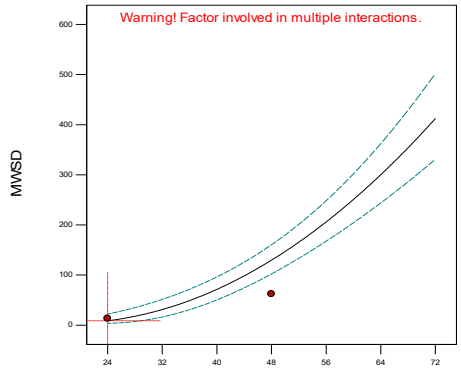
- No relevant effects on S/N over whole design space => assay seems very robust

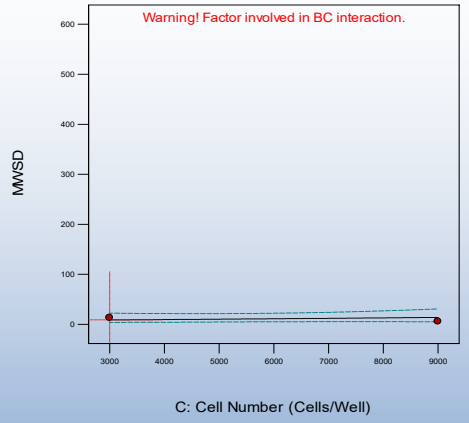
Case study #2: Apoptosis-Assay

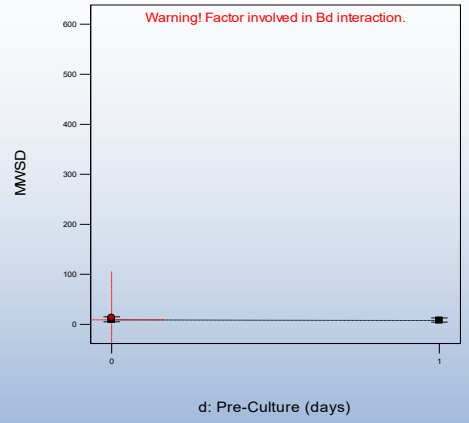
Design-Expert® Software
Factor Coding: Actual
Original Scale
MWSD
● Design Points

Actual Factors
a: Trypsination = 3
B: Incubation time = 24
C: Cell Number = 3000
d: Pre-Culture = 0





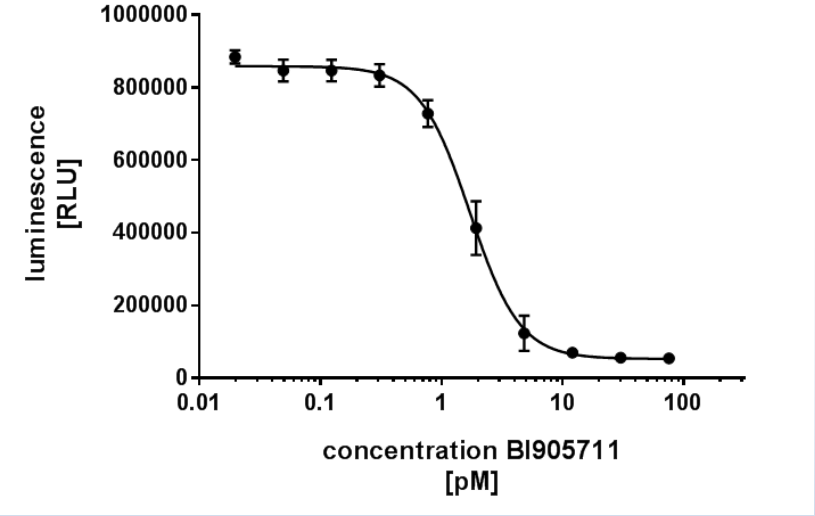


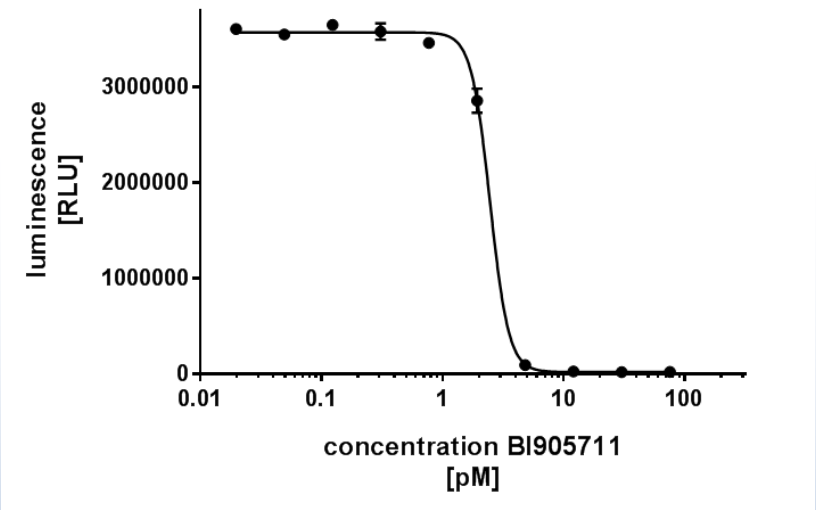


Conclusion:

- No relevant effects on S/N over whole design space => assay seems very robust
- …but not clear why 2 factor interaction of incubation time and cell number highly relevant for MWSD

Case study #2: Apoptosis-Assay





Conclusion:

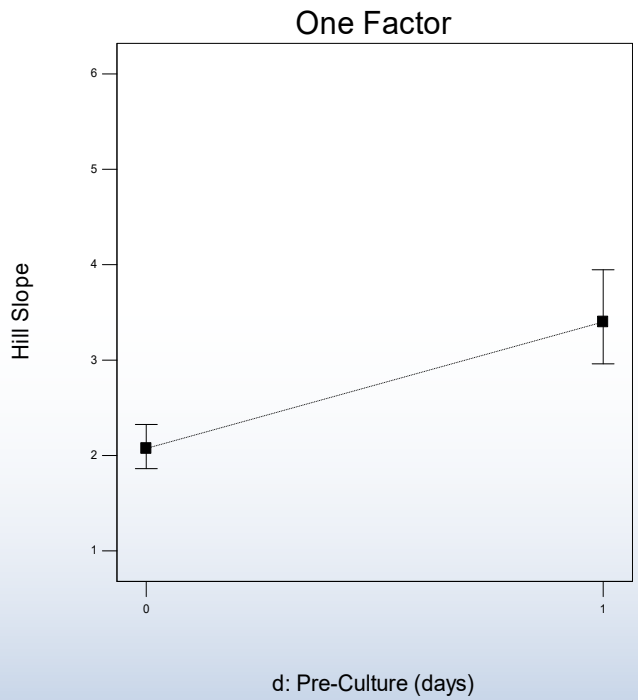
- certain settings caused curves too steep to be fitted

Case study #2: Apoptosis-Assay

Design-Expert® Software
Factor Coding: Actual
Original Scale
Hill Slope

X1 = d: Pre-Culture

Actual Factors
a: Trypsination = 4.5
B: Incubation time = 48
C: Cell Number = 6000



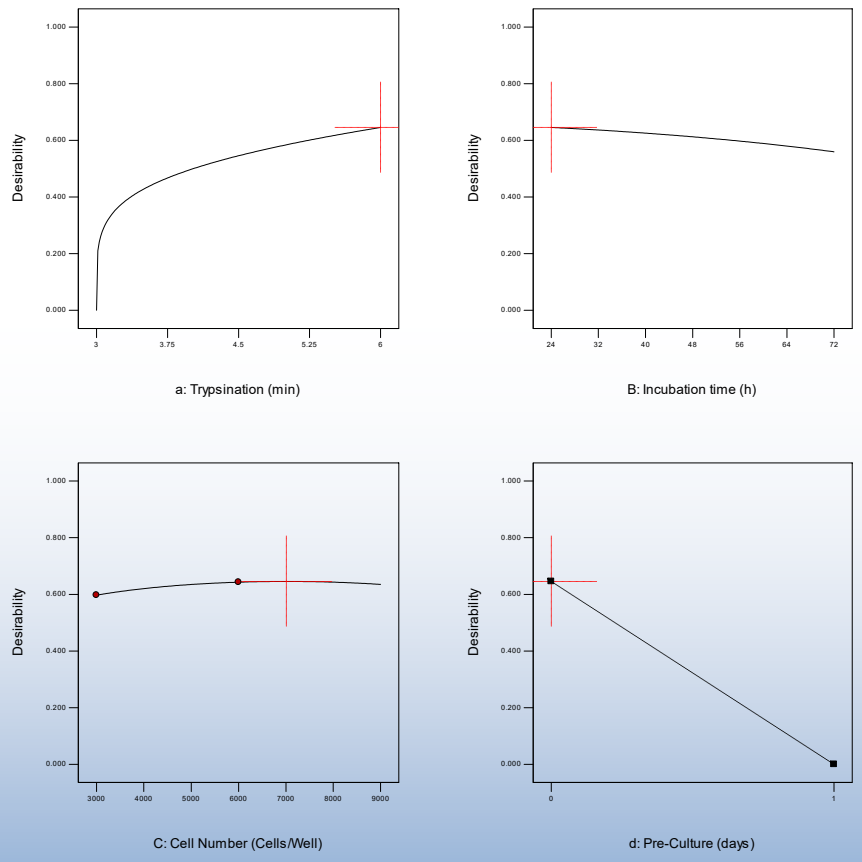
Conclusion:

- using „Hill slope“ as a response clearly showed that Pre-Culture is crucial to get nice curves

Case study #2: Apoptosis-Assay

Design-Expert® Software
Factor Coding: Actual
Desirability

Actual Factors
a: Trypsination = 6
B: Incubation time = 24.0003
C: Cell Number = 7007.47
d: Pre-Culture = 0



Conclusion:

- low „Hill slope“ was ranked as highest desirability, followed by MWSD and S/N to obtain
- desirabity clearly showed that Pre-Culture is crucial to get nice curves

…further procedure…

- confirmation of results during fine-tuning i.e.:
  - if necessary, check wider design space
  - adaptation of starting-concentration and serial dilution and plate-layout at set-point
  - plate-uniformity at set-point
  - pre-testing of accuracy/precission befor assay-validation/qualification at set-point

Capacities

Capacities

		3 in 1 approach	DoE „classic“ approach	OFaT
Planing of experiments		→ ~3h expert	2 DoEs → ~6h expert	Interpretation of every set of experiments → >>3h expert
Capacitiy for experiments (screening & optimization)		40-50 assays => ≤10 days → ~50h technician	>60 assays => >15 days → >75h technician	Preparation and performance of different experiments → >50h technician
Evaluation		→~3h expert	→~9h expert	Interpretation of every set of experiments → >>3h expert
Report		Template → ~3h expert	→ ~8h expert	Individual report → >3h expert
Robustness		Evaluation of existing data → ~1h expert	Separate DoE → ~30h technician → ~6h expert (design & evaluation)	Experiments performed separately → >50h technician → >3h expert (planing and report)
Sum		**~50h technician ~10h expert**	**>100h technician ~29h expert**	**>100h technician >>12h expert**

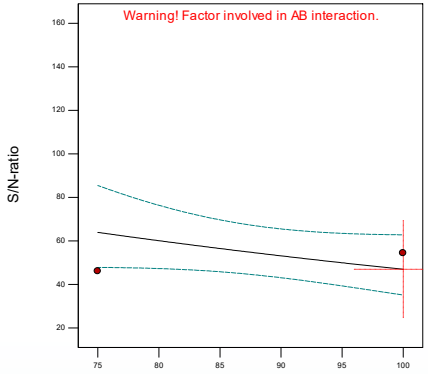
Summary

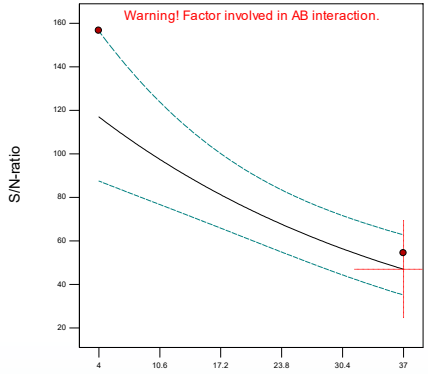
- 2 in 1approach less labor-intense then good OFaT or „classical“ DoE-approach
- robustness via DoE at least for early phase reasonable => 3 in 1
- considering different parameters for assay-quality (S/N often of avail)
- some front-loading required to get statisitcal know-how
- provides much more insight into critical assay parameters => fast break even because better assays during later routine

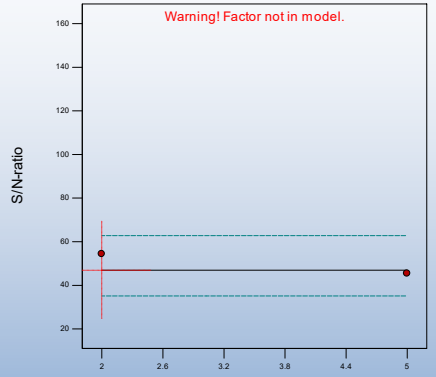
Backup (Effect of Substrate Temperature on Substrate Volume)

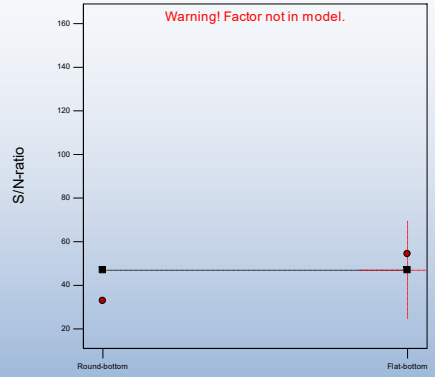
Design-Expert® Software
Factor Coding: Actual
Original Scale
S/N-ratio
● Design Points

Actual Factors
A: Substrate volume = 100
B: Substrate Temperature = 37
C: Cell-Number = 2
D: Plate Type = Flat-bottom





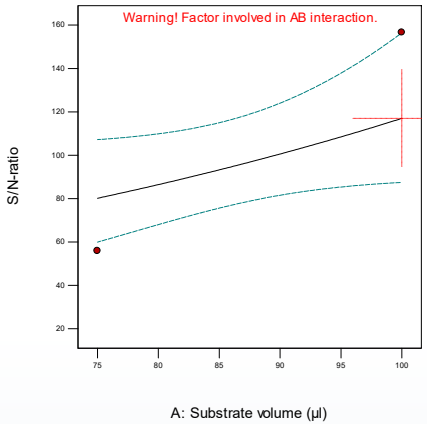


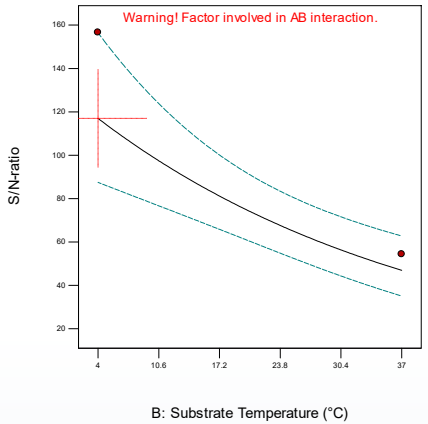


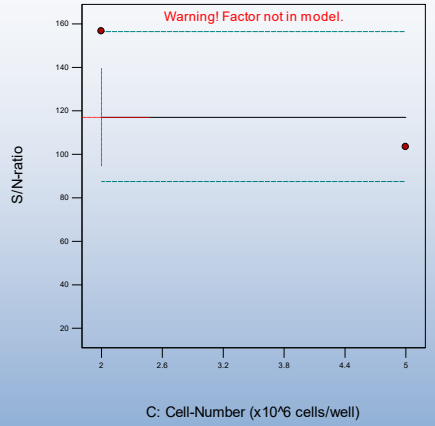
Backup (Effect of Substrate Temperature on Substrate Volume)

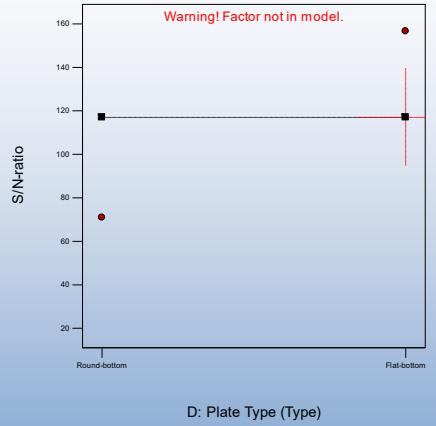
Design-Expert® Software
Factor Coding: Actual
Original Scale
S/N-ratio
● Design Points

Actual Factors
A: Substrate volume = 100
B: Substrate Temperature = 4
C: Cell-Number = 2
D: Plate Type = Flat-bottom







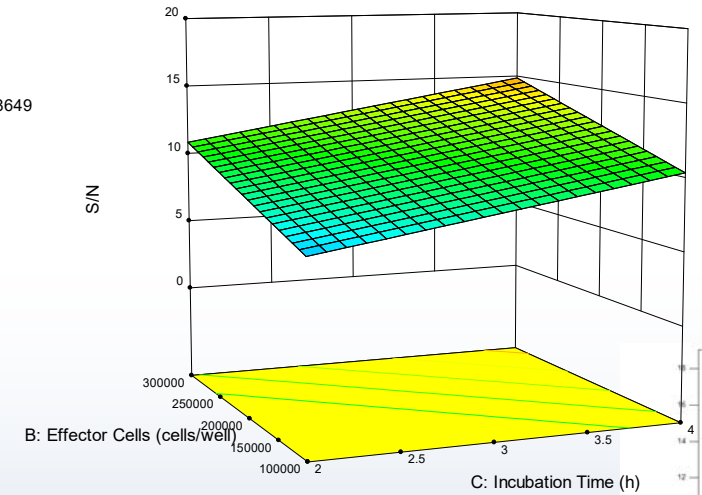


Case study: DoE of ADCC assay

Design-Expert® Software
Factor Coding: Actual
S/N
16.8316
3.69865

X1 = C: Incubation Time
X2 = B: Effector Cells

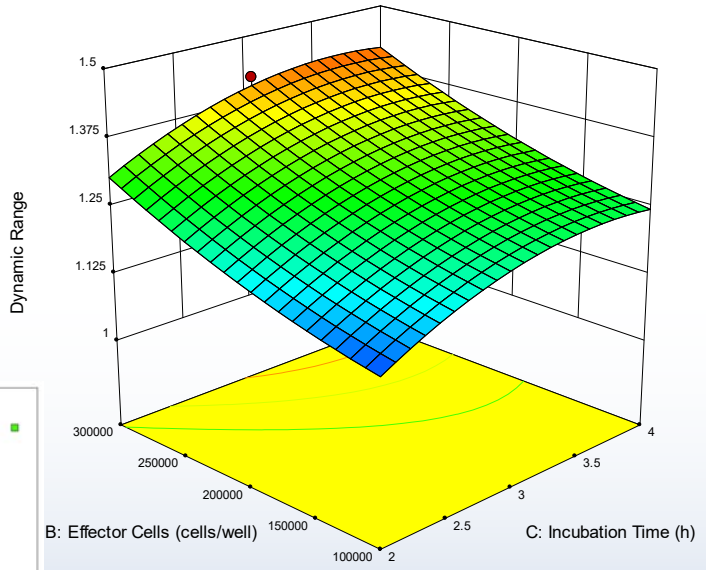
Actual Factors
A: Target Cells = 7500
d: Pre-culture Time = 1.48649
E: Passage = 17.4595

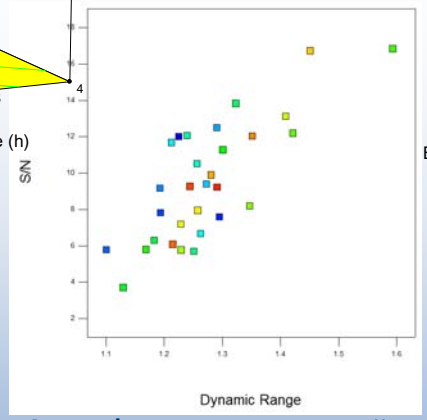


Design-Expert® Software
Factor Coding: Actual
Dynamic Range
Design points above predicted value
1.45141
1.10086

X1 = C: Incubation Time
X2 = B: Effector Cells

Actual Factors
A: Target Cells = 7500
d: Pre-culture Time = 2
E: Passage = 17





R-Squared	0.65	
Adj R-Squared	0.56	
	Coefficient	Standard
Source	Estimate	Error
Intercept	9.43	0.39
Whole-plot Terms:		
d-Pre-culture Time	0.74	0.39
Subplot Terms:		
A-Target Cells	-1.25	0.51
B-Effector Cells	2.24	0.50
C-Incubation Time	2.00	0.51

Conclusion:

- rather poor model for S/N shows „only“ maximum
- finding supported by good model from dynamic range
- for ADCC S/N and dynamic range correlate

R-Squared	0.96	
Adj R-Squared	0.93	
	Coefficient	Standard
Source	Estimate	Error
Intercept	1.28	7.712E-003
Whole-plot Terms:		
d-Pre-culture Time	0.031	4.939E-003
E-Passage	0.024	6.004E-003
Subplot Terms:		
A-Target Cells	1.860E-003	5.106E-003
B-Effector Cells	0.059	4.872E-003
C-Incubation Time	0.057	5.016E-003
AB	-0.012	5.929E-003
Bd	0.014	4.916E-003
B^2	0.019	8.140E-003
C^2	-0.044	8.575E-003