Introduction

The three scripts here (01-make_matrices, 02-make_profile, and 02-make_heatmap) are used to generate visualizations of comparisons between two or more bigwig files.

Bigwig files simply contain genome-wide numerical data. In our ChIP-seq experiments, these numbers are read density. If you open these bigwig files in IGV, you'll see a graph across the entire genome showing the relative number of reads for each "chunk" of bases. (Most of our bigwigs use chunks that are 10 bases wide) The taller the bar in a certain region of the genome, the more reads were attributed to that region.

These scripts, which use the *deeptools* libraries, generate visualizations of our bigwig data (read density) across all genes in the Aedes genome .bed file you use. We typically use one that lacks unassembled regions. (NIGP) Different .bed files are available on the NAS in: NAS_Server_Shared/InformaticsResources/Aedes_aegypti/

There is no difference between running these scripts for input-adjusted vs. non-input-adjusted data. You must simply use the right bigwigs (input-adjusted or not input-adjusted) when running make_matrices.

How to use the make_matrices, make_profile, and make_heatmap scripts to create visualizations:

You must first run the 01-make_matrices script, then you can run 02-make_profile or 02-make_heatmap using the output (.gz) of 01-make_matrices. You will have to edit the scripts themselves to change your output. **Please run all these scripts using slurm.**

For both 02 scripts, the -T option sets the displayed title, but it is set to the second argument when running the script. This is so you can reuse the same script with different inputs while keeping the other settings the same. (Without having to change the scripts each time)

The 01-make_matrices script takes a single argument, that being the path to a text file with the list of bigwigs you want to visualize the comparison of. They can be space- or newline-delimited. As an example:

RVFV_d7_ac_merged.bw BF_d7_ac_merged.bw

and

RVFV_d7_ac_merged.bw
BF_d7_ac_merged.bw

are both valid ways to enter the day 7 acetylation data for RVFV vs BF in a comparison text file.

What values to change:

For the 01-make_matrices script

The --referencePoint option can be changed to focus on different parts of the genes. (options are TSS, TES, and center)

The --samplesLabel option is for properly labeling your different groups. (This can also be done in the make_profile and heatmap scripts.) It takes space-separated strings, so you could enter: --samplesLabel “BF” “RVFV”

The -a and -b options change the number of bases after (a) and before (b) the reference point. This helps you focus on certain regions around the referencePoint.

To run: **sbatch ./01-make_matrices.sh path/to/comparison.txt**

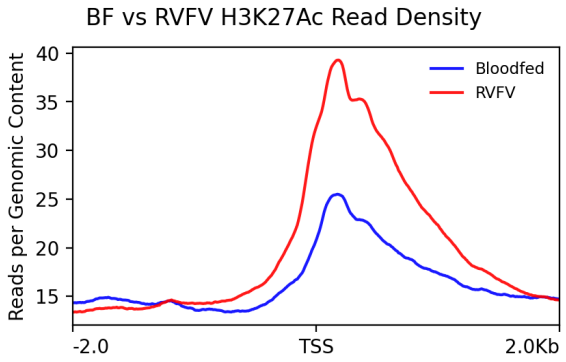
For the 02-make_profile.sh script

The --colors option sets the colors for each group’s graph. You can have space-separated inputs, like “red blue”. (without quotes) If you have three groups, you will need to write 3 colors.

The --refPointLabel option should be set to the name of your referencePoint. (for example, if you chose TSS, then you should set –refPointLabel to “TSS” or “Transcription Start Site”)

The --samplesLabel option can be used to properly label your different groups if you forgot to do so in the 01-make_matrices script.

Example of a profile:



To run: **sbatch ./02-make_profile.sh path/to/matrix.gz “Title of graph in quotes”**

For the 02-make_heatmap.sh script

The --colorMap option sets the color scheme for the heatmaps. You can check the docs (linked above) for your different options.

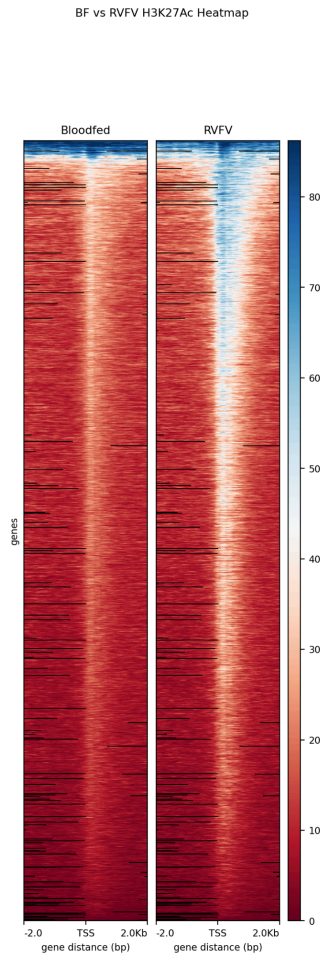
The --whatToShow option specifies what you want to display. (options are plot, heatmap, and/or color legend. Check the docs for more details on how to specify this)

The --refPointLabel option should be set to the name of your referencePoint. (for example, if you chose TSS, then you should set –refPointLabel to “TSS” or “Transcription Start Site”)

The --samplesLabel option can be used to properly label your different groups if you forgot to do so in the 01-make_matrices script.

The --zMin and --zMax values specify the minimum and maximum cutoffs in the heatmap. By default they are set to “auto”.

Example of a heatmap:



To run: **sbatch ./02-make_heatmap.sh path/to/matrix.gz “Title of heatmap in quotes”**

Instructions written by Zoey Mikol, contact me over the Rosenberg Lab Slack or at semechki@colostate.edu with any questions.