**Example 2: assigning completely randomised design to randomised bock design**

**(Phase 1: 6 animals, 2 treatments, Phase 2: 6 MS runs and 4-plex iTRAQ tag system)**

For this case, the optimisation process obtained the best average efficiency factor for the animal information in the within runs stratum is 0.8936755. This average efficiency factor is calculated from the harmonic mean of the canonical efficiency factors of 1.0000, 0.9375, 0.9375, 0.8125 and 0.8125.

The eigenvectors, which corresponds to the five canonical efficiency factors described above, are

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

[1,] -0.4082483 0.4337891 -0.3809991 -0.50534565 -0.2792116

[2,] 0.4082483 0.4337891 -0.3809991 0.50534565 0.2792116

[3,] 0.4082483 0.1130603 0.5661720 -0.01086849 -0.5772480

[4,] 0.4082483 -0.5468495 -0.1851729 -0.49447715 0.2980364

[5,] -0.4082483 -0.5468495 -0.1851729 0.49447715 -0.2980364

[6,] -0.4082483 0.1130603 0.5661720 0.01086849 0.5772480

Based on the current example and the criteria described, the best animal design was found to be the one in a matrix notation below. In this matrix, the rows denote the MS runs and columns denote the tags.

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

[1,] "D" "E" "F" "C"

[2,] "A" "B" "D" "F"

[3,] "F" "A" "B" "E"

[4,] "B" "C" "A" "D"

[5,] "C" "D" "E" "B"

[6,] "E" "F" "C" "A"

The animal incidence matrix for this design can be written as below

> with(design.df, table(Ani, Run))

Run

Ani 1 2 3 4 5 6

A 1 1 0 1 0 1

B 1 0 1 1 0 1

C 1 0 1 1 1 0

D 0 1 1 0 1 1

E 0 1 0 1 1 1

F 1 1 1 0 1 0

The animal concurrence matrix for this design can be written as below

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

> N %\*% t(N)

Ani

Ani A B C D E F

A 4 3 2 2 3 2

B 3 4 3 2 2 2

C 2 3 4 2 2 3

D 2 2 2 4 3 3

E 3 2 2 3 4 2

F 2 2 3 3 2 4

The treatment design for Phase 2 experiment can be made from the animal and treatment allocation of the Phase 1 experiment as shown below

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

[1,] "controlled" "controlled" "controlled" "diseased"

[2,] "controlled" "diseased" "controlled" "diseased"

[3,] "controlled" "controlled" "diseased" "diseased"

[4,] "diseased" "diseased" "diseased" "controlled"

[5,] "diseased" "controlled" "diseased" "controlled"

[6,] "diseased" "diseased" "controlled" "controlled"

The theoretical ANOVA table for this two-phase experiment with the animal and treatment allocation described can be shown as below

$ANOVA

DF e Ani Run

Between Run

Between Ani

Trt 1 1 1/2 4

Residual 3 1 1/2 4

Residual 1 1 0 4

Within

Between Ani

Trt 1 1 81/22 0

Residual 4 1 315/88 0

Residual

Tag 3 1 0 0

Residual 10 1 0 0

$EF

Trt Tag eff.Trt eff.Tag

Between Run

Between Ani

Trt 1 1/12

Residual

Within

Between Ani

Trt 11 11/12

Residual

Tag 6 1

Note this optimisation process, 0.8936755 of animal information is found in the within runs stratum, hence it is expected that treatment information may also be separated across different strata. This theoretical ANOVA table shows **11/12** of treatment information is in the within runs stratum. In addition, the coefficients of animal variance components are not identical for the treatment and the residual in the between animals within runs stratum. Hence, to test for the differences between the treatment groups, the coefficients of animal variance components needs to be adjusted.

**Example 3: assigning completely randomised design to randomised bock design**

**(Phase 1: 9 animals, 3 treatments, Phase 2: 9 MS runs and 4-plex iTRAQ tag system)**

For this case, the optimisation process obtained the best average efficiency factor for the animal information in the within runs stratum is **0.8340019**. This average efficiency factor is calculated from the harmonic mean of the canonical efficiency factors of 0.9924616, 0.9924616, 0.853294, 0.853294, 0.7792444, 0.7792444, 0.75 and 0.75.

The eigenvectors, which corresponds to the canonical efficiency factors described above, are

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

[1,] 0.1925810 -0.43027293 0.44854602 0.1450127 -0.46974696 -0.03949703 0.4670661 -0.06380834

[2,] -0.4689178 0.04835646 -0.09868832 -0.4609586 0.26907891 -0.38706429 0.4670661 -0.06380834

[3,] -0.3902948 -0.26437133 0.25039382 0.3994060 0.42790897 0.19777799 -0.2887927 -0.37258692

[4,] 0.2763368 0.38191647 -0.34985770 0.3159459 0.20066805 0.42656132 0.4670661 -0.06380834

[5,] -0.0338049 0.47019087 0.22069884 -0.4165504 -0.38523525 0.27169105 -0.2887927 -0.37258692

[6,] -0.1290485 -0.45339684 -0.37189812 -0.2896791 -0.12046768 0.45575186 -0.1782734 0.43639525

[7,] 0.4571775 0.11493911 0.43681856 -0.1772336 0.45492653 -0.12354786 -0.1782734 0.43639525

[8,] 0.4240997 -0.20581953 -0.47109266 0.0171444 -0.04267372 -0.46946904 -0.2887927 -0.37258692

[9,] -0.3281289 0.33845773 -0.06492044 0.4669128 -0.33445885 -0.33220401 -0.1782734 0.43639525

Based on the current example and the criteria described, the best animal design was found to be the one in a matrix notation below. In this matrix, the rows denote the MS runs and columns denotes the tags.

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

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

[2,] "A" "C" "E" "D"

[3,] "I" "A" "G" "B"

[4,] "G" "I" "H" "C"

[5,] "F" "D" "A" "I"

[6,] "C" "G" "F" "E"

[7,] "B" "F" "D" "G"

[8,] "H" "E" "I" "F"

[9,] "E" "H" "B" "A"

The animal incidence matrix for this design can be written as below

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

Run

Ani 1 2 3 4 5 6 7 8 9

A 0 1 1 0 1 0 0 0 1

B 1 0 1 0 0 0 1 0 1

C 1 1 0 1 0 1 0 0 0

D 1 1 0 0 1 0 1 0 0

E 0 1 0 0 0 1 0 1 1

F 0 0 0 0 1 1 1 1 0

G 0 0 1 1 0 1 1 0 0

H 1 0 0 1 0 0 0 1 1

I 0 0 1 1 1 0 0 1 0

The animal concurrence matrix for this design can be written as below

> N %\*% t(N)

Ani

Ani A B C D E F G H I

A 4 2 1 2 2 1 1 1 2

B 2 4 1 2 1 1 2 2 1

C 1 1 4 2 2 1 2 2 1

D 2 2 2 4 1 2 1 1 1

E 2 1 2 1 4 2 1 2 1

F 1 1 1 2 2 4 2 1 2

G 1 2 2 1 1 2 4 1 2

H 1 2 2 1 2 1 1 4 2

I 2 1 1 1 1 2 2 2 4

The treatment design for Phase 2 experiment can be made from the animal and treatment allocation of the Phase 1 experiment as shown below

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

[1,] "controlled" "diseased" "treated" "diseased"

[2,] "controlled" "treated" "diseased" "controlled"

[3,] "treated" "controlled" "controlled" "diseased"

[4,] "controlled" "treated" "diseased" "treated"

[5,] "treated" "controlled" "controlled" "treated"

[6,] "treated" "controlled" "treated" "diseased"

[7,] "diseased" "treated" "controlled" "controlled"

[8,] "diseased" "diseased" "treated" "treated"

[9,] "diseased" "diseased" "diseased" "controlled"

The theoretical ANOVA table for this two-phase experiment with the animal and treatment allocation described can be shown as below

$ANOVA

DF e Ani Run

Between Run

Between Ani

Trt 2 1 13/15 4

Residual 6 1 49/90 4

Within

Between Ani

Trt 2 1 1931/559 0

Residual 6 1 4698/1403 0

Residual

Tag 3 1 0 0

Residual 16 1 0 0

$EF

Trt Tag eff.Trt eff.Tag

Between Run

Between Ani

Trt 45/28 15/112

Within

Between Ani

Trt 1677/164 559/656

Residual

Tag 9 1

Note this optimisation process, 0.8340019 of animal information is found in the within runs stratum, hence it is expected that treatment information may also be separated across different strata. This theoretical ANOVA table shows **559/656 (= 0.8521341)** of treatment information is in the within runs stratum. In addition, the coefficients of animal variance components are not identical for the treatment and the residual in the between animals within runs stratum. Hence, to test for the differences between the treatment groups, the coefficients of animal variance components needs to be adjusted.

**Example 4: assigning completely randomised design to randomised bock design**

**(Phase 1: 12 animals, 2 treatments, Phase 2: 9 MS runs and 4-plex iTRAQ tag system)**

The highest average efficiency factor that was found is 0.7833828 and is computed from the harmonic mean of the canonical efficiency factors: 0.8888889, 0.8888889, 0.8888889, 0.75, 0.75, 0.75, 0.75, 0.75, 0.75, 0.75 and 0.75.

Animal design:

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

[1,] "D" "G" "C" "B"

[2,] "A" "K" "D" "F"

[3,] "I" "F" "L" "C"

[4,] "C" "J" "H" "K"

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

[6,] "L" "B" "A" "J"

[7,] "K" "L" "E" "G"

[8,] "H" "I" "G" "A"

[9,] "B" "H" "F" "E"

Animal incidence matrix:

Run

Ani 1 2 3 4 5 6 7 8 9

A 0 1 0 0 0 1 0 1 0

B 1 0 0 0 0 1 0 0 1

C 1 0 1 1 0 0 0 0 0

D 1 1 0 0 1 0 0 0 0

E 0 0 0 0 1 0 1 0 1

F 0 1 1 0 0 0 0 0 1

G 1 0 0 0 0 0 1 1 0

H 0 0 0 1 0 0 0 1 1

I 0 0 1 0 1 0 0 1 0

J 0 0 0 1 1 1 0 0 0

K 0 1 0 1 0 0 1 0 0

L 0 0 1 0 0 1 1 0 0

Animal concurrence matrix:

Ani

Ani A B C D E F G H I J K L

A 3 1 0 1 0 1 1 1 1 1 1 1

B 1 3 1 1 1 1 1 1 0 1 0 1

C 0 1 3 1 0 1 1 1 1 1 1 1

D 1 1 1 3 1 1 1 0 1 1 1 0

E 0 1 0 1 3 1 1 1 1 1 1 1

F 1 1 1 1 1 3 0 1 1 0 1 1

G 1 1 1 1 1 0 3 1 1 0 1 1

H 1 1 1 0 1 1 1 3 1 1 1 0

I 1 0 1 1 1 1 1 1 3 1 0 1

J 1 1 1 1 1 0 0 1 1 3 1 1

K 1 0 1 1 1 1 1 1 0 1 3 1

L 1 1 1 0 1 1 1 0 1 1 1 3

Treatment design:

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

[1,] "b" "a" "a" "b"

[2,] "a" "a" "b" "b"

[3,] "a" "b" "b" "a"

[4,] "a" "b" "b" "a"

[5,] "a" "b" "b" "a"

[6,] "b" "b" "a" "b"

[7,] "a" "b" "a" "a"

[8,] "b" "a" "a" "a"

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

Based on this design, the theoretical ANOVA can be shown as below

$ANOVA

DF e Ani Run

Between Run

Between Ani

Trt 1 1 3/4 4

Residual 7 1 3/4 4

Within

Between Ani

Trt 1 1 87/32 0

Tag 3 1 91/32 0

Residual 7 1 9/4 0

Residual

Tag 3 1 0 0

Residual 13 1 0 0

$EF

Trt Tag eff.Trt eff.Tag

Between Run

Between Ani

Trt 2 1/9

Within

Between Ani

Trt 16 5/8 8/9 5/72

Tag 9/14 1/14

Residual

Tag 8 8/9

The model of the above theoretical ANOVA table is generated with the treatment fitted before the tag for the model. Since there is confounding between the treatment effects and tag effects, it can be shown from the above theoretical ANOVA table that treatment contains 5/72 of the tag information.

If the tag is fitted before the treatment, the theoretical ANOVA table becomes

$ANOVA

DF e Ani Run

Between Run

Between Ani

Trt 1 1 3/4 4

Residual 7 1 3/4 4

Within

Between Ani

Tag 3 1 3 0

Trt 1 1 9/4 0

Residual 7 1 9/4 0

Residual

Tag 3 1 0 0

Residual 13 1 0 0

$EF

Tag Trt eff.Tag eff.Trt

Between Run

Between Ani

Trt 2 1/9

Within

Between Ani

Tag 1 10 1/9 5/9

Trt 6 1/3

Residual

Tag 8 8/9

Despite that treatment does not have any tag information, but now there is only 1/3 of treatment information remains.

Alternatively, I have decided to perform simulated annealing algorithm for 100 times and find a design that has the most amount of the treatment information without confounding the tag.

**New design**

This design only has average efficiency factor of animal to run\*tag of 0.772973, which is lower than the previous design of 0.7833828. The average efficiency factor is computed from the harmonic mean of the canonical efficiency factors: 0.9912405, 0.9912405, 0.8888889 0.75, 0.75, 0.75, 0.75, 0.75, 0.75, 0.6476484 and 0.6476484.

Animal design:

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

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

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

[3,] "K" "J" "F" "B"

[4,] "L" "K" "G" "I"

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

[6,] "H" "I" "A" "F"

[7,] "J" "C" "L" "A"

[8,] "E" "L" "B" "H"

[9,] "A" "B" "D" "G"

Animal incidence matrix:

Run

Ani 1 2 3 4 5 6 7 8 9

A 0 0 0 0 0 1 1 0 1

B 0 0 1 0 0 0 0 1 1

C 1 1 0 0 0 0 1 0 0

D 1 0 0 0 1 0 0 0 1

E 0 1 0 0 1 0 0 1 0

F 0 1 1 0 0 1 0 0 0

G 0 1 0 1 0 0 0 0 1

H 1 0 0 0 0 1 0 1 0

I 0 0 0 1 1 1 0 0 0

J 0 0 1 0 1 0 1 0 0

K 1 0 1 1 0 0 0 0 0

L 0 0 0 1 0 0 1 1 0

Animal concurrence matrix:

Ani

Ani A B C D E F G H I J K L

A 3 1 1 1 0 1 1 1 1 1 0 1

B 1 3 0 1 1 1 1 1 0 1 1 1

C 1 0 3 1 1 1 1 1 0 1 1 1

D 1 1 1 3 1 0 1 1 1 1 1 0

E 0 1 1 1 3 1 1 1 1 1 0 1

F 1 1 1 0 1 3 1 1 1 1 1 0

G 1 1 1 1 1 1 3 0 1 0 1 1

H 1 1 1 1 1 1 0 3 1 0 1 1

I 1 0 0 1 1 1 1 1 3 1 1 1

J 1 1 1 1 1 1 0 0 1 3 1 1

K 0 1 1 1 0 1 1 1 1 1 3 1

L 1 1 1 0 1 0 1 1 1 1 1 3

Based on this design, the theoretical ANOVA can be shown as below

$ANOVA

DF e Ani Run

Between Run

Between Ani

Trt 1 1 3/4 4

Residual 7 1 3/4 4

Within

Between Ani

Trt 1 1 87/32 0

Tag 3 1 715837/283360 0

Residual 7 1 39029/16357 0

Residual

Tag 3 1 0 0

Residual 13 1 0 0

$EF

Trt Tag eff.Trt eff.Tag

Between Run

Between Ani

Trt 2 1/9

Within

Between Ani

Trt 16 1/8 8/9 1/72

Tag 26565/23246 8855/69738

Residual

Tag 39/5 13/15

The treatment is still confounded with the tag effects; it can be shown from the above theoretical ANOVA table that treatment contains 1/72 of the tag information. Note that it is lower than the previous design of 5/72.

If the tag is fitted before the treatment, the theoretical ANOVA table becomes

$ANOVA

DF e Ani Run

Between Run

Between Ani

Trt 1 1 3/4 4

Residual 7 1 3/4 4

Within

Between Ani

Tag 3 1 89/35 0

Trt 1 1 94533/35420 0

Residual 7 1 39029/16357 0

Residual

Tag 3 1 0 0

Residual 13 1 0 0

$EF

Tag Trt eff.Tag eff.Trt

Between Run

Between Ani

Trt 2 1/9

Within

Between Ani

Tag 105/89 54/35 35/267 3/35

Trt 506/35 253/315

Residual

Tag 39/5 13/15

With this design, amount of un-confounded treatment information is 253/315, which is much higher than the previous design of 1/3. Hence, this design may be better for performing test of differences between the treatment groups, because it has more treatment information in the between animals within runs stratum where the test is perform.

**Summary table**

**Table for 4-plex system**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Phase 1 Design parameters | | Phase 2 Design parameters | | Average efficiency factors | |
| Treatments | Animals | Tags | Runs | Animal (Eliminate tag effects) | Treatment (Ignore/Eliminate tag effects) |
| 2 | 6 | 4 | 6 | 0.8936755 | 0.9166667 |
| 2 | 10 | 4 | 10 | 0.8231707 | 0.9 |
| 2 | 12 | 4 | 9 | 0.7833828 | 0.8888889/ 0.3333333 |
| 2 | 12 | 4 | 9 | 0.772973 | 0.8888889/ 0.8031746 |
| 2 | 16 | 4 | 8 | 0.7568269 | 0.9375/ 0.8565476 |
|  |  |  |  |  |  |
| 3 | 9 | 4 | 9 | 0.8340019 | 0.8521341 |
| 3 | 12 | 4 | 9 | 0.7833828 | 0.7894737/ 0.6 |
| 3 | 12 | 4 | 9 | 0.7730699 | 0.8690476/ 0.7288772 |
|  |  |  |  |  |  |
| 4 | 12 | 4 | 9 | 0.7833828 | 0.7894737/ 0.6 |
| 4 | 12 | 4 | 9 | 0.7833828 | 0.8289392/ 0.4356436 |
| 4 | 12 | 4 | 9 | 0.7717573 | 0.8289392/ 0.7374482 |
| 4 | 16 | 4 | 8 | 0.7568269 | 0.5591182/ 0.3853879 |
| 4 | 16 | 4 | 8 | 0.6432813 | 0.8371229/0.745885 |