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

Applying the simulated annealing algorithm, some optimal designs have found. This write-up describes the patterns of these designs. From studying these patterns, it can be a good starting point to construct a generic method for designing the two-phase MudPIT-iTRAQ experiments.

The most trivial designs for the MudPIT-iTRAQ two-phase experiments are the designs with 2 treatment groups and two technical replicates with four-plex system. 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.

For the 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 run for the second phase experiments.

For the rest of this write-up, the allocations are illustrated 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.

a) The designs with even number of runs are shown as follows:

**For two biological replicates**

The animal allocation can be shown as follows

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

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

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

The contrast of Animal A and B versus C and D is completely confounded with the tag contrast of 114, 115 versus 116, 117.

Treatment allocation

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

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

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

Theoretical ANOVA table

$ANOVA

DF e Ani Run

Between Run 1 1 0 4

Within

Between Ani

Tag 1 1 2 0

Trt 1 1 2 0

Residual 1 1 2 0

Residual

Tag 2 1 0 0

Residual 1 1 0 0

$EF

Tag Trt eff.Tag eff.Trt

Between Run

Within

Between Ani

Tag 2 1

Trt 4 1

Residual

Tag 2 1

**For four biological replicates:**

Animal allocation

[,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 tag and runs. In particular, the animal effects is completely confounded with run contrasts of 1, 2 versus 3, 4 and tag 114, 115 versus 116, 117.

Treatment allocation

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

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

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

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

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

Theoretical ANOVA table

$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

For six biological replicates

Animal allocation

[,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" "J" "K" "L"

[6,] "J" "I" "L" "K"

describe

Treatment allocation

[,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"

Theoretical ANOVA table

$ANOVA

DF e Ani Run

Between Run

Between Ani 2 1 2 4

Residual 3 1 0 4

Within

Between Ani

Tag 1 1 2 0

Trt 1 1 2 0

Residual 7 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 6 1

Trt 12 1

Residual

Tag 6 1

Additional even number of runs will increase the degrees of freedom associated with residual mean square in the between animals stratum by 3.

The treatment information stays intact in the between animals stratum.

The tag will always confound with one DF of the between animals stratum.

**b) The designs with odd number of biological replicates are shown as follows**

**For three biological replicates**

Animal allocation

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

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

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

[3,] "E" "E" "F" "F"

describe

The animal allocation of the first two run is identical to the previous with two runs.

To perverse the same pattern of disconnectedness between the animals and tags, the animal E is allocated to the group of animal A and B and animal F is allocated to the group of animal C and D. The allocation can be shown in the last run of the experiment.

Treatment allocation

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

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

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

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

With odd number of runs, the treatment effects are unavoidable to be confounded with the tags.

Theoretical ANOVA table

$ANOVA

DF e Ani Run

Between Run

Between Ani 1 1 2 4

Residual 1 1 0 4

Within

Between Ani

Tag 1 1 2 0

Trt 1 1 2 0

Residual 2 1 2 0

Residual

Tag 2 1 0 0

Residual 3 1 0 0

$EF

Tag Trt eff.Tag eff.Trt

Between Run

Between Ani

Residual

Within

Between Ani

Tag 3 2/3 1 1/9

Trt 16/3 8/9

Residual

Tag 3 1

**For five biological replicates**

Animal allocation

[,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"

describe

Treatment allocation

[,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"

Theoretical ANOVA table

$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

**For seven biological replicates**

Animal allocation

[,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" "J" "K" "L"

[6,] "J" "I" "L" "K"

[7,] "M" "M" "N" "N"

describe

Treatment allocation

[,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" "a" "b" "b"

Theoretical ANOVA table

$ANOVA

DF e Ani Run

Between Run

Between Ani 3 1 2 4

Residual 3 1 0 4

Within

Between Ani

Tag 1 1 2 0

Trt 1 1 2 0

Residual 8 1 2 0

Residual

Tag 2 1 0 0

Residual 9 1 0 0

$EF

Tag Trt eff.Tag eff.Trt

Between Run

Between Ani

Residual

Within

Between Ani

Tag 7 2/7 1 1/49

Trt 96/7 48/49

Residual

Tag 7 1

Additional runs should increase the efficiency factor of treatment in the between animals stratum.

The next set of designs to be described are the designs with 2 treatment groups, 3 technical replicates and four tags, the patterns can be as follows

For these set of designs, the number biological replicate has to be even to able to fit into the four-plex experiments.

**For six biological replicates**

Animal allocation

[,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"

[7,] "I" "J" "K" "L"

[8,] "K" "I" "J" "L"

[9,] "J" "K" "I" "L"

describe

Treatment allocation

[,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"

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

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

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

Theoretical ANOVA table

$ANOVA

DF e Ani Run

Between Run

Between Ani 2 1 3 4

Residual 6 1 0 4

Within

Between Ani

Tag 1 1 3 0

Trt 1 1 3 0

Residual 7 1 3 0

Residual

Tag 2 1 0 0

Residual 16 1 0 0

$EF

Tag Trt eff.Tag eff.Trt

Between Run

Between Ani

Residual

Within

Between Ani

Tag 9 2/3 1 1/27

Trt 52/3 26/27

Residual

Tag 9 1

Additional 3 runs

> test.design(2, 6, 4, 4)

, , 1

[,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"

[9,] "I" "J" "K" "L"

[10,] "J" "I" "L" "K"

[11,] "K" "L" "I" "J"

[12,] "L" "K" "J" "I"

, , 2

[,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"

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

[10,] "b" "a" "b" "a"

[11,] "a" "b" "a" "b"

[12,] "b" "a" "b" "a"

$ANOVA

DF e Ani Run

Between Run

Between Ani 2 1 4 4

Residual 9 1 0 4

Within

Between Ani

Trt 1 1 4 0

Residual 8 1 4 0

Residual

Tag 3 1 0 0

Residual 24 1 0 0

$EF

Tag Trt eff.Tag eff.Trt

Between Run

Between Ani

Residual

Within

Between Ani

Trt 24 1

Residual

Tag 12 1

>

3 biological replicates

Any additional biological replicate will need two additional runs in the second phase experiment.

Animal allocation

[,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"

describe

The last two runs of the experiment is somewhat similar in the concepts of odd biological replicates and two technical replicates.

The difference in this case is that that tag should not be confounded with treatment, because the number of run should always be even.

Treatment allocation

[,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"

Theoretical ANOVA table

$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

> test.design(2, 5, 4, 4) #<-

, , 1

[,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"

[9,] "I" "I" "J" "J"

[10,] "J" "J" "I" "I"

, , 2

[,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"

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

[10,] "b" "b" "a" "a"

$ANOVA

DF e Ani Run

Between Run

Between Ani 2 1 4 4

Residual 7 1 0 4

Within

Between Ani

Trt 1 1 4 0

Residual 6 1 4 0

Residual

Tag 3 1 0 0

Residual 20 1 0 0

$EF

Tag Trt eff.Tag eff.Trt

Between Run

Between Ani

Residual

Within

Between Ani

Trt 20 1

Residual

Tag 10 1

>

> test.design(2, 7, 4, 4) #<-

, , 1

[,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"

[9,] "I" "J" "K" "L"

[10,] "J" "I" "L" "K"

[11,] "K" "L" "I" "J"

[12,] "L" "K" "J" "I"

[13,] "M" "M" "N" "N"

[14,] "N" "N" "M" "M"

, , 2

[,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"

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

[10,] "b" "a" "b" "a"

[11,] "a" "b" "a" "b"

[12,] "b" "a" "b" "a"

[13,] "a" "a" "b" "b"

[14,] "b" "b" "a" "a"

$ANOVA

DF e Ani Run

Between Run

Between Ani 3 1 4 4

Residual 10 1 0 4

Within

Between Ani

Trt 1 1 4 0

Residual 9 1 4 0

Residual

Tag 3 1 0 0

Residual 29 1 0 0

$EF

Tag Trt eff.Tag eff.Trt

Between Run

Between Ani

Residual

Within

Between Ani

Trt 28 1

Residual

Tag 14 1