**Introduction**

From the previous chapter, we have learn how to search for the optimal design where the Phase 1 experiment is arranged in completely randomised design (CRD). This chapter increases the complexity of the design for the Phase 1 experiment to randomised block design (RBD). The blocking factor in the Phase 1 experiment is referring the caging of the animals. Note that the term block is referred to any group of experimental units that share a set of characteristics thought to possibly affect the response to be observed after treatment. For current case, the cages should always contain all treatment groups; hence, the test for the treatment effects is conducted in the Between Animals Within Cages stratum.

This chapter considers RBD with $v$ treatments, $r\_b$ biological replicates, $n\_C$ cages and $r\_t$ technical replicates for first phase experiment. The number of animals is denoted by $n\_A$ which equals $v r\_b$. For the design to be RBD, the number of biological replicates should be divisible by the number of cages. The second phase experiment is arranged in the randomised block design (RBD) where the numbers of MudPIT run, $n\_R$, and iTRAQ tag, $n\_\gamma$, correspond to the numbers of blocks and block size, respectively. The total number of observation, denoted by $n$, equals to both $v r\_b r\_t$ and $n\_R n\_gamma$.

The main focus of this chapter is on the construction of the objective function. This is because the additional cage component in the Phase 1 experiment which will change the way on how the average efficiency factors for the animals and treatments effects are calculated. The main idea in the modified simulated annealing algorithm (SA) stays the same from the previous chapter.

The aim of this chapter is to develop a method for generating the optimal two-phase designs where the Phase 1 experiment is arranged in RBD.