**Goal:** This tutorial provides a guide to generating 2 basic plots (Composite plot and Heatmap) using the ScriptManager platform and the data generated by the Yeast Epigenome project.

**Download ScriptManager (v0.12):**

The current version of ScriptManager is available for download here:  
https://github.com/CEGRcode/scriptmanager/releases/download/v0.13/ScriptManager-v0.13.jar

**Notes on running ScriptManager:**

Depending on your system permissions (especially on managed computers), you may run into issues with ScriptManager on macOS 10.15+. The Desktop and Downloads folders will be unavailable to ScriptManager if your administrator has disabled access. It is recommended in this case to create a new folder in your home directory and operate ScriptManager and subsequent analyses from there. For example: /Macintosh HD/Users/***userID***/ScriptManager

ScriptManager requires Java v11+ to run. If not installed, follow the link below to download (Administrator rights typically required):

<https://www.oracle.com/java/technologies/javase-downloads.html>

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**Download Reb1 sample ChIP-exo data:**

1. Navigate to [www.yeastepigenome.org](http://www.yeastepigenome.org) and search for Reb1
2. Select ‘META DATA’

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1. Select ‘Direct Download’

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1. Unzip the resulting file ‘12141\_YEP.zip’ and inspect the contents. It should contain the following files:

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**Generate the Plots**

1. Open ScriptManager by double-clicking on the icon.

|  |
| --- |
| Depending on your system permissions, you may see this error message upon attempting to open ScriptManager the first time.  Graphical user interface, text, application  Description automatically generated  On Mac systems, this can overridden by right-clicking the ScriptManager file and selecting ‘Open’ at the top. You will then be given the option to open ScriptManager.  Graphical user interface, text, application  Description automatically generated |

**ScriptManager landing page:**

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1. Generate BAI index files for each BAM file of interest (i.e., the tag occupancy data you want to plot).

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***This only needs to be done once for each BAM file.***

*The speed of this step scales with the size of the BAM file. Generally this step takes <30 sec for a 100 MB BAM file but may take 1-2 min for a multi-GB BAM file.*

*The BAI index file allows for rapid access of the sorted and aligned sequence reads (BAM file). The recommended SAM/BAM standard is to keep BAI file in same directory as BAM file with the ScriptManager-generated filename.*

1. Resize the Reb1 motif-aligned BED file

* *This is the set of reference coordinates that your heatmap and composite plots will be aligned to, but you’ll need to specify how far upstream and downstream you want your data to be plotted; i.e., “Size of Expansion (bp). If you bed file is defined by more than a 1 bp interval AND you want to add to limits of that interval, then select “Add to Border”)*
* *BED file coordinates often need to be resized for more informative tag pileups. For Reb1 (yeast) 250-500 bp windows are generally sufficient. Mammalian samples may require larger windows (500-2000 bp) based on the amount of indirect-crosslinking*
  1. In this example, use the file ‘12141\_Motif\_1\_bound.bed’

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*Load BED files to expand*

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*Select folder where you want the files output*

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1. Generate the tag pileup

*The default parameters Tag Pileup is set to expect is a sequence-specific strand separated ChIP-exo dataset. Modifications to these parameters are needed for either specific analysis or alternative assays.*

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*Load BED files*

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*Load BAM files*

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*Select Output Directory*

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*Pileup tags*

*Selects which files are output*

Chart

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*The displayed composite plot can be modified by right-clicking and selecting properties. Things such as axis labels, axis range, and colors can be modified here. The final image can then be saved by right-clicking and selecting ‘Save as’. PNG is fine for most cases, but SVG is strongly recommended if this composite plot will be used in Adobe Illustrator later.*

*The raw data used to generate the plot is also available in the second tab labelled ‘Pileup Statistics’.*

*The raw data may also be exported to a tab-delimited file (default ‘composite\_average.out’) that can be visualized in any graphing software.*

1. Generate Heatmaps

*Heatmap Generator can only generate one color at a time, so ‘Sense’ and ‘Anti’ files should be processed separately. The ChIP-exo standard for strand colors is ‘Sense’ == blue and ‘Anti’ == red.*

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*Load files*

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*Choose Output directory*

*Select ‘blue’*

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A picture containing timeline

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*Remove previous file*

*Load opposite strand*

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*Select ‘red’*

A picture containing bar chart

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1. Merge strand-separated heatmaps

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*Choose Output directory*

*Select PNG images to merge*

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Timeline

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*The script will automatically match sense to anti using the standardized naming conventions generated by ScriptManager*

**General Comments**

*Bioinformatic projects should be organized in a uniform and consistent manner as described below:*

[*https://journals.plos.org/ploscompbiol/article/file?id=10.1371/journal.pcbi.1000424&type=printable*](https://journals.plos.org/ploscompbiol/article/file?id=10.1371/journal.pcbi.1000424&type=printable)