RNAge Progress Report

https://github.com/edgeslab/cs418-project-RNAge/blob/master/Progress_Report.ipynb (https://github.com/edgeslab/cs418-project-RNAge/blob/master/Progress_Report.ipynb)

In [10]:
```
#Import necessary packages
import pandas as pd
import numpy as np
import seaborn as sns
import matplotlib.pyplot as plt
from IPython.display import display, HTML,Image
from pathlib import Path
import sklearn
import sklearn.model_selection
import sklearn.feature_selection
from os import listdir
%matplotlib inline
```

In [11]:
```
#Setting up the environment and importing the necessary data

sns.set_style("darkgrid")
data_dir=Path("data")
tissue_dir=Path("tissue-specific")

#!mkdir data && cp merged_meta.tsv data #Needed after cloning repo
manifest={
    "data":"All_Tissue_Site_Details.combined.reads.gct",
    "sample_meta":"GTEx_v7_Annotations_SampleAttributesDS.txt",
    "subject_meta":"GTEx_v7_Annotations_SubjectPhenotypesDS.txt",
    "merged_meta":"merged_meta.tsv"}

meta=pd.read_csv(manifest['merged_meta'],sep="\t",dtype={'SMUBRID':object,'SE
X':object,'DTHHRDY':object})
```

Introduction

The aim of the project is to:

1. Characterize and compare the relationship of different tissues with aging.
2. Find significant genes participating in aging across different tissues.

Additional goal is to:

- Find the differences in aging in healthy and diseased tissues.

Data acquisition

Data source:
The data has been obtained from GTEx portal.

Data source: https://gtexportal.org/home/datasets (https://gtexportal.org/home/datasets)
Data summary: https://gtexportal.org/home/tissueSummaryPage (https://gtexportal.org/home/tissueSummaryPage)

This data can be downloaded and placed under 'data' directory.

Data Pre-processing:
Due to the huge size of the original file, it is split into tissue specific data files for easier processing on local machines.

Changes

The objective presented at the initial checkin has been retained. Albeit, the results of the mid-phase report could influence the feasibility of achieving the final goals.

Data Cleaning

Our *Data Cleaning* has four general steps.

1. Remove samples without age.
2. Choose tissues with counts more than 200.
3. Filter samples with low varuance and low expression level.
4. Remove samples with low row counts.

Our data has some missing *age* information. When we upload CSV files for each tissue, we eliminate all samples with missed age. The following piece of code removes samples without age.

```
In [14]: meta=meta[~(meta['AGE'].isnull())] # removes all samples without age
```

We choose the tissues which are relatively large enough. We assume that if the count number of a tissue is more that 200, we can include that in our analysis, otherwise, we eliminate the tissue.

In [15]:
```
counts=pd.DataFrame(meta['SMTS'].value_counts())
display(counts)
```

	SMTS
Skin	1202
Esophagus	1021
Blood Vessel	913
Adipose Tissue	797
Heart	600
Muscle	564
Blood	537
Colon	507
Thyroid	446
Lung	427
Nerve	414
Brain	331
Breast	290
Stomach	261
Testis	259
Pancreas	248
Adrenal Gland	190
Pituitary	183
Liver	175
Spleen	162
Prostate	152
Small Intestine	137
Ovary	133
Vagina	115
Uterus	111
Salivary Gland	97
Kidney	45
Cervix Uteri	11
Bladder	11
Fallopian Tube	7

This table shows that there are many tissues with >200 samples with age recorded. Only tissues with 200 samples or more will be considered for predictive analysis.

In [5]:
```
df=meta[meta['SMTS'].isin(counts[counts['SMTS']>200].index)]
df=pd.crosstab(index=df['SMTS'],columns=df['AGE'])
display(df)
```

AGE	20-29	30-39	40-49	50-59	60-69	70-79
SMTS						
Adipose Tissue	57	66	131	273	245	25
Blood	50	46	103	169	163	6
Blood Vessel	75	75	160	310	273	20
Brain	12	8	36	112	152	11
Breast	26	32	53	88	81	10
Colon	45	48	95	164	140	15
Esophagus	102	91	191	356	258	23
Heart	33	29	95	220	205	18
Lung	27	30	76	145	139	10
Muscle	46	45	88	188	179	18
Nerve	33	33	70	130	133	15
Pancreas	20	21	53	95	57	2
Skin	98	94	200	398	377	35
Stomach	33	27	52	93	55	1
Testis	25	25	38	90	75	6
Thyroid	30	29	81	151	143	12

Now, all our data includes age of the sample and tissue with more than 200 counts.

In [6]:
```
TISSUE='Colon'
infiles=listdir(data_dir/tissue_dir)
TISSUE_files=[f for f in infiles if  TISSUE in f]
TISSUE_files

cpm=pd.read_csv(data_dir/tissue_dir/str(TISSUE+"_cpm.tsv"),sep="\t",index_col=0)
lcpm=pd.read_csv(data_dir/tissue_dir/str(TISSUE+"_lcpm.tsv"),sep="\t",index_col=0)
cdat=pd.read_csv(data_dir/tissue_dir/str(TISSUE+"_c.tsv"),sep="\t",index_col=0)
```

In [7]:
```
lib_size=np.sum(cdat,axis=1)
#plt.figure(figsize=(16, 6))
#sns.distplot(lib_size,kde=False,rug=True)
#cpm.iloc[0:5,0:6]
```

We now filter data by expression level with the following function

In [8]:
```
# Likely not possible in Python as cpm() is required
def filter_by_expr(counts,min_count=None,min_sample=None,grp=None):
    lib_size=np.sum(counts,axis=1)
    MedianLibSize=np.median(lib_size)
    norm_cutoff=min_count/MedianLibSize*1e6
    print(norm_cutoff)
    gene_counts=np.sum(counts)
```

At the next step, we filter by row count threshold

In [9]:
```
tissue_meta=meta[(meta['SMTS']==TISSUE)]
tissue_meta.iloc[0:3]

cdat_train, cdat_test, y_train, y_test = \
        sklearn.model_selection.train_test_split(cdat, tissue_meta['AGE'], tes
t_size=.3, random_state=1234)
cpm_train, cpm_test, y_train, y_test = \
        sklearn.model_selection.train_test_split(cpm, tissue_meta['AGE'], test
_size=.3, random_state=1234) # random state guarantees that the same split is
 made for a given tissue.
print(cpm_train.shape)
print(cpm_test.shape)
print(y_train.shape)
print(y_test.shape)
#GTEX-P78B-1326-SM-3P611 for reference for liver
print(cdat_train.index[0])
print(cpm_train.index[0]) # Confirms that two different calls to train_test_sp
lit on a different data set produce the same results
sum(cpm_train.iloc[:,0]) # Confirms that the split is the same each time
# Biased against samples with a smaller library size
def simpleExpressionFilter(counts,min_count):
    """accepts raw counts and a minimum sum count per gene across all samples
    return a boolean array of all genes, which can be applied to any transform
ed counts.
    True is associated with passing the test.
    """
    keep=np.sum(counts)>min_count
    print("Pre",counts.shape[1])
    filtered_counts=counts.loc[:,(keep)] # similar to how the boolean array wo
uld be used on any count matrix
    print("Post",filtered_counts.shape[1])
    return(keep)
keep_expr=simpleExpressionFilter(cdat_train,10)
cpm_train_expression_filter=cpm_train.loc[:,(keep_expr)]
cpm_test_expression_filter=cpm_test.loc[:,(keep_expr)]
print(cpm_test_expression_filter.shape) # confirming that both train and test
 set have undergone transformation identically
print(cpm_train_expression_filter.shape)
```

```
(354, 56202)
(153, 56202)
(354,)
(153,)
GTEX-14PHX-1126-SM-5YYA5
GTEX-14PHX-1126-SM-5YYA5
Pre 56202
Post 44995
(153, 44995)
(354, 44995)
```

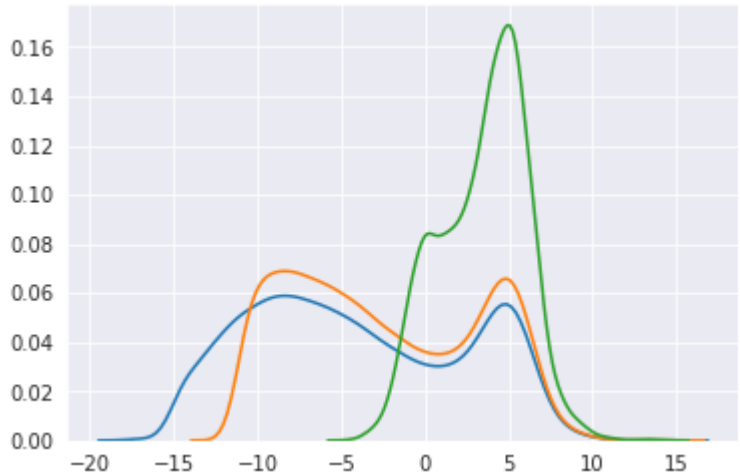
Finally, we can filter by low variance variance and plot mean distribution of genes after each step explained above.

In [10]:
```
selector=sklearn.feature_selection.VarianceThreshold(threshold=.1)
selector.fit(cpm_train_expression_filter)
var_keep=selector.get_support(indices=True)
train_final=cpm_train_expression_filter.iloc[:,var_keep]
test_final=cpm_test_expression_filter.iloc[:,var_keep]
print("Pre",cpm_train_expression_filter.shape[1])
print("Post",train_final.shape[1])

print(train_final.shape) # confirming that both train and test set have underg
one transformation identically
print(test_final.shape)
```

```
Pre 44995
Post 18572
(354, 18572)
(153, 18572)
```

In [11]:
```
ax=sns.kdeplot(np.log2(np.mean(cpm_train,axis=0)))
ax=sns.kdeplot(np.log2(np.mean(cpm_train_expression_filter,axis=0)))
ax=sns.kdeplot(np.log2(np.mean(train_final,axis=0)))
```

```
/home/imlay/programs.installed/anaconda3/envs/cs418env/lib/python3.7/site-pac
kages/ipykernel_launcher.py:1: RuntimeWarning: divide by zero encountered in
log2
  """Entry point for launching an IPython kernel.
/home/imlay/programs.installed/anaconda3/envs/cs418env/lib/python3.7/site-pac
kages/scipy/stats/stats.py:1713: FutureWarning: Using a non-tuple sequence fo
r multidimensional indexing is deprecated; use `arr[tuple(seq)]` instead of `
arr[seq]`. In the future this will be interpreted as an array index, `arr[np.
array(seq)]`, which will result either in an error or a different result.
  return np.add.reduce(sorted[indexer] * weights, axis=axis) / sumval
```



EDA

The objective of our EDA efforts were to explore each useful metadata field. The useful features included:

- Tissue origin (SMTS)
- Tissue sub-origin (SMTSD)
- Sex (SEX)
- Age (AGE)
- Death Classification Hardy Scale (DTHHRDY)

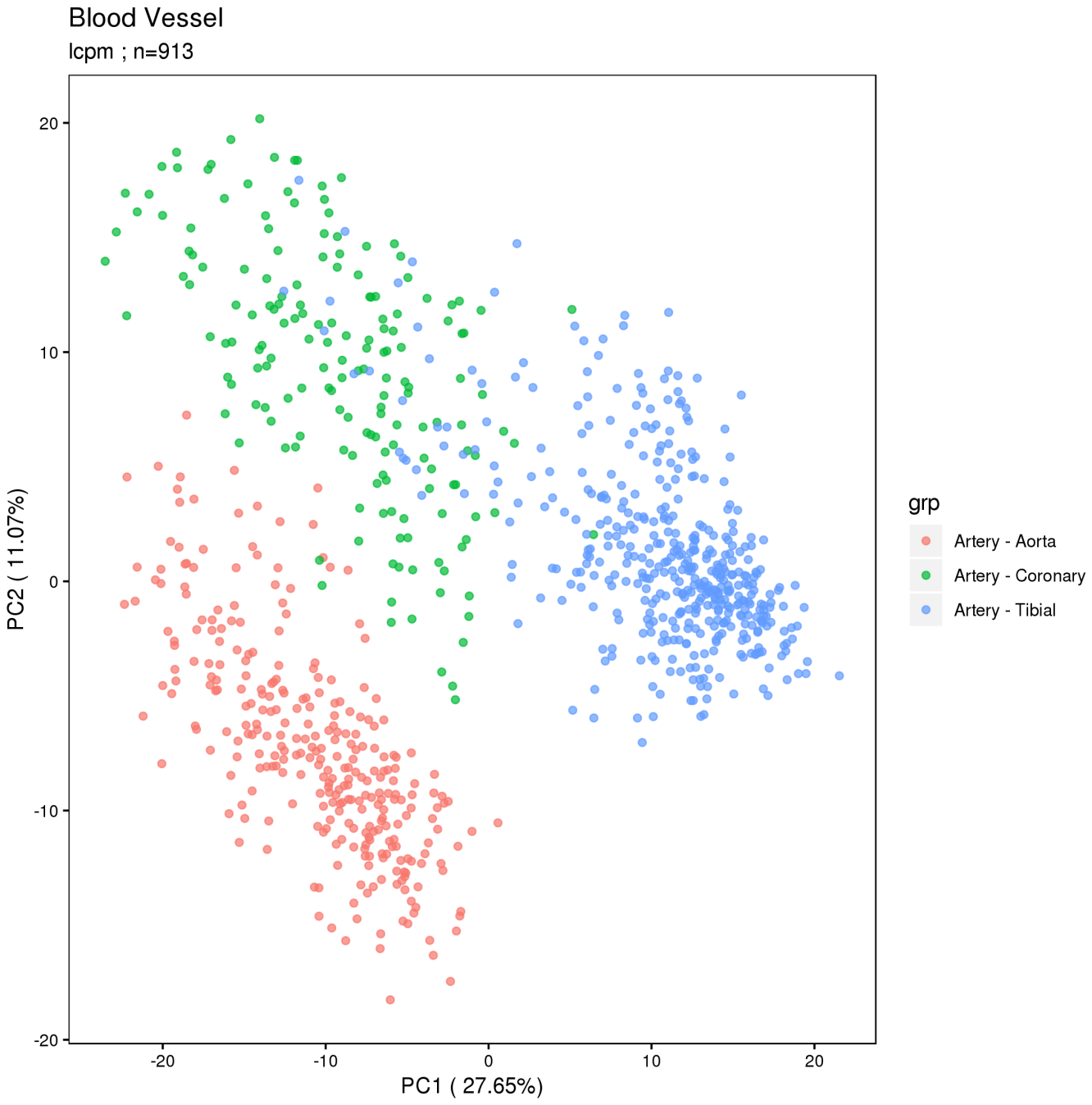
Our first task was to understand how many samples were from each tissue origin. This table shows how many samples are from each tissue. Interestingly, some of the lowest count tissues are sex organs. Some of the most collected tissues seem that they may have been easier to collect.

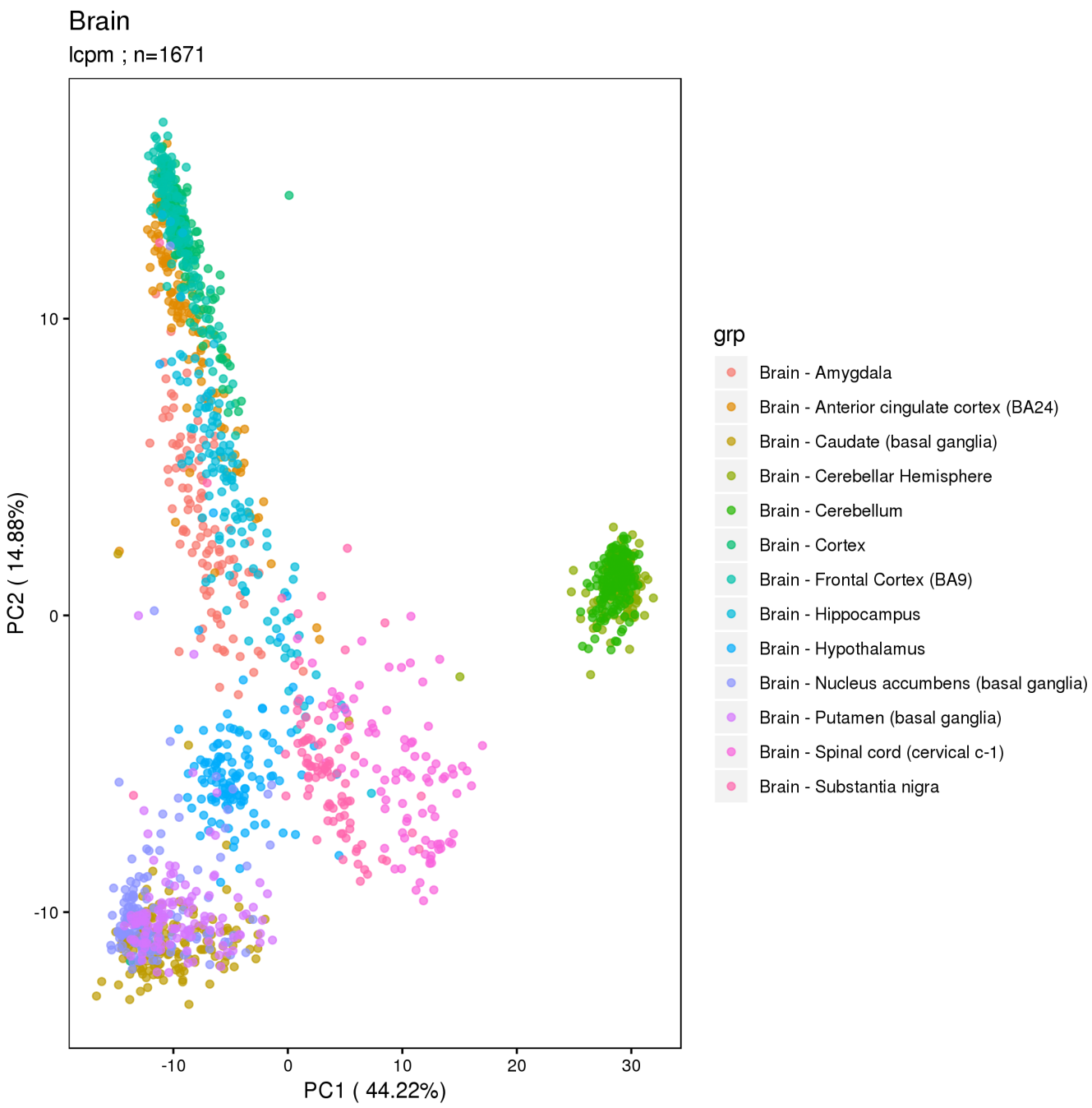
In [18]:
```
meta=pd.read_csv(data_dir/manifest['merged_meta'],sep="\t",dtype={'SMUBRID':ob
ject,'SEX':object,'DTHHRDY':object})
counts=pd.DataFrame(meta['SMTS'].value_counts())
display(counts)
```

	SMTS
Brain	1671
Skin	1203
Esophagus	1021
Blood Vessel	913
Adipose Tissue	797
Heart	600
Muscle	564
Blood	537
Colon	507
Thyroid	446
Lung	427
Nerve	414
Breast	290
Stomach	262
Testis	259
Pancreas	248
Adrenal Gland	190
Pituitary	183
Liver	175
Spleen	162
Prostate	152
Small Intestine	137
Ovary	133
Vagina	115
Uterus	111
Salivary Gland	97
Kidney	45
Cervix Uteri	11
Bladder	11
Fallopian Tube	7

Sub-tissue location

We then sought to explore how SMTSD,sub-tissue location, affects the samples. We generated PCA plots using the top 500 most variable genes following the logCPM transformation. The code for these plots can be found in `GTEx_input.R` . We found that SMTSD seems to account for the largest axis of variance for any tissue where STMSD is recorded. Representative plots from the blood and brain samples are shown.

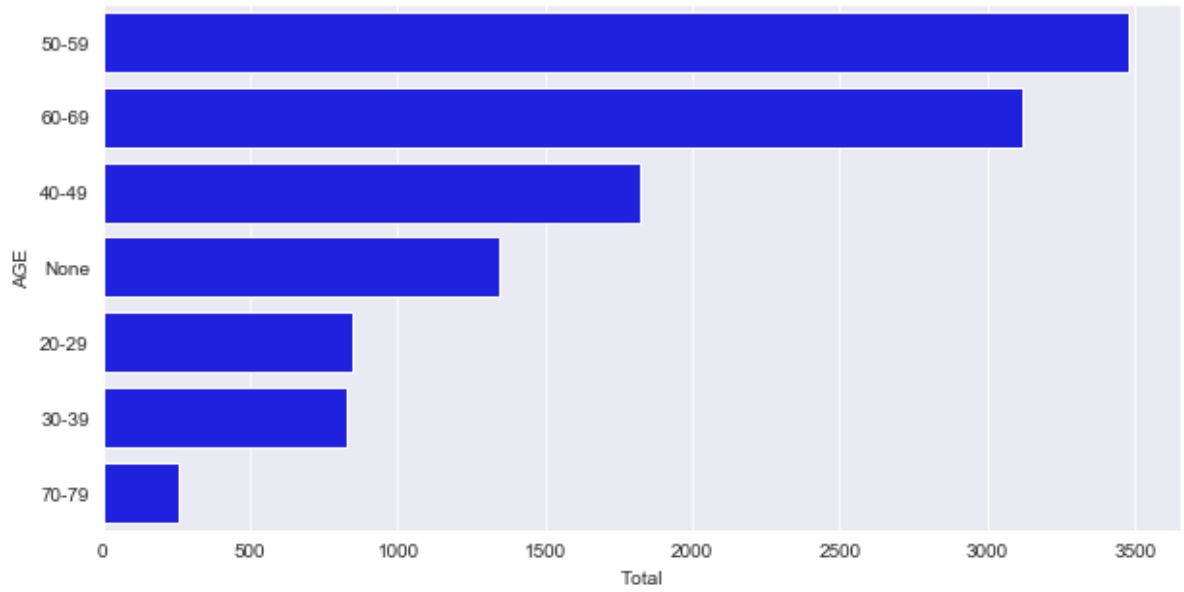




Age

We then explored age, the primary dependent variable in our project. We found that many subjects did not have age recorded, affecting nearly 1500 samples.

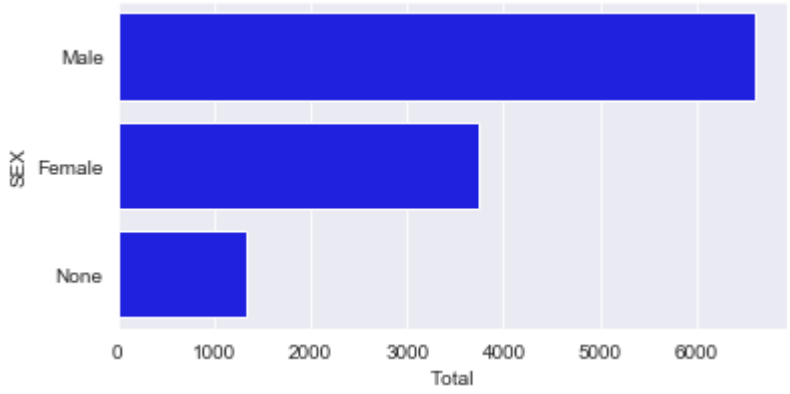
In [5]:
```
sns.set_color_codes("pastel")
AGE=meta['AGE'].copy()
AGE[AGE.isnull()]="None"
counts=pd.DataFrame({"AGE":AGE.value_counts()})
counts['Total']=counts['AGE']
counts['AGE']=counts.index.values
plt.figure(figsize=(10, 5))
ax=sns.barplot(x="Total", y="AGE", data=counts, label="Total", color="blue")
```



Gender

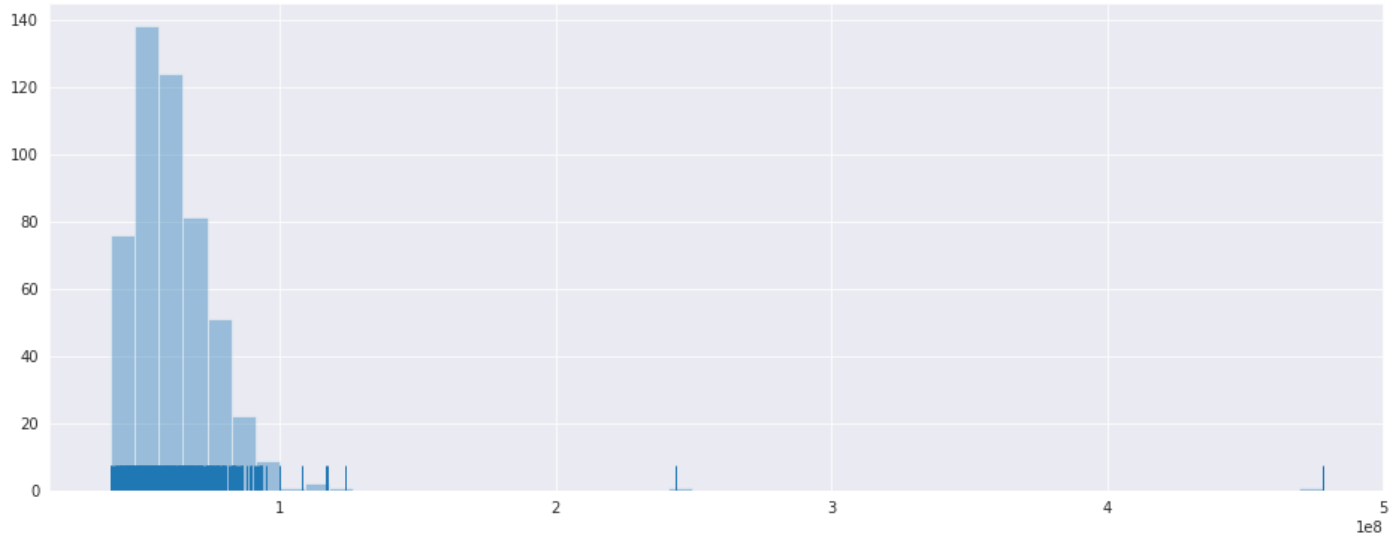
We explored the gender feature (SEX) in the same way. We found that there are many more men than women in the data set. And again, there are nearly 1500 samples with sex recorded.

In [12]:
```
sns.set_color_codes("pastel")
SEX=meta['SEX'].copy()
SEX[SEX.isnull()]="None"
counts=pd.DataFrame({"SEX":SEX.value_counts()})
counts['Total']=counts['SEX']
counts['SEX']=counts.index.values
counts['SEX'].replace({"1.0":"Male","2.0":"Female"},inplace=True)
plt.figure(figsize=(6, 3))
ax=sns.barplot(x="Total", y="SEX", data=counts, label="Total", color="blue")
```



Library size

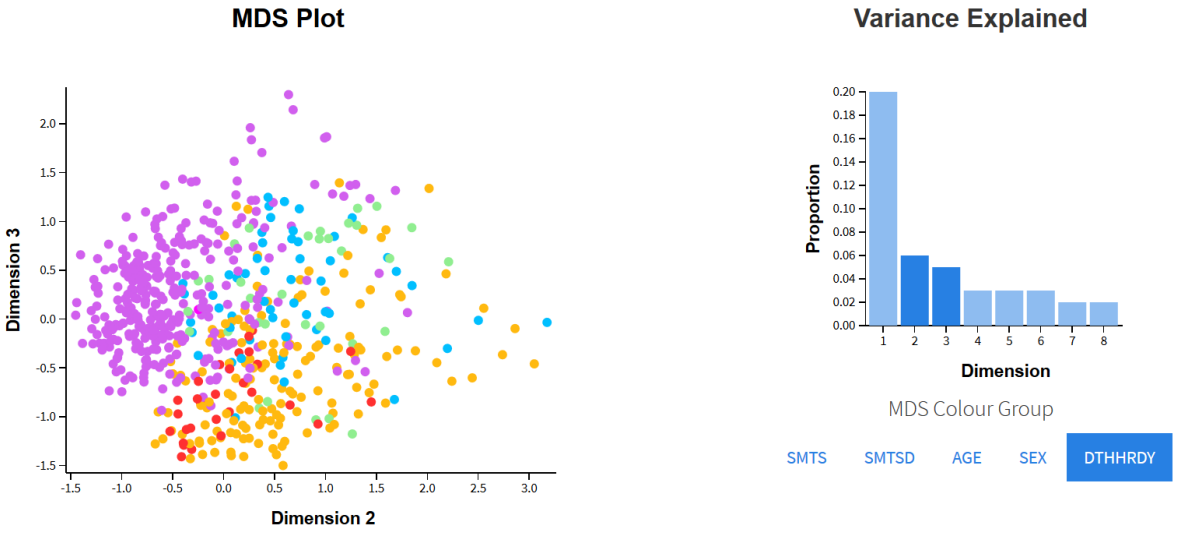
We found that library sizes (the total number of reads sequenced and aligned into counts) were generally around 10 million, which is good, but there are some outliers for each tissue. This plot shows the distribution of library sizes for Colon samples. Code for this plot can be viewed in `perTissue_models.ipynb` .



Death classification

And finally we visualized the effect of the death classification with MDS plots from `Glimma` a specialized R package which utilizes `D3.js` to visualize RNA-seq data. We created MDS plots for every tissue, but the heart is particularly representative of the trends from the metadata features. The plot below can browsed interactively **here (progress_plots/Heart/MDS-Plot.html)**.

A selected view shows that death classification has a major effect in the second and third dimension of MDS. There appears to be major differences between samples that died by vent or quickly by natural causes. This makes intuitive sense because dying while on a vent involves hypoxia. This trend persists for most tissues.



Visualization

We knew that some tissues had very few samples. To begin testing the relationships of the data, we needed to establish a threshold of minimum number of samples per tissue. This table shows the number of people in each age group.

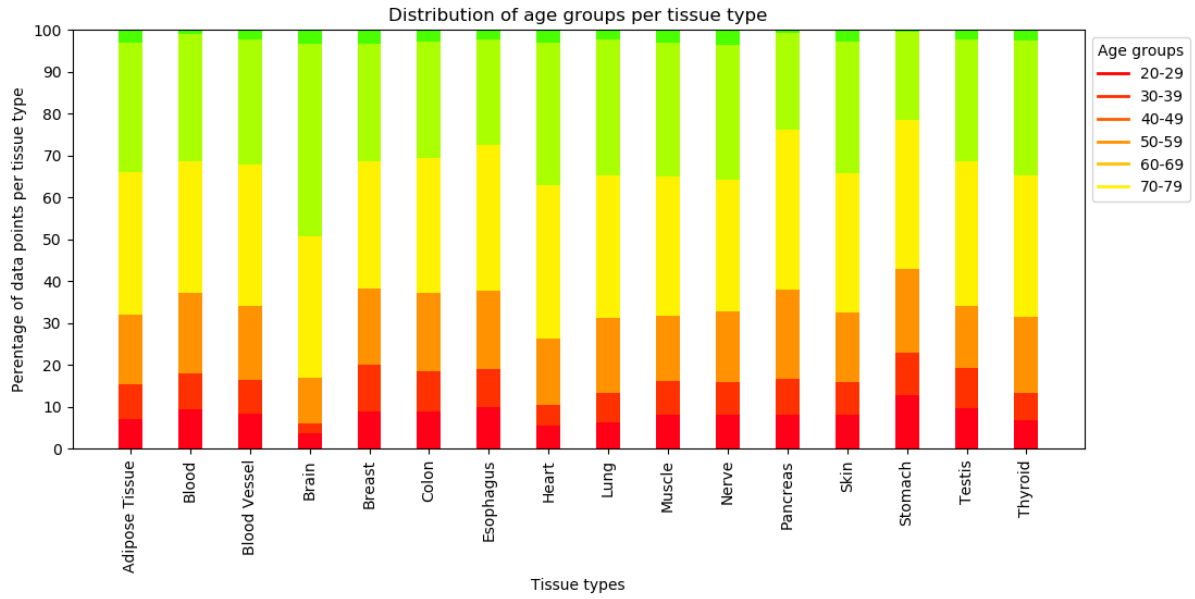
In [16]:
```
counts=pd.DataFrame(meta['SMTS'].value_counts())
df=meta[meta['SMTS'].isin(counts[counts['SMTS']>1].index)]
df=pd.crosstab(index=df['SMTS'],columns=df['AGE'])
sorted_i=df.sum(axis=1).sort_values(ascending=False).index.values
df=df.loc[sorted_i,:]
display(df)
```

AGE SMTS	20-29	30-39	40-49	50-59	60-69	70-79
Skin	98	94	200	398	377	35
Esophagus	102	91	191	356	258	23
Blood Vessel	75	75	160	310	273	20
Adipose Tissue	57	66	131	273	245	25
Heart	33	29	95	220	205	18
Muscle	46	45	88	188	179	18
Blood	50	46	103	169	163	6
Colon	45	48	95	164	140	15
Thyroid	30	29	81	151	143	12
Lung	27	30	76	145	139	10
Nerve	33	33	70	130	133	15
Brain	12	8	36	112	152	11
Breast	26	32	53	88	81	10
Stomach	33	27	52	93	55	1
Testis	25	25	38	90	75	6
Pancreas	20	21	53	95	57	2
Adrenal Gland	17	14	37	67	53	2
Pituitary	7	3	17	59	85	12
Liver	7	10	28	65	62	3
Spleen	11	20	36	60	32	3
Prostate	19	16	27	46	43	1
Small Intestine	17	16	28	45	28	3
Ovary	16	10	29	42	34	2
Vagina	11	8	30	34	28	4
Uterus	16	12	26	33	24	0
Salivary Gland	7	11	20	29	29	1
Kidney	1	4	5	13	21	1
Cervix Uteri	1	1	5	2	2	0
Bladder	2	1	6	2	0	0
Fallopian Tube	1	1	3	2	0	0

In [17]:
```
import matplotlib as mpl
from matplotlib.lines import Line2D

df2=meta[meta['SMTS'].isin(counts[counts['SMTS']>200].index)]
df2_cols = df2.columns.values
df2_cols[0] = 'row_id'
df2.columns = df2_cols
grp_trends = df2[['SMTS', 'AGE', 'row_id']].groupby(['SMTS', 'AGE']).count().r
eset_index()
grp_trends['percentage_per_tissue'] = 1.0
for index, row in grp_trends.iterrows():
    grp_trends.at[index, 'percentage_per_tissue'] = (row['row_id'] / grp_trend
s['row_id'][grp_trends['SMTS']==row['SMTS']].sum()) * 100

unique_age = grp_trends['AGE'].unique()
unique_tissue = grp_trends['SMTS'].unique()

percent_matrix = np.zeros(shape=(len(unique_age), len(unique_tissue)))

i = 0
for entry in unique_age:
    percent_matrix[i] = (grp_trends['percentage_per_tissue'][grp_trends['AGE']
==entry]).as_matrix()
    i += 1

cmap = mpl.cm.get_cmap('hsv', len(unique_tissue)*2)
colorPalette = []
for i in range(cmap.N):
    rgb = cmap(i)[:3] #returns [rgba], hence extracting [rgb]
    colorPalette.append(mpl.colors.rgb2hex(rgb))

mpl.style.use('default')
fig = plt.figure(figsize=(12,5))
ax = fig.add_subplot(111)

configs = percent_matrix[0]
N = len(configs)
ind = np.arange(N)
width = 0.4

i = 0
plt.bar(ind, percent_matrix[i], width, color=colorPalette[i*2-1])
i += 1
while i < len(unique_age):
    j = i-1
    res = np.zeros(shape=(1, len(unique_tissue)))
    while j >= 0:
        res += percent_matrix[j]
        j -= 1
    plt.bar(ind, percent_matrix[i], width, bottom=res[0], color=colorPalette[i
*2-1], tick_label='placeholder')
    i += 1

ax.set_xticklabels(unique_tissue, rotation=90)
ax.set(title='Distribution of age groups per tissue type')
ax.set(xlabel='Tissue types')
```

```
ax.set(ylabel='Perentage of data points per tissue type')
ax.set(ylim=(0,100))
ax.set(yticks=np.arange(0,110,10))

leg_ele = [
    Line2D([0], [0], lw=2, color=colorPalette[0], label=unique_age[0]),
    Line2D([0], [0], lw=2, color=colorPalette[1], label=unique_age[1]),
    Line2D([0], [0], lw=2, color=colorPalette[2], label=unique_age[2]),
    Line2D([0], [0], lw=2, color=colorPalette[3], label=unique_age[3]),
    Line2D([0], [0], lw=2, color=colorPalette[4], label=unique_age[4]),
    Line2D([0], [0], lw=2, color=colorPalette[5], label=unique_age[5])
    ]
ax.legend(handles = leg_ele, loc='upper left', bbox_to_anchor=(1,1), title='Ag
e groups')

plt.show()
```

```
C:\Users\suji1\Anaconda3\envs\cs418env\lib\site-packages\ipykernel_launcher.p
y:20: FutureWarning: Method .as_matrix will be removed in a future version. U
se .values instead.
```



Observations:

- It can be observed that the age group distribution is not uniform.
- The general trend seems to for the age range '70-79' to have very less or no data points at all.
- This has to be accounted for in the ML/DL models built: oversampling/undersampling may be performed if required.

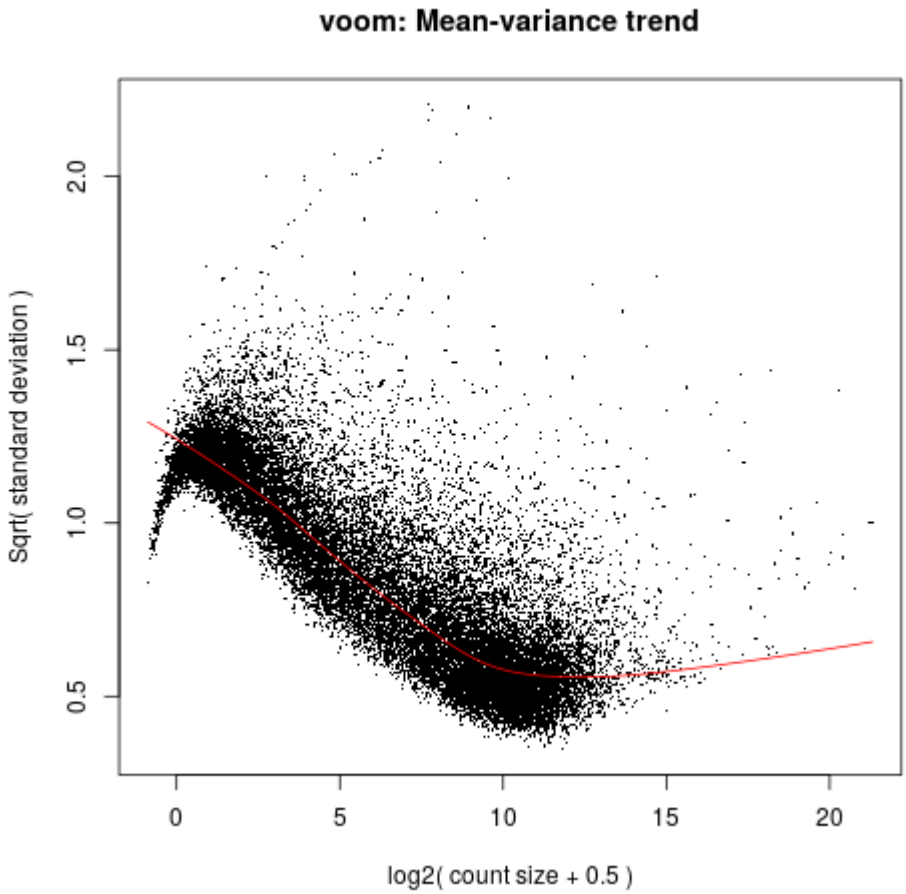
Differential Gene Expression

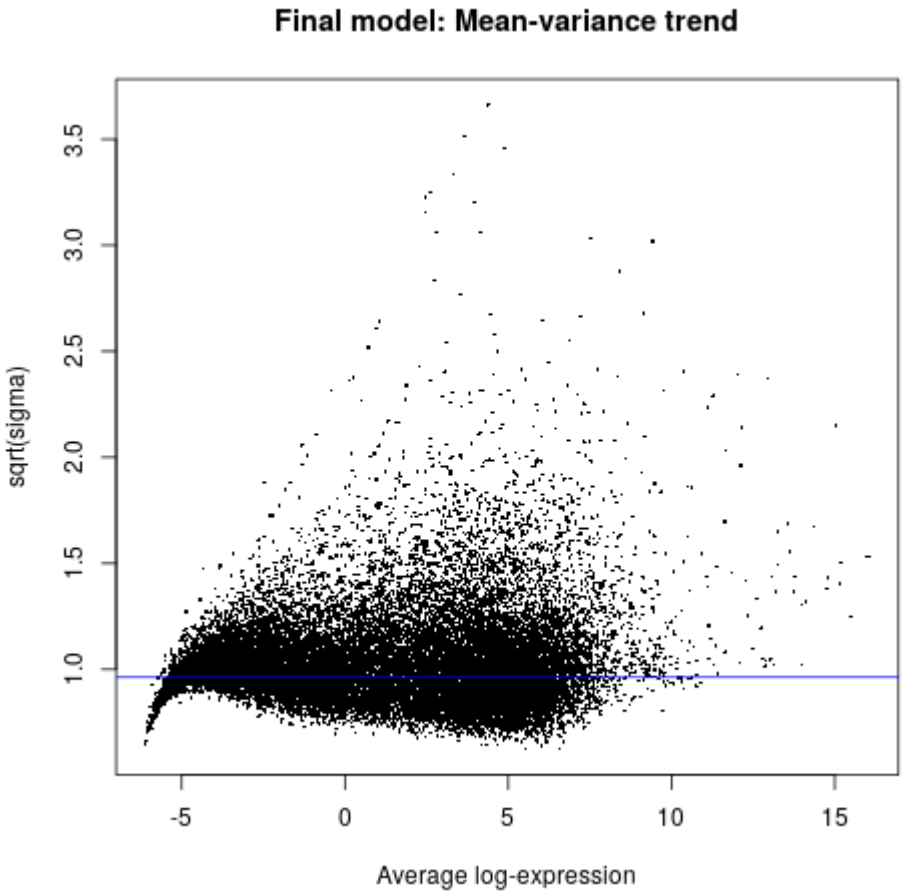
We also wanted to test whether there was differential gene expression (DGE) on the basis of subject age for any tissue. This was done for all tissues, but the results from the Pancreas will be seen here.

Specifically, we tested $LAvs.HA = \frac{(A1+A2+A3)}{3} - \frac{(A4+A5+A6)}{3}$ where LA means low-age and HA means high age, and where our null hypothesis for each gene was there is no difference in the gene expression between the two populations.

Unfortunately, there are no tools in Python to accomplish the mean-dispersion estimation/transformation needed to run statistical tests on RNA-seq count data. Instead, we used `R` and the `edgeR`, `Limma`, and `Glimma` packages. The code to run DGE can be found in `GTEx_DGE_AGE.R`. The edgeR → Limma pipeline is unique insofar that it transforms the right-skewed and heteroskedastic count data into a normal distribution for traditional linear methods.

This process is called voom and can be seen in the following two plots, one from before and after the transformation.





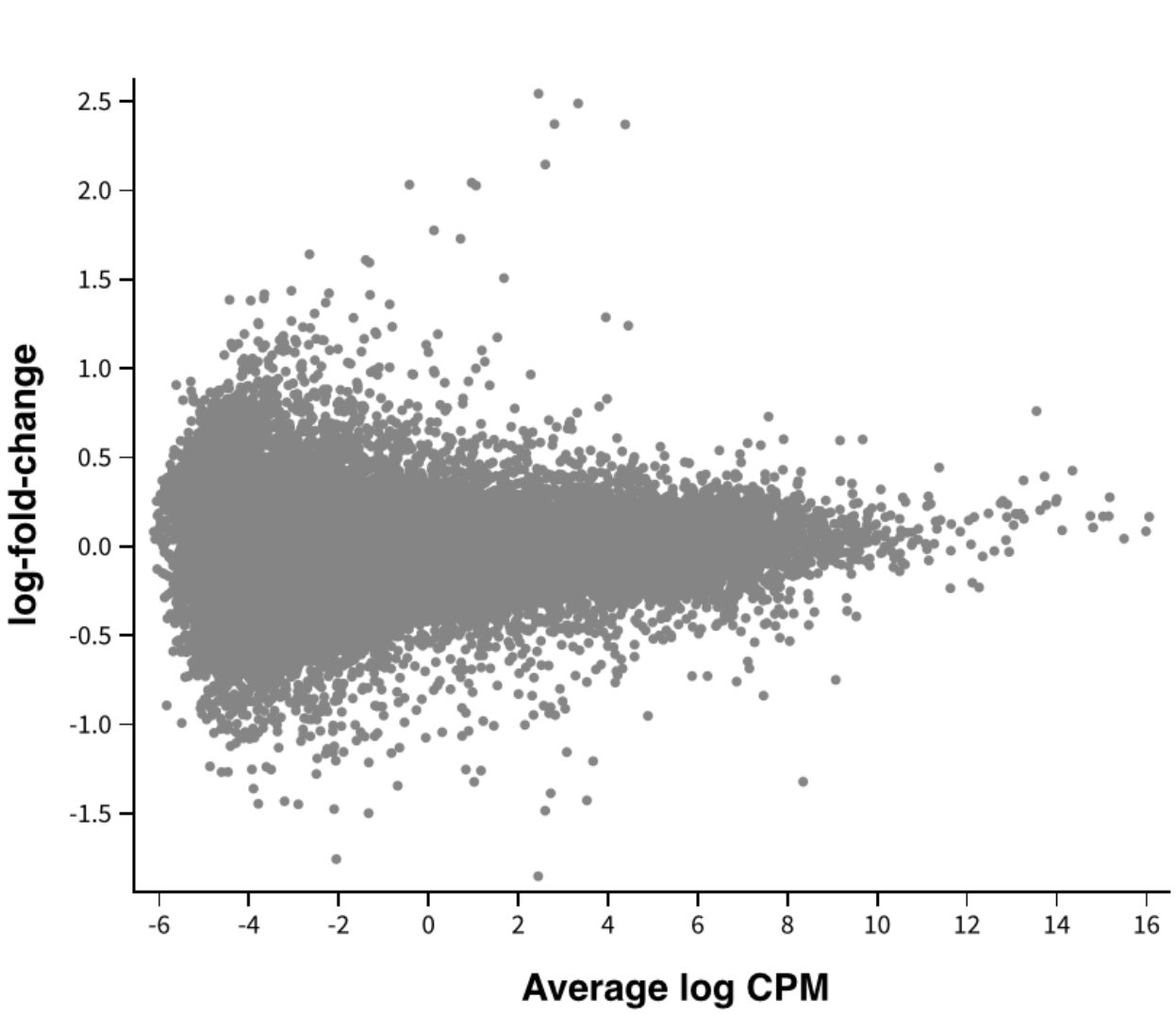
The high dispersion outliers are generally sexually dimorphic genes. This trend occurs to some degree for most tissues. This impacted the normalization and DGE process and indicates it may be a good idea to only work with male samples.

DGE Results (progress_plots/Pancreas/MD-Plot.html) - Pancreas

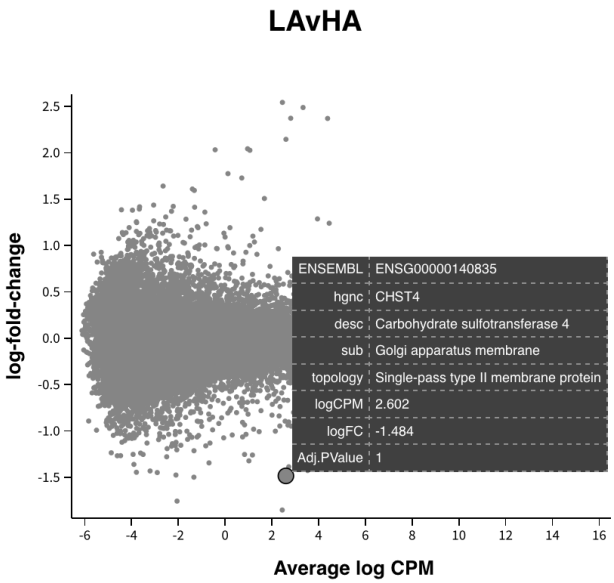
Differential expression analysis in edgeR and Limma involves fitting a linear model to the normalized data to better estimate the dispersion of each gene. This pooled dispersion is then used in the computation of a *moderated* t-statistic between the test groups

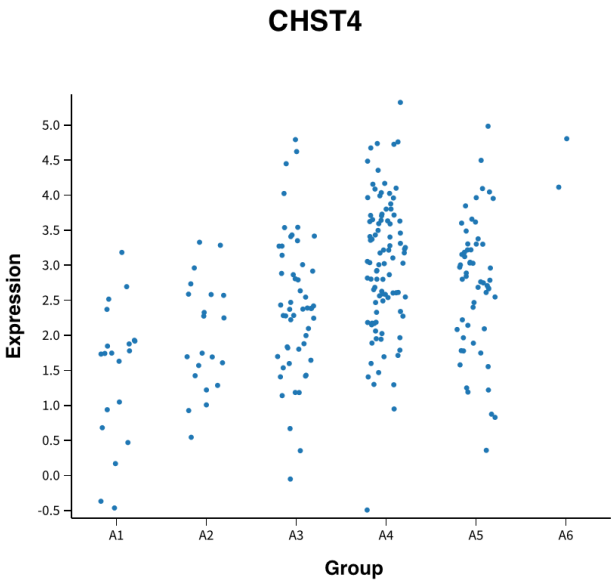
In general, we did not detect differential expression when the lowest three age groups were compared with the highest three age groups. Many genes were marginally differentially expressed, but the BH multiple hypothesis testing correction meant that very few genes were statistically significantly differentially expressed.

Below is a snapshot of the MD plot, which plots mean expression vs. log-fold change between the groups. Usually, differentially expressed genes are highlighted in color, but there are none in this case. This interactive plot can be viewed here (progress_plots/Pancreas/MD-Plot.html).



Visually, there were trends in some genes the seemed to reflect a trend based on age. A strip chart for one such gene can be seen below. For each tissue, there were a handful of anecdotally significant genes with functions relevant to the tissue. For example, the pancreas is a major extracellular exporter, and this gene which appears to be differentially expressed has to do with the Golgi apparatus.





ML

We use the **PyTorch** classifier to build the model and predict the age groups for different tissues. It is a deep learning research platform that provides maximum flexibility and speed.</n> PyTorch provides two main features: </n>

- An n-dimesnional tensor
- Automatic differentiation for building and training neural networks

We build a simple model with all the attributes for the genes as inputs and six output layers(one for each age group).

Baseline classifier

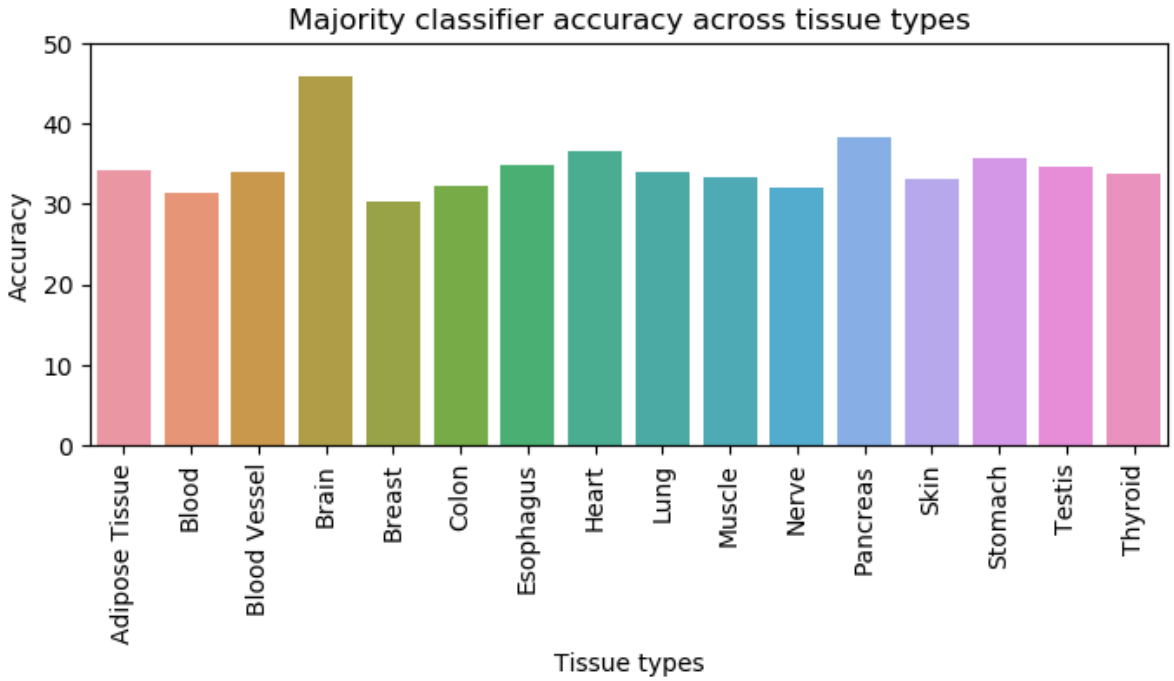
We consider the majority classifier for each tissue type to be the baseline classifier in order to compare the performance of the constructed model.

In [22]:
```
maj_clf = grp_trends[['percentage_per_tissue', 'SMTS']].groupby(['SMTS']).max
().reset_index()
maj_clf
```

Out[22]:

	SMTS	percentage_per_tissue
0	Adipose Tissue	34.253450
1	Blood	31.471136
2	Blood Vessel	33.953998
3	Brain	45.921450
4	Breast	30.344828
5	Colon	32.347140
6	Esophagus	34.867777
7	Heart	36.666667
8	Lung	33.957845
9	Muscle	33.333333
10	Nerve	32.125604
11	Pancreas	38.306452
12	Skin	33.111481
13	Stomach	35.632184
14	Testis	34.749035
15	Thyroid	33.856502

In [32]:
```
import warnings
warnings.filterwarnings('ignore')

mpl.style.use('default')
fig = plt.figure(figsize=(8,3))
ax = fig.add_subplot(111)

model_plot = sns.barplot(x = maj_clf['SMTS'],
            y=maj_clf['percentage_per_tissue'],
            data = maj_clf,
            orient='v',
            estimator=np.mean,
            capsize=0.1)

tick_labels = maj_clf['SMTS'].as_matrix()
ax.set_xticklabels(tick_labels,rotation=90)
ax.set(ylim=(0,50))
ax.set(title='Majority classifier accuracy across tissue types')
ax.set(xlabel='Tissue types')
ax.set(ylabel='Accuracy')

plt.show()
```



In [1]:
```
import pandas as pd
from pathlib import Path
from os import listdir
import sklearn.model_selection
import sklearn.feature_selection
import numpy as np
import pandas as pd
from PIL import Image
import matplotlib.pyplot as plt
import torch
import torch.nn as nn
from torch.autograd import Variable
import torch.utils.data as data

data_dir=Path("data")
tissue_dir=Path("tissue-specific")
manifest={"data":"All_Tissue_Site_Details.combined.reads.gct",
              "sample_meta":"GTEx_v7_Annotations_SampleAttributesDS.txt",
              "subject_meta":"GTEx_v7_Annotations_SubjectPhenotypesDS.txt",
               "merged_meta":"merged_meta.tsv"}
meta=pd.read_csv(data_dir/manifest['merged_meta'],sep="\t",dtype={'SMUBRID':ob
ject,'SEX':object,'DTHHRDY':object})
meta=meta[~(meta['AGE'].isnull())] # removes all samples without age
#meta=meta[~(np.isnan(meta['AGE']))]
#meta.iloc[0:3,:]
counts=pd.DataFrame(meta['SMTS'].value_counts())
df=meta[meta['SMTS'].isin(counts[counts['SMTS']>200].index)]
df=pd.crosstab(index=df['SMTS'],columns=df['AGE'])
tissues = df.index.values
print(tissues)
```

```
['Adipose Tissue' 'Blood' 'Blood Vessel' 'Brain' 'Breast' 'Colon'
 'Esophagus' 'Heart' 'Lung' 'Muscle' 'Nerve' 'Pancreas' 'Skin' 'Stomach'
 'Testis' 'Thyroid']
```

In [2]:
```
class my_points():
    def __init__(self):
        self.data = cpm_new
        self.target = y_train_new.as_matrix()
        self.n_samples = self.data.shape[0]

    def __len__(self):
        return self.n_samples

    def __getitem__(self, index):
        return self.data[index], self.target[index]
```

In [3]:
```
# We build a simple model with the inputs and six output layers(one for each a
ge group).
class my_model(nn.Module):
    def __init__(self,n_in=56201,n_hidden=10,n_out=6):
        super(my_model,self).__init__()
        self.n_in  = n_in
        self.n_out = n_out

        self.linearlinear = nn.Sequential(
            nn.Linear(self.n_in,self.n_out,bias=True),   # Hidden layer.
            )
        self.logprob = nn.LogSoftmax(dim=1)                 # -Log(Softmax pro
bability).

    def forward(self,x):
        x = self.linearlinear(x)
        x = self.logprob(x)
        return x
```

In [4]:
```
infiles=listdir(data_dir/tissue_dir)
tissueList = tissues
accuracies=[]
ts=[]
for TISSUE in tissueList:
    print(TISSUE)
    TISSUE_files=[f for f in infiles if  TISSUE in f]
    cpm=pd.read_csv(data_dir/tissue_dir/TISSUE_files[1],sep="\t",index_col=0)
    lcpm=pd.read_csv(data_dir/tissue_dir/TISSUE_files[0],sep="\t",index_col=0)
    tissue_meta=meta[meta['SMTS']==TISSUE]
    print('Dimensions: ',lcpm.shape)
    if tissue_meta.shape[0] == lcpm.shape[0]:

        cpm_train, cpm_test, y_train, y_test = \
                sklearn.model_selection.train_test_split(cpm, tissue_meta['AG
E'], test_size=.3, random_state=1234)
        # random state guarantees that the same split is made for a given tiss
ue.
        cpm_new = cpm_train.as_matrix()
        cpm_new = np.delete(cpm_new,0,axis=1)
        y_train_new = y_train.map({'20-29':0,'30-39':1,'40-49':2,'50-59':3, '6
0-69':4, '70-79':5})
        my_data = my_points()
        batch_size = 1
        import torch.utils.data as data
        my_loader = data.DataLoader(my_data,batch_size=batch_size,num_workers=
0)
        # Now, we create the mode, the loss function or criterium and the opti
mizer
        # that we are going to use to minimize the loss.

        # Model.
        model = my_model()

        # Negative log likelihood loss.
        criterium = nn.NLLLoss()

        # Adam optimizer with learning rate 0.1 and L2 regularization with wei
ght 1e-4.
        optimizer = torch.optim.Adam(model.parameters(),lr=0.1,weight_decay=1e
-4)
        # Training.
        model.double()
        accuracy=0
        for epoch in range(3):
            truecount=0
            totalcount=0

            for k, (data, target) in enumerate(my_loader):
                model.zero_grad()
                log_p = model(data)
                loss = criterium(log_p,target)
                loss.backward()
                totalcount+=1
                if(target == torch.max(torch.exp(log_p),1)[1]):
                    truecount+=1
```

```
            # Model weight modification based on the optimizer.
            optimizer.step()
        if ((truecount/totalcount)*100)>accuracy:
            accuracy = (truecount/totalcount)*100
            accuracies.append(accuracy)
            ts.append(TISSUE)
        print('Epoch ',epoch+1,':',truecount/totalcount)

    print('Accuracy for ',TISSUE,' tissue: ',accuracy)
```

```
Adipose Tissue
Dimensions:  (797, 56202)
```

```
C:\Users\adity\Anaconda3\envs\cs418env\lib\site-packages\ipykernel_launcher.p
y:20: FutureWarning: Method .as_matrix will be removed in a future version. U
se .values instead.
C:\Users\adity\Anaconda3\envs\cs418env\lib\site-packages\ipykernel_launcher.p
y:4: FutureWarning: Method .as_matrix will be removed in a future version. Us
e .values instead.
  after removing the cwd from sys.path.
```

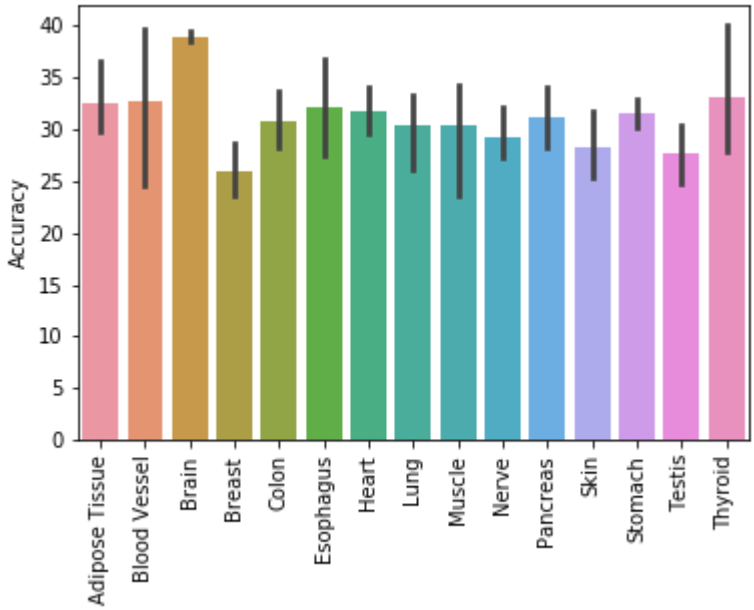
```
Epoch  1 : 0.2980251346499102
Epoch  2 : 0.3123877917414722
Epoch  3 : 0.36624775583482944
Accuracy for  Adipose Tissue  tissue:  36.62477558348294
Blood
Dimensions:  (913, 56202)
Blood Vessel
Dimensions:  (913, 56202)
Epoch  1 : 0.24569640062597808
Epoch  2 : 0.3411580594679186
Epoch  3 : 0.39593114241001565
Accuracy for  Blood Vessel  tissue:  39.593114241001565
Brain
Dimensions:  (331, 56202)
Epoch  1 : 0.3852813852813853
Epoch  2 : 0.329004329004329
Epoch  3 : 0.3939393939393939
Accuracy for  Brain  tissue:  39.39393939393939
Breast
Dimensions:  (290, 56202)
Epoch  1 : 0.23645320197044334
Epoch  2 : 0.2561576354679803
Epoch  3 : 0.2857142857142857
Accuracy for  Breast  tissue:  28.57142857142857
Colon
Dimensions:  (507, 56202)
Epoch  1 : 0.2824858757062147
Epoch  2 : 0.3050847457627119
Epoch  3 : 0.3361581920903955
Accuracy for  Colon  tissue:  33.61581920903955
Esophagus
Dimensions:  (1021, 56202)
Epoch  1 : 0.27450980392156865
Epoch  2 : 0.32072829131652664
Epoch  3 : 0.36694677871148457
Accuracy for  Esophagus  tissue:  36.69467787114846
Heart
Dimensions:  (600, 56202)
Epoch  1 : 0.29523809523809524
Epoch  2 : 0.319047619047619
Epoch  3 : 0.3404761904761905
Accuracy for  Heart  tissue:  34.04761904761905
Lung
Dimensions:  (427, 56202)
Epoch  1 : 0.26174496644295303
Epoch  2 : 0.31543624161073824
Epoch  3 : 0.33221476510067116
Accuracy for  Lung  tissue:  33.22147651006711
Muscle
Dimensions:  (564, 56202)
Epoch  1 : 0.23604060913705585
Epoch  2 : 0.3299492385786802
Epoch  3 : 0.3426395939086294
Accuracy for  Muscle  tissue:  34.263959390862944
Nerve
Dimensions:  (414, 56202)
Epoch  1 : 0.27335640138408307
```

```
Epoch  2 : 0.2837370242214533
Epoch  3 : 0.3217993079584775
Accuracy for  Nerve  tissue:  32.17993079584775
Pancreas
Dimensions:  (248, 56202)
Epoch  1 : 0.2832369942196532
Epoch  2 : 0.34104046242774566
Epoch  3 : 0.3352601156069364
Accuracy for  Pancreas  tissue:  34.104046242774565
Skin
Dimensions:  (1202, 56202)
Epoch  1 : 0.25326991676575505
Epoch  2 : 0.27705112960760997
Epoch  3 : 0.31747919143876335
Accuracy for  Skin  tissue:  31.747919143876334
Stomach
Dimensions:  (261, 56202)
Epoch  1 : 0.3021978021978022
Epoch  2 : 0.3131868131868132
Epoch  3 : 0.32967032967032966
Accuracy for  Stomach  tissue:  32.967032967032964
Testis
Dimensions:  (259, 56202)
Epoch  1 : 0.24861878453038674
Epoch  2 : 0.22099447513812154
Epoch  3 : 0.30386740331491713
Accuracy for  Testis  tissue:  30.386740331491712
Thyroid
Dimensions:  (446, 56202)
Epoch  1 : 0.2467948717948718
Epoch  2 : 0.34294871794871795
Epoch  3 : 0.40064102564102566
Accuracy for  Thyroid  tissue:  40.06410256410257
```

In [5]:
```
import seaborn as sns
ax = sns.barplot(x=ts,y=accuracies)
for item in ax.get_xticklabels():
    item.set_rotation(90)
ax.set(ylabel='Accuracy')
```

```
C:\Users\adity\Anaconda3\envs\cs418env\lib\site-packages\scipy\stats\stats.p
y:1713: FutureWarning: Using a non-tuple sequence for multidimensional indexi
ng is deprecated; use `arr[tuple(seq)]` instead of `arr[seq]`. In the future
this will be interpreted as an array index, `arr[np.array(seq)]`, which will
result either in an error or a different result.
  return np.add.reduce(sorted[indexer] * weights, axis=axis) / sumval
```

Out[5]:
```
[Text(0, 0.5, 'Accuracy')]
```



Reflection

- The size of the data has been extremely prohibitive. The transformed counts are floats, which take much more space. It is best to compute the cpm transformation on the entire data set, meaning that we had to create these floats all in R before moving to Python.
- It can be observed that the DL model is not producing satisfactory results. It seems to enhance the argument that identifying there doesn't seem to be one predominant gene to predict the age.

Next Steps

- The immediate takeaway is to improve the performance of the available models to achieve satisfactory results.
- Identify effective transformations of the gene expression data, if possible.