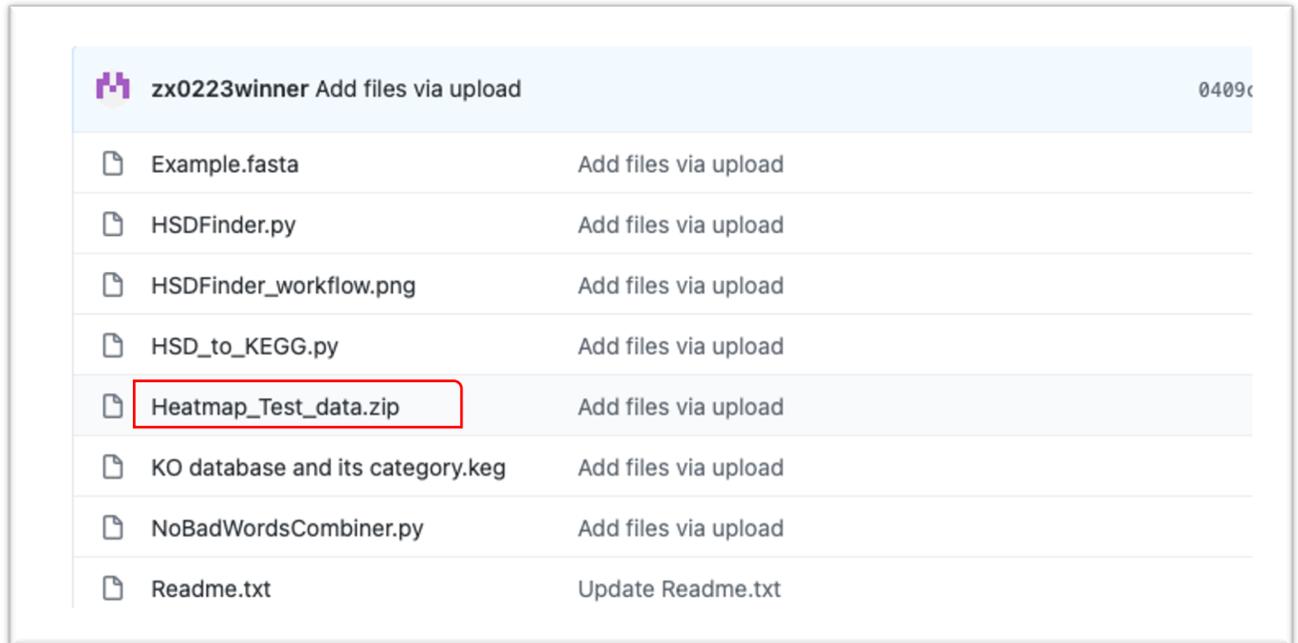
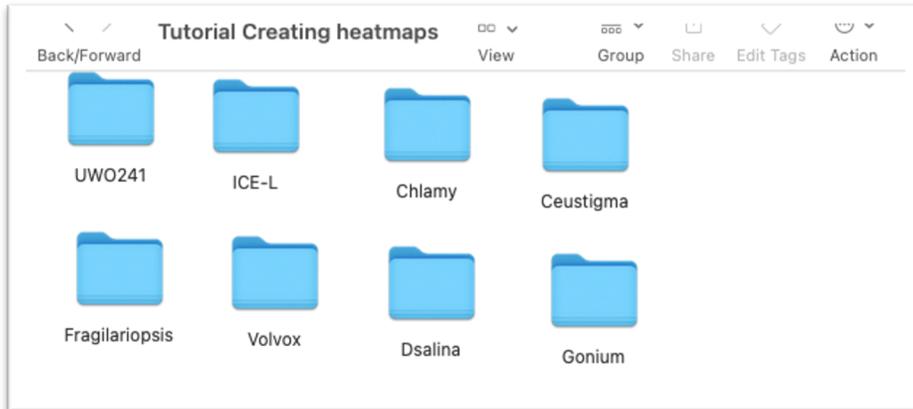


Hands-on protocol to create heatmap with Test data

1. Download the Heatmap_Test_data.zip via the link from GitHub (<https://github.com/zx0223winner/HSDFinder>).



2. We provided the data from eight algae species (*Chlamydomonas* sp. UWO241, *Chlamydomonas* sp. ICE-L, *Chlamydomonas reinhardtii*, *Chlamydomonas eustigma*, *Gonium pectorale*, *Dunaliella salina*, *Volvox carteri* and *Fragilariopsis cylindrus*), user can create the heatmap by selecting some of them.
 - Each folder represents one species, there are two files in each folder. Take Chlamy genome as example, The file named “HSD_Chlamy_90pct_10aa.txt” is the result of highly similar duplicates (HSDs) in Chlamy genome with the filtering option more than 90% amino acid pairwise identity and within 10 amino acid difference. (Check the tutorial for more details about how to acquire this file on your own species)



- Another file named “Chlamy_KO_annotation.txt” is retrieved from the KEGG database documented the correlation of KO accession with each gene model identifier.
- User can upload the respective files to the web server to create the Heatmap (Check the screenshot below). Make sure you have an organism name for the files you chose to upload.

Make sure the files you upload for each species fits the options.

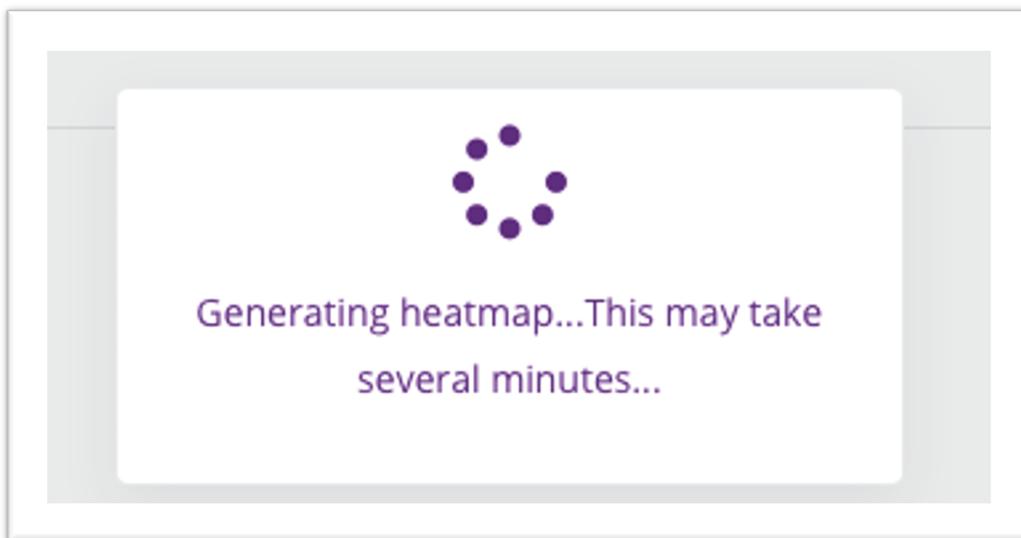
| HSD File | Gene list with KO annotation | Organism name |
|--|---|--|
| <input type="text" value="HSD_File_example.txt"/> <input type="button" value="Choose File"/> HSD_UWO241_...t_10aa.txt | <input type="text" value="Genelist_KO_annotation_example.txt"/> <input type="button" value="Choose File"/> UWO241_KO_a...otation.txt | <input type="text" value="UWO241"/> |
| <input type="text" value="HSD_ICE-L_90pct_10aa.txt"/> <input type="button" value="Choose File"/> | <input type="text" value="Gene list with KO annotation"/> <input type="button" value="Choose File"/> ICE-L_KO_annotation.txt | <input type="text" value="Organism name"/> ICE-L |
| <input type="text" value="HSD_Chlamy_9...ct_10aa.txt"/> <input type="button" value="Choose File"/> | <input type="text" value="Gene list with KO annotation"/> <input type="button" value="Choose File"/> Chlamy_KO_annotation.txt | <input type="text" value="Organism name"/> Chlamy |

3. Once the user finished uploading the files, there is an option below to design the figure size at user's preference. The 'row' option can change the width of the heatmap image. The 'col' option can change the length of the heatmap image.

Figure Size: row col

Creat Heatmap

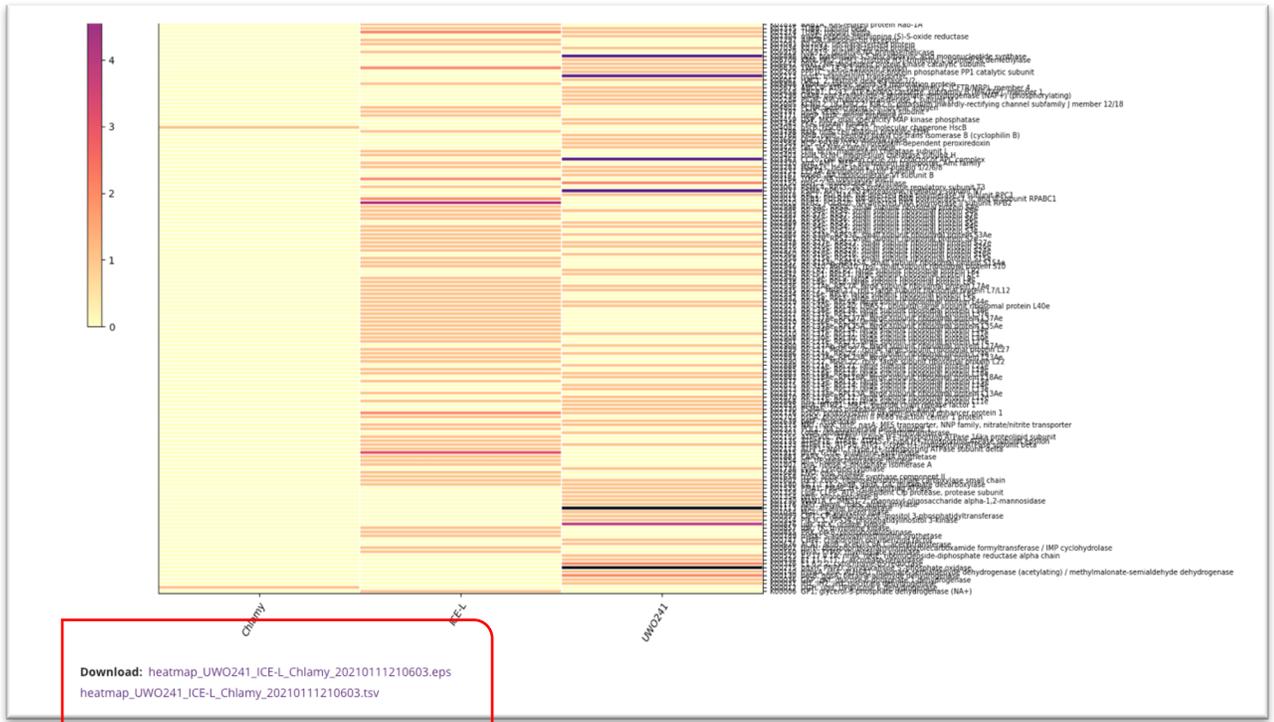
4. Tap the Create Heatmap button, the following gif. Will jump out. It usually takes around less than one minute to run with three to five species.



5. Once heatmap created, the HSDs numbers for each species will be displayed in a heatmap under different KEGG function category. On the left side, the color bar indicates a broad category of HSDs who have pathway function matches, such as carbohydrate metabolism, energy metabolism, translation etc. The color for the matrix indicates the number of HSDs across species.



- User can also find the option below the image where to download the high-resolution version and the text file for user to use in future analysis.



7. Just in case you are not satisfied with the heatmap figure size. E.g., the image texts are overlapped. You can always rerun with more appropriate ‘width and length’ options.

Figure Size: row col

[Creat Heatmap](#)

8. The new heatmap after changing the size of the image.

