Experimental design discussion by Oberg

Oberg and Vitek (2009) have discussed the experimental design specifically for the iTRAQ experiments. The goal of an experimental design is to allocate individual samples iTRAQ tags and MudPIT runs in a way that avoids bias, and reduces the variance of treatment comparison.

Without the usage of tags, the variance of treatment comparison will comprise the variance components of between MudPIT runs. Since the variation between MudPIT runs is known to be large, the usage of iTRAQ tags is essential to enable the comparison of between treatments in the within MudPIT runs.

Oberg and Vitek (2009) recognised that each MudPIT run as a block; thus the treatment allocation to runs can be either arranged as randomised complete block design (RCBD) or balanced incomplete block design (BIBD). The RCBD is when the number of treatment is the same as the number of tags. The BIBD is when the number of treatment is more than the number of tags. Based on the theory in designing microarray experiments, two additional designs were discusses: reference design and loop design. Reference design is where each run contains a reference sample for comparison. Loop design is where the samples are allocated in such a way that they are cycled through the blocks systematically.

The author concluded that the RCBD is most ideal, because it requires the lesser number of runs. In addition, if there is a loss of a run, which is equivalent in reducing of one set of biological replicates. The resultant design is still remained in a balanced structure. On the other hand, if the loss of run occurs in the BIBD and loop design, the resultant design may not preserve the balanced structure. The reference design is most robust in run failure, but it may require the most number of runs for the additional reference sample.

Recall that the number of tags can be either 4 or 8, given the high costs for performing the experiment in each MudPIT run, the biologists are likely to utilise all the tags. Therefore, the RCBD may not be possible in all circumstances, because the biologists are unlikely to compare between 4 or 8 conditions all the time.

Furthermore, the author only consider the allocation of treatments to runs and tags, the allocation of the animals is equally important, because it can also affect the variance of treatment comparison. Therefore, such experiments should always be treated as the two-phase experiments.

Oberg and Mahoney (2012) have also discussed the experimental design for the MudPIT-iTRAQ experiment. They recognised that allocation of samples to runs and tags as a randomised block design. Moreover, the author suggest that multiple MudPIT runs are required to avoid the confounding of tag effects with the treatment effects.