## **Moue to Usable (M2U)**

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***Purpose***

This program takes either the output from the QUINT workflow or a specific file format and allows you to create two types of heatmap that can be used to display the data or to QC the data for further analysis. One heatmap is a normal rectangular heatmap while the other is an anatomical heatmap which displays data on a mouse brain outline. There are a couple output files throughout the app to save your spot for easy return as well as easy download of the heatmaps and data behind them. The app is hosted at: https://vai-bbc.shinyapps.io/Mouse\_to\_Usable\_M2U/.

***Preparation Using Nutil Files from the Quint Workflow***

First, create a folder which will contain all your data for this project and name it whatever you would like. After completing the QUINT workflow, you should have a folder (or more likely, multiple folders) called “Reports” which contains another folder with an ending label of RefAtlasRegions. In this folder there will be at least two files with one of them having the same name as the folder. Ignore that file and copy the rest into the project folder you created in the beginning. Ensure that the file names contain the hemisphere such that you can identify them later. **No two files should have the same name.** Do this for every report folder you have until all the files are in your project folder.

You may now open the app.

***Preparation Using Outside Data***

First, open the app and download the non-nutil template and then fill it in using your data.

***Notes:***

* Replace the value column names with your value type
* The annotations are separate and can contain the following information: **"mouse", "sex", "treatment", "mpi", "genotype", "marker", and "batch"**. If you have this information and are interested in comparing it, please make sure to:
  + Download multiple of the template
  + Ensure the value column names match
  + Name each template such that you will remember what annotations to give it while filling out the annotation file later (ex. “3mpi.csv”, “6mpi.csv”)
  + **It is ideal for each mouse to have a separate file**
* **Please remove any regions you didn’t measure**

Save this file as a **CSV**.

You may now open the app.

***Using M2U for the first time:***

1. Hit the browse button under **“select all files to import”,** find your files, select them all, and then hit open
2. Download the raw data and store it in a folder
3. Move to the “Annotation Creation” sub-tab
   1. If you need to step away from the application at this point, you will need to upload the raw data in the “Annotation Creation” sub-tab upon returning
4. If you only have one hemisphere in your data, select the slider for that
5. Select what annotation options you would like to fill out for further use later
6. If you would like additional options not listed, type them in the “Custom Options” section and hit enter after each option
7. When you are done, click “Create Annotation file”
8. Next, download the blank annotation file and fill it out
   1. Each row will be a different file you uploaded, so make sure you know what each one is

***Using N2U when you have completed filling out the annotation file:***

1. Begin by clicking into the “Merge Data and Annotations” sub-tab
2. Hit the Browse button labeled raw data and select the raw data you saved earlier and click ‘open’
3. Click the browse button to input the completed annotation file you finished creating
4. Once that has completed, the download button will open and at this point you should **download the Checkpoint** somewhere you have easy access to
   1. This will be for if you would like to come back to the app again later or if the app manages to fail at any later step
5. Once that is complete, please move to the “Variable Selection” tab
   1. If you have not just finished making the checkpoint or putting in a checkpoint, this tab will be empty
6. This is the variable selection phase where default variables are common things you may want to see. Below I will have a brief description of your options
7. Annotation(s) of Interest (AoI):
   1. These variables will make up the **rows** of the heatmap with the labels for them showing on the **left side** of the heatmap
   2. They are your annotations from earlier, so you need at least one thing to select from there
8. Region Level:
   1. These variables will be the heatmaps **columns** and will be labeled on the **bottom**
   2. By default, if you have multiple hemispheres, it will be shown here combined with whatever level you wish to look at
   3. ‘Specials’ is a special variable containing the following ABA regions: CTXsp, fiber tracts, HPF, HY, Isocortex, OLF, PAL, STR, TH, MB, P, MY, and CB
9. Select Legend Variables (based on selected region level)
   1. These variables will be displayed in the form of a color key on the **outside** **top** of the heatmap
   2. It will only allow the selected region level or higher here
   3. Please note that the more elements in a variable the less distinguishable the color key is going to be (i.e. don’t use ‘daughter’ and use ‘parent’ instead)
   4. This is a good place to use ‘specials’
10. Row Names and Column Names are both only going to be options from the selected AoIs and Region level
    1. These are the labels mentioned to show on the outside of the heatmap
    2. If you have anything other than none selected (even if none is still there), it will use that instead
    3. Multiple variables will be separated by an underscore
11. Move on to the “Data and Transformation” sub-tab
12. Select a column to get the data from
    1. This column must have been shared across all initial upload files
    2. There may be an option called ‘load’ which is a calculation run in the app only if you have data from nutil. Scroll to the bottom of this document for an in-depth explanation on how to use it
13. Move the following sliders as you see fit
    1. Log is log 10
    2. Trim refers to removing any column/row with more than 50% of the data missing
14. If you would like **to ignore regions** from your heatmap, select any amount of them from the drop down
    1. This does not remove them from the data or anything, it will just not show them in any of the heatmaps
15. Move to the “Color Scheme” sub-tab
16. The minimum and maximum of your data will appear as text after setting the variables on the next page, but you can adjust them now if you would like
17. Select the color palette you wish here as well
    1. The options below will change based on the palette option you choose
18. Move to the “Variable Set and Save” sub-tab
19. Click the “Finalize the Variables” button
20. Once it is done loading, the downloads will open and I highly recommend you download both
    1. The variable save can be uploaded alongside the checkpoint to allow you to skip right to the variable set page rather than going through all the options again (except for the color scheme currently)
21. Upon finalizing the variables, the “Data Preview” tab will open. Navigate there
22. Select from the two sub-tabs based on what type of heatmap you wish to see
    1. You can do both or skip to any
23. Normal Heatmap:
    1. Click the create normal heatmap button
    2. **To save the heatmap**: you have two options you can choose from or download both
       1. Click the button labeled **‘Download the data preview heatmap’** and this will download a heatmap you can see visually
       2. Click the button labeled **‘Download the heatmap without the legend’** and this will download a heatmap without any of the legends for easy editing. Make sure to download it into a directory you can easily access to check if you like your chosen options
24. Anatomical Heatmap:
    1. Select the slices of the mouse brain you wish to see displayed (Alan Brain Atlas designations)
    2. Click the create the anatomical heatmap button
    3. Save the heatmap by downloading it
       1. Please note that you should only click once and wait. It can sometimes take a while to load, and it can’t always stop you from clicking too fast and downloading multiple times
25. Look at your heatmaps and determine **if you need to change anything**. If you do, start from step 5 of this section and check over each step one at a time
    1. **Don’t forget to set your variables before trying to load new heatmaps**

***Measures***

To elaborate the option available in the measurement variable step (step 12) of N2U:

* **Load**: This measure calculates the proportion of pathology occupying each region. Nutil outputs contain a column for object area (segmented pathology) and for region area. The load measure in M2U generates by taking the object area/region area. This proportion can be converted to a percentage by multiplying by 100. It automatically selects the level based on what region level you select. If you select something higher than daughter, it will add up the sub region areas and then divide to get an accurate load for the larger region.