**Pseudo code of the simulated annealing**

Generate a starting design as discussed in Section 3.

Set the initial temperature of 100; then I use the optim function in R which implements the simulated annealing algorithm. The detail of the simulated annealing is described as follow.

Repeat the following process for 50000 times{

Generate a new design using the stage 1 of swapping – swapping is restricted to within run.

Compare the new design to the previous design based on the objective functions.

If the result of the objective function is better for the new design than the previous design, then the new design is accepted for next iteration and store as the best design.

If the result of the objective function is worse for the new design than the previous design, then computes the acceptance probability using formula as follow

where and denote the result from the objective function of the current and previous design, respectively, and denote the temperature that is used for the ith iteration. The acceptance probability is compared to the probability of accepting this design, which is randomly generated between the numbers of zero and one.

If the acceptance probability is higher than the probability of accepting this design, then this design is accepted for next iteration.

If the acceptance probability is lower than the probability of accepting this design, then this design is rejected. Hence, the previous design is retain for the next iteration.

}

Extract the best design from the above search and use this design as the starting design for the next search.

Repeat the following process for 50000 times{

Generate a new design using stage 2 of swapping – swapping is restricted to within tags.

Compare the new design to the previous design based on the objective functions as described above.

}

Extract the best design from the search and use this design as the starting design for the next search.

The next step is to apply the accelerated cooling method, which is to repeat process of these two stages of swapping with the initial temperature of 50, 25, 12.5 and till the tenth level of the simulated annealing algorithm is performed.

The best design is then extracted from the search of the last level of the accelerated cooling method. This best design is then considered as the optimal design for the given experimental design parameter.

**Example**

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 “AA” to “AR”. 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"

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 is composed of two tables; random effects table and fixed effects table. The first table is the random effects table. The first column corresponds the sources of variation, the second column corresponds the degrees of freedom and the remaining columns are the coefficients of the variance components for the expected means squares. The first two columns of the fixed effects table is the coefficients of the treatment parameters and the last two columns correspond to the average efficiency factors of the treatment effects. These two 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.

**Step-by-step illustration**

Note the starting allocation of the animals and treatments to runs and tags are represented in two matrices as,

[,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"

[,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 result of the objective function defined in Section 2 of this design is 97.61799.

The first swap of the first stage and first level is swapping any random pairs of animals and treatment within the same runs under the temperature of 100. For this illustration, the first swap is between Animals “AJ” and “AK”, the allocation of the animals and treatment becomes

[,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" **"AK"** **"AJ"** "AL"

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

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

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

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

[,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" **"e"** **"d"** "f"

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

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

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

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

The result of the objective function of this design is 96.31085, which is lower than the starting design of 97.61799. Therefore, this design is not as good as the previous design based on the objective function. The next step is check whether I should accept this design to continue the searching of the optimal design.

The probability of accepting this design is then randomly generated between the numbers of zero and one, for this case, the number 0.424 is picked.

The acceptance probability is calculated using the following formula,

where and denote the result from the objective function of the current and previous design, respectively, and denote the temperature that is used for the ith iteration. For this example, this formula gives 0.987 and is higher than the probability of accepting this design of 0.424. Hence, this design is accepted to continue the search, but the previous better design is stored to be compared throughout the search.

The second swap is between the animals “AM” and “AO”

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

The objective function of this design gives 96.50794, which is higher than the previous design of 96.31085, but it is still lower than the first design of 97.61799. The probability of accepting this design is then again randomly generated to be 0.752. The acceptance probability is computed to be 1.002; hence, this design is still accepted for the search. However, the best design is still the first design.

After 50000 swaps, the following design is generated

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

The objective function of this design gives 97.67238, which is higher than the first design of 97.61799. This means this search did find a design that is better than the first design.

The best design from the previous search is used as the starting design of the search for the second stage. The second stage of the swapping is restricted to within the same tags. For this example, this first swap is between the animals “AR” and “AN”, which gives the following allocation of animals and treatment to runs and tags,

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

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

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

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

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

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

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

[7,] "AO" **"AR"** "AP" "AM"

[8,] **"AR"** "AO" "AM" "AP"

[9,] **"AN" "AN"** "AQ" "AQ"

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

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

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

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

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

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

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

[7,] "c" **"f"** "d" "a"

[8,] **"f"** "c" "a" "d"

[9,] **"b" "b"** "e" "e"

The objective function of this design gives 97.87345, which is better than the previous design is 97.67238. Hence, this design is both accepted for the search and stored as the best design.

After another 50000 swaps, the following design is generated

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

[1,] "AO" "AB" "AP" "AF"

[2,] "AB" "AO" "AF" "AP"

[3,] "AJ" "AE" "AM" "AI"

[4,] "AE" "AJ" "AI" "AM"

[5,] "AK" "AR" "AC" "AH"

[6,] "AR" "AK" "AH" "AC"

[7,] "AD" "AG" "AL" "AQ"

[8,] "AG" "AD" "AQ" "AL"

[9,] "AN" "AN" "AA" "AA"

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

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

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

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

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

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

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

[7,] "d" "a" "f" "e"

[8,] "a" "d" "e" "f"

[9,] "b" "b" "a" "a"

The objective function of this design gives 98.1892, which is the best design that has been founded so far.

This process of is then repeated with the lower starting temperature of 50, 25, 12.5 and till the tenth level of the simulated annealing algorithm is performed. This is discussed in Section 3 as the method called “accelerated cooling”. However, for this example, the accelerated cooling could not improve the best design founded in the first level with starting temperature of 100. Therefore, the best design founded in the first level can be considered as the optimal design for the experiment of the design parameters of

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

Phase 2 experiment – 9 runs and 4 tags.

**Analysis of this optimal design**

The animals allocation is the resulting design can be represented in matrix notation as follows,

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

[1,] "AO" "AB" "AP" "AF"

[2,] "AB" "AO" "AF" "AP"

[3,] "AJ" "AE" "AM" "AI"

[4,] "AE" "AJ" "AI" "AM"

[5,] "AK" "AR" "AC" "AH"

[6,] "AR" "AK" "AH" "AC"

[7,] "AD" "AG" "AL" "AQ"

[8,] "AG" "AD" "AQ" "AL"

[9,] "AN" "AN" "AA" "AA"

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,] "c" "b" "d" "f"

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

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

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

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

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

[7,] "d" "a" "f" "e"

[8,] "a" "d" "e" "f"

[9,] "b" "b" "a" "a"

For this new design, the treatment pairs are not always identical like before. The treatment pairs are “c” and “b”, “d” and “f”, “a” and “d”, “a” and “c”, “e” and “f”, “c” and “b”, “a” and “d”, “f” and “e” and “b” and “a”. 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 of this design 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.