**This is an example of GO analysis on Tania paper, which can be easily modified for other transcriptomics/proteomics data analysis.**

Section 1: Differentially expressed gene (DEG) analysis

Step 1, download counts file, sample information of RNA-seq and prepare comparison information (here we do all against all); In this example the three files were named them as “counts.txt”, “rpkm.txt” , “sample\_information\_Tania.txt”.

Step 2, modify and run DEseq2 R scripts, “DEseq\_code\_normalize\_all\_samples\_together2.r”;

Step 3, mark the significant genes with R scripts, “RNAseq\_calculate\_statis\_category\_p001\_fold2.r”; The specific cutoff about fold-change and p-value can be adjusted, the default one is p=0.001 fold=2.

Section 2, GO enrichment based on the DEG results (the output from above step 3)

Step 4: manually download protein fasta file, GO annotation from JGI Mycocosm database;

Step 5: modify and run the perl script “reformat\_GO\_references\_A.niger.pl” to get reformatted GO file;

Step 6: put the GO terms and GO relation tables in the same folder. These two files (“all\_GO\_terms.txt”, and “GO\_ancster\_relationship.txt”) could be used for analysis of other species. (This GO data is exported from R package called GO.db, (Carlson M. 2018. GO.db: A set of annotation maps describing the entire Gene Ontology)). The all\_GO\_terms.txt file and GO\_ancster\_relationship.txt are too big and I put it one the reference folder.

Step 7; modify and run the perl script “add\_GO\_references\_ancester.pl”, as the original JGI GO list lack GO ancestor nodes;

There are two approaches for GO analysis and visualization: first one is directly using GOslim for enrichment analysis and visualization as shown step 8 and 9; the other one is do analysis on the full GO list firstly and then filter full GO list to GO slim list and then visualize them as shown in Step 8.2, 9.2 10.

Step 8; modify and run R script (“GOslim\_BP\_analysis.r”, “GOslim\_CC\_analysis.r”, “GOslim\_\_analysis.r”) for GO enrichment analysis based on DEGs results of DEseq2;

Step9; plot the GO slim terms with “GO\_all\_enrichment\_plot - all\_together - v3.r"

Step 8.2: modify and run R script (“GO\_MF\_analysis.r”, “GO\_BP\_analysis.r”, “GO\_CC\_analysis.r”) for GO enrichment analysis based on DEGs results of DEseq2;

Step 9.2: combine all GO enrichment results with perl script, “combine\_GO\_MF\_p001\_size10.pl”, “combine\_GO\_BP\_p001\_size10.pl”, “combine\_GO\_CC\_p001\_size10.pl”;

Step 10.2: select only the GO\_slim terms to plot with R script “GO\_slim\_filter\_bubblePlot.r” based on GO\_slim defined by AspGD database “GO\_slim\_AspGD.xls”;