tissue_specific_rnaseq_analysis_jupyter_notebook

December 19, 2016

1 Supporting Information Notes S1. Documentation of data analysis.

Custom Python code was used in a Jupyter notebook using the Pandas, NumPy, Seaborn, and SciPy packages to organize, process, and display the data.

1.0.1 Files Required in this Notebook and their Source:

Purpose	File	Source
Annotation	Mesculenta_305_v6.1.annotation_info.txt	Phytozome10.3
Gene Expression	gene_exp.diff	Cuffdiff
Gene Expression	genes.read_group_tracking	Cuffdiff
GO Enrichment	4.genes_nodes_mean_exp.txt	SOM Clustering Analysis

```
In [1]: import sys,os

        import pandas as pd                       # version 0.17.0
        import numpy as np                        # version 1.11.0
        import seaborn as sns                     # version 0.7.0
        import matplotlib.pyplot as plt           # version 1.5.1

        from scipy.stats import percentileofscore as percentileofscore
        # version 0.16.0

        %matplotlib inline
```

```
In [2]: os.chdir('tissue_specific_rnaseq/')
```

1.1 Annotation

```
In [3]: # read in annotations for AM560-2 assembly version 6.1
        annot = pd.read_table( 'Mesculenta_305_v6.1.annotation_info.txt',
                              sep='\t', header=None )

        # limit table to specific columns
        #    and drop any duplicates that may be present
        annot = annot[[1,5,9,10,12]].drop_duplicates(subset=1).rename(
            columns={1:'gene',5:'PTHR',9:'go',10:'TAIR',12:'annot'})

        # for the genes that do not have an arabidopsis functional annotation,
        #     use the PANTHER funtional annotation provided where possible
        annot['annot'][annot['annot'].isnull()] = annot['PTHR']
        # drop the PANTHER column
        annot = annot.dropna(axis=0, subset=['annot']).drop(['PTHR'], axis=1)

        df_go = annot[['go','gene']].dropna().set_index('gene')['go']
        df_go = df_go.str.split(',').apply(pd.Series,1).stack()
        df_go.index = df_go.index.droplevel(-1)
        df_go.name = 'go'

        annot.columns

Out[3]: Index(['gene', 'go', 'TAIR', 'annot'], dtype='object')

In [4]: print('Annotated Gene Count: {}'.format(annot.shape[0]) )

Annotated Gene Count: 26015
```

1.2 Read in Data

```
In [5]: # read in data
        df_cuff = pd.read_table( 'gene_exp.diff',
                                sep='\t', index_col=0)

        # Create dataframe to hold all duplicated genes (defined as
        #    genes containing)
        df_dups = df_cuff.drop_duplicates(['gene_id','gene'])['gene']
        df_shiny = df_dups.str.split(',').apply(pd.Series,1).stack()

        df_dups = df_dups[df_dups.str.contains(',')
                          ].str.split(',').apply(pd.Series,1).stack()
        df_dups.index = df_dups.index.droplevel(-1)
        df_shiny.index = df_shiny.index.droplevel(-1)
        df_shiny.name = 'gene'


        df_cuff['gene'] = df_cuff['gene'].str.replace(r',.*', '')

        df_cuff.columns

Out[5]: Index(['gene_id', 'gene', 'locus', 'sample_1', 'sample_2', 'status', 'value_1',
               'value_2', 'log2(fold_change)', 'test_stat', 'p_value', 'q_value',
               'significant'],
              dtype='object')

In [6]: # create dataframe containing each gene in one row with its
        #    expression values in each tissue type
        df_cuff_exp = pd.concat([
                df_cuff[['gene_id',
                         'gene',
                         'locus',
                         'sample_1',
                         'value_1']].rename(columns={'sample_1':'sample',
                                                     'value_1':'value'}),
                df_cuff[['gene_id',
                         'gene',
                         'locus',
                         'sample_2',
                         'value_2']].rename(columns={'sample_2':'sample',
                                                     'value_2':'value'}),
            ]).drop_duplicates().pivot(index='gene_id',
                                       columns='sample',
                                       values='value')

        # reorganize column names with similar tissues near each other
        tissue_order = ['Leaf','Mid_Vein','Petiole','Stem','Lateral_Bud','SAM',
                        'Storage_Root','Fibrous_Root','RAM',
                        'OES','FEC'
                       ]
        df_cuff_exp = df_cuff_exp.loc[:,tissue_order]

        df_cuff_exp.columns

Out[6]: Index(['Leaf', 'Mid_Vein', 'Petiole', 'Stem', 'Lateral_Bud', 'SAM',
               'Storage_Root', 'Fibrous_Root', 'RAM', 'OES', 'FEC'],
              dtype='object', name='sample')

In [7]: # remove all rows with all 0's, with no annotation
        # merge expression data with annotation data and
        #    only keep genes that have been annotated
        df_cuff_ann_all = df_cuff_exp.merge(
            df_cuff[['gene_id','gene','locus']],
            left_index=True,
            right_on='gene_id',
            copy=False).drop_duplicates()

        # drop all denovo genes
        df_cuff_ann_all = df_cuff_ann_all[df_cuff_ann_all['gene'] != '-']

        # add annotation (gene names)
        df_cuff_ann_all = df_cuff_ann_all.merge( annot[['gene','annot']],
                                                how='left',
                                                on='gene',
                                                copy=False)

        # drop all genes that do not have an annotation
        df_cuff_ann = df_cuff_ann_all.dropna()

        # drop genes with lower than 0 mean expression
        df_cuff_ann = df_cuff_ann[df_cuff_ann.mean(axis=1) > 0]
        df_cuff_ann.columns

Out[7]: Index(['Leaf', 'Mid_Vein', 'Petiole', 'Stem', 'Lateral_Bud', 'SAM',
               'Storage_Root', 'Fibrous_Root', 'RAM', 'OES', 'FEC', 'gene_id', 'gene',
               'locus', 'annot'],
              dtype='object', name='sample')

In [8]: # read data from genes.read_group_tracking file
        genes_rgt = pd.read_table('genes.read_group_tracking')
        # merge condition and replicate columns to pivot table on
        genes_rgt['pivot'] = genes_rgt['condition'] + genes_rgt['replicate'].astype(str)

        # pivot table to look at expression values for each replicate per gene
        genes_rgt_piv = genes_rgt.pivot(index='tracking_id',
                                        columns='pivot',
                                        values='FPKM')


        # merge with gene names
        genes_rgt_piv = genes_rgt_piv.merge(df_cuff.loc[:,['gene_id',
                                                             'gene'
                                                            ]].drop_duplicates(),
                                            left_index=True,
                                            right_on='gene_id')

        # Merge with functional annotations
        genes_rgt_piv = genes_rgt_piv.merge( annot,
                                            on='gene',
                                            how='left' ).set_index('gene_id')

        genes_rgt_piv['gene_id'] = genes_rgt_piv.index

        # for later analyses, prep tissue to index dictionary
        tissue_rep_index = {'FEC':[0,1,2],
                            'Fibrous_Root':[3,4,5],
                            'Lateral_Bud':[6,7,8],
                            'Leaf':[9,10,11],
                            'Mid_Vein':[12,13,14],
                            'OES':[15,16,17],
                            'Petiole':[18,19,20],
                            'RAM':[21,22,23],
                            'SAM':[24,25,26],
                            'Stem':[27,28,29],
                            'Storage_Root':[30,31]
                           }

        genes_rgt_piv.columns

Out[8]: Index(['FEC0', 'FEC1', 'FEC2', 'Fibrous_Root0', 'Fibrous_Root1',
               'Fibrous_Root2', 'Lateral_Bud0', 'Lateral_Bud1', 'Lateral_Bud2',
               'Leaf0', 'Leaf1', 'Leaf2', 'Mid_Vein0', 'Mid_Vein1', 'Mid_Vein2',
               'OES0', 'OES1', 'OES2', 'Petiole0', 'Petiole1', 'Petiole2', 'RAM0',
               'RAM1', 'RAM2', 'SAM0', 'SAM1', 'SAM2', 'Stem0', 'Stem1', 'Stem2',
               'Storage_Root0', 'Storage_Root1', 'gene', 'go', 'TAIR', 'annot',
               'gene_id'],
              dtype='object', name='pivot')

In [9]: #########################
        # RSHINY APP FILE PREP ##
        # print files for RShiny App
        df_temp = genes_rgt_piv[genes_rgt_piv.gene != '-'].drop_duplicates().copy()
        del df_temp['gene']
        df_temp = df_temp.merge( pd.DataFrame(df_shiny),
                                  how='left',
                                  left_index=True,
                                  right_index=True )

        df_temp['possible_issues'] = df_temp['gene'].isin(df_dups)

        print(df_temp.shape)
        df_temp.to_csv('mesculenta_v6_output/Rshiny_app_dataset.txt',
                      sep='\t', index=False)


        ## Print genes with multiple associated annotations
        df_dups.to_csv('mesculenta_v6_output/warning_genes.txt',
                      index=False, header=False)
        print( df_dups.shape )
```

```
(35200, 38)
(4531,)
```

2 SOM Clustering with GO Enrichment

2.1 Find all genes with at least one significantly differentially expressed pairwise comparison

This data is for use in an analysis in R to identify gene expression clusters using a self organizing map

```
In [10]: # write to file all gene names with a statistically significant pairwise
         #    difference where one of the pairs is expressed greater than 1 FPKM
         df_gene_ids = pd.DataFrame(df_cuff[(df_cuff['q_value'] <= 0.05)
                             & ((df_cuff['value_1'] > 1) | (df_cuff['value_2'] > 1))
                            ].loc[:,['gene_id','gene']].drop_duplicates().index)
         df_gene_ids.to_csv('mesculenta_v6_output/0.cassava_diff_genes.txt',
                           index=False, sep='\t')
```

```
In [11]: # write to file all genes with at least one tissue expressed
         #     greater than 1 FPKM for use as background in the GO analysis
         # print to file expression values for all genes expressed > 1FPKM
         genes_rgt_piv.iloc[:,:32].to_csv('./mesculenta_v6_output/1.cassava_exp.txt',
                                         sep='\t')

         # print to file expression values with functional annotations
         genes_rgt_piv.drop('go', axis=1
                          ).to_csv('./mesculenta_v6_output/2.cassava_annotation.txt',
                                  sep='\t')
```

3 Parse and Trim goatools GO Terms from R Cluster Analysis

A python tool called goatools was used to create the files being read in this section. The 4 nodes are based on a self organizing map from an R analysis to cluster genes by expression profile

3.0.1 GO PREP

Split Genes into Files by node

```
In [12]: df_pcanodes = pd.read_table('4.genes_nodes_mean_exp.txt',
                                     sep='\t', usecols = ['test_id','node'])

         print(df_pcanodes.shape)

(14426, 2)
```

```
In [13]: ## READ IN NODE DATA FROM DAN'S ANALYSIS
         df_node1 = df_pcanodes[df_pcanodes['node'] == 1
                               ].merge(df_cuff.loc[:,['gene']].drop_duplicates(),
                                       left_on='test_id',
                                       right_index=True
                                      )
         df_node2 = df_pcanodes[df_pcanodes['node'] == 2
                               ].merge(df_cuff.loc[:,['gene']].drop_duplicates(),
                                       left_on='test_id',
                                       right_index=True
                                      )
         df_node3 = df_pcanodes[df_pcanodes['node'] == 3
                               ].merge(df_cuff.loc[:,['gene']].drop_duplicates(),
                                       left_on='test_id',
                                       right_index=True
                                      )
         df_node4 = df_pcanodes[df_pcanodes['node'] == 4
                               ].merge(df_cuff.loc[:,['gene']].drop_duplicates(),
                                       left_on='test_id',
                                       right_index=True
                                      )

         ## PRINT NODE COUNTS TO SCREEN
         print('node1 counts: {}'.format(df_node1.shape[0]) )
         print('node2 counts: {}'.format(df_node2.shape[0]) )
         print('node3 counts: {}'.format(df_node3.shape[0]) )
         print('node4 counts: {}'.format(df_node4.shape[0]) )

         ## PRINT GO PREP GENE NAMES TO FILE
         df_cuff[(df_cuff['q_value'] <= 0.05)
                 & ((df_cuff['value_1'] > 1) | (df_cuff['value_2'] > 1))
                ].loc[:,['gene_id','gene']
                     ].drop_duplicates()['gene'].to_csv('goprep_pcasom_bkgrnd.txt',
                                                        index=False, sep='\t' )

         df_cuff[((df_cuff['value_1'] > 1) | (df_cuff['value_2'] > 1))
                ].loc[:,['gene_id','gene']
                     ].drop_duplicates()['gene'].to_csv('goprep_pcasom_bkgrnd_all.txt',
                                                        index=False, sep='\t' )


         df_node1['gene'].to_csv('goprep_pcasom_node1.txt',
                                 index=False, sep='\t' )
         df_node2['gene'].to_csv('goprep_pcasom_node2.txt',
                                 index=False, sep='\t' )
         df_node3['gene'].to_csv('goprep_pcasom_node3.txt',
                                 index=False, sep='\t' )
         df_node4['gene'].to_csv('goprep_pcasom_node4.txt',
                                 index=False, sep='\t' )
```

```
node1 counts: 2672
node2 counts: 2672
node3 counts: 2914
node4 counts: 3727
```

3.0.2 NODE1

```
In [14]: node1_df = pd.read_table('pcasom_node1_goatools_noprop.txt', skiprows=2)

         # GO Term list before limiting by significance
         print('Before significance filtering')
         print('GO Count: {}'.format(node1_df.shape[0]))
         print('Fraction of enriched genes: {:.2f}'.format(
                 node1_df[node1_df['enrichment'] == 'e'].shape[0]/node1_df.shape[0]))

         # limit GO Terms by FDR corrected p value
         node1_df = node1_df[node1_df['p_fdr'] < 0.001]

         # GO Term list before limiting by significance
         print('\nAfter significance filtering')
         print('GO Count: {}'.format(node1_df.shape[0]))
         print('Fraction of enriched genes: {:.2f}'.format(
                 node1_df[node1_df['enrichment'] == 'e'].shape[0]/node1_df.shape[0]))

         node1_df = node1_df[node1_df['enrichment'] == 'e']

         node1_df.to_csv('pcasom_node1_goenrichment.txt', sep='\t', index=False)

         df_node1_gotags = node1_df.loc[:,'id']

         node1_df.columns
```

```
Before significance filtering
GO Count: 155
Fraction of enriched genes: 0.84

After significance filtering
GO Count: 35
Fraction of enriched genes: 0.80
```

```
Out[14]: Index(['id', 'enrichment', 'description', 'ratio_in_study', 'ratio_in_pop',
                'p_uncorrected', 'p_bonferroni', 'p_holm', 'p_sidak', 'p_fdr'],
               dtype='object')
```

3.0.3 NODE2

```
In [15]: node2_df = pd.read_table('pcasom_node2_goatools_noprop.txt', skiprows=2)
```

```
        # GO Term list before limiting by significance
        print('Before significance filtering')
        print('GO Count: {}'.format(node2_df.shape[0]))
        print('Fraction of enriched genes: {:.2f}'.format(
                node2_df[node2_df['enrichment'] == 'e'].shape[0]/node2_df.shape[0]))

        # limit GO Terms by FDR corrected p value
        node2_df = node2_df[node2_df['p_fdr'] < 0.01]

        # GO Term list before limiting by significance
        print('\nAfter significance filtering')
        print('GO Count: {}'.format(node2_df.shape[0]))
        print('Fraction of enriched genes: {:.2f}'.format(
                node2_df[node2_df['enrichment'] == 'e'].shape[0]/node2_df.shape[0]))

        node2_df = node2_df[node2_df['enrichment'] == 'e']

        node2_df.to_csv('pcasom_node2_goenrichment.txt', sep='\t', index=False)

        df_node2_gotags = node2_df.loc[:,'id']

        node2_df.columns
```

```
Before significance filtering
GO Count: 84
Fraction of enriched genes: 0.58

After significance filtering
GO Count: 6
Fraction of enriched genes: 0.50
```

```
Out[15]: Index(['id', 'enrichment', 'description', 'ratio_in_study', 'ratio_in_pop',
                'p_uncorrected', 'p_bonferroni', 'p_holm', 'p_sidak', 'p_fdr'],
               dtype='object')
```

3.0.4 NODE3

```
In [16]: node3_df = pd.read_table('pcasom_node3_goatools_noprop.txt', skiprows=2)

        # GO Term list before limiting by significance
        print('Before significance filtering')
        print('GO Count: {}'.format(node3_df.shape[0]))
        print('Fraction of enriched genes: {:.2f}'.format(
                node3_df[node3_df['enrichment'] == 'e'].shape[0]/node3_df.shape[0]))

        # limit GO Terms by FDR corrected p value
        node3_df = node3_df[node3_df['p_fdr'] < 0.001]

        # GO Term list before limiting by significance
        print('\nAfter significance filtering')
        print('GO Count: {}'.format(node3_df.shape[0]))
        print('Fraction of enriched genes: {:.2f}'.format(
                node3_df[node3_df['enrichment'] == 'e'].shape[0]/node3_df.shape[0]))
        node3_df = node3_df[node3_df['enrichment'] == 'e']

        node3_df.to_csv('pcasom_node3_goenrichment.txt', sep='\t', index=False)

        df_node3_gotags = node3_df.loc[:,'id']

        node3_df.columns
```

```
Before significance filtering
GO Count: 92
Fraction of enriched genes: 0.71

After significance filtering
GO Count: 10
Fraction of enriched genes: 0.60
```

```
Out[16]: Index(['id', 'enrichment', 'description', 'ratio_in_study', 'ratio_in_pop',
                'p_uncorrected', 'p_bonferroni', 'p_holm', 'p_sidak', 'p_fdr'],
               dtype='object')
```

3.0.5 NODE4

```
In [17]: node4_df = pd.read_table('pcasom_node4_goatools_noprop.txt', skiprows=2)

         # GO Term list before limiting by significance
         print('Before significance filtering')
         print('GO Count: {}'.format(node4_df.shape[0]))
         print('Fraction of enriched genes: {:.2f}'.format(
                 node4_df[node4_df['enrichment'] == 'e'].shape[0]/node4_df.shape[0]))

         # limit GO Terms by FDR corrected p value
         node4_df = node4_df[node4_df['p_fdr'] < 0.001]

         # GO Term list before limiting by significance
         print('\nAfter significance filtering')
         print('GO Count: {}'.format(node4_df.shape[0]))
         print('Fraction of enriched genes: {:.2f}'.format(
                 node4_df[node4_df['enrichment'] == 'e'].shape[0]/node4_df.shape[0]))

         node4_df = node4_df[node4_df['enrichment'] == 'e']

         node4_df.to_csv('pcasom_node4_goenrichment.txt', sep='\t', index=False)

         df_node4_gotags = node4_df.loc[:,'id']

         node4_df.columns
```

```
Before significance filtering
GO Count: 156
Fraction of enriched genes: 0.77

After significance filtering
```

```
GO Count: 19
Fraction of enriched genes: 0.68
```

```
Out[17]: Index(['id', 'enrichment', 'description', 'ratio_in_study', 'ratio_in_pop',
                'p_uncorrected', 'p_bonferroni', 'p_holm', 'p_sidak', 'p_fdr'],
               dtype='object')
```

Plotting the expression values of node clustered genes

```
In [18]: ##############
         ### NODE 1 ###
         ##############
         df_node1_plot = df_node1.copy()
         print('node1 preGO: {}'.format(df_node1_plot.shape[0]))

         df_node1_plot = df_node1_plot.merge(pd.DataFrame(df_go),
                                             left_on='gene',
                                             right_index=True
                                            ).dropna()

         df_node1_plot = df_node1_plot[df_node1_plot['go'].isin(df_node1_gotags)
                                      ].loc[:,['test_id','gene']].drop_duplicates()

         print('node1 postGO: {}'.format(df_node1_plot.shape[0]))

         ##############
         ### NODE 2 ###
         ##############
         df_node2_plot = df_node2.copy()
         print('node2 preGO: {}'.format(df_node2_plot.shape[0]))

         df_node2_plot = df_node2_plot.merge(pd.DataFrame(df_go),
                                             left_on='gene',
                                             right_index=True
                                            ).dropna()

         df_node2_plot = df_node2_plot[df_node2_plot['go'].isin(df_node2_gotags)
                                      ].loc[:,['test_id','gene']].drop_duplicates()

         print('node2 postGO: {}'.format(df_node2_plot.shape[0]))

         ##############
         ### NODE 3 ###
         ##############
         df_node3_plot = df_node3.copy()
         print('node3 preGO: {}'.format(df_node3_plot.shape[0]))

         df_node3_plot = df_node3_plot.merge(pd.DataFrame(df_go),
                                             left_on='gene',
                                             right_index=True
                                            ).dropna()

         df_node3_plot = df_node3_plot[df_node3_plot['go'].isin(df_node3_gotags)
```

```
                                ].loc[:,['test_id','gene']].drop_duplicates()

        print('node3 postGO: {}'.format(df_node3_plot.shape[0]))

        ##############
        ### NODE 4 ###
        ##############
        df_node4_plot = df_node4.copy()
        print('node4 preGO: {}'.format(df_node4_plot.shape[0]))

        df_node4_plot = df_node4_plot.merge(pd.DataFrame(df_go),
                                            left_on='gene',
                                            right_index=True
                                           ).dropna()

        df_node4_plot = df_node4_plot[df_node4_plot['go'].isin(df_node4_gotags)
                                ].loc[:,['test_id','gene']].drop_duplicates()

        print('node4 postGO: {}'.format(df_node4_plot.shape[0]))
```

```
node1 preGO: 2672
node1 postGO: 474
node2 preGO: 2672
node2 postGO: 186
node3 preGO: 2914
node3 postGO: 638
node4 preGO: 3727
node4 postGO: 337
```

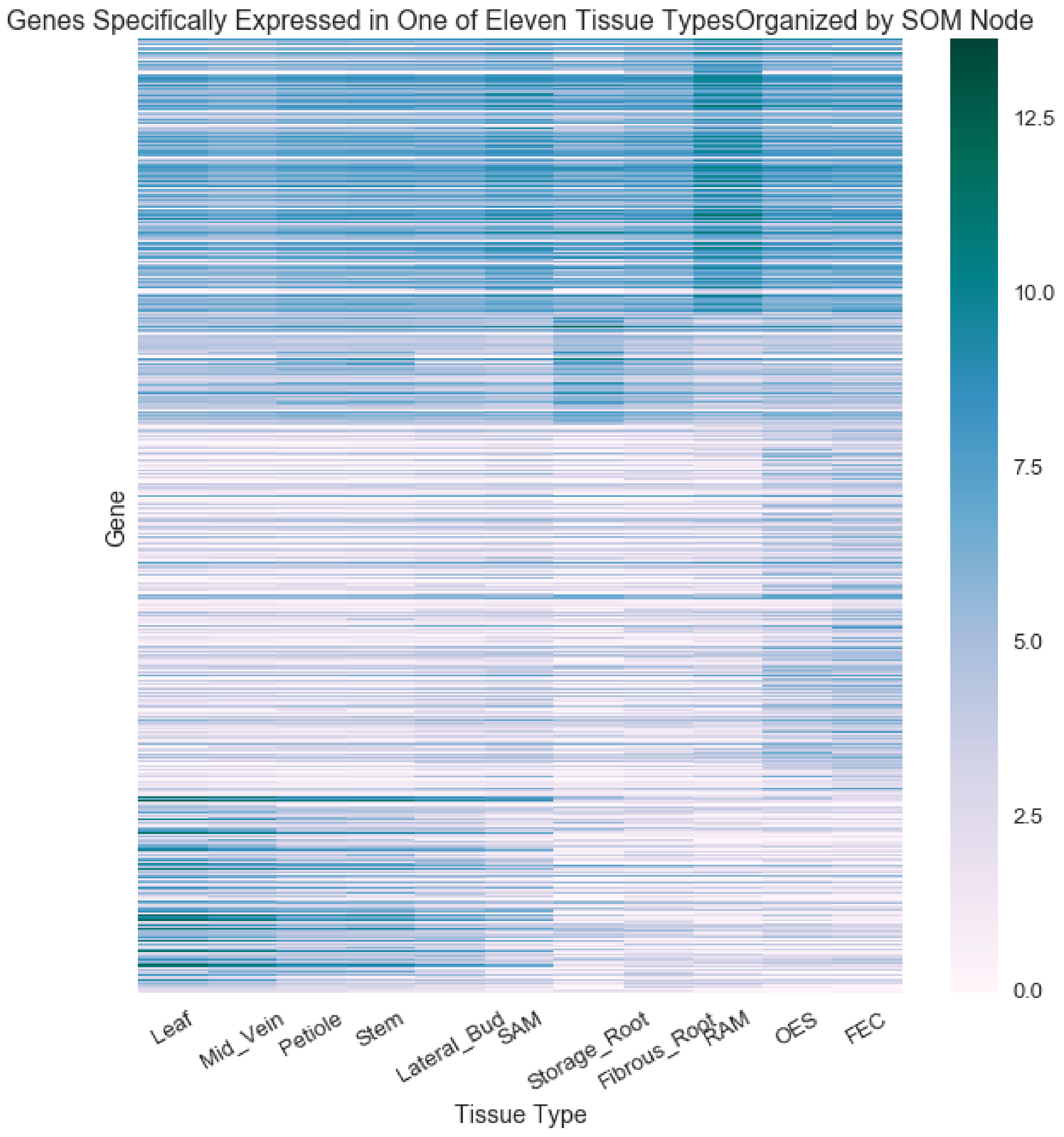
3.0.6 Plot SOM Nodes in order

```
In [19]: df_nodeplot = pd.concat([df_node1_plot,
                                  df_node2_plot,
                                  df_node3_plot,
                                  df_node4_plot])

         df_nodeplot = df_nodeplot.merge(df_cuff_exp,
                                         left_on = 'test_id',
                                         right_index = True)
         print(df_nodeplot.shape)
```

```
(1635, 13)
```

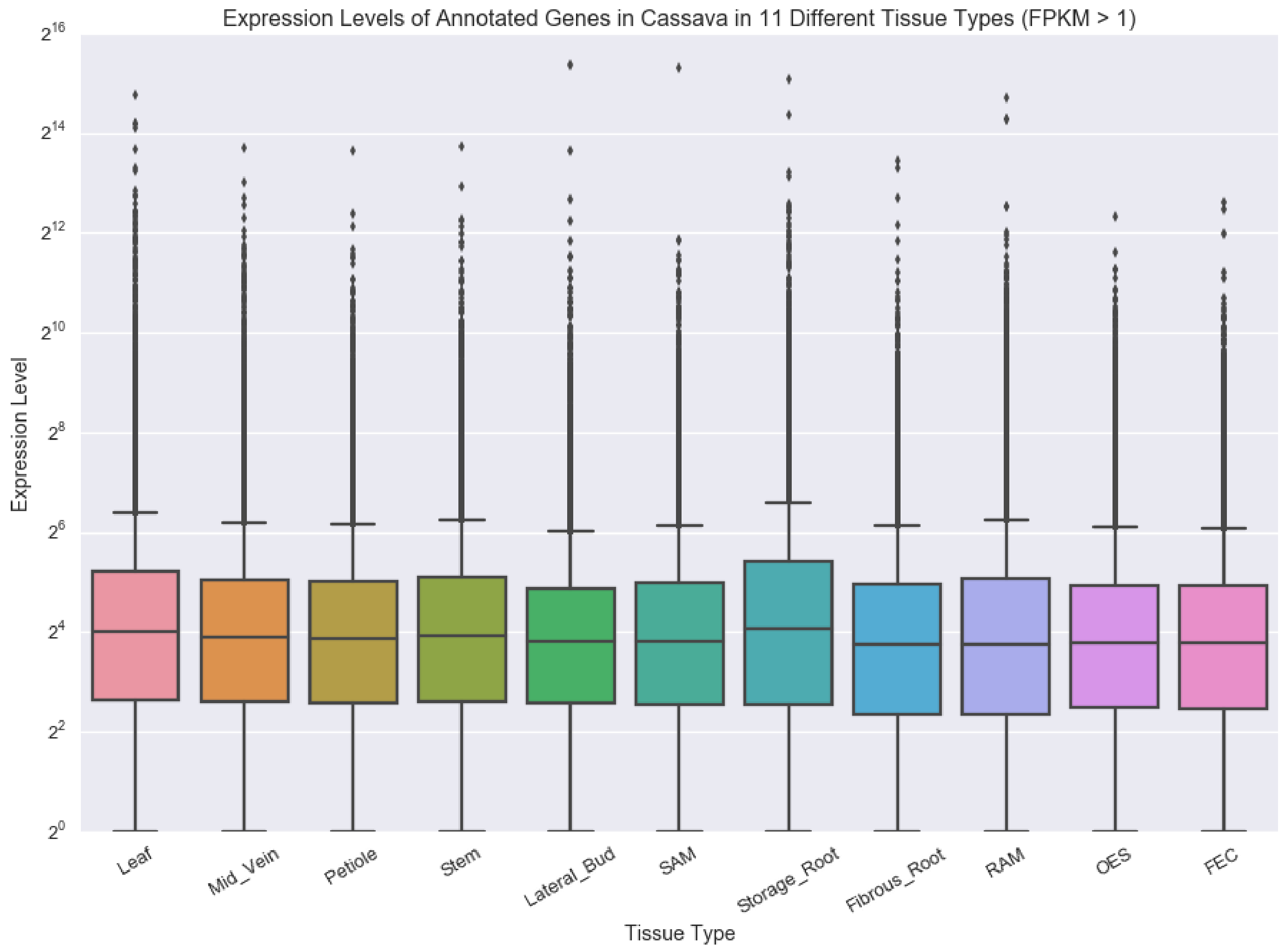
```
In [20]: # Plot log expression values for increased contrast
         df_plot_log = df_nodeplot.drop(['test_id','gene'], axis=1).copy()
         df_plot_log = np.log2(df_plot_log + 1)

         with( sns.plotting_context( 'talk' ) ):
             plt.figure(figsize=(10,10))
             sns.set_style('darkgrid')

             g = sns.heatmap(df_plot_log, cmap='PuBuGn', yticklabels=False)
```

```
    g.set_ylabel('Gene')
    g.set_xlabel('Tissue Type')
    g.set_title('Genes Specifically Expressed in One of Eleven Tissue Types\
Organized by SOM Node')
    g.set_xticklabels(df_plot_log.columns,rotation=30)

    plt.savefig('./mesculenta_v6_output/SOM_go_enrichment_heatmap.pdf',
                bbox_inches='tight')
```



4 Plotting Distribution of FPKM

```
In [21]: # plot distribution of expression values in each tissue type
         with( sns.plotting_context( 'talk' ) ):
             plt.figure(figsize=(15,10))
             sns.set_style('darkgrid')

             g = sns.boxplot(data=df_cuff_ann[df_cuff_ann.iloc[:,:11] > 1].iloc[:,:11])
             g.set_yscale('log', basey=2)
             g.set_ylabel('Expression Level')
             g.set_xlabel('Tissue Type')
             g.set_title('Expression Levels of Annotated Genes \
         in Cassava in 11 Different Tissue Types (FPKM > 1)')
             g.set_xticklabels(df_cuff_ann.iloc[:,:11].columns, rotation=30)

             plt.savefig('./mesculenta_v6_output/genes_exp_dist_1FPKM.pdf',
                         bbox_inches='tight')
```

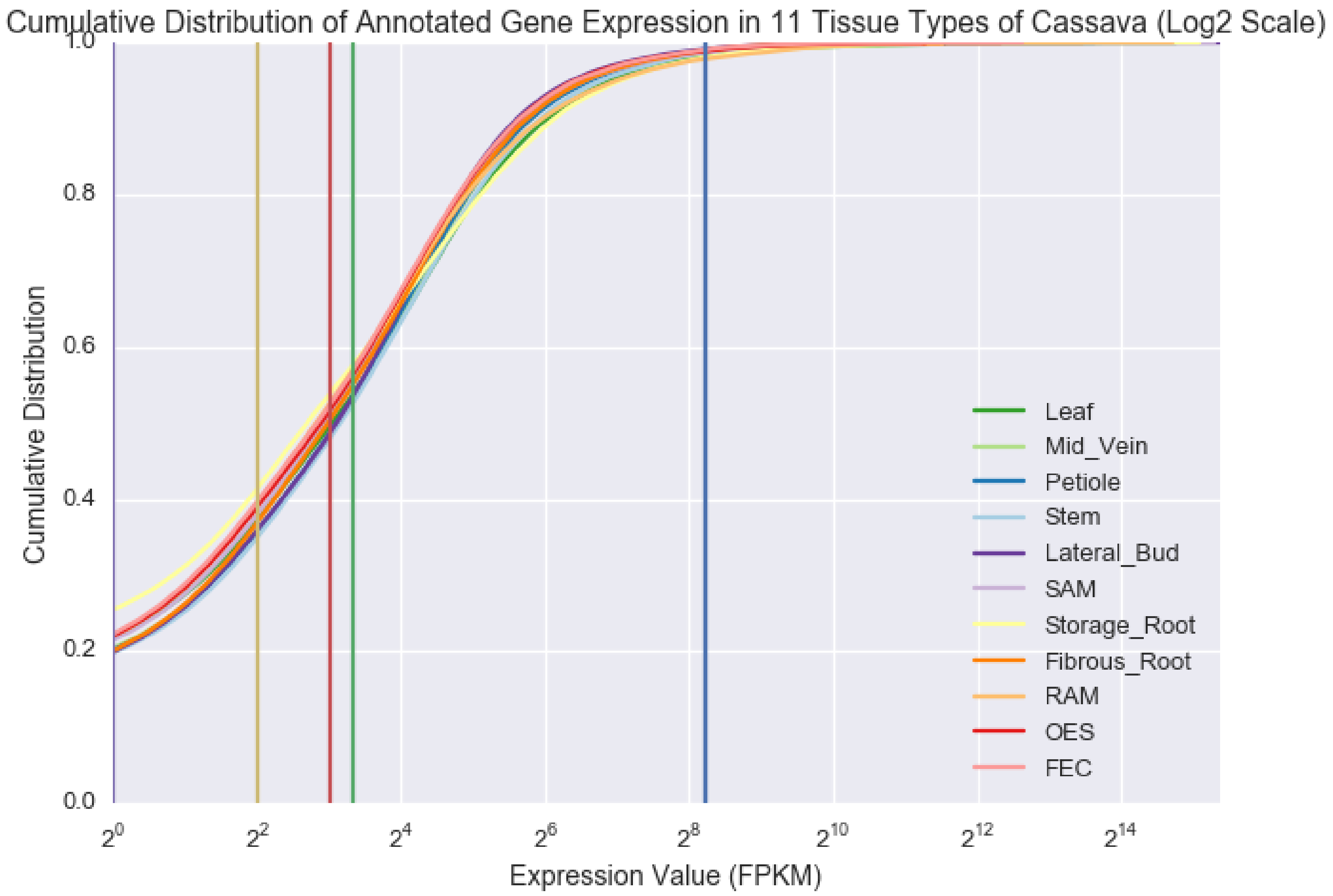


4.1 Gene Expression Density Plot

```
In [22]: with( sns.plotting_context( 'talk' ) ):
             sns.set_style('darkgrid')
```

```
# plot distribution of each tissue type separately
g = sns.kdeplot(df_cuff_ann['Leaf'],clip=(0,3000), color="#33a02c",
                cumulative=True, gridsize=6000)
plt.plot((2000, df_cuff_ann['Leaf'].max()),(1,1), color="#33a02c")

sns.kdeplot(df_cuff_ann['Mid_Vein'],clip=(0,3000), color="#b2df8a",
            cumulative=True, gridsize=6000)
plt.plot((2000, df_cuff_ann['Mid_Vein'].max()),(1,1), color="#b2df8a")

sns.kdeplot(df_cuff_ann['Petiole'],clip=(0,3000), color="#1f78b4",
            cumulative=True, gridsize=6000)
plt.plot((2000, df_cuff_ann['Petiole'].max()),(1,1), color="#1f78b4")

sns.kdeplot(df_cuff_ann['Stem'],clip=(0,3000), color="#a6cee3",
            cumulative=True, gridsize=6000)
plt.plot((2000, df_cuff_ann['Stem'].max()),(1,1), color="#a6cee3")

sns.kdeplot(df_cuff_ann['Lateral_Bud'],clip=(0,3000), color="#6a3d9a",
            cumulative=True, gridsize=6000)
plt.plot((2000, df_cuff_ann['Lateral_Bud'].max()),(1,1), color="#6a3d9a")

sns.kdeplot(df_cuff_ann['SAM'],clip=(0,3000), color="#cab2d6",
            cumulative=True, gridsize=6000)
plt.plot((2000, df_cuff_ann['SAM'].max()),(1,1), color="#cab2d6")

sns.kdeplot(df_cuff_ann['Storage_Root'],clip=(0,3000), color="#ffff99",
            cumulative=True, gridsize=6000)
plt.plot((2000, df_cuff_ann['Storage_Root'].max()),(1,1), color="#ffff99")

sns.kdeplot(df_cuff_ann['Fibrous_Root'],clip=(0,3000), color="#ff7f00",
            cumulative=True, gridsize=6000)
plt.plot((2000, df_cuff_ann['Fibrous_Root'].max()),(1,1), color="#ff7f00")

sns.kdeplot(df_cuff_ann['RAM'],clip=(0,3000), color="#fdbf6f",
            cumulative=True, gridsize=6000)
plt.plot((2000, df_cuff_ann['RAM'].max()),(1,1), color="#fdbf6f")

sns.kdeplot(df_cuff_ann['OES'],clip=(0,3000), color="#e31a1c",
            cumulative=True, gridsize=6000)
plt.plot((2000, df_cuff_ann['OES'].max()),(1,1), color="#e31a1c")

sns.kdeplot(df_cuff_ann['FEC'],clip=(0,3000), color="#fb9a99",
            cumulative=True, gridsize=6000)
plt.plot((2000, df_cuff_ann['FEC'].max()),(1,1), color="#fb9a99")

g.axes.set_xlim(1,max(df_cuff_ann.max(numeric_only=True)))

# plot lines at FPKM cutoff values used in analysis
fpkm_high = 300
fpkm_on = 10
fpkm_on_loose = 8
fpkm_off = 1
```

```
        fpkm_off_loose = 4

        plt.plot((fpkm_high,fpkm_high),(0,1))
        plt.plot((fpkm_on,fpkm_on),(0,1))
        plt.plot((fpkm_on_loose,fpkm_on_loose),(0,1))
        plt.plot((fpkm_off,fpkm_off),(0,1))
        plt.plot((fpkm_off_loose,fpkm_off_loose),(0,1))

        g.set_xscale('log', basex=2)
        g.set_ylabel('Cumulative Distribution')
        g.set_xlabel('Expression Value (FPKM)')
        g.set_title('Cumulative Distribution of Annotated Gene \
    Expression in 11 Tissue Types of Cassava (Log2 Scale)')

        plt.legend(loc=4)

        plt.savefig('./mesculenta_v6_output/CDF_gene_exp_logscale.pdf',
                    bbox_inches='tight')
```



4.2 Percentiles of Expression Values in Tissue Types

```
In [23]: # Create dictionary of percentiles of various expression values
         perc = {i: [percentileofscore( df_cuff_ann.loc[:,[i]].values, 1, kind='weak'),
                     percentileofscore( df_cuff_ann.loc[:,[i]].values, 4, kind='weak' ),
                     percentileofscore( df_cuff_ann.loc[:,[i]].values, 8, kind='weak' ),
                     percentileofscore( df_cuff_ann.loc[:,[i]].values, 10, kind='weak' ),
                     percentileofscore( df_cuff_ann.loc[:,[i]].values, 50, kind='weak' ),
                     percentileofscore( df_cuff_ann.loc[:,[i]].values, 100, kind='weak'),
                     percentileofscore( df_cuff_ann.loc[:,[i]].values, 200, kind='weak'),
                     percentileofscore( df_cuff_ann.loc[:,[i]].values, 300, kind='weak'),
                     percentileofscore( df_cuff_ann.loc[:,[i]].values, 400, kind='weak'),
                     percentileofscore( df_cuff_ann.loc[:,[i]].values, 500, kind='weak')]
                for i in df_cuff_ann.columns[:11] }
         perc_df = pd.DataFrame(perc, index=[1,4,8,10,50,100,200,300,400,500])
         perc_df
```

```
Out[23]:          FEC  Fibrous_Root  Lateral_Bud       Leaf   Mid_Vein        OES  \
         1    28.358090     24.136832    24.873561  28.214727  25.502768  27.947911
         4    41.806380     39.440882    37.879814  40.846641  38.528932  41.348413
         8    52.658198     50.953765    48.807296  50.049779  48.644021  51.961292
         10   57.078571     55.322369    53.864840  54.175461  53.076341  56.509100
         50   90.088009     89.128270    90.402612  86.786667  88.738003  89.992434
         100  95.834495     95.420334    96.133169  93.831389  95.257059  95.993788
         200  98.295568     98.088487    98.498666  97.284059  98.255744  98.255744
         300  99.092031     98.984509    99.143802  98.411055  98.936721  98.940703
         400  99.498228     99.386723    99.474334  98.912827  99.243359  99.342917
         500  99.645574     99.617697    99.657521  99.139819  99.422564  99.561945

              Petiole        RAM        SAM       Stem  Storage_Root
         1    25.032854  27.466051  27.310740  24.606746     34.184222
         4    38.126717  42.069213  40.169647  37.581140     46.238700
         8    48.902871  53.092270  51.308192  48.480745     54.772809
         10   53.773247  57.417068  55.832105  53.048465     58.078133
         50   88.690215  87.634901  89.331369  87.467644     85.767194
         100  95.093784  93.731831  95.404404  94.444666     93.285811
         200  97.992911  96.849986  97.881407  97.590697     96.945562
         300  98.940703  97.869460  98.701764  98.729640     98.116363
         400  99.334953  98.371232  99.127872  99.195572     98.649994
         500  99.522122  98.677870  99.346900  99.438493     98.952650
```

4.3 Highly Expressed Genes Across All Tissue Types

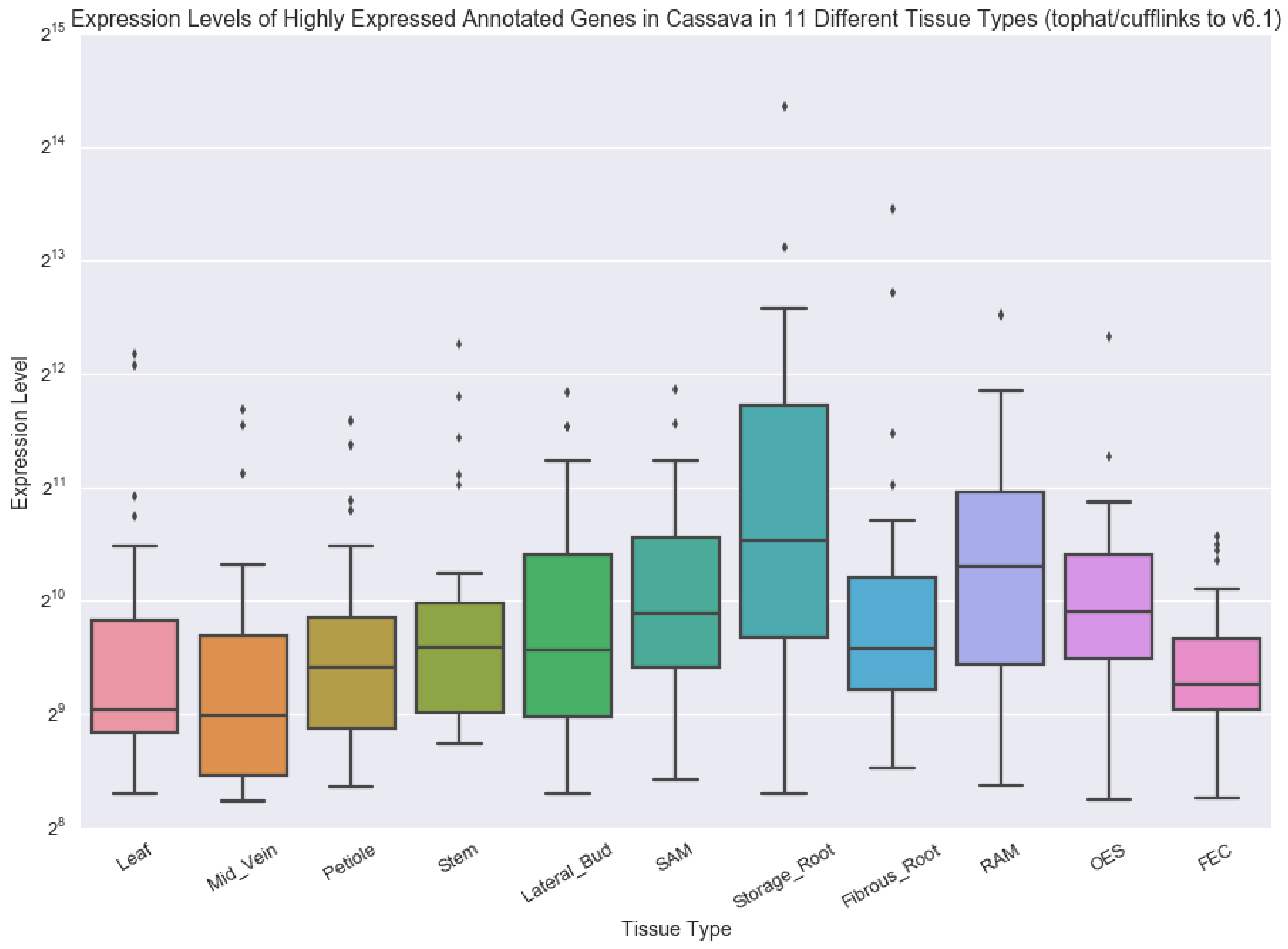
Cutoff of Same Value Determined by Housekeeping Genes (Specifically Max Expression of Manes.09G039900)

```
In [24]: # drop genes that have expression less than 300 in any tissue type
         df_cuff_min = df_cuff_ann[df_cuff_ann.min(axis=1,numeric_only=True) > 300]

         # set gene_id as index
         df_cuff_min = df_cuff_min.set_index('gene_id')

         print('Highly Expressed Genes: {}'.format(df_cuff_min.shape[0]))
```

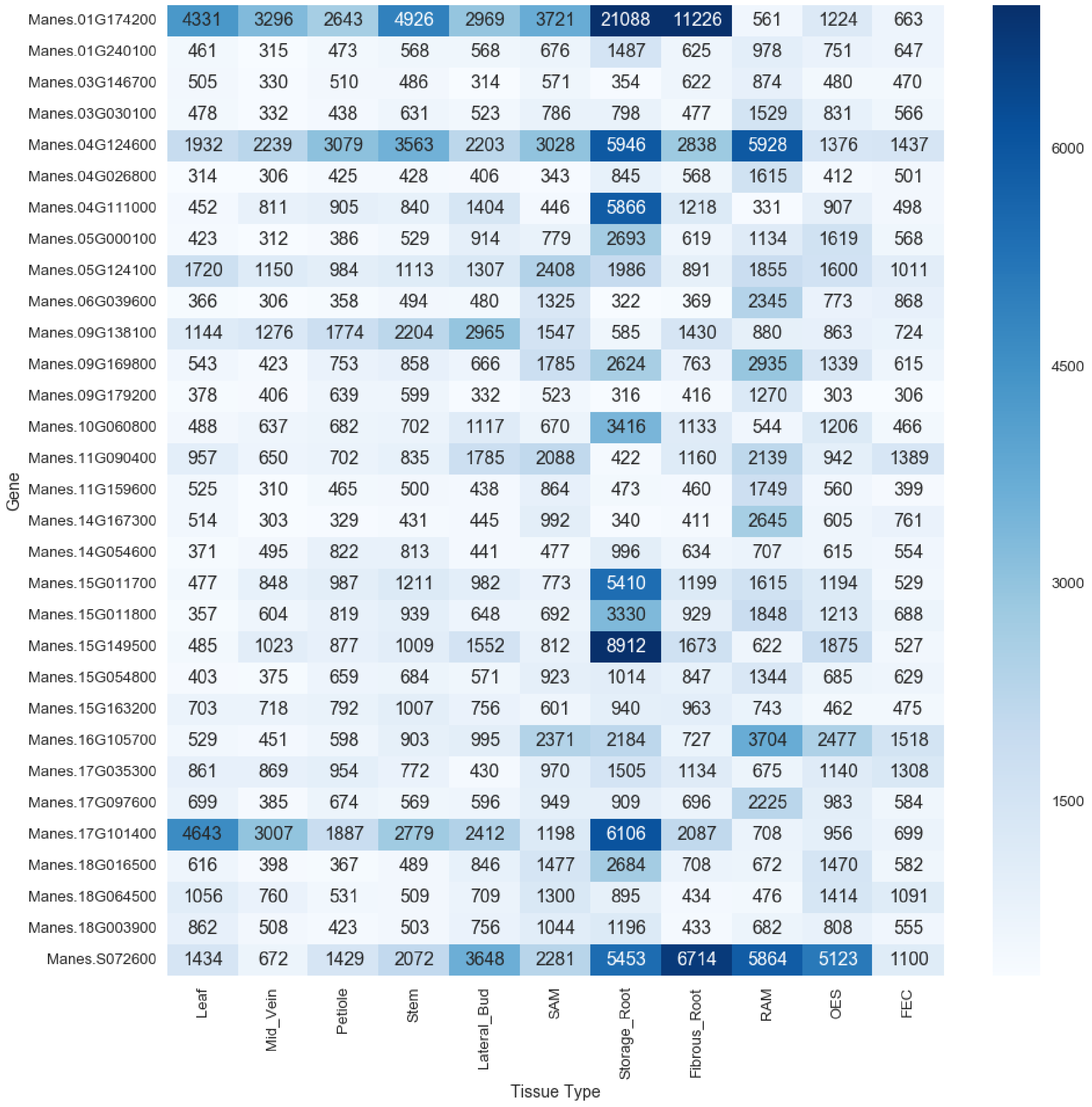
```
Highly Expressed Genes: 31
```

```
In [25]: with( sns.plotting_context( 'talk' ) ):
             plt.figure(figsize=(15,10))
             sns.set_style('darkgrid')
```

```
    g = sns.boxplot(data=df_cuff_min.iloc[:,:11])
    g.set_yscale('log', basey=2)
    g.set_ylabel('Expression Level')
    g.set_xlabel('Tissue Type')
    g.set_title('Expression Levels of Highly Expressed Annotated Genes \
in Cassava in 11 Different Tissue Types (tophat/cufflinks to v6.1)')
    g.set_xticklabels(df_cuff_min.iloc[:,:11].columns, rotation=30)

    plt.savefig('./mesculenta_v6_output/genes_high_exp_dist.pdf',
                bbox_inches='tight')
```



```
In [26]: df_plot = df_cuff_min.drop(['locus','annot'], axis=1).set_index(['gene'])

         with( sns.plotting_context( 'talk' ) ):
             plt.figure(figsize=(15,15))
             sns.set_style('darkgrid')

             g = sns.heatmap(df_plot,
                             cmap='Blues',
                             annot=True,
                                        fmt='.0f',
                                        vmax=7000   # set max for heatmap color scale at
                                                    #     7000FPKM to capture more contrast
                                       )

             g.set_ylabel('Gene')
             g.set_xlabel('Tissue Type')
             plt.savefig('./mesculenta_v6_output/genes_high_exp_annot_heatmap.pdf',
                         bbox_inches='tight')
```



Gene	Leaf	Mid_Vein	Petiole	Stem	Lateral_Bud	SAM	Storage_Root	Fibrous_Root	RAM	OES	FEC
Manes.01G174200	4331	3296	2643	4926	2969	3721	21088	11226	561	1224	663
Manes.01G240100	461	315	473	568	568	676	1487	625	978	751	647
Manes.03G146700	505	330	510	486	314	571	354	622	874	480	470
Manes.03G030100	478	332	438	631	523	786	798	477	1529	831	566
Manes.04G124600	1932	2239	3079	3563	2203	3028	5946	2838	5928	1376	1437
Manes.04G026800	314	306	425	428	406	343	845	568	1615	412	501
Manes.04G111000	452	811	905	840	1404	446	5866	1218	331	907	498
Manes.05G000100	423	312	386	529	914	779	2693	619	1134	1619	568
Manes.05G124100	1720	1150	984	1113	1307	2408	1986	891	1855	1600	1011
Manes.06G039600	366	306	358	494	480	1325	322	369	2345	773	868
Manes.09G138100	1144	1276	1774	2204	2965	1547	585	1430	880	863	724
Manes.09G169800	543	423	753	858	666	1785	2624	763	2935	1339	615
Manes.09G179200	378	406	639	599	332	523	316	416	1270	303	306
Manes.10G060800	488	637	682	702	1117	670	3416	1133	544	1206	466
Manes.11G090400	957	650	702	835	1785	2088	422	1160	2139	942	1389
Manes.11G159600	525	310	465	500	438	864	473	460	1749	560	399
Manes.14G167300	514	303	329	431	445	992	340	411	2645	605	761
Manes.14G054600	371	495	822	813	441	477	996	634	707	615	554
Manes.15G011700	477	848	987	1211	982	773	5410	1199	1615	1194	529
Manes.15G011800	357	604	819	939	648	692	3330	929	1848	1213	688
Manes.15G149500	485	1023	877	1009	1552	812	8912	1673	622	1875	527
Manes.15G054800	403	375	659	684	571	923	1014	847	1344	685	629
Manes.15G163200	703	718	792	1007	756	601	940	963	743	462	475
Manes.16G105700	529	451	598	903	995	2371	2184	727	3704	2477	1518
Manes.17G035300	861	869	954	772	430	970	1505	1134	675	1140	1308
Manes.17G097600	699	385	674	569	596	949	909	696	2225	983	584
Manes.17G101400	4643	3007	1887	2779	2412	1198	6106	2087	708	956	699
Manes.18G016500	616	398	367	489	846	1477	2684	708	672	1470	582
Manes.18G064500	1056	760	531	509	709	1300	895	434	476	1414	1091
Manes.18G003900	862	508	423	503	756	1044	1196	433	682	808	555
Manes.S072600	1434	672	1429	2072	3648	2281	5453	6714	5864	5123	1100

```
In [27]: # associate highly expressed genes with functional annotations
```

```
high_exp_genes = df_cuff_min['gene']
high_exp_genes = high_exp_genes.to_frame().merge(annot.loc[:,['gene','annot']],
                                                  how='left',
                                                  on='gene'
                                                 )

# sort by 'gene' and set 'gene' as index
high_exp_genes = high_exp_genes.sort_values('gene').set_index('gene')

# write to table
high_exp_genes.to_csv('./mesculenta_v6_output/high_exp_gene_annot_table.txt',
                      sep='\t')

high_exp_genes
```

Out[27]:

```
                                                            annot
gene
Manes.01G174200                               metallothionein 2B
Manes.01G240100                60S acidic ribosomal protein family
Manes.03G030100               Ribosomal protein S30 family protein
Manes.03G146700     GTP binding Elongation factor Tu family protein
Manes.04G026800                             ascorbate peroxidase 1
Manes.04G111000                                   polyubiquitin 10
Manes.04G124600              Translation machinery associated TMA7
Manes.05G000100              Ribosomal protein L19e family protein
Manes.05G124100                              high mobility group B2
Manes.06G039600               Ribosomal protein L39 family protein
Manes.09G138100                              dehydrin family protein
Manes.09G169800                              Ribosomal protein S21e
Manes.09G179200                          ADP-ribosylation factor A1E
Manes.10G060800                                   DNAJ homologue 2
Manes.11G090400                                      rotamase CYP 1
Manes.11G159600                60S acidic ribosomal protein family
Manes.14G054600  glyceraldehyde-3-phosphate dehydrogenase C sub...
Manes.14G167300         Zinc-binding ribosomal protein family protein
Manes.15G011700              translationally controlled tumor protein
Manes.15G011800              translationally controlled tumor protein
Manes.15G054800     GTP binding Elongation factor Tu family protein
Manes.15G149500                          ADP-ribosylation factor A1F
Manes.15G163200            cold, circadian rhythm, and rna binding 2
Manes.16G105700               Ribosomal protein S30 family protein
Manes.17G035300                                         ubiquitin 4
Manes.17G097600  translocase of the outer mitochondrial membrane 6
Manes.17G101400            cold, circadian rhythm, and RNA binding 1
Manes.18G003900                              high mobility group B2
Manes.18G016500                         Histone superfamily protein
Manes.18G064500                         Histone superfamily protein
Manes.S072600         Zinc-binding ribosomal protein family protein
```

4.4 Specific Tissue Expression

Single Tissue Expression for Promoters

tissue specific on10 off1

```
In [28]: on_thresh = 10
         off_thresh = 1

         df_tiss_spec = pd.DataFrame( columns = df_cuff_ann.columns )

         # loop through each tissue
         for t in tissue_order:
             t = [t]

             # create index lists of on tisues and off tissues
             index_on = sorted([ i for j in tissue_order
                                   if j in t for i in tissue_rep_index[j] ])
             index_off = sorted([ i for j in tissue_order
                                    if j not in t for i in tissue_rep_index[j] ])

             # subset the replicate dataset for genes matching the tissue
             #    parameters for this iteration of the loop
             df_temp = genes_rgt_piv[(genes_rgt_piv.iloc[:,index_off]
                                      < off_thresh).all(axis=1) &
                                     (genes_rgt_piv.iloc[:,index_on]
                                      > on_thresh).all(axis=1)
                                    ]

             # select for genes with annotations
             df_temp = df_temp[df_temp['gene_id'].isin(df_cuff_ann['gene_id'])]

             # add annotations and use mean expression values for each tissue
             #    instead of replicate data
             df_temp = df_temp['gene_id'].to_frame().merge(df_cuff_ann_all, on='gene_id')

             # sort by max value of each gene
             df_max = df_temp.iloc[:,:11].max(axis=1)
             df_temp = df_temp.reindex( df_max.sort_values(ascending=False).index )

             # keep only the top 3 genes in each tissue
             df_tiss_spec = pd.concat([df_tiss_spec,
                                       df_temp.iloc[:3,:]
                                      ]
                                     )

         df_tiss = df_tiss_spec.copy().set_index('gene')

         print( 'Gene Count: {}'.format(df_tiss.shape[0]) )
```

```
Gene Count: 11
```

tissue specific on10 off1: Grouped Tissues

```
In [29]: on_thresh = 10
         off_thresh = 1
```

```
        df_tiss_spec = pd.DataFrame( columns = df_cuff_ann.columns )

        tissue_groups = [['Leaf','Mid_Vein','Petiole','Stem','Lateral_Bud','SAM'],
                         ['Storage_Root'],['Fibrous_Root','RAM'],
                         ['OES','FEC']
                        ]

        # loop through each tissue
        for t in tissue_groups:
            # create index lists of on tisues and off tissues
            index_on = sorted([ i for j in tissue_order
                                if j in t for i in tissue_rep_index[j] ])
            index_off = list( set(range(32)) - set(index_on) )

            # subset the replicate dataset for genes matching the tissue
            #    parameters for this iteration of the loop
            df_temp = genes_rgt_piv[(genes_rgt_piv.iloc[:,index_off]
                                     < off_thresh).all(axis=1) &
                                    (genes_rgt_piv.iloc[:,index_on]
                                     > on_thresh).all(axis=1)
                                   ]

            # select for genes with annotations
            df_temp = df_temp[df_temp['gene_id'].isin(df_cuff_ann['gene_id'])]

            # add annotations and use mean expression values for each tissue
            #    instead of replicate data
            df_temp = df_temp['gene_id'].to_frame().merge(df_cuff_ann, on='gene_id')

            # sort by max value of each gene
            df_max = df_temp.iloc[:,:11].max(axis=1)
            df_temp = df_temp.reindex( df_max.sort_values(ascending=False).index )

            # keep only the top 3 genes in each tissue
            df_tiss_spec = pd.concat([df_tiss_spec,
                                      df_temp.iloc[:3,:]
                                     ]
                                    )

        df_tissgrps = df_tiss_spec.copy().set_index('gene')

        print( 'Gene Count: {}'.format(df_tissgrps.shape[0]) )
```

```
Gene Count: 9
```

tissue specific on8 off4

```
In [30]: on_thresh = 8
         off_thresh = 4

         df_tiss_spec = pd.DataFrame( columns = df_cuff_ann.columns )

         # loop through each tissue
```

```
        for t in tissue_order:
            # using relaxed parameters, finding single tissue genes in
            #    each tissue without 3 genes with strict parameters
            if t == 'FEC' or t == 'Fibrous_Root' or t == 'RAM':
                continue

            t = [t]

            # create index lists of on tisues and off tissues
            index_on = sorted([ i for j in tissue_order
                                  if j in t for i in tissue_rep_index[j] ])
            index_off = sorted([ i for j in tissue_order
                                   if j not in t for i in tissue_rep_index[j] ])

            # subset the replicate dataset for genes matching the tissue
            #    parameters for this iteration of the loop
            df_temp = genes_rgt_piv[(genes_rgt_piv.iloc[:,index_off]
                                     < off_thresh).all(axis=1) &
                                    (genes_rgt_piv.iloc[:,index_on]
                                     > on_thresh).all(axis=1)
                                   ]

            # select for genes with annotations
            df_temp = df_temp[df_temp['gene_id'].isin(df_cuff_ann['gene_id'])]

            # add annotations and use mean expression values for each tissue
            #    instead of replicate data
            df_temp = df_temp['gene_id'].to_frame().merge(df_cuff_ann, on='gene_id')
            df_temp = df_temp.drop_duplicates('gene')

            # sort by max value of each gene
            df_max = df_temp.iloc[:,:11].max(axis=1)
            df_temp = df_temp.reindex( df_max.sort_values(ascending=False).index )

            # keep only the top 3 genes in each tissue
            df_tiss_spec = pd.concat([df_tiss_spec,
                                      df_temp.iloc[:3,:]
                                     ]
                                    )

        df_tissrlx = df_tiss_spec.copy().set_index('gene')

        print( 'Gene Count: {}'.format(df_tissrlx.shape[0]) )
```

```
Gene Count: 17
```

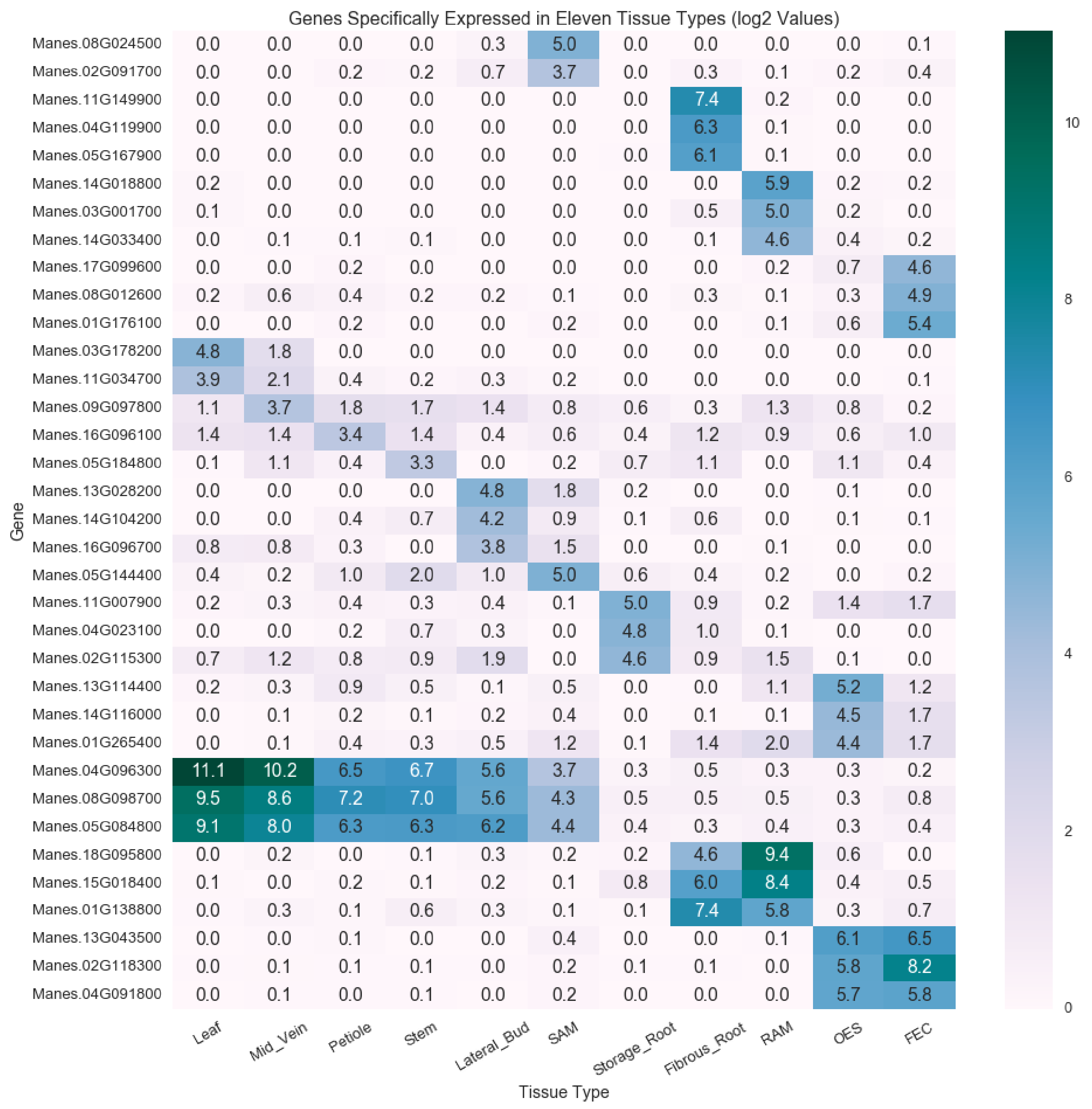
Tissue Specific Plot Prep

```
In [31]: # drop first gene in SAM so it's not duplicated in the plot
         df_tissrlx.drop( df_tissrlx[df_tissrlx['SAM'] > on_thresh].index[1:],
                          inplace=True)

         # concatenate the 3 Tissue Specfic DataFrames for the plot
```

```
        df_plot = pd.concat( [df_tiss, df_tissrlx, df_tissgrps] )
        df_plot['annot'].to_csv('./mesculenta_v6_output/specific_exp_gene_annotonly.txt')

        # Restrict plot DataFrame to expression values
        df_plot = df_plot.iloc[:,:11]

        # Sort Columns in tissue_order as specified earlier
        df_plot = df_plot.loc[:,tissue_order]
        df_tiss_spec = df_tiss_spec.set_index('gene_id')

        print( 'Gene Count: {}'.format(df_plot.shape[0]) )
```

```
Gene Count: 35
```

```
In [32]: df_plot_log = df_plot.copy()
         df_plot_log = np.log2(df_plot_log + 1)

         with( sns.plotting_context( 'talk' ) ):
             plt.figure(figsize=(15,15))
             sns.set_style('darkgrid')

             g = sns.heatmap(df_plot_log, cmap='PuBuGn', annot=True, fmt='.1f')

             g.set_ylabel('Gene')
             g.set_xlabel('Tissue Type')
             g.set_title('Genes Specifically Expressed in \
         Eleven Tissue Types (log2 Values)')
             g.set_xticklabels(df_plot_log.columns,rotation=30)

             plt.savefig('./mesculenta_v6_output/specific_exp_mrg_strict_relaxed_log.pdf',
                         bbox_inches='tight')
```



Gene	Leaf	Mid_Vein	Petiole	Stem	Lateral_Bud	SAM	Storage_Root	Fibrous_Root	RAM	OES	FEC
Manes.08G024500	0.0	0.0	0.0	0.0	0.3	5.0	0.0	0.0	0.0	0.0	0.1
Manes.02G091700	0.0	0.0	0.2	0.2	0.7	3.7	0.0	0.3	0.1	0.2	0.4
Manes.11G149900	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.4	0.2	0.0	0.0
Manes.04G119900	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.3	0.1	0.0	0.0
Manes.05G167900	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.1	0.1	0.0	0.0
Manes.14G018800	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.9	0.2	0.2
Manes.03G001700	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.5	5.0	0.2	0.0
Manes.14G033400	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.1	4.6	0.4	0.2
Manes.17G099600	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.2	0.7	4.6
Manes.08G012600	0.2	0.6	0.4	0.2	0.2	0.1	0.0	0.3	0.1	0.3	4.9
Manes.01G176100	0.0	0.0	0.2	0.0	0.0	0.2	0.0	0.0	0.1	0.6	5.4
Manes.03G178200	4.8	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Manes.11G034700	3.9	2.1	0.4	0.2	0.3	0.2	0.0	0.0	0.0	0.0	0.1
Manes.09G097800	1.1	3.7	1.8	1.7	1.4	0.8	0.6	0.3	1.3	0.8	0.2
Manes.16G096100	1.4	1.4	3.4	1.4	0.4	0.6	0.4	1.2	0.9	0.6	1.0
Manes.05G184800	0.1	1.1	0.4	3.3	0.0	0.2	0.7	1.1	0.0	1.1	0.4
Manes.13G028200	0.0	0.0	0.0	0.0	4.8	1.8	0.2	0.0	0.0	0.1	0.0
Manes.14G104200	0.0	0.0	0.4	0.7	4.2	0.9	0.1	0.6	0.0	0.1	0.1
Manes.16G096700	0.8	0.8	0.3	0.0	3.8	1.5	0.0	0.0	0.1	0.0	0.0
Manes.05G144400	0.4	0.2	1.0	2.0	1.0	5.0	0.6	0.4	0.2	0.0	0.2
Manes.11G007900	0.2	0.3	0.4	0.3	0.4	0.1	5.0	0.9	0.2	1.4	1.7
Manes.04G023100	0.0	0.0	0.2	0.7	0.3	0.0	4.8	1.0	0.1	0.0	0.0
Manes.02G115300	0.7	1.2	0.8	0.9	1.9	0.0	4.6	0.9	1.5	0.1	0.0
Manes.13G114400	0.2	0.3	0.9	0.5	0.1	0.5	0.0	0.0	1.1	5.2	1.2
Manes.14G116000	0.0	0.1	0.2	0.1	0.2	0.4	0.0	0.1	0.1	4.5	1.7
Manes.01G265400	0.0	0.1	0.4	0.3	0.5	1.2	0.1	1.4	2.0	4.4	1.7
Manes.04G096300	11.1	10.2	6.5	6.7	5.6	3.7	0.3	0.5	0.3	0.3	0.2
Manes.08G098700	9.5	8.6	7.2	7.0	5.6	4.3	0.5	0.5	0.5	0.3	0.8
Manes.05G084800	9.1	8.0	6.3	6.3	6.2	4.4	0.4	0.3	0.4	0.3	0.4
Manes.18G095800	0.0	0.2	0.0	0.1	0.3	0.2	0.2	4.6	9.4	0.6	0.0
Manes.15G018400	0.1	0.0	0.2	0.1	0.2	0.1	0.8	6.0	8.4	0.4	0.5
Manes.01G138800	0.0	0.3	0.1	0.6	0.3	0.1	0.1	7.4	5.8	0.3	0.7
Manes.13G043500	0.0	0.0	0.1	0.0	0.0	0.4	0.0	0.0	0.1	6.1	6.5
Manes.02G118300	0.0	0.1	0.1	0.1	0.0	0.2	0.1	0.1	0.0	5.8	8.2
Manes.04G091800	0.0	0.1	0.0	0.1	0.0	0.2	0.0	0.0	0.0	5.7	5.8

5 Pairwise Tissue Comparison

5.1 OES and FEC

5.1.1 Differentially Expressed in OES and FEC

```
In [33]: lfc = 2
         sig = 0.05
         fpkm_cutoff = 1
```

```
        # limit list of genes to significantly differentially
        #       expressed with a abs(log2(fold_change)) value > 2
        #       and an expression value of at least 1 FPKM in one of the tissues
        df_volc = df_cuff[(df_cuff['sample_1'] == 'FEC')
                          & (df_cuff['sample_2'] == 'OES')
                          & (df_cuff['q_value'] < sig )
                          & (np.abs(df_cuff['log2(fold_change)']) > lfc)
                          & ((df_cuff['value_1'] > fpkm_cutoff)
                             | (df_cuff['value_2'] > fpkm_cutoff))
                          & (df_cuff['gene'] != '-')].merge( annot[['gene','annot']],
                                                             how='inner',
                                                             on='gene',
                                                             copy=False)

        df_volc_n = df_cuff[(df_cuff['sample_1'] == 'FEC')
                          & (df_cuff['sample_2'] == 'OES')
                          & ((df_cuff['q_value'] >= sig )
                             | (np.abs(df_cuff['log2(fold_change)']) <= lfc ))
                          & ((df_cuff['value_1'] > fpkm_cutoff)
                             | (df_cuff['value_2'] > fpkm_cutoff))
                          & (df_cuff['gene'] != '-')].merge( annot[['gene','annot']],
                                                             how='inner',
                                                             on='gene',
                                                             copy=False)

        # write genes to file
        df_volc.to_csv('./mesculenta_v6_output/diffexp_oesfec.txt', sep='\t')

        # calculate log score for volcano plot
        df_volc['-log(q_value)'] = -np.log10(df_volc['q_value'])
        df_volc_n['-log(q_value)'] = -np.log10(df_volc_n['q_value'])

        print('OES vs FEC')
        print('Differentially Expressed Genes: {}'.format(df_volc.shape[0]))

        # Reflect foldchange values to convey the transition
        #  from OES to FEC
        df_volc['log2(fold_change)'] *=-1
        df_volc_n['log2(fold_change)'] *=-1
```

```
OES vs FEC
Differentially Expressed Genes: 2022
```

```
In [34]: ############
         ## GO PREP
         ##########
         df_volc[ df_volc['log2(fold_change)'] < -2
                ]['gene'].drop_duplicates().to_csv(
             './mesculenta_v6_output/goprep_diffexp_oesfec_oes.txt', index=False)

         df_volc[ df_volc['log2(fold_change)'] > 2
                ]['gene'].drop_duplicates().to_csv(
             './mesculenta_v6_output/goprep_diffexp_oesfec_fec.txt', index=False)
```

```
df_cuff[(df_cuff['sample_1'] == 'FEC') &
        (df_cuff['sample_2'] == 'OES') &
        ((df_cuff['value_1'] > 1) | (df_cuff['value_2'] > 1)) &
        (df_cuff['gene'] != '-')
       ]['gene'].drop_duplicates().to_csv(
    './mesculenta_v6_output/goprep_bkgrnd_oesfec.txt', index=False)
```

```
In [35]: ########################
         ## goatools ANALYSIS:
         # python ~/src/goatools/scripts/find_enrichment.py \
         #    --fdr --obo ~/src/goatools/go-basic.obo \
         #    goprep_diffexp_oesfec_oes.txt goprep_bkgrnd_oesfec.txt \
         #    Mesculenta_305_v6.1.annotation_info.go_only_uniq.txt \
         #    > oesfec_oes_goatools.txt

         ## Process goatools output
         ### OES
         print( 'OES' )
         df_oesfecgo_oes = pd.read_table(
             './mesculenta_v6_output/oesfec_oes_goatools.txt', comment='#')

         print( 'GO count unfiltered: {}'.format(df_oesfecgo_oes.shape[0]))
         print( 'Enriched GO count, FDR < 0.01: {}'.format(
                 df_oesfecgo_oes[(df_oesfecgo_oes['p_fdr'] < 0.01) &
                                 (df_oesfecgo_oes['enrichment'] == 'e')
                                ].shape[0] )
              )
         print( 'Enriched GO count, FDR < 0.001: {}'.format(
                 df_oesfecgo_oes[(df_oesfecgo_oes['p_fdr'] < 0.001) &
                                 (df_oesfecgo_oes['enrichment'] == 'e')
                                ].shape[0] )
              )

         print()

         ########################
         ## goatools ANALYSIS:
         # python ~/src/goatools/scripts/find_enrichment.py \
         #    --fdr --obo ~/src/goatools/go-basic.obo \
         #    goprep_diffexp_oesfec_fec.txt goprep_bkgrnd_oesfec.txt \
         #    Mesculenta_305_v6.1.annotation_info.go_only_uniq.txt \
         #    > oesfec_fec_goatools.txt

         ### FEC
         print( 'FEC' )
         df_oesfecgo_fec = pd.read_table(
             './mesculenta_v6_output/oesfec_fec_goatools.txt', comment='#')

         print( 'GO count unfiltered: {}'.format(df_oesfecgo_fec.shape[0]))
         print( 'Enriched GO count, FDR < 0.01: {}'.format(
                 df_oesfecgo_fec[(df_oesfecgo_fec['p_fdr'] < 0.01) &
                                 (df_oesfecgo_fec['enrichment'] == 'e')
                                ].shape[0] )
              )
          print( 'Enriched GO count, FDR < 0.001: {}'.format(
                  df_oesfecgo_fec[(df_oesfecgo_fec['p_fdr'] < 0.001) &
                                  (df_oesfecgo_fec['enrichment'] == 'e')
                                ].shape[0] )
              )
```

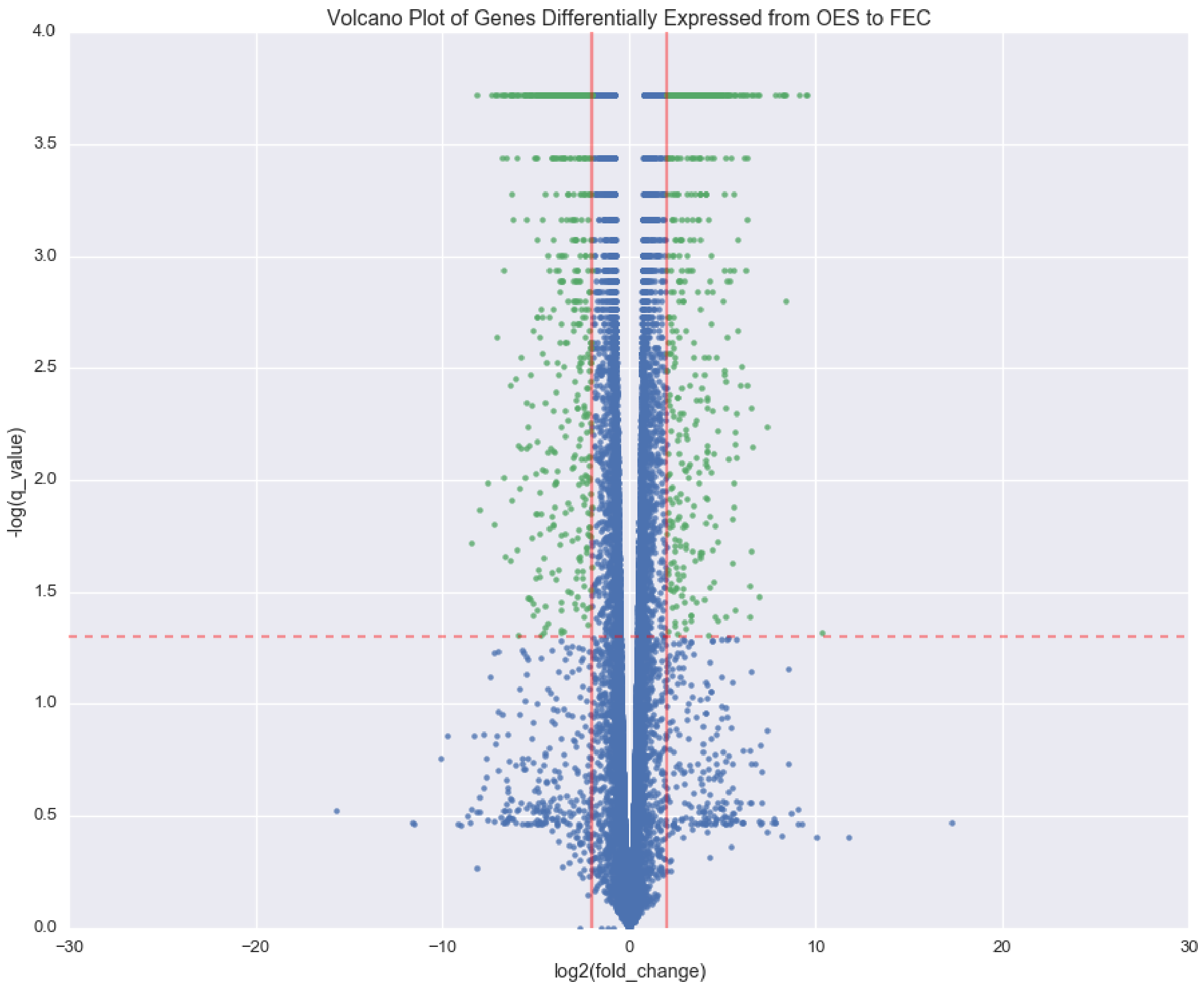
```
OES
GO count unfiltered: 236
Enriched GO count, FDR < 0.01: 43
Enriched GO count, FDR < 0.001: 35

FEC
GO count unfiltered: 275
Enriched GO count, FDR < 0.01: 26
Enriched GO count, FDR < 0.001: 16
```

```
In [36]: print('Genes Upregulated in FEC: {}'.format(
                  df_volc[df_volc['log2(fold_change)'] > 2].shape[0])
              )
         print('Genes Upregulated in OES: {}'.format(
                  df_volc[df_volc['log2(fold_change)'] < -2].shape[0])
              )
```

```
Genes Upregulated in FEC: 937
Genes Upregulated in OES: 1085
```

```
In [37]: lfc = 2
         on_thresh = 10
         off_thresh = 1

         with( sns.plotting_context('talk') ):
             plt.figure(figsize=(15,12))
             sns.set_style('darkgrid')


             g = sns.regplot( y='-log(q_value)',
                              x='log2(fold_change)',
                              data=df_volc_n,
                              scatter=True,
                              fit_reg=False
                            )

             g = sns.regplot( y='-log(q_value)',
                              x='log2(fold_change)',
                              data=df_volc,
                              scatter=True,
                              fit_reg=False
                            )

             y_limit = (0,4)
```

```
    x_limit = (-30,30)
    g.axes.set_ylim(*y_limit)
    g.axes.set_xlim(*x_limit)
    g.set_title( 'Volcano Plot of Genes Differentially \
Expressed from OES to FEC')

    plt.plot( (lfc,lfc), (0,y_limit[1]), color='red', alpha = 0.4 )
    plt.plot( (-lfc,-lfc), (0,y_limit[1]), color='red', alpha = 0.4 )
    plt.plot( (x_limit[0], x_limit[1]), (1.3,1.3),
              linestyle = '--', color='red', alpha = 0.4 )

    plt.savefig('./mesculenta_v6_output/volcano_diffexp_oesfec.pdf',
                 bbox_inches='tight')
```



5.2 Storage Root and Fibrous Root

5.2.1 Differentially Expressed in Storage_Root and Fibrous_Root

```
In [38]: ###################
         ## ROOTS ######
         ##################

         lfc = 2
         sig = 0.05
         fpkm_cutoff = 1

         # limit list of genes to significantly differentially
         #       expressed with a abs(log2(fold_change)) value > 2
         #       and an expression value of at least 1 FPKM in one of the tissues
         df_volc = df_cuff[(df_cuff['sample_1'] == 'Storage_Root')
                          & (df_cuff['sample_2'] == 'Fibrous_Root')
                          & (df_cuff['q_value'] < sig )
                          & (np.abs(df_cuff['log2(fold_change)']) > lfc)
                          & ((df_cuff['value_1'] > fpkm_cutoff)
                             | (df_cuff['value_2'] > fpkm_cutoff))
                          & (df_cuff['gene'] != '-')].merge( annot[['gene','annot']],
                                                         how='inner',
                                                         on='gene',
                                                         copy=False)

         df_volc_n = df_cuff[(df_cuff['sample_1'] == 'Storage_Root')
                          & (df_cuff['sample_2'] == 'Fibrous_Root')
                          & ((df_cuff['q_value'] >= sig )
                             | (np.abs(df_cuff['log2(fold_change)']) <= lfc ))
                          & ((df_cuff['value_1'] > fpkm_cutoff)
                             | (df_cuff['value_2'] > fpkm_cutoff))
                          & (df_cuff['gene'] != '-')].merge( annot[['gene','annot']],
                                                         how='inner',
                                                         on='gene',
                                                         copy=False)

         # write genes to file
         df_volc.to_csv('./mesculenta_v6_output/diffexp_roots.txt', sep='\t')

         # calculate log score for volcano plot
         df_volc['-log(q_value)'] = -np.log10(df_volc['q_value'])
         df_volc_n['-log(q_value)'] = -np.log10(df_volc_n['q_value'])

         print('Storage Root vs Fibrous Root')
         print('Differentially Expressed Genes: {}'.format(df_volc.shape[0]) )
```

```
Storage Root vs Fibrous Root
Differentially Expressed Genes: 3486
```

```
In [39]: ############
         ## GO PREP
         ##########
```

```
        df_volc[ df_volc['log2(fold_change)'] > 2
                ]['gene'].drop_duplicates().to_csv(
            './mesculenta_v6_output/goprep_diffexp_root_fib.txt', index=False)

        df_volc[ df_volc['log2(fold_change)'] < -2
                ]['gene'].drop_duplicates().to_csv(
            './mesculenta_v6_output/goprep_diffexp_root_sto.txt', index=False)

        df_cuff[(df_cuff['sample_1'] == 'Storage_Root') &
                 (df_cuff['sample_2'] == 'Fibrous_Root') &
                 ((df_cuff['value_1'] > 1) | (df_cuff['value_2'] > 1)) &
                 (df_cuff['gene'] != '-')
                ]['gene'].drop_duplicates().to_csv(
            './mesculenta_v6_output/goprep_bkgrnd_root.txt', index=False)

In [40]: ## goatools ANALYSIS:
        # python ~/src/goatools/scripts/find_enrichment.py \
        #     --fdr --obo ~/src/goatools/go-basic.obo \
        #     goprep_diffexp_root_fib.txt goprep_bkgrnd_root.txt \
        #     Mesculenta_305_v6.1.annotation_info.go_only_uniq.txt \
        #     > root_fib_goatools.txt

        ## Process goatools output
        ### FIBROUS ROOT
        print('FIBROUS ROOT')
        df_rootgo_fib = pd.read_table(
            './mesculenta_v6_output/root_fib_goatools.txt', comment='#')

        print( 'GO count unfiltered: {}'.format(df_rootgo_fib.shape[0]))
        print( 'Enriched GO count, FDR < 0.05: {}'.format(
                 df_rootgo_fib[(df_rootgo_fib['p_fdr'] < 0.05) &
                               (df_rootgo_fib['enrichment'] == 'e')
                              ].shape[0] )
             )
        print( 'Enriched GO count, FDR < 0.01: {}'.format(
                 df_rootgo_fib[(df_rootgo_fib['p_fdr'] < 0.01) &
                               (df_rootgo_fib['enrichment'] == 'e')
                              ].shape[0] )
             )

        print()

        ## goatools ANALYSIS:
        # python ~/src/goatools/scripts/find_enrichment.py \
        #     --fdr --obo ~/src/goatools/go-basic.obo \
        #     goprep_diffexp_root_sto.txt goprep_bkgrnd_root.txt \
        #     Mesculenta_305_v6.1.annotation_info.go_only_uniq.txt \
        #     > root_sto_goatools.txt

        ### STORAGE ROOT
        print('STORAGE ROOT')
        df_rootgo_sto = pd.read_table(
            './mesculenta_v6_output/root_sto_goatools.txt', comment='#')
        print( 'GO count unfiltered: {}'.format(df_rootgo_sto.shape[0]))
        print( 'Enriched GO count, FDR < 0.05: {}'.format(
                df_rootgo_sto[(df_rootgo_sto['p_fdr'] < 0.05) &
                              (df_rootgo_sto['enrichment'] == 'e')
                             ].shape[0] )
             )
        print( 'Enriched GO count, FDR < 0.01: {}'.format(
                df_rootgo_sto[(df_rootgo_sto['p_fdr'] < 0.01) &
                              (df_rootgo_sto['enrichment'] == 'e')
                             ].shape[0] )
             )
```

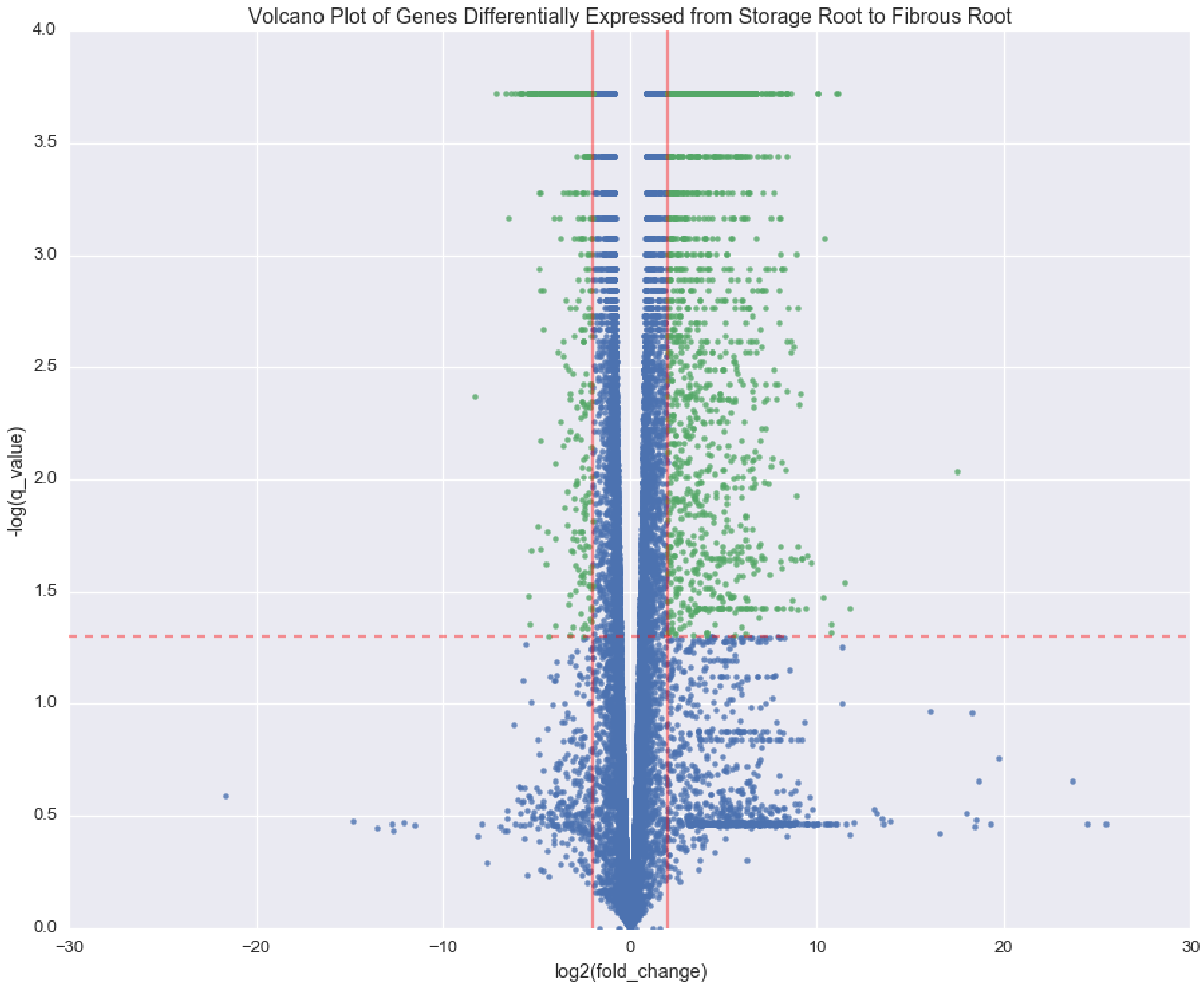
```
FIBROUS ROOT
GO count unfiltered: 414
Enriched GO count, FDR < 0.05: 139
Enriched GO count, FDR < 0.01: 135

STORAGE ROOT
GO count unfiltered: 177
Enriched GO count, FDR < 0.05: 24
Enriched GO count, FDR < 0.01: 4
```

```
In [41]: print('Genes Upregulated in Fibrous Root: {}'.format(
                df_volc[df_volc['log2(fold_change)'] > 0].shape[0])
             )
         print('Genes Upregulated in Storage Root'.format(
                df_volc[df_volc['log2(fold_change)'] < 0].shape[0])
             )
```

```
Genes Upregulated in Fibrous Root: 2524
Genes Upregulated in Storage Root
```

```
In [42]: ###################
         ## ROOTS ######
         ##################

         lfc = 2
         on_thresh = 10
         off_thresh = 1

         with( sns.plotting_context('talk') ):
             plt.figure(figsize=(15,12))
             sns.set_style('darkgrid')


             g = sns.regplot( y='-log(q_value)',
                             x='log2(fold_change)',
                             data=df_volc_n,
                             scatter=True,
                             fit_reg=False
                            )
```

```
    g = sns.regplot( y='-log(q_value)',
                    x='log2(fold_change)',
                    data=df_volc,
                    scatter=True,
                    fit_reg=False
                   )


    y_limit = (0,4)
    x_limit = (-30,30)
    g.axes.set_ylim(*y_limit)
    g.axes.set_xlim(*x_limit)
    g.set_title( 'Volcano Plot of Genes Differentially \
Expressed from Storage Root to Fibrous Root')

    plt.plot( (lfc,lfc), (0,y_limit[1]), color='red', alpha = 0.4 )
    plt.plot( (-lfc,-lfc), (0,y_limit[1]), color='red', alpha = 0.4 )
    plt.plot( (x_limit[0], x_limit[1]), (1.3,1.3),
              linestyle = '--', color='red', alpha = 0.4 )

    plt.savefig('./mesculenta_v6_output/volcano_diffexp_roots.pdf',
                bbox_inches='tight')
```



5.3 Fibrous Root and Leaf

5.3.1 Differentially Expressed in Fibrous_Root and Leaf

```
In [43]: ###################
         ## ROOT/LEAF ######
         ##################

         lfc = 2
         sig = 0.05
         fpkm_cutoff = 1

         # limit list of genes to significantly differentially
         #       expressed with a abs(log2(fold_change)) value > 2
         #       and an expression value of at least 1 FPKM in one of the tissues
         df_volc = df_cuff[(df_cuff['sample_1'] == 'Leaf')
                              & (df_cuff['sample_2'] == 'Fibrous_Root')
                              & (df_cuff['q_value'] < sig )
```

```
                             & (np.abs(df_cuff['log2(fold_change)']) > lfc)
                             & ((df_cuff['value_1'] > fpkm_cutoff)
                                | (df_cuff['value_2'] > fpkm_cutoff))
                             & (df_cuff['gene'] != '-')].merge( annot[['gene','annot']],
                                                                how='inner',
                                                                on='gene',
                                                                copy=False)

         df_volc_n = df_cuff[(df_cuff['sample_1'] == 'Leaf')
                             & (df_cuff['sample_2'] == 'Fibrous_Root')
                             & ((df_cuff['q_value'] >= sig )
                                | (np.abs(df_cuff['log2(fold_change)']) <= lfc ))
                             & ((df_cuff['value_1'] > fpkm_cutoff)
                                | (df_cuff['value_2'] > fpkm_cutoff))
                             & (df_cuff['gene'] != '-')].merge( annot[['gene','annot']],
                                                                how='inner',
                                                                on='gene',
                                                                copy=False)

         # write genes to file
         df_volc.to_csv('./mesculenta_v6_output/diffexp_rootleaf.txt', sep='\t')

         # calculate log score for volcano plot
         df_volc['-log(q_value)'] = -np.log10(df_volc['q_value'])
         df_volc_n['-log(q_value)'] = -np.log10(df_volc_n['q_value'])

         print('Leaf vs Fibrous Root')
         print('Differentially Expressed Genes: {}'.format(df_volc.shape[0]))
```

```
Leaf vs Fibrous Root
Differentially Expressed Genes: 4884
```

```
In [44]: ############
         ## GO PREP
         ##########
         df_volc[df_volc['log2(fold_change)'] > 2
                ]['gene'].drop_duplicates().to_csv(
             './mesculenta_v6_output/goprep_diffexp_rootleaf_fibroot.txt', index=False)

         df_volc[df_volc['log2(fold_change)'] < -2
                ]['gene'].drop_duplicates().to_csv(
             './mesculenta_v6_output/goprep_diffexp_rootleaf_leaf.txt', index=False)

         df_cuff[(df_cuff['sample_1'] == 'Leaf') &
                 (df_cuff['sample_2'] == 'Fibrous_Root') &
                 ((df_cuff['value_1'] > 1) | (df_cuff['value_2'] > 1)) &
                 (df_cuff['gene'] != '-')
                ]['gene'].drop_duplicates().to_csv(
             './mesculenta_v6_output/goprep_bkgrnd_rootleaf.txt', index=False)
```

```
In [45]: ##########################
         ## goatools ANALYSIS:
         # python ~/src/goatools/scripts/find_enrichment.py \
         #    --fdr --obo ~/src/goatools/go-basic.obo \
         #    goprep_diffexp_rootleaf_leaf.txt goprep_bkgrnd_rootleaf.txt \
         #    Mesculenta_305_v6.1.annotation_info.go_only_uniq.txt \
         #    > rootleaf_leaf_goatools.txt

         ## Process goatools output
         ### UPREGULATED IN LEAF
         print( 'LEAF' )
         df_rootgo = pd.read_table(
             './mesculenta_v6_output/rootleaf_leaf_goatools.txt', comment='#')

         print( 'GO count unfiltered: {}'.format(df_rootgo.shape[0]))
         print( 'Enriched GO count, FDR < 0.01: {}'.format(
                 df_rootgo[(df_rootgo['p_fdr'] < 0.01) &
                           (df_rootgo['enrichment'] == 'e')
                          ].shape[0] )
              )
         print( 'Enriched GO count, FDR < 0.001: {}'.format(
                 df_rootgo[(df_rootgo['p_fdr'] < 0.001) &
                           (df_rootgo['enrichment'] == 'e')
                          ].shape[0] )
              )

         print()

         #########################
         ## goatools ANALYSIS:
         # python ~/src/goatools/scripts/find_enrichment.py \
         #    --fdr --obo ~/src/goatools/go-basic.obo \
         #    goprep_diffexp_rootleaf_fibroot.txt goprep_bkgrnd_rootleaf.txt \
         #    Mesculenta_305_v6.1.annotation_info.go_only_uniq.txt \
         #    > rootleaf_fibroot_goatools.txt

         ### UPREGULATED IN FIBROUS ROOT
         print( 'FIBROUS ROOT' )
         df_rootgo = pd.read_table(
             './mesculenta_v6_output/rootleaf_fibroot_goatools.txt', comment='#')

         print( 'GO count unfiltered: {}'.format(df_rootgo.shape[0] ))
         print( 'Enriched GO count, FDR < 0.01: {}'.format(
                 df_rootgo[(df_rootgo['p_fdr'] < 0.01) &
                           (df_rootgo['enrichment'] == 'e')
                          ].shape[0] )
              )
         print( 'Enriched GO count, FDR < 0.001: {}'.format(
                 df_rootgo[(df_rootgo['p_fdr'] < 0.001) &
                           (df_rootgo['enrichment'] == 'e')
                          ].shape[0] )
              )
```

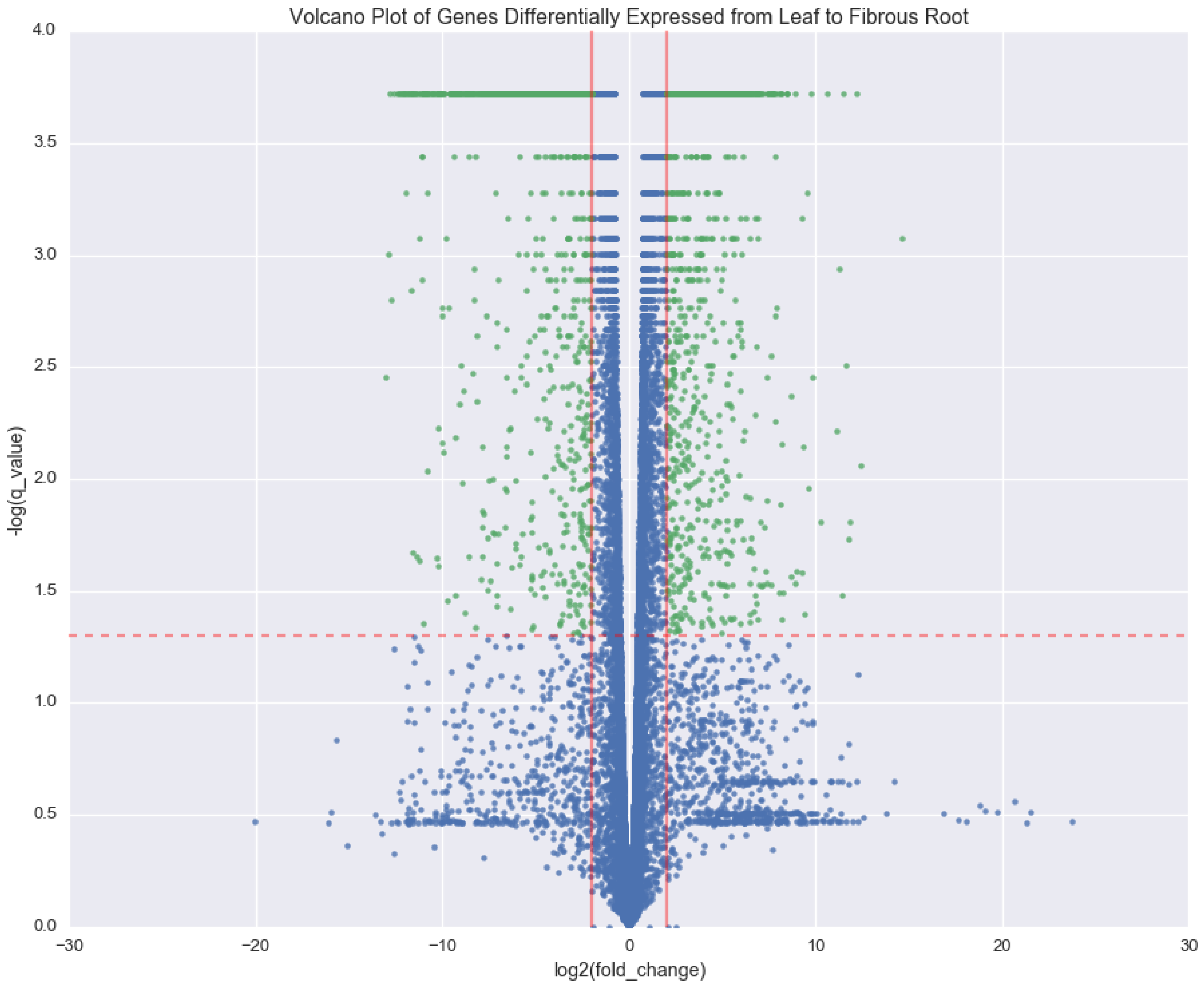
```
LEAF
GO count unfiltered: 398
Enriched GO count, FDR < 0.01: 67
Enriched GO count, FDR < 0.001: 47

FIBROUS ROOT
GO count unfiltered: 408
Enriched GO count, FDR < 0.01: 97
Enriched GO count, FDR < 0.001: 88
```

```
In [46]: print('Genes Upregulated in Fibrous Root: {}'.format(
                 df_volc[df_volc['log2(fold_change)'] > 0].shape[0])
              )
         print('Genes Upregulated in Leaf: {}'.format(
                 df_volc[df_volc['log2(fold_change)'] < 0].shape[0])
              )
```

```
Genes Upregulated in Fibrous Root: 2446
Genes Upregulated in Leaf: 2438
```

```
In [47]: ###################
         ## ROOT/LEAF ######
         ##################

         lfc = 2
         on_thresh = 10
         off_thresh = 1

         with( sns.plotting_context('talk') ):
             plt.figure(figsize=(15,12))
             sns.set_style('darkgrid')


             g = sns.regplot( y='-log(q_value)',
                              x='log2(fold_change)',
                              data=df_volc_n,
                              scatter=True,
                              fit_reg=False
                             )

             g = sns.regplot( y='-log(q_value)',
                              x='log2(fold_change)',
                              data=df_volc,
                              scatter=True,
                              fit_reg=False
                             )

             y_limit = (0,4)
             x_limit = (-30,30)
             g.axes.set_ylim(*y_limit)
             g.axes.set_xlim(*x_limit)
             g.set_title( 'Volcano Plot of Genes Differentially \
         Expressed from Leaf to Fibrous Root')

             plt.plot( (lfc,lfc), (0,y_limit[1]), color='red', alpha = 0.4 )
```

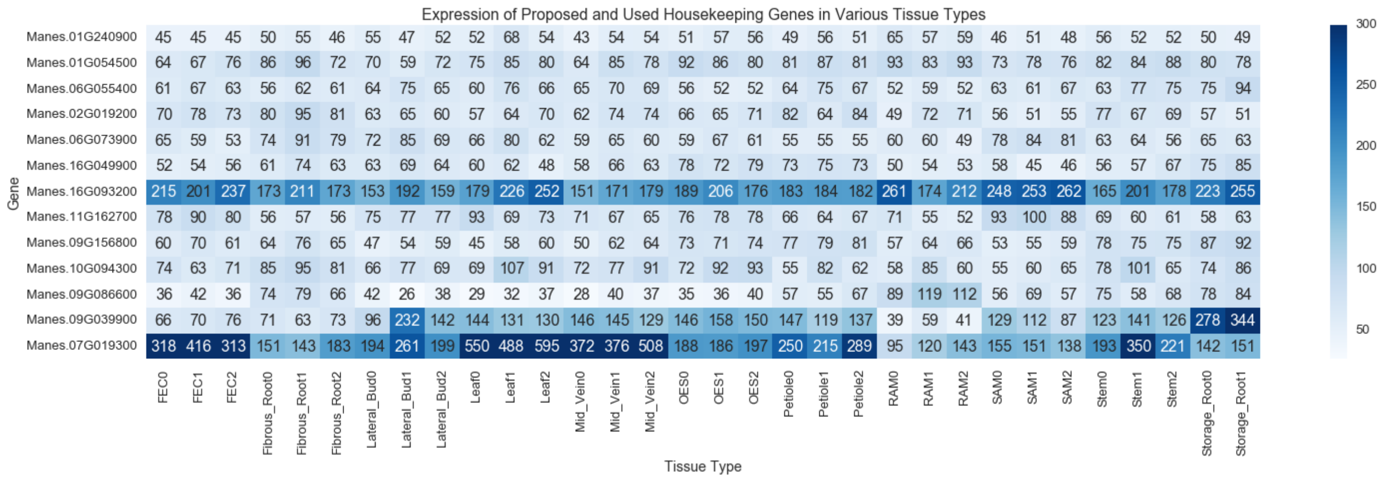
```
    plt.plot( (-lfc,-lfc), (0,y_limit[1]), color='red', alpha = 0.4 )
    plt.plot( (x_limit[0], x_limit[1]), (1.3,1.3),
              linestyle = '--', color='red', alpha = 0.4 )

    plt.savefig('./mesculenta_v6_output/volcano_diffexp_rootleaf.pdf',
                bbox_inches='tight')
```



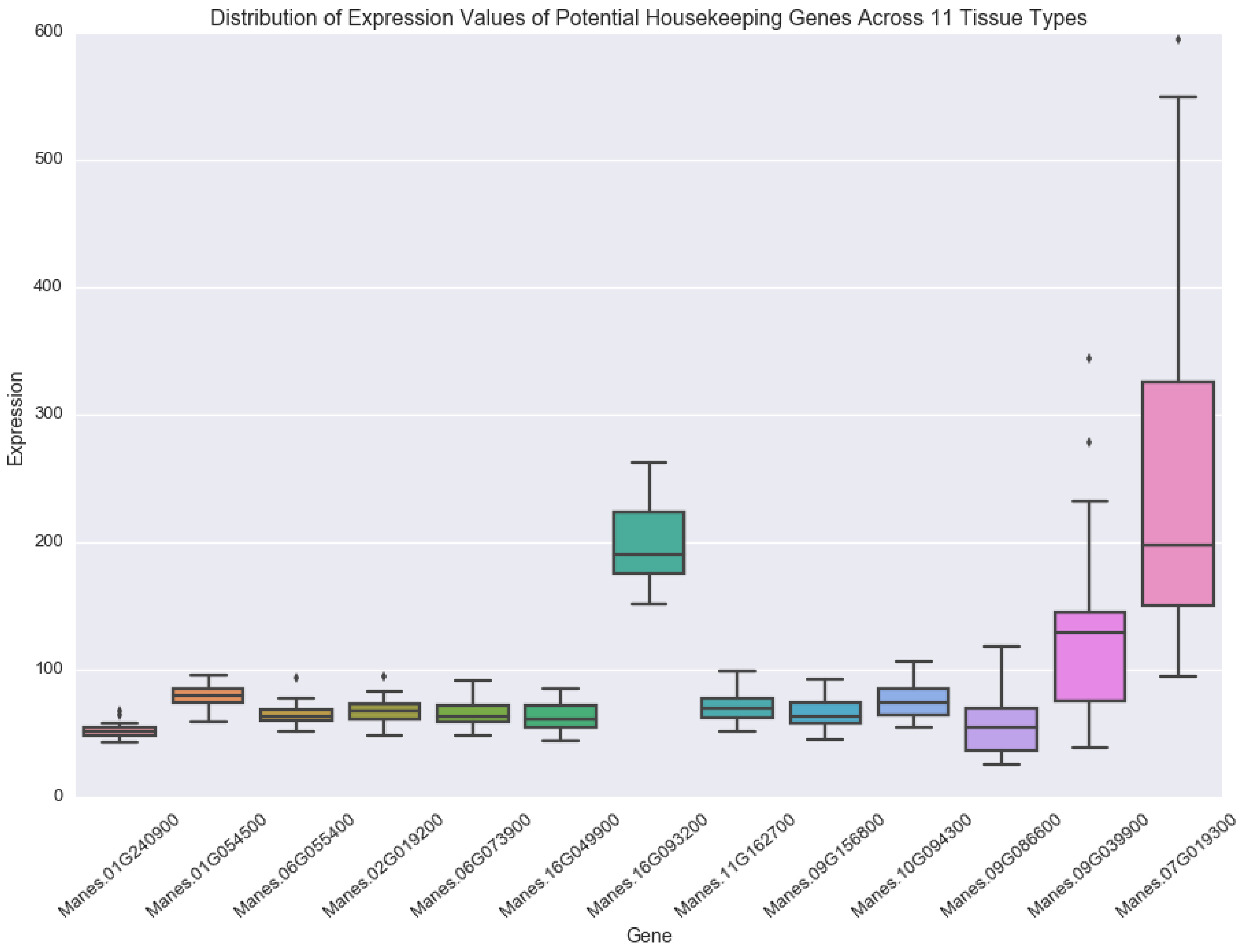
6 Similarly Expressed Genes

```
In [48]: genes_rgt_sim = genes_rgt_piv.copy()

         # limit to genes with a minimum expression of 40 FPKM in all samples
         genes_rgt_sim = genes_rgt_sim[(genes_rgt_sim.min(axis=1) >= 40)]

         # calculate Coefficient of Variation
         genes_rgt_sim['CoV'] = genes_rgt_sim.std(axis=1) / genes_rgt_sim.mean(axis=1)
```

```
        print( 'Gene Count: {}'.format(genes_rgt_sim.shape[0]) )

        # display top ten genes sorted by Coefficient of Variation
        genes_rgt_sim.sort_values('CoV').columns

Gene Count: 994


Out[48]: Index(['FEC0', 'FEC1', 'FEC2', 'Fibrous_Root0', 'Fibrous_Root1',
                'Fibrous_Root2', 'Lateral_Bud0', 'Lateral_Bud1', 'Lateral_Bud2',
                'Leaf0', 'Leaf1', 'Leaf2', 'Mid_Vein0', 'Mid_Vein1', 'Mid_Vein2',
                'OES0', 'OES1', 'OES2', 'Petiole0', 'Petiole1', 'Petiole2', 'RAM0',
                'RAM1', 'RAM2', 'SAM0', 'SAM1', 'SAM2', 'Stem0', 'Stem1', 'Stem2',
                'Storage_Root0', 'Storage_Root1', 'gene', 'go', 'TAIR', 'annot',
                'gene_id', 'CoV'],
               dtype='object', name='pivot')

In [49]: # get functional annotations for top ten similarly
         #    expressed as sorted by Coefficient of Variation
         sim_genes = genes_rgt_sim.sort_values('CoV').head(10)['gene']
         sim_genes = sim_genes.to_frame().merge(annot.loc[:,['gene','annot']],
                                                  how='left', on='gene')

         sim_genes

Out[49]:                gene                                            annot
         0  Manes.01G240900    RNA-binding (RRM/RBD/RNP motifs) family protein
         1  Manes.01G054500                         subunit of exocyst complex 8
         2  Manes.06G055400        SIT4 phosphatase-associated family protein
         3  Manes.02G019200                                PLAC8 family protein
         4  Manes.06G073900                                          decapping 5
         5  Manes.16G049900  cytochrome c oxidase assembly protein CtaG / C...
         6  Manes.16G093200    RNA-binding (RRM/RBD/RNP motifs) family protein
         7  Manes.11G162700    Transducin/WD40 repeat-like superfamily protein
         8  Manes.09G156800       Sec23/Sec24 protein transport family protein
         9  Manes.10G094300                                                  NaN

In [50]: # select expression values for 3 previously used housekeeping genes
         genes_rgt_hskp = pd.concat([
                 genes_rgt_sim.sort_values('CoV').head(10).drop('CoV', axis=1),
                 genes_rgt_piv[genes_rgt_piv['gene'].isin(['Manes.07G019300',
                                                           'Manes.09G086600',
                                                           'Manes.09G039900']
                                                         )]
                                 ])

         # calculate Coefficient of Variation
         genes_rgt_hskp['CoV'] = genes_rgt_hskp.std(axis=1) / genes_rgt_hskp.mean(axis=1)
         genes_rgt_hskp = genes_rgt_hskp.sort_values('CoV')

         # write to file
         genes_rgt_hskp.to_csv(
             './mesculenta_v6_output/similar_exp_gene_annot_table.txt', sep='\t')
        genes_rgt_hskp.loc[:,['annot']].to_csv(
            './mesculenta_v6_output/similar_exp_gene_annotonly.txt', sep='\t')

        genes_rgt_hskp.columns

Out[50]: Index(['FEC0', 'FEC1', 'FEC2', 'Fibrous_Root0', 'Fibrous_Root1',
                'Fibrous_Root2', 'Lateral_Bud0', 'Lateral_Bud1', 'Lateral_Bud2',
                'Leaf0', 'Leaf1', 'Leaf2', 'Mid_Vein0', 'Mid_Vein1', 'Mid_Vein2',
                'OES0', 'OES1', 'OES2', 'Petiole0', 'Petiole1', 'Petiole2', 'RAM0',
                'RAM1', 'RAM2', 'SAM0', 'SAM1', 'SAM2', 'Stem0', 'Stem1', 'Stem2',
                'Storage_Root0', 'Storage_Root1', 'gene', 'go', 'TAIR', 'annot',
                'gene_id', 'CoV'],
              dtype='object', name='pivot')

In [51]: # get top 10 genes sorted by Coefficient of Variation for plotting
         df_plot = genes_rgt_hskp.set_index('gene').iloc[:,:32]

         with( sns.plotting_context( 'talk' ) ):
             plt.figure(figsize=(25,6))
             sns.set_style('darkgrid')

             g = sns.heatmap(data=df_plot,
                             cmap='Blues', annot=True, fmt='.0f', vmax=300)

             g.set_ylabel('Gene')
             g.set_xlabel('Tissue Type')
             g.set_title('Expression of Proposed and Used Housekeeping \
         Genes in Various Tissue Types')
```



```
In [52]: df_plot = genes_rgt_hskp.copy()
         df_plot = df_plot.drop(['annot',
                                 'TAIR',
                                 'go',
                                 'gene_id',
                                 'CoV'], axis=1).set_index('gene')

         # plot distribution of gene expression across all samples of top most
         #     similarly expressed genes and 3 previously used housekeeping genes
         with( sns.plotting_context( 'talk' ) ):
             plt.figure(figsize=(15,10))
             sns.set_style('darkgrid')

             g = sns.boxplot(data=df_plot.transpose())
             g.set_ylabel('Expression')
             g.set_xlabel('Gene')
             g.set_title('Distribution of Expression Values of Potential \
         Housekeeping Genes Across 11 Tissue Types')
             g.set_xticklabels(df_plot.index, rotation=40)

             plt.savefig('./mesculenta_v6_output/similar_exp_allsamp_cov_dist.pdf',
                         bbox_inches='tight')
```



```
In [ ]:
```