**Study of patterns in the animals and treatment allocation of the Phase 1 experiments part 2**

This write-up further discussed the pattern of animal and treatment allocations to the run and tags. Some basic patterns for building up the designs for the 4-plex experiments are described. Only two and four treatments groups are discussed here.

The allocation of the animals and treatments to the runs and tags are described here. The upper case letters denote the Animal ID and the numbers denote the treatments.

For the first phase experiments, the case with two treatment groups, the animals are assigned to the treatment 1 and 2 alternatively, i.e. Animals A, C, E and so on are assigned to Treatment 1 and Animals B, D, F and so on are assigned to Treatment 2. If the design is with four treatment groups, the animals are assigned to the treatment 1, 2, 3 and 4 in a sequential order, i.e. Animals A, E are assigned to treatment 1, Animals B and F are assigned to treatment 2, Animals C and G are assigned to treatment 3 and Animal D and L are assigned to treatment 4.

The simplest design is the experiments with two technical replicates and two biological replicates. If in the first phase experiment with two treatment groups, the Animals A and C are assigned to treatment 1 and the Animals B and D are assigned to treatment 2. The animal allocation to the runs and tags is as follows,

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | A | B | C | D |
| 2 | B | A | D | C |

The black bold box denotes each of the two biological replicates. The treatment allocation is then as follows

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | 1 | 2 | 1 | 2 |
| 2 | 2 | 1 | 2 | 1 |

If the first phase experiment consists of four treatment groups, then the Animals A and E are assigned to treatment 1, the Animals B and F are assigned to treatment 2, the Animals C and G are assigned to treatment 3 and the Animal D and H are assigned to treatment 4. The animal allocation for two biological replicate is as follows,

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | A | B | C | D |
| 2 | B | A | D | C |
| 3 | G | H | E | F |
| 4 | H | G | F | E |

The black bold box denotes each of the biological replicates. The first two runs are identical as the animal allocation for the 2 treatment groups.

The treatment allocation for the 4 treatment groups is then as follows

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | 1 | 2 | 3 | 4 |
| 2 | 2 | 1 | 4 | 3 |
| 3 | 3 | 4 | 1 | 2 |
| 4 | 4 | 3 | 2 | 1 |

For above two cases, if there are additional 2 biological replicates from the first phase experiment, then the patterns are repeated for the additional run. This means the patterns in the black boxes of the both the animals and treatment allocations are repeated for the additional runs. For example, for the designs of two treatment groups, if there are 4 biological replicates from the first phase, then Animals A, C, E and G are assigned to treatment 1 and Animals B, D, F and H are assigned to treatment 2. The animal allocation is as follows,

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | A | B | C | D |
| 2 | B | A | D | C |
| 3 | G | H | E | F |
| 4 | H | G | F | E |

The treatment allocation is as follows,

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | 1 | 2 | 1 | 2 |
| 2 | 2 | 1 | 2 | 1 |
| 3 | 1 | 2 | 1 | 2 |
| 4 | 2 | 1 | 2 | 1 |

Continue with the designs of two technical replicates and two treatment groups, if there are 3 biological replicates from the first phase experiment, then the animal allocation is as follows,

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | A | B | C | D |
| 2 | B | A | D | C |
| 2 | E | E | F | F |

The black bold box denotes each of three biological replicates.

The treatment allocation is then as follows

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | 1 | 2 | 1 | 2 |
| 2 | 2 | 1 | 2 | 1 |
| 2 | 1 | 1 | 2 | 2 |

For the designs with four treatments, the animal allocation for three biological replicate is as follows,

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | A | B | C | D |
| 2 | B | A | D | C |
| 3 | E | F | G | H |
| 4 | F | E | H | G |
| 5 | K | L | I | J |
| 6 | L | K | J | I |

The black bold box denotes each of three biological replicates.

The treatment allocation for the 4 treatment groups is then as follows

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | 1 | 2 | 3 | 4 |
| 2 | 2 | 1 | 4 | 3 |
| 3 | 1 | 2 | 3 | 4 |
| 4 | 2 | 1 | 4 | 3 |
| 5 | 3 | 4 | 1 | 2 |
| 6 | 4 | 3 | 2 | 1 |

The next set of designs is with 4 technical replicates. For the two treatment groups with two biological replicates, the animal allocation is as follows,

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | A | B | C | D |
| 2 | B | A | D | C |
| 3 | C | D | A | B |
| 4 | D | C | B | A |

The treatment allocation is as follows,

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | 1 | 2 | 1 | 2 |
| 2 | 2 | 1 | 2 | 1 |
| 3 | 1 | 2 | 1 | 2 |
| 4 | 2 | 1 | 2 | 1 |

For the four treatment groups with two biological replicates, the animal allocation is as follows,

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 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 black bold box denotes each of two biological replicates.

The treatment allocation is as follows,

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | 1 | 2 | 3 | 4 |
| 2 | 2 | 1 | 4 | 3 |
| 3 | 3 | 4 | 1 | 2 |
| 4 | 4 | 3 | 2 | 1 |
| 5 | 1 | 2 | 3 | 4 |
| 6 | 2 | 1 | 4 | 3 |
| 7 | 3 | 4 | 1 | 2 |
| 8 | 4 | 3 | 2 | 1 |

For above two cases, if there are additional 2 biological replicates from the first phase experiment, then the patterns are again repeated for additional runs. For the example of two treatments, the animal allocation is as follows,

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 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 treatment allocation is as follows,

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | 1 | 2 | 1 | 2 |
| 2 | 2 | 1 | 2 | 1 |
| 3 | 1 | 2 | 1 | 2 |
| 4 | 2 | 1 | 2 | 1 |
| 5 | 1 | 2 | 1 | 2 |
| 6 | 2 | 1 | 2 | 1 |
| 7 | 1 | 2 | 1 | 2 |
| 8 | 2 | 1 | 2 | 1 |

Continue with the designs of four technical replicates and two treatment groups, if there are 3 biological replicates from the first phase experiment, then the animal allocation is as follows,

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 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 runs are identical to the animal allocation of with two biological replicates. The black box for Runs 5 and 6 denotes the last biological replicate of Animals E and F. The treatment allocation is as follows,

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | 1 | 2 | 1 | 2 |
| 2 | 2 | 1 | 2 | 1 |
| 3 | 1 | 2 | 1 | 2 |
| 4 | 2 | 1 | 2 | 1 |
| 5 | 1 | 1 | 2 | 2 |
| 6 | 2 | 2 | 1 | 1 |

For the design with four treatment groups with 3 biological replicates, the animal allocation is as follows,

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 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 |

The black box denotes each of three biological replicates. Note that the patterns are completely identical within each three black boxes. The treatment allocation is as follows,

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | 1 | 2 | 3 | 4 |
| 2 | 2 | 1 | 4 | 3 |
| 3 | 3 | 4 | 1 | 2 |
| 4 | 4 | 3 | 2 | 1 |
| 5 | 1 | 2 | 3 | 4 |
| 6 | 2 | 1 | 4 | 3 |
| 7 | 3 | 4 | 1 | 2 |
| 8 | 4 | 3 | 2 | 1 |
| 9 | 1 | 2 | 3 | 4 |
| 10 | 2 | 1 | 4 | 3 |
| 11 | 3 | 4 | 1 | 2 |
| 12 | 4 | 3 | 2 | 1 |

The last set of design is with 3 technical replicates. For the two treatment groups with two biological replicates, the animal allocation is as follows,

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | A | B | C | D |
| 2 | B | C | A | D |
| 3 | C | A | B | D |

Note the last column of the animal allocation contains only Animal D. This causes the animal to be confounded with one contrast of runs, however, it allows the valid test for the treatment difference can be conducted in the between animals stratum.

The treatment allocation is as follows,

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | 1 | 2 | 1 | 2 |
| 2 | 2 | 1 | 1 | 2 |
| 3 | 1 | 1 | 2 | 2 |

For additional 2 biological replicates, 3 runs are needed to be able to measure all samples. The allocation of animals is as follows,

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | A | B | C | D |
| 2 | B | C | A | D |
| 3 | C | A | B | D |
| 4 | F | G | H | E |
| 5 | G | H | F | E |
| 6 | H | F | G | E |

The black boxes denote 2 of four biological replicates. Note the Animal E is assigned to the last column of Run 4 to 6, because the Animal E is assigned to treatment 1. This allocation allows the treatment to be orthogonal to both runs and tags, because every runs and tags contains equal numbers of each treatment groups.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | 1 | 2 | 1 | 2 |
| 2 | 2 | 1 | 1 | 2 |
| 3 | 1 | 1 | 2 | 2 |
| 1 | 1 | 2 | 2 | 1 |
| 2 | 2 | 2 | 1 | 1 |
| 3 | 2 | 1 | 2 | 1 |

Therefore, for any additional 2 biological replicates, the last column of the treatment allocation of last three runs must be different to its previous three runs.

For the designs with four treatment groups and two biological replicates, the animal allocation is as follows,

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | A | B | C | D |
| 2 | B | C | A | D |
| 3 | C | A | B | D |
| 4 | E | F | H | G |
| 5 | F | H | E | G |
| 6 | H | E | F | G |

The black box here denotes the each of two biological replicates. Note the allocation of animal is similar to the design with two treatments; expect the last column of   
Runs 4 to 6 where Animal G is assigned. This is because the Animal G is assigned to the treatment 3 which is different to the Animal D which is assigned to treatment 4. In fact, the allocation with two treatments can still be used here, because Animal E is assigned to treatment 1. However, I have assigned this way for the sequential reasons. The allocation of treatment is as follows,

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | 1 | 2 | 3 | 4 |
| 2 | 2 | 3 | 1 | 4 |
| 3 | 3 | 1 | 2 | 4 |
| 4 | 1 | 2 | 4 | 3 |
| 5 | 2 | 4 | 1 | 3 |
| 6 | 4 | 1 | 2 | 3 |

If there is an additional biological replicate in the first phase experiment, additional three runs are required to measure all samples. The allocation of animals is as follows,

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | A | B | C | D |
| 2 | B | C | A | D |
| 3 | C | A | B | D |
| 4 | E | F | H | G |
| 5 | F | H | E | G |
| 6 | H | E | F | G |
| 7 | I | K | L | J |
| 8 | K | L | I | J |
| 9 | L | I | K | J |

The black box here denotes the each of three biological replicates. The allocation of treatment is as follows,

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | 1 | 2 | 3 | 4 |
| 2 | 2 | 3 | 1 | 4 |
| 3 | 3 | 1 | 2 | 4 |
| 4 | 1 | 2 | 4 | 3 |
| 5 | 2 | 4 | 1 | 3 |
| 6 | 4 | 1 | 2 | 3 |
| 7 | 1 | 3 | 4 | 2 |
| 8 | 3 | 4 | 1 | 2 |
| 9 | 4 | 1 | 3 | 2 |

Note that the last column of last three runs is assigned to only treatment 2. Hence, if there is another biological replicates from the first phase experiments, then last column of last three runs must assigned to treatment 1.