Homework/Mini Project 3

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Now its time to practice what we have learned in class and learn even more! For this homework/mini project you will do a RNA-seq analysis of the TB/HIV dataset. Note that your homework should be written in R Markdown, and turned in by uploading a tarball with your .Rmd, .html (from Rmarkdown, MultiQC, etc) and other outputs on Canvas.

RNA-sequencing analysis

1. To access the data for Homework 3, you will have to download the data from the Sequence Read Archive (SRA) using the `sratoolkit`. There are 33 fastq files, with the SRR numbers are listed in the `homework3_srr.txt` file.
2. Align the reads to the human genome reference using your choice of the `Rsubread` or the `STAR` aligners.
3. Use the `featureCounts` function in the `Rsubread` package to generate a counts file for this dataset.
4. Generate a `SummarizedExperiment` object for your counts. The `colData` for these data are provided in the `homework3_metadata.txt` file.

5. Preprocess these data by removing `TB-HIV-ART` samples (should be two of them), removing any genes with 0 expression for all samples, and by generating a log counts per million assay.
6. (Extra credit) Create a batch corrected assay in your `SummarizedExperiment` using ComBat-Seq. You can use the `ComBat_Seq` function in the `sva` package, or simply do it in `BatchQC` and then extract the `SummarizedExperiment`. For this example, pretend that `disease_status` is the batch variable. Note that this will remove the disease status variability, so **don't** use this assay in the following analyses! This was merely a practice for cases where you have an actual batch variable.

7. Apply SVA and UMAP to your data and generate dimension reduction plots for the results. Color the TB-HIV to the HIV only samples in different colors. Note that you should be using the log CPM values for this analysis.
8. Use `DESeq2` to do a differential expression analysis (on the counts) comparing the TB-HIV to the HIV only samples. Provide the top 50 most differentially expressed genes.

9. Now conduct the same analysis using `limma` on the log CPM values. How do the `DESeq2` results compare to the `limma` results?
10. Give a heatmap plot of either the `DESeq2` or the `limma` results (top 50). Add a colorbar for disease status.
11. Conduct a pathway analysis of the top 50 genes usign a tools such as enrichR (through R or online). What are the top scoring pathways?
12. (More extra credit) Conduct a TBSignatureProfiler analysis on these data – including signature heatmaps, individual boxplots, and AUC boxplots. Interpret your findings.