The Use of Quality-by-Design and DOE Tools for BioAssay Development: Part 1: Component Optimization

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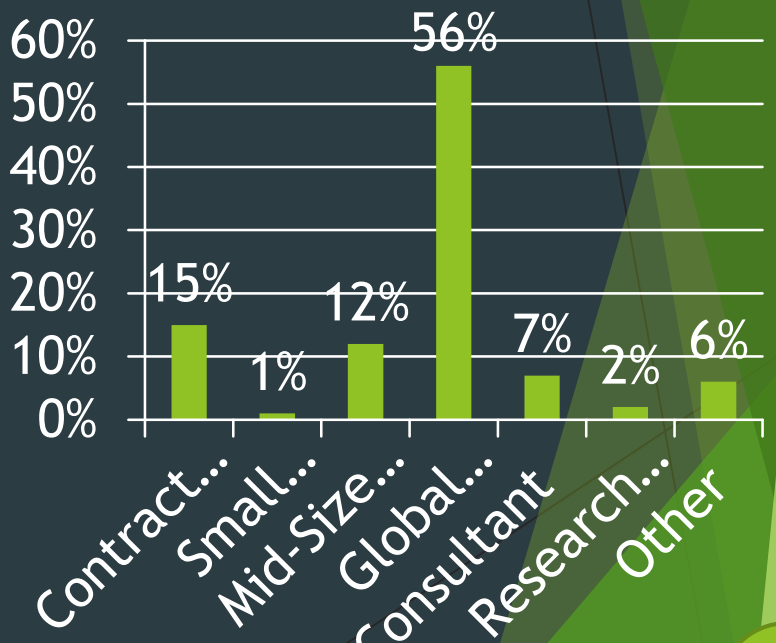
Before we start:

- You have a transceiver. These are to allow us to do some interactive things.
- When the clock appears in the bottom right hand side push a number for your answer.
- A green light will appear. If it remains green and then goes out your answer was accepted. If the light becomes red your answer was not received. Try again.
- If you hit the wrong answer – just answer again. The first answer will be removed and replaced with the most recent answer. (only 1 answer allowed per transceiver.)

About You

What Kind of Company do you work for?

1. Contract Organization
2. Small Biopharmaceutical (< 50 employees)
3. Mid-Size Biopharmaceutical (50 – 300)
4. Global Pharmaceutical
5. Consultant
6. Research Institute
7. Other



About You

EU RESULTS

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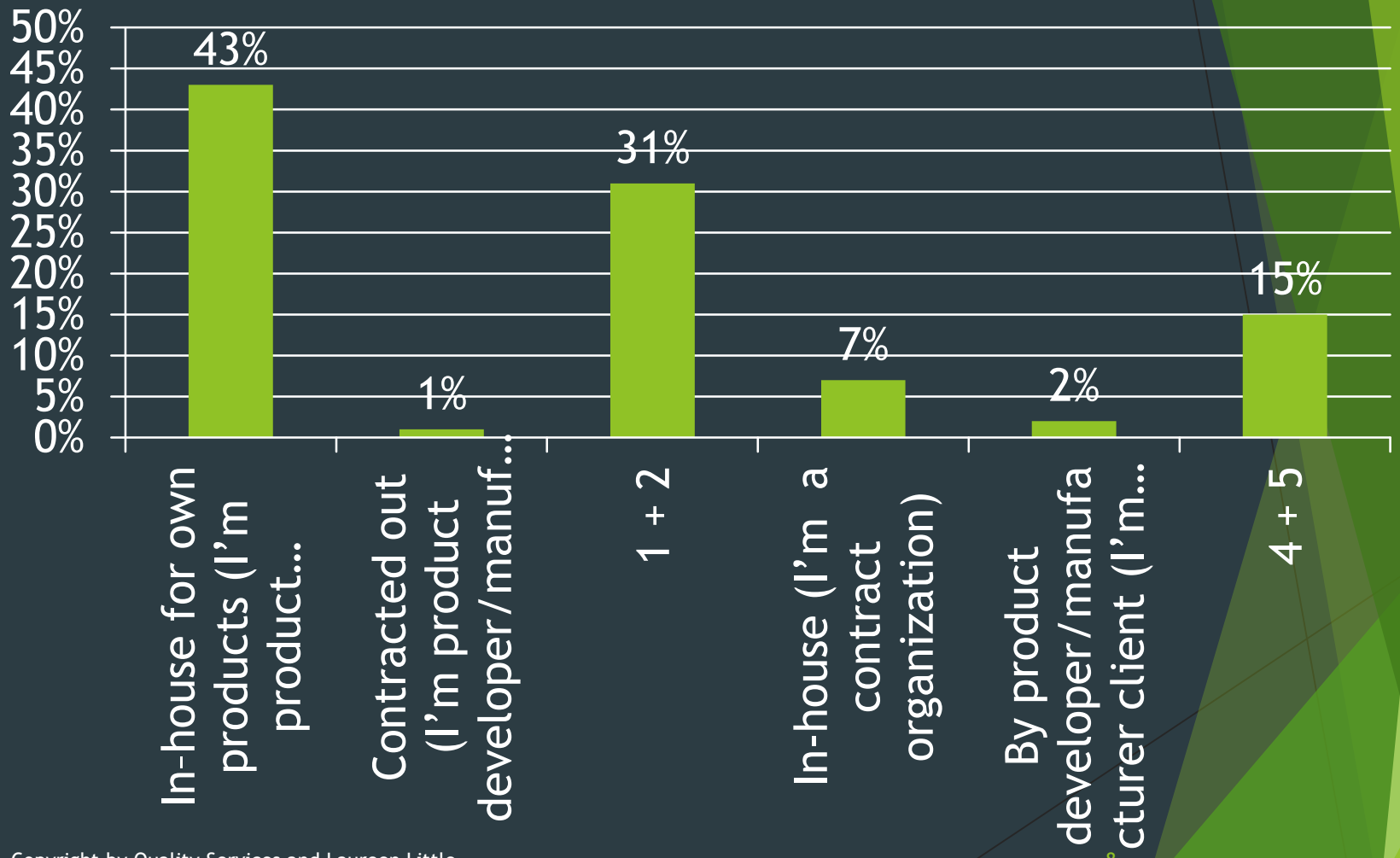
5

6

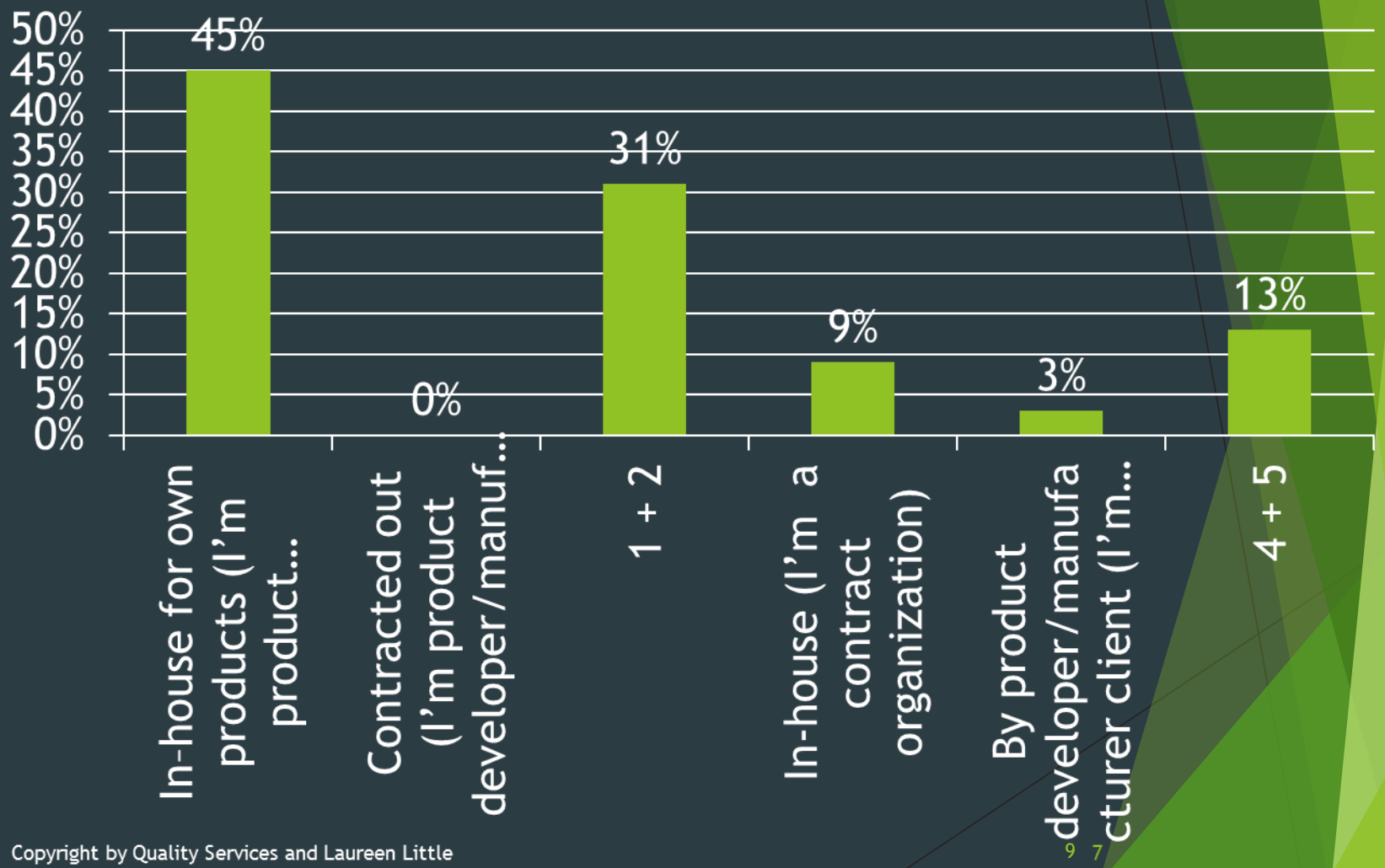
Where are the bioassays developed?

1. In-house for own products (I'm product developer/manufacturer)
2. Contracted out (I'm product developer/manufacturer)
3. 1 + 2
4. In-house (I'm a contract organization)
5. By product developer/manufacturer client (I'm a contract organization)
6. 4 + 5

Where?



Where? EU Responses

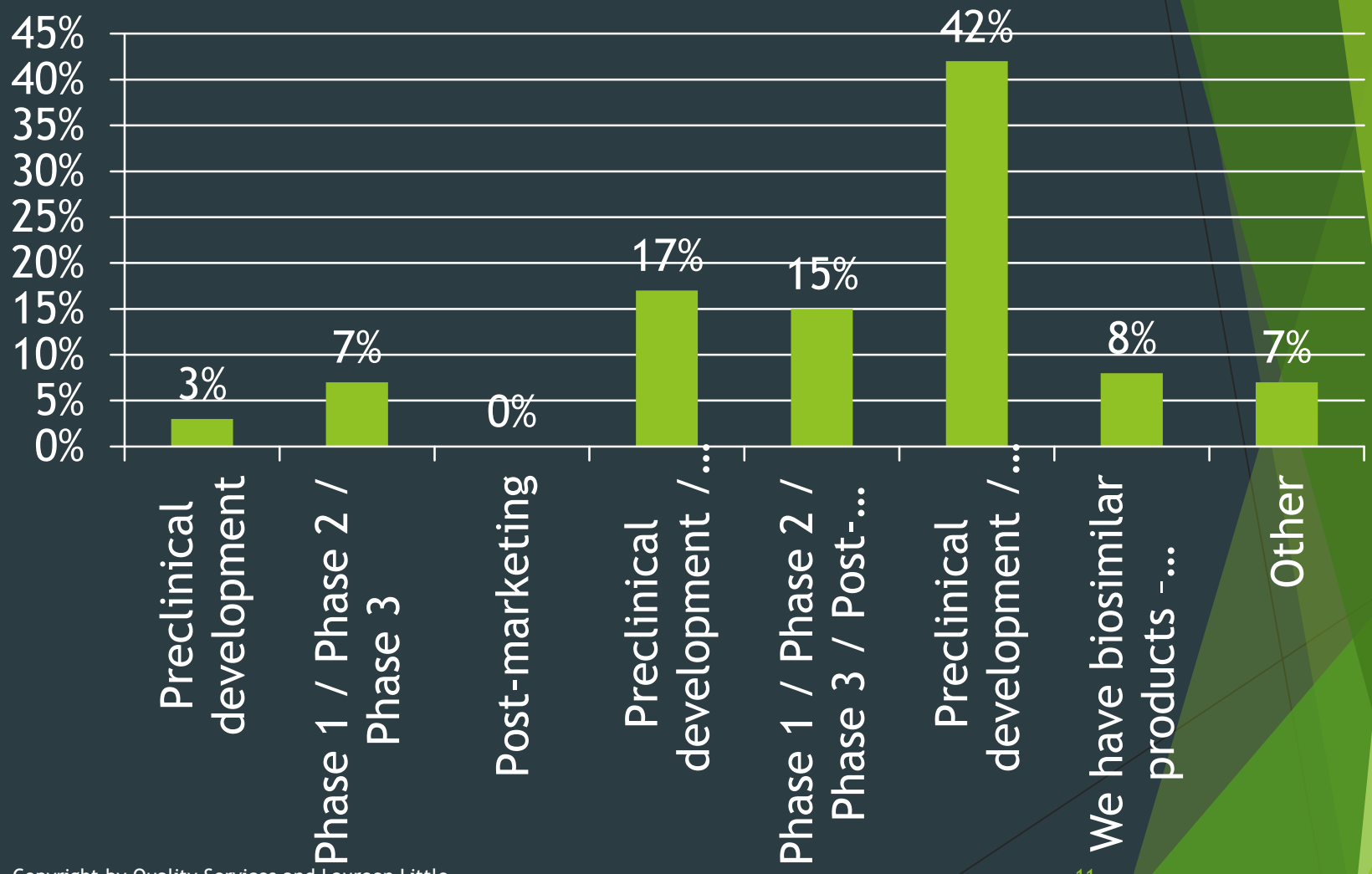


Stages at which assay(s) used

1. Preclinical development
2. Phase 1 / Phase 2 / Phase 3
3. Post-marketing
4. Preclinical development / Phase 1 / Phase 2 / Phase 3
5. Phase 1 / Phase 2 / Phase 3 / Post-marketing
6. Preclinical development / Phase 1 / Phase 2 / Phase 3 / Post-marketing
7. We have biosimilar products – therefore the above doesn't make sense
8. Other

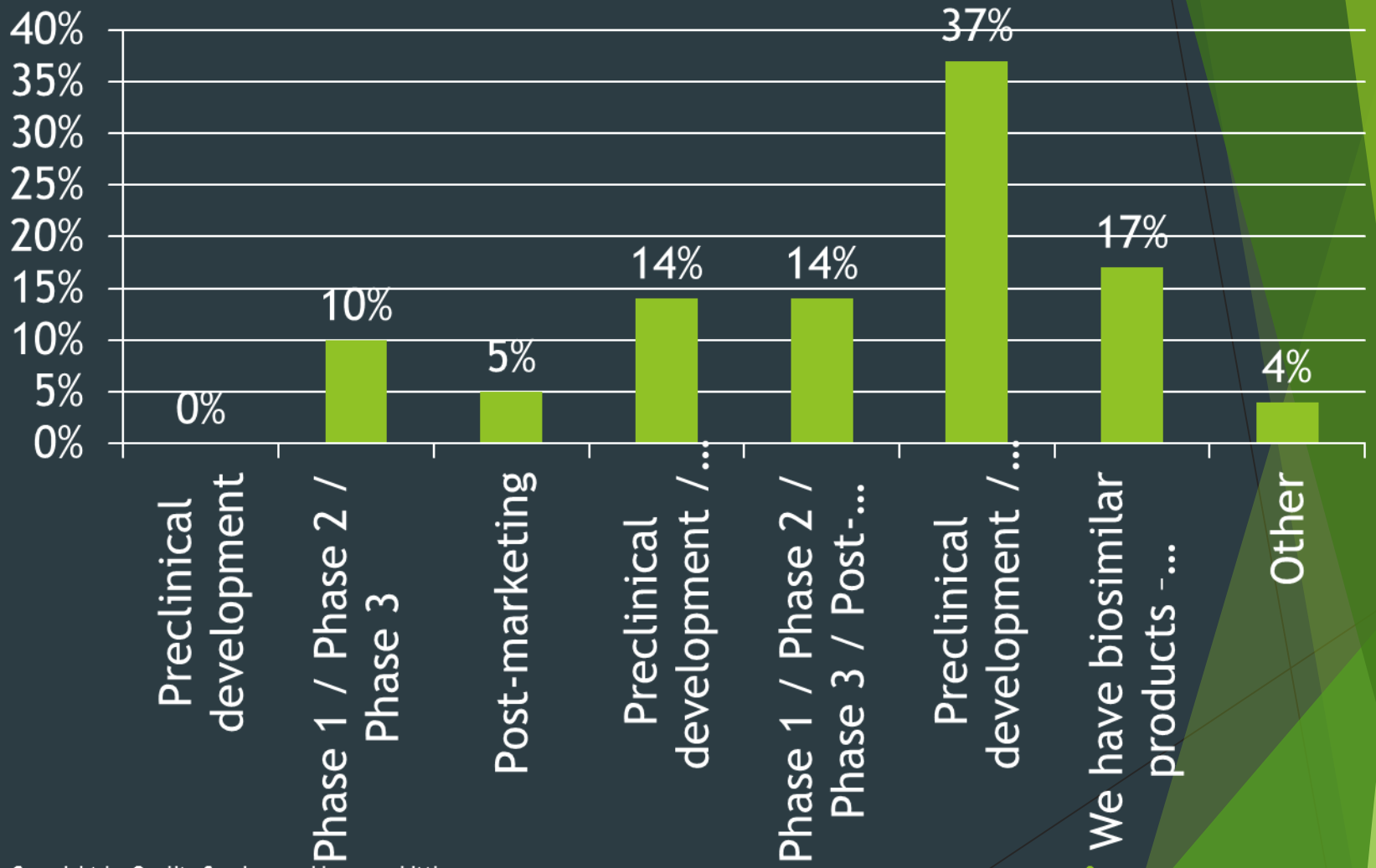
10

When?



When?

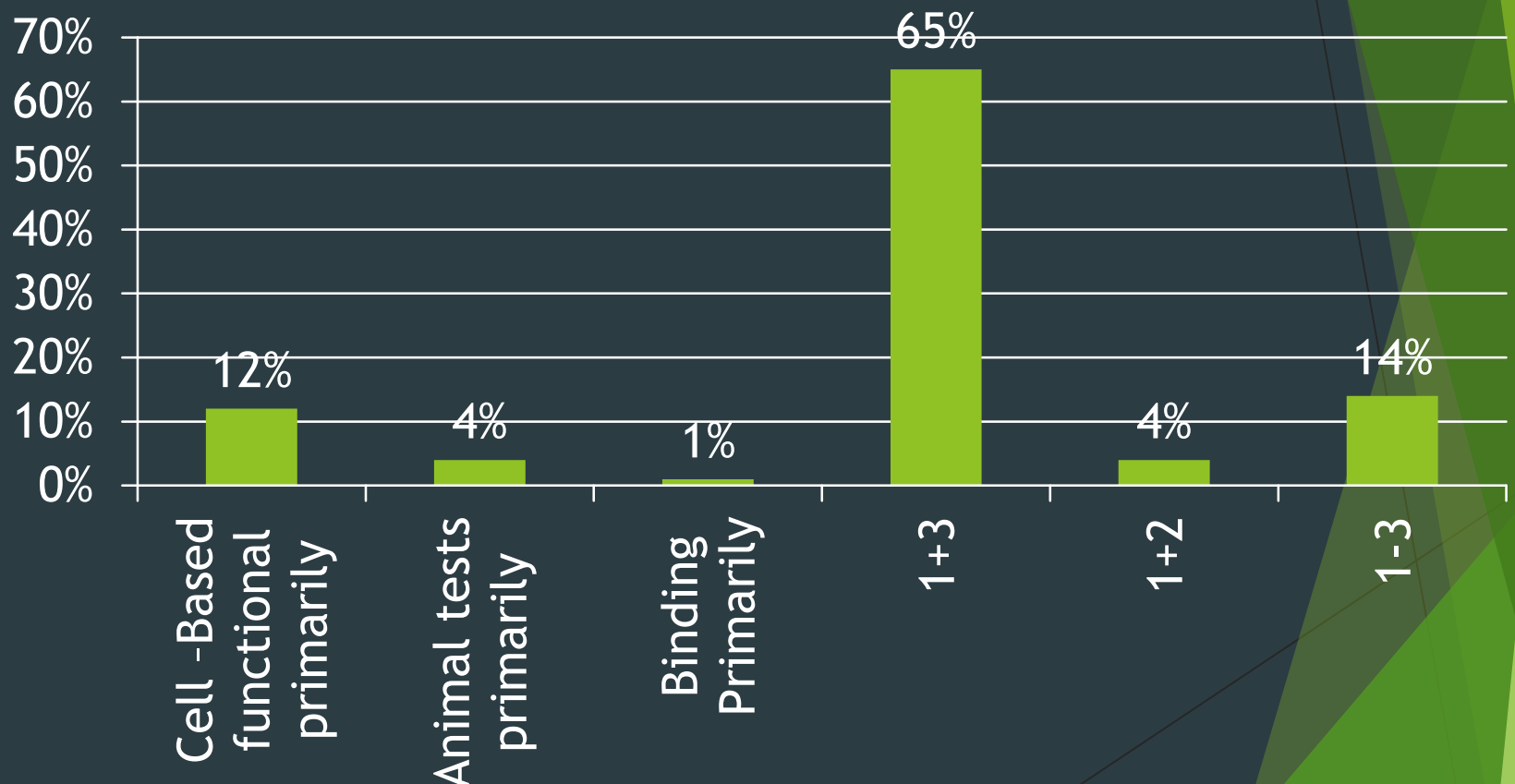
EU Responses



Functional or Ligand Binding?

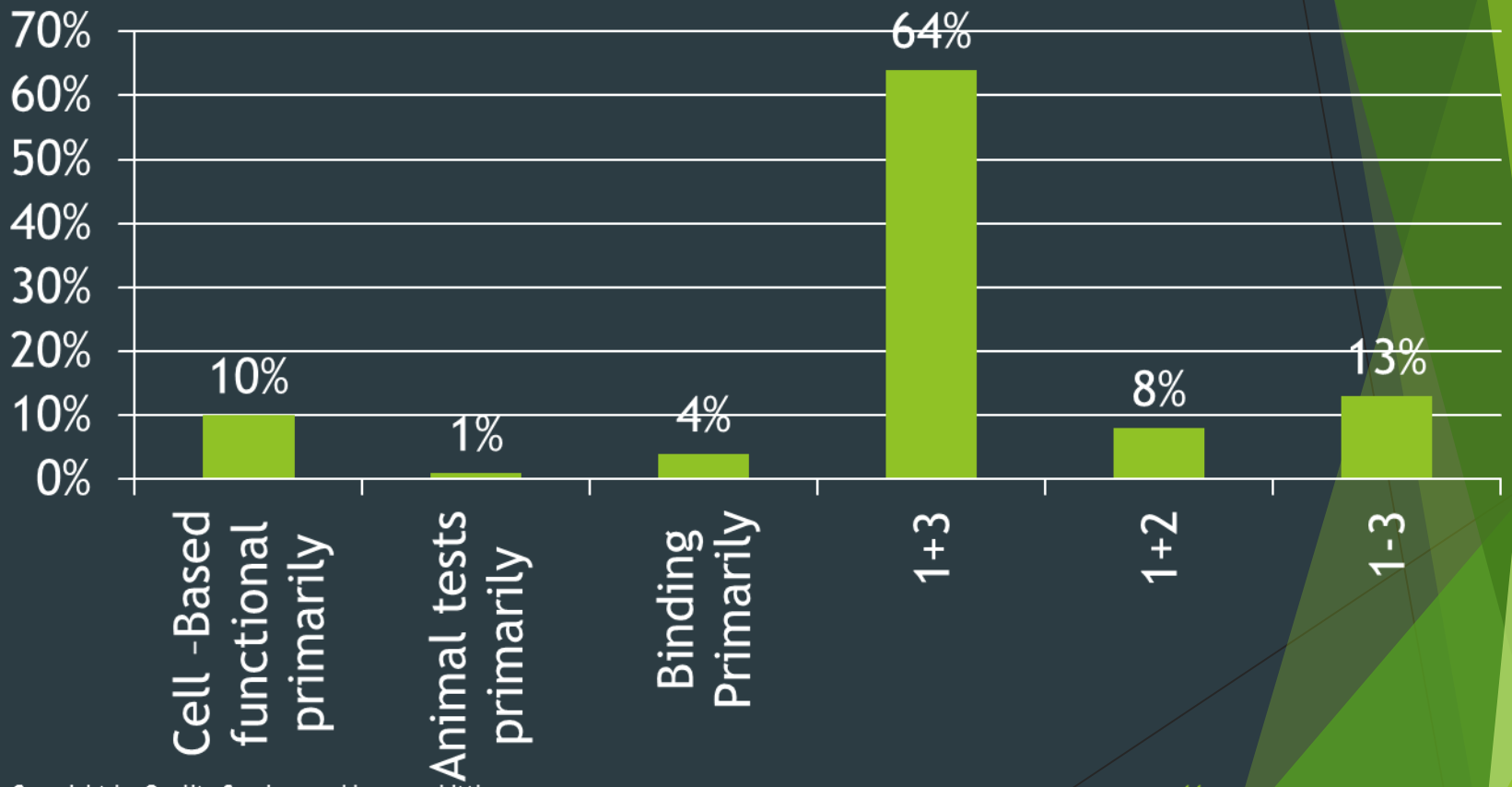
1. Cell –Based functional primarily
2. Animal tests primarily
3. Binding Primarily
4. 1+3
5. 1+2
6. 1-3

Types of Assays



Types of Assays

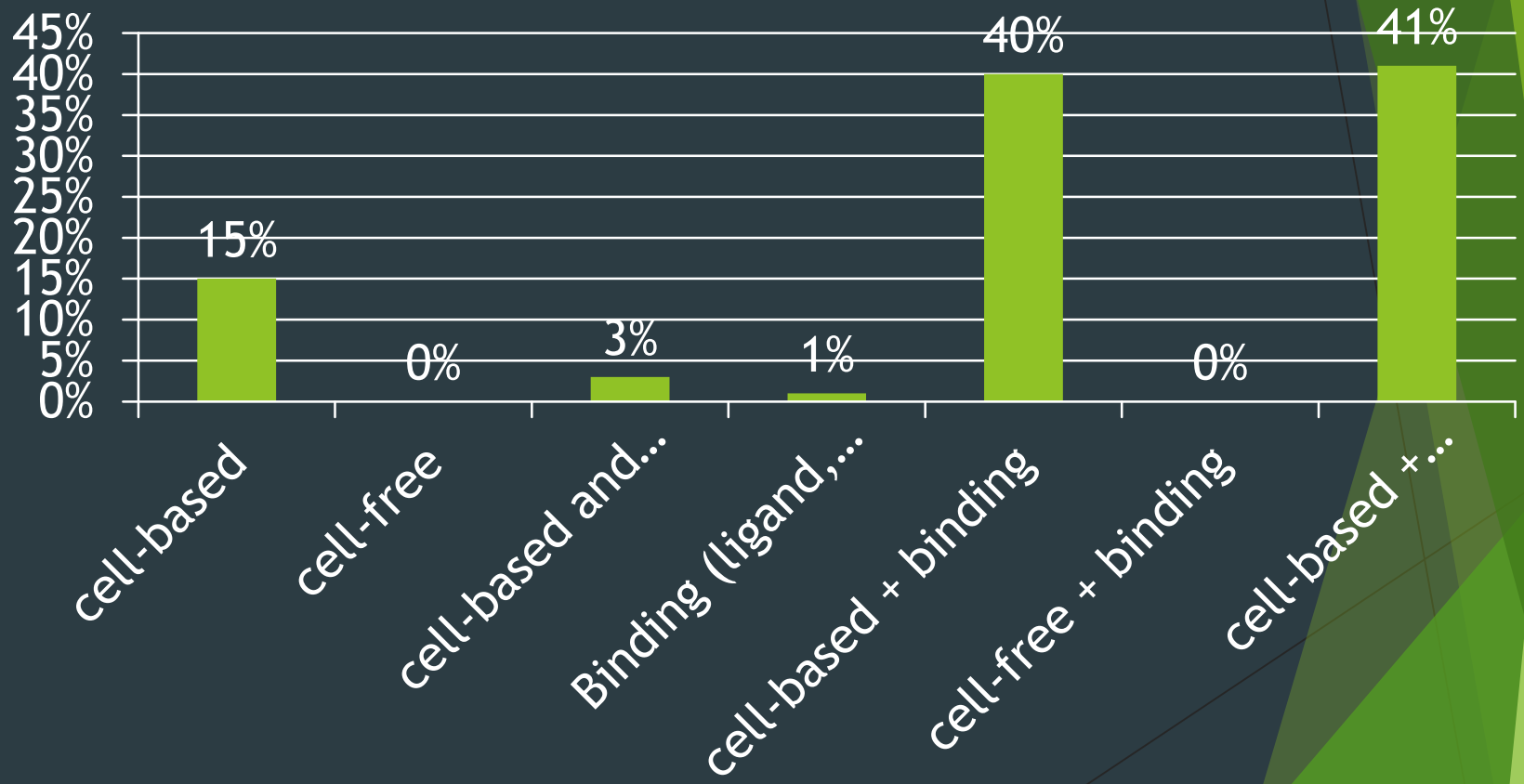
EU Response



Functional Assay types used

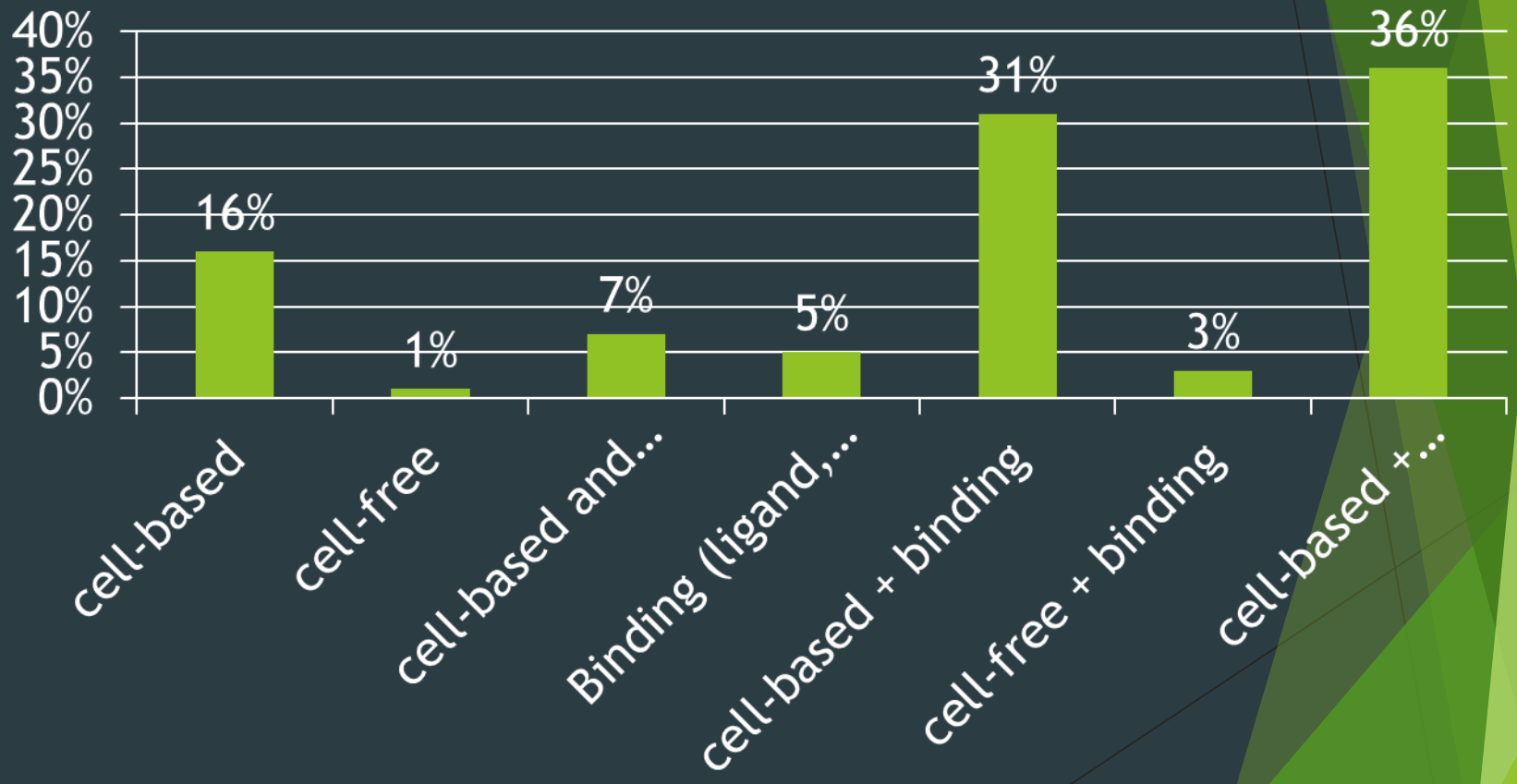
1. cell-based
2. cell-free
3. cell-based and functional cell-free
4. Binding (ligand, receptor, cofactor, ….)
5. cell-based + binding
6. cell-free + binding
7. cell-based + functional cell-free + binding

Functional Assay types used



Functional Assay Types

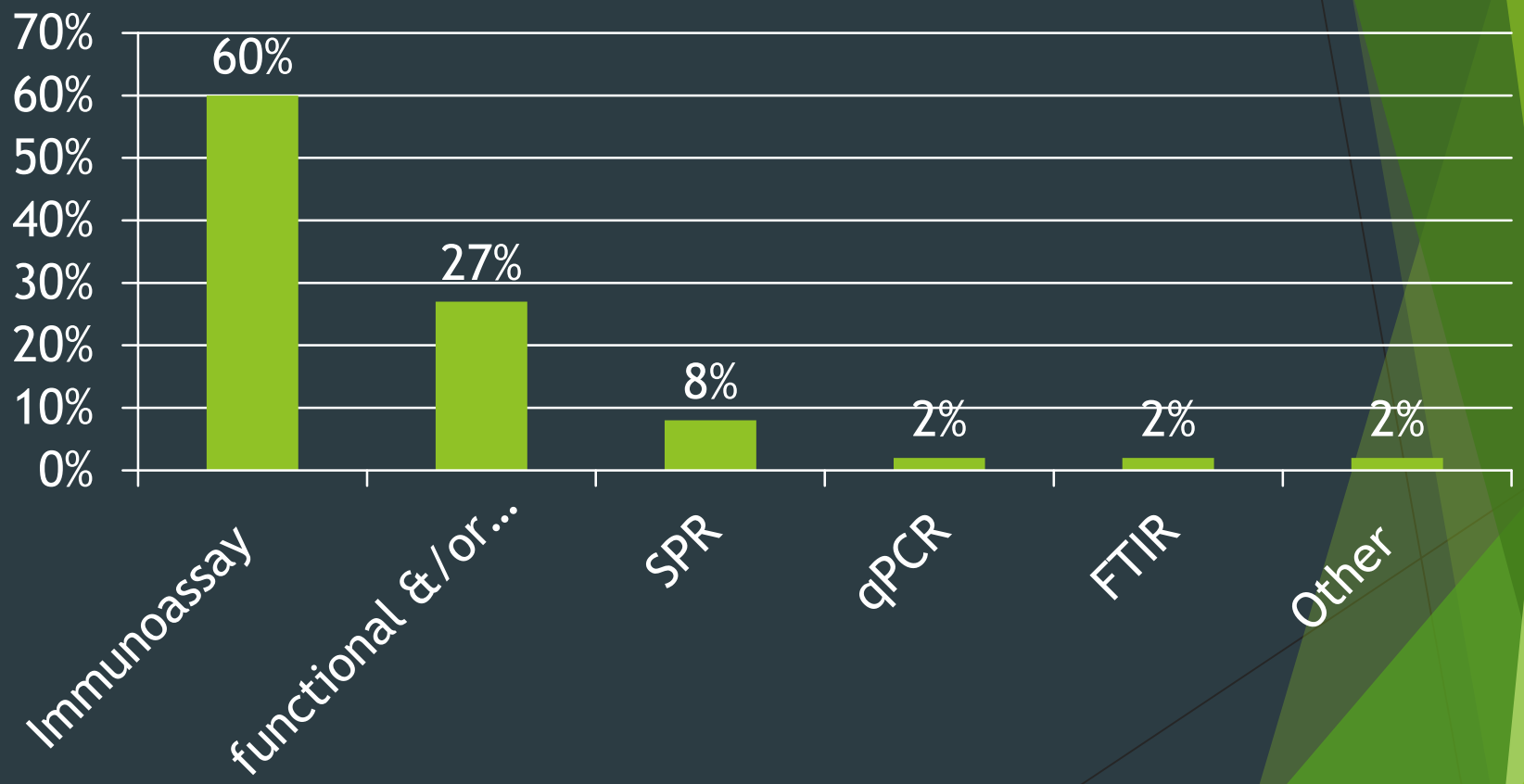
EU Response



Binding Assay type (primarily)

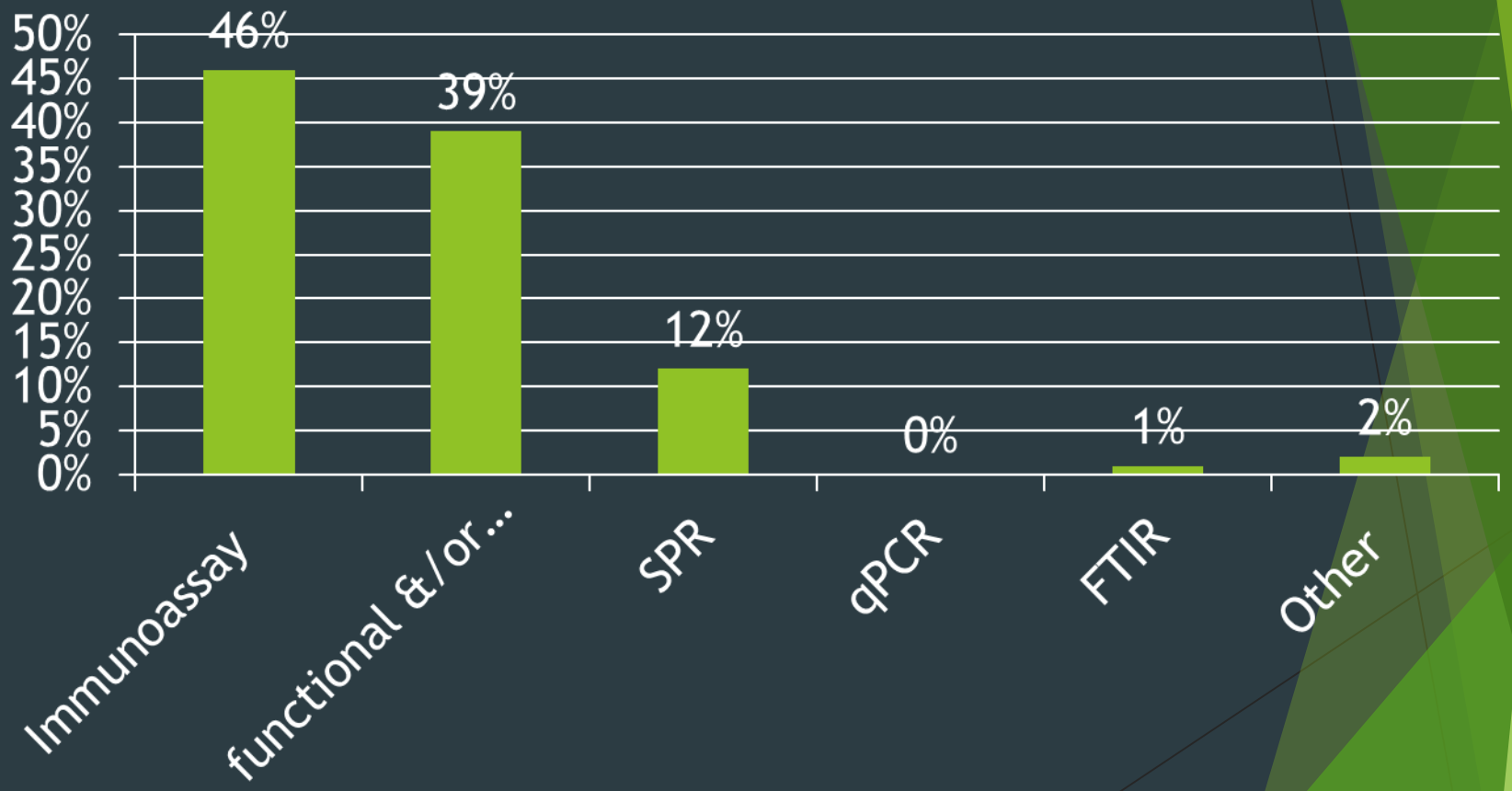
1. Immunoassay
2. functional &/or binding
3. SPR
4. qPCR
5. FTIR
6. Other

Ligand Binding Assays



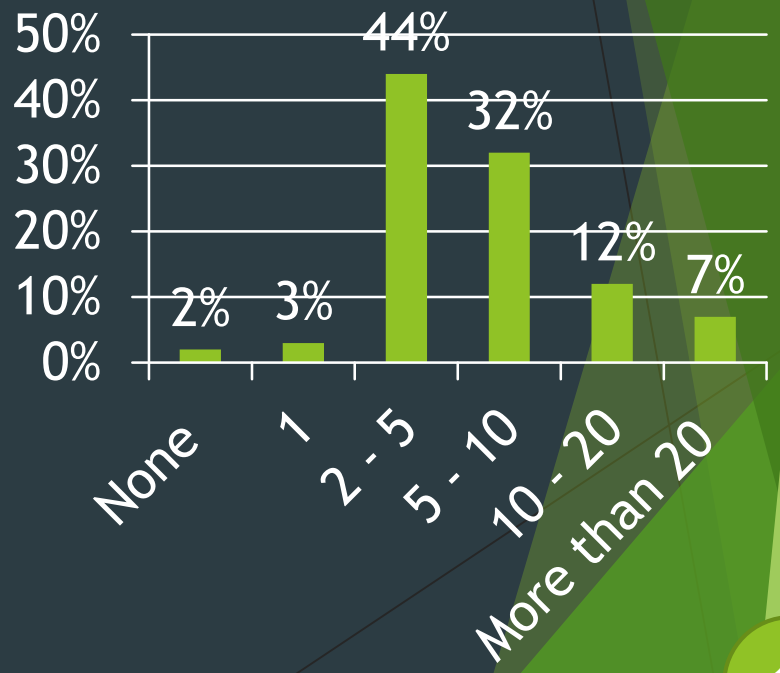
Ligand Binding Assays

EU Responses



How many bioassay systems do you run?

1. None
2. 1
3. 2 - 5
4. 5 - 10
5. 10 - 20
6. More than 20



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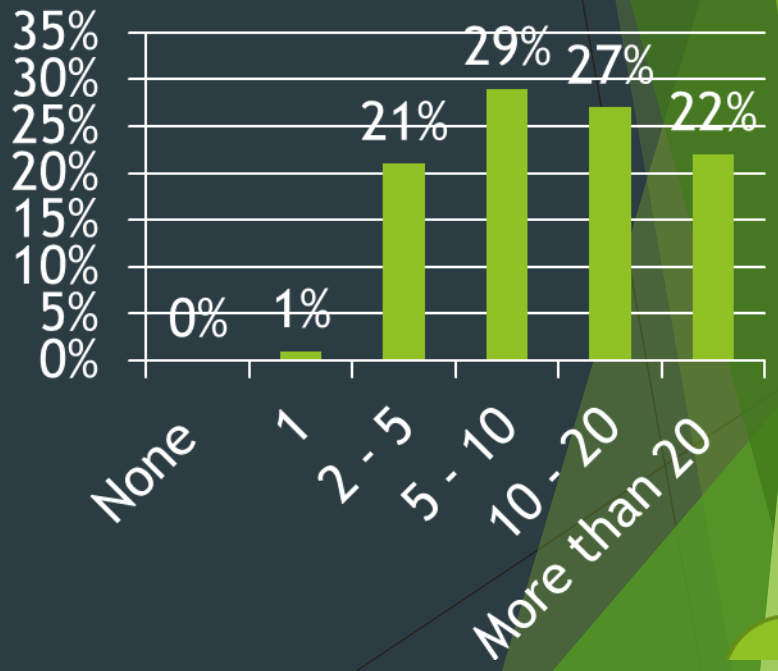
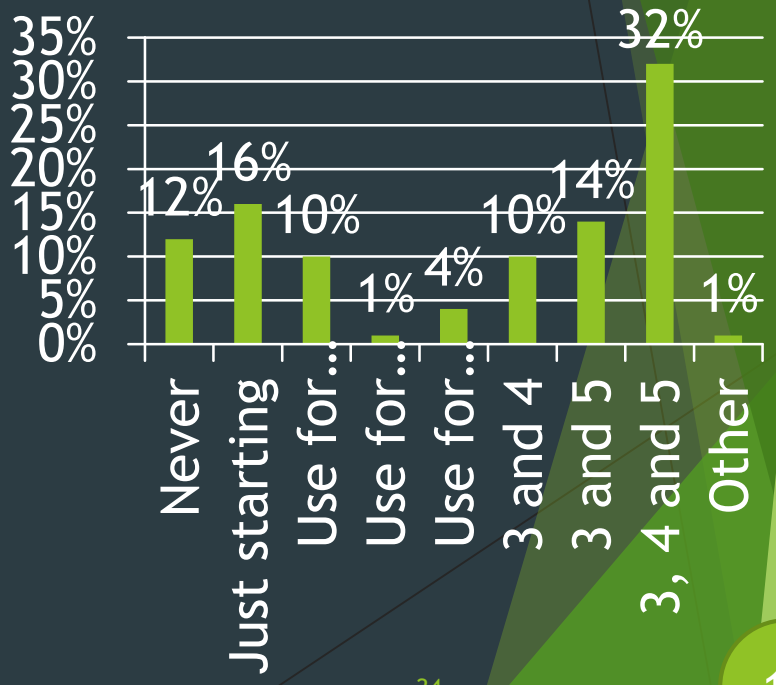


DOE in your lab

What is your current use of DOE?

1. Never
2. Just starting
3. Use for robustness only
4. Use for trouble shooting
5. Use for component optimization
6. 3 and 4
7. 3 and 5
8. 3, 4 and 5
9. Other



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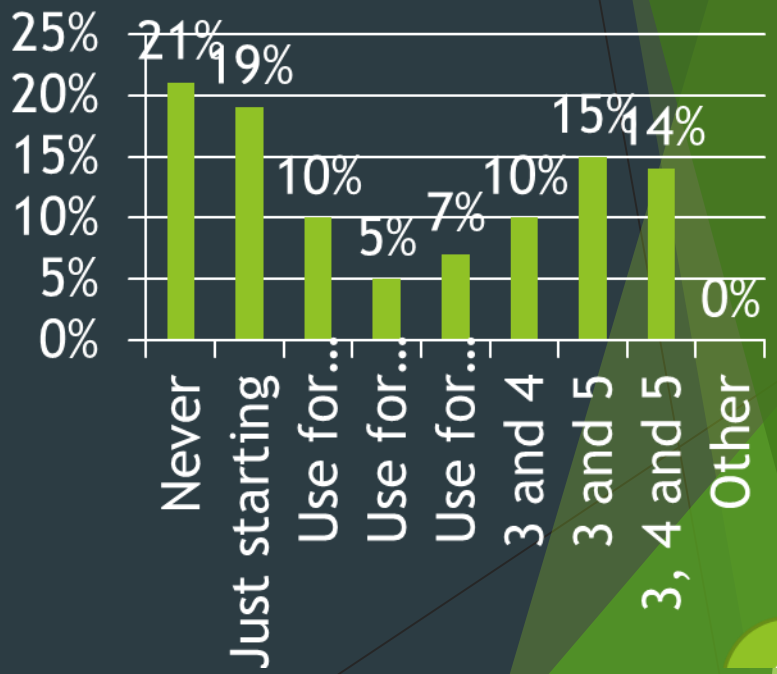


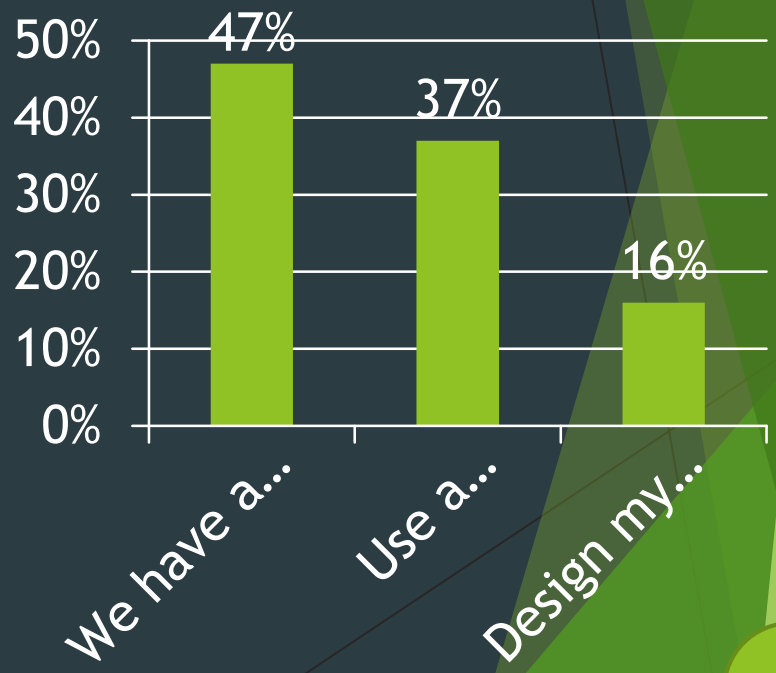
Design of Experiments (DOE) in Bioassays

- 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!

Your Designs

How do you design you DOEs

1. We have a statistician (either employed or consultant)
2. Use a software and design my own.
3. Design my own without software



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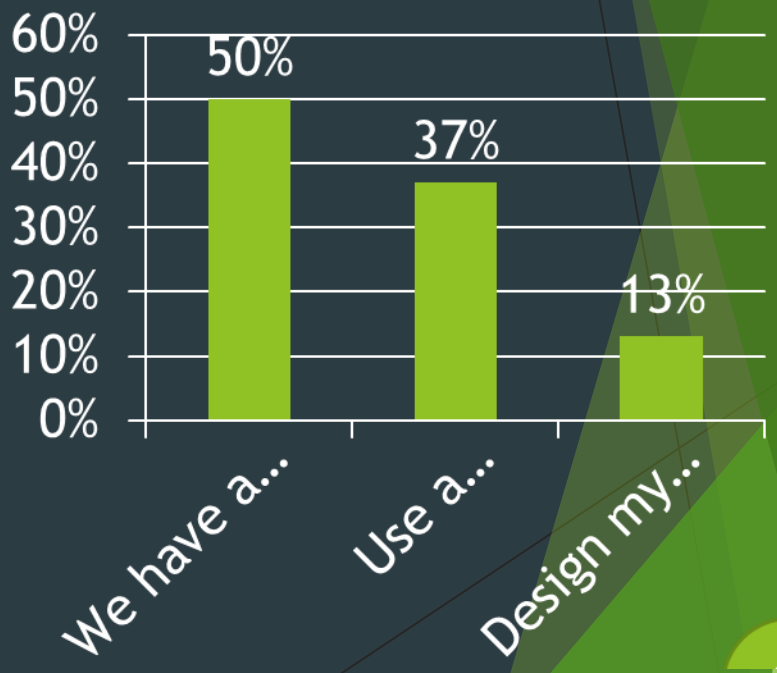


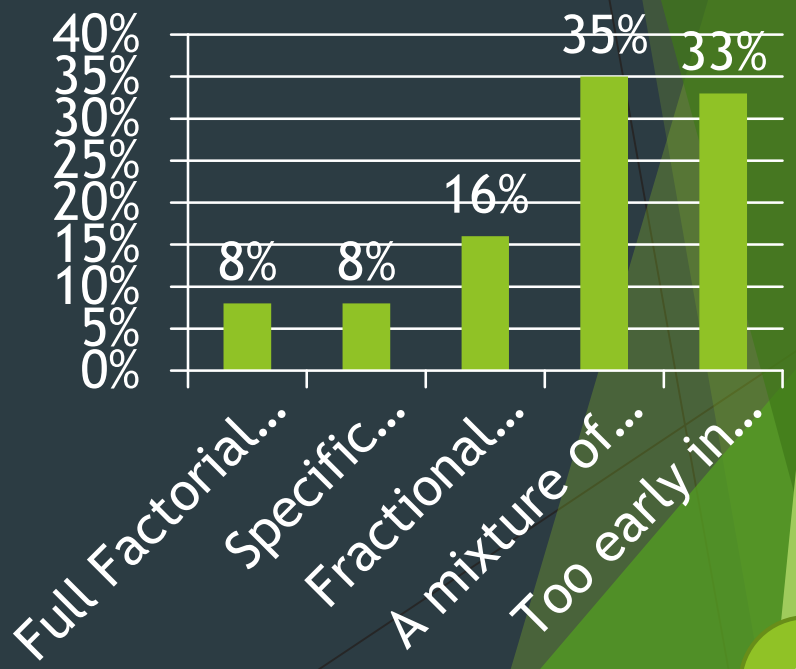
Types of DoE

- Screening methodologies : which are designed to determine what factors are important.
  - Fractional Factorials
  - Specialized designs such as Taguchi (Plackett-Burman)
- Full Factorials: which are designed to determine the best conditions of the factors you know to be important
  - Most common one we see: 2^3 factorial

Your Design (Continued)

What type of designs do you use?

1. Full Factorial only
2. Specific screening design only (such as a placket-burman)
3. Fractional Factorial
4. A mixture of the above
5. Too early in our use of DOE to be able to answer this question



Your Design (Continued)

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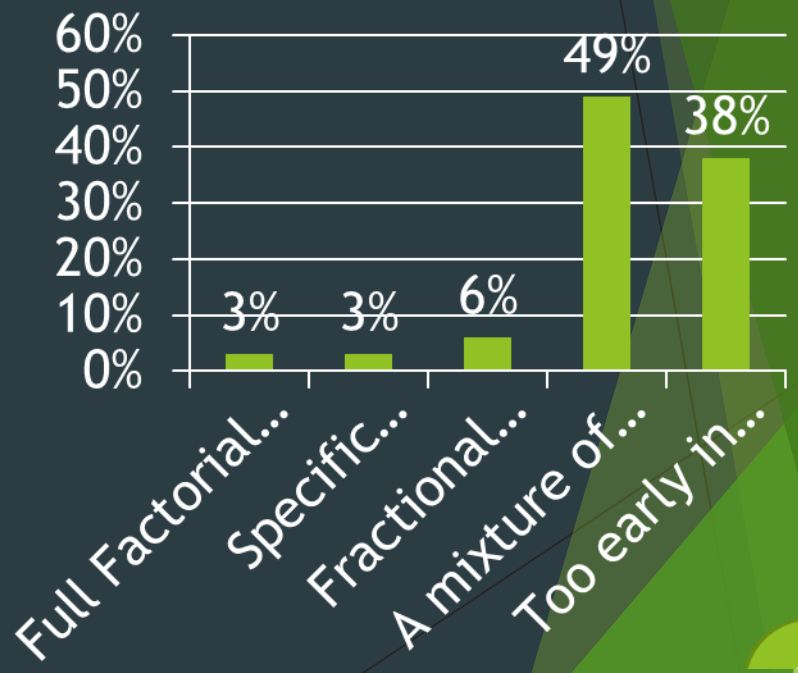

Cell Culture Example

- This was a screening design – we were trying to optimize a component – the cell culture – we didn't know what was important.
- 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 actually impact the method. Therefore it is smart to figure out which factors are critical and then study them.

This is a Sequential DOE Approach

- Following is a sequential method development – using sequential DOE.
- The following example is a design to determine the best growing conditions for a cell-based potency assay. ***Why? It is the most crucial component for achieving low imprecision.***

Choosing the Right Response

- Most of the DOEs that I have seen have not carefully thought through what should be the measured response.
- This is especially important if you are trying to optimize the assay by improving a specific characteristic or component of the assay.
- ***IN THIS EXAMPLE WE DID NOT HAVE DRUG PRESENT!! Since we were only trying to optimize the cells we only looked at a viability dye.***

In Other Assay Systems I would have:

- Perhaps looked at specific receptor expression on the cell
- Looked at zero and high drug concentrations
- Perhaps other viability marker?

- But when optimizing components – you normally do not want to look at the entire assay.

Additional Examples

- ELISA assays
  - We were having a non-specific binding problem. We used sequential DOE to identify blocking reagents and procedures to essentially eliminate this. Our read out was average of 5 blank samples and 5 high samples. We calculated results for both Blank average and Signal/Noise (Z' factor)
- Cell based assay (biomarker)
  - We were having dilutional linearity problems. We used sequential DOE. Here we used 4 single point dilutions of several patient samples and looked at "average" relative bias numbers. We found a sample diluent which completely solved the problem. (This took 5 placket-burman runs, followed by 2 full factorials)

Back to example at Hand: Some Interesting Things about this Design

- It was chosen to do this in 16 plates (based upon the tissue culture analyst that could handle 8 plates)
  - Note the analysts actually informed me they could easily handle 16 plates per day. I assumed that in any type of DOE throughput drops by 50% because of the complexity of the individual assay runs.
- We chose 6 variables.

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		

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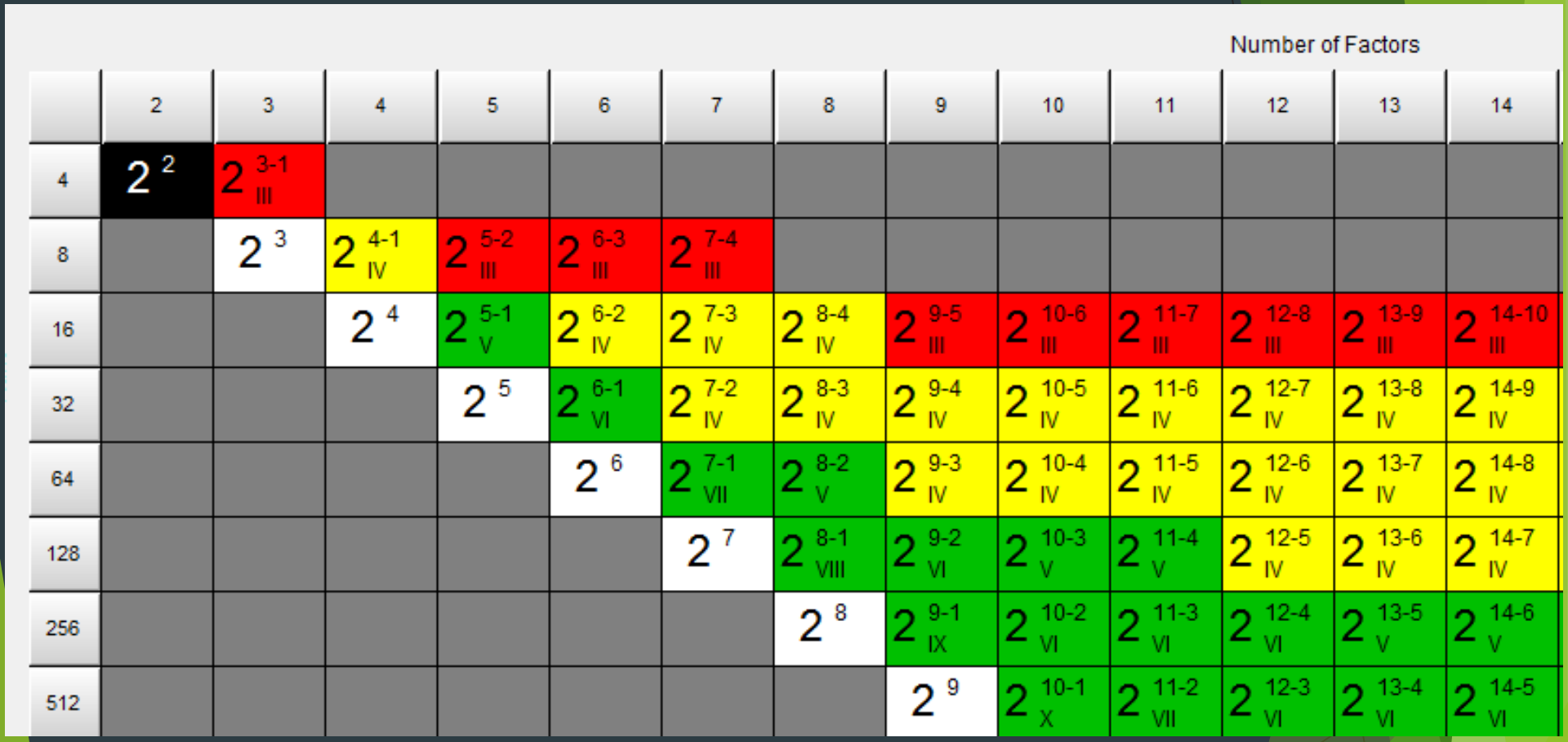
Initial Screen from StatEase



↑ Number of runs

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

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

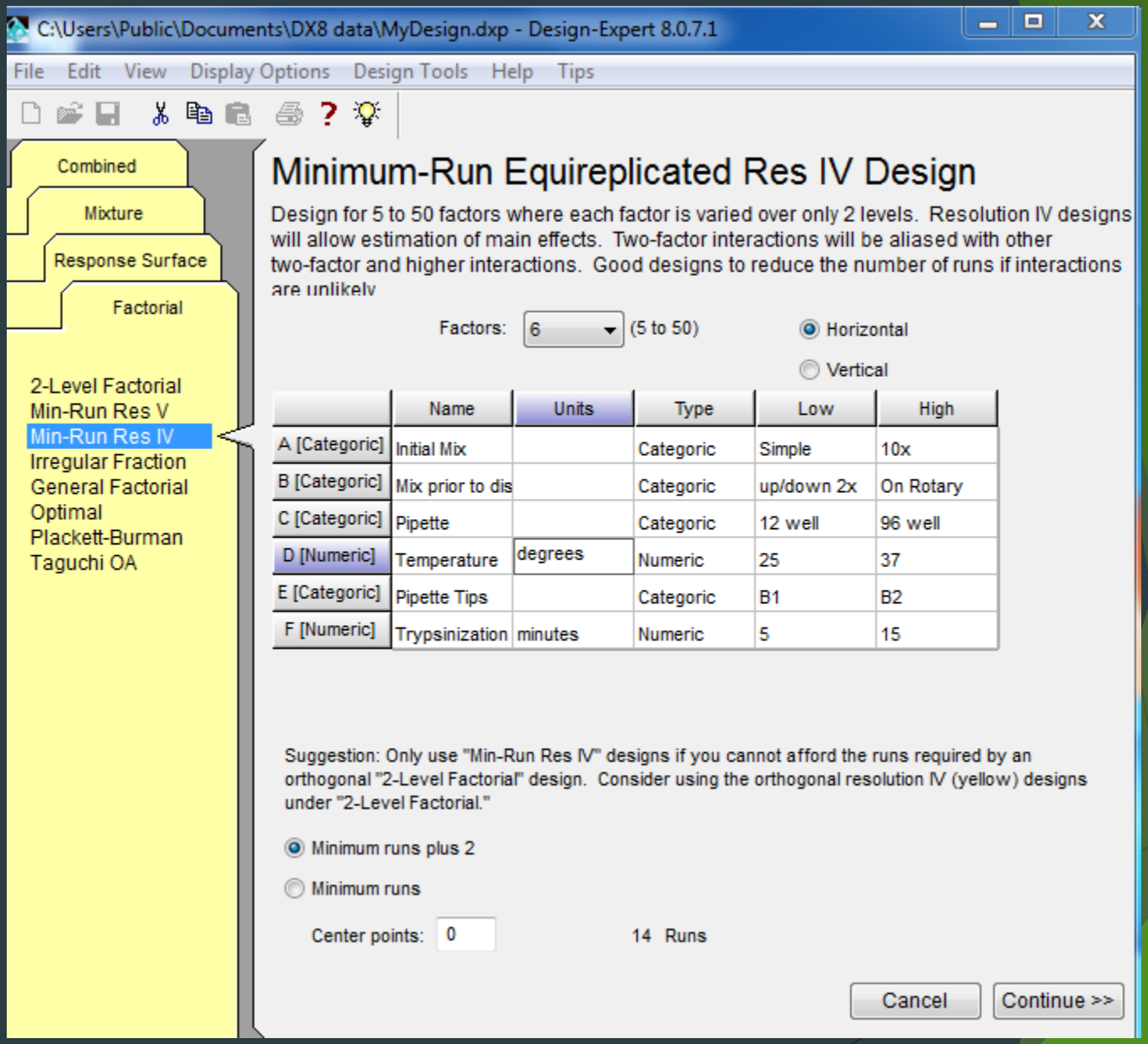
Run Design

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		

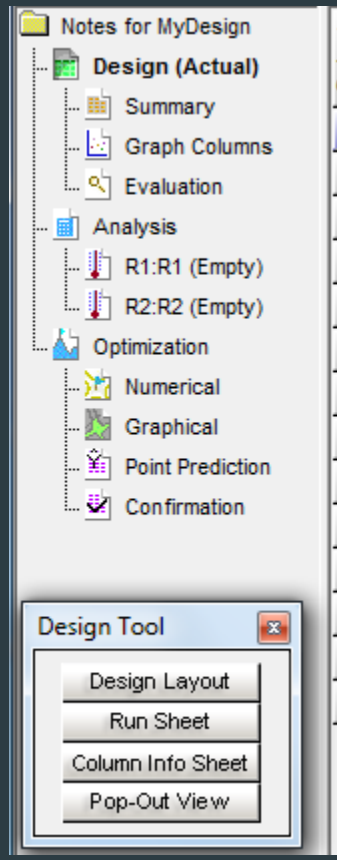
Results

- 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.

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
R1	%CV*	Response		
R2	1st row vs last row	Response		



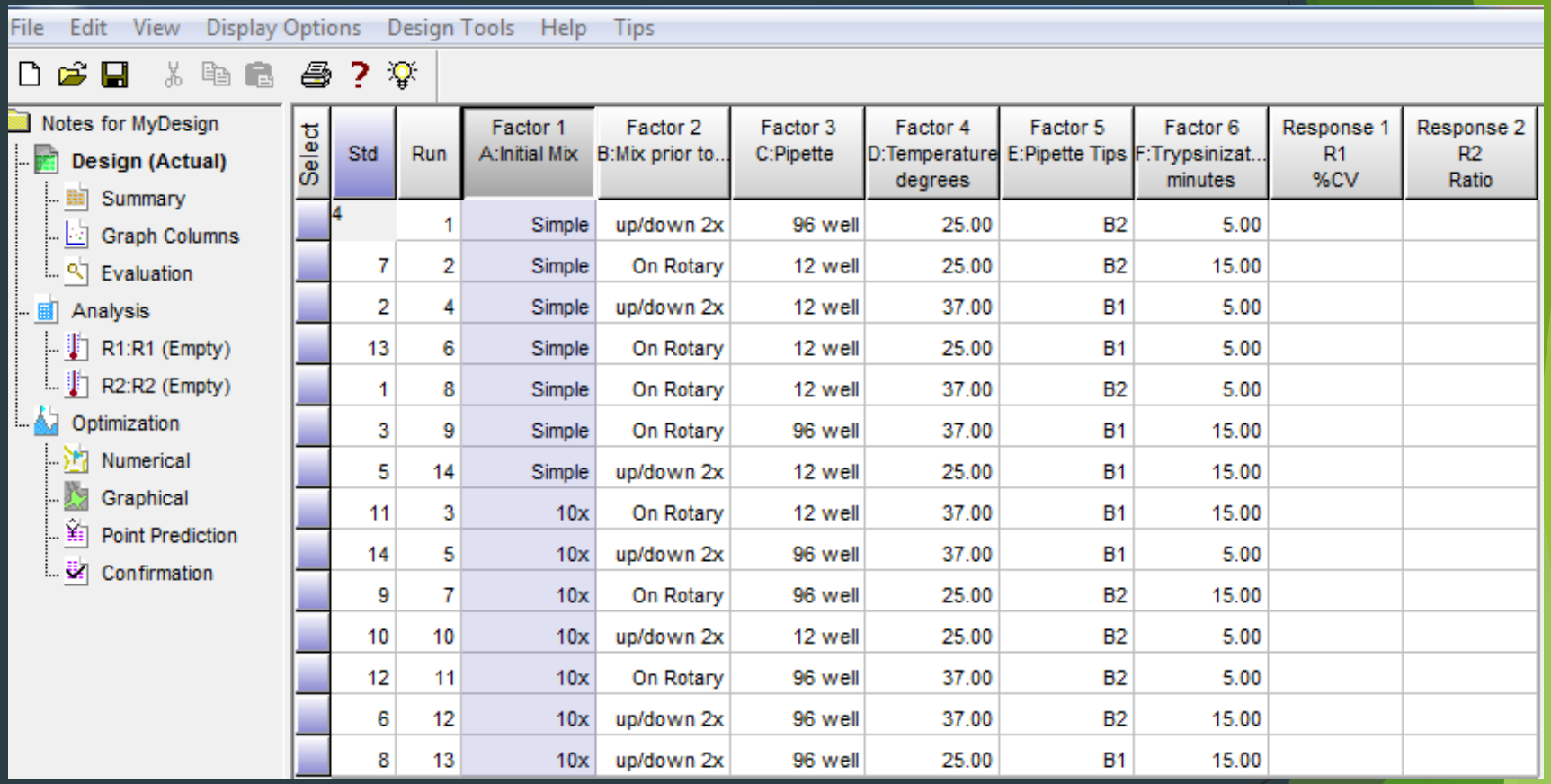
Original Run Lay Out Selected by Software



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		
	7	2	Simple	On Rotary	12 well	25.00	B2	15.00		
	11	3	10x	On Rotary	12 well	37.00	B1	15.00		
	2	4	Simple	up/down 2x	12 well	37.00	B1	5.00		
	14	5	10x	up/down 2x	96 well	37.00	B1	5.00		
	13	6	Simple	On Rotary	12 well	25.00	B1	5.00		
	9	7	10x	On Rotary	96 well	25.00	B2	15.00		
	1	8	Simple	On Rotary	12 well	37.00	B2	5.00		
	3	9	Simple	On Rotary	96 well	37.00	B1	15.00		
	10	10	10x	up/down 2x	12 well	25.00	B2	5.00		
	12	11	10x	On Rotary	96 well	37.00	B2	5.00		
	6	12	10x	up/down 2x	96 well	37.00	B2	15.00		
	8	13	10x	up/down 2x	96 well	25.00	B1	15.00		
	5	14	Simple	up/down 2x	12 well	25.00	B1	15.00		

Problem is that having mixing procedures intertwined is procedurally difficult.

Sorted by Factor 1



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	1	8	Simple	On Rotary	12 well	37.00	B2	5.00		
	3	9	Simple	On Rotary	96 well	37.00	B1	15.00		
	5	14	Simple	up/down 2x	12 well	25.00	B1	15.00		
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	14	5	10x	up/down 2x	96 well	37.00	B1	5.00		
	9	7	10x	On Rotary	96 well	25.00	B2	15.00		
	10	10	10x	up/down 2x	12 well	25.00	B2	5.00		
	12	11	10x	On Rotary	96 well	37.00	B2	5.00		
	6	12	10x	up/down 2x	96 well	37.00	B2	15.00		
	8	13	10x	up/down 2x	96 well	25.00	B1	15.00		

Still had a practical problem: Next sorted by Factor Two

Final Run Lay Out

File Edit View Display Options Design Tools Help Tips

- Notes for MyDesign
- **Design (Actual)**
  - Summary
  - Graph Columns
  - Evaluation
- Analysis
  - R1:R1 (Empty)
  - R2:R2 (Empty)
- Optimization
  - Numerical
  - Graphical
  - Point Prediction
  - Confirmation

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	5	14	Simple	up/down 2x	12 well	25.00	B1	15.00		
	14	5	10x	up/down 2x	96 well	37.00	B1	5.00		
	10	10	10x	up/down 2x	12 well	25.00	B2	5.00		
	6	12	10x	up/down 2x	96 well	37.00	B2	15.00		
	8	13	10x	up/down 2x	96 well	25.00	B1	15.00		
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	1	8	Simple	On Rotary	12 well	37.00	B2	5.00		
	3	9	Simple	On Rotary	96 well	37.00	B1	15.00		
	11	3	10x	On Rotary	12 well	37.00	B1	15.00		
	9	7	10x	On Rotary	96 well	25.00	B2	15.00		
	12	11	10x	On Rotary	96 well	37.00	B2	5.00		

Insert Data for Analysis

File Edit View Display Options Design Tools Help Tips

- Notes for Cells run 2
- Design (Actual)
  - Summary
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  - R2:R2
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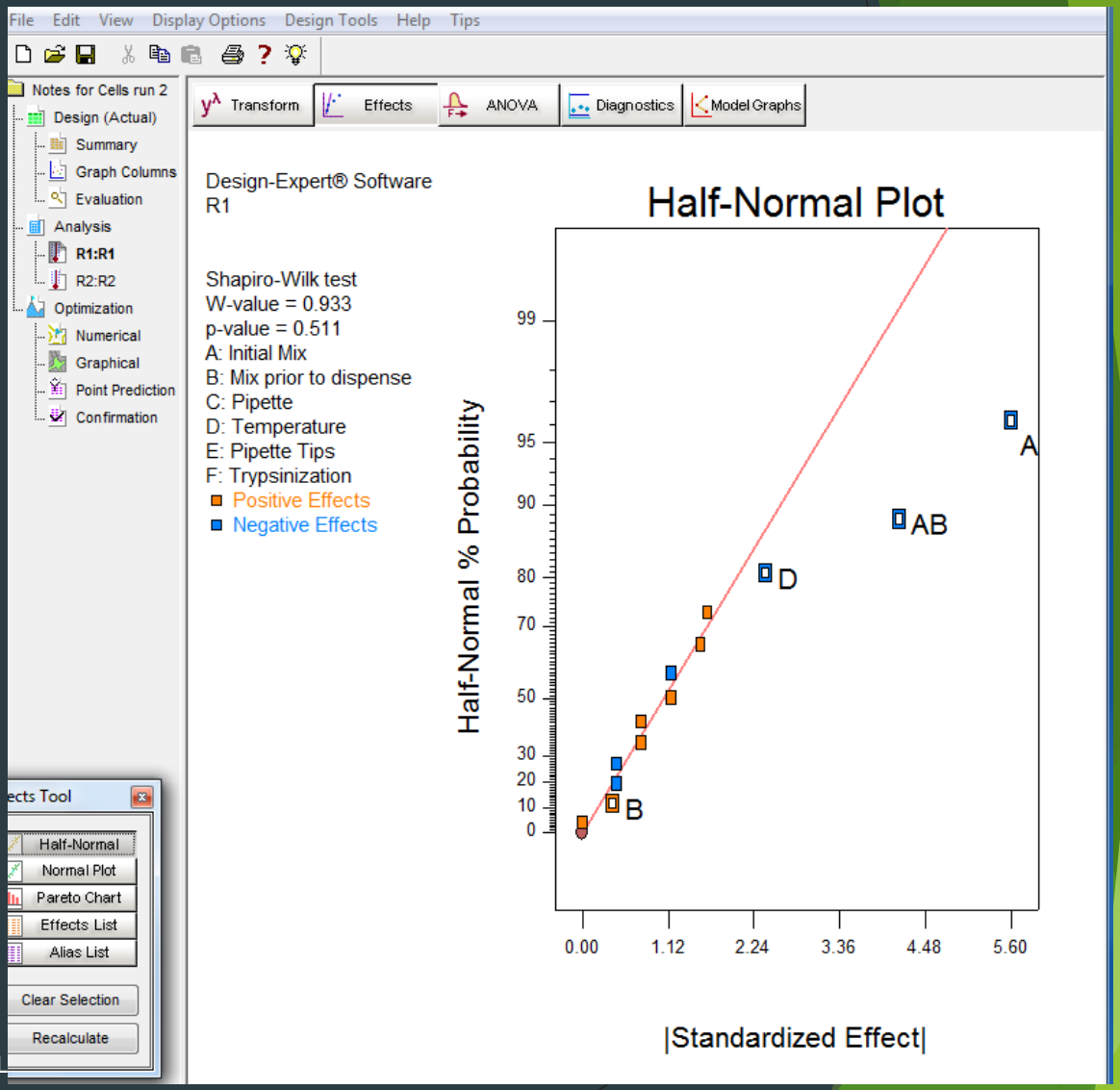
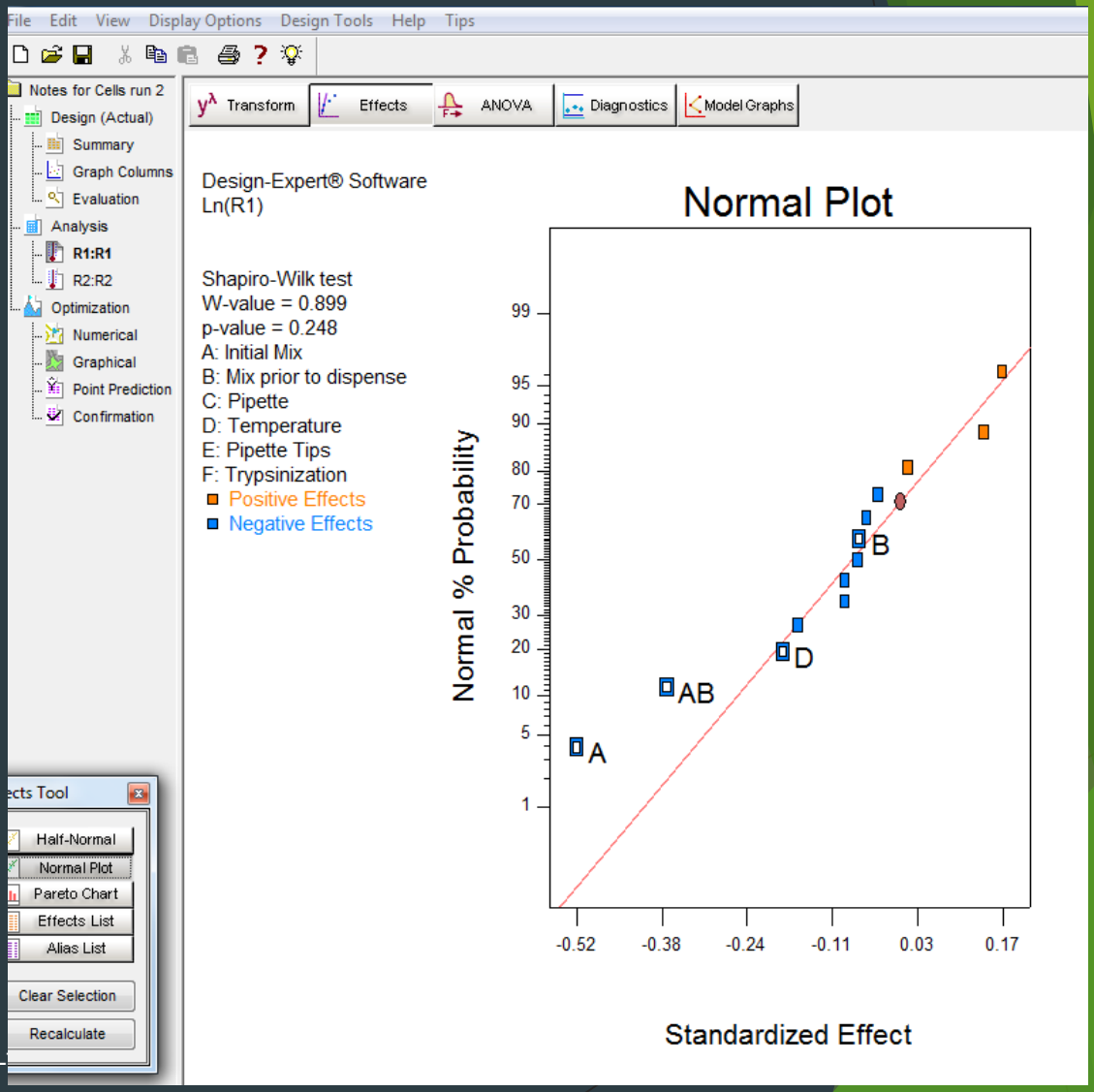
	4	1	Simple	up/down 2x	96 well	25.00	B2	5.00	12	1.5
	7	2	Simple	On Rotary	12 well	25.00	B2	15.00	18	1.6
	11	3	10x	On Rotary	12 well	37.00	B1	15.00	8	1.1
	2	4	Simple	up/down 2x	12 well	37.00	B1	5.00	11	1.4
	14	5	10x	up/down 2x	96 well	37.00	B1	5.00	7	1.2
	13	6	Simple	On Rotary	12 well	25.00	B1	5.00	19	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

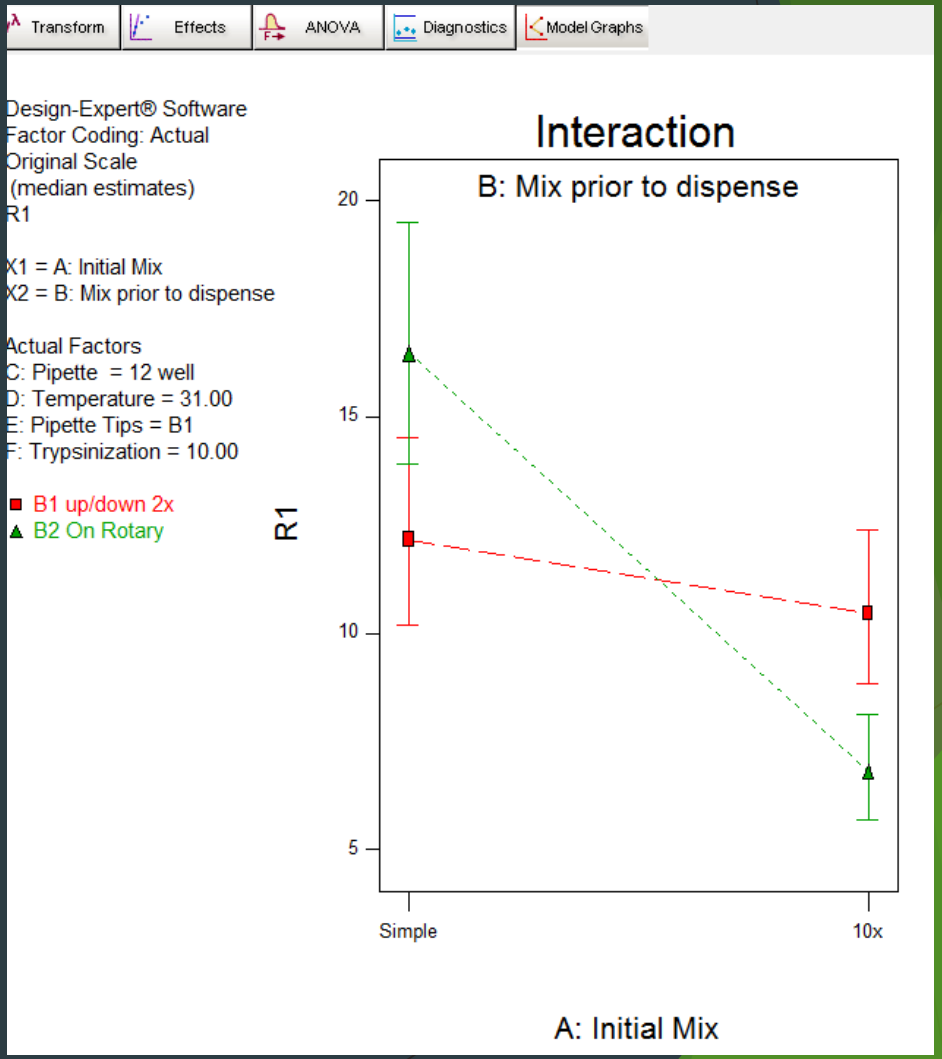
R1 – Diagnostic Plot



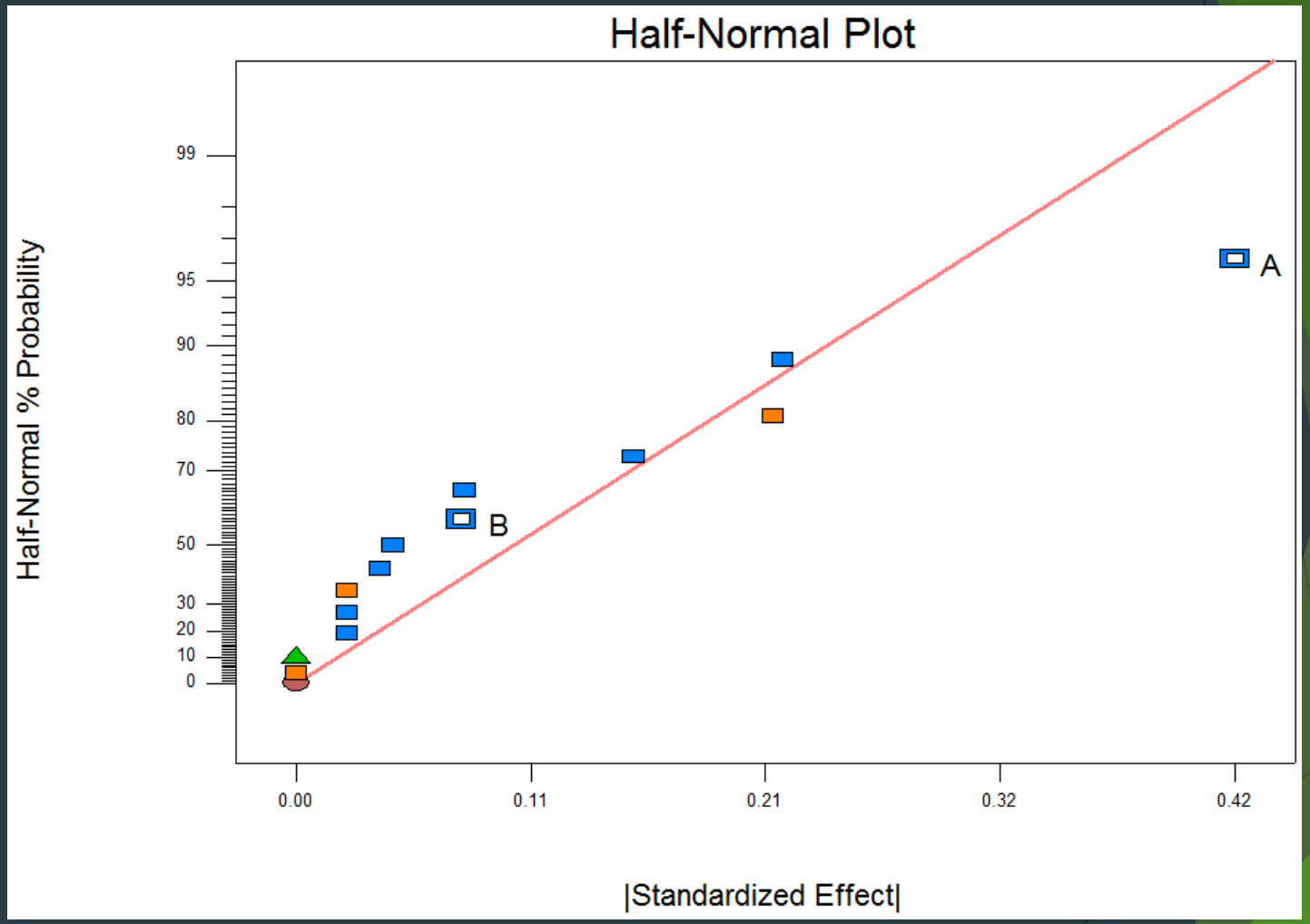
R1 – Diagnostic Plot



This Demonstrates the Importance of the Interaction



Based on R2 (Ratio of 1st to Last Row)



Now Went Back to the First Design

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		

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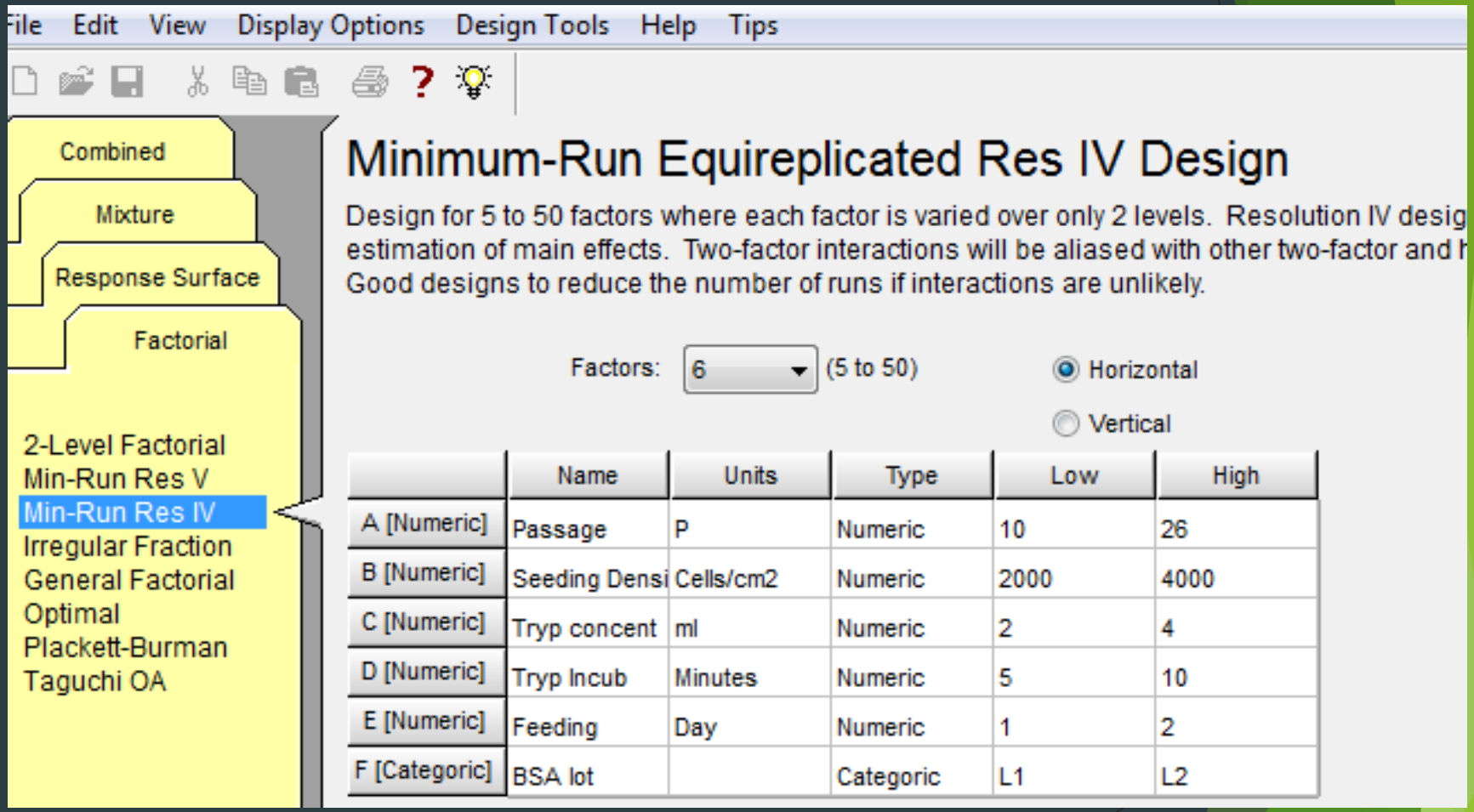
Results

- Found pre-mixing (mixing prior to dispensing) important.
- Repeated the first screening and found passage number and trypsinzation conditions were also important
- Did a 2^3 full factorial and limited the passage number to less than 20 (based on other available data – FACs studies to look at stability of the receptor expression).

Design Selected



	Name	Units	Type	Low	High
A [Numeric]	Passage	P	Numeric	10	26
B [Numeric]	Seeding Densi	Cells/cm2	Numeric	2000	4000
C [Numeric]	Tryp concent	ml	Numeric	2	4
D [Numeric]	Tryp Incub	Minutes	Numeric	5	10
E [Numeric]	Feeding	Day	Numeric	1	2
F [Categoric]	BSA lot		Categoric	L1	L2

Sorted by Passage Number

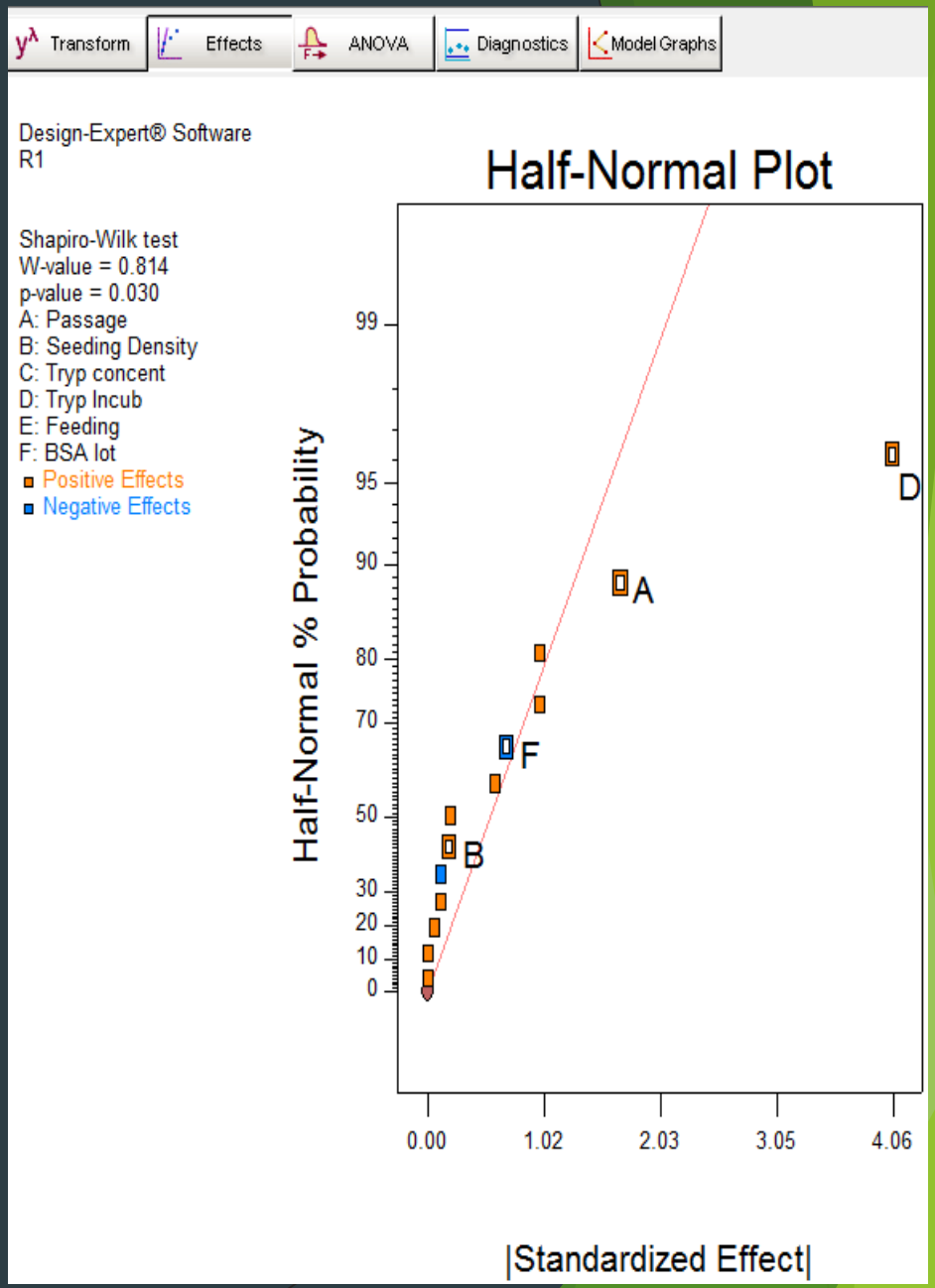
File Edit View Display Options Design Tools Help Tips

- Notes for MyDesign
- **Design (Actual)**
  - Summary
  - Graph Columns
  - Evaluation
- Analysis
  - R1:R1 (Empty)
- Optimization
  - Numerical
  - Graphical
  - Point Prediction
  - Confirmation

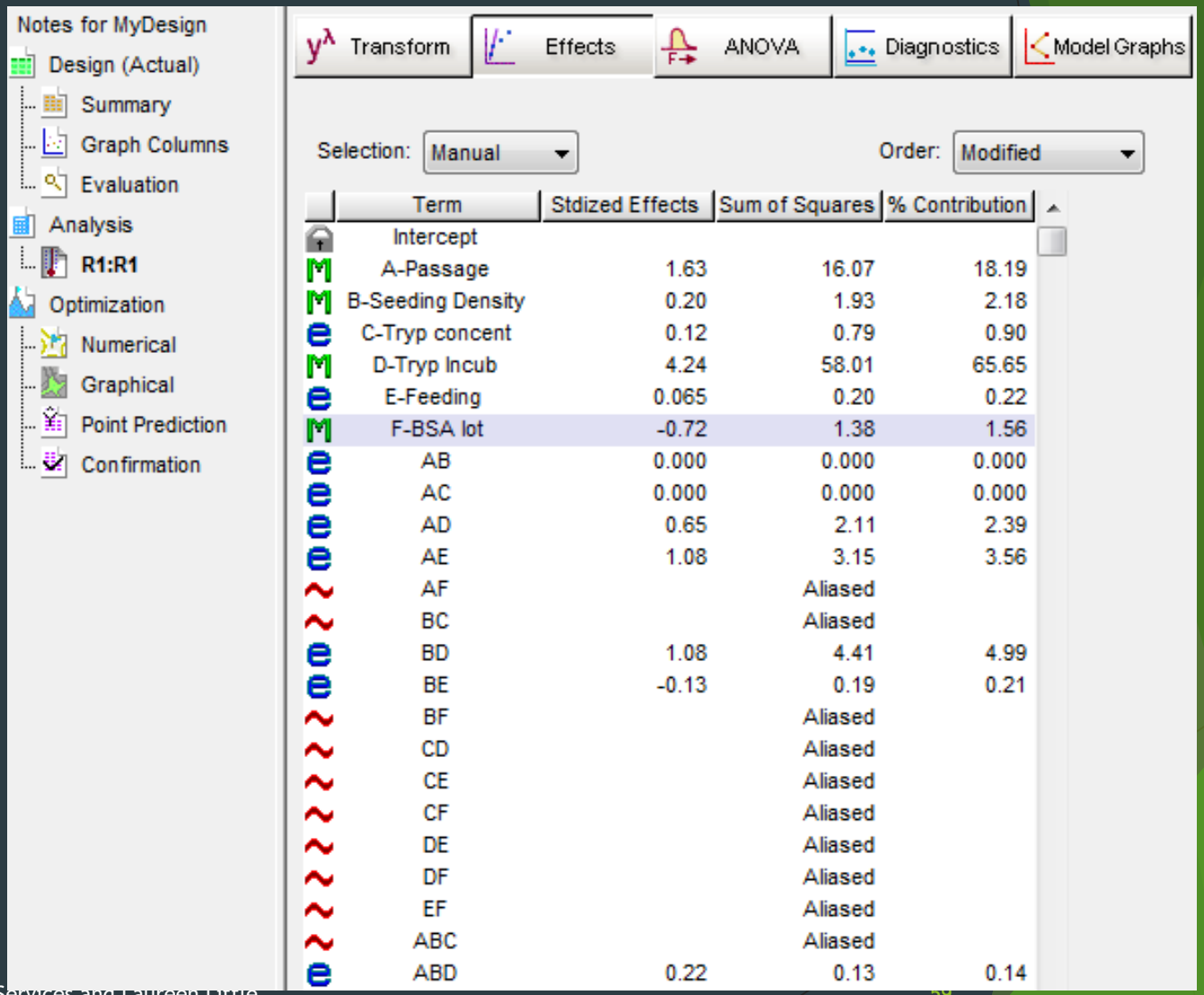
Select	Std	Run	Factor 1 A:Passage P	Factor 2 B:Seeding D... Cells/cm2	Factor 3 C:Tryp conc... ml	Factor 4 D:Tryp Incub Minutes	Factor 5 E:Feeding Day	Factor 6 F:BSA lot	Response 1 R1 %CV
	13	2	10.00	4000.00	2.00	5.00	1.00	L1	6
	4	3	10.00	2000.00	4.00	5.00	2.00	L1	6
	3	8	10.00	4000.00	4.00	10.00	1.00	L2	10
	2	9	10.00	2000.00	2.00	10.00	1.00	L1	9
	7	11	10.00	4000.00	2.00	5.00	2.00	L2	4
	1	12	10.00	4000.00	2.00	10.00	2.00	L1	9
	5	14	10.00	2000.00	2.00	5.00	1.00	L2	6
	11	1	26.00	4000.00	2.00	10.00	1.00	L2	11
	12	4	26.00	4000.00	4.00	10.00	2.00	L1	13
	6	5	26.00	2000.00	4.00	10.00	2.00	L2	11
	10	6	26.00	2000.00	2.00	5.00	2.00	L1	8
	8	7	26.00	2000.00	4.00	5.00	1.00	L2	6
	14	10	26.00	2000.00	4.00	10.00	1.00	L1	10
	9	13	26.00	4000.00	4.00	5.00	2.00	L2	6

Diagnostic Plot

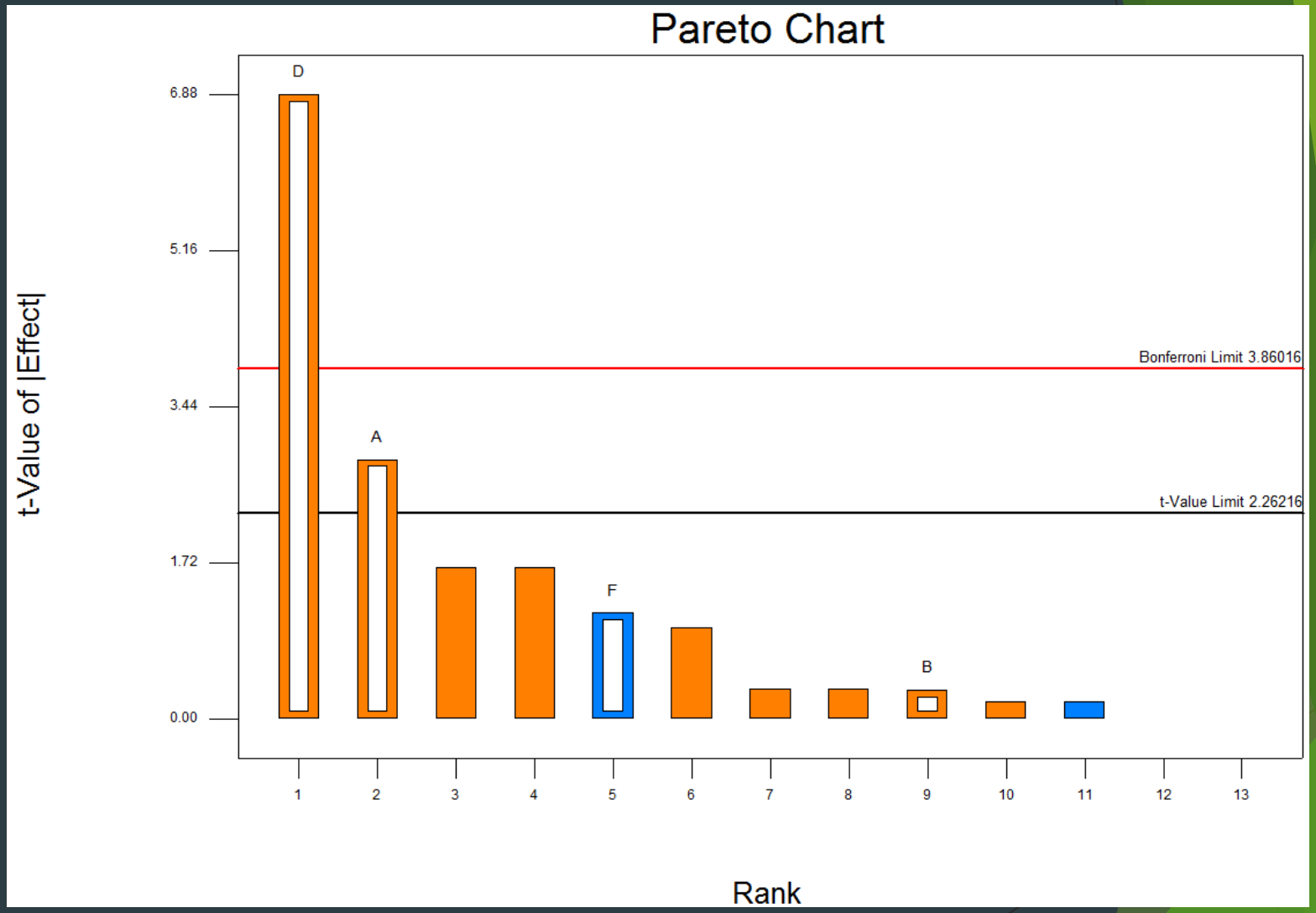
Indicates that the Passage Number and the Length of the Trypsin Incubation have an Effect



Choose Model vs. Error Terms



Term	Stdized Effects	Sum of Squares	% Contribution
Intercept			
A-Passage	1.63	16.07	18.19
B-Seeding Density	0.20	1.93	2.18
C-Tryp concent	0.12	0.79	0.90
D-Tryp Incub	4.24	58.01	65.65
E-Feeding	0.065	0.20	0.22
F-BSA lot	-0.72	1.38	1.56
AB	0.000	0.000	0.000
AC	0.000	0.000	0.000
AD	0.65	2.11	2.39
AE	1.08	3.15	3.56
AF		Aliased	
BC		Aliased	
BD	1.08	4.41	4.99
BE	-0.13	0.19	0.21
BF		Aliased	
CD		Aliased	
CE		Aliased	
CF		Aliased	
DE		Aliased	
DF		Aliased	
EF		Aliased	
ABC		Aliased	
ABD	0.22	0.13	0.14



Take Home Messages

- This example was to indicate that the DOE can be used during optimization – not just final characterization or verification of assay performance.
- Sequential DOE studies is an excellent approach.
- Don’t panic if the first design doesn’t yield “results” – perhaps you didn’t select the appropriate factors to study.
- Although a statistician is a real asset with modern software – you can still use DOE and have it really accelerate your development time.

What to Do?

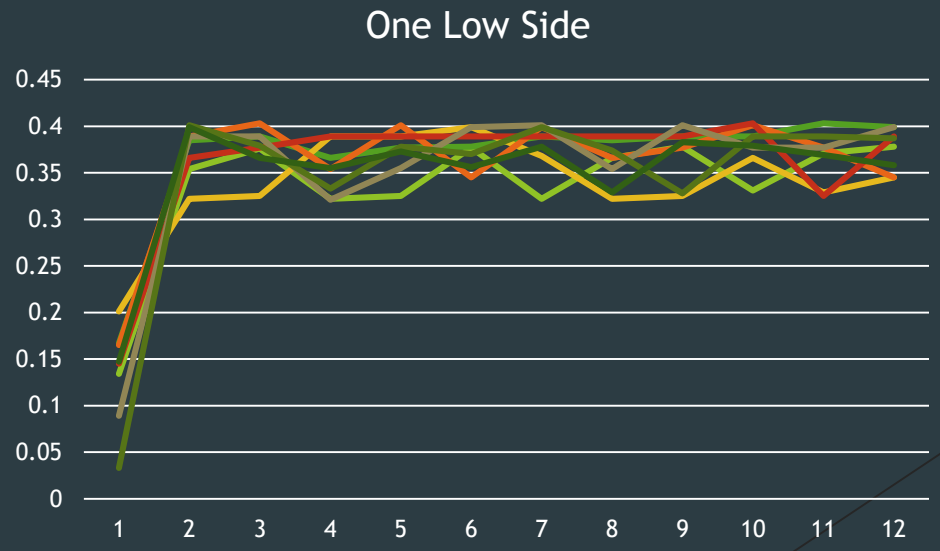
- This specific result should not be taken as a universal decision.
  - Specifically, not every cell line should be pre-mixed as described here.
  - Not every cell line will be sensitive to Tryspinization or have the same passage number restrictions.
- I would suggest instead, that you might be able to come up with a universal ***screening design*** – of factors into which specific levels for a given cell line could be inserted.
- Then, require that as part of development, each new proposed cell-line would be tested in this universal design.

But…..DOE isn't the Panacea for all Component Assay Woes

- It is a great tool to quickly differentiate critical parameters from those which have little impact.
- It doesn't eliminate the need for focused scientific problem solving.
- Always start with potential scientific root causes to performance problems

But…..What about the Following?

0.134	0.354	0.376	0.322	0.325	0.377	0.322	0.366	0.378	0.331	0.371	0.378
0.167	0.385	0.389	0.366	0.377	0.378	0.389	0.385	0.389	0.389	0.403	0.399
0.201	0.322	0.325	0.389	0.389	0.399	0.368	0.322	0.325	0.366	0.329	0.345
0.165	0.389	0.403	0.354	0.401	0.345	0.399	0.366	0.377	0.401	0.377	0.345
0.145	0.366	0.377	0.389	0.389	0.389	0.389	0.389	0.389	0.403	0.325	0.389
0.089	0.389	0.389	0.321	0.355	0.399	0.401	0.354	0.401	0.377	0.377	0.399
0.033	0.401	0.379	0.333	0.378	0.37	0.399	0.374	0.328	0.389	0.389	0.387
0.147	0.399	0.366	0.355	0.373	0.356	0.378	0.329	0.383	0.379	0.369	0.358

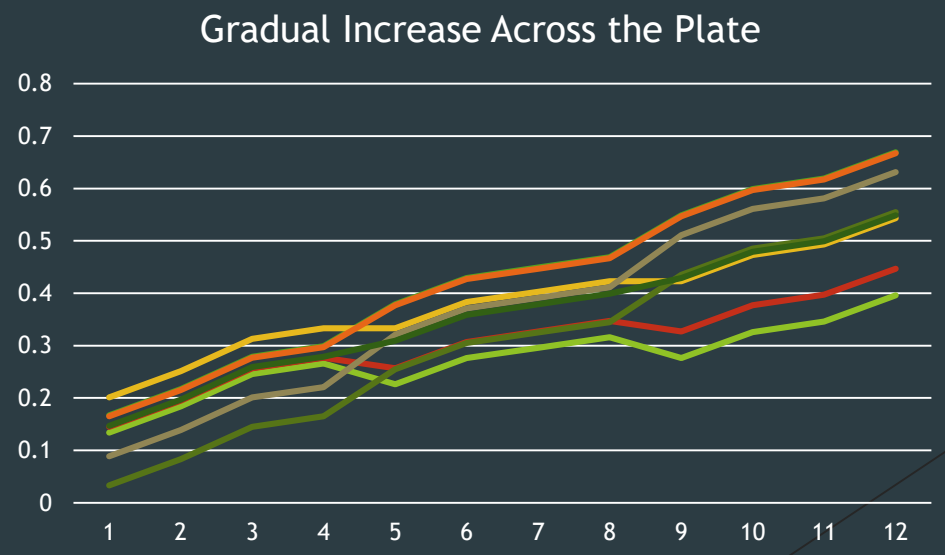


Potential Causes

- Poorly calibrated pipette
- Insufficient Number of Cells
- Too concentrated of some biological component which is killing cells or inhibiting growth
- Reader Problem (off-set detector)

Yet another case…..

0.134	0.184	0.246	0.266	0.226	0.276	0.296	0.316	0.276	0.326	0.346	0.396
0.167	0.217	0.279	0.299	0.379	0.429	0.449	0.469	0.549	0.599	0.619	0.669
0.201	0.251	0.313	0.333	0.333	0.383	0.403	0.423	0.423	0.473	0.493	0.543
0.165	0.215	0.277	0.297	0.377	0.427	0.447	0.467	0.547	0.597	0.617	0.667
0.145	0.195	0.257	0.277	0.257	0.307	0.327	0.347	0.327	0.377	0.397	0.447
0.089	0.139	0.201	0.221	0.321	0.371	0.391	0.411	0.511	0.561	0.581	0.631
0.033	0.083	0.145	0.165	0.255	0.305	0.325	0.345	0.435	0.485	0.505	0.555
0.147	0.197	0.259	0.279	0.309	0.359	0.379	0.399	0.429	0.479	0.499	0.549



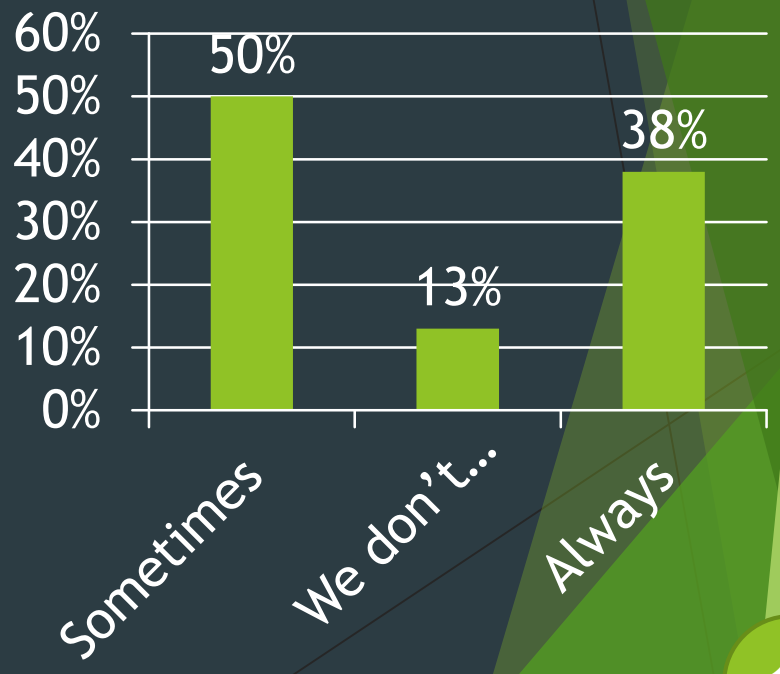
Possible Causes

- Settling of cells during initial pipetting
- Fragile cells breaking during mixing during plating
- Increased or Decreased concentration of a critical component because of dilution scheme
- Time differences due to manipulation of the cells

Your Components

We have talked a lot about cells. Do you optimize well-to-well characteristics of your plates?

1. Sometimes
2. We don't usually need to because we get our cells from a potency group which has optimized our cells
3. Always

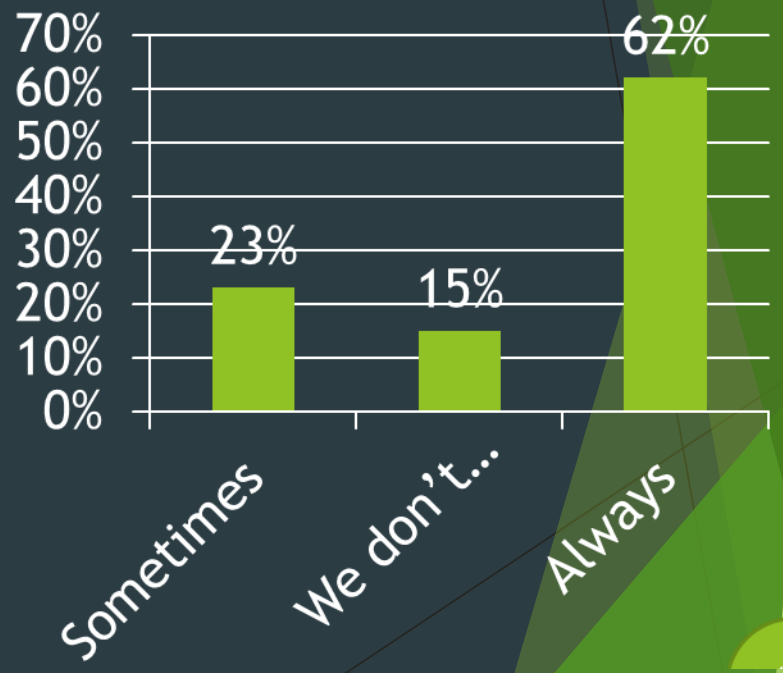


Your Components

EU Response

We have talked a lot about cells. Do you optimize well-to-well characteristics of your plates?

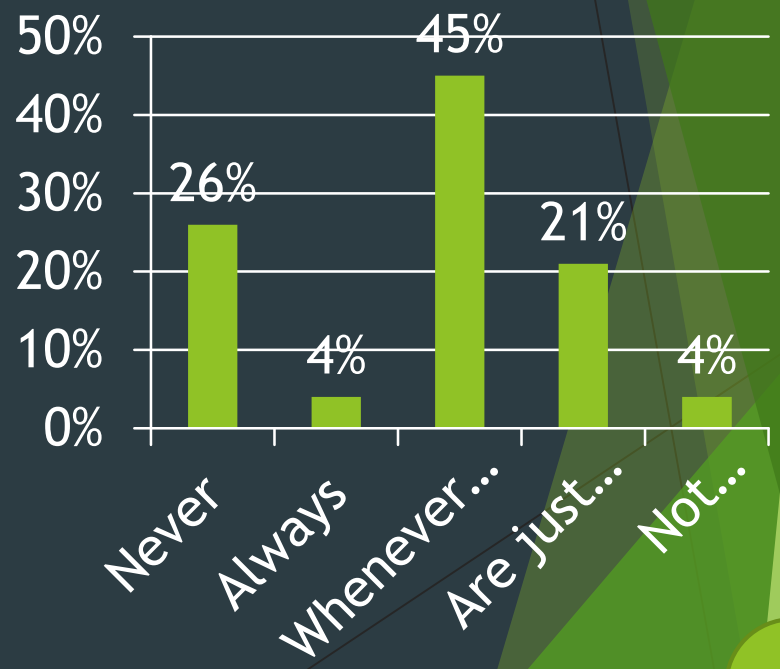
1. Sometimes
2. We don't usually need to because we get our cells from a potency group which has optimized our cells
3. Always



Ready-to-Use

Do you use Ready to Use cells?

1. Never
2. Always
3. Whenever possible
4. Are just implementing
5. Not applicable to our products



Ready-to-Use

EU Response

Do you use Ready to Use cells?

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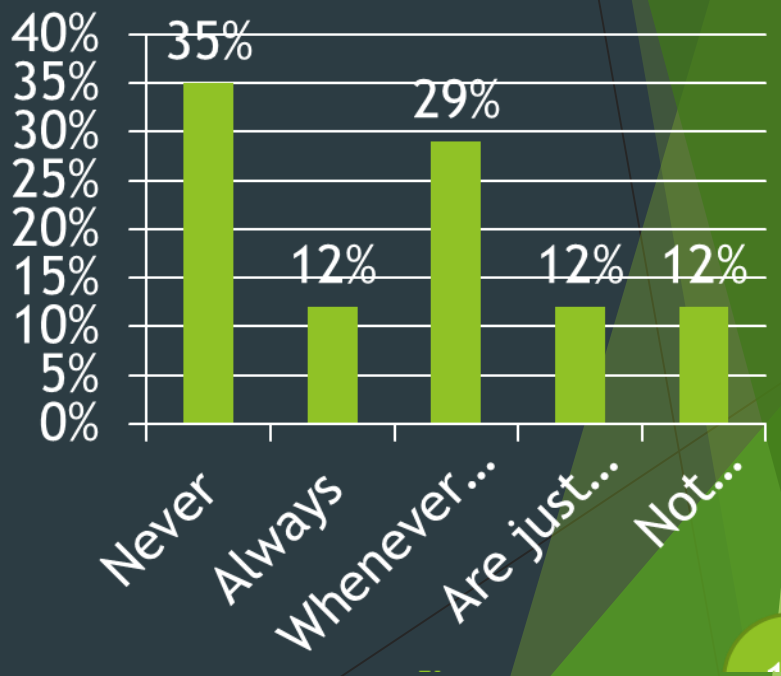
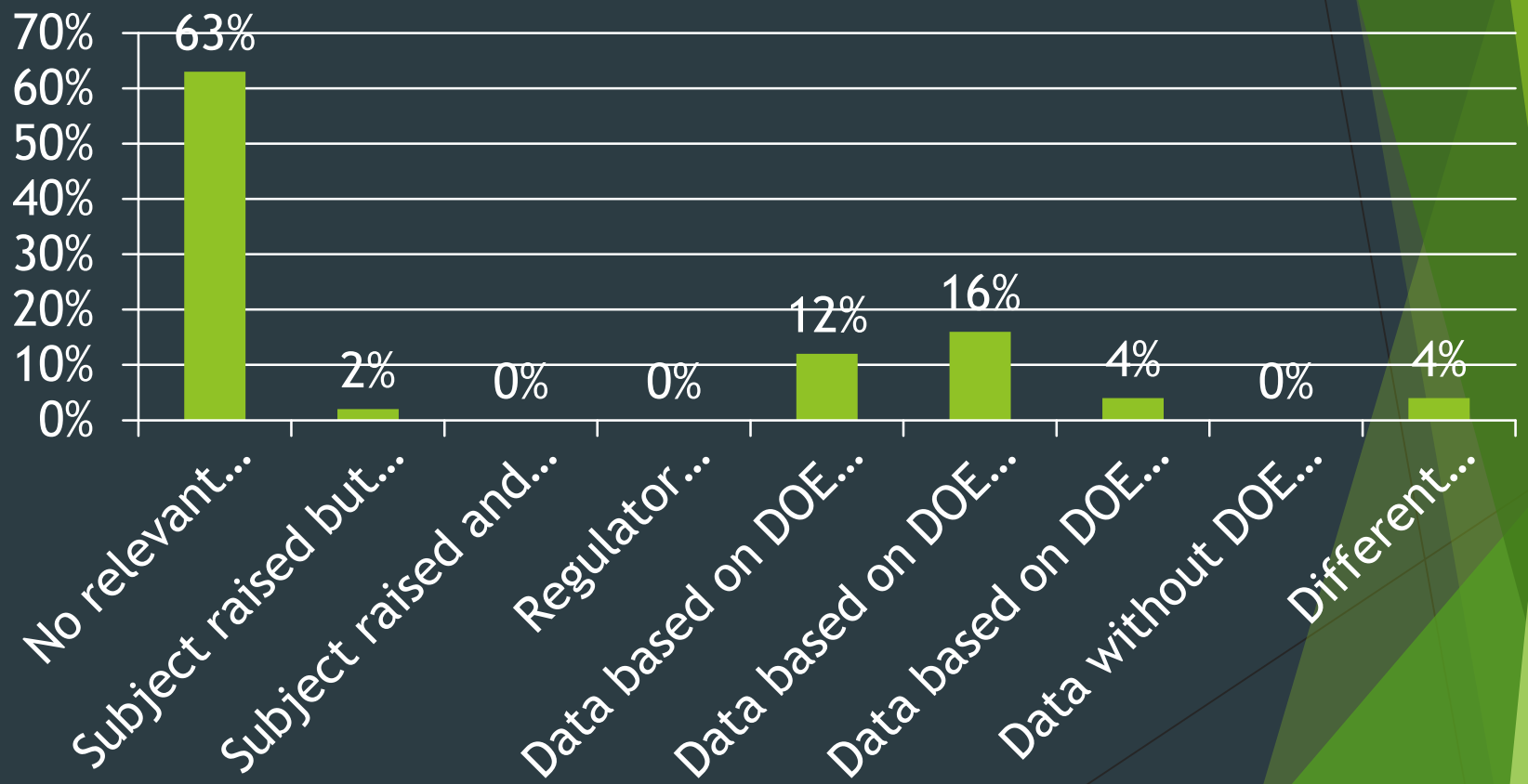


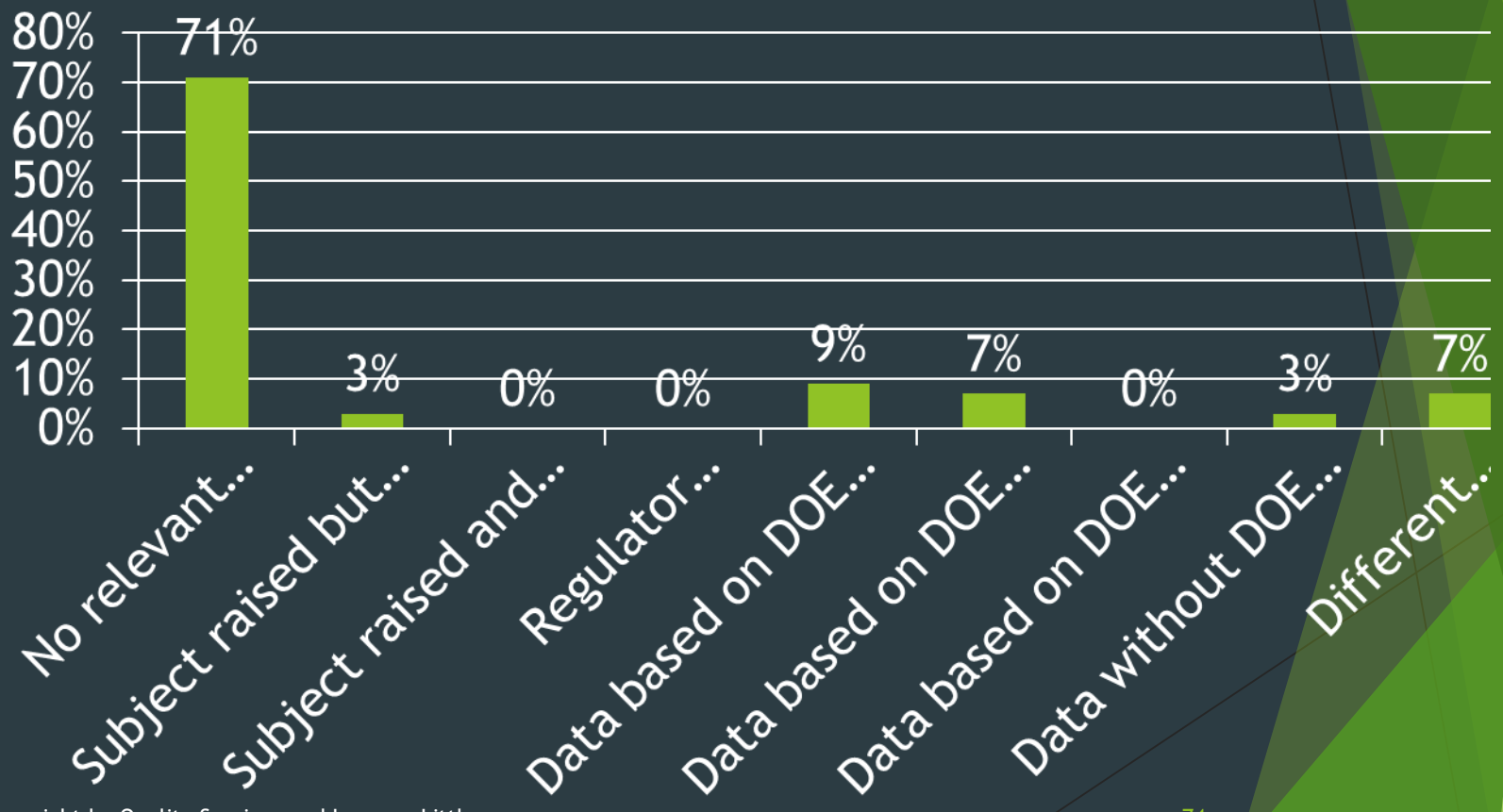
Have you had any discussion on DOE with regulators?

1. No relevant discussion with regulator
2. Subject raised but no comment from regulator
3. Subject raised and regulator suggested use
4. Regulator spontaneously suggested use
5. Data based on DOE submitted – no comment
6. Data based on DOE submitted – favourable response
7. Data based on DOE submitted – modifications suggested
8. Data without DOE submitted – regulator required use
9. Different responses in different cases

Discussion with Regulators



Discussion with Regulators (EU Response)



Appendix: DOE Basics

These are for your convenience and were not covered in the talk.

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**

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)

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Example # 1

- Few Conditions: Therefore = factorial design → look at three factors → at two levels:
- 2^3 → This is three factors
- This tells you there are two levels.

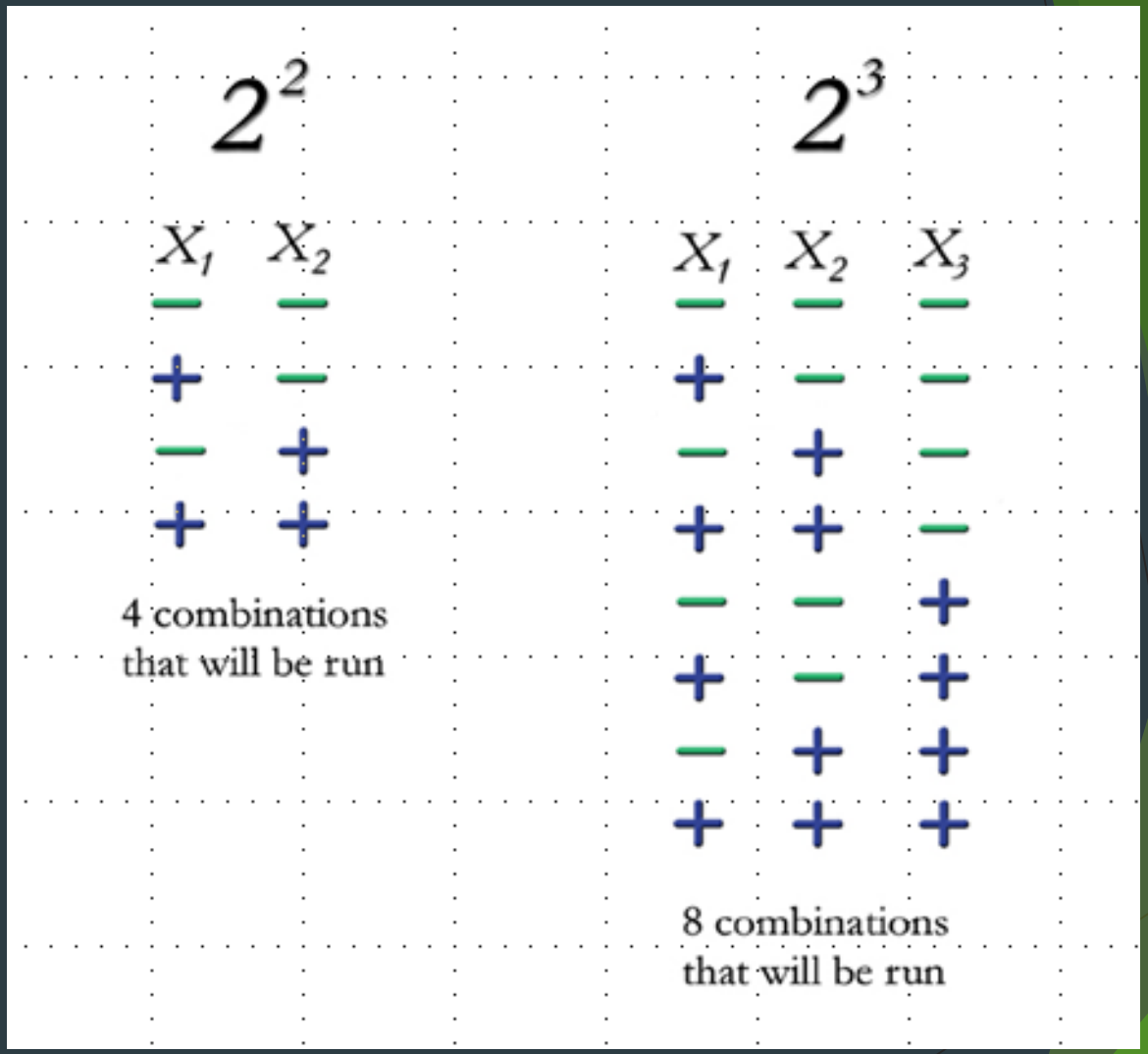
An example would be :

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

What is Factorial Design?

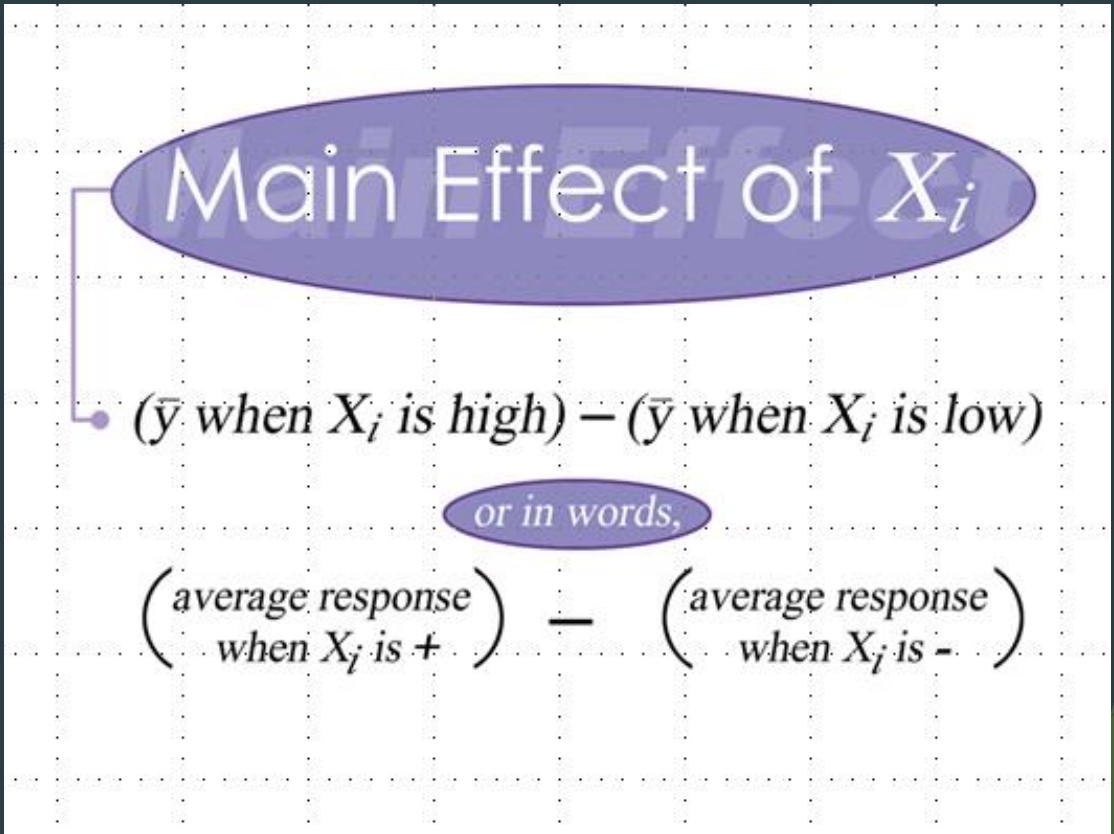
- Set of experiments so that more than one variable can be tested at the same time.
- This is done by running all the possible combinations-of each factor at each level.
- Therefore 2^2= 2*2 experiments: 4 experiments
- And 2^3= 2*2*2 experiments: 8

Note that all of these designs are balanced. This is a key aspect of DOE



This type of design allows us to determine the effect of changing each variable (aka: the main effect):

NB: Y is the measured response



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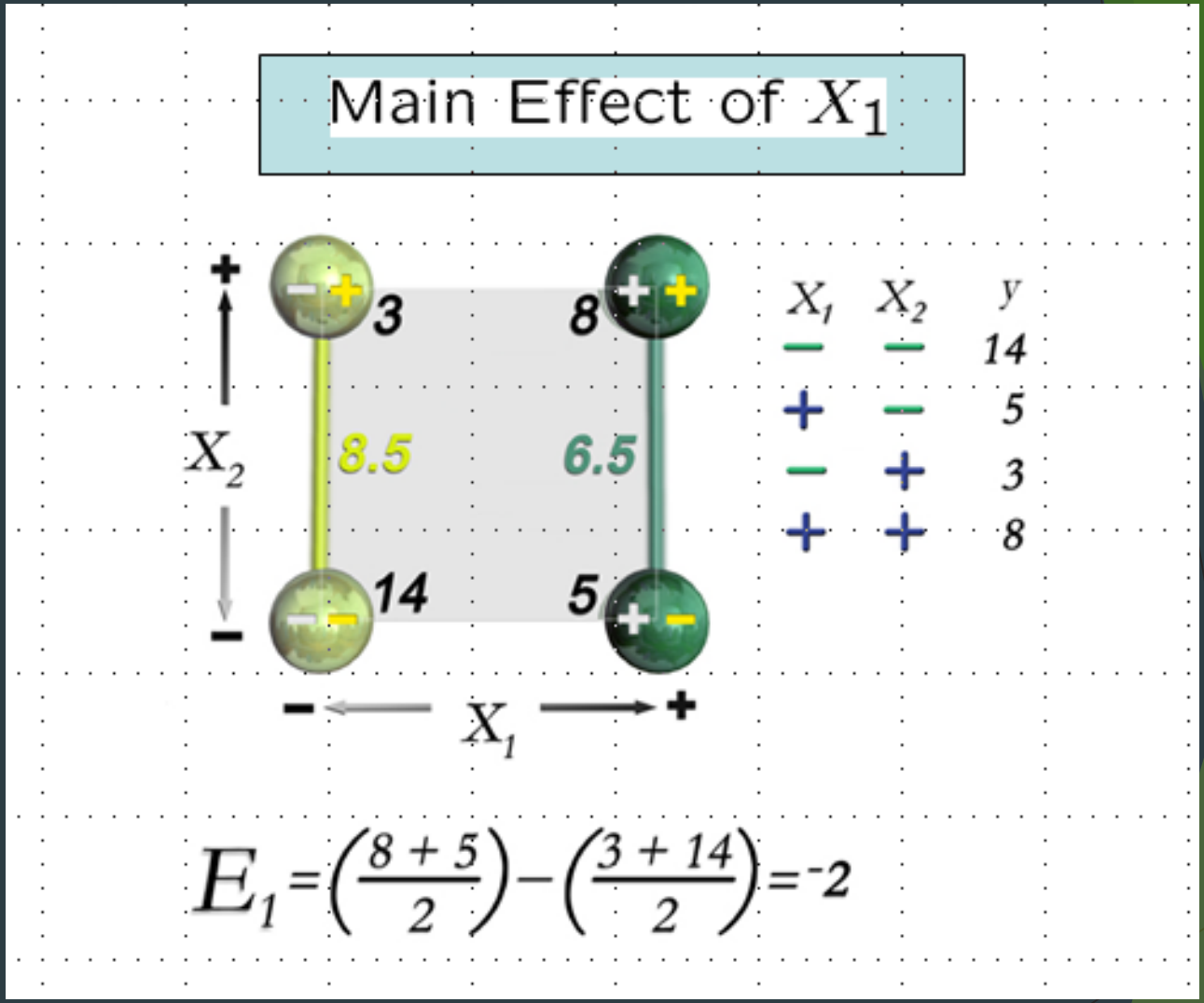
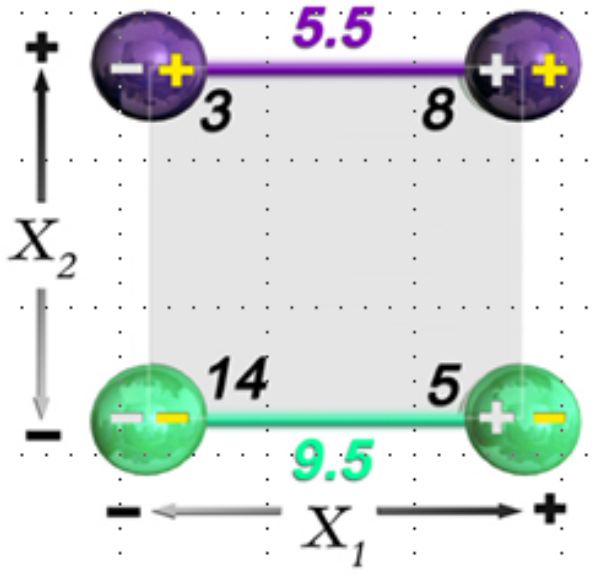
NB: E is a measure of the effect



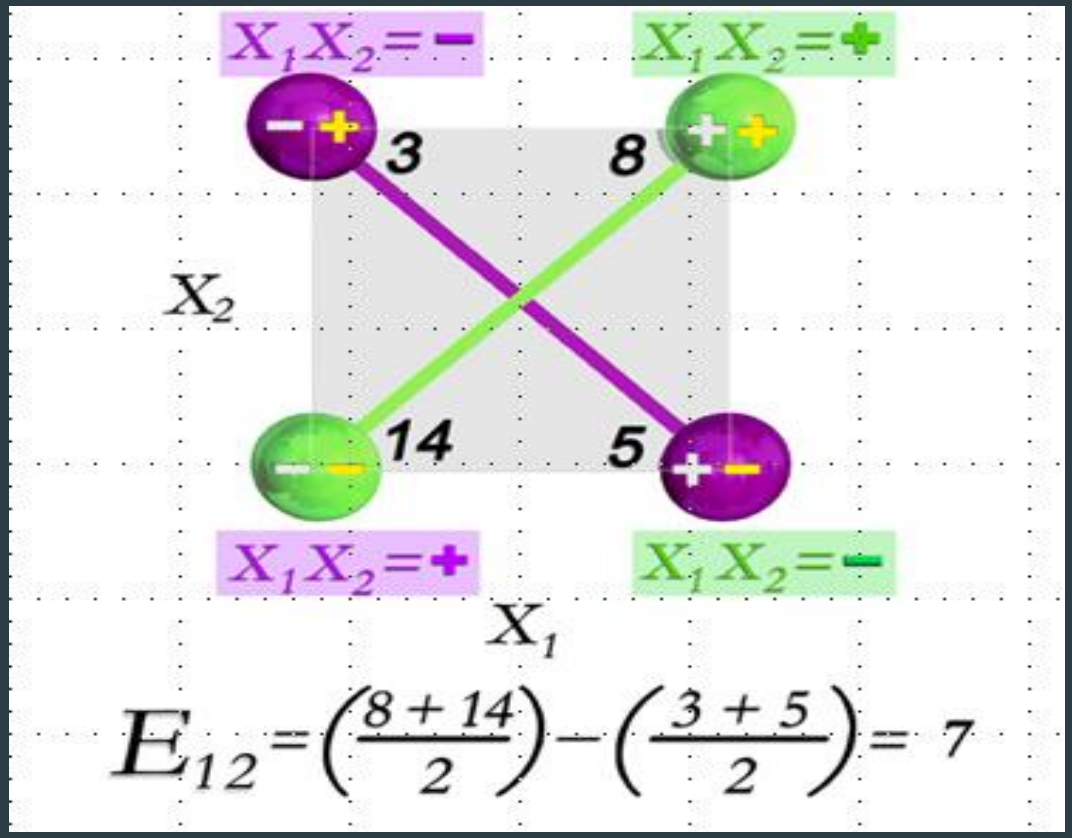
Main Effect of X_2



X_1	X_2	y
−	−	14
+	−	5
−	+	3
+	+	8

$$E_2 = \left(\frac{3+8}{2}\right) - \left(\frac{14+5}{2}\right) = -4$$

The X_1X_2 Interaction



What is the Problem?

- Early in development – we have many variables.
- A full-factorial design for many variables soon becomes too big.
- An example: Cell growth:

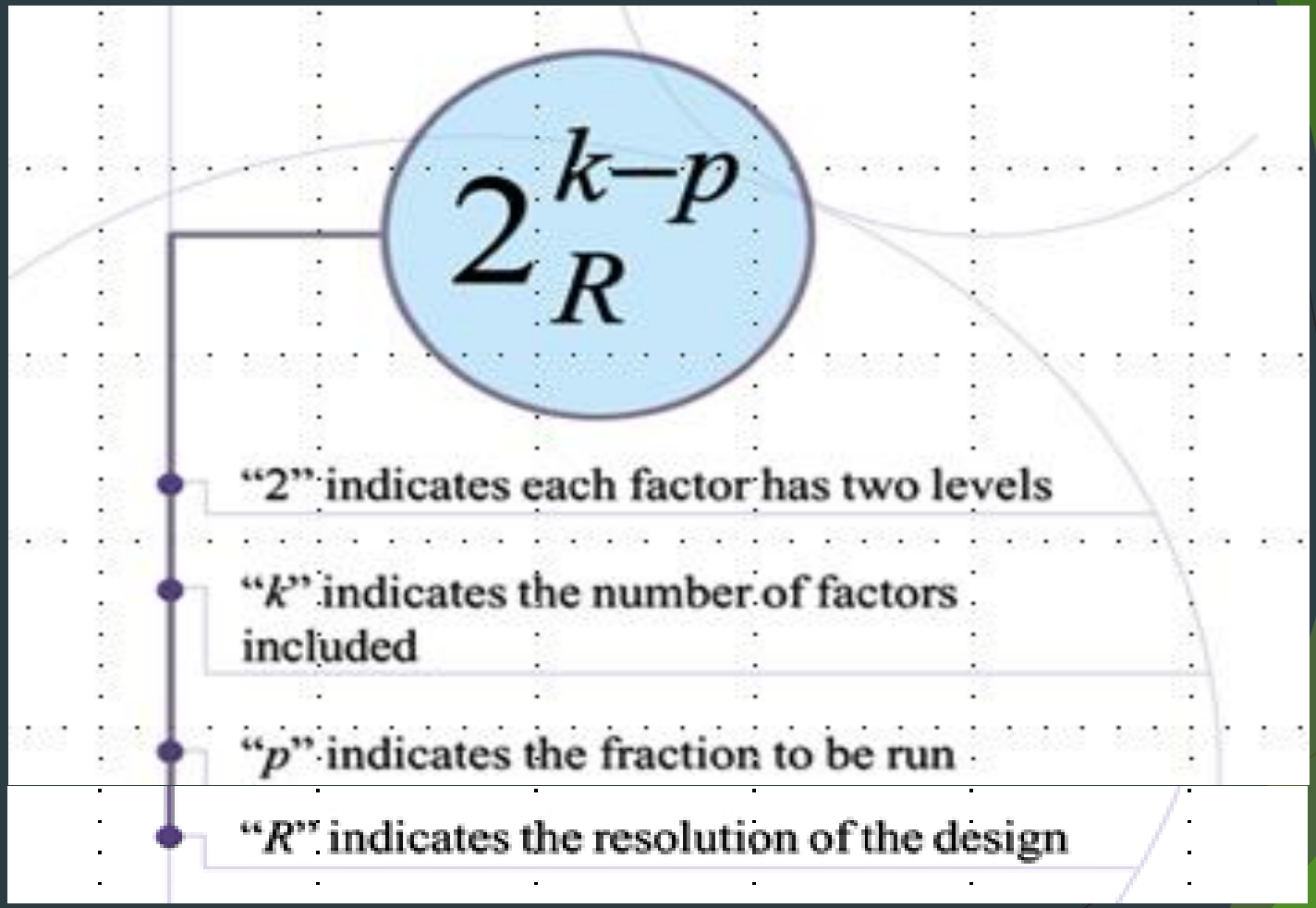
Media Type	Location in incubator
%FBS	Initial thawing temperature
Seeding Density	dispersion technique
Feeding Schedule	Maximum # of passages
Method of removing cells	Culture Time

Problem (continued)

- 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 Design



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Laureen's slide Your Designs(#11)

What kind of designs do you use?

1. Full Factorial only
2. Specific screening design only (such as a placket-burman)
3. Fractional Factorial
4. A mixture of the above
5. Too early in our use of DOE to be able to answer this question

Future use of DOE in your lab

1. No plans to start
2. Plan to start
3. Plan to discontinue
4. Continue with limited use
5. Plan to expand use

Laureen's slide Your Designs(#9)

How do you design your DOEs?

1. We have a statistician (either employed or consultant)
2. Use a software and design my own.
3. Design my own without software

Laureen's slide Your Designs(#11)

What kind of designs do you use?

1. Full Factorial only
2. Specific screening design only (such as a placket-burman)
3. Fractional Factorial
4. A mixture of the above
5. Too early in our use of DOE to be able to answer this question