## Random pooling algorithm

## Background

As collecting a lot of human samples is sometimes not possible, one common situation we face is to have the limited number of the samples for analysis. The sample size is a confounding factor for computing differential gene expression. In order to minimize this effect and false positive caused by the small sample set, we developed "random pooling algorithm" combining to "DESeq2" to obtain reliable and stable differentially expressed genes.

## **Description and details**

The aim of this algorithm is to calculate the differentially expressed genes based on the sub-sampling from the original sample set. The detail of the algorithm is:

- 1) To estimate the sample sizes of the two groups and select the small size group as the random pooling reference group;
- 2) To set up *i*, which contains the range of the size of sub-samples that could be selected from group1 and group2. In our case, *i* is from 3 to 11, the N-1 of samples in the reference group;
- 3) To randomly select same number *i* of the samples from group1 and group2, respectively;
- 4) To calculate the differentially expressed genes by using selected samples;
- 5) To repeat 3)-4) for *j* times.

## **Usage and arguments**

The inputs include:

- 1) Matrix1: the read count matrix of group1, in which the rows are genes and the columns are samples;
- 2) Matrix2: the read count matrix of group2, in which the rows are genes and the columns are samples;
- 3) Sn: it represents "j", which means the repeating times in random pooling process;
- 4) Group1Inf: a dataframe, containing the group1's information;

- 5) Group2Inf: a dataframe, containing the group2's information;
- 6) OutDir: the folder to save the results;
- 7) Parallel\_TF: a logical value. If it is TRUE, the DESeq will run by using BiocParallel; if it is FALSE, the DESeq will run by single core;
- 8) Ncores: the number of cores used when running DESeq;