**Example section**

This section illustrates two examples using the objective function and simulated annealing algorithm described in the previous two sections.

The first example is the same example of the simulated annealing algorithm section. The design parameter for both phases of the experiments is as follow,

Phase 1 experiment - 6 treatments, 3 biological replicates, 2 technical replicates,

Phase 2 experiment – 9 runs and 4 tags.

For the Phase 1 experiment, the 6 treatments are denoted by “a”, “b”, “c”, “d”, “e” and “f”. Since 3 biological replicates are used, this means 3 animals are assigned to each treatment which gives a total of 15 animals. These 15 animals are denoted by upper case letters of “A” to “R”. The theoretical ANOVA of the Phase 1 experiment can be presented as follows,

$ANOVA

DF Ani

Between Ani

Trt 5 1

Residual 12 1

$EF

Trt eff.Trt

Between Ani

Trt 3 1

The allocation of the animal for the starting design can be represented in the matrix notation as follows,

[,1] [,2] [,3] [,4]

[1,] "AA" "AB" "AC" "AD"

[2,] "AB" "AA" "AD" "AC"

[3,] "AE" "AF" "AG" "AH"

[4,] "AF" "AE" "AH" "AG"

[5,] "AI" "AJ" "AK" "AL"

[6,] "AJ" "AI" "AL" "AK"

[7,] "AM" "AN" "AO" "AP"

[8,] "AN" "AM" "AP" "AO"

[9,] "AQ" "AQ" "AR" "AR"

As described in the previous section, the assignment of animals to runs and tags is to group a pair of animals of the identical technical replicates and allocating them in a sector of 2 runs and 2 tags. This design generated 13 canonical efficiency factors all unity, for the animals in the within runs stratum. The average efficiency factor is also 1.

The allocation of the treatment for the starting design is also represented in the matrix notation as follows,

[,1] [,2] [,3] [,4]

[1,] "a" "b" "c" "d"

[2,] "b" "a" "d" "c"

[3,] "e" "f" "a" "b"

[4,] "f" "e" "b" "a"

[5,] "c" "d" "e" "f"

[6,] "d" "c" "f" "e"

[7,] "a" "b" "c" "d"

[8,] "b" "a" "d" "c"

[9,] "e" "e" "f" "f"

The pairs of treatments are always the same where are “a” and “b”, “c” and “d”, and “e” and “f”. This design generated 5 canonical efficiency factors, i.e. 1, 1, 0.976, 0.747 and 0.5, for the treatment elimination tags in the between animals within runs stratum. The average efficiency factor is 0.7856.

The theoretical ANOVA table can be shown as follows,

$ANOVA

DF e Ani Run

Between Run

Between Ani

Trt 2 1 2 4

Residual 2 1 2 4

Residual 4 1 0 4

Within

Between Ani

Tag 1 1 2 0

Trt 5 1 2 0

Residual 7 1 2 0

Residual

Tag 2 1 0 0

Residual 12 1 0 0

$EF

Tag Trt eff.Tag eff.Trt

Between Run

Between Ani

Trt 3/2 1/4

Residual

Within

Between Ani

Tag 9 2/3 1 1/9

Trt 3540/751 590/751

Residual

Tag 9 1

From the random effects table, the formal test for the treatment differences can be conducted as the variance components of the between animals for the treatment and residual mean square in the between animal stratum are identical. From the fixed effects table, the treatment is confounded with runs and tags effects by 1/4 and 1/9 of treatment information, respectively. Hence, there is 590/751 = 0.7856 of pure treatment information remaining, note that this value is also the same as the average efficiency factor computed.

Using the objective function and simulated annealing described, a more improved design has been generated. The animals allocation is the resulting design can be represented in matrix notation as follows,

[,1] [,2] [,3] [,4]

[1,] "AK" "AD" "AG" "AN"

[2,] "AD" "AK" "AN" "AG"

[3,] "AA" "AP" "AQ" "AC"

[4,] "AP" "AA" "AC" "AQ"

[5,] "AI" "AH" "AJ" "AL"

[6,] "AH" "AI" "AL" "AJ"

[7,] "AM" "AR" "AO" "AB"

[8,] "AR" "AM" "AB" "AO"

[9,] "AF" "AF" "AE" "AE"

The arrangement of the animals still follows 2-by-2 setting but with the different animal pairs compare to the starting design. This design also generated 13 canonical efficiency factors all unity, for the animals in the within runs stratum. The average efficiency factor is also 1. This means the test for the treatment differences should be able to conduct.

The treatment allocation to the runs and tags based on the animal allocation and can be represented in matrix notation as follows,

[,1] [,2] [,3] [,4]

[1,] "e" "d" "a" "b"

[2,] "d" "e" "b" "a"

[3,] "a" "d" "e" "c"

[4,] "d" "a" "c" "e"

[5,] "c" "b" "d" "f"

[6,] "b" "c" "f" "d"

[7,] "a" "f" "c" "b"

[8,] "f" "a" "b" "c"

[9,] "f" "f" "e" "e"

For this new design, the treatment pairs are not always identical like before. The treatment pairs are “e” and “d”, “a” and “b”, “a” and “d”, “e” and “c”, “b” and “c”, “d” and “f”, “a” and “f”, “c” and “b” and “e” and “f”. This design also generated 5 canonical efficiency factors, but they are different than before, i.e. 0.9167, 0.9167, 0.8889, 0.75 and 0.75, for the treatment elimination tags in the between animals within runs stratum. The average efficiency factor is 0.837 which is higher than the previous design.

The theoretical ANOVA table can be shown as follows,

$ANOVA

DF e Ani Run

Between Run

Between Ani

Trt 4 1 2 4

Residual 4 1 0 4

Within

Between Ani

Tag 1 1 2 0

Trt 5 1 2 0

Residual 7 1 2 0

Residual

Tag 2 1 0 0

Residual 12 1 0 0

$EF

Tag Trt eff.Tag eff.Trt

Between Run

Between Ani

Trt 3/4 1/8

Residual

Within

Between Ani

Tag 9 2/3 1 1/9

Trt 7920/1577 1320/1577

Residual

Tag 9 1

From the random effects table, notable change compare the random effects table of the previous design is that there are 4 DF associated with treatment effects in the between runs stratum compare the 2 DF for the previous design. However, a valid test for the treatment differences can still be conducted. From the fixed effects table of the new design, the amount of treatment information in the between runs stratum become 1/8 which is lower than before There is still 1/9 of treatment information confounded with the tag. This means there is (1320/1577 =) 0.8370 of pure treatment information remaining in the between animals within runs stratum, that is 0.051 more than the previous design. Therefore, this suggests that the new design is better than the previous design. The treatment pairs have to be different to minimise the confounding of treatment with the runs, which will also maximise the treatment information in the between animals within runs stratum.

The second example uses the 8-plex system for the second phase experiment, the design parameters for both phase of experiment can be described as follows,

Phase 1 experiment - 8 treatments, 2 biological replicates, 2 technical replicates,

Phase 2 experiment – 4 runs and 8 tags.

The theoretical ANOVA table for the Phase 1 experiment can be shown as follows,

$ANOVA

DF Ani

Between Ani

Trt 7 1

Residual 8 1

$EF

Trt eff.Trt

Between Ani

Trt 2 1

The allocation of the animal for the starting design can be represented in the matrix notation as follows,

[,1] [,2] [,3] [,4] [,5] [,6] [,7] [,8]

[1,] "AA" "AB" "AC" "AD" "AE" "AF" "AG" "AH"

[2,] "AB" "AA" "AD" "AC" "AF" "AE" "AH" "AG"

[3,] "AK" "AL" "AI" "AJ" "AO" "AP" "AM" "AN"

[4,] "AL" "AK" "AJ" "AI" "AP" "AO" "AN" "AM"

This design generated 14 canonical efficiency factors all unity, for the animals in the within runs stratum. The average efficiency factor is also 1.

The allocation of the treatment for the starting design is also represented in the matrix notation as follows,

[,1] [,2] [,3] [,4] [,5] [,6] [,7] [,8]

[1,] "a" "b" "c" "d" "e" "f" "g" "h"

[2,] "b" "a" "d" "c" "f" "e" "h" "g"

[3,] "c" "d" "a" "b" "g" "h" "e" "f"

[4,] "d" "c" "b" "a" "h" "g" "f" "e"

This design generated 6 canonical efficiency factors all unity, for the treatment elimination tags in the between animals within runs stratum. The average efficiency factor is also 1.

The theoretical ANOVA table can be shown as follows,

$ANOVA

DF e Ani Run

Between Run

Between Ani 1 1 2 8

Residual 2 1 0 8

Within

Between Ani

Tag 3 1 2 0

Trt 6 1 2 0

Residual 5 1 2 0

Residual

Tag 4 1 0 0

Residual 10 1 0 0

$EF

Tag Trt eff.Tag eff.Trt

Between Run

Between Ani

Residual

Within

Between Ani

Tag 4 4 1 1

Trt 4 1

Residual

Tag 4 1

From the random effect table, the valid test for the treatment differences can be conducted. Note that total number of DF associated with the treatment is seven. From the fixed effects table, both tag and treatment mean squares contain 100% of treatment information. In addition, there are 6 DF associated with the treatment mean square, this means One DF associated with the treatment effects is completely confounded with the tag effects. Therefore, despite there are 100% of the treatment information for conducting the test of the treatment differences, but, it is only based on the 6 DF associated with the treatment effects.

Apply the simulated annealing algorithm with the objective function, a following design was generated. The allocation of the animal for the starting design can be represented in the matrix notation as follows,

[,1] [,2] [,3] [,4] [,5] [,6] [,7] [,8]

[1,] "AL" "AI" "AJ" "AO" "AM" "AN" "AP" "AK"

[2,] "AI" "AL" "AO" "AJ" "AN" "AM" "AK" "AP"

[3,] "AC" "AG" "AD" "AE" "AH" "AA" "AB" "AF"

[4,] "AG" "AC" "AE" "AD" "AA" "AH" "AF" "AB"

This design generated 14 canonical efficiency factors all unity, for the animals in the within runs stratum. The average efficiency factor is also 1.

The allocation of the treatment for the starting design is also represented in the matrix notation as follows,

[,1] [,2] [,3] [,4] [,5] [,6] [,7] [,8]

[1,] "d" "a" "b" "g" "e" "f" "h" "c"

[2,] "a" "d" "g" "b" "f" "e" "c" "h"

[3,] "c" "g" "d" "e" "h" "a" "b" "f"

[4,] "g" "c" "e" "d" "a" "h" "f" "b"

Same as the previous example, for the improved design, the treatment pairs are not consistent like the previous design. This design also generated 7 canonical efficiency factors, but they are different than before, i.e. 1, 1, 1, 1, 0.75, 0.75 and 0.5, for the treatment elimination tags in the between animals within runs stratum. The average efficiency factor is 0.8077 which is lower than the previous design, but it is based on seven canonical efficiency factors.

The theoretical ANOVA table can be shown as follows,

$ANOVA

DF e Ani Run

Between Run

Between Ani 1 1 2 8

Residual 2 1 0 8

Within

Between Ani

Tag 3 1 2 0

Trt 7 1 2 0

Residual 4 1 2 0

Residual

Tag 4 1 0 0

Residual 10 1 0 0

$EF

Tag Trt eff.Tag eff.Trt

Between Run

Between Ani

Residual

Within

Between Ani

Tag 4 6/5 1 3/10

Trt 42/13 21/26

Residual

Tag 4 1

From the random effect table, the valid test for the treatment differences can be conducted. The test is conducted based on the seven DF associated with the treatment effects, however, the DF associated with the residual mean squares of the between animals within runs stratum is reduced to 4 from 5 of the previous design. Form the fixed effects table, there is 3/10 treatment information confounded with the tag effects; hence, there is still 21/26 = 0.8077 of the pure treatment information remaining for conducting the test for the treatment differences.

The second example utilised the last component of the objective function in monitoring the DF associated with the treatment effects.

sdfs

**Summary of the tables…**

**Summary of overall…**

Summary