1. Introduction

MOCHIS is a software that allows the user to perform flexible non-parametric tests of differential gene expression. Such tests include the popular Mann-Whitney (Wilcoxon rank sum) test, which was recently promoted by Li et al. (2022) as an approach to perform differential analysis on RNA-seq data without incurring an inflated false positive rate. In this markdown document, we explore how MOCHIS can detect multiple kinds of differential gene expression signatures, including mean shifts or dispersion shifts. Dispersion shifts have recently been shown to characterize age-related changes in gene expression (see Schaum et al., 2020 and Yamamoto and Chung et al., 2022+). In particular, we:

- perform multiple kinds of two-sample tests on all single-cell tissue data provided in Tabula muris senis
- report and compare findings across the different kinds of tests For Section 3 (Analysis), all our analyses are followed by a summary of key findings, to help the reader quickly grasp the main points.

In [156]:
```
# Setup

import scanpy
import numpy as np
import anndata
import pandas as pd
import matplotlib.pyplot as plt
from main_draft0 import *
import scipy
import statistics
import csv
import os
import seaborn as sns
from matplotlib_venn import venn2
import math

np.random.seed(2022)
scipy.__version__
```

Out[156]:
```
'1.8.0'
```

2. Data

Publicly available *mus musculus* (house mice) single-cell RNA-seq data from the Chan-Zuckerberg Initiative (also known as *Tabula Muris Senis*) is used. We download senescence datasets from here (https://cellxgene.cziscience.com/collections/0b9d8a04-bb9d-44da-aa27-705bb65b54eb). These datasets are made up of single cell gene transcript levels measured using Smart-Seq2, across 22 distinct mice tissues. For each tissue, the cells originate from mice that are either 3 months, 18 months or 24 months old (with the exception of the mammary gland tissue, which has 3 months, 18 months and 21 months). There are also other cell labels like tissue location (identified with guidance from biologists) and mice sex.

Below, we perform the Mann-Whitney test to identify genes that are differentially expressed, also known as differentially expressed genes (DEGs), across age groups. We compare each pair of age group, so that for each gene $\binom{3}{2} = 3$ tests are performed.

We restrict our analysis to those regions where the zero counts are the fewest, using an 80% cut-off. This avoids running tests on genes that have pronounced zero inflation, which hinders the detection of differential expression.

We additionally compute a "ratio of variances" index, which heuristic measures of the difference in dispersion across the pair of age groups. The larger the ratio of variances, the more differentially dispersed the gene expression between the pair of age groups.

```
In [ ]:
```

In [2]:
```
# Perform analysis for each tissue
# There are 22 tissues
all_tissues = sorted(["bone-marrow",
              "brain-myeloid",
              "heart",
              "large-intestine",
              "lung",
              "skin-of-body",
              "thymus",
              "limb-muscle",
              "spleen",
              "subcutaneous-adipose-tissue",
              "tongue",
              "gonadal-fat-pad",
              "pancreas",
              "mammary-gland",
              "trachea",
              "mesenteric-fat-pad",
              "liver",
              "bladder-lumen",
              "brown-adipose-tissue",
              "diaphragm",
              "kidney",
              "aorta"])


for tissue in all_tissues:
    #os.mkdir(os.path.join("tissues/", tissue))


    tissue_smartseq2_data = scanpy.read_h5ad('tissues/' + tissue + '.h5ad')
    transcripts = tissue_smartseq2_data.var.n_cells.index
    ages = np.array(tissue_smartseq2_data.obs['age'].index)
    smartseq2_raw_counts = tissue_smartseq2_data.raw.X.toarray()
    #print(smartseq2_raw_counts.shape)  # 14517 mice cells x 21069 regions

    # Get cutoff and restrict to only those genes
    cutoff = round(0.8*smartseq2_raw_counts.shape[0])

    cell_count_sums_by_region = np.count_nonzero(smartseq2_raw_counts, axis

    highly_expressed_genes_indices = [i for i,v in enumerate(cell_count_sum

    smartseq2_high_exp_sparse_mat = []
    for i in highly_expressed_genes_indices:
        smartseq2_high_exp_sparse_mat.append(smartseq2_raw_counts[:, i])

    print("Found ", len(highly_expressed_genes_indices), " genes out of ",

    highly_expressed_transcripts = [transcripts[i] for i in highly_expresse

    # Grab age labels
    #smartseq2_df = anndata.AnnData(np.transpose(smartseq2_high_exp_sparse_
    smartseq2_df = pd.DataFrame(np.append(np.transpose(smartseq2_high_exp_s

    # Run Mann-Whitney test for genes
    gene_names = smartseq2_df.columns.values[:-1]
    results_df = pd.DataFrame(columns=['TRANSCRIPT', 'MANN_WHITNEY_3_18', '

    print("How many cells of each age group?")
    print(smartseq2_df['ages'].value_counts())


    # Run test for each gene
    for i in range(len(gene_names)):
        to_run_test = smartseq2_df[[gene_names[i], 'ages']]

        if tissue == "mammary-gland":
            print("Reminder that mammary-gland has 3m, 18m and 21m age grou
            age_3m = to_run_test.loc[to_run_test["ages"] == "3m", gene_name
            age_18m = to_run_test.loc[to_run_test["ages"] == "18m", gene_na
            age_24m = to_run_test.loc[to_run_test["ages"] == "21m", gene_na
        else:
            age_3m = to_run_test.loc[to_run_test["ages"] == "3m", gene_name
            age_18m = to_run_test.loc[to_run_test["ages"] == "18m", gene_na
            age_24m = to_run_test.loc[to_run_test["ages"] == "24m", gene_na

        age_3m = [float(i) for i in age_3m]
        age_18m = [float(i) for i in age_18m]
        age_24m = [float(i) for i in age_24m]

        wrs_test_3_18 = scipy.stats.mannwhitneyu(x=age_3m, y=age_18m, alter
        wrs_test_18_24 = scipy.stats.mannwhitneyu(x=age_18m, y=age_24m, alt
        wrs_test_24_3 = scipy.stats.mannwhitneyu(x=age_3m, y=age_24m, alter

        var_3_18 = max(statistics.variance(age_3m)/statistics.variance(age_
        var_18_24 = max(statistics.variance(age_18m)/statistics.variance(ag
        var_24_3 = max(statistics.variance(age_24m)/statistics.variance(age

        results_df = results_df.append({
            'TRANSCRIPT': gene_names[i],
            'MANN_WHITNEY_3_18': wrs_test_3_18.pvalue,
            'MANN_WHITNEY_18_24': wrs_test_18_24.pvalue,
            'MANN_WHITNEY_24_3': wrs_test_24_3.pvalue,
            'VAR_3_18': var_3_18,
            'VAR_18_24': var_18_24,
            'VAR_24_3': var_24_3
        }, ignore_index=True)



    print("Saving results for ", tissue)
    results_df.to_csv("tissues/"+tissue+"/p_val_table.csv")
```

```
Name: ages, dtype: int64
Saving results for  diaphragm
```

```
Found  288  genes out of  3406  genes meeting the cutoff threshold...
How many cells of each age group?
3m     1464
24m    1067
18m     875
Name: ages, dtype: int64
Saving results for  gonadal-fat-pad
Found  186  genes out of  9669  genes meeting the cutoff threshold...
How many cells of each age group?
3m     4433
24m    3185
18m    2051
Name: ages, dtype: int64
Saving results for  heart
Found  67  genes out of  1833  genes meeting the cutoff threshold...
How many cells of each age group?

18m    668
```

2.1 Mann-Whitney DEGs

Given we have the tables of p-values and ratios of variances from the previous step, we now select genes whose p-values, after a Benjamini-Hochberg adjustment procedure, lie below or equal to a 0.05 significance level. These are Mann-Whitney significant genes that would be flagged as potentially carrying biological signal in a typical differential expression analysis procedure.

In [3]:
```
def p_adjust_bh(p):
    """Benjamini-Hochberg p-value correction for multiple hypothesis testin
    p = np.asfarray(p)
    by_descend = p.argsort()[::-1]
    by_orig = by_descend.argsort()
    steps = float(len(p)) / np.arange(len(p), 0, -1)
    q = np.minimum(1, np.minimum.accumulate(steps * p[by_descend]))
    return q[by_orig]
```

In [4]:
```
tissue_transcript_3_18 = pd.DataFrame(columns=['TRANSCRIPT', 'MANN_WHITNEY'
tissue_transcript_18_24 = pd.DataFrame(columns=['TRANSCRIPT', 'MANN_WHITNEY
tissue_transcript_24_3 = pd.DataFrame(columns=['TRANSCRIPT', 'MANN_WHITNEY'


for tissue in all_tissues:
    print("Reading in summary of p-values and ratios of variances for ", ti
    tissue_mann_whitney_df = pd.read_csv("tissues/"+tissue+"/p_val_table.cs

    # Pick genes where one of the three pairs (3m, 18m, 24m) has significan
    selected_genes_3_18 = tissue_mann_whitney_df[p_adjust_bh(tissue_mann_wh
    selected_genes_3_18 = selected_genes_3_18[["TRANSCRIPT", "MANN_WHITNEY_
    selected_genes_3_18= selected_genes_3_18.rename(columns={"MANN_WHITNEY_
    selected_genes_3_18["TISSUE"] = [tissue for i in range(selected_genes_3
    tissue_transcript_3_18 = pd.concat([tissue_transcript_3_18, selected_ge

    selected_genes_18_24 = tissue_mann_whitney_df[p_adjust_bh(tissue_mann_w
    selected_genes_18_24 = selected_genes_18_24[["TRANSCRIPT", "MANN_WHITNE
    selected_genes_18_24 = selected_genes_18_24.rename(columns={"MANN_WHITN
    selected_genes_18_24["TISSUE"] = [tissue for i in range(selected_genes_
    tissue_transcript_18_24 = pd.concat([tissue_transcript_18_24, selected_

    selected_genes_24_3 = tissue_mann_whitney_df[p_adjust_bh(tissue_mann_wh
    selected_genes_24_3 = selected_genes_24_3[["TRANSCRIPT", "MANN_WHITNEY_
    selected_genes_24_3 = selected_genes_24_3.rename(columns={"MANN_WHITNEY
    selected_genes_24_3["TISSUE"] = [tissue for i in range(selected_genes_2
    tissue_transcript_24_3 = pd.concat([tissue_transcript_24_3, selected_ge

tissue_transcript_3_18.to_csv("tissues/mw_sig_3m_18m.csv")
tissue_transcript_18_24.to_csv("tissues/mw_sig_18m_24m.csv")
tissue_transcript_24_3.to_csv("tissues/mw_sig_24m_3m.csv")
```

```
Reading in summary of p-values and ratios of variances for  aorta
Reading in summary of p-values and ratios of variances for  bladder-lumen
Reading in summary of p-values and ratios of variances for  bone-marrow
Reading in summary of p-values and ratios of variances for  brain-myeloid
Reading in summary of p-values and ratios of variances for  brown-adipose
-tissue
Reading in summary of p-values and ratios of variances for  diaphragm
Reading in summary of p-values and ratios of variances for  gonadal-fat-p
ad
Reading in summary of p-values and ratios of variances for  heart
Reading in summary of p-values and ratios of variances for  kidney
Reading in summary of p-values and ratios of variances for  large-intesti
ne
Reading in summary of p-values and ratios of variances for  limb-muscle
Reading in summary of p-values and ratios of variances for  liver
Reading in summary of p-values and ratios of variances for  lung
Reading in summary of p-values and ratios of variances for  mammary-gland
Reading in summary of p-values and ratios of variances for  mesenteric-fa
```

```
t-pad
Reading in summary of p-values and ratios of variances for  pancreas
Reading in summary of p-values and ratios of variances for  skin-of-body
Reading in summary of p-values and ratios of variances for  spleen
Reading in summary of p-values and ratios of variances for  subcutaneous-
adipose-tissue
Reading in summary of p-values and ratios of variances for  thymus
Reading in summary of p-values and ratios of variances for  tongue
Reading in summary of p-values and ratios of variances for  trachea
```

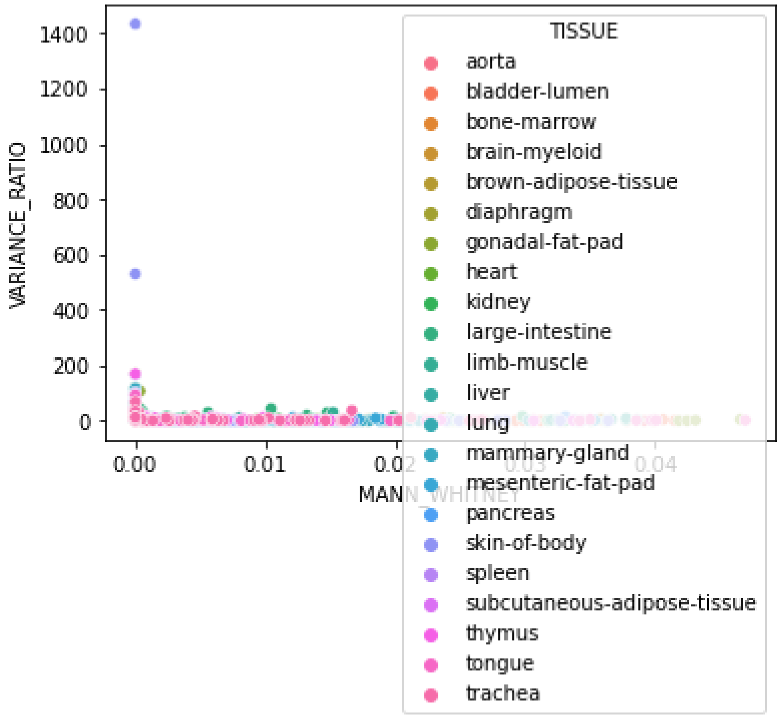
Let us visualize the raw p-values and variance ratios of the Mann-Whitney DEGs fished out from the above procedure.

In [5]:
```
df_3_18 = pd.read_csv("tissues/mw_sig_3m_18m.csv")
df_18_24 = pd.read_csv("tissues/mw_sig_18m_24m.csv")
df_24_3 = pd.read_csv("tissues/mw_sig_24m_3m.csv")


df_3_18["PAIR"] = ["3m vs 18m" for i in range(df_3_18.shape[0])]
df_18_24["PAIR"] = ["18m vs 24m" for i in range(df_18_24.shape[0])]
df_24_3["PAIR"] = ["3m vs 24m" for i in range(df_24_3.shape[0])]



'''
groups = df_3_18.groupby("TISSUE")
for name, group in groups:
    plt.plot(group["MANN_WHITNEY"], group["VARIANCE_RATIO"], marker="o", li
plt.legend()

groups = df_18_24.groupby("TISSUE")
for name, group in groups:
    plt.plot(group["MANN_WHITNEY"], group["VARIANCE_RATIO"], marker="o", li
plt.legend()

groups = df_24_3.groupby("TISSUE")
for name, group in groups:
    plt.plot(group["MANN_WHITNEY"], group["VARIANCE_RATIO"], marker="o", li
plt.legend()


grand_df = pd.concat(pd.concat(df_3_18, df_18_24), df_24_3)

g = sns.FacetGrid([grand_df], col="PAIR")
g.map(sns.scatterplot, x='MANN_WHITNEY', y='VARIANCE_RATIO', hue='TISSUE',

#sns.scatterplot(data=grand_df, x='MANN_WHITNEY', y='VARIANCE_RATIO', hue='

sns.scatterplot(data=df_3_18, x='MANN_WHITNEY', y='VARIANCE_RATIO', hue='TI
sns.show()
sns.scatterplot(data=df_3_18, x='MANN_WHITNEY', y='VARIANCE_RATIO', hue='TI
'''
```

Out[5]:
```
'\ngroups = df_3_18.groupby("TISSUE")\nfor name, group in groups:\n    pl
t.plot(group["MANN_WHITNEY"], group["VARIANCE_RATIO"], marker="o", linest
yle="", label=name, alpha=0.5)\nplt.legend()\n\ngroups = df_18_24.groupby
("TISSUE")\nfor name, group in groups:\n    plt.plot(group["MANN_WHITNE
Y"], group["VARIANCE_RATIO"], marker="o", linestyle="", label=name, alpha
=0.5)\nplt.legend()\n\ngroups = df_24_3.groupby("TISSUE")\nfor name, grou
p in groups:\n    plt.plot(group["MANN_WHITNEY"], group["VARIANCE_RATI
O"], marker="o", linestyle="", label=name, alpha=0.5)\nplt.legend()\n\n\n
grand_df = pd.concat(pd.concat(df_3_18, df_18_24), df_24_3)\n\ng = sns.Fa
cetGrid([grand_df], col="PAIR")\ng.map(sns.scatterplot, x=\'MANN_WHITNEY
\', y=\'VARIANCE_RATIO\', hue=\'TISSUE\', alpha=0.5)\n\n#sns.scatterplot
(data=grand_df, x=\'MANN_WHITNEY\', y=\'VARIANCE_RATIO\', hue=\'TISSUE\',
alpha=0.5).FacetGrid\n\nsns.scatterplot(data=df_3_18, x=\'MANN_WHITNEY\',
y=\'VARIANCE_RATIO\', hue=\'TISSUE\')\nsns.show()\nsns.scatterplot(data=d
f_3_18, x=\'MANN_WHITNEY\', y=\'VARIANCE_RATIO\', hue=\'TISSUE\')\n'
```

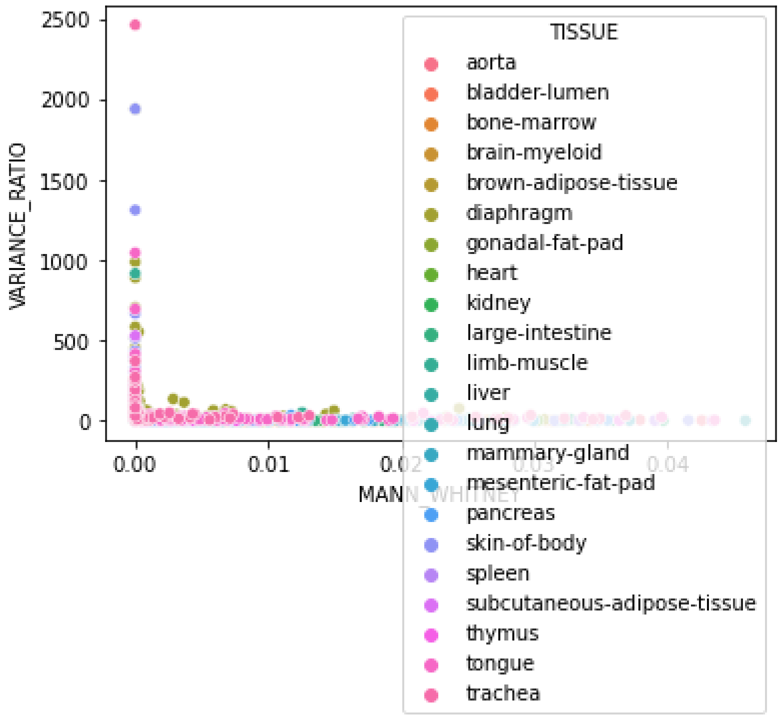
In [6]:
```
sns.scatterplot(data=df_18_24, x='MANN_WHITNEY', y='VARIANCE_RATIO', hue='T
```

Out[6]:
```
<AxesSubplot:xlabel='MANN_WHITNEY', ylabel='VARIANCE_RATIO'>
```



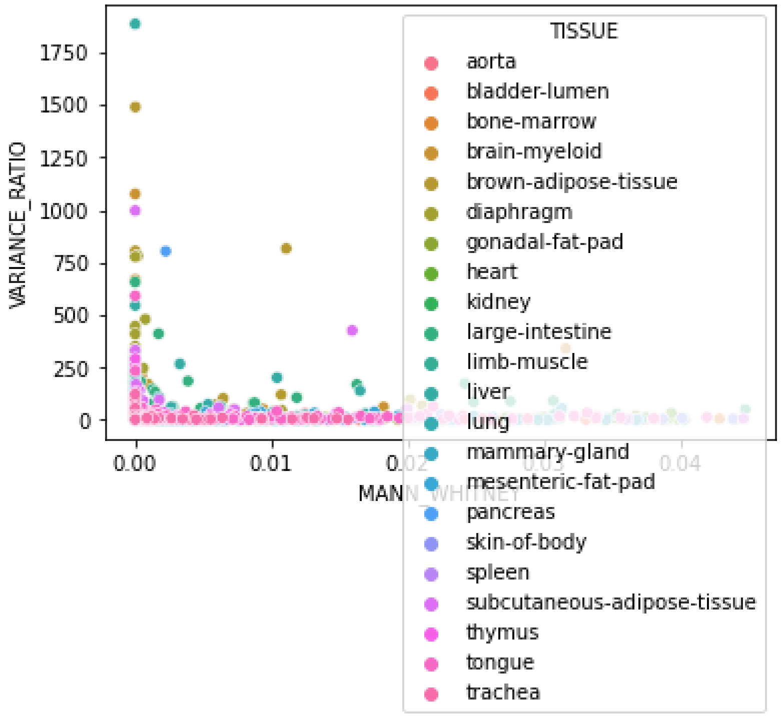
In [7]:
```
sns.scatterplot(data=df_3_18, x='MANN_WHITNEY', y='VARIANCE_RATIO', hue='TI
```

Out[7]:
```
<AxesSubplot:xlabel='MANN_WHITNEY', ylabel='VARIANCE_RATIO'>
```



In [8]:
```
sns.scatterplot(data=df_24_3, x='MANN_WHITNEY', y='VARIANCE_RATIO', hue='TI
```

Out[8]:
```
<AxesSubplot:xlabel='MANN_WHITNEY', ylabel='VARIANCE_RATIO'>
```

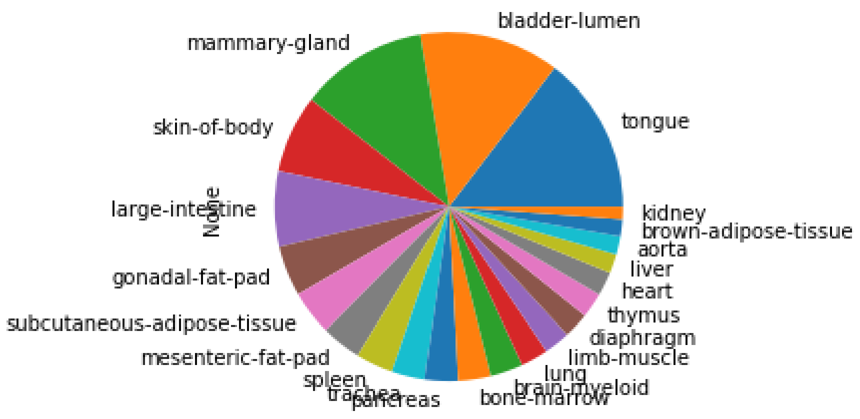


Next we look at the distribution, across tissues, of Mann-Whitney significant genes.

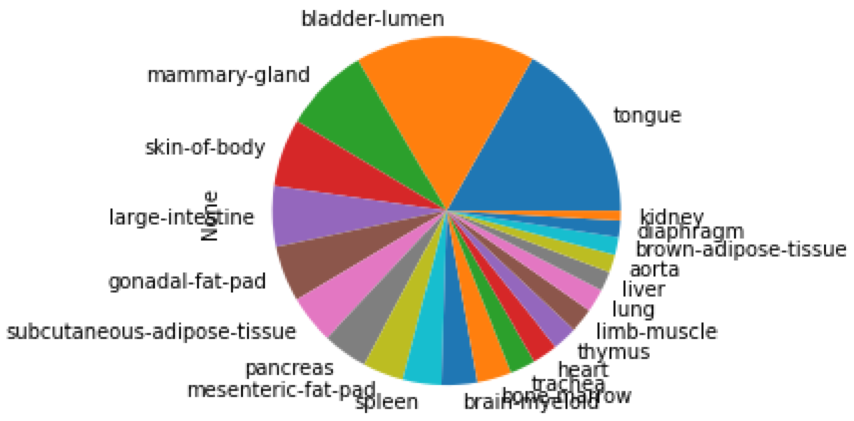
In [9]:
```
print("No. MW significant genes for 3m vs 18m: ", df_3_18.shape[0])
print("No. MW significant genes for 18m vs 24m: ", df_18_24.shape[0])
print("No. MW significant genes for 24m vs 3m: ", df_24_3.shape[0])
```

```
No. MW significant genes for 3m vs 18m:  5571
No. MW significant genes for 18m vs 24m:  5305
No. MW significant genes for 24m vs 3m:  5634
```

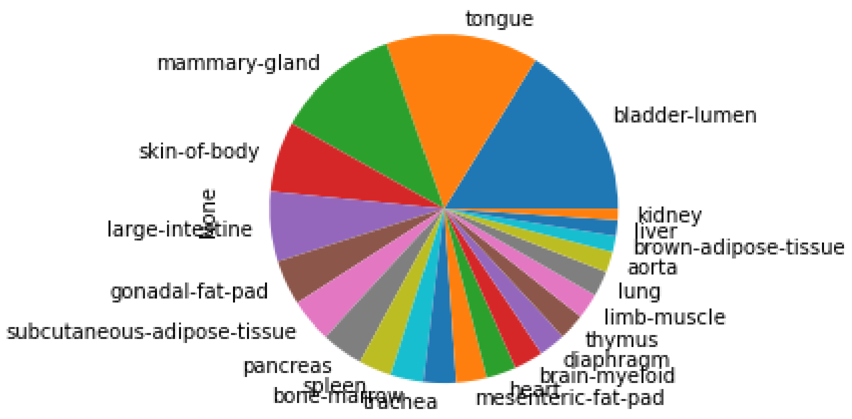
```
In [10]: df_3_18.value_counts("TISSUE").plot(kind="pie")

Out[10]: <AxesSubplot:ylabel='None'>
```



```
In [11]: df_18_24.value_counts("TISSUE").plot(kind="pie")

Out[11]: <AxesSubplot:ylabel='None'>
```



```
In [12]: df_24_3.value_counts("TISSUE").plot(kind="pie")

Out[12]: <AxesSubplot:ylabel='None'>
```



2.2 MOCHIS

We now repeat the DEG identification procedure above, now using our flexible non-parametric testing software MOCHIS. We run MOCHIS with test statistic $||S_{n,k}||_{p,\boldsymbol{w}}^{p}$. We choose the following parametrization:

- $p = 1$
- $\boldsymbol{w} = \left((\frac{j}{k} - \frac{1}{2})^2 : j = 1, \ldots, k\right)$

This parametrization optimizes detection of dispersion shifts between two samples.

Step 1. Compute p-values.

When computing the p-values, we apply a tie-breaking routine (adding noise ranging from -0.25 to 0.25, which is less than the minimum spacing width of integer counts). To ensure that this routine does not overly contaminate the data, we also compute Mann-Whitney p-values and check that the Mann-Whitney DEGs identified after applying the tie-breaking routine are not markedly different from the original DEGs identified in Section 2.1. We report this latter comparison between post-contamination and original DEGs in Section 2.3. (Heads up: We find little difference.)

In [14]:
```
tissue in all_tissues:


#os.mkdir(os.path.join("tissues/", tissue))




tissue_smartseq2_data = scanpy.read_h5ad('tissues/' + tissue + '.h5ad')
transcripts = tissue_smartseq2_data.var.n_cells.index
ages = np.array(tissue_smartseq2_data.obs['age'].index)
smartseq2_raw_counts = tissue_smartseq2_data.raw.X.toarray()
print(smartseq2_raw_counts.shape)  # 14517 mice cells x 21069 regions

# Get cutoff and restrict to only those genes
cutoff = round(0.8*smartseq2_raw_counts.shape[0])

cell_count_sums_by_region = np.count_nonzero(smartseq2_raw_counts, axis=0)

highly_expressed_genes_indices = [i for i,v in enumerate(cell_count_sums_by

smartseq2_high_exp_sparse_mat = []
for i in highly_expressed_genes_indices:
    smartseq2_high_exp_sparse_mat.append(smartseq2_raw_counts[:, i])

print("Found ", len(highly_expressed_genes_indices), " genes out of ", smar

highly_expressed_transcripts = [transcripts[i] for i in highly_expressed_ge

# Grab age labels
#smartseq2_df = anndata.AnnData(np.transpose(smartseq2_high_exp_sparse_mat,
smartseq2_df = pd.DataFrame(np.append(np.transpose(smartseq2_high_exp_spars


# Run Mann-Whitney test for genes
gene_names = smartseq2_df.columns.values[:-1]
results_df = pd.DataFrame(columns=['TRANSCRIPT',
                                   'MOCHIS_3_18',
                                   'MW_3_18',
                                   'MOCHIS_18_24',
                                   'MW_18_24',
                                   'MOCHIS_24_3',
                                   'MW_24_3',
                                   'VAR_3_18',
                                   'INV_3_18',
                                   'VAR_18_24',
                                   'INV_18_24',
                                   'VAR_24_3',
                                   'INV_24_3'])
print("How many cells of each age group?")
print(smartseq2_df['ages'].value_counts())

# Run test for each gene
for i in range(len(gene_names)):
    to_run_test = smartseq2_df[[gene_names[i], 'ages']]

    if tissue == "mammary-gland":
        print("Reminder that mammary-gland has 3m, 18m and 21m age groups,
        age_3m = to_run_test.loc[to_run_test["ages"] == "3m", gene_names[i
        age_18m = to_run_test.loc[to_run_test["ages"] == "18m", gene_names
        age_24m = to_run_test.loc[to_run_test["ages"] == "21m", gene_names
    else:
        age_3m = to_run_test.loc[to_run_test["ages"] == "3m", gene_names[i
        age_18m = to_run_test.loc[to_run_test["ages"] == "18m", gene_names
        age_24m = to_run_test.loc[to_run_test["ages"] == "24m", gene_names

    age_3m = [float(i) for i in age_3m]
    age_18m = [float(i) for i in age_18m]
    age_24m = [float(i) for i in age_24m]


    # Add noise to break ties

    noisy_age_3m = np.sort([value + np.random.uniform(-1/4, 1/4) for value
    noisy_age_18m = np.sort([value + np.random.uniform(-1/4, 1/4) for value
    noisy_age_24m = np.sort([value + np.random.uniform(-1/4, 1/4) for value


    wrs_test_3_18 = scipy.stats.mannwhitneyu(x=noisy_age_3m, y=noisy_age_18
    wrs_test_18_24 = scipy.stats.mannwhitneyu(x=noisy_age_18m, y=noisy_age_
    wrs_test_24_3 = scipy.stats.mannwhitneyu(x=noisy_age_3m, y=noisy_age_24




    if len(noisy_age_3m) > len(noisy_age_18m):
        #print("3 > 18")

        k = len(age_18m) + 1
        mochis_weights = [(i/k-0.5)**2 for i in range(1,k+1)]
        mochis_test_3_18 = mochis_py(x = noisy_age_18m,
                                     p = 1,
                                     wList = mochis_weights,
                                     alternative = "two.sided",
                                     approx = "chebyshev",
                                     n_mom = 100,
                                     y = noisy_age_3m)

    else:
        #print(" 18 > 3")

        k = len(age_3m) + 1
        mochis_weights = [(i/k-0.5)**2 for i in range(1,k+1)]
        mochis_test_3_18 = mochis_py(x = noisy_age_3m,
                                     p = 1,
                                     wList = mochis_weights,
                                     alternative = "two.sided",
                                     approx = "chebyshev",
                                     n_mom = 100,
                                     y = noisy_age_18m)
```

```
if len(noisy_age_18m) > len(noisy_age_24m):
    #print("18 > 24")

    k = len(noisy_age_24m) + 1
    mochis_weights = [(i/k-0.5)**2 for i in range(1,k+1)]
    mochis_test_18_24 = mochis_py(x = noisy_age_24m,
                                  p = 1,
                                  wList = mochis_weights,
                                  alternative = "two.sided",
                                  approx = "chebyshev",
                                  n_mom = 100,
                                  y = noisy_age_18m)

else:
    #print("24 > 18")

    k = len(noisy_age_18m) + 1
    mochis_weights = [(i/k-0.5)**2 for i in range(1,k+1)]
    mochis_test_18_24 = mochis_py(x = noisy_age_18m,
                                  p = 1,
                                  wList = mochis_weights,
                                  alternative = "two.sided",
                                  approx = "chebyshev",
                                  n_mom = 100,
                                  y = noisy_age_24m)


if len(noisy_age_3m) > len(noisy_age_24m):
    #print("3 > 24")

    k = len(noisy_age_24m) + 1
    mochis_weights = [(i/k-0.5)**2 for i in range(1,k+1)]
    mochis_test_24_3 = mochis_py(x = noisy_age_24m,
                                  p = 1,
                                  wList = mochis_weights,
                                  alternative = "two.sided",
                                  approx = "chebyshev",
                                  n_mom = 100,
                                  y = noisy_age_3m)

else:
    #print(" 24 > 3")

    k = len(noisy_age_3m) + 1
    mochis_weights = [(i/k-0.5)**2 for i in range(1,k+1)]
    mochis_test_24_3 = mochis_py(x = noisy_age_3m,
                                  p = 1,
                                  wList = mochis_weights,
                                  alternative = "two.sided",
                                  approx = "chebyshev",
                                  n_mom = 100,
                                  y = noisy_age_24m)



var_3_18 = max(statistics.variance(age_3m)/statistics.variance(age_18m
var_18_24 = max(statistics.variance(age_18m)/statistics.variance(age_2
    var_24_3 = max(statistics.variance(age_24m)/statistics.variance(age_3m

    invert_3_18 = False
    invert_18_24 = False
    invert_24_3 = False
    if var_3_18 == statistics.variance(age_3m)/statistics.variance(age_18m
        invert_3_18 = True
    if var_18_24 == statistics.variance(age_18m)/statistics.variance(age_2
        invert_18_24 = True
    if var_24_3 == statistics.variance(age_3m)/statistics.variance(age_24m
        invert_24_3 = True

    results_df = pd.concat([results_df, pd.DataFrame([{
        "TRANSCRIPT": gene_names[i],
        "MOCHIS_3_18": mochis_test_3_18,
        "MW_3_18": wrs_test_3_18.pvalue,
        "MOCHIS_18_24": mochis_test_18_24,
        "MW_18_24": wrs_test_18_24.pvalue,
        "MOCHIS_24_3": mochis_test_24_3,
        "MW_24_3": wrs_test_24_3.pvalue,
        "VAR_3_18": var_3_18,
        "INV_3_18": invert_3_18,
        "VAR_18_24": var_18_24,
        "INV_18_24": invert_18_24,
        "VAR_24_3": var_24_3,
        "INV_24_3": invert_24_3
    }])])

print("Saving results for ", tissue)
results_df.to_csv("tissues/"+tissue+"/mochis_p_val_table.csv")
```

```
Sample sizes, n and k, large enough such that k/n > 0; p = 1 or p = 2. Ap
plying Gaussian asymptotics...
Normalizing weight vector...
The test statistic for the data is  0.3035284096840164
Sample sizes, n and k, large enough such that k/n > 0; p = 1 or p = 2. Ap
plying Gaussian asymptotics...
Normalizing weight vector...
The test statistic for the data is  0.31246434402568957
Sample sizes, n and k, large enough such that k/n > 0; p = 1 or p = 2. Ap
plying Gaussian asymptotics...
Normalizing weight vector...
The test statistic for the data is  0.2832503999497358
Sample sizes, n and k, large enough such that k/n > 0; p = 1 or p = 2. Ap
plying Gaussian asymptotics...
Normalizing weight vector...
The test statistic for the data is  0.27425320312879486
```

```
Sample sizes, n and k, large enough such that k/n > 0; p = 1 or p = 2. Ap
plying Gaussian asymptotics...
Normalizing weight vector...
```

Step 2. Identify MOCHIS significant genes (with FDR control at 0.05)

In [15]:
```
tissue_transcript_3_18 = pd.DataFrame(columns=['TRANSCRIPT', 'MOCHIS', 'VARI
tissue_transcript_18_24 = pd.DataFrame(columns=['TRANSCRIPT', 'MOCHIS', 'VAR
tissue_transcript_24_3 = pd.DataFrame(columns=['TRANSCRIPT', 'MOCHIS', 'VARI


for tissue in all_tissues:
    print("Reading in summary of p-values and ratios of variances for ", tis
    tissue_mochis_df = pd.read_csv("tissues/"+tissue+"/mochis_p_val_table.cs

    # Pick genes where one of the three pairs (3m, 18m, 24m) has significant
    selected_genes_3_18 = tissue_mochis_df[p_adjust_bh(tissue_mochis_df['MOC
    selected_genes_3_18 = selected_genes_3_18[["TRANSCRIPT", "MOCHIS_3_18",
    selected_genes_3_18= selected_genes_3_18.rename(columns={"MOCHIS_3_18":'
    selected_genes_3_18["TISSUE"] = [tissue for i in range(selected_genes_3_
    tissue_transcript_3_18 = pd.concat([tissue_transcript_3_18, selected_gen

    selected_genes_18_24 = tissue_mochis_df[p_adjust_bh(tissue_mochis_df['MO
    selected_genes_18_24 = selected_genes_18_24[["TRANSCRIPT", "MOCHIS_18_24
    selected_genes_18_24 = selected_genes_18_24.rename(columns={"MOCHIS_18_2
    selected_genes_18_24["TISSUE"] = [tissue for i in range(selected_genes_1
    tissue_transcript_18_24 = pd.concat([tissue_transcript_18_24, selected_g

    selected_genes_24_3 = tissue_mochis_df[p_adjust_bh(tissue_mochis_df['MOC
    selected_genes_24_3 = selected_genes_24_3[["TRANSCRIPT", "MOCHIS_24_3",
    selected_genes_24_3 = selected_genes_24_3.rename(columns={"MOCHIS_24_3":
    selected_genes_24_3["TISSUE"] = [tissue for i in range(selected_genes_24
    tissue_transcript_24_3 = pd.concat([tissue_transcript_24_3, selected_gen

tissue_transcript_3_18.to_csv("tissues/mochis_sig_3m_18m.csv")
tissue_transcript_18_24.to_csv("tissues/mochis_sig_18m_24m.csv")
tissue_transcript_24_3.to_csv("tissues/mochis_sig_24m_3m.csv")
```

```
Reading in summary of p-values and ratios of variances for  aorta
Reading in summary of p-values and ratios of variances for  bladder-lumen
Reading in summary of p-values and ratios of variances for  bone-marrow
Reading in summary of p-values and ratios of variances for  brain-myeloid
Reading in summary of p-values and ratios of variances for  brown-adipose
-tissue
Reading in summary of p-values and ratios of variances for  diaphragm
Reading in summary of p-values and ratios of variances for  gonadal-fat-p
ad
Reading in summary of p-values and ratios of variances for  heart
Reading in summary of p-values and ratios of variances for  kidney
Reading in summary of p-values and ratios of variances for  large-intesti
ne
Reading in summary of p-values and ratios of variances for  limb-muscle
Reading in summary of p-values and ratios of variances for  liver
Reading in summary of p-values and ratios of variances for  lung
Reading in summary of p-values and ratios of variances for  mammary-gland
Reading in summary of p-values and ratios of variances for  mesenteric-fa
t-pad
Reading in summary of p-values and ratios of variances for  pancreas
Reading in summary of p-values and ratios of variances for  skin-of-body
Reading in summary of p-values and ratios of variances for  spleen
Reading in summary of p-values and ratios of variances for  subcutaneous-
adipose-tissue
```

```
Reading in summary of p-values and ratios of variances for  thymus
Reading in summary of p-values and ratios of variances for  tongue
Reading in summary of p-values and ratios of variances for  trachea
```

In [ ]:

```
```

Step 3. Visualization.

First, let us visualize the raw p-values and variance ratios of the MOCHIS DEGs fished out from the above procedure.

In [16]:

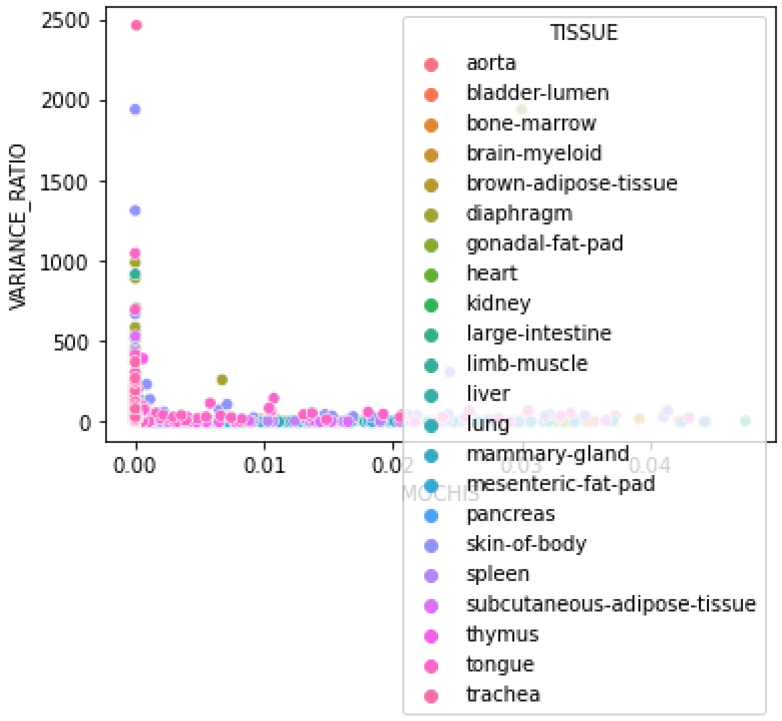
```
df_3_18 = pd.read_csv("tissues/mochis_sig_3m_18m.csv")
df_18_24 = pd.read_csv("tissues/mochis_sig_18m_24m.csv")
df_24_3 = pd.read_csv("tissues/mochis_sig_24m_3m.csv")


df_3_18["PAIR"] = ["3m vs 18m" for i in range(df_3_18.shape[0])]
df_18_24["PAIR"] = ["18m vs 24m" for i in range(df_18_24.shape[0])]
df_24_3["PAIR"] = ["3m vs 24m" for i in range(df_24_3.shape[0])]
```

In [17]:

```
sns.scatterplot(data=df_3_18, x='MOCHIS', y='VARIANCE_RATIO', hue='TISSUE')
```

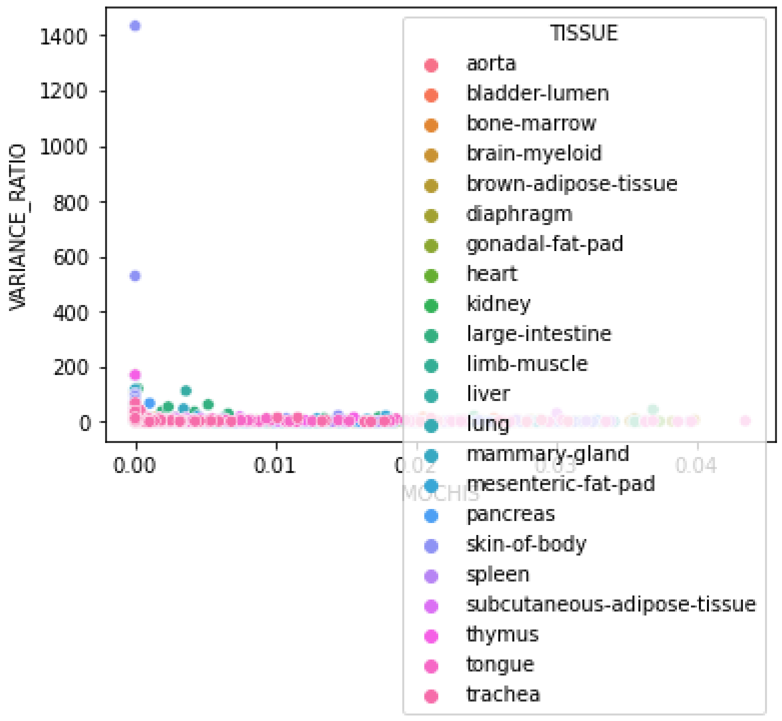
Out[17]:

```
<AxesSubplot:xlabel='MOCHIS', ylabel='VARIANCE_RATIO'>
```



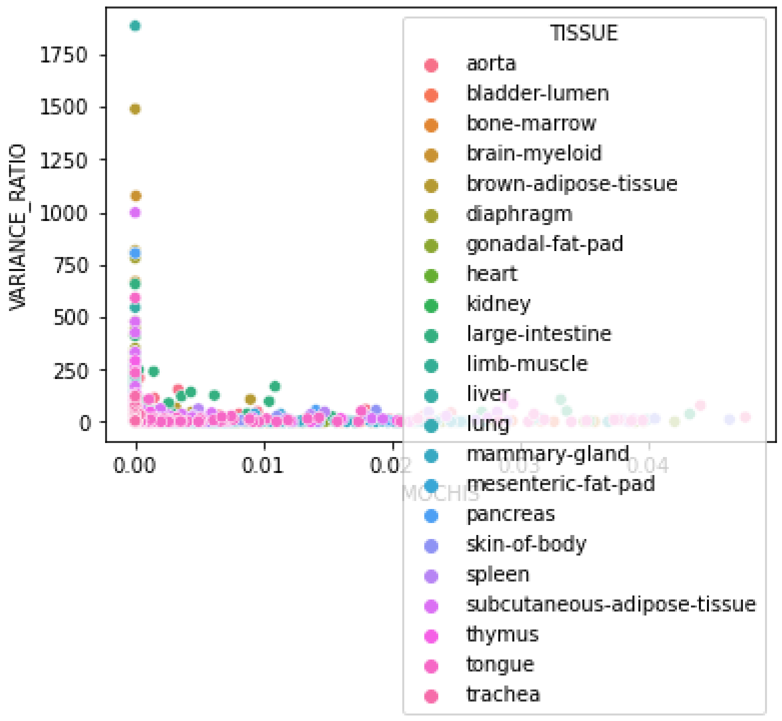
In [18]:
```
sns.scatterplot(data=df_18_24, x='MOCHIS', y='VARIANCE_RATIO', hue='TISSUE'
```

Out[18]:
```
<AxesSubplot:xlabel='MOCHIS', ylabel='VARIANCE_RATIO'>
```



In [19]:
```
sns.scatterplot(data=df_24_3, x='MOCHIS', y='VARIANCE_RATIO', hue='TISSUE')
```

Out[19]:
```
<AxesSubplot:xlabel='MOCHIS', ylabel='VARIANCE_RATIO'>
```



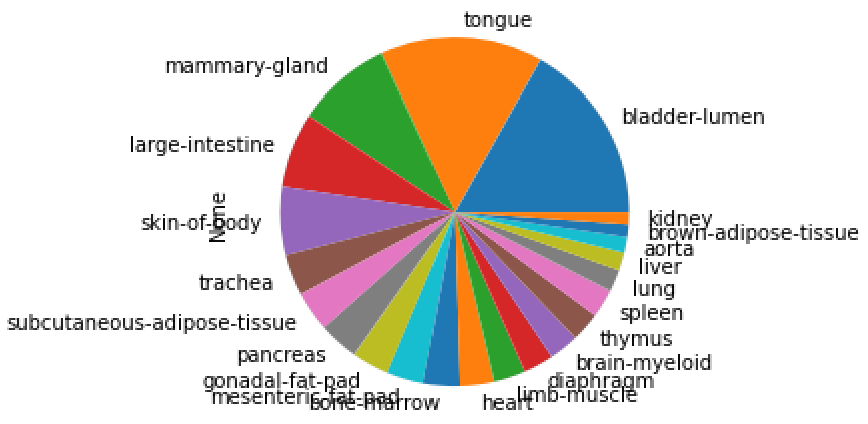
Next we look at the distribution, across tissues, of MOCHIS significant genes.

In [20]:
```
print("No. MOCHIS significant genes for 3m vs 18m: ", df_3_18.shape[0])
print("No. MOCHIS significant genes for 18m vs 24m: ", df_18_24.shape[0])
print("No. MOCHIS significant genes for 24m vs 3m: ", df_24_3.shape[0])
```

```
No. MOCHIS significant genes for 3m vs 18m:  5732
No. MOCHIS significant genes for 18m vs 24m:  4761
No. MOCHIS significant genes for 24m vs 3m:  5479
```

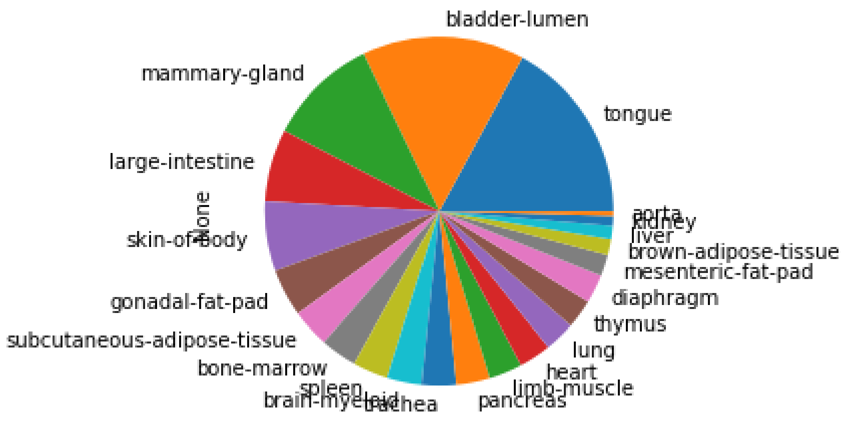
In [21]:
```
df_3_18.value_counts("TISSUE").plot(kind="pie")
```

Out[21]:
```
<AxesSubplot:ylabel='None'>
```



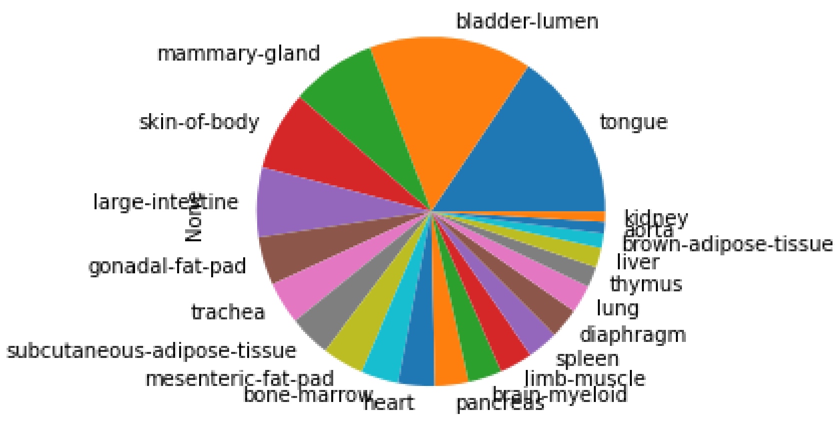
In [22]:
```
df_18_24.value_counts("TISSUE").plot(kind="pie")
```

Out[22]:
```
<AxesSubplot:ylabel='None'>
```



In [23]:
```
df_24_3.value_counts("TISSUE").plot(kind="pie")
```

Out[23]:
```
<AxesSubplot:ylabel='None'>
```



2.3 Impact of Tie Breaking on Mann-Whitney DEGs

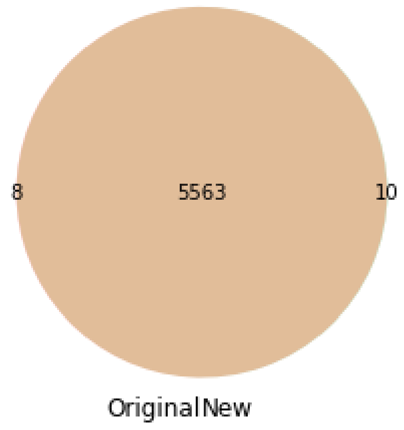
In Section 2.2, we raised the issue of tie breaking potentially affecting the significance of Mann-Whitney DEGs. Here, we check for difference between post-contaminated Mann-Whitney DEGs (this Section) and original DEGs (Section 2.1).

In [24]:
```
tissue_transcript_3_18 = pd.DataFrame(columns=['TRANSCRIPT', 'NEW_MANN_WHIT
tissue_transcript_18_24 = pd.DataFrame(columns=['TRANSCRIPT', 'NEW_MANN_WHI
tissue_transcript_24_3 = pd.DataFrame(columns=['TRANSCRIPT', 'NEW_MANN_WHIT

for tissue in all_tissues:
    print("Reading in summary of p-values and ratios of variances for ", ti

    tissue_mann_whitney_df = pd.read_csv("tissues/"+tissue+"/mochis_p_val_t

    # Pick genes where one of the three pairs (3m, 18m, 24m) has significan
    selected_genes_3_18 = tissue_mann_whitney_df[p_adjust_bh(tissue_mann_wh
    selected_genes_3_18 = selected_genes_3_18[["TRANSCRIPT", "MW_3_18"]]
    selected_genes_3_18= selected_genes_3_18.rename(columns={"MW_3_18":"NEW
    selected_genes_3_18["TISSUE"] = [tissue for i in range(selected_genes_3
    tissue_transcript_3_18 = pd.concat([tissue_transcript_3_18, selected_ge

    selected_genes_18_24 = tissue_mann_whitney_df[p_adjust_bh(tissue_mann_w
    selected_genes_18_24 = selected_genes_18_24[["TRANSCRIPT", "MW_18_24"]]
    selected_genes_18_24= selected_genes_18_24.rename(columns={"MW_18_24":"
    selected_genes_18_24["TISSUE"] = [tissue for i in range(selected_genes_
    tissue_transcript_18_24 = pd.concat([tissue_transcript_18_24, selected_

    selected_genes_24_3 = tissue_mann_whitney_df[p_adjust_bh(tissue_mann_wh
    selected_genes_24_3 = selected_genes_24_3[["TRANSCRIPT", "MW_24_3"]]
    selected_genes_24_3 = selected_genes_24_3.rename(columns={"MW_24_3":"NE
    selected_genes_24_3["TISSUE"] = [tissue for i in range(selected_genes_2
    tissue_transcript_24_3 = pd.concat([tissue_transcript_24_3, selected_ge

# Compare against original MW significant genes
og_transcript_3_18 = pd.read_csv("tissues/mw_sig_3m_18m.csv")
og_transcript_18_24 = pd.read_csv("tissues/mw_sig_18m_24m.csv")
og_transcript_24_3 = pd.read_csv("tissues/mw_sig_24m_3m.csv")
```

```
Reading in summary of p-values and ratios of variances for  aorta
Reading in summary of p-values and ratios of variances for  bladder-lumen
Reading in summary of p-values and ratios of variances for  bone-marrow
Reading in summary of p-values and ratios of variances for  brain-myeloid
Reading in summary of p-values and ratios of variances for  brown-adipose
-tissue
Reading in summary of p-values and ratios of variances for  diaphragm
Reading in summary of p-values and ratios of variances for  gonadal-fat-p
ad
Reading in summary of p-values and ratios of variances for  heart
Reading in summary of p-values and ratios of variances for  kidney
Reading in summary of p-values and ratios of variances for  large-intesti
ne
Reading in summary of p-values and ratios of variances for  limb-muscle
Reading in summary of p-values and ratios of variances for  liver
Reading in summary of p-values and ratios of variances for  lung
Reading in summary of p-values and ratios of variances for  mammary-gland
Reading in summary of p-values and ratios of variances for  mesenteric-fa
t-pad
Reading in summary of p-values and ratios of variances for  pancreas
Reading in summary of p-values and ratios of variances for  skin-of-body
Reading in summary of p-values and ratios of variances for  spleen
Reading in summary of p-values and ratios of variances for  subcutaneous-
adipose-tissue
Reading in summary of p-values and ratios of variances for  thymus
Reading in summary of p-values and ratios of variances for  tongue
Reading in summary of p-values and ratios of variances for  trachea
```

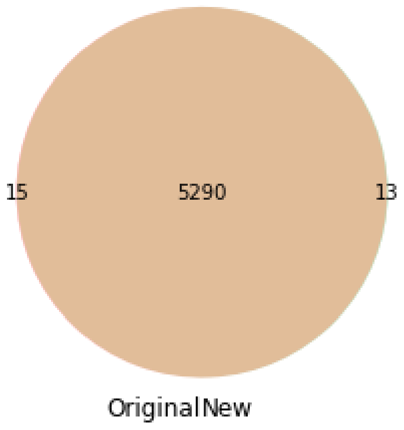
In [26]:
```
set1 = set(og_transcript_3_18['TRANSCRIPT'] + "_" + og_transcript_3_18['TIS
set2 = set(tissue_transcript_3_18['TRANSCRIPT'] + "_" + tissue_transcript_3
venn2([set1, set2], set_labels = ('Original', 'New'))
```

Out[26]:
```
<matplotlib_venn._common.VennDiagram at 0x7f92bbd11c10>
```

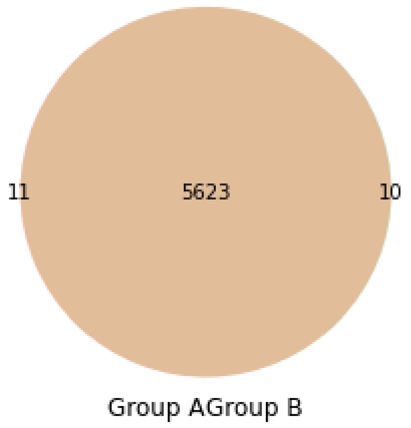


In [27]:
```
# Compare 24m vs 3m
set1 = set(og_transcript_18_24['TRANSCRIPT'] + "_" + og_transcript_18_24['T
set2 = set(tissue_transcript_18_24['TRANSCRIPT'] + "_" + tissue_transcript_
venn2([set1, set2], set_labels = ('Original', 'New'))
```

Out[27]:
```
<matplotlib_venn._common.VennDiagram at 0x7f936360b460>
```



```
In [28]: # Compare 24m vs 3m
         set1 = set(og_transcript_24_3['TRANSCRIPT'] + "_" + og_transcript_24_3['TIS
         set2 = set(tissue_transcript_24_3['TRANSCRIPT'] + "_" + tissue_transcript_2
         venn2([set1, set2], set_labels = ('Group A', 'Group B'))

Out[28]: <matplotlib_venn._common.VennDiagram at 0x7f93633e8af0>
```



We see that there are very few original Mann-Whitney DEGs that are no longer significant after tie breaking, and conversely there are also very few new Mann-Whitney DEGs that were originally non-significant. This suggests that the tie-breaking procedure hardly affected the gene expression distributions between age groups.

3 Analysis

We examine more closely the differences between Mann-Whitney DEGs and MOCHIS DEGs. Recall that Mann-Whitney DEGs are genes that are typically picked up by standard differential analysis routines, whereas MOCHIS DEGs are genes that are differentially expressed owing to shifts in dispersion. Below, we perform some analyses to answer the following questions.

• How many MOCHIS DEGs were previously not detected by Mann-Whitney?

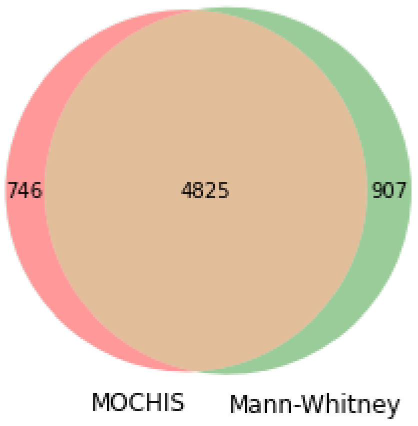
• Does MOCHIS really pick up shifts in dispersion?

• Are there other interesting questions we may answer with our newly detected MOCHIS DEGs?

In [31]:
```
## Compare counts
# Load original DEGs from Section 2.1

og_transcript_3_18 = pd.read_csv("tissues/mw_sig_3m_18m.csv")
og_transcript_18_24 = pd.read_csv("tissues/mw_sig_18m_24m.csv")
og_transcript_24_3 = pd.read_csv("tissues/mw_sig_24m_3m.csv")

# Load MOCHIS DEGs from Section 2.2
tissue_transcript_3_18 = pd.read_csv("tissues/mochis_sig_3m_18m.csv")
tissue_transcript_18_24 = pd.read_csv("tissues/mochis_sig_18m_24m.csv")
tissue_transcript_24_3 = pd.read_csv("tissues/mochis_sig_24m_3m.csv")
```

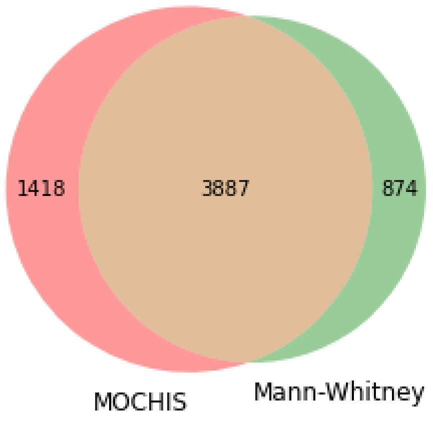
In [32]:
```
# Compare 3m vs 18m
set1 = set(og_transcript_3_18['TRANSCRIPT'] + "_" + og_transcript_3_18['TIS
set2 = set(tissue_transcript_3_18['TRANSCRIPT'] + "_" + tissue_transcript_3
venn2([set1, set2], set_labels = ('MOCHIS', 'Mann-Whitney'))
```

Out[32]:
```
<matplotlib_venn._common.VennDiagram at 0x7f92b96b6d90>
```



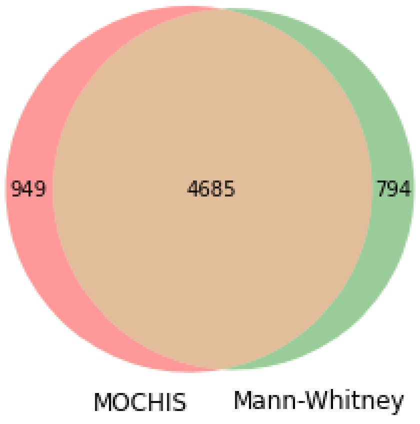
In [33]:
```
# Compare 18m vs 24m
set1 = set(og_transcript_18_24['TRANSCRIPT'] + "_" + og_transcript_18_24['T
set2 = set(tissue_transcript_18_24['TRANSCRIPT'] + "_" + tissue_transcript_
venn2([set1, set2], set_labels = ('MOCHIS', 'Mann-Whitney'))
```

Out[33]:
```
<matplotlib_venn._common.VennDiagram at 0x7f92c5844760>
```



In [34]:
```
# Compare 3m vs 24m
set1 = set(og_transcript_24_3['TRANSCRIPT'] + "_" + og_transcript_24_3['TIS
set2 = set(tissue_transcript_24_3['TRANSCRIPT'] + "_" + tissue_transcript_2
venn2([set1, set2], set_labels = ('MOCHIS', 'Mann-Whitney'))
```

Out[34]:
```
<matplotlib_venn._common.VennDiagram at 0x7f92c583afa0>
```



Summary of Findings

1. In general, there are considerable differences in the genes picked up by Mann-Whitney and MOCHIS. For any pair of age groups, MOCHIS picks up at least 750 DEGs that were not picked up by Mann-Whitney.
2. The number of new genes picked up by MOCHIS is the largest for the pair “3m vs 18m” (= 905), and smallest for the pair “3m vs 21m” (= 794).

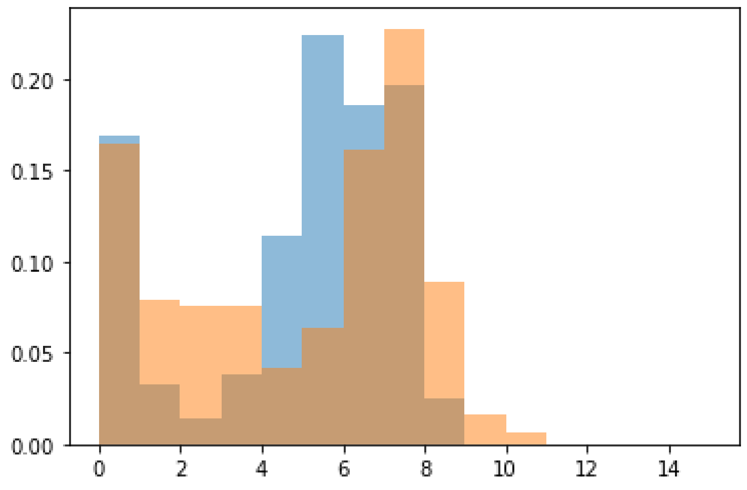
3. The number of Mann-Whitney significant genes that are not MOCHIS significant is greatest for the pair “18m vs 24m” (= 1397) and smallest for the pair “3m vs 18m” (= 753).

3.2 Visualizing Changes in Dispersion

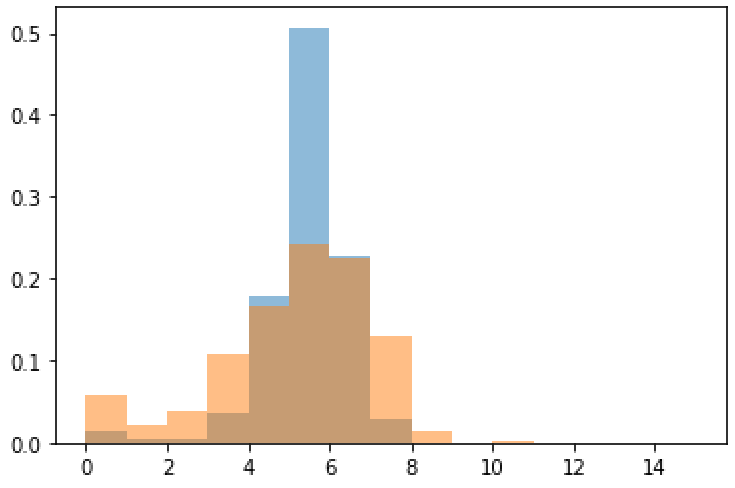
The skeptical reader may wonder if MOCHIS is really picking up a shift in dispersion between the two age groups. Since we realistically cannot compare gene expression distributions between age groups for each MOCHIS significant gene, here we show some gene expression visualizations of MOCHIS significant genes. We focus on MOCHIS DEGs that were not detected by Mann-Whitney. We show visualizations for each pair of age groups (“3m vs 18m”, “18m vs 24m” and “3m vs 24m”).

In [209]:
```
set1 = set(og_transcript_3_18['TRANSCRIPT'] + "_" + og_transcript_3_18['TIS
set2 = set(tissue_transcript_3_18['TRANSCRIPT'] + "_" + tissue_transcript_3
mochis_unique = pd.DataFrame()
for elem in set2:
    if elem not in set1:
        tc = elem.split("_")[0]
        ts = elem.split("_")[1]
        mochis_unique = pd.concat([mochis_unique,
                                   tissue_transcript_3_18.loc[(tissue_trans
        mochis_unique.index = [i for i in range(1, len(mochis_unique)+1)]

# Pick genes by hand (I choose the ones with biggest variance ratio in each
curated_degs_df = pd.DataFrame()
mu_aorta = mochis_unique[mochis_unique['TISSUE']=='aorta']
curated_degs_df = pd.concat([curated_degs_df, mu_aorta[mu_aorta['VARIANCE_R
mu_bladder_lumen = mochis_unique[mochis_unique['TISSUE']=='bladder-lumen']
curated_degs_df = pd.concat([curated_degs_df, mu_bladder_lumen[mu_bladder_l
mu_bone_marrow = mochis_unique[mochis_unique['TISSUE']=='bone-marrow']
curated_degs_df = pd.concat([curated_degs_df, mu_bone_marrow[mu_bone_marrow
mu_brain_myeloid = mochis_unique[mochis_unique['TISSUE']=='brain-myeloid']
curated_degs_df = pd.concat([curated_degs_df,mu_brain_myeloid[mu_brain_myel
mu_heart = mochis_unique[mochis_unique['TISSUE']=='heart']
curated_degs_df = pd.concat([curated_degs_df,mu_heart[mu_heart['VARIANCE_RA
mu_pancreas = mochis_unique[mochis_unique['TISSUE']=='pancreas']
mu_pancreas[mu_pancreas['VARIANCE_RATIO'] == max(mu_pancreas['VARIANCE_RATI
curated_degs_df = pd.concat([curated_degs_df,mu_pancreas[mu_pancreas['VARIA

# Generate plots
for i in range(len(curated_degs_df)):

    tissue = curated_degs_df.iloc[i]['TISSUE']
    transcript = curated_degs_df.iloc[i]['TRANSCRIPT']

    tissue_smartseq2_data = scanpy.read_h5ad('tissues/' + tissue + '.h5ad')
    transcripts = tissue_smartseq2_data.var.n_cells.index
    ages = np.array(tissue_smartseq2_data.obs['age'].values)
    smartseq2_raw_counts = tissue_smartseq2_data.raw.X.toarray()

    this_gene_exp_level = pd.DataFrame({
        'TRANSCRIPT': smartseq2_raw_counts[:, np.where(transcripts==transcr
        'AGE': ages
    })

    if tissue == 'bone-marrow':
        print(len(smartseq2_raw_counts))


    this_gene_exp_level_3m = this_gene_exp_level[this_gene_exp_level['AGE']
    this_gene_exp_level_18m = this_gene_exp_level[this_gene_exp_level['AGE'

    # Visualize


    bins = [i for i in range(16)]

    print(transcript + " in " + tissue)
    plt.hist([math.log(i+1) for i in this_gene_exp_level_3m['TRANSCRIPT'].v
    plt.hist([math.log(i+1) for i in this_gene_exp_level_18m['TRANSCRIPT'].
    plt.show()


    #plt.legend(loc='upper right')
```

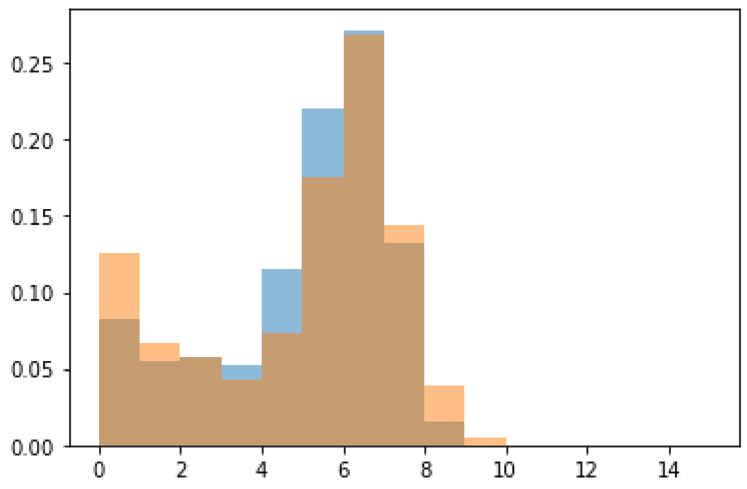
```
ENSMUSG00000032562 in aorta
```



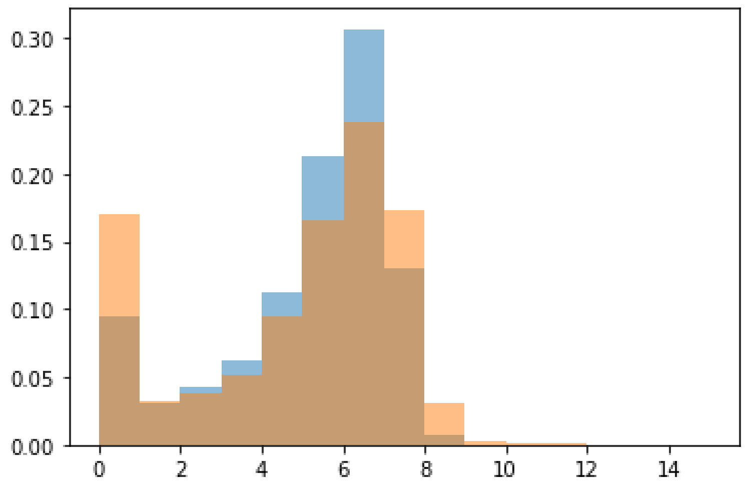
```
ENSMUSG00000020048 in bladder-lumen
```



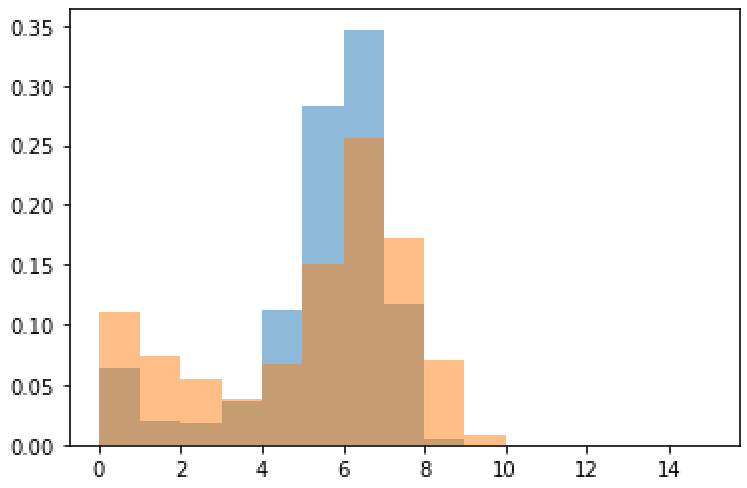
```
14517
ENSMUSG00000036438 in bone-marrow
```



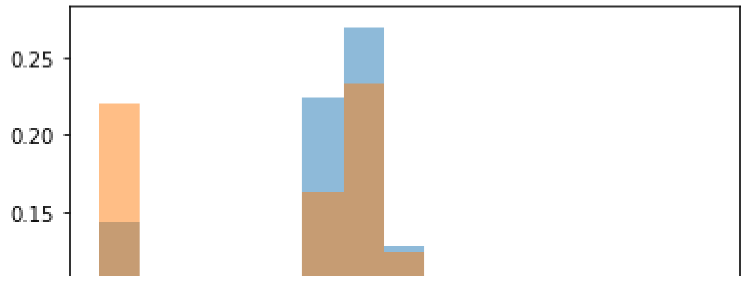
```
ENSMUSG00000029919 in brain-myeloid
```



```
ENSMUSG00000027523 in heart
```

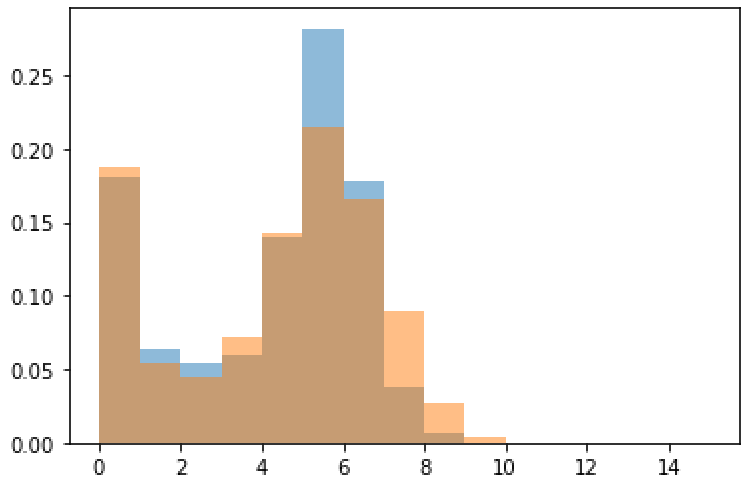


```
ENSMUSG00000027712 in pancreas
```

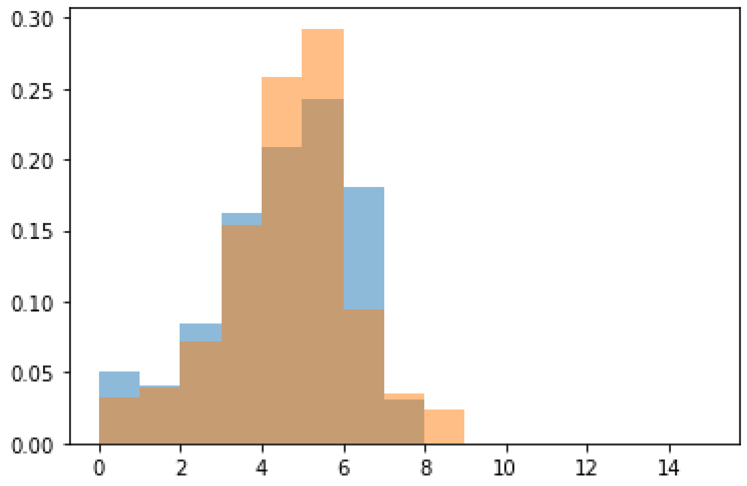

0.25
0.20
0.15

In [211]:
```
set1 = set(og_transcript_18_24['TRANSCRIPT'] + "_" + og_transcript_18_24['T
set2 = set(tissue_transcript_18_24['TRANSCRIPT'] + "_" + tissue_transcript_
mochis_unique = pd.DataFrame()
for elem in set2:
    if elem not in set1:
        tc = elem.split("_")[0]
        ts = elem.split("_")[1]
        mochis_unique = pd.concat([mochis_unique,
                                   tissue_transcript_18_24.loc[(tissue_tran
        mochis_unique.index = [i for i in range(1, len(mochis_unique)+1)]


# Pick genes by hand (I choose the ones with biggest variance ratio in each
curated_degs_df = pd.DataFrame()
mu_aorta = mochis_unique[mochis_unique['TISSUE']=='aorta']
curated_degs_df = pd.concat([curated_degs_df, mu_aorta[mu_aorta['VARIANCE_R
mu_bladder_lumen = mochis_unique[mochis_unique['TISSUE']=='bladder-lumen']
curated_degs_df = pd.concat([curated_degs_df, mu_bladder_lumen[mu_bladder_l
mu_bone_marrow = mochis_unique[mochis_unique['TISSUE']=='bone-marrow']
curated_degs_df = pd.concat([curated_degs_df, mu_bone_marrow[mu_bone_marrow
mu_diaphragm = mochis_unique[mochis_unique['TISSUE']=='diaphragm']
curated_degs_df = pd.concat([curated_degs_df,mu_diaphragm[mu_diaphragm['VAR
mu_large_intestine = mochis_unique[mochis_unique['TISSUE']=='large-intestin
curated_degs_df = pd.concat([curated_degs_df,mu_large_intestine[mu_large_in
mu_limb_muscle = mochis_unique[mochis_unique['TISSUE']=='limb-muscle']
mu_limb_muscle[mu_limb_muscle['VARIANCE_RATIO'] == max(mu_limb_muscle['VARI
curated_degs_df = pd.concat([curated_degs_df,mu_limb_muscle[mu_limb_muscle[


# Generate plots
for i in range(len(curated_degs_df)):
    tissue = curated_degs_df.iloc[i]['TISSUE']
    transcript = curated_degs_df.iloc[i]['TRANSCRIPT']

    tissue_smartseq2_data = scanpy.read_h5ad('tissues/' + tissue + '.h5ad')
    transcripts = tissue_smartseq2_data.var.n_cells.index
    ages = np.array(tissue_smartseq2_data.obs['age'].values)
    smartseq2_raw_counts = tissue_smartseq2_data.raw.X.toarray()

    this_gene_exp_level = pd.DataFrame({
        'TRANSCRIPT': smartseq2_raw_counts[:, np.where(transcripts==transcr
        'AGE': ages
    })


    this_gene_exp_level_18m = this_gene_exp_level[this_gene_exp_level['AGE'
    this_gene_exp_level_24m = this_gene_exp_level[this_gene_exp_level['AGE'

    # Visualize


    bins = [i for i in range(16)]


    print(transcript + " in " + tissue)
    plt.hist([math.log(i+1) for i in this_gene_exp_level_18m['TRANSCRIPT'].
    plt.hist([math.log(i+1) for i in this_gene_exp_level_24m['TRANSCRIPT'].
    plt.show()


    #plt.legend(loc='upper right')
```

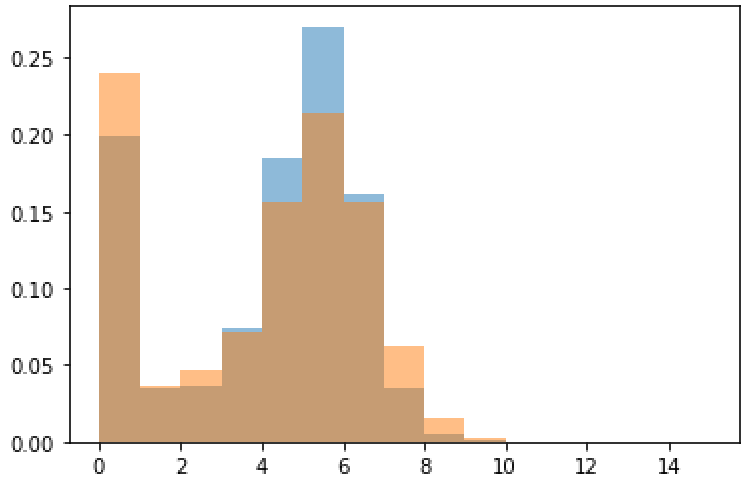
```
ENSMUSG00000058546 in aorta
```



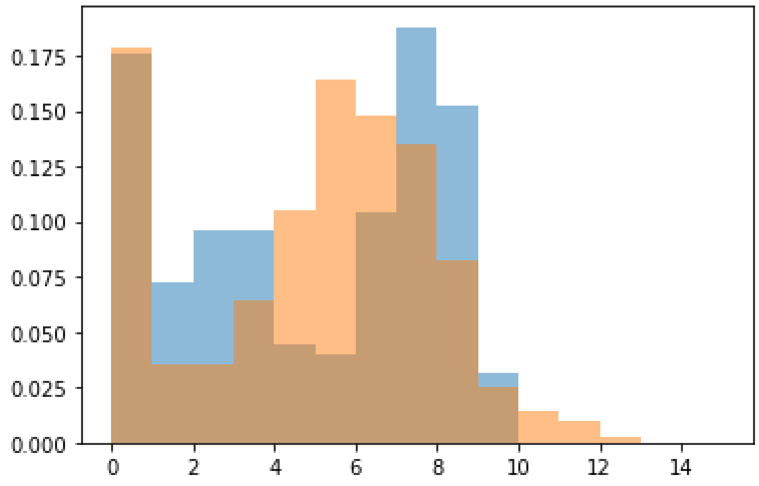
```
ENSMUSG00000018476 in bladder-lumen
```



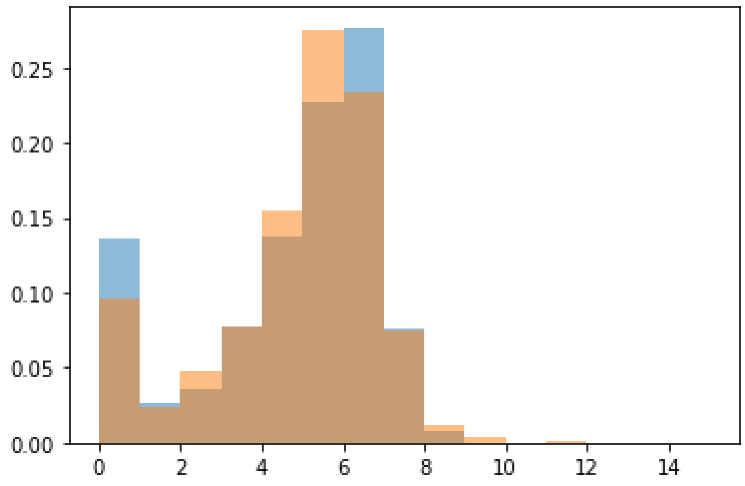
```
ENSMUSG00000022205 in bone-marrow
```



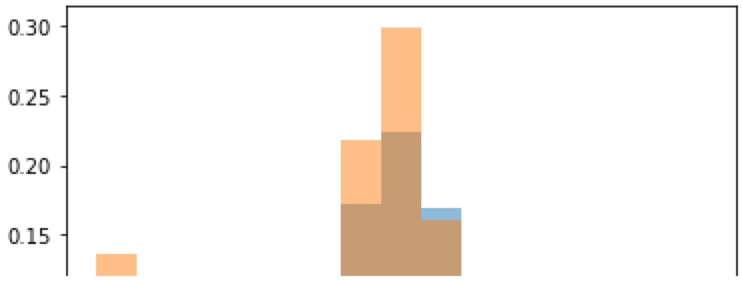
```
ENSMUSG00000071076 in diaphragm
```



```
ENSMUSG00000090862 in large-intestine
```

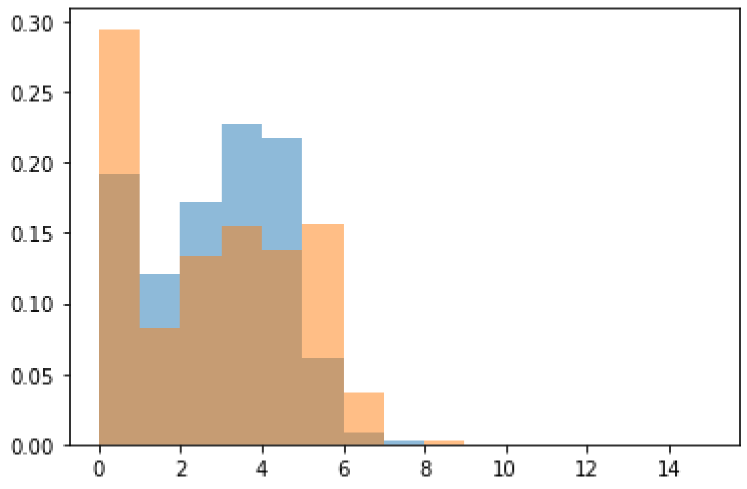


```
ENSMUSG00000025492 in limb-muscle
```

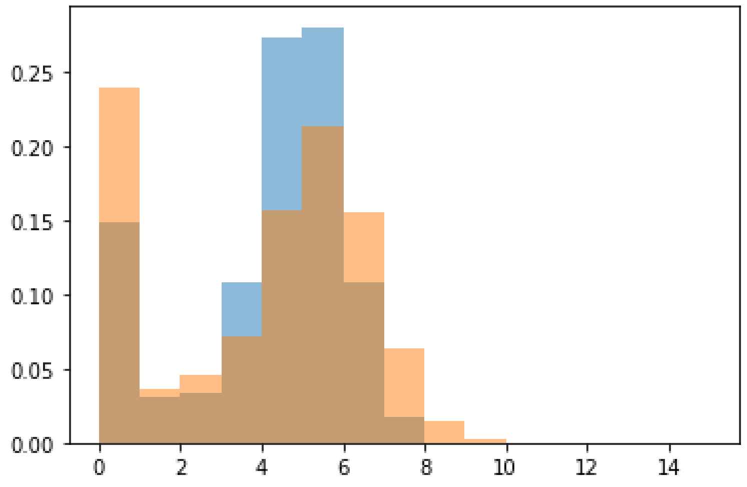

0.30
0.25
0.20
0.15

In [212]:
```
set1 = set(og_transcript_24_3['TRANSCRIPT'] + "_" + og_transcript_24_3['TIS
set2 = set(tissue_transcript_24_3['TRANSCRIPT'] + "_" + tissue_transcript_2
mochis_unique = pd.DataFrame()
for elem in set2:
    if elem not in set1:
        tc = elem.split("_")[0]
        ts = elem.split("_")[1]
        mochis_unique = pd.concat([mochis_unique,
                                   tissue_transcript_24_3.loc[(tissue_trans
        mochis_unique.index = [i for i in range(1, len(mochis_unique)+1)]


# Pick genes by hand (I choose the ones with biggest variance ratio in each
curated_degs_df = pd.DataFrame()

mu_bladder_lumen = mochis_unique[mochis_unique['TISSUE']=='bladder-lumen']
curated_degs_df = pd.concat([curated_degs_df, mu_bladder_lumen[mu_bladder_l

mu_bone_marrow = mochis_unique[mochis_unique['TISSUE']=='bone-marrow']
curated_degs_df = pd.concat([curated_degs_df, mu_bone_marrow[mu_bone_marrow

mu_brain_myeloid = mochis_unique[mochis_unique['TISSUE']=='brain-myeloid']
curated_degs_df = pd.concat([curated_degs_df, mu_brain_myeloid[mu_brain_mye

mu_brown_adipose_tissue = mochis_unique[mochis_unique['TISSUE']=='brown-adi
curated_degs_df = pd.concat([curated_degs_df,mu_brown_adipose_tissue[mu_bro

mu_spleen = mochis_unique[mochis_unique['TISSUE']=='spleen']
curated_degs_df = pd.concat([curated_degs_df,mu_spleen[mu_spleen['VARIANCE_
mu_thymus = mochis_unique[mochis_unique['TISSUE']=='thymus']
curated_degs_df = pd.concat([curated_degs_df,mu_thymus[mu_thymus['VARIANCE_


# Generate plots
for i in range(len(curated_degs_df)):
    tissue = curated_degs_df.iloc[i]['TISSUE']
    transcript = curated_degs_df.iloc[i]['TRANSCRIPT']

    tissue_smartseq2_data = scanpy.read_h5ad('tissues/' + tissue + '.h5ad')
    transcripts = tissue_smartseq2_data.var.n_cells.index
    ages = np.array(tissue_smartseq2_data.obs['age'].values)
    smartseq2_raw_counts = tissue_smartseq2_data.raw.X.toarray()

    this_gene_exp_level = pd.DataFrame({
        'TRANSCRIPT': smartseq2_raw_counts[:, np.where(transcripts==transcr
        'AGE': ages
    })


    this_gene_exp_level_3m = this_gene_exp_level[this_gene_exp_level['AGE']
    this_gene_exp_level_24m = this_gene_exp_level[this_gene_exp_level['AGE'

    # Visualize


    bins = [i for i in range(16)]
    print(transcript + " in " + tissue)
    plt.hist([math.log(i+1) for i in this_gene_exp_level_3m['TRANSCRIPT'].v
    plt.hist([math.log(i+1) for i in this_gene_exp_level_24m['TRANSCRIPT'].
    plt.show()


    #plt.legend(loc='upper right')
```

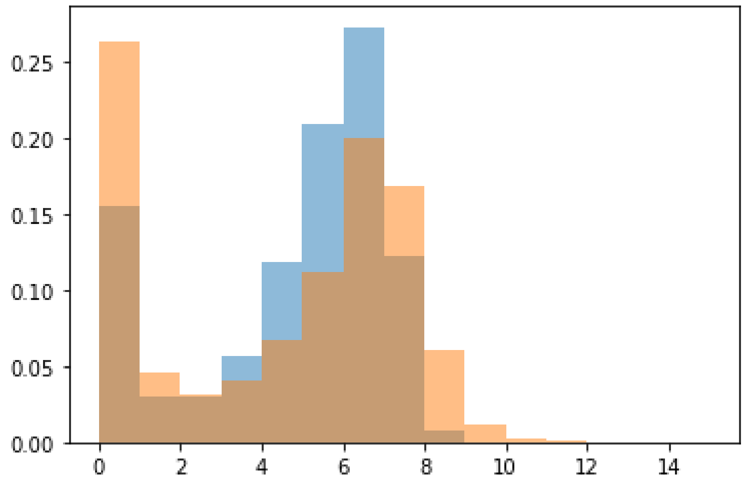
```
ENSMUSG00000020745 in bladder-lumen
```



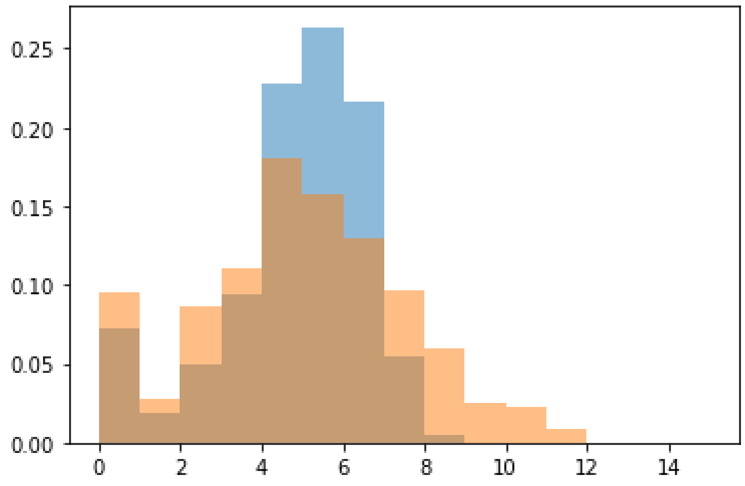
```
ENSMUSG00000022205 in bone-marrow
```



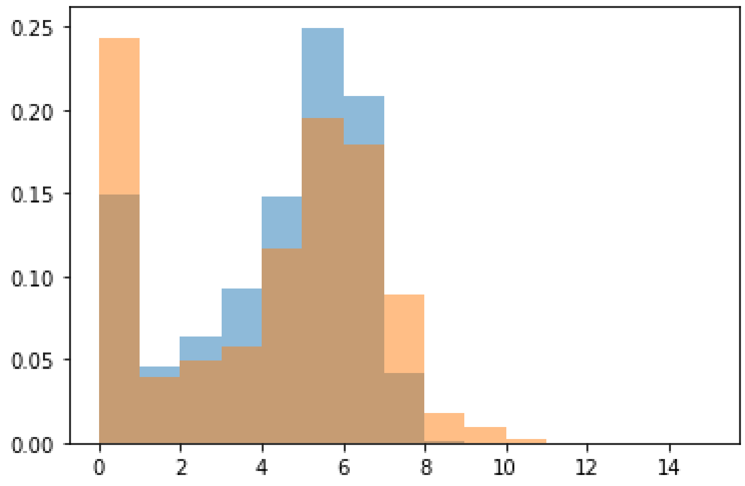
```
ENSMUSG00000000326 in brain-myeloid
```



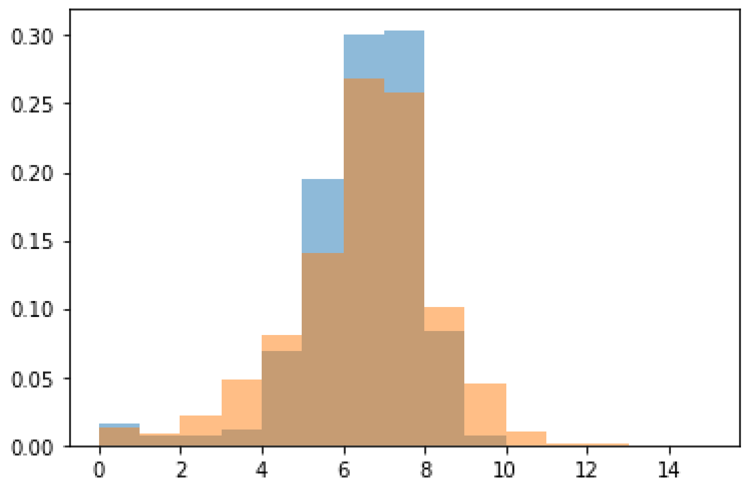
ENSMUSG00000056201 in brown-adipose-tissue



ENSMUSG00000030067 in spleen



ENSMUSG00000050708 in thymus



Summary of Findings

We find that

1. MOCHIS detects shifts in dispersions. These shifts can be in either direction (positive or negative).
2. Some of the shifts can be attributed to more pronounced zero inflation in one age group than another (based on post-analysis visualizations). This raises an important caveat in our analysis, namely, that our first step of filtering out genes that have more than 20% zero-inflation rate effectively removes all contribution by technical noise to the data. If we are skeptical, then we must find other ways to effectively remove contribution by technical noise.

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In [ ]:
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In [ ]:
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In [ ]:
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In [ ]:
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In [94]:
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In [95]:
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In [96]:
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In [97]:
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In [98]:

In [100]:

In [ ]:

In [ ]:

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