**Studying the patterns of the designs with 2 treatment groups with 4-plex experiments**

Using simulated annealing, some optimal designs have found [for which set of design parameters? You need to say this upfront.]. This write-up describes the patterns [in which part(s) of the designs?] of these designs. If patterns are identfied, it can be a good starting point [why only a starting point? Recall our discussion about the design search space?] to develop and define a generic method for designing the two-phase MudPIT-iTRAQ experiments.

The most trivial designs for the MudPIT-iTRAQ two-phase experiments are those with 2 treatment groups and two technical replicates [no mention of biological reps??? As written, what does this mean?]with four-plex system. The patterns [which patterns? You haven’t yet said that you’ve found any patterns. The way your first paragraph is written it doesn’t say that you actually have found any patterns.] can then be divided into two main groups based on number of biological replicates from the first phase experiments: a) designs with even numbers of biological replicates [now you mention biological replicates but you haven’t mentioned them before.] and b) designs odd number of biological replicates.

For designs with 2 treatment groups and two technical replicates with four-plex system, the number of biological replicates is identical to the number of MudPIT runs for the second phase experiments.

For the rest of this write-up, the allocations [which allocations are you talking about?] are shown as a matrix where the rows correspond to the runs and columns correspond to the tags. In addition, the upper case letter denotes the animal ID and the lower case letter denotes the treatments. [This sentence is meaningless if you haven’t explained what you mean by allocations.]

a) An example of a design with an even number of biological replicates is four biological replicates. Animals A, C, E and G are assigned to treatment “a” and Animals B, D, F and H are assigned to treatment “b”.

The animal allocation is as follows:

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

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

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

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

[4,] "F" "E" "H" "G"

The animal effects are confounded with both tags and runs. [Explain why! You are expecting your reader to see this immediately.] In particular, the animal effects is completely confounded with the run contrast of 1, 2 versus 3, 4 and the tag contrast of 114, 115 versus 116, 117.

The treatment allocation is as follows:

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

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

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

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

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

The treatment effects are orthogonal to both runs and tags. [Why? Explain to your reader.]

The theoretical ANOVA table for the animal and treatment designs shown above is as follows:

$ANOVA

DF e Ani Run

Between Run

Between Ani 1 1 2 4

Residual 2 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 4 1 0 0

$EF

Tag Trt eff.Tag eff.Trt

Between Run

Between Ani

Residual

Within

Between Ani

Tag 4 1

Trt 8 1

Residual

Tag 4 1

All the treatment information stays intact in the between animals stratum. [Explain in words what it is in the above anova that allows you to say this!] However, 1 DF associated with animal effects is in the between runs stratum and 1 DF of tag [fix as shown for animal] is confounded with 1 DF of the between animals stratum.

b) An example of design with odd number of biological replicates is five biological replicates. Animal A, C, E, G and I are assigned to treatment “a” and Animal B, D, F, H and J are assigned to treatment “b”.

The animal allocation can be shown as follows,

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

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

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

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

[4,] "F" "E" "H" "G"

[5,] "I" "I" "J" "J"

The first four rows of the animal allocation are identical to the previous design with four biological replicates. The animal allocation of the last row is assigned by “preserving” the confounding between the tags and the animals, i.e. Animal I is assigned with the Animal A, B and E and Animal J is assigned with Animal C, D and H.

The treatment allocation can be shown as follows,

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

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

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

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

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

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

The treatment effect is confounded with the contrast of tag 114, 115 versus 116, 117.

The theoretical ANOVA table is shown as follows,

$ANOVA

DF e Ani Run

Between Run

Between Ani 2 1 2 4

Residual 2 1 0 4

Within

Between Ani

Tag 1 1 2 0

Trt 1 1 2 0

Residual 5 1 2 0

Residual

Tag 2 1 0 0

Residual 6 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

There is 1/25 of treatment information confounded with tag; hence, there is 24/25 of pure treatment information remaining. In addition, two DF of animal is in the between runs stratum and one DF of tag is confound with one DF of the between animals stratum.

The next set of designs to be described is the designs with 2 treatment groups, 3 technical replicates and four tags. For this set of designs, the number biological replicate has to be even to able to fit into the four-plex experiments.

The first design contains two biological replicates where Animal A and C are assigned to treatment “a” and Animal B and D are assigned to treatment “b”.

The animal allocation is shown as follows,

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

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

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

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

The animal is orthogonal to the runs. However, one tag contrast of 114, 115 versus 116, 117 is confounded with animals. The treatment allocation is then shown as follows,

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

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

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

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

The treatment effect is also confounded with the tag contrast of 114, 115 versus 116, 117.

The theoretical ANOVA table is shown as follows,

$ANOVA

DF e Ani Run

Between Run 2 1 0 4

Within

Between Ani

Tag 1 1 3 0

Trt 1 1 3 0

Residual 1 1 3 0

Residual

Tag 2 1 0 0

Residual 4 1 0 0

$EF

Tag Trt eff.Tag eff.Trt

Between Run

Within

Between Ani

Tag 3 2 1 1/3

Trt 4 2/3

Residual

Tag 3 1

There is 1/3 of treatment information confounded with tag; hence, there is 2/3 of pure treatment information remaining. In addition, one DF of tag is confound with one DF of the between animals stratum.

The second design contains four biological replicates where Animal A, C, E and G are assigned to treatment “a” and Animal B, D, F and H are assigned to treatment “b”.

The animal allocation is shown as follows,

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

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

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

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

[4,] "F" "G" "H" "E"

[5,] "H" "F" "G" "E"

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

The first three rows are identical to the previous animal allocation. The next three rows are assigned in a same way expect the last column, where the Animal E is assigned. This is because Animal E is perturbed by treatment “b” and Animal D is perturbed by treatment “a”. Hence, the treatment effect is orthogonal to the tag which can be shown in the treatment allocation as follows,

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

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

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

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

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

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

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

The theoretical ANOVA table is shown as follows,

$ANOVA

DF e Ani Run

Between Run

Between Ani 1 1 3 4

Residual 4 1 0 4

Within

Between Ani

Tag 1 1 3 0

Trt 1 1 3 0

Residual 4 1 3 0

Residual

Tag 2 1 0 0

Residual 10 1 0 0

$EF

Tag Trt eff.Tag eff.Trt

Between Run

Between Ani

Residual

Within

Between Ani

Tag 6 1

Trt 12 1

Residual

Tag 6 1

All the treatment information stays intact in the between animals stratum. However, one DF of animal is in the between runs stratum and one DF of tag is confound with one DF of the between animals stratum.

The next set of designs to be described is the designs with 2 treatment groups, 4 technical replicates and four tags. The patterns can then be divided into two main groups based on number of biological replicates from the first phase experiments: a) designs with even number of biological replicates and b) designs odd number of biological replicates.

a) An example of design with even number of biological replicates is with 2 biological replicates where Animal A and C are assigned to treatment “a” and Animal B and D are assigned to treatment “b”.

The animal allocation is shown as follows,

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

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

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

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

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

This design is perfect design for allocating 4 animals into a four runs and four tags, where the animal is orthogonal to both runs and tags. The treatment allocation is shown as follows,

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

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

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

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

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

The theoretical ANOVA table can be written as follows,

$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

Tag Trt eff.Tag eff.Trt

Between Run

Within

Between Ani

Trt 8 1

Residual

Tag 4 1

All the treatment information stays intact in the between animals stratum. In addition, all three DF of animals are in within runs stratum.

Another example of design with even number of biological replicates is with 4 biological replicates where Animal A, C, E and G are assigned to treatment “a” and Animal B, D, F and H are assigned to treatment “b”. The animal allocation is shown as follows

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

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

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

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

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

[5,] "E" "F" "G" "H"

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

[7,] "G" "H" "E" "F"

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

The first four rows are identical to the previous design with two biological replicates. The next four rows of design are assigned in a same way as the first four rows with Animal E, F, G and H.

The treatment allocation is shown as follows,

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

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

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

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

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

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

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

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

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

The theoretical ANOVA table can be written as follows,

$ANOVA

DF e Ani Run

Between Run

Between Ani 1 1 4 4

Residual 6 1 0 4

Within

Between Ani

Trt 1 1 4 0

Residual 5 1 4 0

Residual

Tag 3 1 0 0

Residual 15 1 0 0

$EF

Tag Trt eff.Tag eff.Trt

Between Run

Between Ani

Residual

Within

Between Ani

Trt 16 1

Residual

Tag 8 1

All the treatment information stays intact in the between animals stratum. However, one DF of animal is in the between runs stratum.

b) An example of design with odd number of biological replicates is with 3 biological replicates where Animal A, C and E are assigned to treatment “a” and Animal B, D and F are assigned to treatment “b”. The animal allocation is shown as follows,

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

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

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

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

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

[5,] "E" "E" "F" "F"

[6,] "F" "F" "E" "E"

The first four rows are identical to the previous design with two biological replicates. The remaining Animal E and F are assigned to the last two rows while making sure the treatment effects is still orthogonal to runs and tags. The treatment allocation is shown as follows,

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

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

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

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

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

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

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

The theoretical ANOVA table can be written as follows,

$ANOVA

DF e Ani Run

Between Run

Between Ani 1 1 4 4

Residual 4 1 0 4

Within

Between Ani

Trt 1 1 4 0

Residual 3 1 4 0

Residual

Tag 3 1 0 0

Residual 11 1 0 0

$EF

Tag Trt eff.Tag eff.Trt

Between Run

Between Ani

Residual

Within

Between Ani

Trt 12 1

Residual

Tag 6 1

All the treatment information stays intact in the between animals stratum. However, one DF of animal is in the between runs stratum.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Phase 1 Experiment | | Technical Rep | Number of observation | Phase 2 Experiment | | DF of Animal in the between Runs stratum | Tag orthogonal to Animal in the within runs stratum | DF of residual in between animals stratum | Tag orthogonal to Treatment | Animal | | Treatment |
| Treatment | Bio Rep | Runs | Tags | Canonical Eff Factor | Average Eff Factor | Average Eff Factor |
| 2 | 2 | 2 | 8 | 2 | 4 | 0 | No (1 DF) | 1 | Yes | 1 (3) | 1 | 1 |
| 3 | 12 | 3 | 1 | No (1 DF) | 2 | No | 1 (4) | 1 | 0.8889 |
| 4 | 16 | 4 | 1 | No (1 DF) | 4 | Yes | 1 (6) | 1 | 1 |
| 5 | 20 | 5 | 2 | No (1 DF) | 5 | No | 1 (7) | 1 | 0.96 |
| 6 | 24 | 6 | 2 | No (1 DF) | 7 | Yes | 1 (9) | 1 | 1 |
| 7 | 28 | 7 | 3 | No (1 DF) | 8 | No | 1 (10) | 1 | 0.9796 |
| 8 | 32 | 8 | 3 | No (1 DF) | 10 | Yes | 1 (12) | 1 | 1 |
| 9 | 38 | 9 | 4 | No (1 DF) | 11 | No | 1 (13) | 1 | 0.9877 |
| 10 | 40 | 10 | 4 | No (1 DF) | 13 | Yes | 1 (15) | 1 | 1 |
| 2 | 3 | 12 | 3 | 0 | No (1 DF) | 1 | No | 1 (3) | 1 | 0.6667 |
| 4 | 24 | 6 | 1 | No (1 DF) | 4 | Yes | 1 (6) | 1 | 1 |
| 6 | 36 | 9 | 2 | No (1 DF) | 7 | No | 1 (9) | 1 | 0.9630 |
| 8 | 48 | 12 | 3 | No (1 DF) | 10 | Yes | 1 (12) | 1 | 1 |
| 10 | 50 | 15 | 4 | No (1 DF) | 13 | No | 1 (15) | 1 | 0.9867 |
| 2 | 4 | 16 | 4 | 0 | Yes | 2 | Yes | 1 (3) | 1 | 1 |
| 3 | 24 | 6 | 1 | Yes | 3 | Yes | 1 (4) | 1 | 1 |
| 4 | 32 | 8 | 1 | Yes | 5 | Yes | 1 (6) | 1 | 1 |
| 5 | 40 | 10 | 2 | Yes | 6 | Yes | 1 (7) | 1 | 1 |
| 6 | 48 | 12 | 2 | Yes | 8 | Yes | 1 (9) | 1 | 1 |
| 7 | 56 | 14 | 3 | Yes | 9 | Yes | 1 (10) | 1 | 1 |
| 8 | 64 | 16 | 3 | Yes | 11 | Yes | 1 (12) | 1 | 1 |
| 9 | 72 | 18 | 4 | Yes | 12 | Yes | 1 (13) | 1 | 1 |
| 10 | 80 | 20 | 4 | Yes | 13 | Yes | 1 (15) | 1 | 1 |

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Phase 1 Experiment | | Technical Rep | Number of observation | Phase 2 Experiment | | DF of Animal in the between Runs stratum | Tag orthogonal to Animal in the within runs stratum | DF of residual in between animals stratum | Tag orthogonal to Treatment | Animal | | Treatment |
| Treatment | Bio Rep | Runs | Tags | Canonical Eff Factor | Average Eff Factor | Average Eff Factor |
| 2 | 4 | 2 | 16 | 2 | 8 | 0 | No (3 DF) | 3 | Yes | 1 (7) | 1 | 1 |
| 6 | 24 | 3 | 1 | No (3 DF) | 6 | No | 1 (10) | 1 | 0.8889 |
| 8 | 32 | 4 | 1 | No (3 DF) | 10 | Yes | 1 (14) | 1 | 1 |
| 10 | 40 | 5 | 2 | No (3 DF) | 13 | Yes | 1 (17) | 1 | 0.96 |
| 4 | 3 | 24 | 3 | 0 | No (3 DF) | 3 | No | 1(7) | 1 | 0.6667 |
| 8 | 48 | 6 | 1 | No (3 DF) | 10 | Yes | 1(14) | 1 | 1 |
| 12 | 72 | 9 | 2 | No (3 DF) | 17 | No | 1(21) | 1 | 0.9630 |
| 2 | 4 | 16 | 2 | 0 | No (1 DF) | 1 | Yes | 1 (3) | 1 | 1 |
| 3 | 24 | 3 | 1 | No (1 DF) | 2 | No | 1 (4) | 1 | 0.8889 |
| 4 | 32 | 4 | 0 | No (1 DF) | 5 | Yes | 1(7) | 1 | 1 |
| 5 | 40 | 5 | 1 | No (1 DF) | 6 | No | 1(8) | 1 | 0.96 |
| 6 | 48 | 6 | 1 | No (1 DF) | 8 | Yes | 1(10) | 1 | 1 |
| 7 | 56 | 7 | 2 | No (1 DF) | 9 | No | 1 (11) | 1 | 0.9796 |
| 8 | 64 | 8 | 1 | No (1 DF) | 12 | Yes | 1 (14) | 1 | 1 |
| 9 | 72 | 9 | 2 | No (1 DF) | 13 | No | 1 (15) | 1 | 0.9877 |
| 10 | 80 | 10 | 2 | No (1 DF) | 15 | Yes | 1 (17) | 1 | 1 |