Cancer Type Classification using Deep-Learning

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This document will explain how to use genomic expression data for classifying different cancer/tumor sites/types. This workshop is a follow-up to the NCI-DOE Pilot1 benchmark also called TC1. You can read about the project here, https://github.com/ECP-CANDLE/Benchmarks/tree/master/Pilot1/TC1 (https://github.com/ECP-CANDLE/Benchmarks/tree/master/Pilot1/TC1)

For classification, we use a Deep-Learning procedure called 1D-Convolutional Neural Network (CONV1D; https://en.wikipedia.org/wiki/Convolutional_neural_network (https://en.wikipedia.org/wiki/Convolutional_neural_network). NCI Genomic Data Commons (GDC; https://gdc.cancer.gov/ (https://gdc.cancer.gov/)) is the source of RNASeq expression data.

First we will start with genomic data preparation and then we will show how to use the data to build CONV1D model that can classify different cancer types. Please note that there are more than ways to extract data from GDC. What I am describing is one possible way.

This is a continuation of data preparation which can be accessed from here, https://github.com/ravichas/ML-TC1 (https://github.com/ravichas/ML-TC1)

Part-2: Convolutional Neural Network

Load some libraries

In [1]:
```
from __future__ import print_function
import os, sys, gzip, glob, json, time, argparse
import warnings
warnings.simplefilter(action='ignore', category=FutureWarning)
import pandas as pd
from pandas.io.json import json_normalize
import numpy as np

from sklearn import preprocessing
from sklearn.model_selection import train_test_split
from sklearn.metrics import accuracy_score
from sklearn.preprocessing import StandardScaler, MinMaxScaler, MaxAbsScaler
from sklearn.preprocessing import LabelEncoder, OneHotEncoder

from keras.utils import to_categorical
from keras import backend as K
from keras.layers import Input, Dense, Dropout, Activation, Conv1D, MaxPooling
1D, Flatten
from keras import optimizers
from keras.optimizers import SGD, Adam, RMSprop
from keras.models import Sequential, Model, model_from_json, model_from_yaml
from keras.utils import np_utils
from keras.callbacks import ModelCheckpoint, CSVLogger, ReduceLROnPlateau
from keras.callbacks import EarlyStopping
```

```
Using TensorFlow backend.
```

Let us read the input data and outcome class data

In [2]:
```
# Read features and output files
TC1data3 = pd.read_csv("Data/TC1-data3stypes.tsv", sep="\t", low_memory = Fals
e)
outcome = pd.read_csv("Data/TC1-outcome-data3stypes.tsv", sep="\t", low_memory
=False, header=None)
```

In [3]:
```
TC1data3.iloc[[0,1,2,3,4],[0,1,2,3,4,5,6,7,8,9,60400,60401,60482]]
```

Out[3]:

	0	1	2	3	4	5	6	7	8	9
0	1.716923	0.0	1.951998	1.167483	0.667981	1.274099	1.258272	1.837351	1.000251	1.99182
1	1.979573	0.0	1.939303	0.946014	0.828050	1.338521	1.215231	2.298950	1.974058	1.74489
2	1.681222	0.0	2.016686	0.789298	0.930981	1.167504	1.026718	2.058239	1.776646	1.51048
3	1.640044	0.0	1.669994	0.821958	0.426876	1.214174	1.673027	1.904529	0.867674	1.52644
4	1.800725	0.0	2.013062	0.743211	0.652487	0.935054	1.102839	2.068075	1.405575	1.67471

In [4]:
```
# outcome[0].value_counts()
outcome = outcome[0].values
```

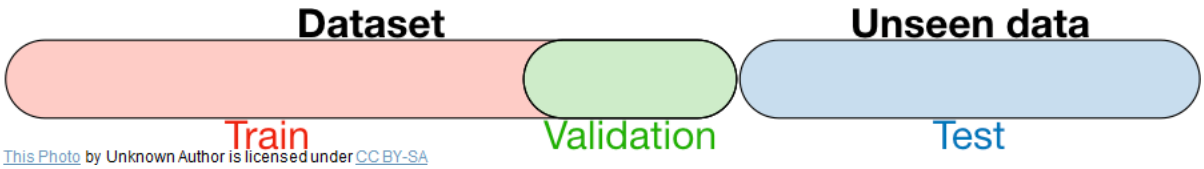
In [5]:
```
def encode(data):
    print('Shape of data (BEFORE encode): %s' % str(data.shape))
    encoded = to_categorical(data)
    print('Shape of data (AFTER  encode): %s\n' % str(encoded.shape))
    return encoded
```

In [6]:
```
# One hot encoding
# Done run more than once
outcome = encode(outcome)
```

```
Shape of data (BEFORE encode): (150,)
Shape of data (AFTER  encode): (150, 3)
```

In [7]:
```
from IPython.core.display import Image
Image(filename='Img/Train-Test.png',width = 600, height = 800 )
```

Out[7]:



You can use the Test data for validatation.

Split the data into training and test set

In [8]:
```
# train/test split
X_train, X_test, Y_train, Y_test = train_test_split(TC1data3, outcome,
                                                    train_size=0.75,
                                                    test_size=0.25,
                                                    random_state=123,
                                                    stratify = outcome)
```

Let us define some parameters

- activation to be RELU
- batch_size is set to 20
- number of classes is three (chosen a small number for performace) for this exercise. The code that is available from NIH FTP site will model 15 cancer site outputs.

In [9]:
```
# parameters
activation='relu'
batch_size=20
# Number of sites
classes=3

drop = 0.1
feature_subsample = 0
loss='categorical_crossentropy'

# metrics='accuracy'
out_act='softmax'

shuffle = False
```

Note epochs should be greather than 10. For hands-on, I have chosen a smaller number

In [10]:
```
epochs=5
optimizer = optimizers.SGD(lr=0.1)
metrics = ['acc']
```

In [11]:
```
x_train_len = X_train.shape[1]

X_train = np.expand_dims(X_train, axis=2)
X_test = np.expand_dims(X_test, axis=2)
```

Please note that the `filters = 128` gave the best results. For the purpose of demonstration via cloud, I might choose a smaller number.

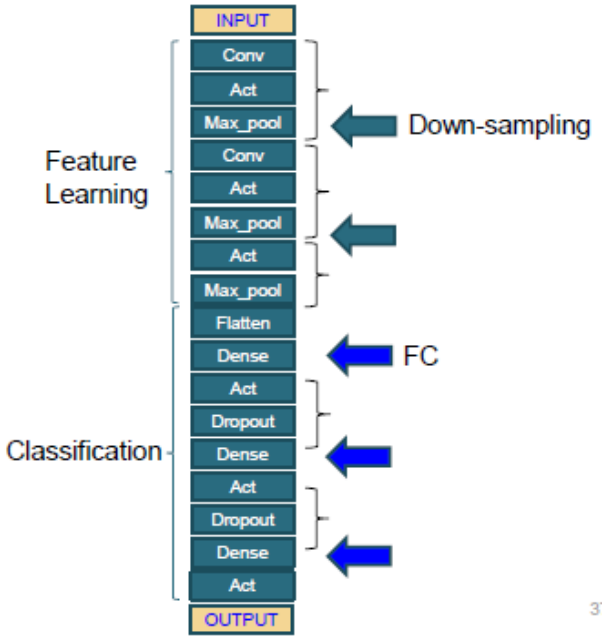
In [12]:
```
# filters = 128
filters = 128
filter_len = 20
stride = 1

K.clear_session()
```

Create and initialize the model

In [13]:
```
from IPython.core.display import Image
Image(filename='Img/TC1-arch.png',width = 300, height = 400 )
```

Out[13]:



In [14]:
```
model = Sequential()

# model.add  1. CONV1D
model.add(Conv1D(filters = filters,
                 kernel_size = filter_len,
                 strides = stride,
                 padding='valid',
                 input_shape=(x_train_len, 1)))
```

Create the topology of the architecture

In [15]:
```
# 2. Activation
model.add(Activation('relu'))

# 3. MaxPooling
model.add(MaxPooling1D(pool_size = 1))

# 4. Conv1D: filters:128, filter_len=20, stride=1
model.add(Conv1D(filters=filters,
                 kernel_size=filter_len,
                 strides=stride,
                 padding='valid'))

# 5. Activation
model.add(Activation('relu'))

# 6. MaxPooling
model.add(MaxPooling1D(pool_size = 10))

# 7. Flatten
model.add(Flatten())

# 8. Dense
model.add(Dense(200))

# 9. activation
model.add(Activation('relu'))

# 10. dropout
model.add(Dropout(0.1))

#11. Dense
model.add(Dense(20))

#12. Activation
model.add(Activation('relu'))

#13. dropout
model.add(Dropout(0.1))

# 14. dense
model.add(Dense(3))

# 15. Activation
model.add(Activation(out_act))
```

Compile and show the model summary

In [16]:
```
model.compile( loss= loss,
              optimizer = optimizer,
              metrics = metrics )

es = EarlyStopping(monitor='val_loss', mode='min', verbose=1, patience=10)
model.summary()
```

```
Model: "sequential_1"
_________________________________________________________________
Layer (type)                 Output Shape              Param #
=================================================================
conv1d_1 (Conv1D)            (None, 60464, 128)        2688
_________________________________________________________________
activation_1 (Activation)    (None, 60464, 128)        0
_________________________________________________________________
max_pooling1d_1 (MaxPooling1 (None, 60464, 128)        0
_________________________________________________________________
conv1d_2 (Conv1D)            (None, 60445, 128)        327808
_________________________________________________________________
activation_2 (Activation)    (None, 60445, 128)        0
_________________________________________________________________
max_pooling1d_2 (MaxPooling1 (None, 6044, 128)         0
_________________________________________________________________
flatten_1 (Flatten)          (None, 773632)            0
_________________________________________________________________
dense_1 (Dense)              (None, 200)               154726600
_________________________________________________________________
activation_3 (Activation)    (None, 200)               0
_________________________________________________________________
dropout_1 (Dropout)          (None, 200)               0
_________________________________________________________________
dense_2 (Dense)              (None, 20)                4020
_________________________________________________________________
activation_4 (Activation)    (None, 20)                0
_________________________________________________________________
dropout_2 (Dropout)          (None, 20)                0
_________________________________________________________________
dense_3 (Dense)              (None, 3)                 63
_________________________________________________________________
activation_5 (Activation)    (None, 3)                 0
=================================================================
Total params: 155,061,179
Trainable params: 155,061,179
Non-trainable params: 0
_________________________________________________________________
```

In [17]:
```
# save
save = '.'
output_dir = "Model"

if not os.path.exists(output_dir):
        os.makedirs(output_dir)

model_name = 'tc1'
path = '{}/{}.autosave.model.h5'.format(output_dir, model_name)
checkpointer = ModelCheckpoint(filepath=path,
                               verbose=1,
                               save_weights_only=True,
                               save_best_only=True)

csv_logger = CSVLogger('{}/training.log'.format(output_dir))
```

In [18]:
```
# SR: change epsilon to min_delta
reduce_lr = ReduceLROnPlateau(monitor='val_loss',
                              factor=0.1,
                              patience=10,
                              verbose=1, mode='auto',
                              min_delta=0.0001,
                              cooldown=0,
                              min_lr=0)
```

This is a time-consuming step and smaller sample sizes will not result in good model.

Here are the commands for training and evaluating test accuracy score.

In [19]:
```
# batch_size = 20; epochs=5
history = model.fit(X_train, Y_train, batch_size=batch_size,
                    epochs=epochs, verbose=1, validation_data=(X_test, Y_test
),
                    callbacks = [checkpointer, csv_logger, reduce_lr])
```

```
Train on 112 samples, validate on 38 samples
Epoch 1/5
112/112 [==============================] - 216s 2s/step - loss: 2.2790 - acc:
0.2946 - val_loss: 1.0991 - val_acc: 0.3158

Epoch 00001: val_loss improved from inf to 1.09910, saving model to Model/tc
1.autosave.model.h5
Epoch 2/5
112/112 [==============================] - 214s 2s/step - loss: 1.1005 - acc:
0.3304 - val_loss: 1.0992 - val_acc: 0.3158

Epoch 00002: val_loss did not improve from 1.09910
Epoch 3/5
112/112 [==============================] - 206s 2s/step - loss: 1.1000 - acc:
0.3214 - val_loss: 1.0985 - val_acc: 0.3158

Epoch 00003: val_loss improved from 1.09910 to 1.09846, saving model to Mode
l/tc1.autosave.model.h5
Epoch 4/5
112/112 [==============================] - 210s 2s/step - loss: 1.0981 - acc:
0.3393 - val_loss: 1.0934 - val_acc: 0.6053

Epoch 00004: val_loss improved from 1.09846 to 1.09340, saving model to Mode
l/tc1.autosave.model.h5
Epoch 5/5
112/112 [==============================] - 208s 2s/step - loss: 1.4125 - acc:
0.4375 - val_loss: 1.1013 - val_acc: 0.3158

Epoch 00005: val_loss did not improve from 1.09340
```

In [20]:
```
score = model.evaluate(X_test, Y_test, verbose=0)

print('Test score:', score[0])
print('Test accuracy:', score[1])
```

```
Test score: 1.101348876953125
Test accuracy: 0.31578946113586426
```

Word of caution about the accuracy

The output loss and accuracy from smalller sample sizes (for example, n = 50) will not reflect the real learning. For good accuracy, we need to use the whole dataset. Here are few epochs from the original dataset modeling (Train: 3375; Validate: 1125).

In [21]:
```
from IPython.core.display import Image
Image(filename='Img/TC1-Acc.PNG',width = 1000, height = 1000 )
```

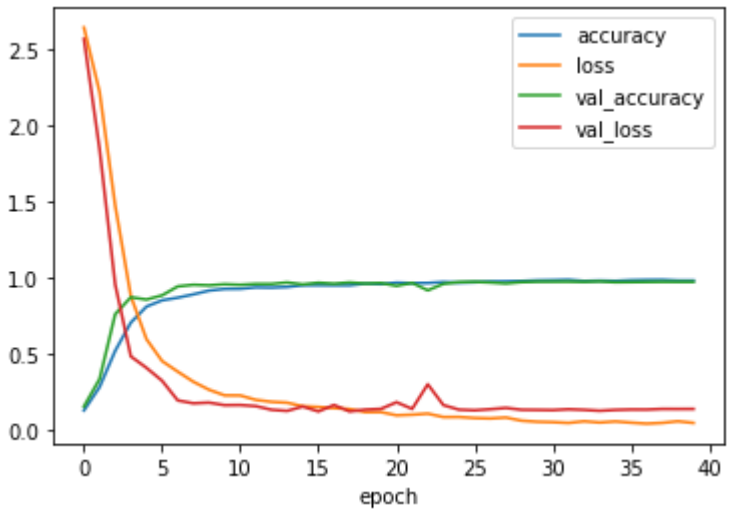
Out[21]:
```
Epoch 00001: val_loss improved from inf to 1.92207, saving model to ./tc1.autosave.model.h
Epoch 2/400 3375/3375 [==============================] - 212s 63ms/step - loss: 1.9032 - acc: 0.3570 - val_loss: 0.9284 - val_acc: 0.6880
Epoch 3/400 3375/3375 [==============================] - 213s 63ms/step - loss: 0.7621 - acc: 0.7336 - val_loss: 0.6930 - val_acc: 0.7938
Epoch 4/400 3375/3375 [==============================] - 214s 63ms/step - loss: 0.4254 - acc: 0.8459 - val_loss: 0.2777 - val_acc: 0.9298
Epoch 5/400 3375/3375 [==============================] - 214s 63ms/step - loss: 0.2689 - acc: 0.9117 - val_loss: 0.3172 - val_acc: 0.9049
Epoch 6/400 3375/3375 [==============================] - 214s 63ms/step - loss: 0.2149 - acc: 0.9333 - val_loss: 0.1892 - val_acc: 0.9404
Epoch 7/400 3375/3375 [==============================] - 214s 63ms/step - loss: 0.1729 - acc: 0.9455 - val_loss: 0.1871 - val_acc: 0.9591
Epoch 8/400 3375/3375 [==============================] - 214s 63ms/step - loss: 0.1359 - acc: 0.9609 - val_loss: 0.2292 - val_acc: 0.9298
Epoch 9/400 3375/3375 [==============================] - 214s 63ms/step - loss: 0.1199 - acc: 0.9630 - val_loss: 0.2630 - val_acc: 0.9387
Epoch 10/400 3375/3375[==============================] - 214s 63ms/step - loss: 0.0894 - acc: 0.9710 - val_loss: 0.3390 - val_acc: 0.9244
Epoch 11/400 3375/3375[==============================] - 214s 63ms/step - loss: 0.1038 - acc: 0.9710 - val_loss: 0.1836 - val_acc: 0.9520
Epoch 12/400 3375/3375[==============================] - 214s 63ms/step - loss: 0.0619 - acc: 0.9813 - val_loss: 0.1905 - val_acc: 0.9609
Epoch 13/400 3375/3375[==============================] - 214s 64ms/step - loss: 0.0654 - acc: 0.9799 - val_loss: 0.1644 - val_acc: 0.9609
Epoch 14/400 3375/3375[==============================] - 213s 63ms/step - loss: 0.0481 - acc: 0.9861 - val_loss: 0.1987 - val_acc: 0.9644
```

In [22]:
```
import numpy as np
import matplotlib.pyplot as plt
import pandas as pd

tc1results = pd.read_csv("Output/tc1results.txt", index_col='epoch')
```

In [23]:
```
tc1results.plot()
```

Out[23]:
```
<matplotlib.axes._subplots.AxesSubplot at 0x1b430fa4448>
```



How to save the model/weights?

In [24]:
```
# JSON JSON
# serialize model to json
json_model = model.to_json()

# save the model architecture to JSON file
with open('Model/tc1.model.json', 'w') as json_file:
    json_file.write(json_model)


# YAML YAML
# serialize model to YAML
model_yaml = model.to_yaml()

# save the model architecture to YAML file
with open("{}/{}.model.yaml".format(output_dir, model_name), "w") as yaml_file
:
    yaml_file.write(model_yaml)


# WEIGHTS HDF5
# serialize weights to HDF5
model.save_weights("{}/{}.model.h5".format(output_dir, model_name))
print("Saved model to disk")
```

```
Saved model to disk
```

Inference

The calculation was carried out on a NIH Biowulf GPU node. Model weights were saved in Python HDF5 grid format. HDF5 is ideal for storing multi-dimensional arrays of numbers. You can read about HDF5 here. http://www.h5py.org/ (http://www.h5py.org/)

In [25]:
```
from keras.models import model_from_json

# Open the handle
json_file = open('Model/tc1.model.json', 'r')

# load json and create model
loaded_model_json = json_file.read()
json_file.close()

loaded_model = model_from_json(loaded_model_json)

# load weights into new model
loaded_model.load_weights('Model/tc1.model.h5')
print("Loaded model from disk")
# loaded_model_json
```

```
Loaded model from disk
```

Mimicking the process of external set

Note this is a demonstration of how to use external data for inference.

When you bring in an external dataset. Make sure you follow the following steps:

a) Make sure you do the same operations that you had done to the data set b) scale the inference dataset in the same way as the training data

In [26]:
```
import numpy as np
chosen_idx = np.random.choice(38, replace=False, size=5)
# X_test[chosen_idx].shape
# Y_test[chosen_idx].shape
# Y_test.shape
```

In [27]:
```
X_mini = X_test[chosen_idx]
y_mini = Y_test[chosen_idx]
# df_trimmed = X_mini.drop(X_mini.columns[[0]], axis=1, inplace=False)
# X_mini = df_trimmed
print('X_mini.shape', X_mini.shape)
print('len(y_minip)', len(y_mini))
```

```
X_mini.shape (5, 60483, 1)
len(y_minip) 5
```

In [28]:
```
print('X_mini.shape', X_mini.shape)
print('y_mini.shape', y_mini.shape)
```

```
X_mini.shape (5, 60483, 1)
y_mini.shape (5, 3)
```

In [29]:
```
# evaluate loaded model on test data
loaded_model.compile(loss='categorical_crossentropy', optimizer='sgd',
                     metrics=['accuracy'])
score = loaded_model.evaluate(X_mini, y_mini, verbose=0)
print("%s: %.2f%%" % (loaded_model.metrics_names[1], score[1]*100))
```

```
accuracy: 0.00%
```

Unsupervised learning plots (PCA and tSNE)

This section was based on Dr. Andrew Weissman's code template. Check out Andrew's Gihub here, https://github.com/andrew-weisman (https://github.com/andrew-weisman)

Load the custom `tc1_library.py` file, which contains the unsupervised learning plotting function

In [30]:
```
import tc1_library
import importlib

importlib.reload(tc1_library);
```

Decode the outcome matrix back into a single vector (remember earlier that we one-hot-encoded it)

In [31]:
```
outcome[0:5]
```

Out[31]:
```
array([[0., 1., 0.],
       [0., 1., 0.],
       [0., 1., 0.],
       [0., 1., 0.],
       [0., 1., 0.]], dtype=float32)
```

Let us explore some outcome values

In [32]:
```
outcome.shape # 150,3
outcome[0:3]
outcome[50:53]
outcome[75:80]
```

Out[32]:
```
array([[1., 0., 0.],
       [1., 0., 0.],
       [1., 0., 0.],
       [1., 0., 0.],
       [1., 0., 0.]], dtype=float32)
```

Let us explore some outcome_decoded values

In [33]:
```
outcome_decoded = [x.argmax() for x in outcome]
```

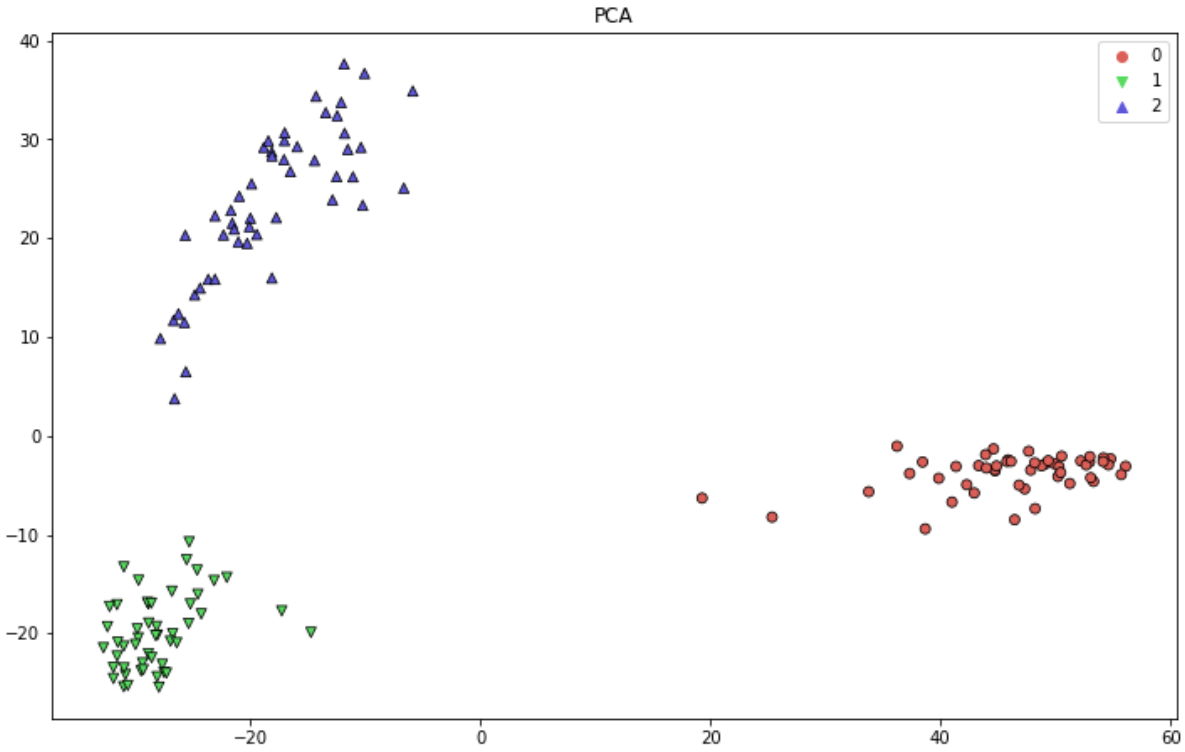
In [34]:
```
outcome_decoded[0:3] #[1,1,1]
outcome_decoded[50:53] #[2,2,0]
outcome_decoded[75:80] # [0, 0, 0, 0]
```

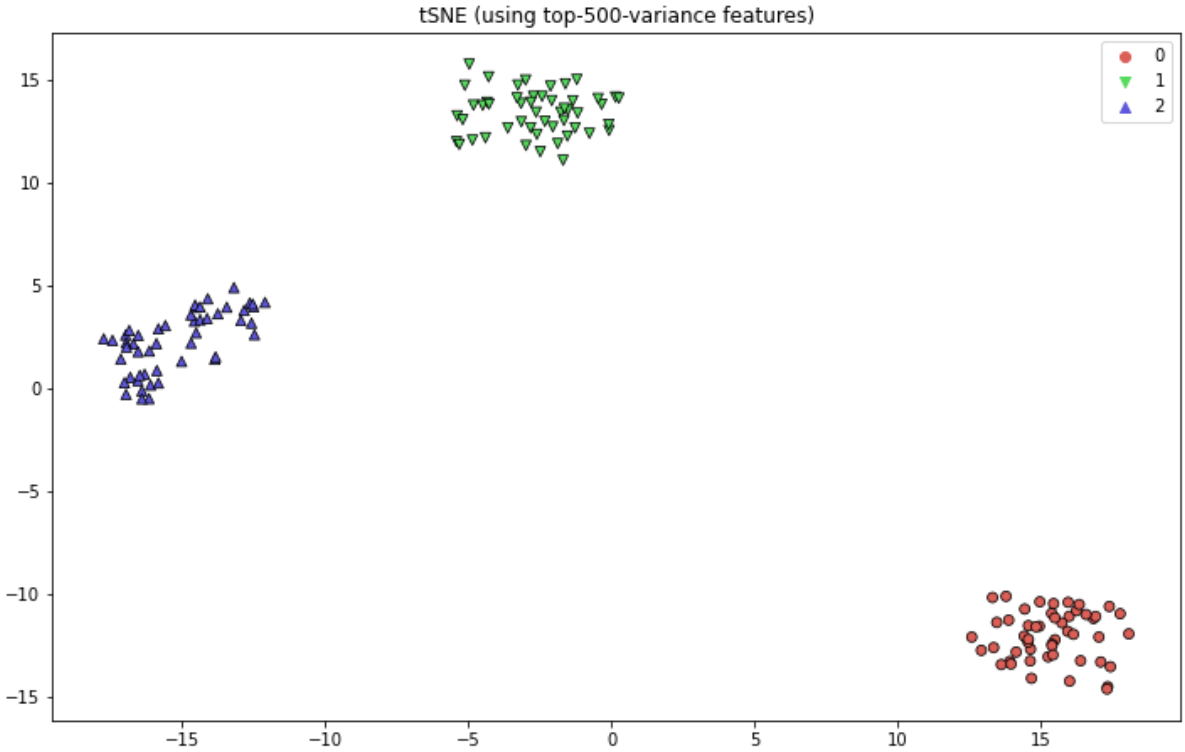
Out[34]:
```
[0, 0, 0, 0, 0]
```

Perform the PCA and t-SNE using scikit-learn

In [35]:
```
import sklearn.decomposition as sk_decomp
tc1_library.run_and_plot_pca_and_tsne(TC1data3, outcome_decoded)
```

```
Top 10 PCA explained variance ratios: [0.38811713 0.12041275 0.0499922  0.030
27331 0.02528322 0.02271728
 0.01852503 0.01623522 0.01457019 0.01271466]
```





Create a binary dataset instead of a multi-class one

Current outcome distribution of the 150 samples

In [36]:
```
pd.value_counts(outcome_decoded)
```

Out[36]:
```
2    50
1    50
0    50
dtype: int64
```

Get the indexes of the data that correspond to classes 0 or 1 only (excluding class 2)

In [37]:
```
binary_indexes = np.where(np.array(outcome_decoded)!=2)[0]
```

Recreate the data structures of the same types that was used in the original analysis above, except this time with just two classes

In [38]:
```
TC1data2 = TC1data3.iloc[binary_indexes,:]
outcome2 = outcome[binary_indexes,:]
```

Decode the new outcome matrix just like we did above, and print out the outcome distribution of the new set of samples

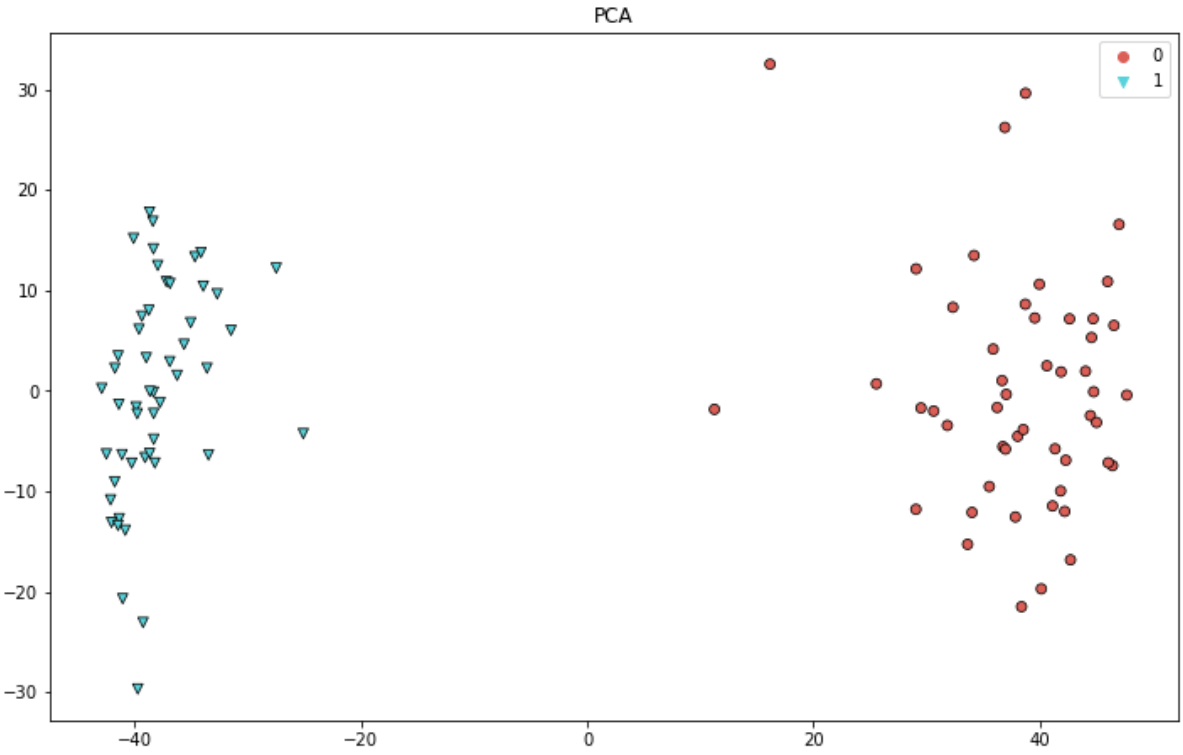
In [39]:
```
outcome_decoded2 = [x.argmax() for x in outcome2]
pd.value_counts(outcome_decoded2)
```

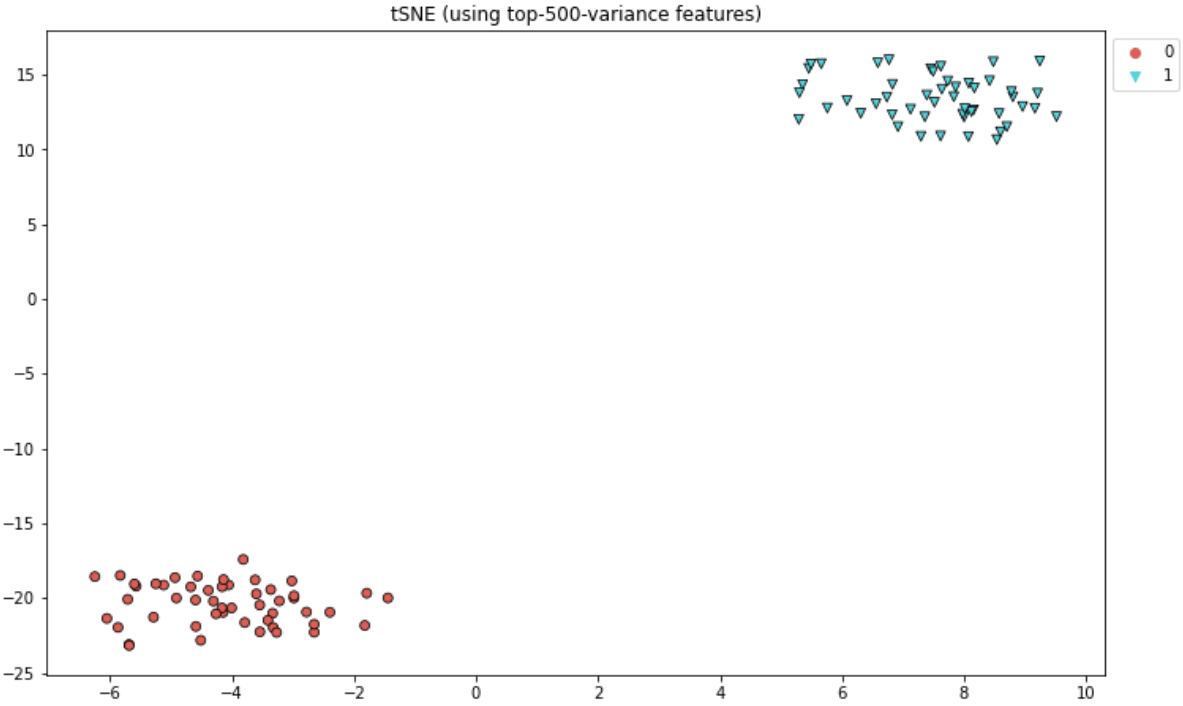
Out[39]:
```
1    50
0    50
dtype: int64
```

Check our new dataset by performing the same unsupervised learning that we did above

In [40]:
```
tc1_library.run_and_plot_pca_and_tsne(TC1data2, outcome_decoded2)
```

```
Top 10 PCA explained variance ratios: [0.49686232 0.04021868 0.03354306 0.032
21509 0.02649848 0.0183547
 0.01706743 0.01658734 0.01308181 0.01172459]
```





Next Steps

- Pick up with the Jupyter notebook from the cell that splits the data into the training and test sets, but now replacing TC1data3 with TC1data2 and outcome with outcome2
- Work through the notebook until at least the model has been trained (the cell with history = model.fit(...)), resulting in a binary classifier
- Apply gene/feature importance tools to the resulting model in order to determine which genes best contribute to discriminating between cancer classes 0 and 1

Saving two-class datafiles to disk

Save the data and labels to CSV format in the repository's data directory

In [41]:
```
TC1data2.reset_index(drop=True).to_csv('Data/X_two_classes.csv')

pd.Series(outcome_decoded2).to_csv('Data/y_two_classes.csv')
```

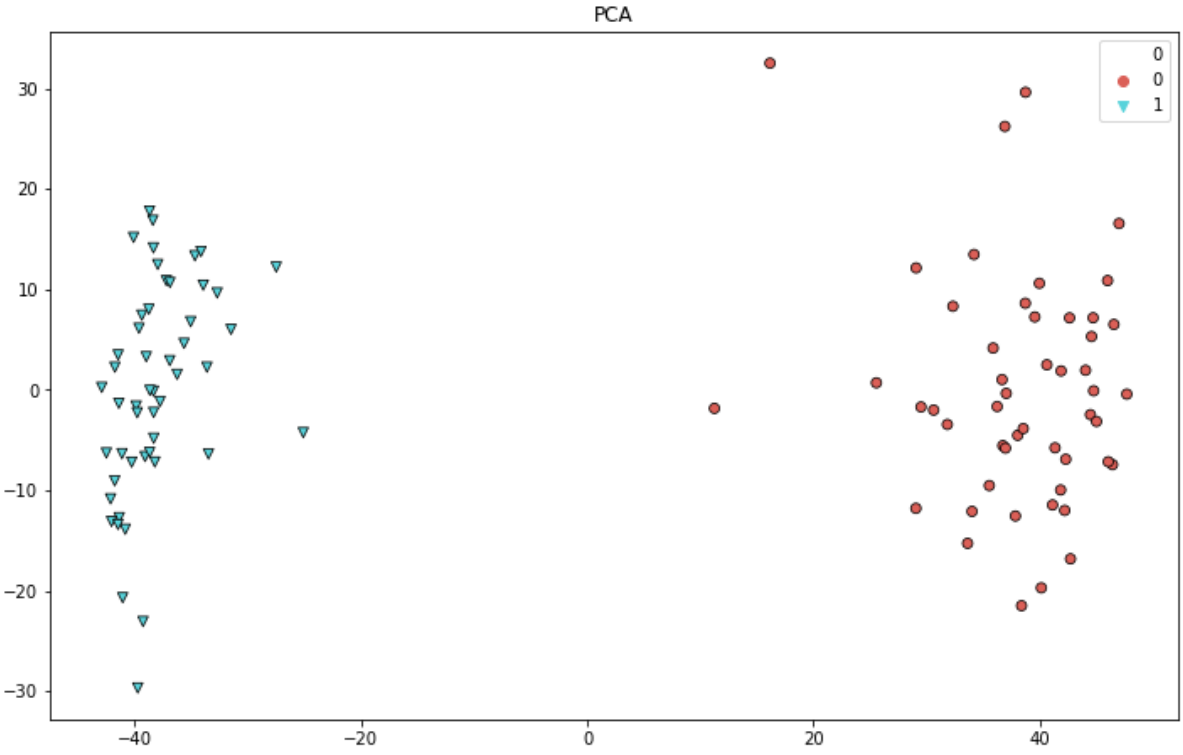
Test that we've exported the data correctly by reading them back in and running the unsupervised learning analyses

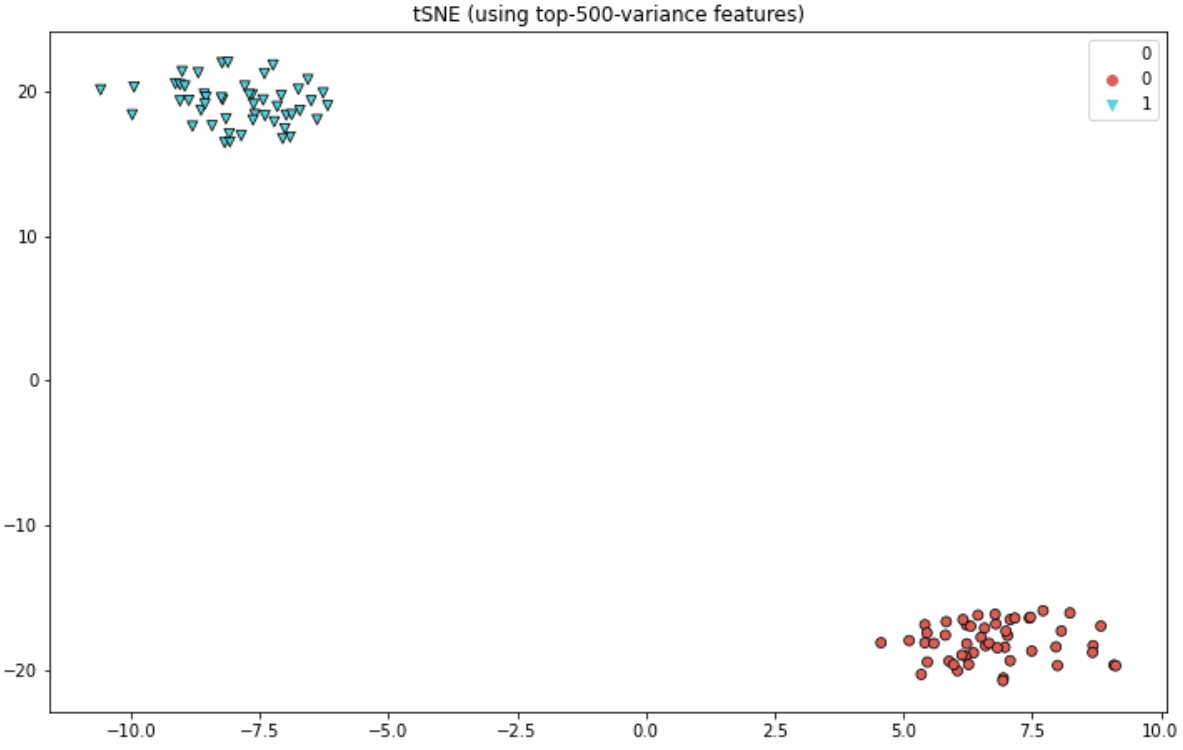
In [42]:
```
X = pd.read_csv('Data/X_two_classes.csv', index_col=0)

y = pd.read_csv('Data/y_two_classes.csv', index_col=0, squeeze=True)

tc1_library.run_and_plot_pca_and_tsne(X, y)
```

```
Top 10 PCA explained variance ratios: [0.49686232 0.04021867 0.03354307 0.032
21509 0.02649846 0.01835562
 0.01706924 0.01658787 0.01310717 0.01186719]
```





In [ ]:
```
You are viewing the Jupyter Notebook from ML-TC1 GitHub repository, https://gi
thub.com/ravichas/ML-TC1
```