**Write up on searching the optimal designs**

The current objective function for the simulated annealing method is to find the design with the highest average efficiency factor when allocating the animals of Phase 1 experiment to the runs and tags of the Phase 2 experiments.

The orthogonal projector for the within runs and tags stratum can be written as

The information matrix can be written as

where denotes the animal design matrix.

Since the design criterion is to maximise the average efficiency factor, the aim is to find that can compute the highest the average efficiency factor from the information matrix.

The simulated annealing method is repeated 10 times with different starting designs. The design with the highest treatment information is used as the optimal designs.

The rest of this write-up is on comparing two competing designs that were found using the method described. Both of these two designs have 2 treatments, 5 biological replicates, 2 technical replicates, 4 tags and 5 runs.

**Design 1:**

The animal allocation for this design is shown below

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

[1,] "C" "B" "J" "A"

[2,] "G" "G" "D" "D"

[3,] "F" "F" "I" "I"

[4,] "H" "H" "E" "E"

[5,] "B" "C" "A" "J"

the rows and columns are corresponding the runs and tags, respectively.

The incidence matrix of animal and run is

> print((N = with(design.df, table(Ani, Run))))

Run

Ani 1 2 3 4 5

A 1 0 0 0 1

B 1 0 0 0 1

C 1 0 0 0 1

D 0 2 0 0 0

E 0 0 0 2 0

F 0 0 2 0 0

G 0 2 0 0 0

H 0 0 0 2 0

I 0 0 2 0 0

J 1 0 0 0 1

This matrix shows that this design is a non-binary design. The binary design is a design where no treatment, in this case animal, occurs more than once in any block, or in this case runs. The non-binary design can be observed for Animal D, E, F, G and H. The binary design can be observed for Animal A, B, C and J. Hence, animals can be split into two groups, which mean this design is disconnected.

The disconnectedness can also be observed in the concurrence matrix of animals and run which is

> cat("Animal concurrence matrix:\n")

Animal concurrence matrix:

> print(N %\*% t(N))

Ani

Ani A B C D E F G H I J

A 2 2 2 0 0 0 0 0 0 2

B 2 2 2 0 0 0 0 0 0 2

C 2 2 2 0 0 0 0 0 0 2

D 0 0 0 4 0 0 4 0 0 0

E 0 0 0 0 4 0 0 4 0 0

F 0 0 0 0 0 4 0 0 4 0

G 0 0 0 4 0 0 4 0 0 0

H 0 0 0 0 4 0 0 4 0 0

I 0 0 0 0 0 4 0 0 4 0

J 2 2 2 0 0 0 0 0 0 2

where the grouping of the Animal A, B, C and J and Animal D, E, F, G and H can be seen.

This design generated 6 canonical efficiency factors all unity, for the animals in the within runs stratum. The average efficiency factor is also 1. This also means 3 out of 9 degrees of freedom (DF) for animals are in the between runs stratum.

The six eigenvectors that corresponding to the canonical efficiency factors are

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

[1,] 0.0000000 0.0000000 0.0000000 0.8660254 0.0000000 0.0000000

[2,] -0.2677175 0.0000000 0.0000000 -0.2886751 0.0000000 0.7713586

[3,] -0.5341574 0.0000000 0.0000000 -0.2886751 0.0000000 -0.6175294

[4,] 0.0000000 0.0000000 0.7071068 0.0000000 0.0000000 0.0000000

[5,] 0.0000000 0.0000000 0.0000000 0.0000000 -0.7071068 0.0000000

[6,] 0.0000000 0.7071068 0.0000000 0.0000000 0.0000000 0.0000000

[7,] 0.0000000 0.0000000 -0.7071068 0.0000000 0.0000000 0.0000000

[8,] 0.0000000 0.0000000 0.0000000 0.0000000 0.7071068 0.0000000

[9,] 0.0000000 -0.7071068 0.0000000 0.0000000 0.0000000 0.0000000

[10,] 0.8018748 0.0000000 0.0000000 -0.2886751 0.0000000 -0.1538291

These eigenvectors show which contrasts are used to define the between animals stratum in the within runs stratum, these contrasts are:

Animal J versus Animal B and C,

Animal F versus Animal I,

Animal D versus Animal G,

Animal A versus Animal B, C and J,

Animal H versus Animal E,

Animal B versus Animal C and J.

The theoretical ANOVA table that is generated from this design is as follows

> summary.aov.twoPhase(design.df, blk.str2 = "Run", blk.str1 = "Ani", trt.str = "Tag + Trt")

$ANOVA

DF e Ani Run

Between Run

Between Ani 3 1 2 4

Residual 1 1 0 4

Within

Between Ani

Tag 1 1 2 0

Trt 1 1 2 0

Residual 4 1 2 0

Residual

Tag 2 1 0 0

Residual 7 1 0 0

$EF

Tag Trt eff.Tag eff.Trt

Between Run

Between Ani

Residual

Within

Between Ani

Tag 5 2/5 1 1/25

Trt 48/5 24/25

Residual

Tag 5 1

A valid F-test for the treatment differences can be directly conducted for this design. The treatment is still partially confounded with tags, and there is 24/25 of treatment information remaining.

In summary, this design provides a valid F-test for the treatment differences, because both the treatment and residual EMS, in the between animals within runs stratum, contains variance components of . The variances of the treatment effects can also be estimated directly from the residual mean squares divided by the treatment replication number. However, the treatment effects are still confounded with tag effects, but this shows that there is still 96% of pure treatment information remaining. In addition, this design is disconnected, because 3 DF of animals are in the between runs stratum which leaves 6 DF of animals in the with runs stratum.

**Design 2:**

The animal allocation of this design is shown below

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

[1,] "H" "D" "C" "A"

[2,] "C" "B" "F" "G"

[3,] "J" "H" "E" "F"

[4,] "A" "E" "I" "B"

[5,] "I" "G" "D" "J"

the rows and columns are corresponding the runs and tags, respectively.

The incidence matrix of animals and runs is

> print((N = with(design.df, table(Ani, Run))))

Run

Ani 1 2 3 4 5

A 1 0 0 1 0

B 0 1 0 1 0

C 1 1 0 0 0

D 1 0 0 0 1

E 0 0 1 1 0

F 0 1 1 0 0

G 0 1 0 0 1

H 1 0 1 0 0

I 0 0 0 1 1

J 0 0 1 0 1

The incidence matrix shows that this design is a binary design.

The concurrence matrix of animals and runs is

> cat("Animal concurrence matrix:\n")

Animal concurrence matrix:

> print(N %\*% t(N))

Ani

Ani A B C D E F G H I J

A 2 1 1 1 1 0 0 1 1 0

B 1 2 1 0 1 1 1 0 1 0

C 1 1 2 1 0 1 1 1 0 0

D 1 0 1 2 0 0 1 1 1 1

E 1 1 0 0 2 1 0 1 1 1

F 0 1 1 0 1 2 1 1 0 1

G 0 1 1 1 0 1 2 0 1 1

H 1 0 1 1 1 1 0 2 0 1

I 1 1 0 1 1 0 1 0 2 1

J 0 0 0 1 1 1 1 1 1 2

From this concurrence matrix, we cannot identify any grouping for the animals. Hence, the design of assigning the animals to the runs is connected. This design generated 9 canonical efficiency factors, of animals in the in the within runs stratum, which are 1, 1, 1, 1, 1, 0.625, 0.625, 0.625 and 0.625. The average efficiency factor is 0.7895. This means all 9 DF for animals are all in the within runs stratum for this design, which means this design of assigning animals to runs is connected. However, 4 out of 9 DF for animal only have 0.625 of the information in the within runs stratum.

The 5 eigenvectors that corresponds to the canonical efficiency factors of 1 are as follows

[,1] [,2] [,3] [,4] [,5]

[1,] 0.00000000 0.00000000 0.7071068 0.00000000 0.000000000

[2,] 0.02623528 -0.60295100 -0.2357023 0.04603466 -0.279440609

[3,] -0.56604347 0.21818314 -0.2357023 0.02459607 0.275373170

[4,] 0.40109722 -0.18645157 -0.2357023 0.48364488 0.122020110

[5,] -0.05338366 0.45203485 -0.2357023 0.47523027 -0.106842450

[6,] 0.42824561 -0.03553542 0.2357023 -0.03762005 0.508303169

[7,] 0.11156259 0.42030329 0.2357023 -0.03301068 -0.504235729

[8,] 0.16494625 -0.03173156 -0.2357023 -0.50824095 -0.397393280

[9,] 0.02714839 0.15091615 -0.2357023 -0.52126493 0.386283059

[10,] -0.53980820 -0.38476787 0.2357023 0.07063073 -0.004067439

The 4 eigenvectors that corresponds to the canonical efficiency factors of 0.625 are as follows

[,6] [,7] [,8] [,9]

[1,] 0.0000000 0.6324555 0.00000000 0.000000000

[2,] 0.3958888 0.1054093 -0.08432074 -0.474395389

[3,] 0.5302496 0.1054093 -0.01415851 0.327908233

[4,] -0.2008050 0.1054093 -0.34479577 0.479251608

[5,] -0.2622641 0.1054093 0.39403539 -0.406008030

[6,] 0.2679854 -0.4216370 0.37987688 -0.078099797

[7,] 0.1950837 -0.4216370 -0.42911651 0.004856219

[8,] -0.1279033 0.1054093 0.46419762 0.396295591

[9,] -0.3351659 0.1054093 -0.41495800 -0.323052013

10,] -0.4630692 -0.4216370 0.04923963 0.073243578

The theoretical ANOVA table that is generated from this design is as follows

$ANOVA

DF e Ani Run

Between Run

Between Ani

Trt 1 1 3/4 4

Residual 3 1 3/4 4

Within

Between Ani

Tag 3 1 24/13 0

Trt 1 1 1999/1092 0

Residual 5 1 641/420 0

Residual

Tag 3 1 0 0

Residual 3 1 0 0

$EF

Tag Trt eff.Tag eff.Trt

Between Run

Between Ani

Trt 1 1/10

Within

Between Ani

Tag 78/47 12/13 78/235 6/65

Trt 105/13 21/26

Residual

Tag 477/152 477/760

The theoretical ANOVA table shows that the F-test for the treatment group differences may not be valid here, because the coefficients of the animal variance components are not identical. The treatment is also partially confounded with tags, which leaves (21/26 =) 0.8076 of treatment information remaining.

In summary, design 2 does not provide a valid F-test for the treatment differences, because the coefficients of between animals variance components, , are not identical. A valid F-test can be conducted by adjusted the coefficients of between animals variance components from the linear combination of the residual in the within animals within runs stratum. The DF for the newly estimated EMS is approximated using the mean squares from the experimental results. Hence, the variances of the treatment effects cannot be estimated directly for this design. This design is connected design where all nine DF of animals are in the within runs stratum. However, four of nine DF only have 0.625 animal information in the within runs stratum. In additional, treatment is also confounded with tag, from the theoretical ANOVA table, there is 0.8076 of pure treatment information which is lower than the previous design.

**Design 2 with manually fitted the contrasts:**

For the second design, we now fit the ANOVA model using the eigenvectors that were computed while calculating the canonical efficiency factors. Note that the canonical efficiency factors are 5 1’s and 4 0.625’s. Hence, we can construct two contrast matrices, denote by A1 and A2, which corresponds to the canonical efficiency factors of 1 and 0.625, respectively, by combining their associated eigenvectors. These two contrast matrices are then fitted into the ANOVA model to facilitate the decomposition of the Phase 1 block stratum. The new theoretical ANOVA table is shown below.

> summary.aov.twoPhase(design.df, blk.str2 = "Run", blk.str1 = "Ani",

+ trt.str = "Tag + Trt", blk.contr = blk.contr)

$ANOVA

DF e Ani Run

Between Run

Between Ani.A2

Trt 1 1 3/4 4

Residual 3 1 3/4 4

Within

Between Ani.A1

Tag 3 1 2 0

Trt 1 1 2 0

Residual 1 1 2 0

Between Ani.A2

Tag 1 1 5/4 0

Trt 1 1 5/4 0

Residual 2 1 5/4 0

Residual

Tag 3 1 0 0

Residual 3 1 0 0

$EF

Tag Trt eff.Tag eff.Trt

Between Run

Between Ani.A2

Trt 1 1/10

Within

Between Ani.A1

Tag 18/17 4/3 18/85 2/15

Trt 6 3/5

Between Ani.A2

Tag 16/15 16/75

Trt 5/3 1/6

Residual

Tag 477/152 477/760

The theoretical ANOVA table shows that a valid F-test for the treatment group differences can be conducted. The treatment is still partially confounded with tags, which leaves (3/5 + 1/6 =) 0.7667 of pure treatment information.

In summary, fitting the ANOVA model by the contrast matrices using the eigenvectors provides a valid F-test for the treatment group differences, because the coefficients of the between animals variance component is identical. Hence, the variance of treatment difference can be estimated based on this theoretical ANOVA table. However, there are total of 3 DF for the residuals for conducting the F-test and estimate the variances, compare to the first design which has 4 DF. In additional, treatment is also confounded with tag, from the theoretical ANOVA table, there is 0.7667of pure treatment information which is still lower than the previous design.

Therefore, despite the first design is disconnected, for the purpose of conducting an experiment and testing for the treatment differences, the first design is more preferable. In addition, the variance component estimates of the animals in the between runs stratum can be recovered using the restricted maximum likelihood method.

**MS-optimality criterion**

The MS-optimality criterion is not used, because disconnected designs are also considered

The formula v(k-1)/k for computing the trace of the information matrix can only be used for the designs that are connected. Using this formula, the trace of both design 1 and 2 should be 7.5, but the trace of the information matrix for the disconnected design, i.e. design1, is 6.5. In addition, this disconnected design would be screen out based on the MS-optimality criterion, because its trace of the information matrix is lower than the trace of the information matrix for the connected design.

Therefore, I believe that I should search for the designs only base on A-optimality criterion which is the designs with the highest average efficiency factor.

**The modified objective function**

The modified objective function, which I believe can find the Design 1 relatively quickly, for the simulated annealing method is to find the design with the highest average efficiency factor when allocating the animals and treatments of Phase 1 experiment to the runs and tags of the Phase 2 experiments.

The orthogonal projector for the within runs and tags stratum can be written as

The information matrix of the animals in the within runs and tags stratum can be written as

where denotes the animal design matrix.

The information matrix of the treatments in the within runs and tags stratum can be written as

where denotes the treatment design matrix.

Since the design criterion is to maximise the average efficiency factor, the aim is to find and that can compute the highest the average efficiency factor from both the information matrix. Note the order of matrix will affect the order of matrix. These two average efficiency factors can be combined as , where and are the average efficiency factors of treatments and animals, and are weights of treatments and animals for calculating the combined average efficiency factors. I have decided to have to be 0.75 and to be 0.25, because I believe more emphasis should be on the average efficiency factor for assigning the animals to runs and tags to be close to 1 as much as possible. However, I still need to look into these weights further with different designs.

This method will maximise the amount of both the animal and treatment information in the within runs and tags stratum, this should in fact minimise the confounding of animals and treatments effects with runs and tags effects.

This simulated annealing method is repeated 10 times with different starting designs. The initial temperature for the simulated annealing is 0.1. Number of iteration is 100000.

The table of the optimal designs that were found using the searching method described above is in a separate document called “new Summary table of optimal designs.docx”.

The table consists the design parameters of two treatment groups and 2-9 biological replicates in the first phase experiments and further divided to two and three technical replicates for the 4-plex system for the second phase experiments.

I found that when the experiment uses two technical replicates and the 4-plex system, it is best to have even number of biological replicates to be able to conduct the test with the 100% of the treatment information in the within runs stratum.

The experiments comparing two treatment groups with three technical replicates using the 4-plex system does not work very well except with two biological replicates, because the canonical efficiency factors of animals in the within runs stratum are not identical. Therefore, a valid test for the treatment difference cannot be directly conducted.