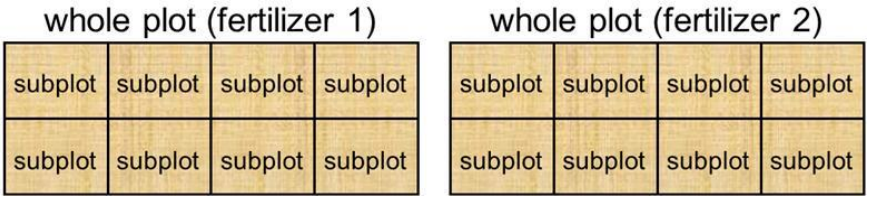
Split-Plot Tutorial for Design-Expert® Software

Introduction

Very often, experimenters set up two-level factorial designs with the best intentions of running them in random order, but they find that a given factor, such as temperature, cannot be easily changed. In this case, the analysis should be done by the split-plot method.

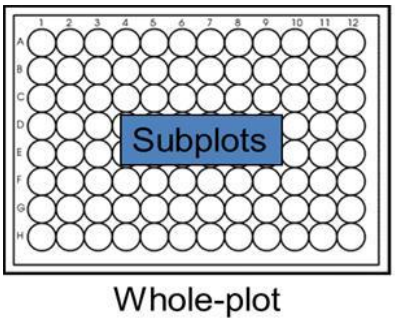
Split-plot designs originated in the field of agriculture where experimenters applied one treatment to a large area of land, called a "whole-plot," and other treatments to smaller areas of land within the whole-plot—called "subplots". For example, the whole-plot treatment might be fertilizer 1 vs. fertilizer 2, with the subplot treatment being seed type 1 through 8 (see picture below).



A field sectioned into two whole plots (fertilizer type) and eight subplots (seed type)

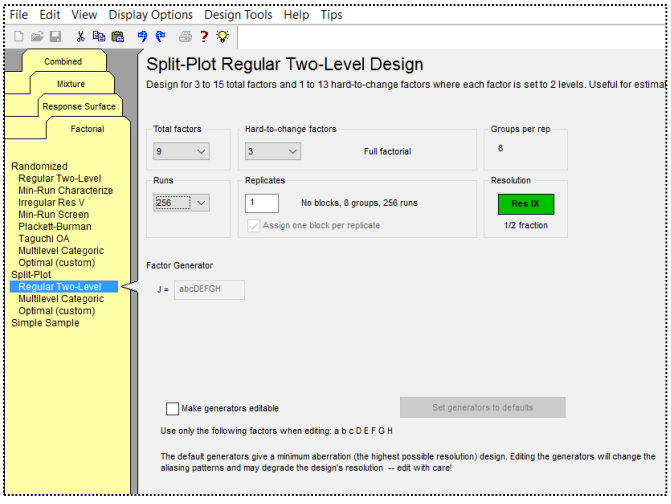
Case study: Polymerase Chain Reaction (PCR)

This example is based on a polymerase chain reaction—a biochemical technology that amplifies DNA for diagnosing hereditary diseases and other purposes. Due to equipment limitations, it is not convenient to fully randomize the treatments, so the biochemists chose a split-plot design. In this case the whole plots are actually plates that are subjected to varying conditions of time and temperature. The subplots fall into the wells within each plate, within which experimenters can randomly apply the remaining factors.



Building the Design

1) From the **Factorial** tab, **Split-Plot** section, choose a **Regular Two-Level Factorial** design. (To bypass the design build, open the file titled "PCR with data.dxpx". Rebuild via File, New Design, clicking Yes to "Use previous design info?", to work through the steps on your screen.)
   a) Change the total number of factors to **9**. This includes both the whole-plot and subplot factors.
   b) Change the Hard-to-change factors to **3**. These are the three thermocycler (whole-plot) factors.
   c) Choose the hard to change factor fraction. In this case there is no option other than accepting the default or **Full** factorial. However, for 5 or more factors the program provides options.



d) Change the number of runs to **256**. This specifies a 2^{9-1} factorial design for all 9 factors, which is resolution IX. Note that the box changes green for any design that is Res V or better, meaning you can fit main effects and two-factor interactions (2FI). Click **Continue** to see aliases and **Continue** again.

2) Note here that the hard-to-change factors are labeled a, b, and c (lowercase), while the easy-to-change factors are E, F, G, H, and J (uppercase). Enter the factor names and levels. (To avoid all the typing, go Back a few screens and Cancel—then open the file noted earlier and rebuild to this point: Then the factor information will be prefilled.)

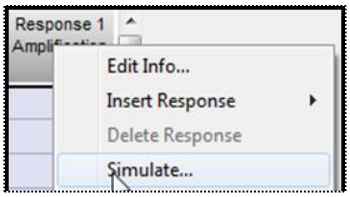
	Name	Units	Change	Type	Low	High
a [Numeric]	Anneal_Temp	deg C	Hard	Numeric	55	65
b [Numeric]	Denature_Temp	deg C	Hard	Numeric	90	97
c [Numeric]	Denature_time	sec	Hard	Numeric	10	20
D [Numeric]	FOR_primer	nM	Easy	Numeric	200	900
E [Numeric]	REV_primer	nM	Easy	Numeric	200	900
F [Numeric]	Probe	nM	Easy	Numeric	100	400
G [Numeric]	MG_C 12	nM	Easy	Numeric	3	9
H [Numeric]	Tween	%	Easy	Numeric	0.005	0.02
J [Numeric]	Taq_Gold	U/ul	Easy	Numeric	0.01	0.04

Click **Continue**.

3) Enter the response (Accept the defaults for delta and sigma). Click **Continue**. Note that the whole plot and subplots have different power. We are only running 8 runs (groups) for the whole-plot factors (a, b & c), so the power is lower than if it were a completely randomized design. Click **Finish**.

	Name	Units	Diff. to detect Delta("Signal")	Est. Std. Dev. Sigma("Noise")	Delta/Sigma (Signal/Noise Ratio)
	Amplification		2	1	2

4) On the **Response 1 – Amplification** column header **right-click** and choose **Simulate**.
5) Choose **Load an existing simulation** and click **Next**.
6) Find the file called **PCR.simx** and click **Open**.

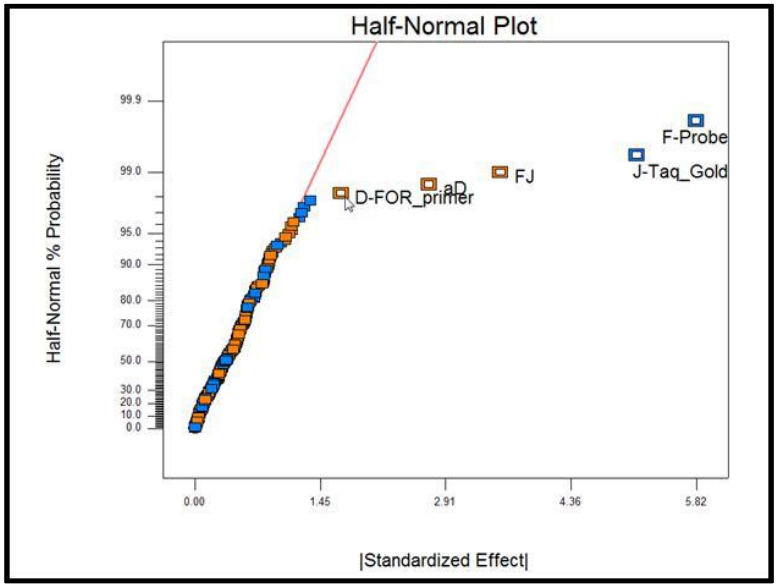


Now you have built the design, ran the experiment (actually, simulated it) and entered the data. Continue on for instruction for analyzing this split-plot DOE.

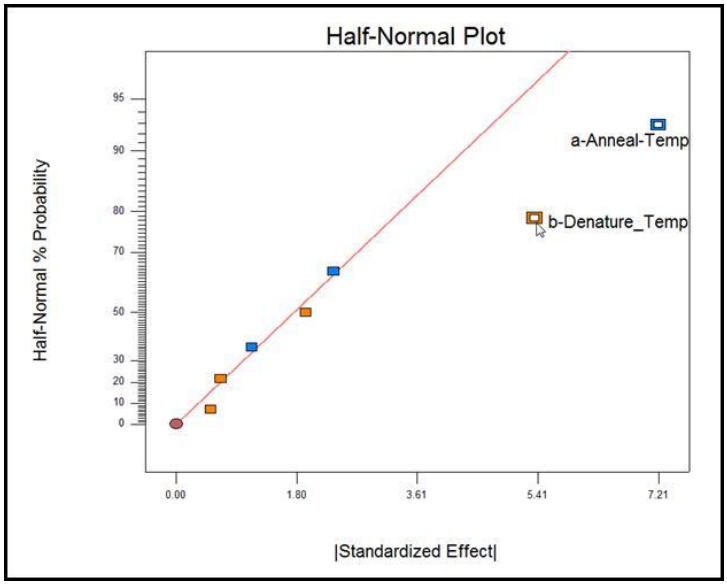
Analyzing the Design

On the design layout screen you will see your 256 run design split up into 8 whole-plot groups. Analysis is the same as for a factorial design in Design-Expert 8, except for one key difference: The subplot and whole-plot effects are analyzed separately. They each get their own half-normal plot. To get started on analyzing the Amplification response, just click on the **R1-Amplification** node.

1) No known transform is needed yet, so click the **Sub-plot Effects** tab.
2) Select the significant effects (those that stand out to the right) by clicking them as shown below.



3) Click on the whole-plot effects button and click **Yes** when asked the question about hierarchy. As shown below, select the significant whole-plot effects not already chosen for hierarchy.



4) Click on the **ANOVA (REML)** tab. The restricted maximum likelihood (REML) analysis is necessary to properly identify the significant effects and calculate p-values for split-plot designs. In this case, all the whole-plot and sub-plot terms are significant at the 0.05 level.
5) Click on the **Diagnostics** tab and analyze the diagnostics as you normally would. There is one possible outlier (outside the red lines), but in a design with 256 runs, that is not unexpected, so we'll leave that run in.

The model graphs, numerical optimization and other post-analysis options are all available and work as they do for all two-level factorial designs in Design-Expert. Explore the graphs for conditions that maximize the "Amplification" response. Then go to the Optimization Numerical node and ask the program to find the optimal settings for the polymerase chain reaction (PCR).