NIAPythonDay4_July2017

July 20, 2017

1 Day 1: IDE, basic data types, operators

2 Day 2: Slicing, NumPy array

3 Day 3: Pandas DataFrame

4 Day 4: Case Study: Microarray Data analysis and Visualization

- file: ExampleMicroarrayData.xls
- Data: brain region microarray data (measures gene expression levels in a tissue)
- Experimental variables = tissue type
  - hippocampus & cerebral cortex
- Columns: 4 repeats for each tissue type
- Rows: 1 row per read; 1 or more rows per gene. Redundant reads need to be merged.

4.1 Analysis Method

1. Import file
2. For a given gene with redundant reads, take maximum value (collapse multiple rows into one row)
3. Log transform
4. Z-score
5. For each comparison, take difference of means of repeats and p-value
6. Create ribbon plot for genes with fold change of 1 and p-value < 0.05

4.2 Import libraries

```
In [1]: import pandas as pd
        import numpy as np
```

4.3 Optional: set Pandas display precision

Use TAB key to show you what options are available

```
In [2]: pd.options.display
```

```
Out[2]: <pandas.core.config.DictWrapper at 0x112830fd0>

In [3]: pd.options.display.precision = 3
```

4.4 Read in Excel File

```
In [4]: microa = pd.read_excel( 'ExampleMicroarrayData.xls')
```

4.5 See what we got

```
In [ ]: microa.head()

In [5]: microa.shape

Out[5]: (59734, 10)

In [6]: microa.columns

Out[6]: Index(['ArrayID', 'Symbol', 'BR1_1', 'BR1_2', 'BR1_3', 'BR1_4', 'BR2_1',
               'BR2_2', 'BR2_3', 'BR2_4'],
              dtype='object')
```

4.6 Review: Get basic statistics across all columns using .describe()

```
In [ ]: microa.describe()
```

4.7 Review: subselect rows by boolean criterion using brackets []

```
In [ ]: microa[ microa.Symbol == 'Mfsd9' ]
```

4.8 Review: subselect one or more columns using brackets []

```
In [ ]: microa[ 'Symbol' ]

In [ ]: microa[ ['ArrayID', 'Symbol' ] ]
```

4.9 For subselecting rows and columns at the same time, use .loc[] (or.iloc[])

```
In [ ]: microa.loc[ microa.Symbol == 'Mfsd9', ['BR1_1', 'BR2_1' ] ]
```

4.10 Note: Difference between .loc[] and .iloc[]

- Use .loc[] to slice by row/column *NAMES* or *BOOLEANS*
- Use .iloc[] to slice by row/column *INDICES* (Like we used with NumPy)

```
In [ ]: microa.loc[ :10, -4: ]

In [ ]: microa.iloc[ :10, -4: ]
```

4.11 How many unique gene symbols do we have?

```
In [7]: len( microa.Symbol )
```

```
Out[7]: 59734
```

```
In [8]: microa.Symbol.unique()
```

```
Out[8]: array(['NA1', 'NA2', 'NA3', ..., 'NA11372', 'NA11373', 'NA11374'], dtype=object)
```

```
In [9]: len(microa.Symbol.unique())
```

```
Out[9]: 29784
```

```
In [ ]: microa.Symbol.value_counts()
```

4.12 Group rows by gene symbol using .groupby()

- Pass the function the name of the column containing the values you want to group by

```
In [10]: grouped = microa.groupby('Symbol')
```

4.12.1 Get a certain group using .get_group()

```
In [ ]: grouped.get_group( 'Mfsd9' )
```

```
In [ ]: grouped.agg( 'max')
```

4.13 Combine reads

Take maximum expression level for a given gene.

```
In [12]: max_expression = grouped.max()
```

```
In [13]: max_expression.shape
```

```
Out[13]: (29784, 9)
```

```
In [ ]: max_expression
```

4.14 .apply() a log transformation function over all expression values

```
In [ ]: np.log?
```

```
In [14]: np.log( [1, 10, 100, 1000] )
```

```
Out[14]: array([ 0.        ,  2.30258509,  4.60517019,  6.90775528])
```

```
In [15]: log_trans = max_expression.apply( np.log )
```

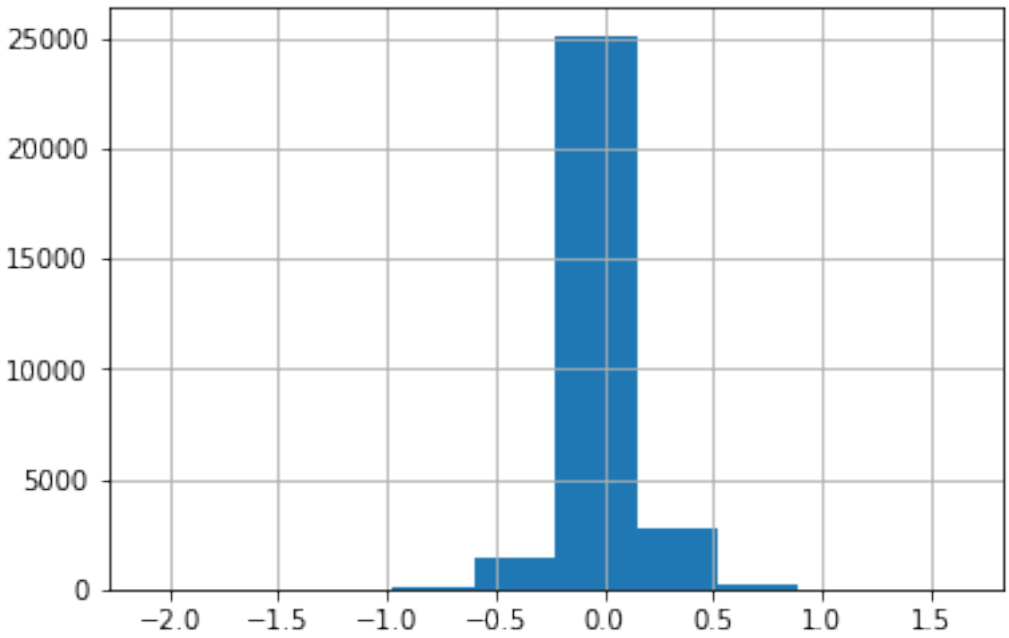
```
In [ ]: log_trans
```

4.15 .apply() a Z-score transformation function over all genes

```
In [16]: from scipy.stats import zscore

In [17]: zscore( [ 1,2,3,4,5,6,7,8,9,10] )

Out[17]: array([-1.5666989 , -1.21854359, -0.87038828, -0.52223297, -0.17407766,
                 0.17407766,  0.52223297,  0.87038828,  1.21854359,  1.5666989 ])

In [18]: z_trans = log_trans.apply(zscore)

In [ ]: log_trans.apply?

In [19]: z_trans.shape

Out[19]: (29784, 9)

In [ ]: z_trans
```

4.16 Compare: Make a histogram of fold change

```
In [20]: br1 = z_trans[ ['BR1_1','BR1_2', 'BR1_3', 'BR1_4' ] ]

In [21]: br1.shape

Out[21]: (29784, 4)

In [22]: br2 = z_trans[ ['BR2_1','BR2_2', 'BR2_3', 'BR2_4' ] ]

In [23]: br2.shape

Out[23]: (29784, 4)

In [24]: br1_mean = br1.mean(axis=1)
         br2_mean = br2.mean(axis=1)

In [25]: br2_mean.shape

Out[25]: (29784,)

In [26]: diff = br2_mean - br1_mean

In [27]: diff.shape

Out[27]: (29784,)

In [28]: # tell Jupyter Notebook to show the figure after it made it
         %matplotlib inline

In [29]: diff.hist()

Out[29]: <matplotlib.axes._subplots.AxesSubplot at 0x112e2a5f8>
```



4.17 Subselect genes based on having fold change > 1

```
In [ ]: diff[ diff.abs() > 1 ]
```

4.18 Get gene expression difference and p-value for desired comparisons

Use Wilcoxon rank-sum statistic for two samples to test if the reads for a given gene across comparison groups come from different distributions.

```
In [30]: from scipy.stats import ranksums

In [31]: ranksums( [1,2,3,4], [5,6,7,8])

Out[31]: RanksumsResult(statistic=-2.3094010767585034, pvalue=0.020921335337794014)

In [32]: ranksums( [1,2,3,4], [50,60,70,80])

Out[32]: RanksumsResult(statistic=-2.3094010767585034, pvalue=0.020921335337794014)

In [33]: ranksums( [1,2,3,5], [4,6,7,8])

Out[33]: RanksumsResult(statistic=-2.0207259421636903, pvalue=0.043308142810791955)
```

4.18.1 Go row-by-row doing significance test

```
In [34]: import time

In [35]: # create an empty list onto which we can append p-values
         pval_list = []

In [36]: t1 = time.time()

         # iterate over every gene
         for gene in br1.index:

             # subselect the expression values for the corresponding gene
             vals1 = br1.loc[ gene ]
             vals2 = br2.loc[ gene ]

             # Do the statistical test for this gene
             statistic, pvalue = ranksums( vals1, vals2 )

             # save the p-value
             pval_list.append( pvalue )

         t2 = time.time()
         print( "This operation took", t2-t1, "seconds.")
```

```
This operation took 11.919003009796143 seconds.
```

```
In [37]: len(pval_list)
```

```
Out[37]: 29784
```

```
In [38]: # One-liner!
         t1 = time.time()
         pval_list = [ ranksums(a,b)[1] for a, b in zip( br1.as_matrix(), br2.as_matrix() ) ]
         t2 = time.time()
         print( "This operation took", t2-t1, "seconds.")
```

```
This operation took 4.959620952606201 seconds.
```

4.19 Combine fold change and p-value into one DataFrame

- Problem 1: the diff object is just one column (a Pandas "Series" object), not a full-fledged DataFrame
- Problem 2: the p-values are stored in a simple Python list
- Solution: use the to_frame() function to turn the Series into a DataFrame, then add the p-values as a new column to that DataFrame.

```
In [39]: type( diff )
```

```
Out[39]: pandas.core.series.Series

In [ ]: diff.head()

In [40]: combined = diff.to_frame()

In [ ]: combined.head()

In [41]: combined = diff.to_frame( name='fold')

In [ ]: combined.head()

In [42]: combined[ 'pvals' ] = pval_list

In [ ]: combined.head()

In [43]: combined.shape

Out[43]: (29784, 2)
```

4.20 Subselect genes with fold change > 1

```
In [ ]: combined[ combined.fold.abs() > 1 ]
```

4.21 Subselect genes with fold change > 1 AND p-value < 0.05

```
In [ ]: combined[ (combined.fold.abs() > 1) & (combined.pvals < 0.05) ]

In [44]: plot_these = combined[ (combined.fold.abs() > 1) & (combined.pvals < 0.05) ]

In [45]: len(plot_these)

Out[45]: 36
```

Turn the row labels into variables in their own right:

```
In [46]: plot_these['Gene'] = plot_these.index

/usr/local/lib/python3.6/site-packages/ipykernel_launcher.py:1: SettingWithCopyWarning:
A value is trying to be set on a copy of a slice from a DataFrame.
Try using .loc[row_indexer,col_indexer] = value instead

See the caveats in the documentation: http://pandas.pydata.org/pandas-docs/stable/indexing.html
  """Entry point for launching an IPython kernel.


In [47]: plot_these.sort_values(by='fold', inplace=True)

/usr/local/lib/python3.6/site-packages/ipykernel_launcher.py:1: SettingWithCopyWarning:
A value is trying to be set on a copy of a slice from a DataFrame

See the caveats in the documentation: http://pandas.pydata.org/pandas-docs/stable/indexing.html
  """Entry point for launching an IPython kernel.
```

4.22 Generate RibbonPlot

4.22.1 Load some of Python's figure-making libraries:

```
In [48]: import seaborn as sns
         import matplotlib.pyplot as plt
```

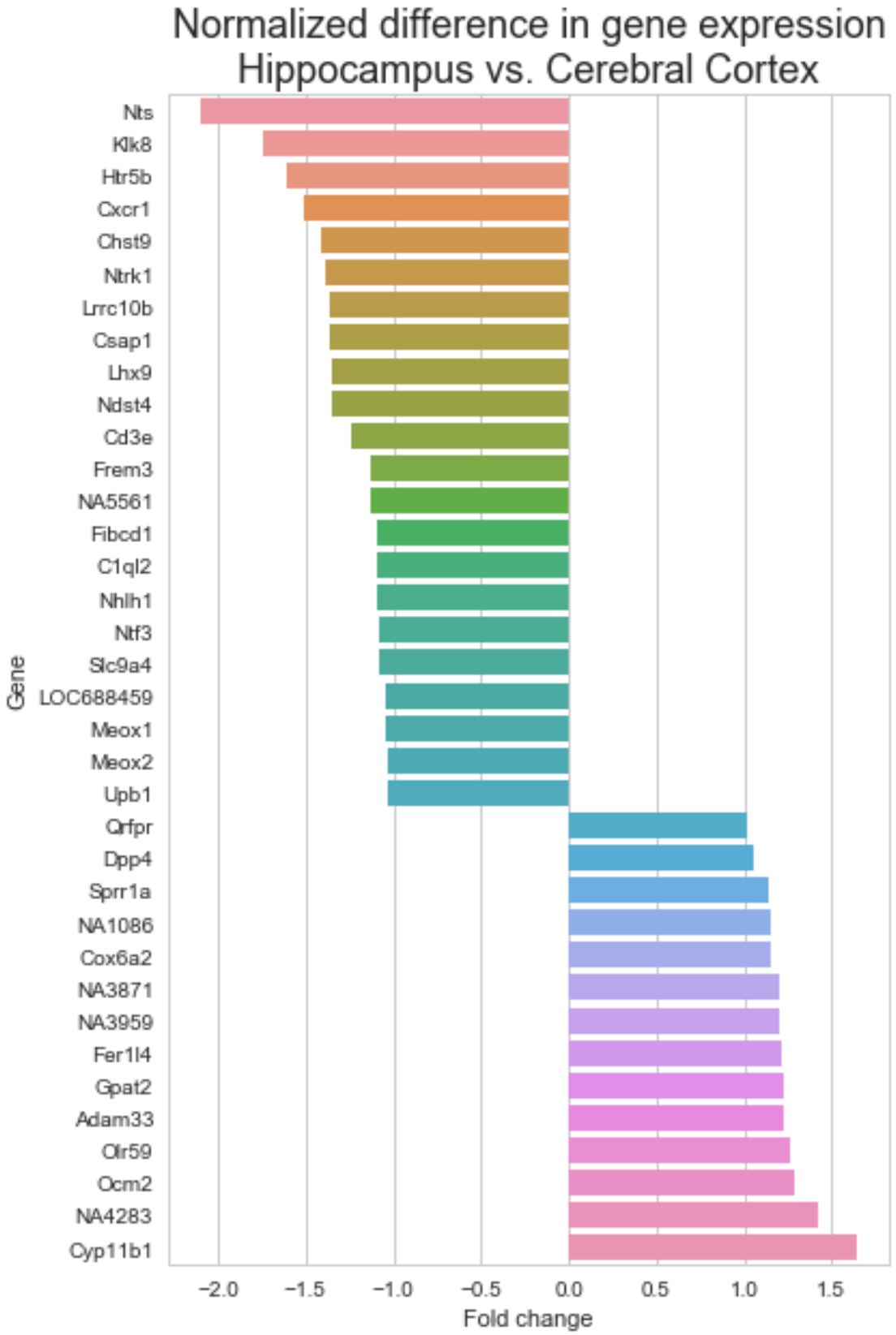
4.22.2 Set the style of the figure

When using the plotting package seaborn, there are five figure styles to choose from: 1. darkgrid 2. whitegrid 3. dark 4. white 5. ticks

```
In [49]: sns.set( style="whitegrid" )
```

4.22.3 Making the figure

```
In [50]: # I want this figure to be 6 inches wide and 10 inches tall
         fig_dimensions=(6, 10)

         # Create a blank figure to hang the data off of
         figure, axes = plt.subplots( figsize=fig_dimensions )

         # Plot the data onto the figure
         sns.barplot( data=plot_these, x="fold", y="Gene" )

         # Assign a title to the figure
         chart_title = """Normalized difference in gene expression
         Hippocampus vs. Cerebral Cortex"""
         axes.set_title( chart_title, size=18 )

         # Assign a label to the x-axis
         axes.set_xlabel( "Fold change" )

Out[50]: <matplotlib.text.Text at 0x1133b9320>
```


Normalized difference in gene expression
Hippocampus vs. Cerebral Cortex
Gene
Nts
Klk8
Htr5b
Cxcr1
Chst9
Ntrk1
Lrrc10b
Csap1
Lhx9
Ndst4
Cd3e
Frem3
NA5561
Fibcd1
C1ql2
Nhlh1
Ntf3
Slc9a4
LOC688459
Meox1
Meox2
Upb1
Qrfpr
Dpp4
Sprr1a
NA1086
Cox6a2
NA3871
NA3959
Fer1l4
Gpat2
Adam33
Olr59
Ocm2
NA4283
Cyp11b1
−2.0
−1.5
−1.0
−0.5
0.0
0.5
1.0
1.5
Fold change

4.22.4 Save the figure as a PDF:

```
In [ ]: figure.savefig( "ribbonplot.pdf")
```