

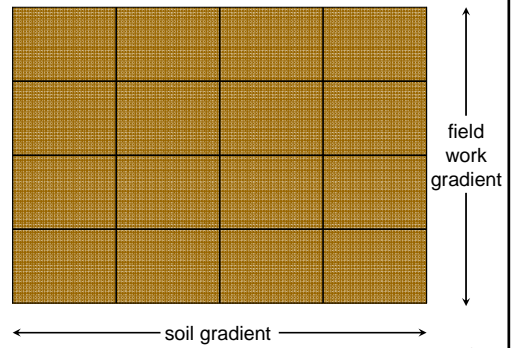
Latin Square Experimental Designs

1/30/2007

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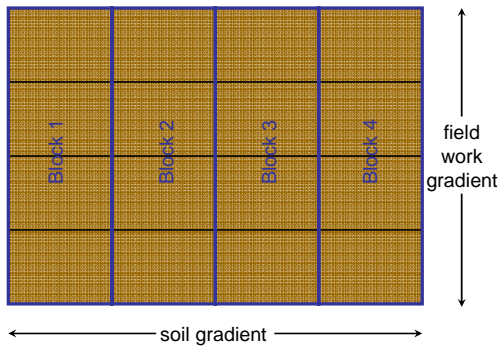
1

A Need for Blocking in Two Directions



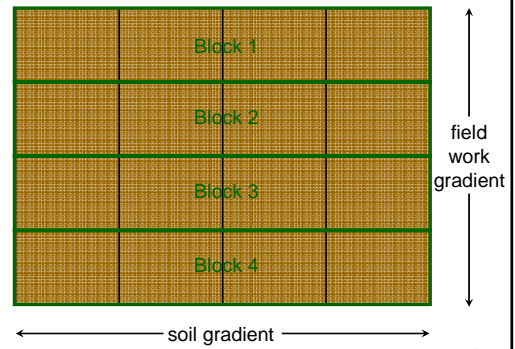
2

Blocks That Vary in Soil Quality



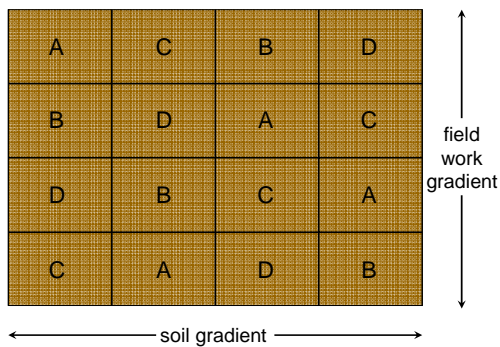
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Blocks That Vary Due to Field Work Variables



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Assignment of Treatments to Experimental Units Using a Latin Square Design



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Assignment of Treatments to Experimental Units Using a Latin Square Design

- Each treatment appears exactly once in each row.
- Each treatment appears exactly once in each column.
- Among all possible assignments of treatments to experimental units that satisfy the above properties, one is selected at random.
- All possibilities are equally likely.
- In the 4 x 4 case, there are 576 to choose from*.
- 5 x 5: 161,280 possibilities, 6 x 6: 812,851,200 possibilities*

*Alter, R. 1975. How Many Latin Squares Are There? *American Mathematical Monthly*, 82: 632-634.

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SAS Code for Obtaining Random Latin Squares

```
proc plan seed=725;
  factors row=4 ordered column=4 ordered / noprint;
  treatments trt=4 cyclic;
  output out=one
    row cvals=('row 1' 'row 2' 'row 3' 'row 4') ordered
    column cvals=('col 1' 'col 2' 'col 3' 'col 4') ordered
    trt cvals=('A' 'B' 'C' 'D') random;
run;

proc print;
run;
```

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Output Produced by SAS Code

Obs	row	column	trt
1	row 1	col 1	C
2	row 1	col 2	B
3	row 1	col 3	D
4	row 1	col 4	A
5	row 2	col 1	B
6	row 2	col 2	D
7	row 2	col 3	A
8	row 2	col 4	C
9	row 3	col 1	D
10	row 3	col 2	A
11	row 3	col 3	C
12	row 3	col 4	B
13	row 4	col 1	A
14	row 4	col 2	C
15	row 4	col 3	B
16	row 4	col 4	D

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Latin Square Generated by SAS Code

C	B	D	A
B	D	A	C
D	A	C	B
A	C	B	D

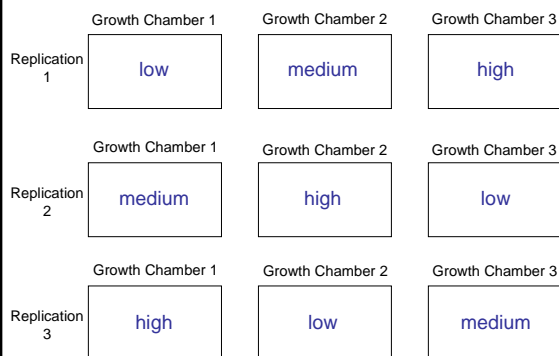
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How would you design the experiment?

- Suppose a researcher would like to study the effects of three temperatures (low, medium, high) on gene expression in three plant genotypes (A, B, C).
- Three growth chambers whose temperature can be set to a constant value are available for use in the experiment.
- Each growth chamber can hold three trays of plants. Each tray holds 18 plants in a 3 x 6 arrangement.
- The researcher will grow plants at a constant temperature for two weeks, harvest plants, and extract RNA for hybridization on Affymetrix GeneChips.
- A total of 27 GeneChips are available for the experiment.
- The researcher is willing to replicate the process of growing plants and extracting RNA in three different two-week periods using all three chambers each time.

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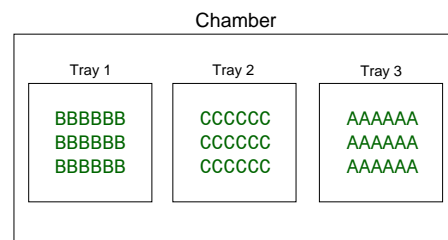
Assign Temperatures to Chambers Using a Latin Square



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Separate Random Arrangements of Plant Genotypes within a Chamber

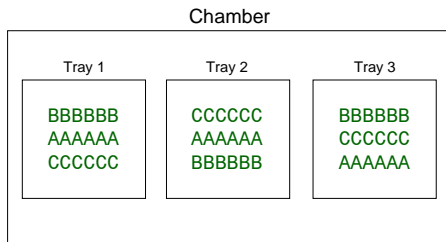
Simplest Strategy



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Separate Random Arrangements of Plant Genotypes within a Chamber

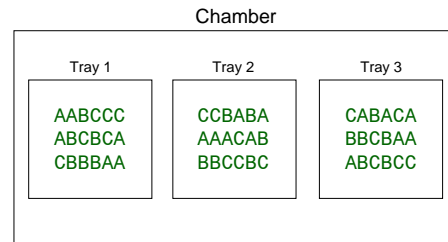
If large tray-to-tray variation within a chamber is expected, we might consider using trays as blocks.



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Separate Random Arrangements of Plant Genotypes within a Chamber

If we expect large tray-to-tray variation within a chamber and large position-to-position variation within a tray...



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There are a variety of possibilities.

- Regardless of how we assign genotypes to positions in each chamber, the plants of the same genotype within any chamber will be pooled for measurement with a single GeneChip.
- The strategy for assigning genotypes to positions within each chamber should consider major sources of variation within each chamber but should also consider the practical challenges of keeping track of which plants are which genotype.

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Our Design is a Split-Plot Design

- The whole-plot factor is temperature.
- The split-plot factor is genotype.
- The whole-plot experimental units are chambers.
- Each split-plot experimental unit is a group of 18 plants of a single genotype that share a growth chamber.
- The whole-plot portion of the experiment is designed as a Latin square with reps and chambers as the two blocking criteria.

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