**Using Simulated annealing to search for optimal two-phase experimental designs**

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**Using Simulated annealing to search for optimal two-phase experimental designs**

**Introduction**

Studying the properties of different two-phase experiments can take a long time. This is because for a given experiment with a given number of treatments, blocks and replicates, there can be many different ways of assigning treatments and blocks from the first phase experiment to the blocks in the second phase. We refer to the numbers of treatments, blocks and replicates as the *design parameters*.

With a given set of the design parameters, we should be able to generate at least one design that is optimal with respect to some statistical criteria. This design is known as the optimal design. There are a number of different optimality criteria available for selecting the “best” two-phase design for a given set of design parameters. These criteria are ….

We will focus on identifying designs that satisfy the A-optimal criterion.

The searching method is based on simulated annealing algorithm ….

Finding the optimal designs for two-phase experiments

The aim is to develop a method for generating the optimal two-phase experimental design focusing on the multiplex proteomics experiments. In particular, we will consider experiments using the iTRAQ isobaric labelling technology which enables the simultaneous assaying of up to eight proteomic samples.

**Searching Methods**

**Simulated annealing**

This write up is on using the simulated annealing algorithm to find the optimal designs.

Simulated annealing was first described by …. metal annealing and temperature control.

Simulated annealing is a good method to solve the global optimization problem of locating a good approximation of the global optimum of a given function in a large space.

During the process of optimization of this method, the worsen case compare to the previous case may be accepted based on the acceptance probability defined by the user.

R function optim

Despite using the optim function, the optimization process is still very sensitive based on the temperature given by the user. This sensitivity issue can be noted when comparing the average efficiency factors, because the differences of the average efficiency factors can be very small.

Formula that described the simulated annealing

exp(-differences/temp)

The acceptance probability, i.e. the probability of the step is taken, from the optim function in R is chosen from a random number between 0 and 1.

Base on the formula, the temperature that is set is the amount of the difference from the wrong direction that can be tolerated.

Another parameter in optim is maxTemp, which is number of function that can be repeated under a same temperature,

**Optimisation Procedure**

Start with a treatment design matrix where the treatments are equally replicated.

The interchange algorithm is then randomly choose two rows of the treatment design matrix, i.e. two observations, then check the effect on the objection function from the this interchange take place.

Only keep the new design is the average efficiency factor is greater than the previous design.

The process repeats until the average efficiency factor cannot be maximised any further using the simulated annealing algorithm.

The simulated annealing also need to restart many times as like performing “jump” on the search space, which is also another way avoiding trap in the local maximum.

Hence, the method is finding the A-optimal design.

Maximise the average efficiency factors

Interchange algorithm is

The current simulated annealing algorithm search from the optimal design by random swapping any two rows of the treatment design matrix and maximised the average efficiency factors.

During the assignment for our MudPIT-iTRAQ experiment, note that number of the animals from Phase 1 need to be greater or equal to the number of tags. This is to avoid all the degrees of freedom from the animals to be confounded with the tags.

Repeat 100 times, this allows the preforming of the jump for the simulated annealing algorithm.

In additional, the average efficiency factors of the treatments can also ne compare out of these 100 design from the

**CRD with a single treatment factor under 4- and 8-plex proteomics experiments**

**Example 1: assigning completely randomised design to randomised bock design**

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

Phase 1 design consists of 4 animals randomly assigned to 2 treatment groups: controlled and diseased groups, where animals A and C are assigned to controlled group and animals B and D are assigned to diseased group.

The theoretical ANOVA table showing the coefficients of the variance components for the Phase 1 experiment can be written as

DF Ani

Between Ani

Trt 1 1

Residual 2 1

The design for the second phase experiment aims to assign the animal in the first phase to the block structure in the second phase.

Suppose the second phase experiment uses 4 MS runs and 4-plex iTRAQ tag system.

The aim of the animal allocation is to minimise the level of confounding between animal effects with both runs and tag effects.

Another way of describing the above point is to maximise the amount of information in the within runs and tags stratum, hence the second phase design can be considered as a row-column design.

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, hence the aim is to find that can compute the highest the average efficiency factor from the information matrix.

Based on the current example and the criteria described, the best animal design was found to be the one below

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 1 | 2 | 3 | 4 |
| 1 | D | C | B | A |
| 2 | A | B | C | D |
| 3 | B | D | A | C |
| 4 | C | A | D | B |

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

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 1 | 2 | 3 | 4 |
| 1 | Diseased | Controlled | Diseased | Controlled |
| 2 | Controlled | Diseased | Controlled | Diseased |
| 3 | Diseased | Diseased | Controlled | Controlled |
| 4 | Controlled | Controlled | Diseased | Diseased |

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 3 1 0 4

Within

Between Ani

Trt 1 1 4 0

Residual 2 1 4 0

Residual

Tag 3 1 0 0

Residual 6 1 0 0

$EF

Trt Tag eff.Trt eff.Tag

Between Run

Within

Between Ani

Trt 8 1

Residual

Tag 4 1

This theoretical ANOVA table shows all the animal and treatment information are in the within runs stratum from the optimisation procedure described here. In addition, tag does not confound with the animals.

**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 |
| 3 | 9 | 4 | 9 | 0.8340019 | 0.8521341 |
| 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 |
|  |  |  |  |  |  |
| 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 |
|  |  |  |  |  |  |
| 2 | 16 | 4 | 8 | 0.7568269 | 0.9375/ 0.8565476 |
|  |  |  |  |  |  |
| 4 | 16 | 4 | 8 | 0.7568269 | 0.5591182/ 0.3853879 |
| 4 | 16 | 4 | 8 | 0.6432813 | 0.8371229/0.745885 |

**Table for 8-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 | 8 | 6 | 0.9746386 | 0.9791667 |
| 3 | 6 | 8 | 6 | 0.9746386 | 0.9763125 |
| 2 | 10 | 8 | 5 | 0.8576644 | 0.975/ 0.1875 |
| 2 | 10 | 8 | 5 | 0.8546439 | 0.95/ 0.7281949 |
|  |  |  |  |  |  |
| 2 | 12 | 8 | 6 | 0.8597194 | 0.875/ 0.5416667 |
| 2 | 12 | 8 | 6 | 0.8585185 | 0.9583333/ 0.7808727 |
|  |  |  |  |  |  |
| 3 | 12 | 8 | 6 | 0.8597194 | 0.9601753/ 0.5846875 |
| 3 | 12 | 8 | 6 | 0.8584483 | 0.9375/ 0.6662183 |
|  |  |  |  |  |  |
| 4 | 12 | 8 | 6 | 0.8597194 | 0.9259127/ 0.2620968 |
| 4 | 12 | 8 | 6 | 0.858276 | 0.9301471/ 0.4636582 |
|  |  |  |  |  |  |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| 2 | 16 | 8 | 6 | 0.8076017 | 0.9375/ 0.2859926 |
| 4 | 16 | 8 | 6 | 0.8076017 | 0.9213397/0.3345515 |
| 4 | 16 | 8 | 8 | 0.8610785 | 0.9313921/0.4318623 |
| 4 | 16 | 8 | 8 | 0.8614311 | 0.909049/0.3706903 |
|  |  |  |  |  |  |

RBD to RBD

Cage/Ani to Run Tag