LATIN SQUARE DESIGN (LS)

Facts about the LS Design

-With the Latin Square design you are able to control variation in two directions.

-Treatments are arranged in rows and columns

-Each row contains every treatment.

-Each column contains every treatment.

-The most common sizes of LS are 5x5 to 8x8

Advantages of the LS Design

1. You can control variation in two directions.

2. Hopefully you increase efficiency as compared to the RCBD.

Disadvantages of the LS Design

1. The number of treatments must equal the number of replicates.

2. The experimental error is likely to increase with the size of the square.

3. Small squares have very few degrees of freedom for experimental error.

4. You can't evaluate interactions between:
   a. Rows and columns
   b. Rows and treatments
   c. Columns and treatments.

Effect of the Size of the Square on Error Degrees of Freedom

SOV	Df	2x2	3x3	4x4	5x5	8x8
Rows	r-1	1	2	3	4	7
Columns	r-1	1	2	3	4	7
Treatments	r-1	1	2	3	4	7
Error	(r-1)(r-2)	0	2	6	12	42
Total	$r^2 - 1$	3	8	15	24	63

Where r = number of rows, columns, and treatments.

-One way to increase the Error df for small squares is to use more than one square in the experiment (i.e. repeated squares).

Example
Two 4x4 Latin squares.

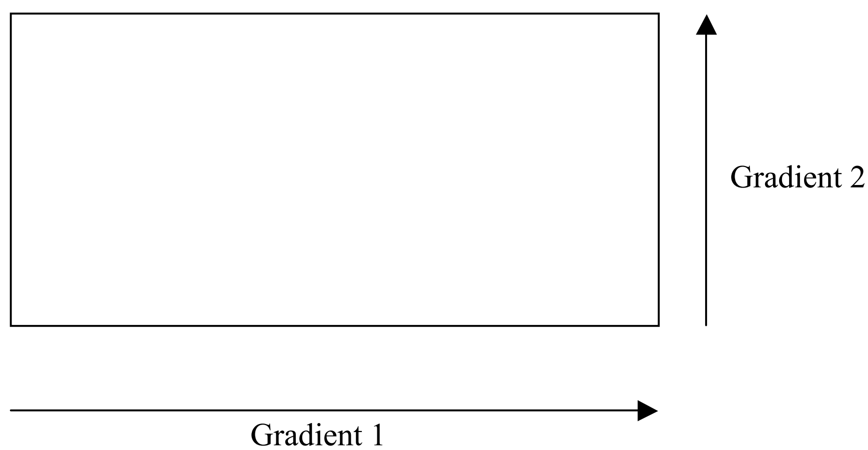
	SOV	Df
	Squares	sq – 1 = 1
*	**Row(square)**	sq(r-1) = 6
*	**Column(square)**	sq(r-1) = 6
	Treatment	r-1 = 3
	Square x Treatment	(sq-1)(r-1) = 3
*	**Error**	sq(r-1)(r-2) = 12
	Total	$sqr^2 - 1 = 31$

*Additive across squares.

Where sq = number of squares.

Examples of Uses of the Latin Square Design

1. Field trials in which the experimental error has two fertility gradients running perpendicular each other or has a unidirectional fertility gradient but also has residual effects from previous trials.



2. Animal science feed trials.

3. Insecticide field trial where the insect migration has a predictable direction that is perpendicular to the dominant fertility gradient of the experimental field.

4. Greenhouse trials in which the experimental pots are arranged in a straight line perpendicular to the glass walls, such that the difference among rows of pots and distace from the glass wall are expected to be the major sources of variability.

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

Randomization Procedure

-Depends on the type of Latin Square you use.

3x3 Latin Square

-Start with the standard square and randomize all columns and all but the first row.

	1	2	3
1	A	B	C
2	B	C	A
3	C	A	B

Standard square

Randomize columns

3	1	2
C	A	B
A	B	C
B	C	A

Randomize all but the first row

C	A	B
B	C	A
A	B	C

4x4 Latin Square

-Randomly choose a standard square.
-Randomize all columns and all but the first row.

5x5 Latin Square

-Randomly choose a standard square.
-Randomize all columns and rows.

Analysis of a Single Latin Square

Example
Grain yield of three maize hybrids (A, B, and D) and a check (C).

Row	Column 1	Column 2	Column 3	Column 4	Row $\left(\sum R\right)$
1	1.640 (B)	1.210 (D)	1.425 (C)	1.345 (A)	5.620
2	1.475 (C)	1.185 (A)	1.400 (D)	1.290 (B)	5.350
3	1.670 (A)	0.710 (C)	1.665 (B)	1.180 (D)	5.225
4	1.565 (D)	1.290 (B)	1.655 (A)	0.660 (C)	5.170
Column total $\left(\sum C\right)$	6.350	4.395	6.145	4.475	21.365

Step 1. Calculate treatment totals.

Treatment	Total
A	5.855
B	5.885
C	4.270
D	5.355

Step 2. Compute the Correction Factor (CF).

$$CF = \frac{Y_{..}^2}{r^2}$$

$$= \frac{21.365^2}{4^2}$$

$$= 28.53$$

Step 3. Calculate the Total SS

$$TotalSS = \sum Y_{ij}^2 - CF$$

$$= (1.64^2 + 1.210^2 + 1.425^2 + ... + 0.66^2) - CF$$

$$= 1.4139$$

Step 4. Calculate the Row SS

$$RowSS = \frac{\sum Row^2}{r} - CF$$

$$= \frac{(5.62^2 + 5.35^2 + 5.225^2 + 5.17^2)}{4} - CF$$

$$= 0.0302$$

Step 5. Calculate the Column SS.

$$Col.SS = \frac{\sum Col^2}{r} - CF$$

$$= \frac{(6.35^2 + 4.395^2 + 6.145^2 + 4.475^2)}{4} - CF$$

$$= 0.8273$$

Step 6. Calculate the Treatment SS

$$TrtSS = \frac{\sum Y_{i.}^2}{r} - CF$$

$$= \frac{(5.855^2 + 5.885^2 + 4.270^2 + 5.355^2)}{4} - CF$$

$$= 0.4268$$

Step 7. Calculate the Error SS

Error SS = Total SS – Row SS – Column SS – Trt SS

= 0.1296

Step 8. Complete the ANOVA table

SOV	Df	SS	MS	F
Row	r-1 = 3	0.030		
Column	r-1 = 3	0.827		
Trt	r-1 = 3	0.427	0.142	Trt MS/Error MS = 6.60*
Error	(r-1)(r-2) = 6	0.129	0.0215	
Total	r^2-1 = 15	1.414		

Step 9. Calculate the LSD.

$$LSD = t_{\alpha/2}\sqrt{\frac{2ErrorMS}{r}}$$

$$= 2.447\sqrt{\frac{2(.0215)}{4}}$$

$$= 0.254$$

Linear Model

$$Y_{ij(t)} = \mu + \beta_i + \kappa_j + \tau_t + \varepsilon_{ij(t)}$$

where: μ = the experiment mean.
β_i = the row effect,
κ_j = the column effect,
τ_t = the treatment effect, and
$\varepsilon_{ij(t)}$ = the random error.

Latin Square - Combined Analysis Across Squares

-The squares can be at the same location, or three different locations, or three different years, etc.

Example

Three 3x3 Latin squares

Square 1

				$\sum R$
	41 (B)	25 (C)	15 (A)	81
	20 (A)	32 (B)	24 (C)	76
	22 (C)	12 (A)	21 (B)	55
$\sum C$	83	69	60	212

SS Row_1 = 126.89
SS $Column_1$ = 89.55
SS $Treatment_1$ = 368.22
SS $Error_1$ = 21.56

Square 2

				$\sum R$
	27 (C)	28 (B)	3 (A)	58
	4 (A)	17 (C)	9 (B)	30
	22 (B)	4 (A)	17 (C)	43
$\sum C$	53	49	29	131

SS Row_2 = 130.89
SS $Column_2$ = 110.22
SS $Treatment_2$ = 534.22
SS $Error_2$ = 14.89

Square 3

				$\sum R$
	43 (B)	27 (C)	17 (A)	87
	22 (A)	34 (B)	26 (C)	82
	24 (C)	14 (A)	23 (B)	61
$\sum C$	89	75	66	230

SS Row_3 = 126.89
SS $Column_3$ = 89.55
SS $Treatment_3$ = 368.22
SS $Error_3$ = 21.56

Step 1. Test the homogeneity of the Error MS from each square using Bartlett's Chi-square test.

Step 1.1 Calculate the Error SS for each square.

Step 1.2 Calculate the Error MS for each square.

Step 1.3 Calculate the Log of each Error MS

Square	Error SS	Error df	Error MS	Log Error MS
1	21.56	2	10.78	1.0326
2	14.89	2	7.45	0.8722
3	21.56	2	10.78	1.0326
			$\sum s_i^2 = 29.01$	$\sum \log s_i^2 = 2.9374$

Step 1.4 Calculate the Pooled Error MS (s_p^2)

$$s_p^2 = \frac{\sum s_i^2}{\#sq} = \frac{29.01}{3} = 9.67$$

Step 1.5 Calculate Bartlett's χ^2

$$\chi^2 = \frac{2.3026(Errordf)\left[\left(sq \log s_p^2\right) - \sum \log s_i^2\right]}{1 + \left[\dfrac{(sq+1)}{3 * sq * Errordf}\right]}$$

Where Error df = df for one square.

$$\chi^2 = \frac{2.3026(2)\left[\left(3 \log 9.67\right) - 2.9374\right]}{1 + \left[\dfrac{(3+1)}{3*3*2}\right]}$$

$$= \frac{0.0869}{1.2222}$$

$$= 0.0711$$

Step 1.6 Look up the Table χ^2-value at the 99.5% level of confidence and df = #sq-1.

$$\chi^2_{0.005;2df} = 10.6$$

Step 1.7 Make conclusions

Since $\chi^2{}_{calc} < \chi^2_{table}$ we fail to reject $H_o : \sigma_1^2 = \sigma_2^2 = \sigma_3^2$ at the 99.5% level of confidence; thus, we can do the combined analysis across squares

Step 2. Calculate Treatment Totals for each square.

Treatment	Square 1	Square 2	Square 3	$\sum TRT$
A	47	11	53	111
B	94	59	100	253
C	71	61	77	209
$\sum Square$	212	131	230	573

Step 3. Calculate the Correction Factor (CF).

$$CF = \frac{Y_{...}^2}{sq * r^2}$$

$$= \frac{573^2}{3 * 3^2}$$

$$= 12{,}160.333$$

Step 4. Calculate the Total SS

$$TotalSS = \left(41^2 + 25^2 + 15^2 + ... + 23^2\right) - CF$$
$$= 2{,}620.67$$

Step 5. Calculate the Square SS

$$SquareSS = \frac{\sum Sq^2}{r^2} - CF$$

$$= \frac{\left(212^2 + 131^2 + 230^2\right)}{3^2} - CF$$

$$= 618.0$$

Step 6. Calculate the Row(Square) SS (Additive across squares)

Row(Square) SS = Row_1 SS + Row_2 SS + Row_3 SS

= 384.67

Step 7. Calculate the Column(Square) SS (Additive across squares)

Column(Square) SS = Column_1 SS + Column_2 SS + Column_3 SS

= 289.32

Step 8. Calculate the Treatment SS

$$TrtSS = \frac{\sum TRT_i^2}{sq * r} - CF$$

$$= \frac{\left(111^2 + 253^2 + 209^2\right)}{3 * 3} - CF$$

$$= 1{,}174.22$$

Step 9. Calculate the Square X Treatment SS.

$$Sq * TrtSS = \frac{\sum (SqXTrt)^2}{r} - CF - SquareSS - TrtSS$$

$$= \frac{\left(47^2 + 94^2 + 71^2 + ... + 77^2\right)}{3} - CF - SquareSS - TrtSS$$

$$= 96.45$$

Step 10. Calculate Error SS (Additive across squares)

Error SS = Error_1 SS + Error_2 SS + Error_3 SS

Error SS = 58.01

Step 11. Complete the ANOVA Table.

SOV	Df	SS	MS	F (Squares and Trt are Fixed effects)
Square	Sq-1 = 2	618.0		Non-valid F-test
Row(Sq)	Sq(r-1) = 6	384.67		Non-valid F-test
Column(Sq)	Sq(r-1) = 6	289.32		Non-valid F-test
Trt	r-1 = 2	1174.22	587.11	Trt MS/Error MS = 60.73^{**}
Sq X Trt	(sq-1)(r-1) = 4	96.45	24.11	Sq X Trt MS/Error MS = 2.49^{ns}
Error	Sq(r-1)(r-2) = 6	58.01	9.67	
Total	Sqr^2-1 = 26	2620.67		

Conclusions:

1. The non-significant Square X Treatment interaction indicates that treatments responded similarly in all squares.

Table 1. Mean for the square x treatment interaction.

	Treatment		
Square	A	B	C
1	15.7	31.3	23.7
2	3.7	19.7	20.3
3	17.7	33.3	25.7
LSD(0.05)	-------------------ns--------------------		

2. The significant F-test for Treatment indicates that averaged across all squares, there were differences between treatments.
3.

Table 2. Mean for the treatment main effect averaged Across squares.

Treatment	Mean
A	12.3
B	28.1
C	23.2
LSD(0.05)	3.6

Step 12. Calculate LSD's

Square X Trt: Normally, you would not calculate this LSD because the F-test for the interaction was non-significant. However, if it would have been significant, you would have calculated the LSD using the following method:

$$LSD_{SqXTrt} = t_{a/2;errordf}\sqrt{\frac{2ErrorMS}{r}}$$

$$= 2.447\sqrt{\frac{2(9.67)}{3}}$$

$$= 6.2$$

This LSD would be used for comparisons only in Table 1.

Treatment:

$$LSD_{Trt} = t_{a/2;errordf}\sqrt{\frac{2ErrorMS}{sq*r}}$$

$$= 2.447\sqrt{\frac{2(9.67)}{3*3}}$$

$$= 3.6$$

This LSD would only be used for comparisons in Table 2.

SAS Commands for the Latin Square (individual squares and combined across squares).

```
options pageno=1;
data lscmb;
input square row column trt $ yield;
datalines;
1 1 1 b 41
1 1 2 c 25
1 1 3 a 15
1 2 1 a 20
1 2 2 b 32
1 2 3 c 24
1 3 1 c 22
1 3 2 a 12
1 3 3 b 21
2 1 1 c 27
2 1 2 b 28
2 1 3 a 3
2 2 1 a 4
2 2 2 c 17
2 2 3 b 9
2 3 1 b 22
2 3 2 a 4
2 3 3 c 17
3 1 1 b 43
3 1 2 c 27
3 1 3 a 17
3 2 1 a 22
3 2 2 b 34
3 2 3 c 26
3 3 1 c 24
3 3 2 a 14
3 3 3 b 23
;;
ods graphics off;
ods rtf file='latin.rtf';
proc print;
```

```
title 'printout of data';
run;
proc sort;
by square;
*Comment The previous statements are needed to do the ANOVA for each
individual square;
run;
proc anova;
by square;
class row column trt;
model yield=row column trt;
title 'anova of each individual square';
run;
proc anova;
class square row column trt;
model yield=square row(square) column(square) trt square*trt;
means trt/lsd;
means square*trt;
*Comment  Note that there is no LSD command since SAS will not
calculate the LSD values for interactions.  SAS only calculates LSD
values for the main effects;
title 'anova combined across squares assuming square and trt are fixed
effects';
run;
proc anova;
class square row column trt;
model yield=square row(square) column(square) trt square*trt;
test h=trt e=square*trt;
*Comment The previous statement is needed since square is a random
effect and treatment is a fixed effect.  Squre*trt is the denominator
of the F-test to test treatment;
means trt/lsd e=square*trt;
means square*trt;
*Comment  Note that there is no LSD command since SAS will not
calculate the LSD values for interactions.  SAS only calculates LSD
values for the main effects;
title 'anova combined across squares assuming square random and trt
fixed';
run;
ods rtf close;
```

Printout of Data for the Latin Square

Obs	square	row	column	trt	yield
1	1	1	1	b	41
2	1	1	2	c	25
3	1	1	3	a	15
4	1	2	1	a	20
5	1	2	2	b	32
6	1	2	3	c	24
7	1	3	1	c	22
8	1	3	2	a	12
9	1	3	3	b	21
10	2	1	1	c	27
11	2	1	2	b	28
12	2	1	3	a	3
13	2	2	1	a	4
14	2	2	2	c	17
15	2	2	3	b	9
16	2	3	1	b	22
17	2	3	2	a	4
18	2	3	3	c	17
19	3	1	1	b	43
20	3	1	2	c	27
21	3	1	3	a	17
22	3	2	1	a	22
23	3	2	2	b	34
24	3	2	3	c	26
25	3	3	1	c	24
26	3	3	2	a	14
27	3	3	3	b	23

ANOVA of each individual square

The ANOVA Procedure

square=1

Class Level Information		
Class	**Levels**	**Values**
row	3	1 2 3
column	3	1 2 3
trt	3	a b c

Number of Observations Read	9
Number of Observations Used	9

ANOVA of each individual square

The ANOVA Procedure

Dependent Variable: yield

square=1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	584.6666667	97.4444444	9.04	0.1029
Error	2	21.5555556	10.7777778		
Corrected Total	8	606.2222222			

R-Square	Coeff Var	Root MSE	yield Mean
0.964443	13.93706	3.282953	23.55556

Source	DF	Anova SS	Mean Square	F Value	Pr > F
row	2	126.8888889	63.4444444	5.89	0.1452
column	2	89.5555556	44.7777778	4.15	0.1940
trt	2	368.2222222	184.1111111	17.08	0.0553

ANOVA of each individual square

The ANOVA Procedure

square=2

Class Level Information		
Class	**Levels**	**Values**
row	3	1 2 3
column	3	1 2 3
trt	3	a b c

Number of Observations Read	9
Number of Observations Used	9

ANOVA of each individual square

The ANOVA Procedure

Dependent Variable: yield

square=2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	775.3333333	129.2222222	17.36	0.0555
Error	2	14.8888889	7.4444444		
Corrected Total	8	790.2222222			

R-Square	Coeff Var	Root MSE	yield Mean
0.981159	18.74508	2.728451	14.55556

Source	DF	Anova SS	Mean Square	F Value	Pr > F
row	2	130.8888889	65.4444444	8.79	0.1021
column	2	110.2222222	55.1111111	7.40	0.1190
trt	2	534.2222222	267.1111111	35.88	0.0271

ANOVA of each individual square

The ANOVA Procedure

square=3

Class Level Information		
Class	**Levels**	**Values**
row	3	1 2 3
column	3	1 2 3
trt	3	a b c

Number of Observations Read	9
Number of Observations Used	9

ANOVA of each individual square

The ANOVA Procedure

Dependent Variable: yield

square=3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	584.6666667	97.4444444	9.04	0.1029
Error	2	21.5555556	10.7777778		
Corrected Total	8	606.2222222			

R-Square	Coeff Var	Root MSE	yield Mean
0.964443	12.84634	3.282953	25.55556

Source	DF	Anova SS	Mean Square	F Value	Pr > F
row	2	126.8888889	63.4444444	5.89	0.1452
column	2	89.5555556	44.7777778	4.15	0.1940
trt	2	368.2222222	184.1111111	17.08	0.0553

ANOVA combined across squares assuming square and trt are fixed effects

The ANOVA Procedure

Class Level Information		
Class	**Levels**	**Values**
square	3	1 2 3
row	3	1 2 3
column	3	1 2 3
trt	3	a b c

Number of Observations Read	27
Number of Observations Used	27

ANOVA combined across squares assuming square and trt are fixed effects

The ANOVA Procedure

Dependent Variable: yield

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	20	2562.666667	128.133333	13.26	0.0021
Error	6	58.000000	9.666667		
Corrected Total	26	2620.666667			

R-Square	Coeff Var	Root MSE	yield Mean
0.977868	14.65033	3.109126	21.22222

Source	DF	Anova SS	Mean Square	F Value	Pr > F
square	2	618.000000	309.000000	31.97	0.0006
row(square)	6	384.666667	64.111111	6.63	0.0183
column(square)	6	289.333333	48.222222	4.99	0.0357
trt	2	1174.222222	587.111111	60.74	0.0001
square*trt	4	96.444444	24.111111	2.49	0.1522

ANOVA combined across squares assuming square and trt are fixed effects

The ANOVA Procedure

t Tests (LSD) for yield

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	9.666667
Critical Value of t	2.44691
Least Significant Difference	3.5863

Means with the same letter are not significantly different.			
t Grouping	**Mean**	**N**	**trt**
A	28.111	9	b
B	23.222	9	c
C	12.333	9	a

ANOVA combined across squares assuming square and trt are fixed effects

The ANOVA Procedure

Level of square	Level of trt	N	yield Mean	yield Std Dev
1	a	3	15.6666667	4.0414519
1	b	3	31.3333333	10.0166528
1	c	3	23.6666667	1.5275252
2	a	3	3.6666667	0.5773503
2	b	3	19.6666667	9.7125349
2	c	3	20.3333333	5.7735027
3	a	3	17.6666667	4.0414519
3	b	3	33.3333333	10.0166528
3	c	3	25.6666667	1.5275252

anova combined across squares assuming square random and trt fixed

The ANOVA Procedure

Class Level Information		
Class	**Levels**	**Values**
square	3	1 2 3
row	3	1 2 3
column	3	1 2 3
trt	3	a b c

Number of Observations Read	27
Number of Observations Used	27

anova combined across squares assuming square random and trt fixed

The ANOVA Procedure

Dependent Variable: yield

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	20	2562.666667	128.133333	13.26	0.0021
Error	6	58.000000	9.666667		
Corrected Total	26	2620.666667			

R-Square	Coeff Var	Root MSE	yield Mean
0.977868	14.65033	3.109126	21.22222

Source	DF	Anova SS	Mean Square	F Value	Pr > F
square	2	618.000000	309.000000	31.97	0.0006
row(square)	6	384.666667	64.111111	6.63	0.0183
column(square)	6	289.333333	48.222222	4.99	0.0357
trt	2	1174.222222	587.111111	60.74	0.0001
square*trt	4	96.444444	24.111111	2.49	0.1522

Tests of Hypotheses Using the Anova MS for square*trt as an Error Term					
Source	**DF**	**Anova SS**	**Mean Square**	**F Value**	**Pr > F**
trt	2	1174.222222	587.111111	24.35	0.0058

ANOVA combined across squares assuming square random and trt fixed

The ANOVA Procedure

t Tests (LSD) for yield

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	4
Error Mean Square	24.11111
Critical Value of t	2.77645
Least Significant Difference	6.4268

Means with the same letter are not significantly different.			
t Grouping	**Mean**	**N**	**trt**
A	28.111	9	b
A			
A	23.222	9	c
B	12.333	9	a

ANOVA combined across squares assuming square random and trt fixed

The ANOVA Procedure

t Tests (LSD) for yield

Level of square	Level of trt	N	yield Mean	yield Std Dev
1	**a**	3	15.6666667	4.0414519
1	**b**	3	31.3333333	10.0166528
1	**c**	3	23.6666667	1.5275252
2	**a**	3	3.6666667	0.5773503
2	**b**	3	19.6666667	9.7125349
2	**c**	3	20.3333333	5.7735027
3	**a**	3	17.6666667	4.0414519
3	**b**	3	33.3333333	10.0166528
3	**c**	3	25.6666667	1.5275252