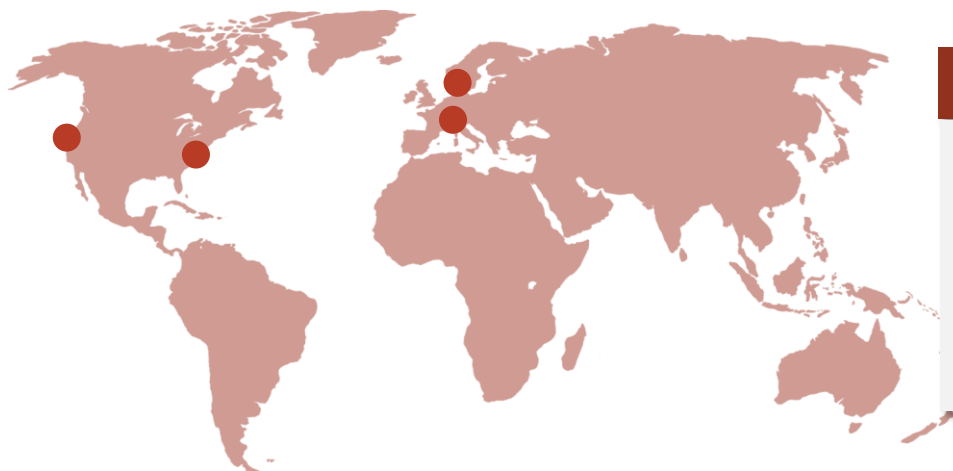


Split-split plot designs in JMP

Stine Fangel, Statistician at Bavarian Nordic

Bavarian Nordic

Cancer Immunotherapies and Vaccines for Infectious Diseases



FACTS

Founded 1994, IPO 1998

Listed on NASDAQ OMX Copenhagen: BAVA

400 employees worldwide

Market Cap DKK 9.2bn

BAVARIAN NORDIC IN BRIEF

- Vertically integrated multinational biotech company
- Revenue-generating
- Leader in vector-based active immunotherapy
- First product approved in 2013, smallpox
- Commercial scale cGMP manufacturing facility
- Long-term R&D and delivery contracts with the US government



Clinical Pipeline

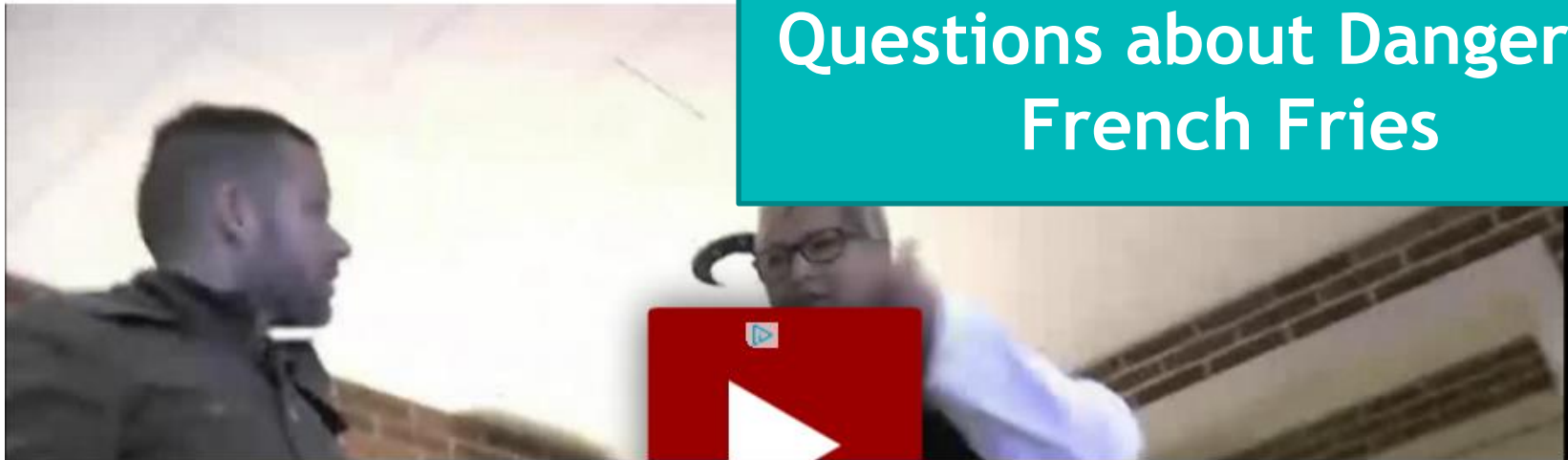
			Phase 1	Phase 2	Phase 3	Market
Product	Indication	Partner				
IMVANEX/ INVAMUNE ¹⁻⁴⁾	Smallpox	BARDA				
INVAMUNE freeze-dried ¹⁾	Smallpox	BARDA				
PROSTVAC	Prostate Cancer	Bristol-Myers Squibb				
PROSTVAC + enzalutamide	Prostate Cancer	NCI				
PROSTVAC + ipilimumab	Prostate Cancer	NCI				
CV-301 Bladder Combo ¹⁾	Bladder Cancer	NCI				
MVA-BN Brachyury ¹⁾	Metastatic Tumors	NCI				
MVA-BN Filo + AdVac [®] ¹⁾	Ebola/Marburg	Janssen, NIH				
MVA-BN RSV	RSV			In 2015		

Case

Tv: Kartoffeldronning flygter fra spørgsmål om farlige fritter

Flensteds direktør er afsløret i at producere farlige pommes frites, men stikker af fra Ekstra Bladet

Af: **Christian Kloster**



Potato Queen Runs from Questions about Dangerous French Fries

Case



How would I suggest this analysis was done?

- **Temperature:** 175° C, 187.5° C, 200° C (hard-to-change)
- **Types of Potato** organic, non organic
- **Two Days** (block)

- **Responses:** **Acrylamide** and **Taste**



One oven with room for two
baking trays

Requests

- Balanced design
- Estimations of block variation

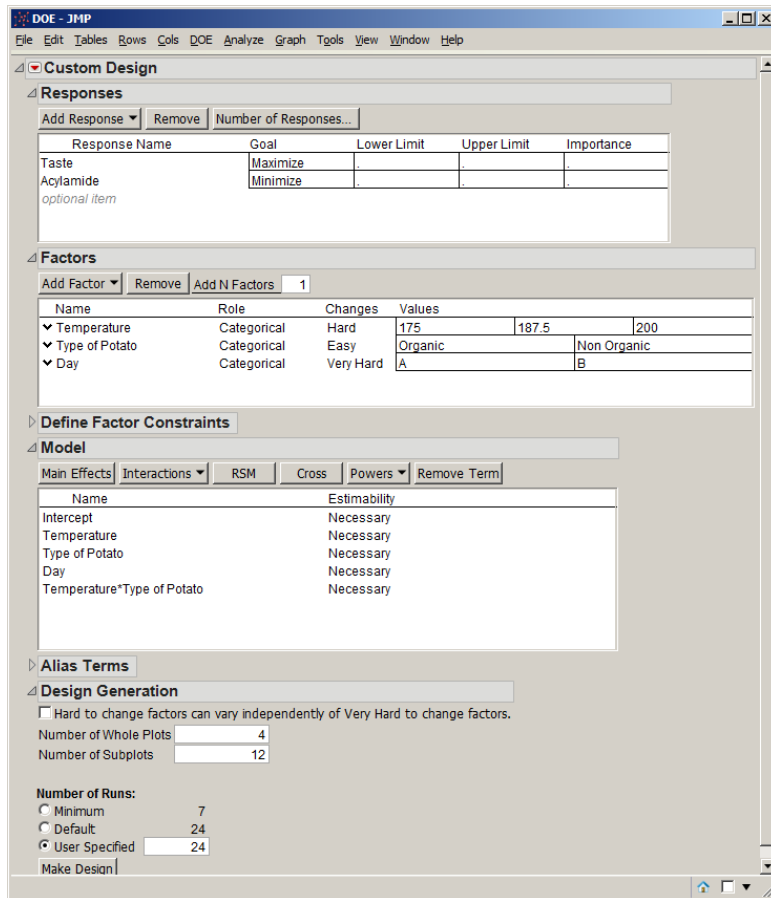
- Montgomery, Design and Analysis of Experiments, eq. 13-16 ed. 5th.

$$y_{ijk} = \mu + \text{Block } Day_i + \text{Whole plot error } Temp_j + (Day \cdot Temp)_{ij} + Type_k + (Temp \cdot Type)_{jk} + \text{Subplot error } \epsilon_{ijk}$$

$\left\{ \begin{array}{l} i = A, B \\ j = 175, 187.5, 200 \\ k = Organic, Non Organic \end{array} \right.$

Design in JMP

The trick is to ask for twice as many runs, twice as many whole plots and twice as many subplots as needed



	Whole Plots	Subplots	Temperature	Type of Potato	Day
1	1	1	187.5	Organic	A
2	1	1	187.5	Non Organic	A
3	1	2	200	Organic	A
4	1	2	200	Non Organic	A
5	1	3	175	Non Organic	A
6	1	3	175	Organic	A
7	2	4	175	Organic	B
8	2	4	175	Non Organic	B
9	2	5	187.5	Organic	B
10	2	5	187.5	Non Organic	B
11	2	6	200	Organic	B
12	2	6	200	Non Organic	B
13	3	7	200	Non Organic	A
14	3	7	200	Organic	A
15	3	8	187.5	Non Organic	A
16	3	8	187.5	Organic	A
17	3	9	175	Organic	A
18	3	9	175	Non Organic	A
19	4	10	200	Organic	B
20	4	10	200	Non Organic	B
21	4	11	175	Organic	B
22	4	11	175	Non Organic	B
23	4	12	187.5	Organic	B
24	4	12	187.5	Non Organic	B

Model specified in JMP

Report: Fit Model - JMP

Model Specification

Select Columns: Temperature, Type of Potato, Day, Taste, Acylamide

Pick Role Variables: Y (Acylamide), Weight (optional numeric), Freq (optional numeric), By (optional)

Personality: Standard Least Squares
Emphasis: Effect Screening
Method: REML (Recommended)

Unbounded Variance Components:
Estimate Only Variance Components:

Buttons: Help, Run, Recall, Remove, Keep dialog open (checked)

Construct Model Effects

Add: Day & Random, Temperature & Random, Temperature*Day & Random, Type of Potato, Temperature*Type of Potato

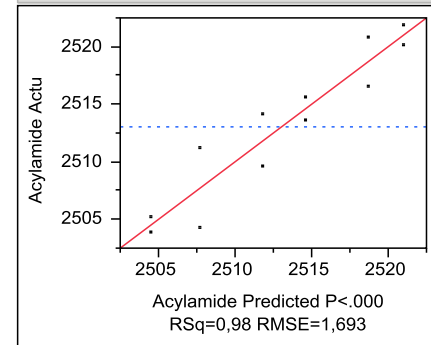
Cross, Nest, Macros

Degree: 2

Attributes, Transform, No Intercept:

Response Acylamide

Actual by Predicted Plot



Summary of Fit

RSquare	0,975025
RSquare Adj	0,954212
Root Mean Square Error	1,693529
Mean of Response	2513,036
Observations (or Sum Wgts)	12

REML Variance Component Estimates

Random Effect	Var Ratio	Component	Std Error	95% Lower	95% Upper	Pct of Total
Day	0,998062	2,8624833	5,8899104	-8,681529	14,406495	36,071
Temperature*Da	0,7688405	2,2050667	3,8228133	-5,28751	9,6976431	27,787
Residual		2,8680417	2,3417462	0,9203844	39,871701	36,141
Total		7,9355917	6,3620993	2,5840847	102,3942	100,000

-2 LogLikelihood = 40,066882331

Note: Total is the sum of the positive variance components

Total including negative estimates = 7,9355917

Fixed Effect Tests

Source	Nparm	DF	DFDen	F Ratio	Prob > F
Temperature	2	2	2	0,0203	0,9801
Type of Potato	1	1	3	06,3165	0,0019 *
Temperature*Type of Potat	2	2	3	10,7392	0,0429 *

Check the design

- Can I estimate what I want?
- I usually also take a look at VIF (variance inflation factor)
- VIF describes how much multicollinearity (correlation between predictors) exists in a regression analysis

Demo in JMP

Case: Time added to the design



- **Temperature:** 175° C, 187.5° C, 200° C (very-hard to change)
- **Types of Potato** organic, non organic
- **Time** 25 minutes and 30 minutes (hard-to-change)
- **Two Days** (block factor)

- **Responses: Acrylamide and Taste**



One oven with room for two plates

Requests

- Balanced design
- Estimations of block variation
- Montgomery, Design and Analysis of Experiments, paragraph 13-5.2, reduced

Block Whole plot error Subplot error

$$y_{ijk} = \mu + Day_i + (Day \cdot Temp)_{ij} + (Day \cdot Temp \cdot Time)_{ijl} + Temp_j + Time_l + Type_k \\ + (Temp \cdot Type)_{jk} + (Temp \cdot Time)_{jl} + (Time \cdot Type)_{kl} + (Temp \cdot Time \cdot Type)_{jkl}$$

$$+ \epsilon_{ijkl} \begin{cases} i = A, B \\ j = 175, 187.5, 200 \\ k = Organic, Non Organic \\ l = 20, 25 \end{cases}$$

Sub-subplot error



2nd Design in JMP

DOE - JMP

File Edit Tables Rows Cols DOE Analyze Graph Tools View Window Help

Custom Design

Responses

Factors

Name	Role	Changes	Values
Temperature	Categorical	Hard	175 187.5 200
Time	Categorical	Easy	20 25
Day	Categorical	Very Hard	A B

Define Factor Constraints

Model

Main Effects Interactions RSM Cross Powers Remove Term

Name	Estimability
Intercept	Necessary
Temperature	Necessary
Time	Necessary
Day	Necessary
Temperature*Time	Necessary

Alias Terms

Design Generation

Hard to change factors can vary independently of Very Hard to change factors.

Number of Whole Plots: 4

Number of Subplots: 12

Number of Runs:

Minimum 7

Default 24

User Specified 24

Make Design

Custom Design - JMP

File Edit Tables Rows Cols DOE Analyze Graph Tools View Window Help

5/0 Cols

24/12

	Whole Plots	Subplots	Temperature	Time	Day
1	1	1	175	20	A
2	1	1	175	25	A
3	1	2	187.5	25	A
4	1	2	187.5	20	A
5	1	3	200	25	A
6	1	3	200	20	A
7	2	4	200	20	A
8	2	4	200	25	A
9	2	5	175	25	A
10	2	5	175	20	A
11	2	6	187.5	20	A
12	2	6	187.5	25	A
13	3	7	175	20	B
14	3	7	175	25	B
15	3	8	200	20	B
16	3	8	200	25	B
17	3	9	187.5	25	B
18	3	9	187.5	20	B
19	4	10	200	25	B
20	4	10	200	20	B
21	4	11	187.5	25	B
22	4	11	187.5	20	B
23	4	12	175	20	B
24	4	12	175	25	B

Add *Type of Potato* to the design, double and then randomize

The screenshot shows the JMP interface with a data table and a Sort dialog box. The data table has columns: Temperature, Time, Day, Type of Potato, Order, and Random. The Sort dialog box is open, showing the 'Sort rows by specified columns' window. The 'By' list contains 'Order' and 'Random (optional)'. The 'Replace table' checkbox is checked.

	Temperature	Time	Day	Type of Potato	Order	Random
1	200	20	A	Organic	1	0,4737168204
2	200	25	A	Organic	2	0,242281988
3	175	25	A	Organic	3	0,4911673993

Sort - JMP

Sort rows by specified columns.

Select Columns

- Day
- Taste
- Acylamide
- Type of Potato
- Order
- Random

By: Order, Random (optional)

Replace table:

Keep dialog open:

21	200	20	B	Non Organic	9	0,3816158161
22	200	25	B	Non Organic	10	0,9684270401
23	187.5	25	B	Non Organic	11	0,8177753647
24	187.5	20	B	Non Organic	12	0,6766095655

The screenshot shows the JMP interface with a data table. The data table has columns: Day, Temperature, Time, Type of Potato, Taste, and Acylamide. The table is sorted by Day, then Temperature, then Time, then Type of Potato, then Taste, and finally Acylamide.

	Day	Temperature	Time	Type of Potato	Taste	Acylamide
1	A	200	20	Organic	1,3	57,4
2	A	200	20	Non Organic	0,5	59,0
3	A	200	25	Organic	5,6	62,2
4	A	200	25	Non Organic	4,2	65,0
5	A	175	25	Organic	5,0	54,4
6	A	175	25	Non Organic	4,3	55,3
7	A	175	20	Non Organic	1,9	40,5
8	A	175	20	Organic	2,6	40,1
9	A	187.5	20	Organic	4,2	52,2
10	A	187.5	20	Non Organic	4,0	52,8
11	A	187.5	25	Non Organic	6,6	62,9
12	A	187.5	25	Organic	6,7	60,3
13	B	175	20	Organic	2,7	24,0
14	B	175	20	Non Organic	2,4	25,5
15	B	175	25	Non Organic	3,1	35,3
16	B	175	25	Organic	4,2	34,8
17	B	200	20	Non Organic	5,8	34,1
18	B	200	20	Organic	7,1	33,1
19	B	200	25	Organic	9,4	39,7
20	B	200	25	Non Organic	8,9	40,9
21	B	187.5	25	Organic	8,2	40,2
22	B	187.5	25	Non Organic	7,6	41,2
23	B	187.5	20	Organic	6,7	34,6
24	B	187.5	20	Non Organic	6,5	35,7

Model specified in JMP

Report: Fit Model - JMP

Model Specification

Select Columns: Whole Plots etc. (6/C), Day, Temperature, Time, Type of Potato, Taste, Acylamide

Pick Role Variables: Y: Taste, Acylamide; optional: Weight, Freq, By

Personality: Standard Least Squares; Emphasis: Effect Screening; Method: EMS (Traditional)

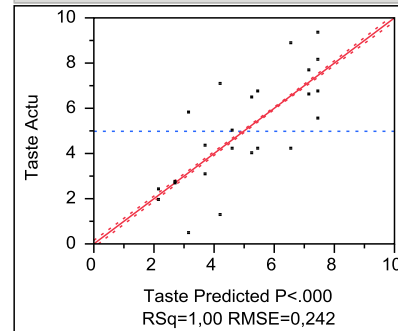
Buttons: Help, Run, Recall, Remove, Keep dialog open (checked)

Construct Model Effects: Add, Cross, Nest, Macros, Degree: 2, Attributes, Transform, No Intercept

Model Effects: Day & Random, Day*Temperature*Random, Day*Temperature*Time*Random, Temperature, Time, Temperature*Time, Type of Potato, Temperature*Type of Potato, Time*Type of Potato, Temperature*Time*Type of Potato

Response Taste

Actual by Predicted Plot



REML Variance Component Estimates

Random Effect	Var Ratio	Component	Std Error	95% Lower	95% Upper	Pct of Total
Day	18,584761	1,0930056	3,4056578	0,1128147	6,62e+14	22,544
Day*Temperature	55,897805	3,2874577	3,5111957	0,84172	202,89929	67,808
Day*Temperature*Tim	6,9534062	0,4089432	0,3583131	0,123914	7,7916059	8,435
Residual		0,0588119	0,0339551	0,0244212	0,2851846	1,213
Total		4,8482186	3,9660612	1,553479	67,934185	100,000

-2 LogLikelihood = 58,984486717

Note: Total is the sum of the positive variance components

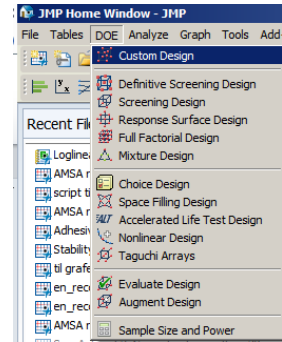
Total including negative estimates = 4,8482186

Fixed Effect Tests

Source	Nparm	DF	DFDen	F Ratio	Prob > F
Temperature	2	2	2	1,3599	0,4237
Time	1	1	3	37,4461	0,0088 *
Temperature*Time	2	2	3	1,7288	0,3167
Type of Potato	1	1	6	43,4652	0,0006 *
Temperature*Type of Potato	2	2	6	4,1577	0,0736
Time*Type of Potato	1	1	6	0,3175	0,5935
Temperature*Time*Type of Potato	2	2	6	0,5645	0,5962

Demo in JMP

Summary / Conclusion



- I have very often used these text books designs, also at different workplaces
- And yes you do have less power, for the factor you split on, however, not less power for the interactions

Wish list for developments in JMP DOE menu

- Possibility to add hierarchy block factors, so these workarounds are not necessary