**Supplementary**

 **Other Integrated analyses**

1. ***mRNA-mRNA (gene-gene) co-expression network analysis***

Gene with similar expression patterns tends to have similar functions, and co-expressed gene modules are found to be involved in the same metabolic pathway. The co-expression network study emphasizes building novel hypotheses or looking for potential interactors in the pathway. Usually, there are two types of co-expression network analyses, i.e., gene co-expression network (GCN), Gene regulatory network (GRN). GCN does not help determine the direction and type of the co-expression, whereas GRN is a directed network representing biochemical processes such as activation, inhibition, or reaction. Co-expression measures are the requirement for this analysis, and various methods can be used for this measurement, such as Pearson’s correlation coefficient, Mutual Information, Spearman’s rank correlation coefficient, Kendall correlation, and Euclidean distance. In R, cor function is the basic function for the calculations of correlation. Preferably transcript per million (TPM) count matrix of gene expression is used as an input. The p-value can be calculated by using the cor.test function. The ‘psych’ R package [1] comes with inbuilt features of p-values, FDR calculation, and ‘corplot’ library. [2] is useful for the visualization plots. For standard un-weighted analyses practice, usually hard or soft thresholds are applied to reduce the network’s complexity and better interpretation, but it could lose information with hard thresholds. Applying the weights on the edges helps preserve most of the information, and this network approach is called Weighted Gene Co-expression Network Analysis (WGCNA) [3]. WGCNA tools provide robust module preservation statistics that define gene clusters, which perform similar functions, and these modules can also be compared between different networks. Detailed guidelines on co-expression network analysis can be found in a review published by Dam et. Al [4].

1. ***miRNA-mRNA co-expression network analysis***

miRNA-mRNA co-expression related studies are also emerging. Unlike gene-gene co-expression, researchers are more interested in studying the miRNA expression and mRNA’s anti-correlation because miRNA expression has an inverse relationship with its targeted mRNA expression. [5] anamiR [6] is an integrated package of the R to study miRNA-mRNA expression profiling. However, our detailed investigation has discovered a few pitfalls, such as it lacks the feature for the calculation of correlation significance, and it comes with inbuilt differential expression analysis and does not take differential expressed mRNA-miRNA files directly.

1. ***Meta-analysis: RNAseq***

Increased number expression studies have collected a lot of data in public databases, e.g. GEO and SRA. Many researchers have focused on integrating the datasets from a range of biological studies with similar biological questions because the conclusions drawn for the same question differ among different studies. The usual reason is inter-study heterogeneity. This heterogeneity could be due to different platforms uses or due to variance in biological samples expression. Meta-analyses combine information from multiple studies of a related hypothesis to improve statistical power, accuracy and reproducibility beyond individual study analysis. Among various preprocessing steps, Normalisation and batch effect correction are important steps to minimise non-biological variations. Three main types of meta-analysis methods have been defined, i.e., a meta-analysis based on effect sizes, a meta-analysis based on P-values combination and meta-analysis based on a rank combination. According to the different platforms (technical variability) and numerous studies conditions (Biological variability) of the selected datasets, Previous studies [7, 8] provide well-defined guidelines for choosing meta-analysis methods. Suppose the datasets belong to the same platforms or conditions; In that case, effect size combination-based tools are the best choice. Where datasets are from different platforms or conditions, rank-based approaches (if the sample size is small) or the p-value combination approach should be preferred. RankerGUI [9], RankProd [10], RankAggreg [11] and Orderedlist [12] tools and packages use rank-based methods. metaRNASeq [7] methods are p-value based. NetworkAnalyst [13], has both effect size and p-values combinations options.

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