Predicting Pregnancy Stage from Cell free mRNA Levels

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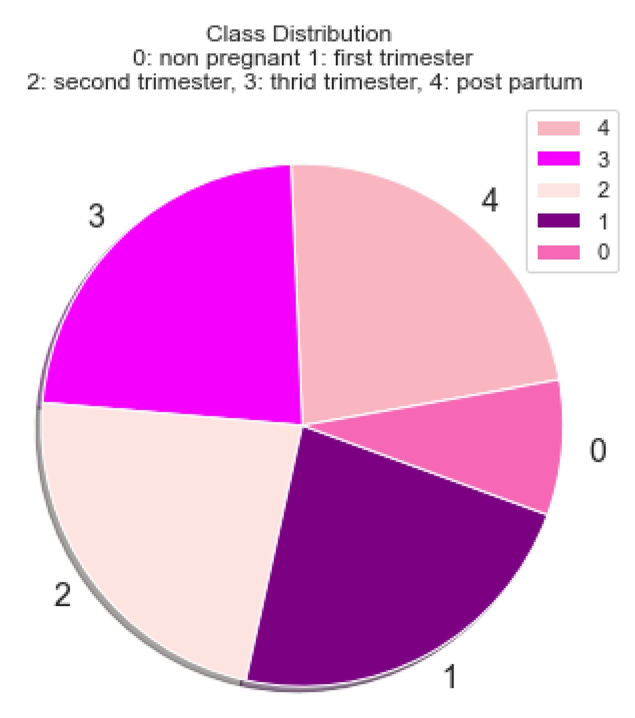


Introduction-

The dataset from the study titled, "Microarray Data of cell-free RNA across pregnancy time course" (Koh et al., 2014) was downloaded from NCBI GEO database. The dataset includes cell-free RNA expression of 12 patients (including a non-pregnant control) over 3 trimesters and post-partum. 33,297 transcripts are measured in the dataset. By analyzing the expression and presence of cf-RNA in the blood, an overview of tissue development and overall health of a fetus can be determined (2014). This study aimed to provide a non-invasive technique for examining fetal tissue development.

Our goal with this dataset is to determine what trimester a patient is in given the expression levels. From this analysis, we are hoping to find which transcript has the highest differential expression during the final stages of pregnancy. Using various Machine Learning models to classify the data to learn and understand how and when these transcripts are expressed, and what up-regulate genes they code for during pregnancy.

Methods -

Overview: To determine which machine learning method worked best for our dataset we begin by downloading the dataset into a pandas dataframe. We next ran various standard data preprocessing steps to get the data ready for classification modeling. We created some charts and graphs to visualize our data. Finally, we created a final dataframe that included all the changes made during our preprocessing steps, we then split that data into a feature matrix (X) and a target matrix (y), and lastly we ran various classification models to see which model was best able to predict which class a patient was in.

Import the Data:

The data consists of 48 samples (12 patients) over the course of 5 stages of pregnancy: first, second, and third trimester, postpartem, and control/non-pregnant microarray data of cell free RNA was collected across 33,297 genes for each patient during each of these time courses

ID_REF	7892501	7892502	7892503	7892504	7892505	7892506	7892507	7892508	7892509	7892510	...	8180409	8180410	8180411
count	48.000000	48.000000	48.000000	48.000000	48.000000	48.000000	48.000000	48.000000	48.000000	48.000000	...	48.000000	48.000000	48.000000
mean	5.004943	2.482006	2.561178	7.310324	2.787235	2.605373	3.165746	2.243372	10.500638	2.562259	...	11.946849	10.312863	8.729550
std	1.853566	0.287033	0.247941	1.055773	0.425361	0.404084	0.727252	0.186912	1.742822	0.266118	...	0.645432	0.727021	1.149331
min	2.089713	1.938133	1.982890	5.428908	2.102378	2.122335	2.367414	1.923975	2.718209	2.022346	...	7.643652	6.219645	5.253879
25%	3.286410	2.287814	2.390578	6.533863	2.540726	2.351941	2.791254	2.106138	10.338530	2.390800	...	11.976620	10.136710	8.073634
50%	5.519676	2.446147	2.514482	7.021481	2.696597	2.464081	2.975391	2.231955	10.952625	2.561447	...	12.035485	10.439085	9.131304
75%	6.390771	2.619133	2.759148	8.047929	2.965962	2.745546	3.281732	2.346940	11.409250	2.682029	...	12.119032	10.698903	9.442569
max	9.105850	3.351691	3.142344	10.706420	4.643989	4.226218	7.068907	2.800776	11.993470	3.234456	...	12.279910	11.043520	10.496080

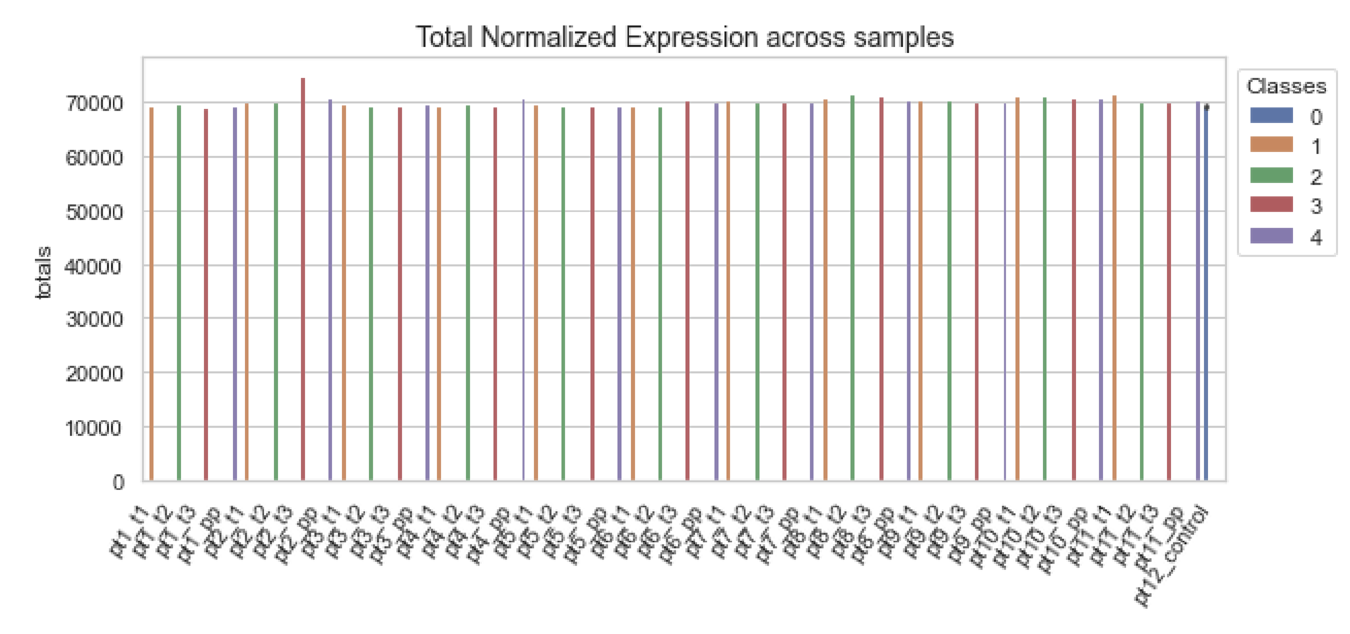
8 rows × 33297 columns

Data Prepocessing :

We started with the standard data preprocessing steps; removing any missing values in the data, and transposing the dataset to be the proper shape needed for running our models

Normalize the data across samples:

In order to be able to compare our samples against each other we needed to normalize the data or account for any outliers. Here we used Log2 base to normalize the data. Log2 fold change was suggested by a classmate during our presentation. Log2 fold is a great tool to help differentiate between original and fold change of a sample using a log2 base for all samples to hover around 0 and 1.



Further data processing:

After reviewing the data in more detail, we were able to determine that additional preprocessing was need.

We decided to select the genes with the highest variability between samples and/or between time periods of the same sample in order to make any meaningful analysis. We noted that the highest variablity between the classes were between the third trimester and post-partem.

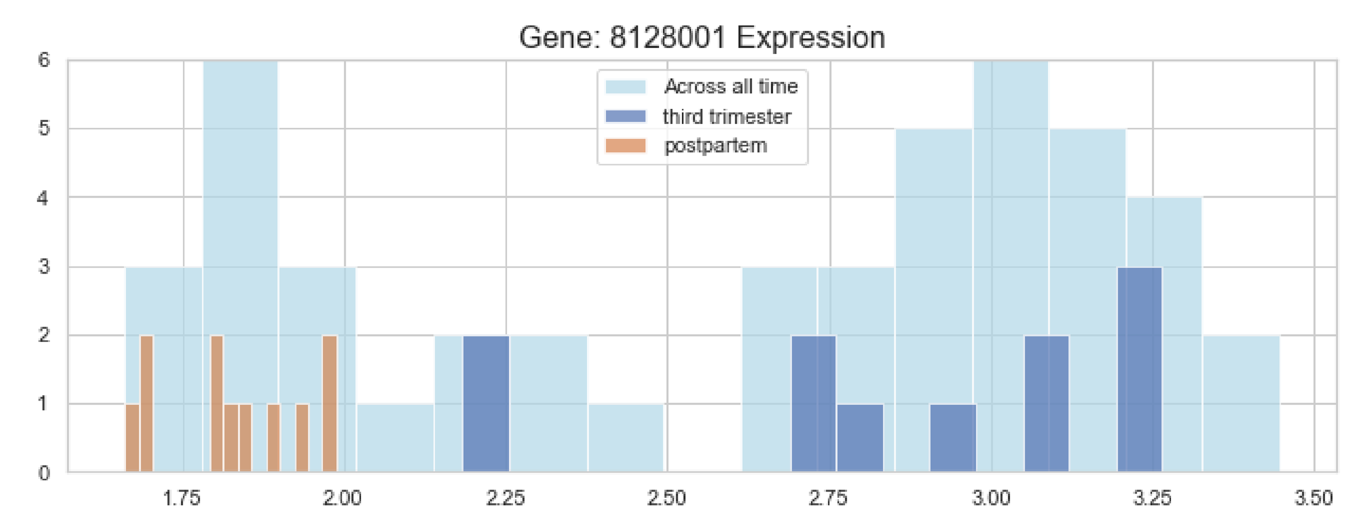
Next, we seperated the data by patient, and then caclculated the differential expression values for each patient from the thirs trimester and post-partem. Note; we eliminated the control patient 12 from our study at this point.

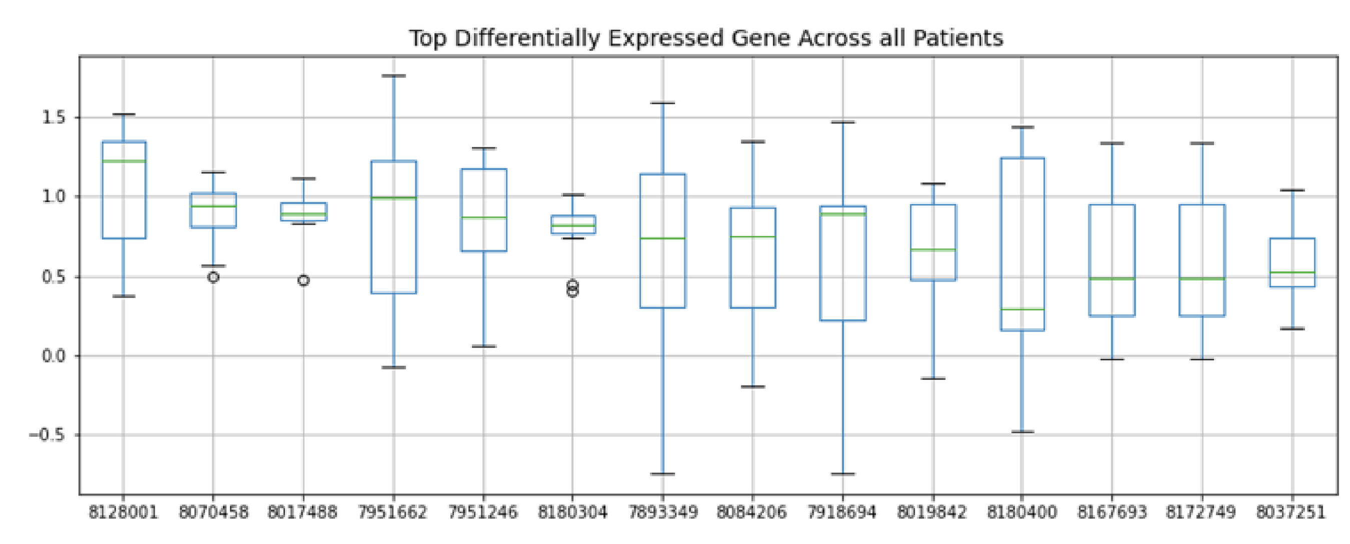
Determine genes with highest differential expression between the two classes :

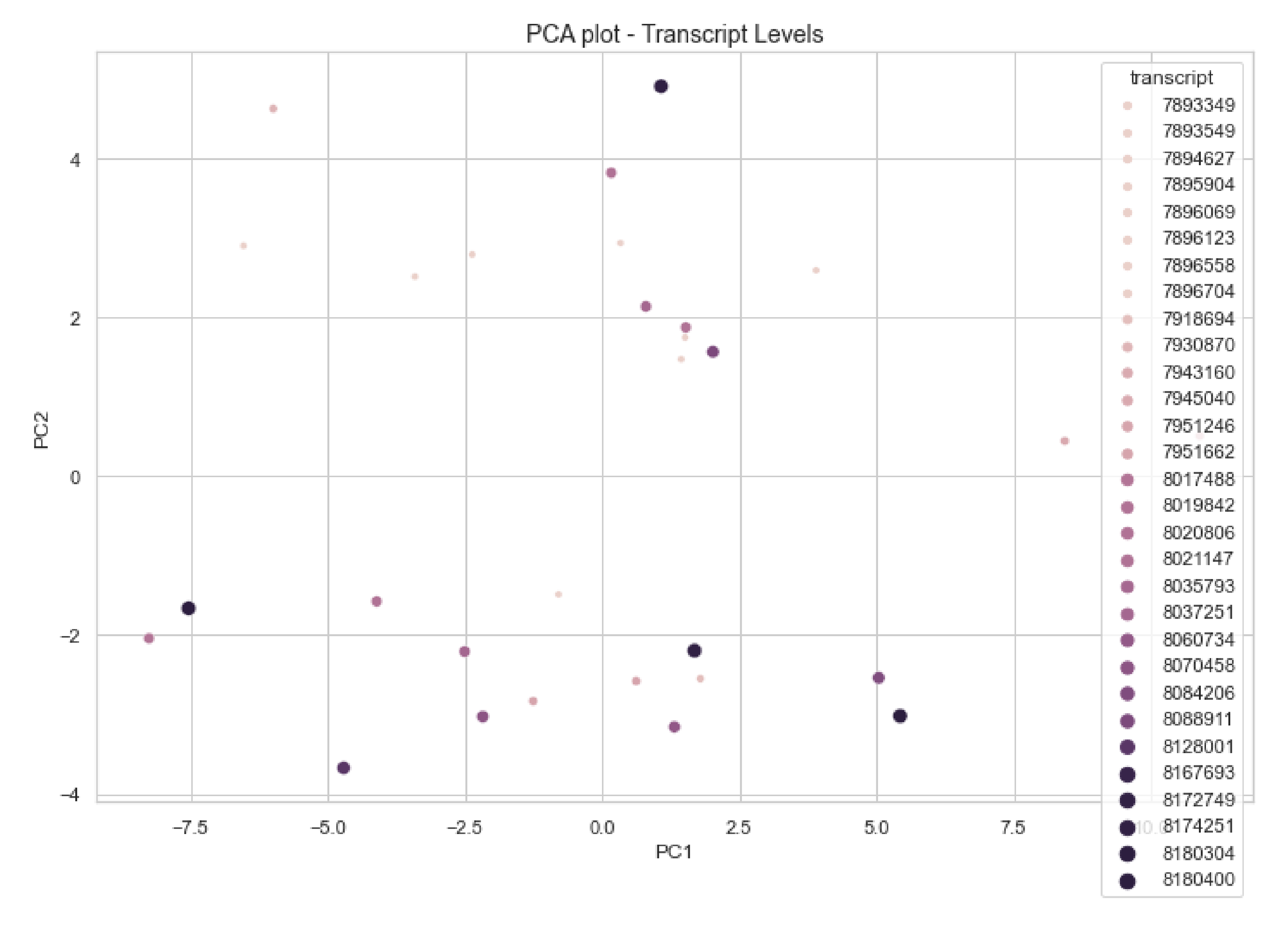
Our dataset contains over 30,000 genes, this makes predictions challenging. In order to reduce the number of genes in our dataset we decided to focus on just the top 30 genes with the highest differential expression between the thrid trimester and post-partem samples for each patient.

Data Visualization:

The below graphs shows how the expression levels vary between between the third trimester compared to post-partem samples. The first graph shows the variation in expression between the two groups for the top differentially expressed gene in the dataset. The second graph shows the top 15 genes that are differentially expressed between the two groups. The last image shows the proportion of expression for the top genes in our dataset.







Putting it all together- Creating our final dataframe:

At this point we finished with the data preprocessing steps and are almost ready to start creating our models. First we have to create our final dataframe with the new curated data from the above steps. Our dataframe will now only include the top 30 genes and sample data from the third trimester and post-partem.

ID_REF	8128001	8070458	8017488	7951662	7951246	8180304	7893349	8084206	7918694	8019842	...	7930870	7896069	7896704	8088911	8035793
pt1_t3	3.110749	2.788043	3.399286	2.982963	2.819630	3.334697	2.896474	2.467626	2.920777	2.927450	...	3.377118	2.534738	2.713673	2.562716	2.295309
pt1_pp	1.660869	1.844100	2.431636	1.538719	1.633755	2.511137	2.318703	1.475046	1.677346	1.941945	...	3.060299	3.222969	3.091271	2.757907	2.958503
pt2_t3	2.737647	2.789186	3.313469	2.594676	3.441163	3.054447	2.676358	2.808282	3.135397	3.346357	...	3.380631	2.930025	2.756968	2.485736	2.432636
pt2_pp	1.988295	2.218284	2.466469	1.645306	2.403199	2.606033	1.085504	1.464668	2.187160	2.601413	...	2.970468	3.381897	2.290621	2.211037	2.562911
pt3_t3	3.204279	3.087972	3.474054	3.010974	3.255985	3.453194	2.761356	2.424237	3.098977	3.080086	...	3.084699	2.622824	2.383003	1.655158	2.674994

5 rows × 31 columns

Splitting our data:

We split the final dataframe into our target values (y), and feature values (X). Then we further split the data into a training dataset and a testing dataset which we will be using to train and test our models.

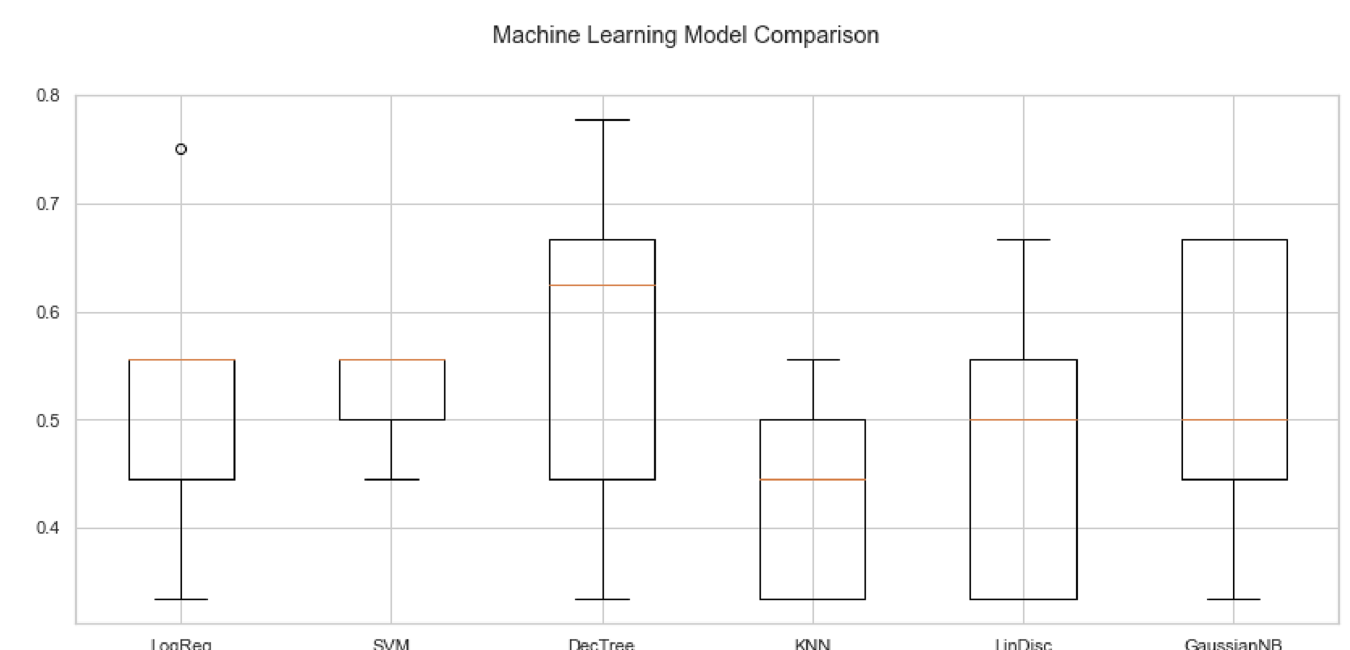
Results-

Models: Classification and Prediction -

Testing models on our inital dataset with all classes and all genes:

Below see a summary of the few models we tested on our full dataset; across all patients, during all time intervals, and including all 33,297 genes.

Note that the results for all models hover around 50% with the decision tree model performing the best on this dataset.



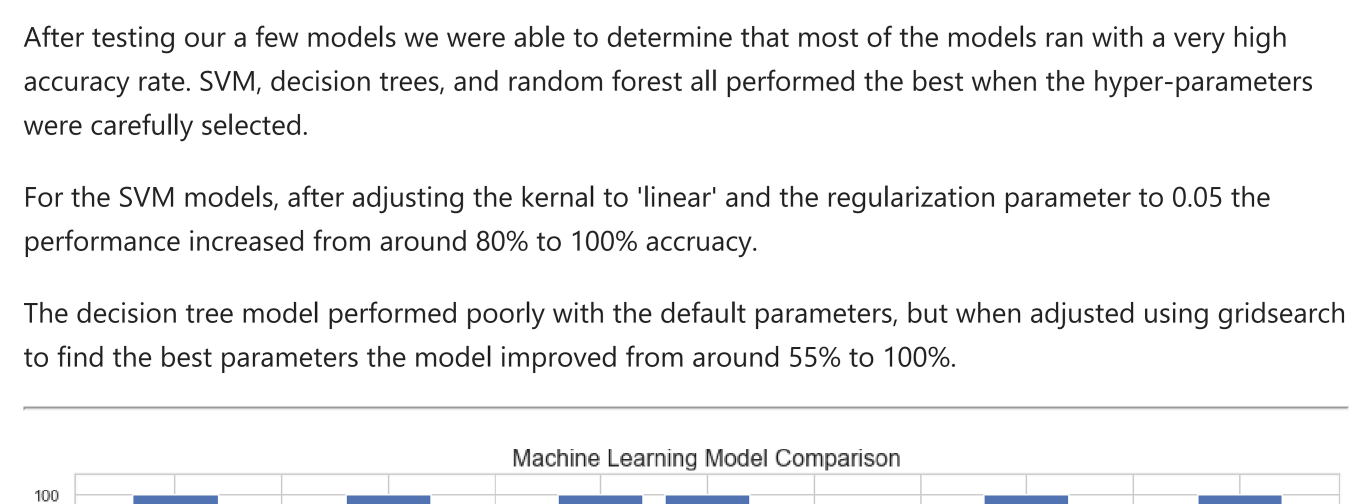
Testing on the updated dataset: two classes - Third trimester vs post-partem and top genes only:

Now our data is are ready to be used to begin testing various machine learning classification models.

After testing our a few models we were able to determine that most of the models ran with a very high accuracy rate. SVM, decision trees, and random forest all performed the best when the hyper-parameters were carefully selected.

For the SVM models, after adjusting the kernal to 'linear' and the regularization parameter to 0.05 the performance increased from around 80% to 100% accruacy.

The decision tree model performed poorly with the default parameters, but when adjusted using gridsearch to find the best parameters the model improved from around 55% to 100%.



Example: Updated SVM model -

Model improved from 77% to 100% with adjusted parameter

Updated the below parameters: Kernal = 'linear' C / regularization = 0.05

1.00 accuracy with a standard deviation of 0.00

```
Predicted  3  4
Actual
3          4  0
4          0  5
Accuracy = 1.0

          precision    recall  f1-score   support

       3       1.00      1.00      1.00         4
       4       1.00      1.00      1.00         5

accuracy                           1.00         9
```

Discussion -

It is clear to see that the performance of each model improved in the seconard part of our report after we adjusted the dataframe to test between just two classes. Determining the top genes with the most variance between these two classes also greatly improved the performance of our models.

Most models performed at nearly 100% accuracy when predicting between class 3 and class 4 (3rd trimester vs postpartem). It is important to note that model parameter tuning is an important part of creating any machine learning models. All datasets will require different model tuning and therefore it is often not known which model and which parameters will be the best choice. Thus, it is important to experiment with running different models and to utilize methods like grid search to find the best parameter for a given model.

All of the models that we ran on our final dataset can be useful for determining the expected expression levels for patients at later stages of pregnancy vs patients who are not pregnant. Note that the postpartem samples represent a baseline level due to the immediate drop in fetal cfRNA after delivery. These models can also be used to train the expected levels during early pregnancy and middle pregnancy as well, and then could be used to predict if a woman is pregnant and at which stage she is in. More data processing would need to be done first to determine the top differentiated genes in each of those stages to see if the fluctuate during different stages of pregnancy.

The models could then be adjusted and potentially used to determine the health of the fetus based on the expected level of transcript expression vs the acutal level for an individual at a specific point and time during her pregnancy.

After running all the models, we have learned that there are various ways you can look at the dataset. While the paper observed the data differently, we both have a similar consensus. The data shows more genes are expressed towards the end of pregnancy. Even from our PCA plot there is a clear distinction between third and post-partum classes. It would have been interesting to have the demographics of the pregnant woman to see how a variable such as aging effects expression. From the 10 differentially expressed genes we observed, most are involved in brain and epithelial tissue development.

References -

Koh W, Pan W, Gawad C, Fan HC et al. Noninvasive in vivo monitoring of tissue-specific global gene expression in humans. Proc Natl Acad Sci U S A 2014 May 20;111(20):7361-6. PMID: 24799715

Scikit-learn: Machine Learning in Python, Pedregosa et al., JMLR 12, pp. 2825-2830, 2011.

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Supplementary document: Python code -

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Load the libraries

In [5]:
```
import pandas as pd
import numpy as np
from sklearn.metrics import accuracy_score
import matplotlib.pyplot as plt
%matplotlib inline
import seaborn as sns
import warnings
import scipy.stats as stats
from sklearn.preprocessing import MinMaxScaler
from sklearn import svm
from sklearn.svm import SVC
from sklearn.linear_model import LogisticRegression
from sklearn import metrics
from sklearn.model_selection import ShuffleSplit
from sklearn.preprocessing import scale
from sklearn.model_selection import cross_val_score
from sklearn.metrics import classification_report
from sklearn.metrics import accuracy_score
from sklearn.svm import SVC
from sklearn.neighbors import KNeighborsClassifier
from sklearn.naive_bayes import GaussianNB
from sklearn.tree import DecisionTreeClassifier
from sklearn.discriminant_analysis import LinearDiscriminantAnalysis
from sklearn import model_selection
from sklearn.metrics import accuracy_score, plot_confusion_matrix
from sklearn.linear_model import LinearRegression
from sklearn.neural_network import MLPClassifier
```

In [6]:
```
# Ignore unimportant warnings
def fxn():
    warnings.warn("deprecated", DeprecationWarning)
warnings.filterwarnings("ignore")
with warnings.catch_warnings():
    warnings.simplefilter("ignore")
    fxn()
```

Import the Data

The data consists of 48 samples (12 patients) over the course of 5 stages of pregnancy: first, second, and third trimester, postpartem, and control/non-pregnant

Microarray data of cell free RNA was collected across 33,297 genes for each patient during each of these time courses

In [36]:
```
# import the data
df = pd.read_csv('GSE56899_series_matrix.txt',sep = '\t')
# define the column names
columns = ['ID_REF','pt1_t1', 'pt1_t2', 'pt1_t3', 'pt1_pp', 'pt2_t1', 'pt2_t2', 'pt2_t
           'pt11_t3', 'pt11_pp', 'pt12_control', 'pt12_control', 'pt12_control', 'pt12

# set the column names
df.columns = columns

# set the index column
df.set_index('ID_REF', inplace = True)
```

Data Prepocessing

We started with the standard data preprocessing steps; removing any missing values in the data, and transposing the dataset to be the proper shape needed for running our models

In [37]:
```
# remove any missing values
df.dropna(inplace=True)
# transpose the dataframe
df_t = df.transpose()
df_t.describe()
```

Out[37]:

ID_REF	7892501	7892502	7892503	7892504	7892505	7892506	7892507	7892508	7892509	78
count	48.000000	48.000000	48.000000	48.000000	48.000000	48.000000	48.000000	48.000000	48.000000	48.
mean	5.004943	2.482006	2.561178	7.310324	2.787235	2.605373	3.165746	2.243372	10.500638	2.
std	1.853566	0.287033	0.247941	1.055773	0.425361	0.404084	0.727252	0.186912	1.742822	0.
min	2.089713	1.938133	1.982890	5.428908	2.102378	2.122335	2.367414	1.923975	2.718209	2.
25%	3.286410	2.287814	2.390578	6.533863	2.540726	2.351941	2.791254	2.106138	10.338530	2.
50%	5.519676	2.446147	2.514482	7.021481	2.696597	2.464081	2.975391	2.231955	10.952625	2.
75%	6.390771	2.619133	2.759148	8.047929	2.965962	2.745546	3.281732	2.346940	11.409250	2.
max	9.105850	3.351691	3.142344	10.706420	4.643989	4.226218	7.068907	2.800776	11.993470	3.

8 rows × 33297 columns

Normalize the data across samples -

In order to be able to compare our samples against each other we needed to normalize the data or account for any outliers. Here we used Log2 to normalize the data.

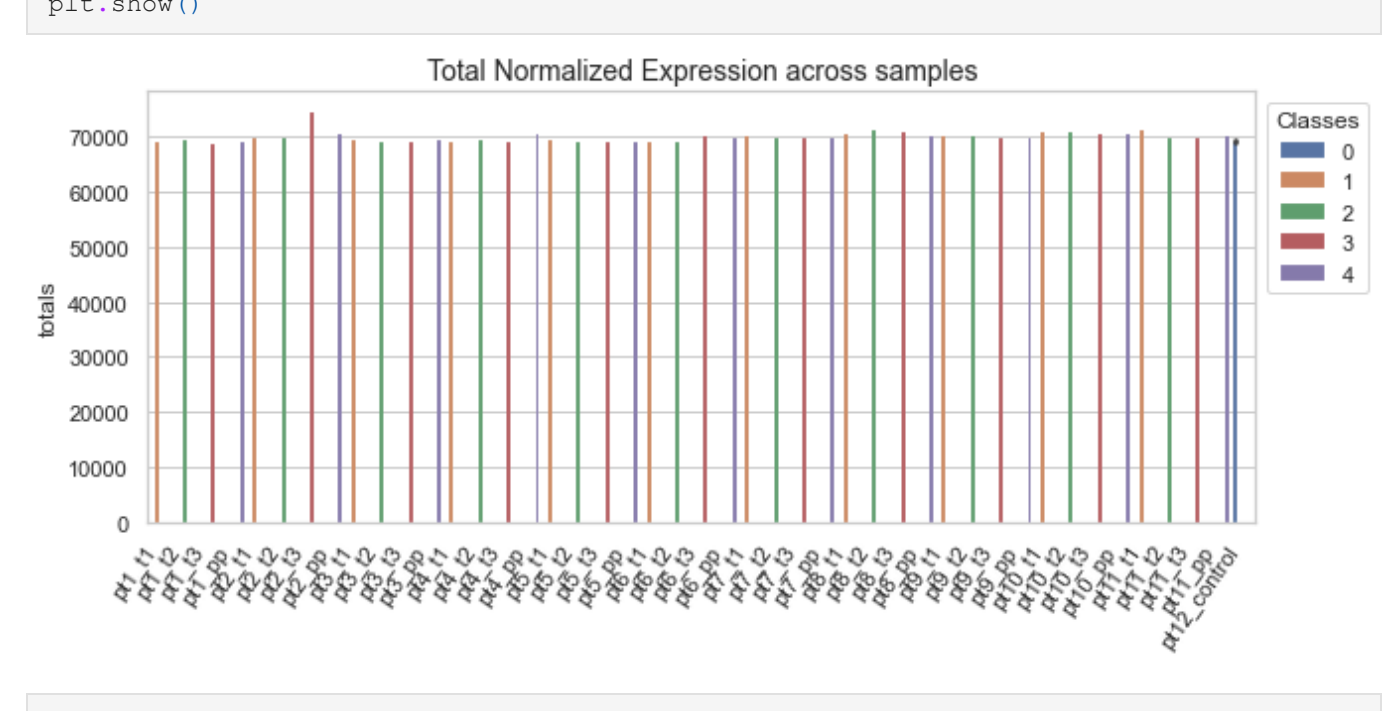
In [38]:
```
# normalize across the data to see how the samples compare to each other (look for ou
df_t = df_t.applymap(np.log2)
```

In [39]:
```
# Add our class/target column - trimester 1, trimester 2, trimester 3, post-partem, a
y = [1, 2, 3, 4] * 11
y1 = [0, 0, 0, 0]
y = y + y1

# add our classes/ taget col to the df_t dataframe
df_t['y'] = y
y = df_t['y'].values

# get our X and y values
X_labels = df_t.columns[0:33297]
X = np.array(df_t[X_labels].values)
print(X.shape)
print(y.shape)
```

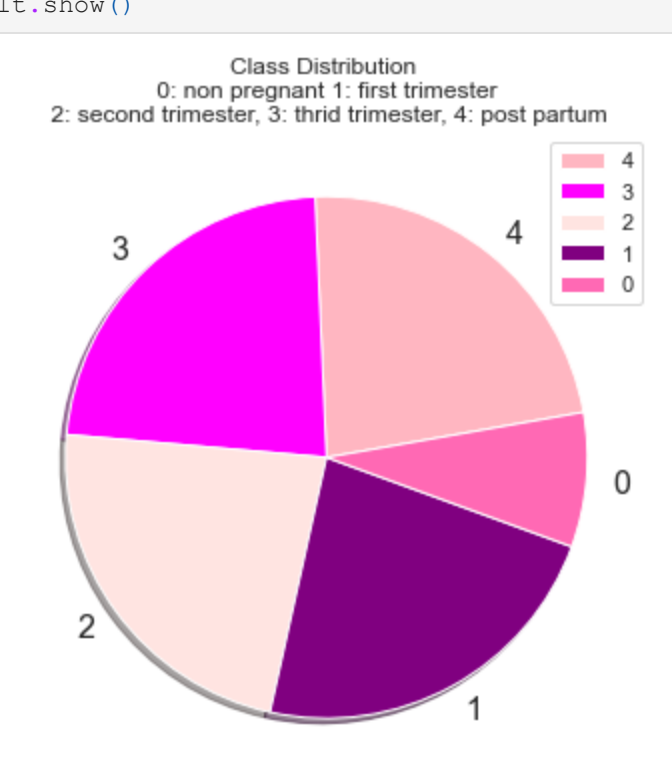
```
(48, 33297)
(48,)
```

In [40]:
```
# get the totals by row
df_t['totals'] = df_t.sum(axis=1)
```

In [41]:
```
# Plot the normalized differences between samples
plt.figure(figsize=(10,4))
sns.set_theme(style = 'whitegrid')
sns.barplot(x=df_t.index, y='totals', hue = 'y', data=df_t,
            edgecolor='1', ci = 'sd', linewidth= .1)
plt.xlabel='samples'
plt.ylabel='total expression'
plt.title('Total Normalized Expression across samples', fontsize='14')
plt.xticks(rotation=55, ha='right')
plt.legend(title = 'Classes', bbox_to_anchor = (1, 1))
plt.show()
```



In [42]:
```
# see our class distribution
colors = ['lightpink','magenta', 'mistyrose', 'purple', 'hotpink']
plt.figure(figsize = (6,6))
classes = (df_t[df_t.y != -1])['y'].value_counts()
classes.plot.pie(colors = colors, shadow = True, startangle = 10,
                 title = 'Class Distribution \n 0: non pregnant 1: first trimester \n
plt.legend()
plt.show()
```



Further data processing

After reviewing the data in more detail, we were able to determine that additional preprocessing was need.

We decided to select the genes with the highest variability between samples and/or between time periods of the same sample in order to make any meaningful analysis. We noted that the highest variablity between the classes were between the third trimester and post-partem.

Next, we seperated the data by patient, and then caclculated the differential expression values for each patient from the thirs trimester and post-partem. Note; we eliminated the control patient 12 from our study at this point.

In [43]:
```
# seperate out info for each patient
pt1 = df_t[:4]
pt2 = df_t[4:8]
pt3 = df_t[8:12]
pt4 = df_t[12:16]
pt5 = df_t[16:20]
pt6 = df_t[20:24]
pt7 = df_t[24:28]
pt8 = df_t[28:32]
pt9 = df_t[32:36]
pt10 = df_t[36:40]
pt11 = df_t[40:44]
#pt12 = X[44:48] # non-pregnant/control samples
```

In [44]:
```
# differential expressions between for each pt - from trimester 3 and postpartum
pt1.loc['1'] = pt1.loc['pt1_t3']-pt1.loc['pt1_pp']
pt2.loc['2'] = pt2.loc['pt2_t3']-pt2.loc['pt2_pp']
pt3.loc['3'] = pt3.loc['pt3_t3']-pt3.loc['pt3_pp']
pt4.loc['4'] = pt4.loc['pt4_t3']- pt4.loc['pt4_pp']
pt5.loc['5'] = pt5.loc['pt5_t3']-pt5.loc['pt5_pp']
pt6.loc['6'] = pt6.loc['pt6_t3']-pt6.loc['pt6_pp']
pt7.loc['7'] = pt7.loc['pt7_t3']-pt7.loc['pt7_pp']
pt8.loc['8'] = pt8.loc['pt8_t3']-pt8.loc['pt8_pp']
pt9.loc['9'] = pt9.loc['pt9_t3']-pt9.loc['pt9_pp']
pt10.loc['10'] = pt10.loc['pt10_t3']-pt10.loc['pt10_pp']
pt11.loc['11'] = pt11.loc['pt11_t3']-pt11.loc['pt11_pp']
```

In [45]:
```
#filter out the differential expression
p1 = pt1.filter(['1'], axis=0)
p2 = pt2.filter(['2'], axis=0)
p3 = pt3.filter(['3'], axis=0)
p4 = pt4.filter(['4'], axis=0)
p5 = pt5.filter(['5'], axis=0)
p6 = pt6.filter(['6'], axis=0)
p7 = pt7.filter(['7'], axis=0)
p8 = pt8.filter(['8'], axis=0)
p9 = pt9.filter(['9'], axis=0)
p10 = pt10.filter(['10'], axis=0)
p11 = pt11.filter(['11'], axis=0)
```

In [46]:
```
#combine dataframes back together
frames = [p1,p2,p3,p4,p5,p6,p7,p8,p9,p10,p11]
de = pd.concat(frames)

#remove 'y' from the dataframe
de = de.drop('y', axis = 1)
```

Determine genes with highest differential expression between the two classes -

Our dataset contains over 30,000 genes, this makes predictions challenging. In order to reduce the number of genes in our dataset we decided to focus on just the top 30 genes with the highest differential expression between the thrid trimester and post-partem samples for each patient.

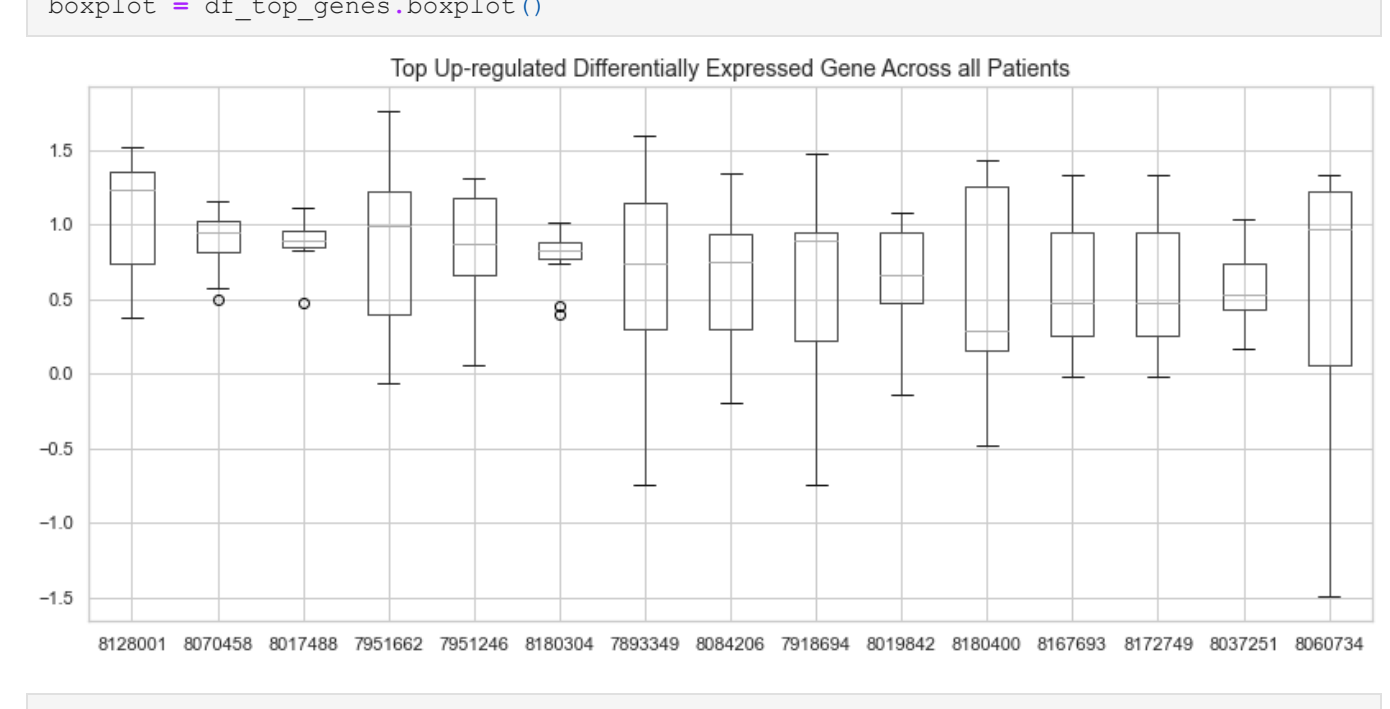
In [47]:
```
# remove any missing values
de.dropna(inplace=True)

df_4 = de.sum()
print(df_4.nlargest(15).index)

df_5 = de.sum()
print(df_4.nsmallest(15).index)
```

```
Index(['totals',  8128001,  8070458,  8017488,  7951662,  7951246,  8180304,
        7893349,  8084206,  7918694,  8019842,  8180400,  8167693,  8172749,
        8037251],
      dtype='object', name='ID_REF')
Index([8174251, 7894627, 8021147, 7895904, 7945040, 7943160, 7930870, 7896069,
       7896704, 8088911, 8035793, 7896123, 7893549, 8020806, 7896558],
      dtype='object', name='ID_REF')
```

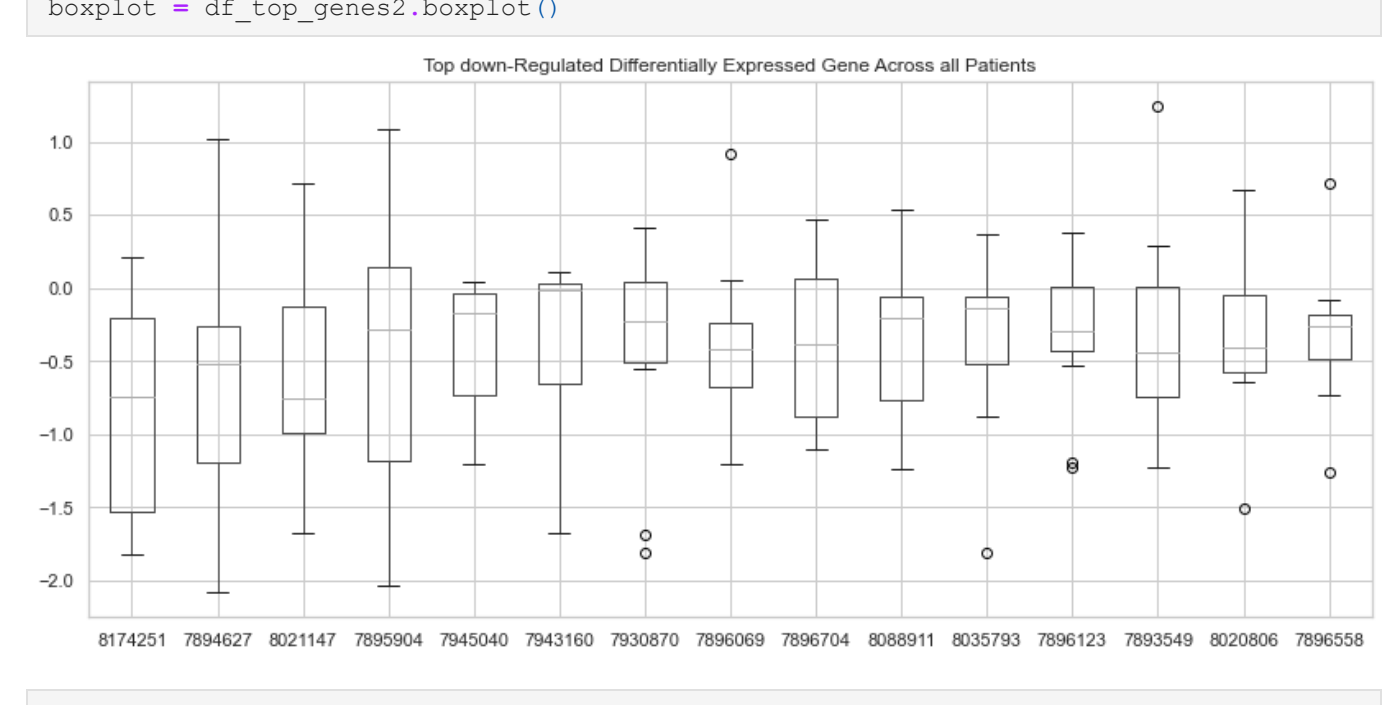
Data Visualization

Lets see how the top genes are expressed across the samples

In [48]:
```
#sorting for all normalized highest and lowest DE genes
df_top_genes = de[[8128001, 8070458, 8017488, 7951662, 7951246, 8180304, 7893349, 808
        7918694, 8019842, 8180400, 8167693, 8172749, 8037251, 8060734]]
df_top_genes = df_top_genes.assign(pt = ['1', '2', '3', '4','5','6','7','8','9','10',
#plot boxplot for each gene
import seaborn as sns
fig = plt.figure(figsize =(14, 6))
plt.title('Top Up-regulated Differentially Expressed Gene Across all Patients', fonts
boxplot = df_top_genes.boxplot()
```

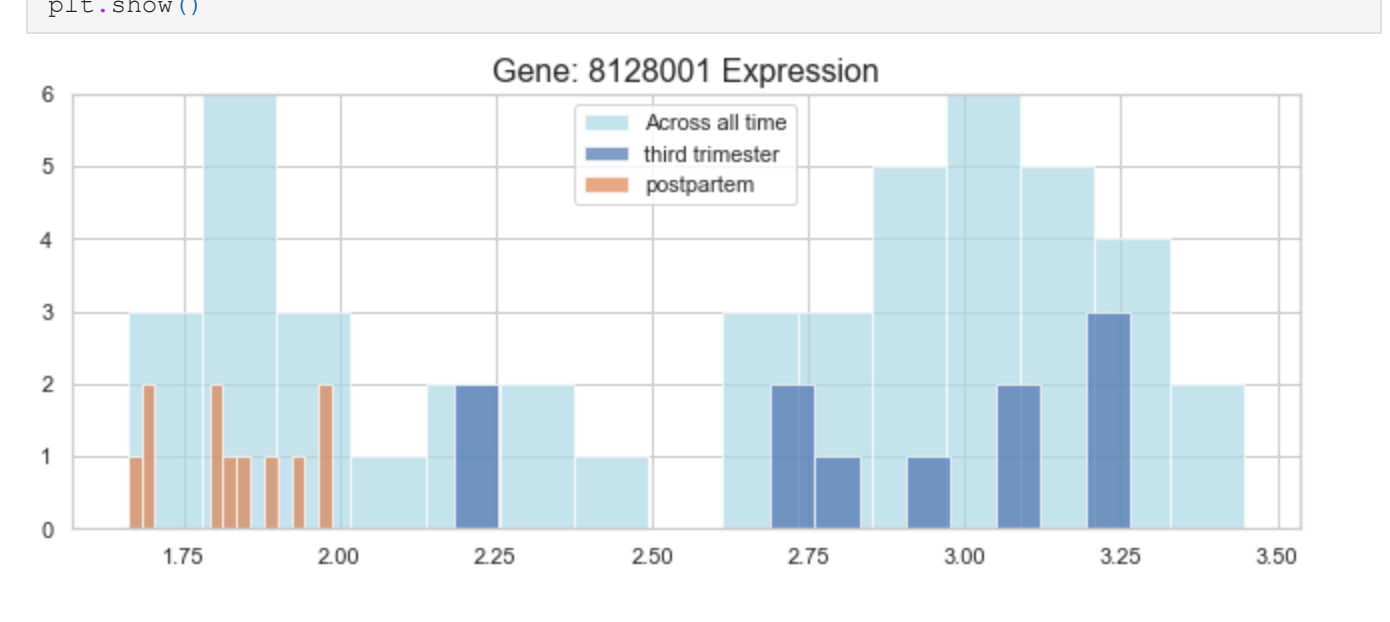


In [49]:
```
#sorting for all normalized highest expression genes
df_top_genes2 = de[[8174251, 7894627, 8021147, 7895904, 7945040, 7943160, 7930870, 78
        7896704, 8088911, 8035793, 7896123, 7893549, 8020806, 7896558]]
df_top_genes2 = df_top_genes2.assign(pt = ['1', '2', '3', '4','5','6','7','8','9','10
#plot boxplot for each gene
import seaborn as sns
fig = plt.figure(figsize =(14, 6))
plt.title('Top down-Regulated Differentially Expressed Gene Across all Patients')
boxplot = df_top_genes2.boxplot()
```



In [50]:
```
# sort the dataframe by y
sorted_df = df_t.sort_values(by=['y'], ascending=False)

# break down the data by time interval
pp = sorted_df[:11]
tri3 = sorted_df[11:22]
tri2 = sorted_df[22:33]
tri1 = sorted_df[33:44]
control = sorted_df[44:48]
```

In [51]:
```
gene1 = df_t[8128001]
plt.figure(figsize = (11,4))
plt.hist(gene1.values, bins=15, color='lightblue', alpha=0.7, label='Across all time')
#plt.hist(tri1[8128001], bins=15, alpha=0.7, label='First trimester')
#plt.hist(tri2[8128001], bins=15, alpha=0.7, label='second trimester')
plt.hist(tri3[8128001], bins=15, alpha=0.7, label='third trimester')
plt.hist(pp[8128001], bins=15, alpha=0.7, label='postpartem')
plt.ylim(0,6) # Cut off at N=100 for visual clarity
plt.xlabel='samples' # label our axes
plt.ylabel='8128001 expression values'
plt.title('Gene: 8128001 Expression', fontsize='16')
plt.legend()
plt.show()
```



Putting it all together- Creating our final dataframe:

At this point we finished with the data preprocessing steps and are almost ready to start creating our models. First we have to create our final dataframe with the new curated data from the above steps. Our dataframe will now only include the top 22 genes and sample data from the third trimester and post-partem.

In [52]:
```
# Now recreate the dataframe with just the top 30 DE genes included
top_de_df = df_t[[8128001, 8070458, 8017488, 7951662, 7951246, 8180304, 7893349, 8084
              7918694, 8019842, 8180400, 8167693, 8172749, 8037251, 8060734, 8174251,
              7894627, 8021147, 7895904, 7945040, 7943160, 7930870, 7896069,
              7896704, 8088911, 8035793, 7896123, 7893549, 8020806, 7896558]]

top_de_df.head(3)
```

Out[52]:

ID_REF	8128001	8070458	8017488	7951662	7951246	8180304	7893349	8084206	7918694	8019842
pt1_t1	3.183323	2.059436	2.760217	1.796702	1.81552	2.704527	2.721869	1.536305	1.675357	2.657084
pt1_t2	3.095638	2.937659	3.260248	1.680922	1.81646	3.238912	2.351149	1.602896	1.993895	2.578314
pt1_t3	3.110749	2.788043	3.399286	2.982963	2.81963	3.334697	2.896474	2.467626	2.920777	2.927450

3 rows × 30 columns

In [53]:
```
# get rid of the control/non-preg sample
top_de_df = top_de_df.iloc[:-4]
```

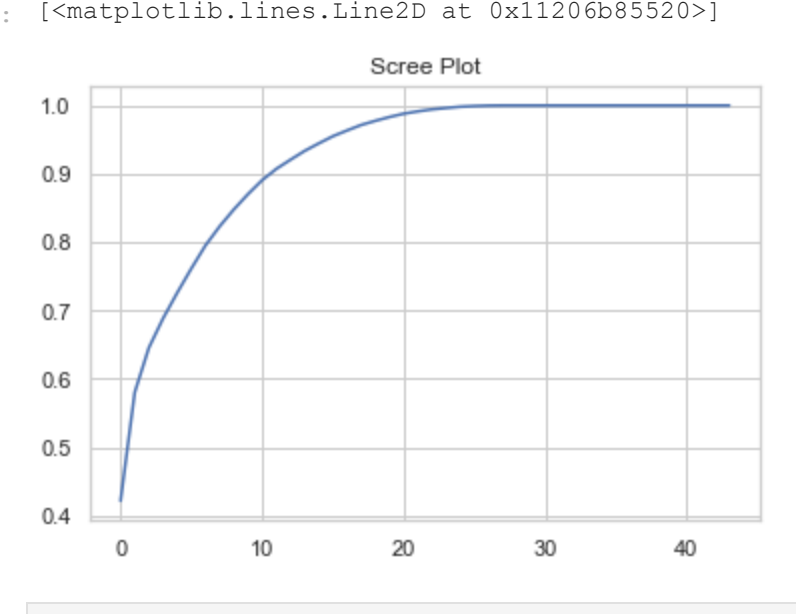
Some additional processing/ visualization step - PCA

In [54]:
```
from sklearn.preprocessing import StandardScaler
from sklearn.decomposition import PCA

# top_de_df.reset_index()
top_transformed=top_de_df.T
#top_transformed
scaler = StandardScaler()
X = scaler.fit_transform(top_de_df.T)
cmat=np.cov(X.T)
eigen_vals, eigen_vecs = np.linalg.eig(cmat)
print(eigen_vals)
```

```
[ 1.91794621e+01+0.00000000e+00j  7.22504289e+00+0.00000000e+00j
  2.97617710e+00+0.00000000e+00j  1.94886231e+00+0.00000000e+00j
  1.70941887e+00+0.00000000e+00j  1.62042093e+00+0.00000000e+00j
  1.53895311e+00+0.00000000e+00j  1.26707144e+00+0.00000000e+00j
  1.12455424e+00+0.00000000e+00j  1.02149032e+00+0.00000000e+00j
  9.29185145e-01+0.00000000e+00j  7.52639578e-01+0.00000000e+00j
  6.08469534e-01+0.00000000e+00j  5.88395084e-01+0.00000000e+00j
  5.01804496e-01+0.00000000e+00j  4.78543077e-01+0.00000000e+00j
  3.82559259e-01+0.00000000e+00j  3.67303979e-01+0.00000000e+00j
  2.76026382e-01+0.00000000e+00j  2.53171743e-01+0.00000000e+00j
  2.21283132e-01+0.00000000e+00j  1.54559109e-01+0.00000000e+00j
  1.32628186e-01+0.00000000e+00j  8.54943873e-02+0.00000000e+00j
  9.21131315e-02+0.00000000e+00j  4.36182217e-02+0.00000000e+00j
  2.89340086e-02+0.00000000e+00j  9.05960517e-03+0.00000000e+00j
 -6.47347131e-16+0.00000000e+00j -5.02974430e-16+1.18285119e-16j
 -5.02974430e-16-1.18285119e-16j  6.20435408e-16+0.00000000e+00j
  4.70849549e-16+7.08798937e-17j  4.70849549e-16-7.08798937e-17j
 -3.60555704e-16+0.00000000e+00j  2.37258584e-16+1.52561931e-16j
  2.37258584e-16-1.52561931e-16j  3.11860466e-16+0.00000000e+00j
  2.48295314e-16+0.00000000e+00j  1.37718457e-16+0.00000000e+00j
 -3.85135051e-17+0.00000000e+00j -1.34593128e-16+4.86629820e-17j
 -1.34593128e-16-4.86629820e-17j -1.85932496e-16+0.00000000e+00j]
```

In [55]:
```
tot = sum(eigen_vals)
ve = [(i/tot) for i in sorted(eigen_vals, reverse=True)]
cum_var_exp = np.cumsum(ve)

#plt.ylabel('% Variance Explained')
#plt.xlabel('# of Features')
plt.title('Scree Plot')

plt.plot(cum_var_exp)
```

Out[55]: `[<matplotlib.lines.Line2D at 0x11206b85520>]`

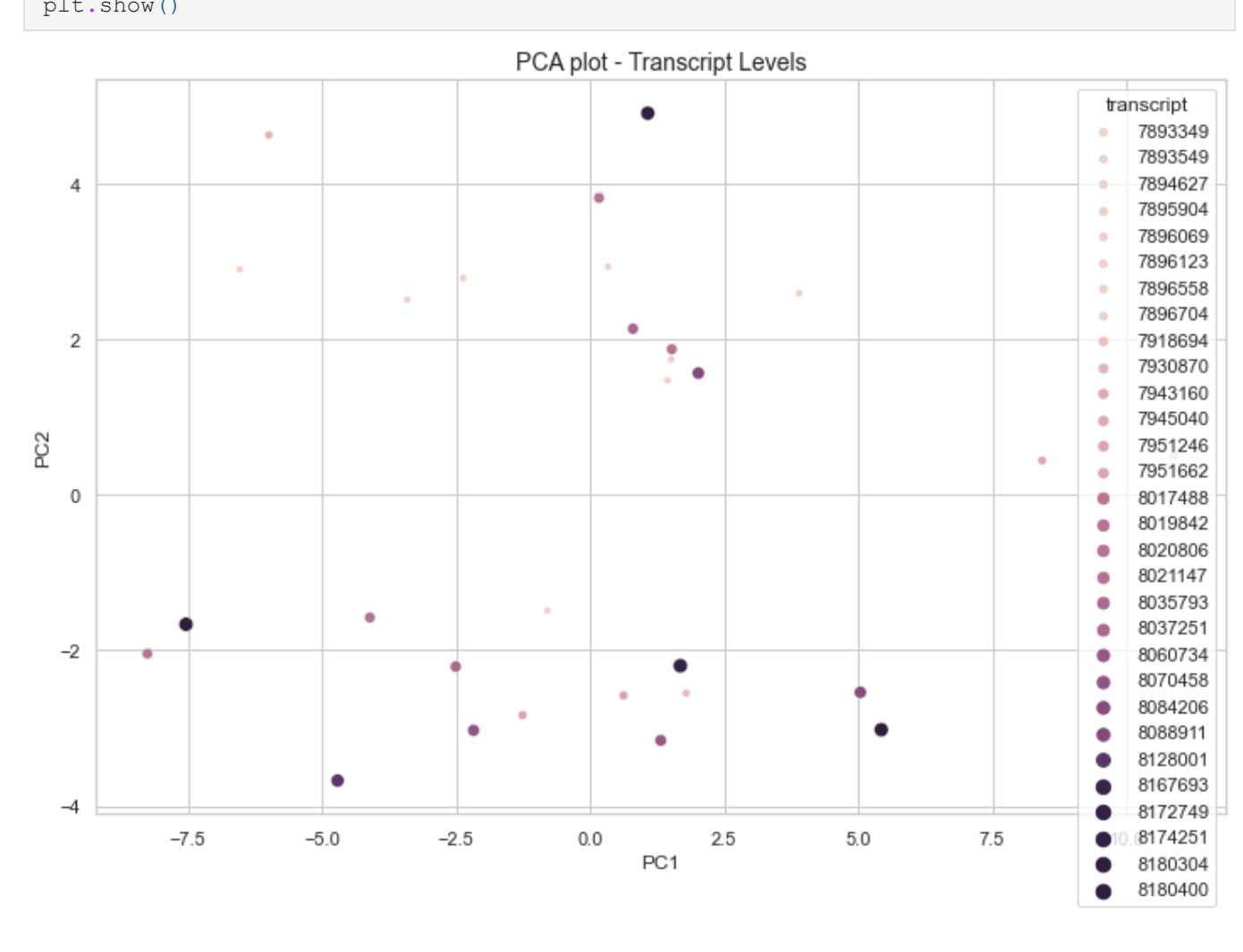


In [56]:
```
eigen_pairs = [(np.abs(eigen_vals[i]),eigen_vecs[:,i]) for i in range(len(eigen_vals))
eigen_pairs.sort(key=lambda k: k[0],reverse=True)

arr = np.hstack((eigen_pairs[0][1][:,np.newaxis],
            eigen_pairs[1][1][:,np.newaxis]))
#arr
```

In [57]:
```
X_pca = X.dot(arr)
pca = PCA(n_components=2)
Pc=pca.fit_transform(X)
pca_df = pd.DataFrame(data = Pc, columns = ['PC1', 'PC2'])
y=[8128001, 8070458, 8017488, 7951662, 7951246, 8180304, 7893349, 8084206,
              7918694, 8019842, 8180400, 8167693, 8172749, 8037251, 8060734, 8174251,
              7894627, 8021147, 7895904, 7945040, 7943160, 7930870, 7896069,
              7896704, 8088911, 8035793, 7896123, 7893549, 8020806, 7896558]

d = pd.DataFrame(data=y, columns = ['transcript'])
pca_df1 = pd.concat([d, pca_df], axis=1)
print(pca_df1)
```

```
    transcript        PC1       PC2
0      8128001  -4.708409 -3.674894
1      8070458  -2.173753 -3.028606
2      8017488  -8.253939 -2.043587
3      7951662   0.620275 -2.581843
4      7951246  -1.257806 -2.833163
5      8180304  -7.533641 -1.666000
6      7893349  -0.793591 -1.491318
7      8084206   5.038818 -2.540674
8      7918694   1.790848 -2.551993
9      8019842  -4.105855 -1.579047
10     8180400   5.428413 -3.021046
11     8167693   1.682236 -2.199051
12     8172749   1.682236 -2.199051
13     8037251  -2.506357 -2.209810
14     8060734   1.316472 -3.157806
15     8174251   1.073885  4.904768
16     7894627   3.896112  2.589029
17     8021147   0.165358  3.816341
18     7895904   1.511030  1.741815
19     7945040   8.428839  0.441460
20     7943160  10.886346  0.503732
21     7930870  -5.990301  4.623091
22     7896069  -3.410216  2.507395
23     7896704  -2.366791  2.785648
24     8088911   2.017833  1.562973
25     8035793   0.797581  2.133118
26     7896123  -6.534514  2.897198
27     7893549   0.334561  2.931906
28     8020806   1.523395  1.870350
29     7896558   1.440934  1.469068
```

In [58]:
```
plt.figure(figsize = (12,8))
sns.scatterplot( x="PC1", y="PC2",
                data=pca_df1,
                hue = 'transcript',
                size='transcript',
                legend='full')
plt.title('PCA plot - Transcript Levels', fontsize=(14))
plt.show()
```



Models: Testing on the full dataset - all classes (minus control class)

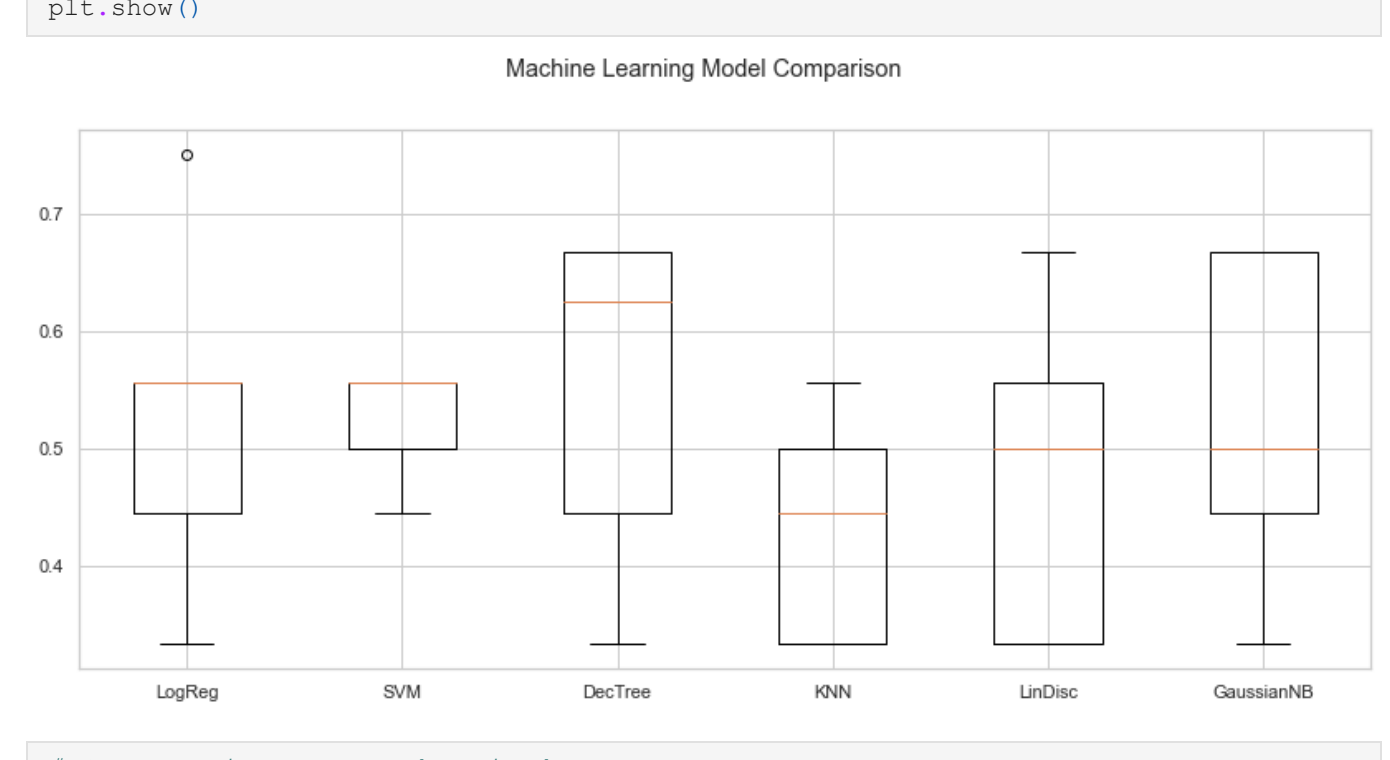
We begin by running various classification models on all the classes to see how they perform, and what areas we might need to improve upon.

In [59]:
```
# add in our y
y1 = [1,2,3, 4] * 11

# add our classes/ taget col to the new dataframe
top_de_df['y'] = y1
y1 = top_de_df['y'].values



# get our X and y values
X_labels = top_de_df.columns[0:30]
X1 = np.array(top_de_df[X_labels].values)
```

In [60]:
```
# run a few standard classification models with default parameters and kfold cv
outcome = []
model_names = []
models = [('LogReg', LogisticRegression()),
          ('SVM', SVC()),
          ('DecTree', DecisionTreeClassifier()),
          ('KNN', KNeighborsClassifier()),
          ('LinDisc', LinearDiscriminantAnalysis()),
          ('GaussianNB', GaussianNB())]

for model_name, model in models:
    k_fold_validation = model_selection.KFold(n_splits=5)
    results = model_selection.cross_val_score(model, X1, y1, cv=k_fold_validation, sco
    outcome.append(results)
    model_names.append(model_name)
    output_message = "%s| Mean=%f STD=%f" % (model_name, results.mean(), results.std()
    print(output_message)
```

```
LogReg| Mean=0.527778 STD=0.138332
SVM| Mean=0.522222 STD=0.044444
DecTree| Mean=0.547222 STD=0.134830
KNN| Mean=0.433333 STD=0.088889
LinDisc| Mean=0.477778 STD=0.129577
GaussianNB| Mean=0.522222 STD=0.129577
```

In [61]:
```
fig = plt.figure(figsize=(14,6))
fig.suptitle('Machine Learning Model Comparison')
ax = fig.add_subplot(111)
plt.boxplot(outcome)
ax.set_xticklabels(model_names)
plt.show()
```



In [62]:
```
# Seperate into test and train data
from sklearn.model_selection import train_test_split

X_train, X_test, y_train, y_test = train_test_split(
    X1, y1, test_size=0.3)
```

In [63]:
```
# scaling the features - standardize
from sklearn.preprocessing import StandardScaler

scaler = StandardScaler()
scaler.fit(X_train)
X_train_std = scaler.transform(X_train)
X_test_std = scaler.transform(X_test)
```

In [43]:
```
svc = svm.SVC(kernel='linear', C =0.5)
svc.fit(X_train_std, y_train)

y_hat = svc.predict(X_test_std)

# Calculate accuracy
print(pd.crosstab(y_test, y_hat, rownames=['Actual'], colnames=['Predicted']))
print('Accuracy =', accuracy_score(y_test, y_hat))

print(classification_report (y_test, y_hat))
```

```
Predicted  1  2  4
Actual
1          2  1  0
2          1  1  0
3          2  3  0
4          0  1  3
Accuracy = 0.42857142857142855
              precision    recall  f1-score   support

           1       0.40      0.67      0.50         3
           2       0.17      0.50      0.25         2
           3       0.00      0.00      0.00         5
           4       1.00      0.75      0.86         4

    accuracy                           0.43        14
   macro avg       0.39      0.48      0.40        14
weighted avg       0.40      0.43      0.39        14
```

In [44]:
```
# Update the SVC model to see if we can get a better performance
svc1 = svm.SVC(kernel='linear', C =.05, gamma='auto')  # changed regularization, kern
svc1.fit(X_train_std, y_train)

# add in cross validation
scores1 = cross_val_score(svc1, X_test_std, y_test, cv=4)
print(scores1)
print("%0.2f accuracy with a standard deviation of %0.2f" % (scores1.mean(), scores1.s

y_hat1 = svc1.predict(X_test_std)

# Calculate accuracy
print(pd.crosstab(y_test, y_hat1, rownames=['Actual'], colnames=['Predicted']))
print('Accuracy =', accuracy_score(y_test, y_hat1))

print(classification_report (y_test, y_hat1))
```

```
[0.5 0.5 0.  1. ]
0.50 accuracy with a standard deviation of 0.35
Predicted  1  2  4
Actual
1          2  1  0
2          1  1  0
3          2  3  0
4          0  1  3
Accuracy = 0.42857142857142855
              precision    recall  f1-score   support

           1       0.40      0.67      0.50         3
           2       0.17      0.50      0.25         2
           3       0.00      0.00      0.00         5
           4       1.00      0.75      0.86         4

    accuracy                           0.43        14
   macro avg       0.39      0.48      0.40        14
weighted avg       0.40      0.43      0.39        14
```

In [45]:
```
# Create decision tree with adaboost classifer
from sklearn.ensemble import AdaBoostClassifier
from sklearn.tree import DecisionTreeClassifier
from sklearn.metrics import confusion_matrix

# Create a decision tree object
tree = DecisionTreeClassifier()

# Fit on training data
tree.fit(X_train_std, y_train)
abc = AdaBoostClassifier(base_estimator=tree)

# Train Adaboost Classifer
model = abc.fit(X_train_std, y_train)

#Predict the response with test dataset
y_pred = model.predict(X_test_std)

print("Accuracy for Decision Tree model: ", accuracy_score(y_test,y_pred)*100) ## Sti

# Make a Confusion Matrix
print(pd.crosstab(y_test, y_pred, rownames=['Actual'], colnames=['Predicted']))
```

```
Accuracy for Decision Tree model:  42.857142857142854
Predicted  1  2  3  4
Actual
1          2  1  0  0
2          1  1  0  0
3          0  4  1  0
4          2  0  0  2
```

In [59]:
```
# Try Gridsearch to tune the hyperparameters to see if we get better accuracy
from sklearn.model_selection import GridSearchCV
tree_param = {'criterion':['gini','entropy'],
              'max_depth':[2,3,4,5,6,7,8,9,10,11,12,15,20],
              'min_samples_split':[3,4,5,10,20,30,40,50,60,70,80,90,100],
              'min_samples_leaf':[2,3,4,5,6,7,8,9,10],
              'max_features':list(range(1,X_train_std.shape[1])),
              'max_features': ['auto', 'sqrt', 'log2'],
              'presort':[True, False]
             }
grid = GridSearchCV(DecisionTreeClassifier(), tree_param, cv=4)
grid.fit(X_train_std, y_train)
#best_predict = grid.best_score_(y_test)
print('Best Accuracy = ',grid.best_score_)
print(grid.best_params_)
print(grid.best_estimator_)
#print(pd.crosstab(y_test, best_predict, rownames=['Actual'], colnames=['Predicted']))
```

```
Best Accuracy =  0.6696428571428572
{'criterion': 'gini', 'max_depth': 4, 'max_features': 'sqrt', 'min_samples_leaf': 3,
'min_samples_split': 5, 'presort': False}
DecisionTreeClassifier(max_depth=4, max_features='sqrt', min_samples_leaf=3,
                       min_samples_split=5, presort=False)
```

In [60]:
```
# Now use adaboost on the gridsearch results to see if we can get an even better pred
tree = grid.best_estimator_
tree.fit(X_train_std, y_train)

# Create adaboost classifer object
abc = AdaBoostClassifier(n_estimators=100,
                         learning_rate=10,
                         base_estimator=tree)
# Train Adaboost Classifer
tree2 = abc.fit(X_train_std, y_train)

# Predict the response for test dataset
y_hat2 = tree2.predict(X_test_std)
print("Accuracy:",accuracy_score(y_test, y_hat2))
```

```
Accuracy: 0.21428571428571427
```

In [61]:
```
from sklearn.ensemble import RandomForestClassifier
from sklearn.datasets import make_classification

rfc = RandomForestClassifier(n_estimators = 20,
                             criterion = 'entropy')
rfc.fit(X_train_std, y_train)
y_pred = rfc.predict(X_test_std)

# Making the Confusion Matrix
print(pd.crosstab(y_test, y_pred, rownames=['Actual'], colnames=['Predicted']))
print('Accuracy =', accuracy_score(y_test, y_pred))
```

```
Predicted  1  2  4
Actual
1          1  2  0
2          0  2  0
3          0  5  0
4          0  0  4
Accuracy = 0.5
```

In [48]:
```
lrc = LogisticRegression().fit(X_train_std, y_train)
y_hat4 = lrc.predict(X_test_std)

# Making the Confusion Matrix
print(pd.crosstab(y_test, y_hat4, rownames=['Actual'], colnames=['Predicted']))
print('Accuracy =', accuracy_score(y_test, y_hat4))
```

```
Predicted  1  2  3  4
Actual
1          2  1  0  0
2          1  1  0  0
3          0  4  1  0
4          0  1  0  3
Accuracy = 0.5
```

Testing on just the two classes - Third trimester vs post-partem

In [64]:
```
# Keep just samples from 3rd trimester and post-partem
array = [3, 4]
df_3 = top_de_df.loc[top_de_df['y'].isin(array)]
df_3.head()
```

Out[64]:

ID_REF	8128001	8070458	8017488	7951662	7951246	8180304	7893349	8084206	7918694	8019842
pt1_t3	3.110749	2.788043	3.399286	2.982963	2.819630	3.334697	2.896474	2.467626	2.920777	2.927450
pt1_pp	1.660869	1.844100	2.431636	1.538719	1.633755	2.511137	2.318703	1.475046	1.677346	1.941945
pt2_t3	2.737647	2.789186	3.313469	2.594676	3.441163	3.054447	2.676358	2.808282	3.135397	3.346357
pt2_pp	1.988295	2.218284	2.466469	1.645306	2.403199	2.606033	1.085504	1.464668	2.187160	2.601413
pt3_t3	3.204279	3.087972	3.474054	3.010974	3.255985	3.453194	2.761356	2.424237	3.098977	3.080086

5 rows × 31 columns

Splitting our data -

We split the final dataframe into our target values (y), and feature values (X). Then we further split the data into a training dataset and a testing dataset which we will be using to train and test our models.

In [65]:
```
# Re-create our X and y matrices
y1 = [3, 4] * 11

# add our classes/ taget col to the dataframe
df_3['y'] = y1
y1 = df_3['y'].values

# get our X and y values
X_labels = df_3.columns[0:30]
X1 = np.array(df_3[X_labels].values)

print(y1.shape)
print(X1.shape)
```

```
(22,)
(22, 30)
```

PCA Across patients from 3rd trimester and post-partem

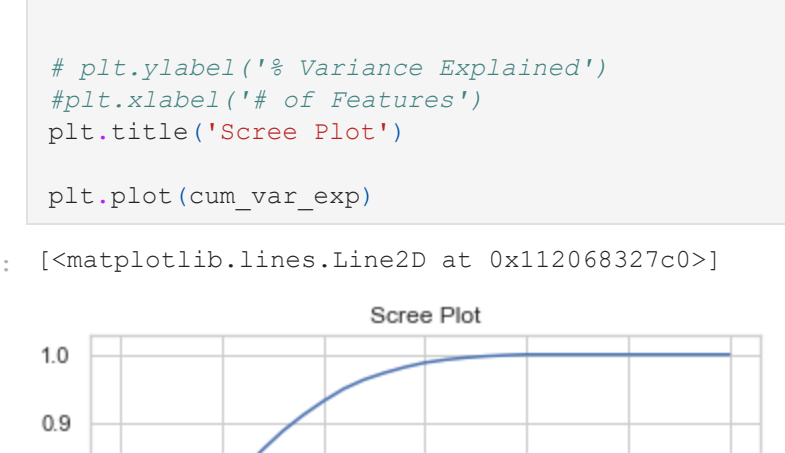
In [66]:
```
scaler = StandardScaler()
X = scaler.fit_transform(df_3)
cmat=np.cov(X.T)
eigen_vals, eigen_vecs = np.linalg.eig(cmat)
print(eigen_vals)
```

```
[ 1.37001268e+01+0.00000000e+00j  3.71963720e+00+0.00000000e+00j
  2.80786931e+00+0.00000000e+00j  2.12881897e+00+0.00000000e+00j
  1.73468595e+00+0.00000000e+00j  1.50600447e+00+0.00000000e+00j
  1.34679103e+00+0.00000000e+00j  1.01050781e+00+0.00000000e+00j
```

```
 8.91575337e-01+0.00000000e+00j  7.54832407e-01+0.00000000e+00j
 6.63000059e-01+0.00000000e+00j  5.84984929e-01+0.00000000e+00j
 4.33279985e-01+0.00000000e+00j  3.20753156e-01+0.00000000e+00j
 2.70728330e-01+0.00000000e+00j  2.15162526e-01+0.00000000e+00j
 1.36730472e-01+0.00000000e+00j  9.88228027e-02+0.00000000e+00j
 7.09368449e-02+0.00000000e+00j  4.75666910e-02+0.00000000e+00j
 3.33754136e-02+0.00000000e+00j  1.05100450e-15+0.00000000e+00j
-4.73778712e-16+0.00000000e+00j -4.49437527e-16+0.00000000e+00j
 2.99865877e-16+0.00000000e+00j  1.25413641e-16+1.55879276e-16j
 1.25413641e-16-1.55879276e-16j  1.26445382e-17+9.08685342e-17j
 1.26445382e-17-9.08685342e-17j -1.23028549e-16+0.00000000e+00j
-2.20538142e-16+0.00000000e+00j]
```

In [67]:
```python
tot = sum(eigen_vals)
ve = [(i/tot) for i in sorted(eigen_vals, reverse=True)]
cum_var_exp = np.cumsum(ve)


# plt.ylabel('% Variance Explained')
#plt.xlabel('# of Features')
plt.title('Scree Plot')

plt.plot(cum_var_exp)
```

Out[67]: [<matplotlib.lines.Line2D at 0x112068327c0>]



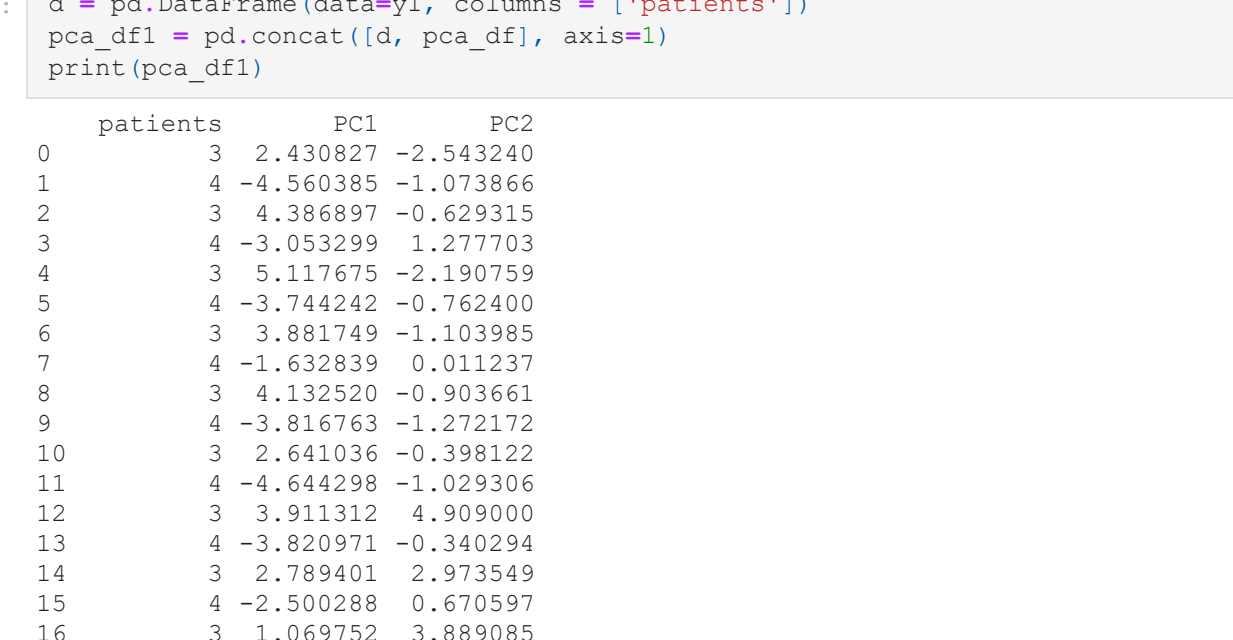
In [68]:
```python
eigen_pairs = [(np.abs(eigen_vals[i]),eigen_vecs[:,i]) for i in range(len(eigen_vals))
eigen_pairs.sort(key=lambda k: k[0],reverse=True)

arr = np.hstack((eigen_pairs[0][1][:,np.newaxis],
           eigen_pairs[1][1][:,np.newaxis]))

#arr
```

In [69]:
```python
# PCA across patient groups
X_pca = X.dot(arr)
pca = PCA(n_components=2)
Pc=pca.fit_transform(X)
pca_df = pd.DataFrame(data = Pc, columns = ['PC1', 'PC2'])
```

In [70]:
```python
d = pd.DataFrame(data=y1, columns = ['patients'])
pca_df1 = pd.concat([d, pca_df], axis=1)
print(pca_df1)
```

```
    patients       PC1       PC2
0          3  2.430827 -2.543240
1          4 -4.560385 -1.073866
2          3  4.386897 -0.629315
3          4 -3.053299  1.277703
4          3  5.117675 -2.190759
5          4 -3.744242 -0.762400
6          3  3.881749 -1.103985
7          4 -1.632839  0.011237
8          3  4.132520 -0.903661
9          4 -3.816763 -1.272172
10         3  2.641036 -0.398122
11         4 -4.644298 -1.029306
12         3  3.911312  4.909000
13         4 -3.820971 -0.340294
14         3  2.789401  2.973549
15         4 -2.500288  0.670597
16         3  1.069752  3.889085
17         4 -2.324165  0.816336
18         3  4.356599 -0.676356
19         4 -4.156642 -0.828046
20         3  3.473014 -2.328394
21         4 -3.936892  1.532410
```

In [72]:
```python
fig = plt.figure(figsize=(10,6))
sns.scatterplot( x="PC1", y="PC2",
                data=pca_df1,
                hue = 'patients',
                legend='full') # specify the point size
plt.show()
```

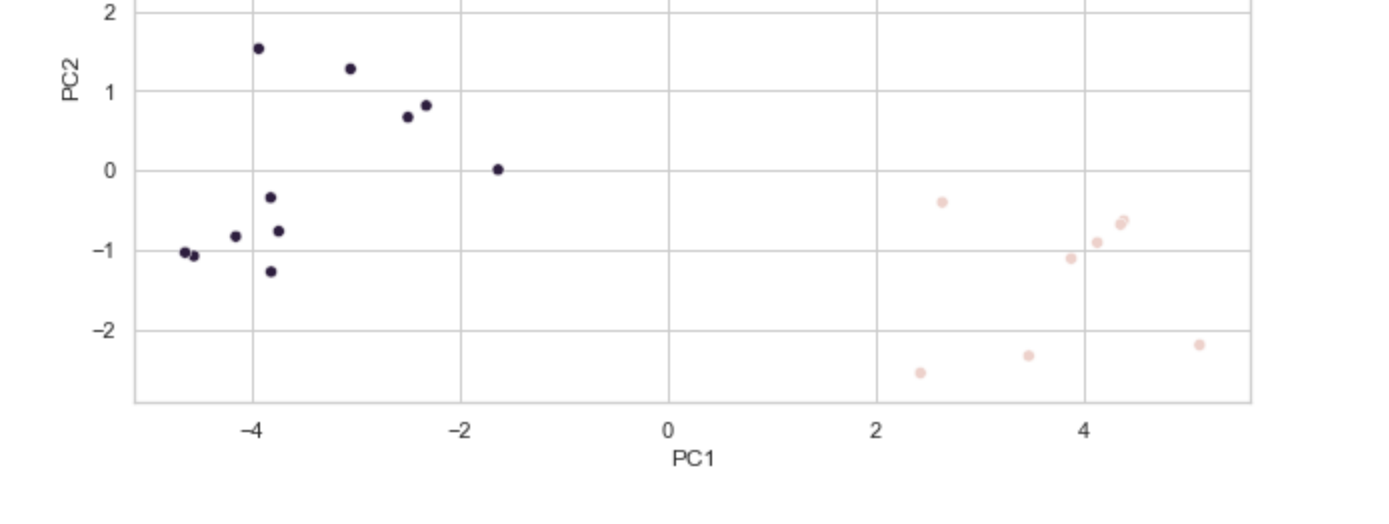


Test some classification models on new dataset -

In [77]:
```python
# run a few standard classification models with default parameters and kfold cv on ou
outcome = []
model_names = []
models = [('LogReg', LogisticRegression()),
          ('SVM', SVC()),
          ('DecTree', DecisionTreeClassifier()),
          ('KNN', KNeighborsClassifier()),
          ('LinDisc', LinearDiscriminantAnalysis()),
          ('GaussianNB', GaussianNB()),
          ('RFC', RandomForestClassifier()),
          ('AdaBoost', AdaBoostClassifier()),
          ('MLP', MLPClassifier())]

for model_name, model in models:
    k_fold_validation = model_selection.KFold(n_splits=5)
    results = model_selection.cross_val_score(model, X1, y1, cv=k_fold_validation, sco
    outcome.append(results)
    model_names.append(model_name)
    output_message = "%s| Mean=%f STD=%f" % (model_name, results.mean(), results.std(
    print(output_message)
```

```
LogReg| Mean=1.000000 STD=0.000000
SVM| Mean=0.960000 STD=0.080000
DecTree| Mean=1.000000 STD=0.000000
KNN| Mean=1.000000 STD=0.000000
LinDisc| Mean=1.000000 STD=0.000000
GaussianNB| Mean=1.000000 STD=0.000000
RFC| Mean=1.000000 STD=0.000000
AdaBoost| Mean=1.000000 STD=0.000000
MLP| Mean=1.000000 STD=0.000000
```

In [76]:
```python
fig = plt.figure(figsize=(12,4))
ax = fig.add_axes([0,0,1,1])
models1 = ['LogReg','SVM', 'SVM-1','DecTree','DT_Grid','KNN','LinDisc', 'GaussianNB',
results = [100,80,100,60, 100, 100,95,95,100,90,100]
ax.bar(models1,results)
plt.title('Machine Learning Model Comparison', fontsize=(16))
plt.show()
```



Results:

Models: Classification and Prediction

Now our data is are ready to be used to begin testing various machine learning classification models.

After testing our a few models we were able to determine that most of the models ran with a very high accuracy rate. SVM, decision trees, and random forest all performed the best when the hyper-parameters were carefully selected.

For the SVM models, after adjusting the kernal to 'linear' and the regularization parameter to 0.05 the performance increased from around 88% to 100% accruacy.

The decision tree model performed poorly with the default parameters, but when adjusted using gridsearch to find the best parameters the model improved from around 55% to 100%.

In [79]:
```python
# Seperate into test and train data
from sklearn.model_selection import train_test_split

X_train, X_test, y_train, y_test = train_test_split(
    X1, y1, test_size=0.4)
```

In [80]:
```python
# scaling the features - standardize
from sklearn.preprocessing import StandardScaler

scaler = StandardScaler()
scaler.fit(X_train)
X_train_std = scaler.transform(X_train)
X_test_std = scaler.transform(X_test)
```

In [81]:
```python
svc = svm.SVC(kernel='rbf', C =1)
svc.fit(X_train_std, y_train)

y_hat = svc.predict(X_test_std)

# Calculate accuracy
print(pd.crosstab(y_test, y_hat, rownames=['Actual'], colnames=['Predicted']))
print('Accuracy =', accuracy_score(y_test, y_hat))

print(classification_report (y_test, y_hat))
```

```
Predicted  3  4
Actual
3          6  0
4          0  3
Accuracy = 1.0
              precision    recall  f1-score   support

           3       1.00      1.00      1.00         6
           4       1.00      1.00      1.00         3

    accuracy                           1.00         9
   macro avg       1.00      1.00      1.00         9
weighted avg       1.00      1.00      1.00         9
```

In [82]:
```python
# Update the SVC model to see if we can get a better performance
svc1 = svm.SVC(kernel='linear', C =0.05, gamma='auto')  # changed regularization, kern
svc1.fit(X_train_std, y_train)

# add in cross validation
scores1 = cross_val_score(svc1, X_test_std, y_test, cv=5)
print(scores1)
print("%0.2f accuracy with a standard deviation of %0.2f" % (scores1.mean(), scores1.s

y_hat1 = svc1.predict(X_test_std)

# Calculate accuracy
print(pd.crosstab(y_test, y_hat1, rownames=['Actual'], colnames=['Predicted']))
print('Accuracy =', accuracy_score(y_test, y_hat1))

print(classification_report (y_test, y_hat1))
```

```
[1. 1. 1. 1. 1.]
1.00 accuracy with a standard deviation of 0.00
Predicted  3  4
Actual
3          6  0
4          0  3
Accuracy = 1.0
              precision    recall  f1-score   support

           3       1.00      1.00      1.00         6
           4       1.00      1.00      1.00         3

    accuracy                           1.00         9
   macro avg       1.00      1.00      1.00         9
weighted avg       1.00      1.00      1.00         9
```

In [83]:
```python
# Create decision tree with adaboost classifer
from sklearn.ensemble import AdaBoostClassifier
from sklearn.tree import DecisionTreeClassifier
from sklearn.metrics import confusion_matrix

# Create a decision tree object
tree = DecisionTreeClassifier()

# Fit on training data
tree.fit(X_train_std, y_train)
abc = AdaBoostClassifier(base_estimator=tree)

# Train Adaboost Classifer
model = abc.fit(X_train_std, y_train)

#Predict the response with test dataset
y_pred = model.predict(X_test_std)

print("Accuracy for Decision Tree model: ", accuracy_score(y_test,y_pred)*100) ## Sti

# Make a Confusion Matrix
print(pd.crosstab(y_test, y_pred, rownames=['Actual'], colnames=['Predicted']))
```

```
Accuracy for Decision Tree model:  88.88888888888889
Predicted  3  4
Actual
3          5  1
4          0  3
```

In [84]:
```python
# Try Gridsearch to tune the hyperparameters to see if we get better accuracy
from sklearn.model_selection import GridSearchCV
tree_param = {'criterion':['gini','entropy'],
             'max_depth':[3,4,5,6,7,8,9,10,11,12,15,20],
             'min_samples_split':[5,10,20,30,40,50,60,70,80,90,100],
             'min_samples_leaf':[2,3,4,5,6,7,8,9,10],
             'max_features':list(range(1,X_train_std.shape[1])),
             'max_features': ['auto', 'sqrt', 'log2'],
             'presort':[True, False]
            }
grid = GridSearchCV(DecisionTreeClassifier(), tree_param, cv=4)
grid.fit(X_train_std, y_train)
#best_predict = grid.best_score_(y_test)
print('Best Accuracy = ',grid.best_score_)
print(grid.best_params_)
print(grid.best_estimator_)
#print(pd.crosstab(y_test, best_predict, rownames=['Actual'], colnames=['Predicted'])).
```

```
Best Accuracy =  1.0
{'criterion': 'gini', 'max_depth': 3, 'max_features': 'auto', 'min_samples_leaf': 3,
'min_samples_split': 5, 'presort': True}
DecisionTreeClassifier(max_depth=3, max_features='auto', min_samples_leaf=3,
                       min_samples_split=5, presort=True)
```

In [85]:
```python
# Now use adaboost on the gridsearch results to see if we can get an even better pred
tree = grid.best_estimator_
tree.fit(X_train_std, y_train)

# Create adaboost classifer object
abc1 = AdaBoostClassifier(n_estimators=100,
                         learning_rate=5,
                         base_estimator=tree)
# Train Adaboost Classifer
model3 = abc1.fit(X_train_std, y_train)

# Predict the response for test dataset
y_hat2 = model3.predict(X_test_std)
print("Accuracy:",accuracy_score(y_test, y_hat2))
```

```
Accuracy: 0.8888888888888888
```

In [86]:
```python
from sklearn.ensemble import RandomForestClassifier
from sklearn.datasets import make_classification
classifier = RandomForestClassifier(n_estimators = 20,
                                    criterion = 'entropy')
classifier.fit(X_train_std, y_train)
y_pred = classifier.predict(X_test_std)

# Making the Confusion Matrix
print(pd.crosstab(y_test, y_pred, rownames=['Actual'], colnames=['Predicted']))
print('Accuracy =', accuracy_score(y_test, y_pred))
```

```
Predicted  3  4
Actual
3          5  1
4          0  3
Accuracy = 0.8888888888888888
```

In [87]:
```python
model4 = LogisticRegression().fit(X_train_std, y_train)
y_hat4 = model4.predict(X_test_std)

# Making the Confusion Matrix
print(pd.crosstab(y_test, y_hat4, rownames=['Actual'], colnames=['Predicted']))
print('Accuracy =', accuracy_score(y_test, y_hat4))
```

```
Predicted  3  4
Actual
3          6  0
4          0  3
Accuracy = 1.0
```

References -

Koh W, Pan W, Gawad C, Fan HC et al. Noninvasive in vivo monitoring of tissue-specific global gene expression in humans. Proc Natl Acad Sci U S A 2014 May 20;111(20):7361-6. PMID: 24799715

Scikit-learn: Machine Learning in Python, Pedregosa et al., JMLR 12, pp. 2825-2830, 2011.