main

July 10, 2021

1 Calculate RXE for public dataset, females only based on Apua's script

[1]:

```
import re
import functools
import numpy as np
import pandas as pd
from os import environ
from plotnine import *
from scipy.stats import variation
from warnings import filterwarnings
from scipy.stats import mannwhitneyu
from matplotlib.cbook import mplDeprecation
```

[2]:

```
filterwarnings("ignore",category=mplDeprecation)
filterwarnings('ignore', category=UserWarning, module='plotnine.*')
filterwarnings('ignore', category=DeprecationWarning, module='plotnine.*')
```

[3]:

```
environ['NUMEXPR_MAX_THREADS'] = '10'
```

[4]:

```
config = {
    'pheno_file': 'cmc_phenotypes.csv',
    'cpm_file': 'cmc_log2tpm.tsv',
    'tpm_file': '/ceph/users/jbenja13/projects/sex_sz_ria/input/commonMind/
 ↪counts/gene_tpm/_m/log2tpm.csv',
    'annot_file': 'cmc_gene_annotation.tsv',
}
```

[5]:

```
@functools.lru_cache()
def get_pheno():
    return pd.read_csv(config['pheno_file'], index_col=0)


@functools.lru_cache()
def get_annotation():
    return pd.read_csv(config['annot_file'], index_col=0, sep='\t')
```

[6]:

```
def chrom_type(chrom):
    if chrom=='X':
        return 'X'
    elif re.search('^\d+', chrom):
        return 'autosome'
    else:
        return 'other'


def save_ggplot(p, fn):
    for ext in ['.pdf', '.png', '.svg']:
        p.save(fn+ext, width=10, height=7)
```

[7]:

```
gene_annot = get_annotation()
gene_annot.head(2)
```

[7]:

```
                      ensembl_gene_id hgnc_symbol chromosome_name
gene_id
ENSG00000000003.14  ENSG00000000003      TSPAN6               X
ENSG00000000005.5   ENSG00000000005        TNMD               X
```

[8]:

```
gene_annot.shape
```

[8]:

```
(57765, 3)
```

[9]:

```
log2tpm = pd.read_csv(config['tpm_file'], index_col=0, sep=',', engine='c')
log2tpm.iloc[0:2, 0:5]
```

[9]:

```
                    MSSM_RNA_BP_PFC_2  MSSM_RNA_BP_PFC_4  MSSM_RNA_BP_PFC_5  \
gene_id
ENSG00000000003.14           1.927755           1.341998           1.531728
ENSG00000000005.5            0.089456           0.036288           0.030684

                    MSSM_RNA_BP_PFC_6  MSSM_RNA_BP_PFC_7
gene_id
ENSG00000000003.14           1.565244           2.178456
ENSG00000000005.5            0.088621           0.235076
```

[10]:

```
log2tpm.shape
```

[10]:

```
(50689, 606)
```

[11]:

```
df0 = gene_annot.merge(log2tpm, right_index=True, left_index=True).
 ↪drop_duplicates(subset=['ensembl_gene_id'])
df0.shape
```

[11]:

```
(50097, 609)
```

[12]:
```
df0['chrom_type'] = df0['chromosome_name'].apply(chrom_type)
df0.shape
```

[12]:
```
(50097, 610)
```

[13]:
```
df0.groupby('chrom_type').size()
```

[13]:
```
chrom_type
X             1970
autosome     47618
other          509
dtype: int64
```

[14]:
```
samples = np.array(log2tpm.columns[log2tpm.columns.str.contains("RNA|CMC",␣
 ↪regex=True)])
df = df0[(df0[samples].sum(axis=1) >= 0.2 * len(samples)) & (df0['chrom_type'] !
 ↪= 'other')]
df1  = df.groupby('chrom_type').mean().transpose()
df1['sample'] = df1.index
df1['RXE'] = df1['X'] - df1['autosome']
df1.head(2)
```

[14]:
```
chrom_type                X  autosome             sample       RXE
MSSM_RNA_BP_PFC_2  2.200941  2.124278  MSSM_RNA_BP_PFC_2  0.076663
MSSM_RNA_BP_PFC_4  1.666506  1.628365  MSSM_RNA_BP_PFC_4  0.038141
```

[15]:
```
df2 = df1.merge(get_pheno(), left_index=True, right_index=True)
df2.head(2)
```

[15]:
```
                      X  autosome          sample       RXE Individual_ID  \
MSSM_RNA_PFC_1  2.594985  2.529523  MSSM_RNA_PFC_1  0.065462  CMC_MSSM_025
MSSM_RNA_PFC_2  2.672613  2.594684  MSSM_RNA_PFC_2  0.077929  CMC_MSSM_136

               Institution Reported_Gender       Dx  Age_of_Death
MSSM_RNA_PFC_1        MSSM            Male      SCZ          68.0
MSSM_RNA_PFC_2        MSSM            Male  Control          76.0
```

[16]:
```
df2.loc[:, ['Reported_Gender', 'Institution', 'RXE']].groupby(["Institution",␣
 ↪'Reported_Gender']).describe()
```

[16]:
```
                              RXE                                          \
                            count      mean       std       min       25%
Institution Reported_Gender
MSSM        Female          126.0  0.065413  0.067790 -0.090430  0.012126
            Male            181.0  0.058109  0.061735 -0.122148  0.028566
Penn        Female           54.0  0.089080  0.050252 -0.061299  0.057125
            Male             40.0  0.087665  0.060816 -0.053713  0.051894
Pitt        Female           41.0  0.097466  0.031784  0.035957  0.074324
```

```
            Male                 106.0  0.088188  0.038642  0.013731  0.061899


                                     50%       75%       max
Institution Reported_Gender
MSSM        Female           0.077704  0.109618  0.255605
            Male             0.071640  0.100297  0.192727
Penn        Female           0.088885  0.118559  0.238989
            Male             0.090865  0.115655  0.243992
Pitt        Female           0.096307  0.126792  0.157051
            Male             0.085425  0.115341  0.186669
```

[17]:
```
for institution in df2.Institution.unique():
    for sex in ["Female", "Male"]:
        var = variation(df2[(df2['Institution']==institution) &
                            (df2['Reported_Gender']==sex)].RXE)
        print("There is %.3f variation for %s in %s." % (var, sex, institution))
```

```
There is 1.032 variation for Female in MSSM.
There is 1.059 variation for Male in MSSM.
There is 0.559 variation for Female in Penn.
There is 0.685 variation for Male in Penn.
There is 0.322 variation for Female in Pitt.
There is 0.436 variation for Male in Pitt.
```

[18]:
```
df2.loc[:, ['Reported_Gender', 'Institution', 'RXE']].
 ↪groupby(['Reported_Gender']).describe()
```

[18]:
```
                   RXE                                                      \
                 count      mean       std       min       25%       50%
Reported_Gender
Female           221.0  0.077142  0.059949 -0.090430  0.047601  0.085898
Male             327.0  0.071475  0.057008 -0.122148  0.044566  0.077639


                      75%       max
Reported_Gender
Female           0.113375  0.255605
Male             0.106715  0.243992
```

[19]:
```
for sex in ["Female", "Male"]:
    var = variation(df2[(df2['Reported_Gender']==sex)].RXE)
    print("There is %.3f variation for %s." % (var, sex)) # Not sure if this␣
 ↪variation is real
```

```
There is 0.775 variation for Female.
There is 0.796 variation for Male.
```

1.0.1 Separate out tissues

[20]:

```
for institution in df2.Institution.unique():
    dft = df2[(df2['Institution'] == institution)].copy()
    stat, pval = mannwhitneyu(dft[(dft['Reported_Gender']=="Female")].RXE,
                              dft[(dft['Reported_Gender']=="Male")].RXE)
    print("Mann-WhitneyU for female vs male (RXE) for %s: %.4f" % (institution,␣
 ↪pval))
```

```
Mann-WhitneyU for female vs male (RXE) for MSSM: 0.1548
Mann-WhitneyU for female vs male (RXE) for Penn: 0.4137
Mann-WhitneyU for female vs male (RXE) for Pitt: 0.0719
```

[21]:

```
stat, pval = mannwhitneyu(df2[(df2['Reported_Gender']=="Female")].RXE,
                          df2[(df2['Reported_Gender']=="Male")].RXE)
print("Mann-WhitneyU for female vs male (RXE) for %s: %.4f, %.1f" % ("DLPFC",␣
 ↪pval, stat))
```

```
Mann-WhitneyU for female vs male (RXE) for DLPFC: 0.0734, 33495.0
```

[22]:

```
for institution in df2.Institution.unique():
    dft = df2[(df2['Institution'] == institution)].copy()
    stat, pval = mannwhitneyu(dft[(dft['Dx']=='Control')].RXE,
                              dft[(dft['Dx']=='SCZ')].RXE)
    print("Mann-WhitneyU for ctl vs sz (RXE) for %s: %.4e" % (institution,␣
 ↪pval))
```

```
Mann-WhitneyU for ctl vs sz (RXE) for MSSM: 1.6429e-02
Mann-WhitneyU for ctl vs sz (RXE) for Penn: 1.6475e-01
Mann-WhitneyU for ctl vs sz (RXE) for Pitt: 1.7510e-01
```

[23]:

```
stat, pval = mannwhitneyu(df2[(df2['Dx']=="Control")].RXE,
                          df2[(df2['Dx']=="SCZ")].RXE)
print("Mann-WhitneyU for female vs male (RXE) for %s: %.4e, %.1f" % ("DLPFC",␣
 ↪pval, stat))
```

```
Mann-WhitneyU for female vs male (RXE) for DLPFC: 3.1883e-02, 33911.0
```

[24]:

```
for institution in df2.Institution.unique():
    dft = df2[(df2['Institution'] == institution)].copy()
    female = dft[(dft['Reported_Gender']=='Female')].copy()
    male = dft[(dft['Reported_Gender']=='Male')].copy()
    stat_f, pval_f = mannwhitneyu(female[(female['Dx']=='Control')].RXE,
                                  female[(female['Dx']=='SCZ')].RXE)
    stat_m, pval_m = mannwhitneyu(male[(male['Dx']=='Control')].RXE,
                                  male[(male['Dx']=='SCZ')].RXE)
    print("Mann-WhitneyU of female, ctl vs sz (RXE) for %s: %.4f" %␣
 ↪(institution, pval_f))
```

```
    print("Mann-WhitneyU of male, ctl vs sz (RXE) for %s: %.4f" % (institution,␣
 ↪pval_m))
```

```
Mann-WhitneyU of female, ctl vs sz (RXE) for MSSM: 0.1330
Mann-WhitneyU of male, ctl vs sz (RXE) for MSSM: 0.0499
Mann-WhitneyU of female, ctl vs sz (RXE) for Penn: 0.3124
Mann-WhitneyU of male, ctl vs sz (RXE) for Penn: 0.0294
Mann-WhitneyU of female, ctl vs sz (RXE) for Pitt: 0.3120
Mann-WhitneyU of male, ctl vs sz (RXE) for Pitt: 0.1254
```

[25]:

```
female = df2[(df2['Reported_Gender']=='Female')].copy()
male = df2[(df2['Reported_Gender']=='Male')].copy()
stat_f, pval_f = mannwhitneyu(female[(female['Dx']=='Control')].RXE,
                                  female[(female['Dx']=='SCZ')].RXE)
stat_m, pval_m = mannwhitneyu(male[(male['Dx']=='Control')].RXE,
                                  male[(male['Dx']=='SCZ')].RXE)
print("Mann-WhitneyU of female, ctl vs sz (RXE) for %s: %.4f, %.2f" % ("DLPFC",␣
 ↪pval_f, stat_f))
print("Mann-WhitneyU of male, ctl vs sz (RXE) for %s: %.4f, %.2f" % ("DLPFC",␣
 ↪pval_m, stat_m))
```

```
Mann-WhitneyU of female, ctl vs sz (RXE) for DLPFC: 0.2171, 5601.00
Mann-WhitneyU of male, ctl vs sz (RXE) for DLPFC: 0.0487, 11943.00
```

1.0.2 Interaction model

[26]:

```
df2.Reported_Gender = df2.Reported_Gender.astype("category").cat.
 ↪reorder_categories(["Female", "Male"], ordered=True)
df2.Dx = df2.Dx.astype("category").cat.rename_categories({"SCZ": "SZ",␣
 ↪"Control": "CTL"})
df2.head(2)
```

[26]:

```
                       X  autosome          sample       RXE Individual_ID  \
MSSM_RNA_PFC_1  2.594985  2.529523  MSSM_RNA_PFC_1  0.065462  CMC_MSSM_025
MSSM_RNA_PFC_2  2.672613  2.594684  MSSM_RNA_PFC_2  0.077929  CMC_MSSM_136

               Institution Reported_Gender   Dx  Age_of_Death
MSSM_RNA_PFC_1        MSSM            Male   SZ          68.0
MSSM_RNA_PFC_2        MSSM            Male  CTL          76.0
```

[27]:

```
df3 = df2.loc[:, ['RXE', 'Reported_Gender', "Dx", 'Institution']]
df3.loc[:, "Reported_Gender"] = df3.Reported_Gender.cat.codes
df3.Dx = df3.Dx.cat.codes
df3.loc[:, "Institution"] = df3.Institution.astype("category").cat.codes
df3.head(2)
```

[27]:

```
                     RXE  Reported_Gender  Dx  Institution
MSSM_RNA_PFC_1  0.065462                1   1            0
```

```
MSSM_RNA_PFC_2  0.077929                1   0             0
```

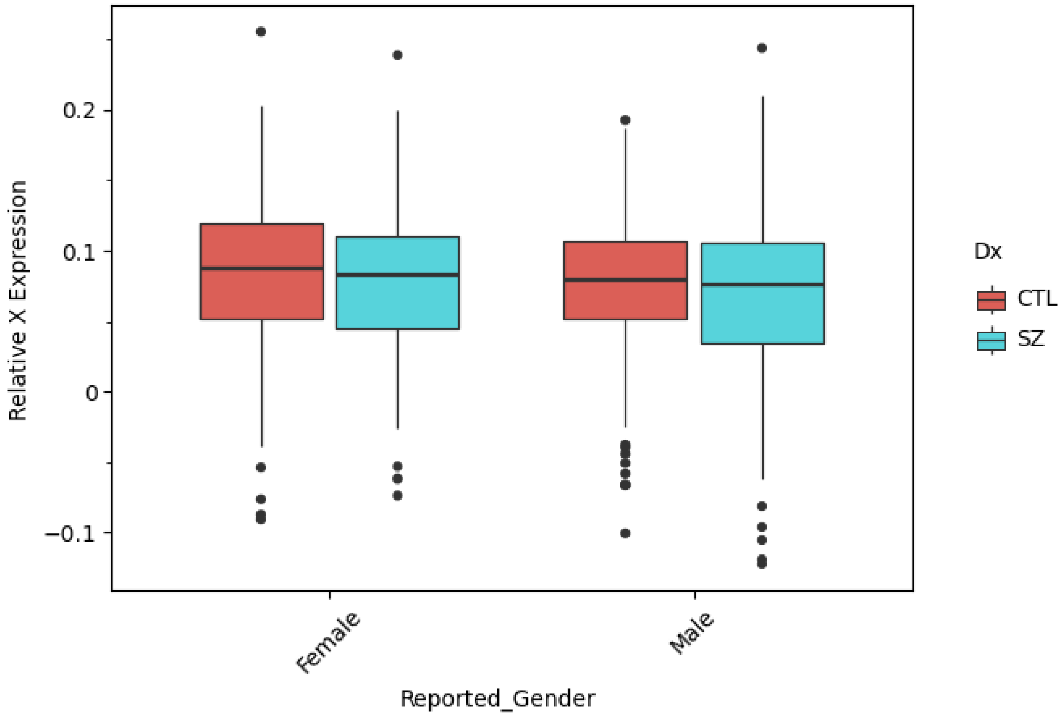
[28]:
```
import statsmodels.api as sm
from statsmodels.formula.api import ols

anova_df = df2.loc[:,['RXE','Reported_Gender', "Dx", 'Institution']]

# ANOVA results with combinations of 2 groups:
formula = 'RXE ~ C(Reported_Gender) + C(Dx) + C(Institution) +␣
 ↪C(Reported_Gender):C(Dx) + C(Reported_Gender):C(Institution) + C(Dx):
 ↪C(Institution)'
lm = ols(formula, anova_df).fit()
table = sm.stats.anova_lm(lm, typ=2)
print(table)
```

```
                                      sum_sq     df          F        PR(>F)
C(Reported_Gender)                  0.002992    1.0   0.938861  3.330063e-01
C(Dx)                               0.008155    1.0   2.559338  1.102315e-01
C(Institution)                      0.111778    2.0  17.540066  4.173517e-08
C(Reported_Gender):C(Dx)            0.000445    1.0   0.139511  7.089152e-01
C(Reported_Gender):C(Institution)   0.000732    2.0   0.114794  8.915716e-01
C(Dx):C(Institution)                0.013260    2.0   2.080725  1.258432e-01
Residual                            1.714256  538.0        NaN           NaN
```

There is a massive difference between institutions, but no interaction between institution and sex or sex and diagnosis

1.0.3 Plot RXE

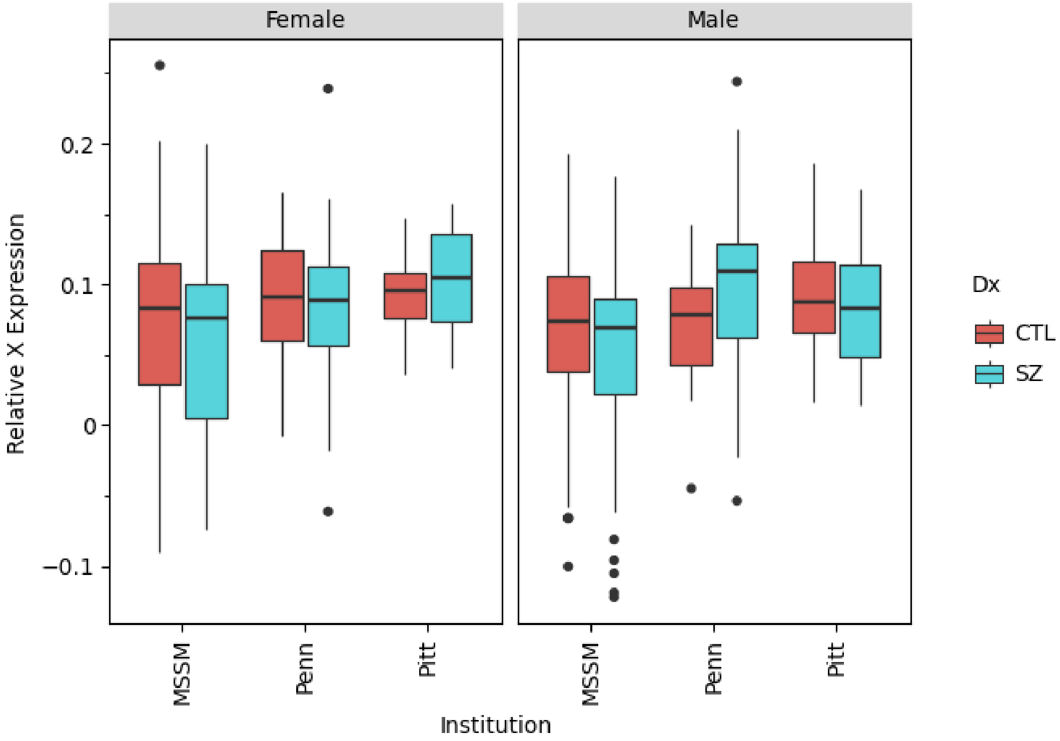
[29]:
```
p = ggplot(df2, aes(x='Reported_Gender', y='RXE', fill='Dx')) \
+ geom_boxplot()\
+ ylab("Relative X Expression")\
+ theme_matplotlib()
p + theme(axis_text_x = element_text(angle = 45))
```



```
[29]: <ggplot: (8781457543801)>
```

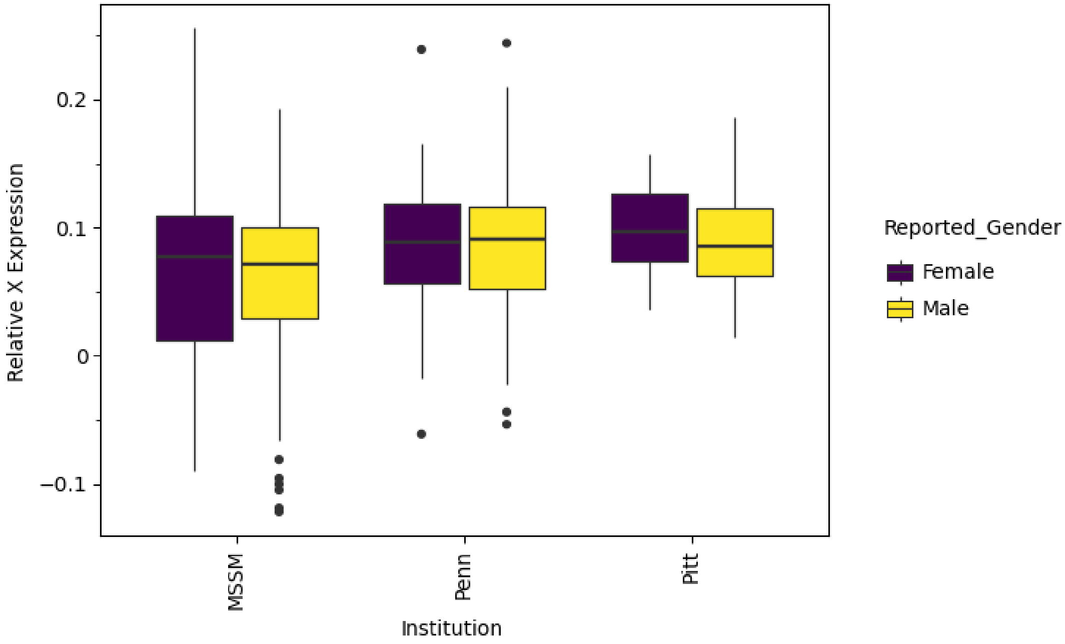
```
[30]: p = ggplot(df2, aes(x='Institution', y='RXE', fill='Dx')) \
      + geom_boxplot()\
      + ylab("Relative X Expression")\
      + facet_grid("~Reported_Gender")\
      + theme_matplotlib()
      p + theme(axis_text_x = element_text(angle = 90))
```

```
/home/jbenja13/.local/lib/python3.9/site-
packages/plotnine/facets/facet_grid.py:136: FutureWarning: Index.__and__
operating as a set operation is deprecated, in the future this will be a logical
operation matching Series.__and__.  Use index.intersection(other) instead
/home/jbenja13/.local/lib/python3.9/site-
packages/plotnine/facets/facet_grid.py:137: FutureWarning: Index.__and__
operating as a set operation is deprecated, in the future this will be a logical
operation matching Series.__and__.  Use index.intersection(other) instead
```



```
[30]: <ggplot: (8781457543774)>
```

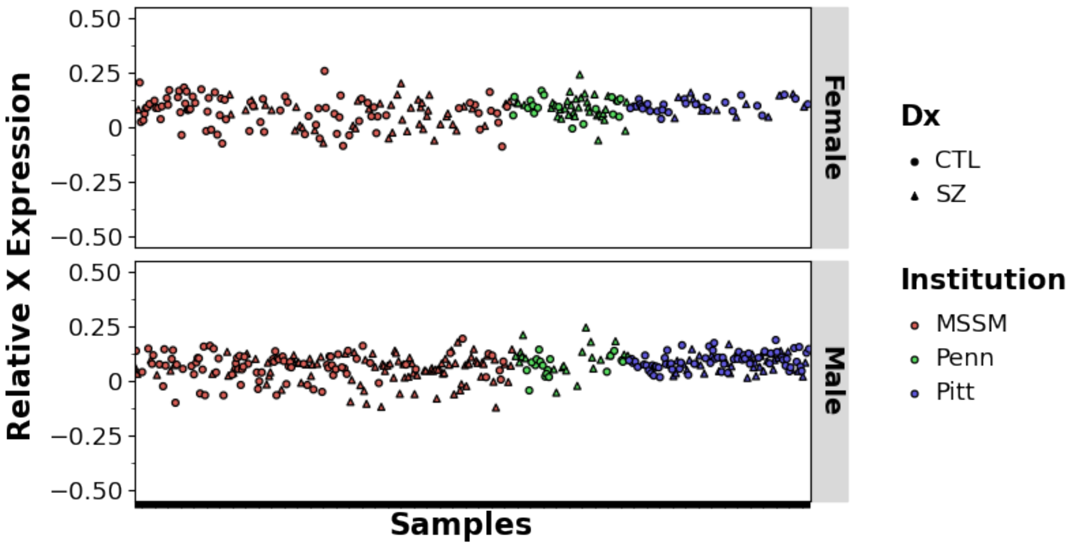
```
[31]: p = ggplot(df2, aes(x='Institution', y='RXE', fill='Reported_Gender')) \
      + geom_boxplot()\
      + ylab("Relative X Expression")\
      + theme_matplotlib()
      p + theme(axis_text_x = element_text(angle = 90))
```



[31]: <ggplot: (8781458928014)>

[32]:
```
p = ggplot(df2, aes(x='sample', y='RXE', shape="Dx", fill='Institution')) \
+ geom_point() + ylim([-0.5, 0.5]) + xlab("Samples")\
+ ylab("Relative X Expression") + facet_grid("Reported_Gender~.")\
+ theme_matplotlib() \
+ theme(axis_text_x=element_blank(),
        axis_text=element_text(size=13),
        legend_title=element_text(size=15, face="bold"),
        legend_text=element_text(size=13),
        axis_title=element_text(size=16, face="bold"),
        strip_text=element_text(size=14, face="bold"))
p
```

```
/home/jbenja13/.local/lib/python3.9/site-
packages/plotnine/facets/facet_grid.py:136: FutureWarning: Index.__and__
operating as a set operation is deprecated, in the future this will be a logical
operation matching Series.__and__.  Use index.intersection(other) instead
/home/jbenja13/.local/lib/python3.9/site-
packages/plotnine/facets/facet_grid.py:137: FutureWarning: Index.__and__
operating as a set operation is deprecated, in the future this will be a logical
operation matching Series.__and__.  Use index.intersection(other) instead
```



```
[32]: <ggplot: (8781458553733)>
```

```
[33]: df2.to_csv('RXE_public.csv')
      save_ggplot(p, "RXE_public_bySex")
```

```
/home/jbenja13/.local/lib/python3.9/site-
packages/plotnine/facets/facet_grid.py:136: FutureWarning: Index.__and__
operating as a set operation is deprecated, in the future this will be a logical
operation matching Series.__and__.  Use index.intersection(other) instead
/home/jbenja13/.local/lib/python3.9/site-
packages/plotnine/facets/facet_grid.py:137: FutureWarning: Index.__and__
operating as a set operation is deprecated, in the future this will be a logical
operation matching Series.__and__.  Use index.intersection(other) instead
/home/jbenja13/.local/lib/python3.9/site-
packages/plotnine/facets/facet_grid.py:136: FutureWarning: Index.__and__
operating as a set operation is deprecated, in the future this will be a logical
operation matching Series.__and__.  Use index.intersection(other) instead
/home/jbenja13/.local/lib/python3.9/site-
packages/plotnine/facets/facet_grid.py:137: FutureWarning: Index.__and__
operating as a set operation is deprecated, in the future this will be a logical
operation matching Series.__and__.  Use index.intersection(other) instead
/home/jbenja13/.local/lib/python3.9/site-
packages/plotnine/facets/facet_grid.py:136: FutureWarning: Index.__and__
operating as a set operation is deprecated, in the future this will be a logical
operation matching Series.__and__.  Use index.intersection(other) instead
/home/jbenja13/.local/lib/python3.9/site-
packages/plotnine/facets/facet_grid.py:137: FutureWarning: Index.__and__
operating as a set operation is deprecated, in the future this will be a logical
operation matching Series.__and__.  Use index.intersection(other) instead
```

[ ]:

```
```