Welcome to BEBPA's

Sneak Peek Webinar

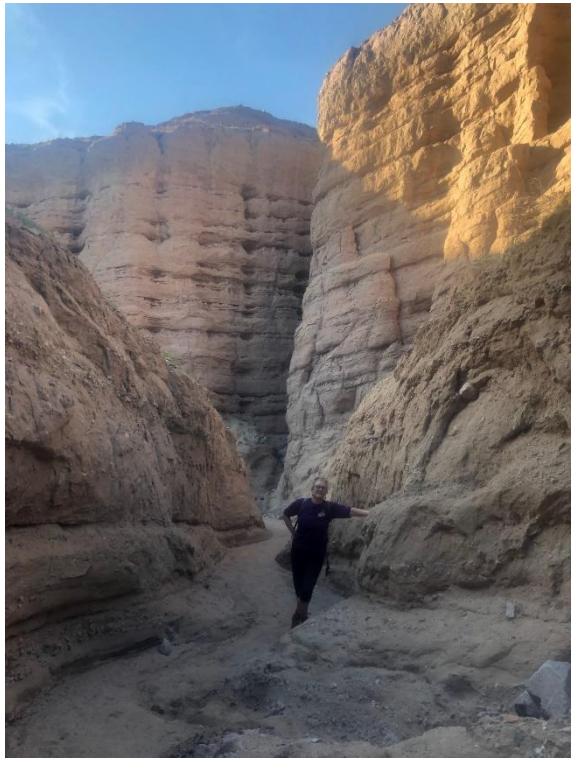
DOE Basics for Potency Assay Workshop

Begins at :

Korean Time 9:00 AM

USA 5:00 PM (PT)

Welcome & Introduction



Introduction

- Experience: Working in industrial biotechnology since 1986. Consulting for small and large biopharmaceutical firms since 1993. Working on Analytical Test Procedures for Commercial Release, CMC issues, Establishing GLP facilities and the founder of BEBPA which produces high quality conferences for the biopharmaceutical industry. I also founded and published BioQuality, a newsletter which monitored the regulatory and compliance happenings in the industry.

Who is BEBPA?

Welcome to the Biopharmaceutical Emerging Best Practices Association (BEBPA), a non-profit organization dedicated to advancing patient safety through the collaborative efforts of the biopharmaceutical scientific community.

- 3+ Annual Hybrid Events
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- White Papers
- Free Scientific Webinars
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- Journal Club
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"Launch" by Hejum Ba (Whistle)

Logistics

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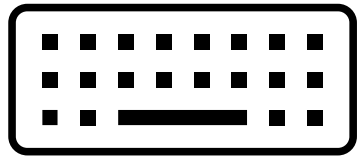
Ask Questions in the Question Box



You can always email questions later

Just a warning: This is not a full course in DOE

Hopefully, you are using these tools or this will give you the desire to use the tools.

The Quiet We Made
Jagannath Pau

What is Design of Experiments (DoE)?

- It is a way of designing experiments which allows more than one factor to be changed.
- This can seem heretical as we are always taught if you change more than one factor in an experiment, than you will not know which change is causing the change in the result.
- In DOE terms the traditional one factor at a time is called an ***OFAT*** experiment and a DoE is called an ***MFAT*** for Multiple factor at a time.

DOE - Basics

- Factors = The assay conditions or reagents that you vary (e.g.: incubation temperature, dilution, incubation time, etc. Usually assign these letters
- Level = The condition of the factor which you test (e.g.: 25 minutes vs. 35 minutes, 1:1000 vs. 1:2000 dilution, etc.) In the following example:

High level = Plus (+) (or can be capital letters)

Low Level = Minus (-) (or can be small letters)

DOE Designs

- If you only have a few conditions (such as with stability) – typically do **full-factorial designs**
- If you have many conditions and you are interested in finding which (if any) are important, you will do partial factorial (screening) designs
    - E.g. **Plackett-Burman, Fractional Factorials**

Example # 1 – Full Factorial

Few Conditions: Therefore = factorial design → look at three factors → at two levels:

2^3 → This is three factors

2^3 → This tells you there are two levels.

An example would be :

Factor	-	+
Time	2h	4h
Temperature	20°C	37°C
pH	6.5	8

What is Factorial Design?

- Set of experiments so that more than one variable can be tested at the same time.
- This is done by running all the possible combinations-of each factor at each level.
- Therefore 2^2= 2*2 experiments: 4 experiments
- And 2^3= 2*2*2 experiments: 8

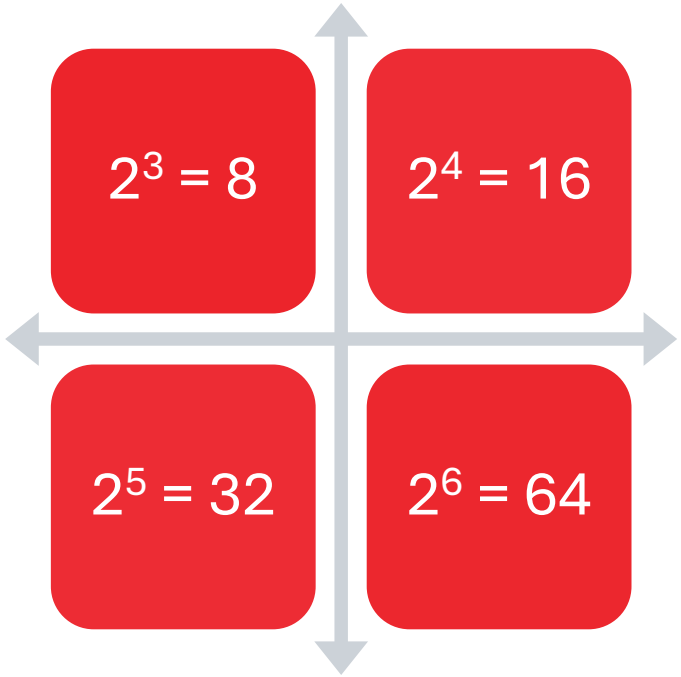
	Factor	-	+
X1	Time	2h	4h
X2	Temperature	20°C	37°C
X3	pH	6.5	8

A 2^3 factorial experiment will have 8 runs.

X1	X2	X3
-	-	-
+	-	-
-	+	-
+	+	-
-	-	+
+	-	+
-	+	+
+	+	+

Time	Temperature	pH
2H	20°C	6.5
4h	20°C	6.5
2H	37°C	6.5
4h	37°C	6.5
2H	20°C	8
4h	20°C	8
2H	37°C	8
4h	37°C	8

The Number of Experimental Runs is defined by the Number of Runs and Levels



- In assay development we only use full factorial experiments when there are very few conditions to study.
- When we are in early development there are usually many conditions we want to study. For these studies we typically used partial factorial design

Example of the Problem

Early in development – we have many variables.

A full-factorial design for many variables soon becomes too big.

An example: Cell growth:

Media Type	Location in incubator
%FBS	Initial thawing temperature
Seeding Density	dispersion technique
Feeding Schedule	Maximum # of passages
Method of removing cells	Culture Time

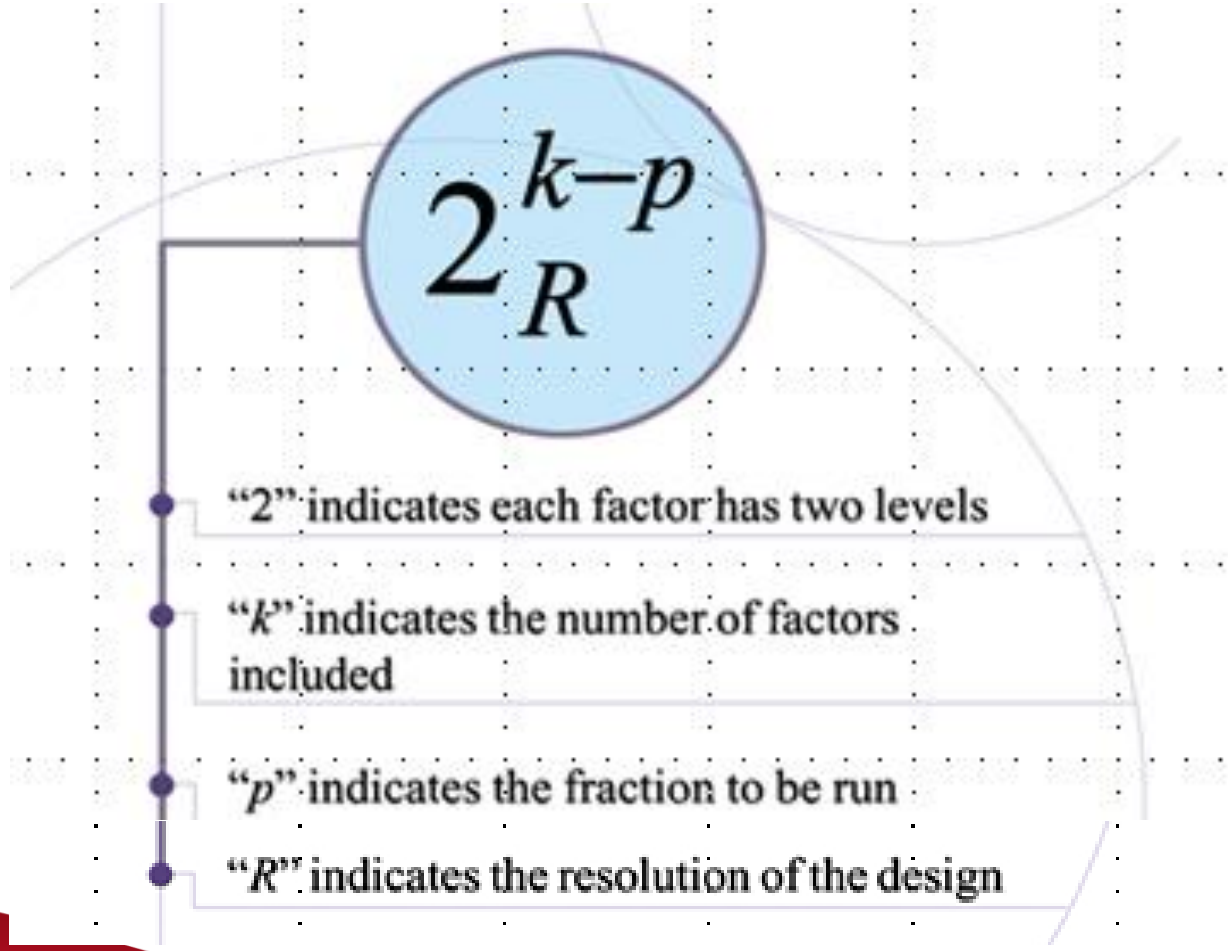
The Problem

This simple example leads to: 2^{10} =1024 experiments!!!!

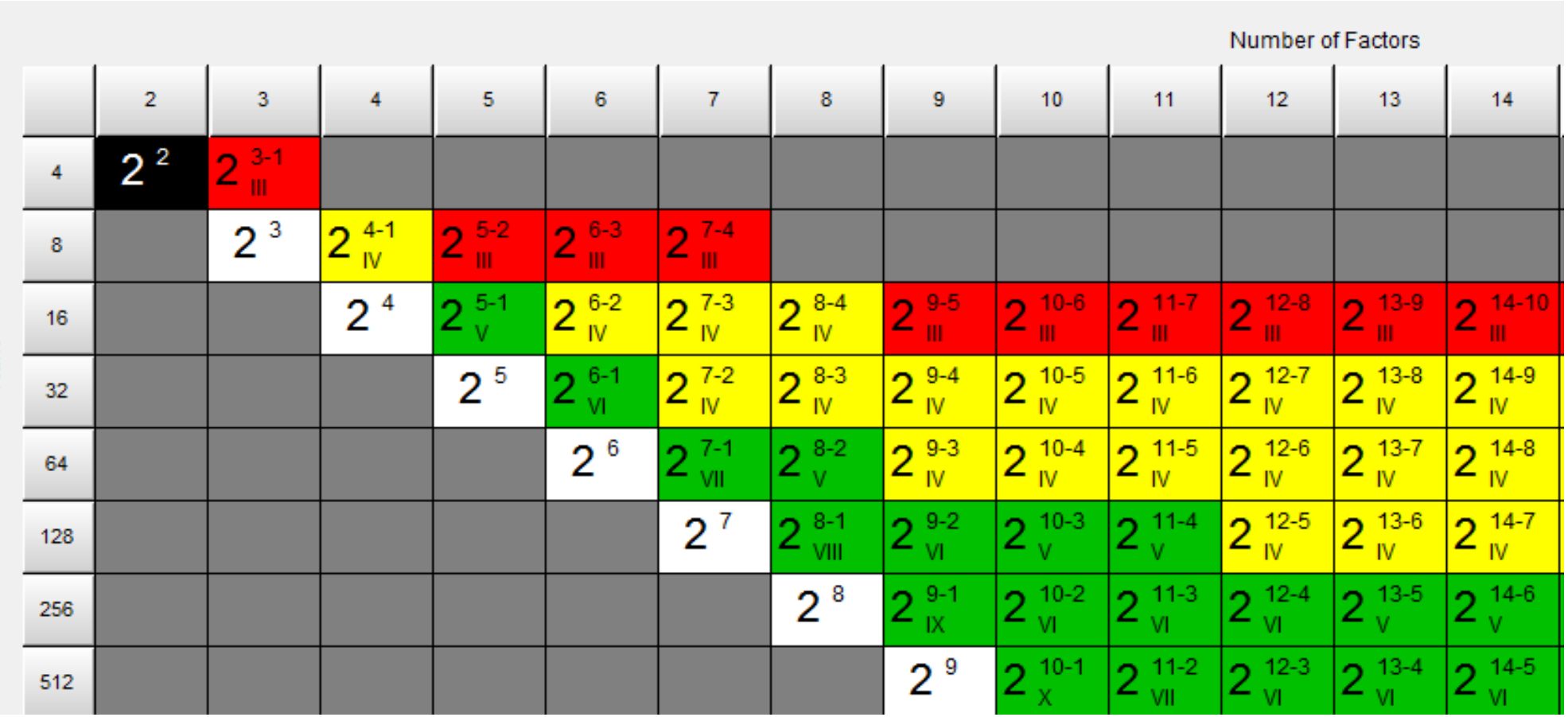
This is where the fractional factorial comes in.

A Fractional Factorial Design runs a subset of the full factorial runs. If chosen correctly, we can still estimate the main effects but may lose the higher order interactions.

Fractional Factorial



Initial Screen from StatEase



This gave us a 2^{6-2} Fractional Factorial. This is a level 4 resolution. What does this mean?

Cell Culture Example

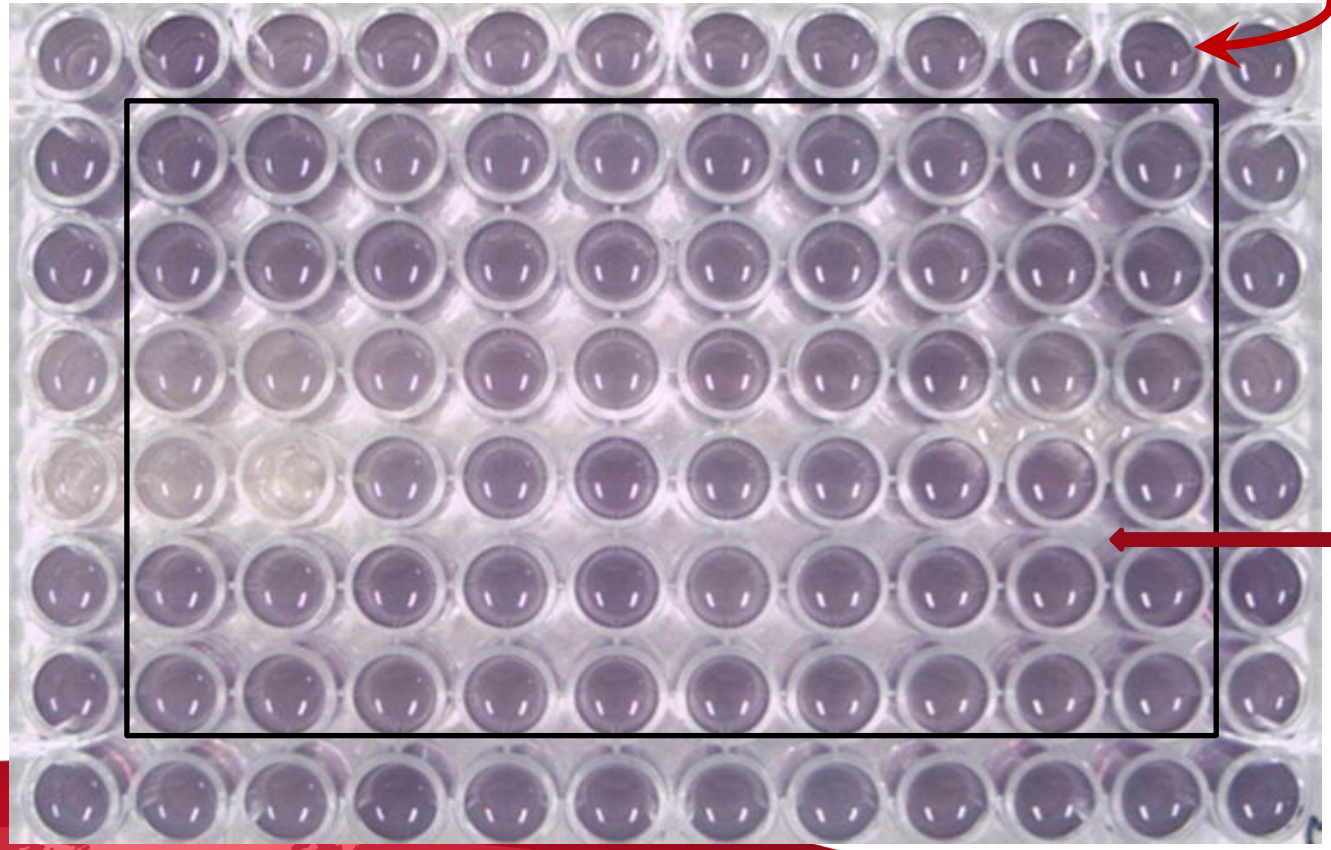
- Choose 5-6 factors and design a Fractional Factorial.
- This can be done twice.
- We may find that most of the factors we think we should study – don't impact the cell culture. Therefore, it is smart to figure out which factors are critical and then study them.

First Select the Factors and Levels

Name	Units	Type	Low	High
Passage #	passage	Factor	<P10	>P26
Seeding Density	cells per cm2	Factor	2000	4000
Tryp concentration	ml	Factor	2	4
Tryp Incub	Minutes	Factor	5	10
FBS lots	Lot	Factor	B1	B2
Day Feeding	times per week	Factor	1	2
R1	%CV*	Response		
R2	Bowl Ratio**	Response		

Bowl Ratio

Bowl ratio = Average outside/Average inside



Average OD of outside wells

Average OD of inside wells

Resolution	Ability
II	Not useful: main effects are confounded with other main effects
III	Estimate main effects, but these may be confounded with two-factor interactions
IV	Estimate main effects unconfounded by two-factor interactions Estimate two-factor interaction effects, but these may be confounded with other two-factor interactions
V	Estimate main effects unconfounded by three-factor (or less) interactions Estimate two-factor interaction effects unconfounded by two-factor interactions Estimate three-factor interaction effects, but these may be confounded with other two-factor interactions
VI	Estimate main effects unconfounded by four-factor (or less) interactions Estimate two-factor interaction effects unconfounded by three-factor (or less) interactions Estimate three-factor interaction effects, but these may be confounded with other three-factor interactions

What is the Response?

This is component optimization

– not assay optimization.

Therefore, what are the characteristics we would like to see?

Well-to-well consistency of growth (This can be measured by an Alamar Blue dye, cell-titer glo, whatever viability assay you have – then reported out as an average and %CV.)

Lack of systematic bias: Experience tells us that the bowl ratio is the most common growth pattern: Therefore, let's take Avg OD outer/Avg OD inner

Run Design (Fractional Factorial 2^{6-2})

Std	Run	Passage	Seeding	[Tryp]	Try Incub	FBS	Feeding	R1	R2
		P#	cells per cm2	ml	Minutes	Lot	X /week	%CV	Bowl Ratio
1	1	<P6	5000	2	10	B2	2		
9	2	<P6	5000	4	10	B1	1		
2	3	<P6	5000	2	10	B2	2		
10	4	<P6	5000	4	10	B1	1		
13	5	<P6	10000	4	5	B1	2		
5	6	<P6	10000	2	5	B2	1		
14	7	<P6	10000	4	5	B1	2		
6	8	<P6	10000	2	5	B2	1		
11	9	>P12	5000	4	5	B2	1		
3	10	>P12	5000	2	5	B1	2		
12	11	>P12	5000	4	5	B2	1		
4	12	>P12	5000	2	5	B1	2		
7	13	>P12	10000	2	10	B1	1		
15	14	>P12	10000	4	10	B2	2		
8	15	>P12	10000	2	10	B1	1		
16	16	>P12	10000	4	10	B2	2		

Results

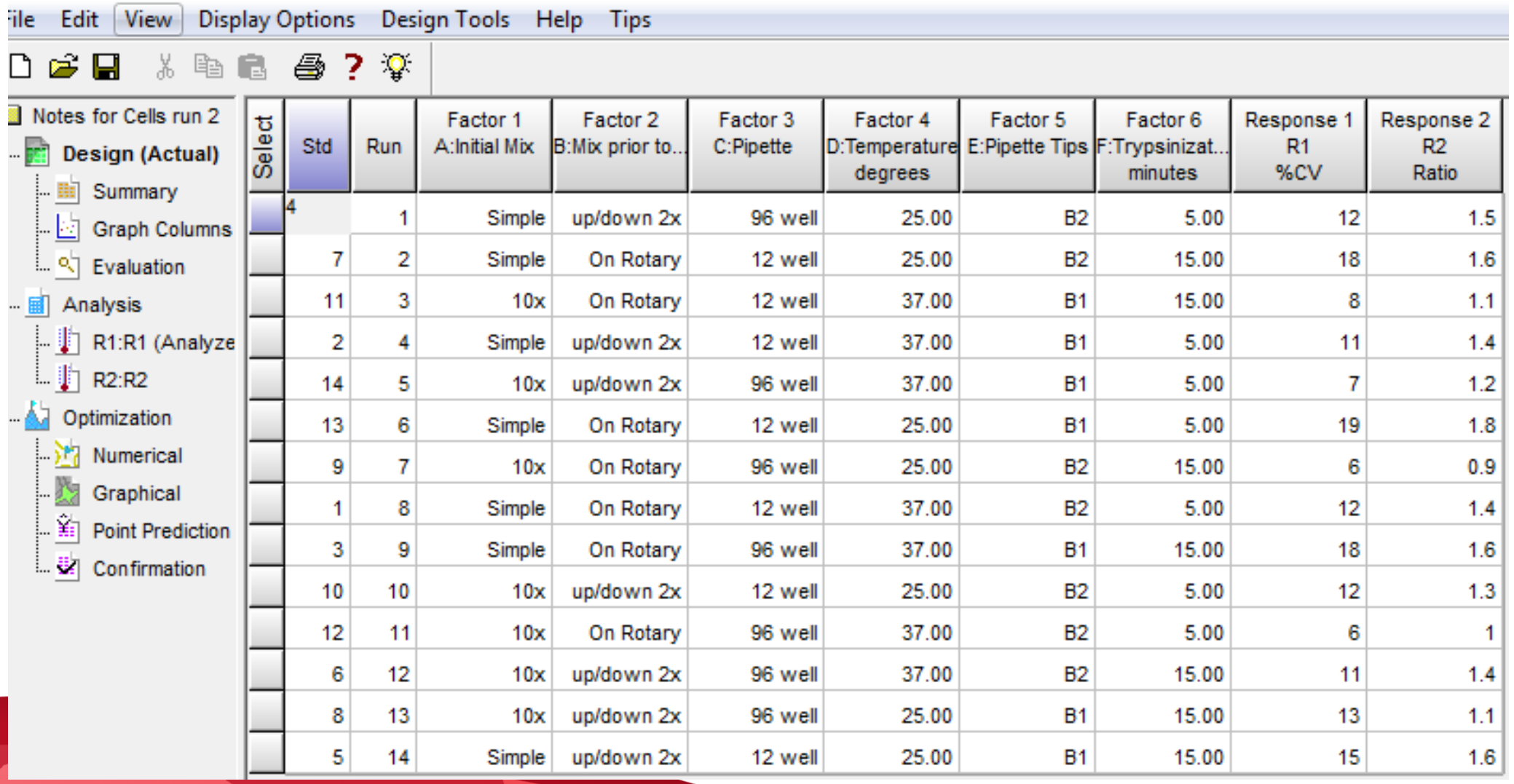
All of the results had a serious positional problem.

None of the factors studied had an impact.

Did a second round of experimentation looking at more of the technique issues.

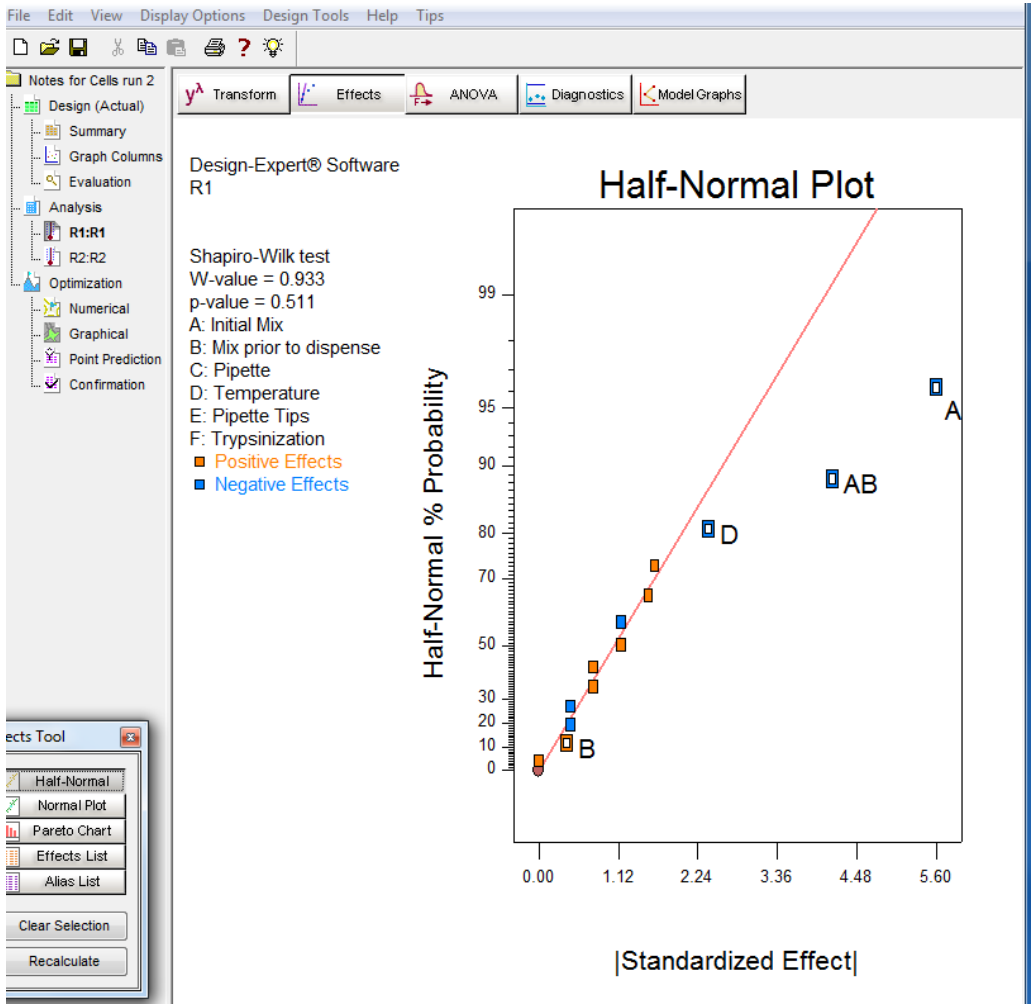
We did a second round of experimentation with different factors (conditions)

Name	Units	Type	Low	High
Initial mixing of cells	Y/N	Factor	Simple Inversion	10 x inversion
Mixing prior to dispensing	Y/N	Factor	up/down 2 x in pipette	on rotary
pipette type used	pipette type	Factor	12 well	96-well
Temperature (media)	degrees	Factor	25	37
pipette tips	brand	Factor	B1	B2
Trypsinzation	time (minutes)	Factor	5	15



Select	Std	Run	Factor 1 A:Initial Mix	Factor 2 B:Mix prior to...	Factor 3 C:Pipette	Factor 4 D:Temperature degrees	Factor 5 E:Pipette Tips	Factor 6 F:Trypsinizat... minutes	Response 1 R1 %CV	Response 2 R2 Ratio
	4	1	Simple	up/down 2x	96 well	25.00	B2	5.00	12	1.5
	7	2	Simple	On Rotary	12 well	25.00	B2	15.00	18	1.6
	11	3	10x	On Rotary	12 well	37.00	B1	15.00	8	1.1
	2	4	Simple	up/down 2x	12 well	37.00	B1	5.00	11	1.4
	14	5	10x	up/down 2x	96 well	37.00	B1	5.00	7	1.2
	13	6	Simple	On Rotary	12 well	25.00	B1	5.00	19	1.8
	9	7	10x	On Rotary	96 well	25.00	B2	15.00	6	0.9
	1	8	Simple	On Rotary	12 well	37.00	B2	5.00	12	1.4
	3	9	Simple	On Rotary	96 well	37.00	B1	15.00	18	1.6
	10	10	10x	up/down 2x	12 well	25.00	B2	5.00	12	1.3
	12	11	10x	On Rotary	96 well	37.00	B2	5.00	6	1
	6	12	10x	up/down 2x	96 well	37.00	B2	15.00	11	1.4
	8	13	10x	up/down 2x	96 well	25.00	B1	15.00	13	1.1
	5	14	Simple	up/down 2x	12 well	25.00	B1	15.00	15	1.6

The Software does a series of calculations, but I like the visual which allows us to see which variables fall away from the predicted standardized effect. In this case it was Factor A – which was the pre-mixing of the cells just prior to plating.



R1 – Diagnostic Plot

Design of Experiments (DOE) in Bioassays

DOE is a tool which can be used throughout the entire development cycle.

- It is best used sequentially (i.e. don't try to design ***one*** experiment to ask all your development questions).

- Current bioassay field uses DOE to determine robustness. While this is a fabulous tool – if it is your only use, then you are starting too late!

Crows at Sunset by Zeshin

Any Last Questions?

This was just a quick overview of DoE and not meant to allow you to go out and utilize this great tool. However, the full workshop at the upcoming bioassay conference will the 3.5 hours and provide more of an in-depth discussion!

Thank You!

Asia Bioassay Conference

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