Carrying on from the previous write-up for the experiments with 6 treatments, 2 technical replicates and 3 biological replicates

I have found out that the design with the higher average efficiency of treatment in the between animals within runs stratum was hard to find using the current simulated annealing algorithm.

Therefore, I believe I need to modify the swapping method of the simulated annealing algorithm to enable me to find the optimal design quickly and efficiently. The current swapping method is to swap any random pair of observations throughout the design. Note that the way how I have designed the experiments with 2 technical replicates is to group a pair of animals and treatments and assigned them to a quadrant comprising 2 runs and 2 tags. Hence, the new swapping method is to swap any two random pairs of animals and treatments.

In addition, I have also modified the objective function of the simulated annealing algorithm. The current objection function gives a weighted sum of the average efficiency factors of animals and treatments. However, for an experiment consisting of more than 2 treatments, there will be more than 1 degree of freedom (DF) associated with the treatment effects in some stratum of the ANOVA table. Furthermore, for iTRAQ experiments, if I only try to find the design with the highest average efficiency factor for treatments in the between animals within runs stratum, then I can obtain a design that has 100% of average efficiency factor for the treatments, but some DF of treatments are lost to the other strata. This situation is also known as *disconnected design*. Therefore, to avoid generating a such the disconnected designs when searching for the optimal design using the simulated annealing algorithm, the modified objection function gives a weighted sum of the average efficiency factors of animals and treatments and the proportion of the DF of treatment of current design to the total DF of treatment, i.e.

Using this new simulated annealing algorithm, I have be able find the designs with higher average efficiency factors of treatment as well as for the experiments with even biological replicates. The results can be shown in a table as follows,

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 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 | Can Eff Factor | Ave Eff Factor | Can Eff Factor | Ave Eff Factor |
| 6 | 2 | 2 | 24 | 6 | 4 | 2 (2 Trt) | No (1 DF) | 3 | Yes | 1 (9) | 1 | 1(3), 0.75 (2) | 0.8823 |
| 3 | 36 | 9 | 4 (4 Trt) | No (1 DF) | 2 | No | 1 (4) | 1 | 0.9167 (2), 0.8889, 0.75 (2) | 0.8370 |
| 4 | 48 | 12 | 5 (4 Trt) | No (1 DF) | 12 | Yes | 1 (18) | 1 | 1, 0.9375(2), 0.8125(2) | 0.8937 |
| 5 | 60 | 15 | 7 (5 Trt) | No (1 DF) | 16 | No | 1 (22) | 1 | 0.9523, 0.9, 0.8836, 0.8235, 0.8 | 0.8759 |
| 6 | 72 | 18 | 8 (4 Trt) | No (1 DF) | 21 | Yes | 1 (27) | 1 | 1,  0.875 (4) | 0.8974 |
| 7 | 84 | 21 | 10 (5 Trt) | No (1 DF) | 25 | No | 1 (31) | 1 | 0.9286, 0.9164, 0.8571(2), 0.8489 | 0.8803 |
| 8 | 96 | 24 | 11 (5 Trt) | No (1 DF) | 30 | Yes | 1 (36) | 1 | 0.9375 (2), 0.875 (3) | 0.8990 |
| 9 | 108 | 27 | 13 (5 Trt) | No (1 DF) | 34 | No | 1 (40) | 1 | 0.9272, 0.9167, 0.8872, 0.8611, 0.8399 | 0.8852 |
| 10 | 120 | 30 | 14 (5 Trt) | No (1 DF) | 39 | Yes | 1 (45) | 1 | 0.9 (5) | 0.9 |

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 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 | Can Eff Factor | Ave Eff Factor | Can Eff Factor | Ave Eff Factor |
| 8 | 2 | 2 | 32 | 8 | 4 | 3 (3 Trt) | No (1 DF) | 4 | Yes | 1 (12) | 1 | 1(4), 3/4(2), 1/2 | 0.8077 |
| 3 | 48 | 12 | 5 (6 Trt) | No (1 DF) | 10 | No | 1 (18) | 1 | 1, 11/12(2), 8/9, 3/4(2), 2/3 | 0.8261 |
| 4 | 64 | 16 | 7 (7 Trt) | No (1 DF) | 16 | Yes | 1 (24) | 1 | 0.963 (2), 0.875 (2), 0.7866 (2), 0.75 | 0.8498 |
| 5 | 80 | 20 | 9 (7 Trt) | No (1 DF) | 22 | No | 1 (30) | 1 | 9/10(3), 43/50, 4/5(3) | 0.8489 |
| 6 | 96 | 24 | 11 (6 Trt) | No (1 DF) | 28 | Yes | 1 (36) | 1 | 1, 5/6 (6) | 0.8537 |
| 7 | 112 | 28 | 13 (7 Trt) | No (1 DF) | 34 | No | 1 (42) | 1 | 6/7(6), 41/49 | 0.8542 |
| 8 | 128 | 32 | 15 (7 Trt) | No (1 DF) | 40 | Yes | 1 (48) | 1 | 0.9192(2), 0.875, 0.8308(2), 0.8125(2) | 0.8545 |
| 9 | 144 | 36 | 17 (7 Trt) | No (1 DF) | 46 | NO | 1 (54) | 1 | 8/9(2), 71/81 , 5/6 (3) | 0.8546 |
| 10 | 160 | 40 | 19 (7 Trt) | No (1 DF) | 52 | Yes | 1 (60) | 1 | 0.9, 0.8854(2), 0.85(2), 0.8146(2) | 0.8559 |