Mouse-brain-atlas_cell-expr-signal

June 29, 2022

1 Plotting of scRNA-seq signal

Author: Daniel Levings
Date: 2022-06-29

This is the second of two **Jupyter Notebooks** created to document my comparison of a mouse brain snATAC-seq (single nuclei assay for transposase accessible chromatin with sequencing) data set from CATlas with a scRNA-seq (single cell RNA sequencing) data set from the Linnarsson lab's Mouse brain atlas. Specifically, in this code, I identify differences in neuronal vs other cell types in the mouse brain based on gene expression (scRNA-seq signal aggregated by cell type/cluster).

Datasets accompany the following papers:

Li, Y.E., Preissl, S., Hou, X. et al. An atlas of gene regulatory elements in adult mouse cerebrum. (2021). *Nature* 598, 129–136. https://doi.org/10.1038/s41586-021-03604-1

La Manno, G., Siletti, K., Furlan, A., et al. Molecular architecture of the developing mouse brain. Nature. 2021 Aug;596(7870):92-96. Epub 2021 Jul 28. https://doi.org/10.1038/s41586-021-03775-x

1.1 Set up environment

First import relevant *Python* modules. If not installed, please install the following with *pip* or *conda*:

- *numpy*
- *pandas*
- *loompy*
- *matplotlib*
- *plotnine*

[1]:
```
import os
import sys
import requests
import itertools
from datetime import date
import numpy as np
```

```
import pandas as pd
import loompy
import plotnine as p9
from matplotlib import rc
%matplotlib inline
```

Now check the currently loaded environment:

[2]:
```
print(sys.version_info)
modulenames = set(sys.modules) & set(globals())
print(modulenames)
del(modulenames)
```

```
sys.version_info(major=3, minor=9, micro=13, releaselevel='final', serial=0)
{'itertools', 'requests', 'sys', 'loompy', 'os'}
```

1.2 Download and import the data

Download the scRNA-seq data for adolescent brain from Linnarsson lab's Mouse brain atlas. Loompy.org has more detailed information on the file format specifications (Loom files) as well as how to use various API to access these files.

[3]:
```
URL = "https://storage.googleapis.com/linnarsson-lab-loom/l5_all.agg.loom"
response = requests.get(URL)
open("l5_all.agg.loom", "wb").write(response.content)
```

[3]:
```
114859798
```

[4]:
```
# Check to make sure it's in current directory
os.listdir()
```

[4]:
```
['.ipynb_checkpoints',
 'Mouse-brain-CATlas_celltype-clustering.pdf',
 'Mouse-brain-atlas_cell-expr-signal.ipynb',
 'l5_all.agg.loom',
 'bigwigs',
 'Mouse-brain-atlas_cell-expr-signal.pdf',
 'Mouse-brain-CATlas_celltype-clustering.ipynb',
 'Cell annotation.xlsx']
```

Import the data using *loompy*.

[5]:
```
mba = loompy.connect("l5_all.agg.loom")
```

Check the data/make sure it was imported properly.

[6]:
```
mba[0:6, 0:6]
```

[6]:
```
array([[13.97633136,  0.42268041,  0.12711864,  0.08494208,  0.03870968,
         0.        ],
```

```
[ 0.39053254,  0.        ,  0.        ,  0.        ,  0.        ,
  0.03225806],
[ 7.60946746,  1.3814433 ,  0.18644068,  0.51737452,  0.41290323,
  1.32258065],
[ 0.92899408,  0.09278351,  0.19491525,  0.12741313,  0.1483871 ,
  0.19354839],
[ 8.73964497,  9.        ,  3.60169492,  1.75289575,  1.10322581,
  1.87096774],
[ 1.59763314,  0.22680412,  0.04237288,  0.05405405,  0.03225806,
  0.29032258]])
```

[7]:
```
mba.shape
```

[7]:
```
(27998, 265)
```

Check what kind of gene-level (row) information can be accessed.

[55]:
```
mba.ra.keys()
```

[55]:
```
['Accession', 'Gene', '_LogCV', '_LogMean', '_Selected', '_Total', '_Valid']
```

Now, check what kind of sample-level (column) information can be accessed.

[56]:
```
tmp_list = []
idx = list(itertools.chain.
 ↪from_iterable([[0,1,2,3],[*range(22,42)],[200,201,202],[*range(190,214)]]))
for i in range(len(mba.ca.keys())):
    if any(i == j for j in idx):
        tmp_list.append(mba.ca.keys()[i])
print(tmp_list)
del(i, idx, tmp_list)
```

```
['Age_6w', 'Age_?', 'Age_p12, p35', 'Age_p16, p24', 'Bucket', 'Class',
'ClusterName', 'ClusterScore', 'Clusters', 'Comment', 'Description',
'Developmental_compartment', 'LeafOrder', 'Location_based_on', 'MarkerGenes',
'MarkerRobustness', 'MarkerSelectivity', 'MarkerSpecificity', 'NCells',
'Neurotransmitter', 'OriginalClusters', 'Outliers', 'Probable_location',
'Region', 'Tissue_Amygd', 'Tissue_CA1', 'Tissue_CB', 'Tissue_Ctx1',
'Tissue_Ctx1.5', 'Tissue_Ctx2', 'Tissue_Ctx3', 'Tissue_DRG', 'Tissue_DentGyr',
'Tissue_ENS', 'Tissue_HC', 'Tissue_Hypoth', 'Tissue_MBd', 'Tissue_MBv',
'Tissue_Medulla', 'Tissue_OB', 'Tissue_Pons', 'Tissue_SC', 'Tissue_SScortex',
'Tissue_StriatDor', 'Tissue_StriatVent', 'Tissue_Sympath', 'Tissue_Thal',
'_Total']
```

Now import the gene-level and sample-level data of interest into list objects, and the actual gene-by-cluster (scRNA-seq data aggregated by cluster/cell-type) expression matrix into a *pandas* data frame.

[9]:
```
# Get basic cell types/classes
agg_Class = mba.ca.Class.astype('U')

# Get the clusters
agg_Clusters = mba.ca.Clusters
agg_ClusterName = mba.ca.ClusterName.astype('U')

# Get number of cells per cluster
agg_NCells = mba.ca.NCells

# Get other possibly useful info: description for each cell,
# OriginalClusters, region and outliers
agg_Description = mba.ca.Description.astype('U')
agg_OriginalClusters = mba.ca.OriginalClusters
agg_Region = mba.ca.Region.astype('U')

# Now download row attributes- gene names, etc
agg_row_ENSMUSG = mba.ra.Accession.astype('U')
agg_row_GeneName = mba.ra.Gene.astype('U')
agg_row_Total = mba.ra._Total
agg_row_Selected = mba.ra._Selected
agg_row_LogCV = mba.ra._LogCV
agg_row_LogMean = mba.ra._LogMean

# Now, convert the dataset array and these data into dataframes
expr_df = pd.DataFrame(mba[:,:], index = agg_row_ENSMUSG, columns =␣
 ↪agg_ClusterName)
```

Check data (overall size) and sample.

[10]:
```
expr_df.shape
```

[10]:
```
(27998, 265)
```

[11]:
```
expr_df.iloc[0:6, 0:6]
```

[11]:

	ENT9	ENT8	ENT6	ENT5	ENT4	ENT7
ENSMUSG00000024647	13.976331	0.422680	0.127119	0.084942	0.038710	0.000000
ENSMUSG00000041544	0.390533	0.000000	0.000000	0.000000	0.000000	0.032258
ENSMUSG00000029503	7.609467	1.381443	0.186441	0.517375	0.412903	1.322581
ENSMUSG00000039942	0.928994	0.092784	0.194915	0.127413	0.148387	
ENSMUSG00000059187	8.739645	9.000000	3.601695	1.752896	1.103226	
ENSMUSG00000017756	1.597633	0.226804	0.042373	0.054054	0.032258	

```
ENSMUSG00000039942  0.193548
ENSMUSG00000059187  1.870968
ENSMUSG00000017756  0.290323
```

Finally, make 2 data frames, one with all the cluster/cell-type data and one with all the gene-level data.

[12]:
```
col_df = pd.DataFrame({"Cluster_Name" : agg_ClusterName, "Cluster" :␣
↪agg_Clusters,
   "Class" : agg_Class, "Description" : agg_Description, "Region" : agg_Region,
   "Number_of_cells" : agg_NCells})
```

[13]:
```
row_df = pd.DataFrame({"ENSMUSG" : agg_row_ENSMUSG, "Gene_symbol" :␣
↪agg_row_GeneName,
   "LogCV" : agg_row_LogCV, "LogMean" : agg_row_LogMean, "Selected" :␣
↪agg_row_Selected,
   "Total" : agg_row_Total})
```

1.3 Extract *Nsg2* and make plotting data frame

Identify the row corresponding to the gene *Neuron-specific gene 2* (*Nsg2*), and pull the expression data.

[14]:
```
Nsg2_idx = int(np.transpose(np.where(agg_row_GeneName == "Nsg2")))
```

[15]:
```
row_df.iloc[Nsg2_idx, :]
```

[15]:
```
ENSMUSG        ENSMUSG00000020297
Gene_symbol                  Nsg2
LogCV                    0.802589
LogMean                  0.881825
Selected                        0
Total                      2589.0
Name: 15421, dtype: object
```

[16]:
```
expr_df.iloc[Nsg2_idx, 0:6]
```

[16]:
```
ENT9    10.550296
ENT8     9.030928
ENT6     4.813559
ENT5     2.467181
ENT4     2.993548
ENT7     5.645161
Name: ENSMUSG00000020297, dtype: float64
```

Combine expression data with the cluster/cell type-level data.

[17]:
```
plotting_df = pd.concat([pd.DataFrame({"expression" : expr_df.iloc[Nsg2_idx,:].
↪to_numpy()}),
   col_df], axis = 1, ignore_index=False)
```

Check the basic cell types annotated in this data set.

[18]:
```
pd.unique(plotting_df["Class"])
```

[18]:
```
array(['Neurons', 'PeripheralGlia', 'Vascular', 'Oligos', 'Astrocytes',
       'Ependymal', 'Immune'], dtype=object)
```

Now, make basic cell classes even more simple: separate into neuron and non-neuron (broadly called 'support') cells. Add this classification back to *plotting_df*.

[19]:
```
basic_class=[]
for i in plotting_df["Class"]:
    if(i == "Neurons"):
        basic_class.append("Neuron")
    else:
        basic_class.append("Support")

plotting_df["Basic class"] = basic_class
```

Check *plotting_df* sample.

[20]:
```
plotting_df.head()
```

[20]:

	expression	Cluster_Name	Cluster	Class	Description	Region	Number_of_cells	Basic class
0	10.550296	ENT9	0	Neurons	Cholinergic enteric neurons	Enteric nervous system	169	Neuron
1	9.030928	ENT8	1	Neurons	Cholinergic enteric neurons, VGLUT2	Enteric nervous system	97	Neuron
2	4.813559	ENT6	2	Neurons	Cholinergic enteric neurons	Enteric nervous system	118	Neuron
3	2.467181	ENT5	3	Neurons	Cholinergic enteric neurons	Enteric nervous system	259	Neuron
4	2.993548	ENT4	4	Neurons	Cholinergic enteric neurons	Enteric nervous system	155	Neuron

Now, summarize the expression data by the new basic cell classes I designated. Specifically, calculate the median and mean expression in each of these basic cell classes.

[22]:
```
summ_df = plotting_df.drop(['Cluster_Name', 'Cluster', 'Class', 'Region',␣
↪'Description'], axis = 1).groupby(['Basic class'], axis = 0, ).
↪agg(['mean','median'])
```

Flatten the summarized data so I have a 'simple' data frame again (and reset index). Then check the summarized values.

[23]:
```
summ_df.columns = ['_'.join(col) for col in summ_df.columns.values]
summ_df.reset_index(inplace = True)
```

[24]:
```
summ_df
```

[24]:
```
  Basic class  expression_mean  expression_median  Number_of_cells_mean  \
0      Neuron         3.546293           2.871154            348.313084
1     Support         0.239595           0.041005           1691.313725

   Number_of_cells_median
0                   137.5
1                  1025.0
```

1.4 Plot *Nsg2* expression using *plotnine*

First, set up the theme/aesthetics I want to use for my plot in *plotnine*.

[25]:
```
p9.themes.theme_set(p9.theme_bw(base_family = "Arial") +
    p9.theme(plot_title = p9.element_text(ha="center", va="baseline",
             size = 14, margin = {'t' : 5, 'b' : 0, 'l' : 5, 'r' : 5}),
        axis_text = p9.element_text(color="black", size = 11),
        axis_title = p9.element_text(color="black", size = 12),
        axis_ticks = p9.element_line(color="black"),
        legend_title = p9.element_text(face = "italic", size = 12),
        legend_text = p9.element_text(size = 11),
        panel_border = p9.element_rect(size=0.75, color="black"),
        legend_position = "right",
        panel_grid_major = p9.element_line(color = "silver", linetype="solid",␣
↪size=0.2),
        panel_grid_minor = p9.element_line(color = "silver", linetype="dashed",␣
↪size=0.1)
    ))
```

Now use *plotnine* to generate a 'dot-plot' where the dots are constrained within a 'violin plot-like' object to show the distribution of the data for each group. This is called a *sina* plot. Also, I will color by the basic (but more specific that what I annotated) cell types given with the data.

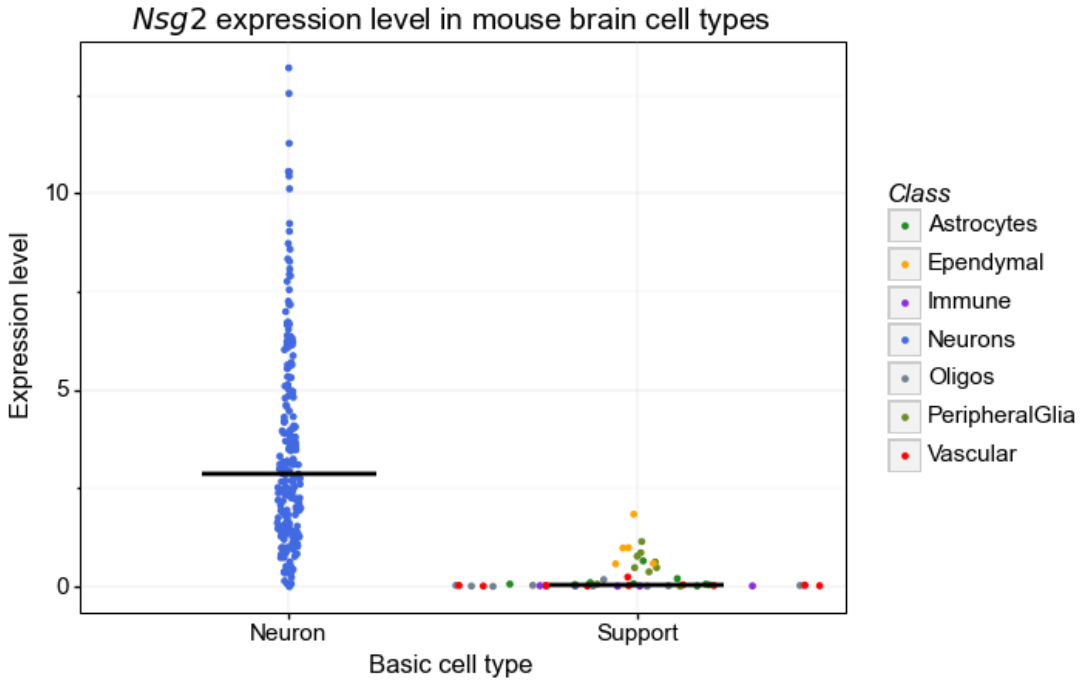
[32]:
```
# make plot

Nsg2_expr_plot = (
    p9.ggplot(data = plotting_df, mapping = p9.aes(x="Basic class",␣
↪y="expression")) +
```

```
    p9.geom_sina(mapping = p9.aes(group = "Basic class", color = "Class"),␣
 ↪maxwidth = 1.1, size = 0.9,
         method = "density", scale = "area") +
    p9.scales.scale_color_manual(values = ({'Neurons' : "royalblue",␣
 ↪'PeripheralGlia' : "olivedrab",
        'Vascular' : "red", 'Oligos' : "slategrey", 'Astrocytes' : "forestgreen",
        'Ependymal' : "orange", 'Immune' : "blueviolet"})) +
    p9.labs(x = "Basic cell type", y = "Expression level",
         title = "$Nsg2$ expression level in mouse brain cell types") +
    p9.geom_errorbar(data = summ_df, inherit_aes = False, size = 1,
         mapping = p9.aes(x="Basic class", ymin="expression_median",␣
 ↪ymax="expression_median"))
    )

# output
Nsg2_expr_plot
```

```
/home/daniel/anaconda3/envs/loompy/lib/python3.9/site-
packages/plotnine/positions/position.py:204: PlotnineWarning: position_dodge
requires non-overlapping x intervals
```



[32]: <ggplot: (8790027644938)>

If you want to save this plot to it's own PDF file, uncomment the next lines and run:

[27]:
```
# Nsg2_expr_plot.save(filename =␣
 ↪"Nsg2-scRNAseq-expression-levels_Mouse-brain-atlas.pdf",
#                     format = "pdf", width = 7, height = 5.5, units = "in",␣
 ↪dpi = 250)
```

Wrap up by closing the *loompy* object.

[28]:
```
mba.close()
```