TC1-dataprep

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1 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

For classification, we use a Deep-Learning procedure called 1D-Convolutional Neural Network (CONV1D; https://en.wikipedia.org/wiki/Convolutional_neural_network. NCI Genomic Data Commons (GDC; 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.

2 Part-1: Genomic data preparation

2.1 Load some libraries (keras, scikit-learn, pandas, numpy etc.)

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

from pandas.io.json import json_normalize
from keras.utils import to_categorical
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,␣
↪MaxPooling1D, 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
```

```
Using TensorFlow backend.
```

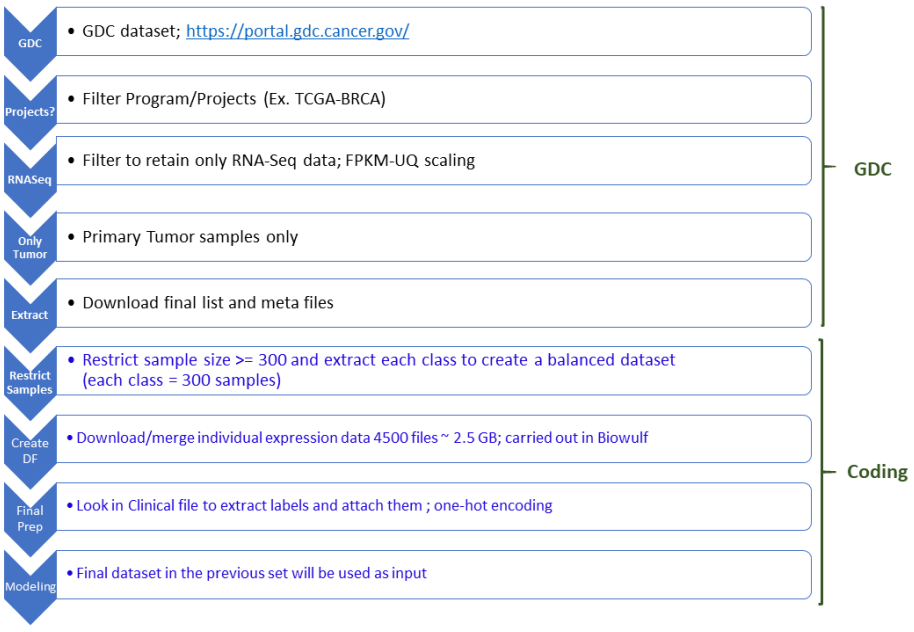
2.2 What type of data we need and where can we get it?

- We will be using RNASeq data
- Genomic Data Commons (www.gdc.org) is the data source.
- We used FPKM-UQ scaled RNASeq expression data for tumor cases. Check here for information on scaling, https://docs.gdc.cancer.gov/Encyclopedia/pages/HTSeq-FPKM-UQ

2.3 List of data preparation steps

[2]:
```
Image(filename='Img/OverallProcedure.png')
```

[2]:



2.4 Data gathering from GDC

Follow GDC tutorial, https://docs.gdc.cancer.gov/Data_Portal/Users_Guide/Getting_Started/, for information about how to download data. Here is a rough procedure:

- search for projects of your interest (BRCA from TCGA, non-tumor samples etc.)
- filter the type of data you need (RNASeq, mutation etc)
- add it to Cart
- download files relevant to your data (Manifest file (contain ids of the data that you want to download), meta data, clinical data etc.)

You can use GDC tool, gdc-client, to download the data. Please, read details about gdc-clieant from the GDC website, https://docs.gdc.cancer.gov/Data_Transfer_Tool/Users_Gu
For this tutorial, I will be using a GDC RNASeq tool (https://github.com/cpreid2/gdc-rnaseq-too that will download the GDC expression data and merge the expression files into one single dataframe.

NIH Biowulf HPC systems have access to gdc-tools. Please read for details here, https://hpc.nih.gov/apps/gdc-client.html

2.5 Manual data gathering steps from GDC

Please click here to see the steps for exploring RNAseq expression data

2.6 Genomic data is complex

Due to complexity of the data, the expression and meta data are kept in different files For example, MetaData, SampleSheet, Manifest etc.

Let us explore Metadata, Clinical, Biospecimen, Manifest and sample_sheet data Please note that the following files are available for each GDC search. You can access them by first adding the data to the Cart. Let us read the following files:

- **Manifest (list of RNASeq data for download):** gdc_manifest_20200309_162520.txt
- **Metadata data:** metadata.cart.2020-03-09.json
- **Clinical data:** clinical.cart.2020-03-09.json
- **Biospecimen data:** biospecimen.cart.2020-03-09.json **(131 MB; due to Github files size limit, this file is not available in the repository)**
- **Sample_sheet data:** gdc_sample_sheet.2020-03-09.tsv

[3]:
```
gdc_manifest = pd.read_csv("Data/gdc_manifest_20200309_162520.txt", sep="\t",␣
↪low_memory=False)
print("gdc_manifest")
gdc_manifest.iloc[0:4,0:3]
```

gdc_manifest

[3]:
```
                                     id  \
0  00086b37-ad3a-4e4b-b44d-ea0cc657f48b
```

```
1  000cf9c6-1373-4fb0-b759-5fd8c3799030
2  000e76af-9529-4e0b-b300-c602f5f717d2
3  0012eb83-a0ab-4abe-bd9b-8706d613ad9c

                                          filename  \
0  bdc49eae-31d4-425b-b7d1-49f1cf14df44.FPKM-UQ.t…
1  b9e7b7b5-54e8-4459-8a21-ea3472b013d7.FPKM-UQ.t…
2  b836a8d2-7c37-4af5-9f2f-7bf0121717f9.FPKM-UQ.t…
3  42489a2e-a77b-43e2-a51e-dc341af3ae19.FPKM-UQ.t…

                               md5
0  a26913ae01c41c8a69661573f201df88
1  6d41923b59f979df27888573ecaf8eaf
2  9e947e256131eee23678eb4e37a25c39
3  a41f8e12aef9dcb10113170eb4d6c89e
```

[4]:
```
metadata = pd.read_json("Data/metadata.cart.2020-03-09.json", encoding='utf-8')
print("metadata")
metadata.iloc[0:3, [0,3,4]]
```

```
metadata
```

[4]:
```
                                          file_name  \
0  d1b25b91-db55-4c0a-a973-2a6c229c2b03.FPKM-UQ.t…
1  875c2a27-9732-4b8a-affc-8ea591595a43.FPKM-UQ.t…
2  35c4cc14-5096-4da8-9190-359338fdb365.FPKM-UQ.t…

                        data_type                               file_id
0  Gene Expression Quantification  d8d316fd-0c47-48ce-9ee0-e41b6f96fcc6
1  Gene Expression Quantification  f4885085-7300-4623-b7cd-3afd10808815
2  Gene Expression Quantification  90b463e6-d18a-4907-a427-ecc054dabb11
```

[5]:
```
clinical = pd.read_json("Data/clinical.cart.2020-03-09.json", encoding='utf-8')
print("clinical data")
clinical.iloc[0:3, [0,2,3]]
```

```
clinical data
```

[5]:
```
                                          diagnoses  \
0  [{'ajcc_pathologic_t': 'T3a', 'synchronous_mal…
1  [{'ajcc_pathologic_t': 'T1b', 'synchronous_mal…
2  [{'ajcc_clinical_t': 'T2c', 'ajcc_pathologic_t…

                               case_id  \
0  5338d435-68fb-4f0d-a3e6-c843f703f75f
1  22b6724c-a59f-4796-8166-992253e8caf1
2  deceb7df-6edc-41f0-99aa-c4ac7e764074
```

```
                                                  demographic
0  {'gender': 'male', 'vital_status': 'Alive', 'u…
1  {'gender': 'male', 'vital_status': 'Alive', 'u…
2  {'gender': 'male', 'vital_status': 'Alive', 'u…
```

[6]:
```
# biospecimen = pd.read_json("Data/biospecimen.cart.2020-03-09.json",␣
 ↪encoding='utf-8')
# print("biospecimen")
# biospecimen.iloc[0:3,0:2]
```

[7]:
```
sample_sheet = pd.read_csv("Data/gdc_sample_sheet.2020-03-09.tsv", sep="\t")
print("sample_sheet")
sample_sheet.iloc[1:4,1:6]
```

```
sample_sheet
```

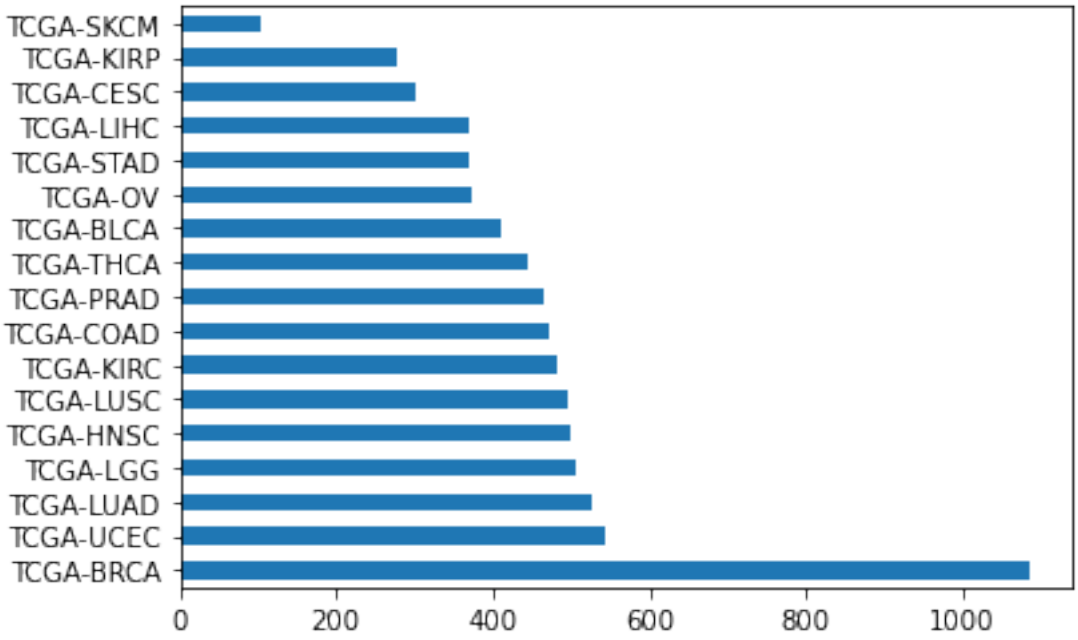
[7]:
```
                                          File Name            Data Category  \
1  875c2a27-9732-4b8a-affc-8ea591595a43.FPKM-UQ.t…  Transcriptome Profiling
2  35c4cc14-5096-4da8-9190-359338fdb365.FPKM-UQ.t…  Transcriptome Profiling
3  b4e20023-e2cd-4b02-b6c3-16133a6d3ca7.FPKM-UQ.t…  Transcriptome Profiling

                        Data Type Project ID       Case ID
1  Gene Expression Quantification    TCGA-OV  TCGA-09-1659
2  Gene Expression Quantification  TCGA-UCEC  TCGA-AX-A1C5
3  Gene Expression Quantification  TCGA-THCA  TCGA-FY-A3R8
```

2.7 Let us count/plot the Project_IDs from sample_sheet

[8]:
```
sample_sheet['Project ID'].value_counts()
# sample_sheet
tab = sample_sheet['Project ID'].value_counts()
tab.plot(kind='barh' )
tab
```

[8]:
```
TCGA-BRCA    1087
TCGA-UCEC     544
TCGA-LUAD     528
TCGA-LGG      506
TCGA-HNSC     499
TCGA-LUSC     496
TCGA-KIRC     483
TCGA-COAD     472
TCGA-PRAD     466
TCGA-THCA     445
TCGA-BLCA     412
TCGA-OV       374
```

```
TCGA-STAD     370
TCGA-LIHC     369
TCGA-CESC     301
TCGA-KIRP     276
TCGA-SKCM     103
Name: Project ID, dtype: int64
```



Here are the Cancer (types) Project codes:

```
BRCA    Breast invasive carcinoma
UCEC    Uterine Corpus Endometrial Carcinoma
LUAD    Lung adenocarcinoma
LGG     Brain Lower Grade Glioma
HNSC    Head and Neck squamous cell carcinoma
LUSC    Lung squamous cell carcinoma
KIRP    Cervical Kidney renal papillary cell carcinoma
SKCM    Skin Cutaneous Melanoma
KIRC    Kidney renal clear cell carcinoma
PRAD    Prostate adenocarcinoma
COAD    Colon adenocarcinoma
THCA    Thyroid carcinoma
BLCA    Bladder Urothelial Carcinoma
OV      Ovarian serous cystadenocarcinoma
STAD    Stomach adenocarcinoma
LIHC    Liver hepatocellular carcinoma
CESC    Cervical squamous cell carcinoma and endocervical adenocarcinoma
```

2.8 Check to see we have only tumor samples

[9]:
```
sum(sample_sheet['Sample Type'] == 'Primary Tumor')
sample_sheet["Sample Type"].value_counts()
```

[9]:
```
Primary Tumor    7731
Name: Sample Type, dtype: int64
```

2.9 Retain only one replicate

Create a **nr_sample_sheet**. I am following this step to reduce size of the data.

[10]:
```
# sample sheet no duplicates ; no replicates
sample_sheet_nr = sample_sheet.drop_duplicates(subset='Case ID', keep="first")
```

2.10 Count the number of samples

[11]:
```
sum(sample_sheet_nr['Project ID'].value_counts())
```

[11]:
```
7654
```

To keep the file size within the github size limit (< 100 MB), I have decided to keep only the following three classes.

TCGA-LGG
TCGA-LUAD
TCGA-THCA

For research projects, you should retain all the available sites/types that have enough (example >= 300) samples.

[12]:
```
df = sample_sheet_nr.loc[sample_sheet_nr['Project ID'].
 ↪isin(['TCGA-THCA','TCGA-LUAD','TCGA-LGG'])]
df['Project ID'].value_counts()
```

[12]:
```
TCGA-LUAD    508
TCGA-LGG     506
TCGA-THCA    445
Name: Project ID, dtype: int64
```

2.11 Pick a stratified sample (of 50) from each class

Create a dataframe, df, that will contain a sample of 50 entries from group. For this exercise, we are restricting samples of size <= 50. ***Please note that the sample_size of 50 is chosen for the hands-on. For research projects, you should change this to a bigger number (ex 300).***

[13]:
```
df = df.groupby('Project ID').apply(lambda s: s.sample(50))
num_of_classes = len(df['Project ID'].value_counts().index)
df = df.reset_index(drop = True)
print("Number of Cancer Types: ", num_of_classes)
```

```
Number of Cancer Types:  3
```

2.12 Filter the manifest file (read in previously) to create a new manifest file

Create a list to slice out a new GDC manifest file. Note this new manifest file will retain only the filtered (only for tumor; smaller sample size etc.) sample ids. This will then become the input for **GDC-toolkit** software (available in Biowulf). The output will be the corresponding RNAseq expression files. The expression files (one for each sample) have to be merged to create the final dataset.

Here is the code to accomplish the tasks:

[14]:
```
list = df['File Name'].values
print(len(list)) # should be 50 * 17 = 850 for this exercise
```

```
150
```

[15]:
```
mgdc = gdc_manifest[gdc_manifest['filename'].isin(list)]
mgdc.head(3)
```

[15]:

	id	filename	md5	size	state
5	0024bbc6-a956-47f3-a926-3dbe50c27ead	0e94f470-f240-474e-867d-bde7c803b6a4.FPKM-UQ.t…	9703e828d8acdc3b7c317964638c4b89	512774	validated
15	00749066-a4b1-4f5a-bca9-5e4699fb179f	ecff92ce-841b-42cd-8a80-9066a44ab9fe.FPKM-UQ.t…	eeb64317bc275ff866f084e915feccb1	524988	validated
47	016f3cc3-0aa7-4a0d-bbf5-67fa6f9c75c2	7cb874e2-6e35-4f1e-b340-2ab8a261e0b5.FPKM-UQ.t…	d2c5ba4aa45b281671337d2ca53c3a27	532645	validated

2.13 Write out the new mgdc dataframe

mgdc filename, **mgdc_manifest_20200309_162520_50_estypes.txt** file. We previously executed the following code chunk to create a filtered manifest file, mgdc_manifest_20200309_162520_50_3stypes (available from the Data sub-folder).

[16]:
```
# We ran the following command to produce the output. No need to run it again.
# mgdc.to_csv('Data/mgdc_manifest_20200309_162520_50_3stypes.txt', sep='\t',␣
 ↪index=False)
```

2.14 Use the Newly created manifest file to download/merge the expression data

I used NIH HPC for this step. For a sample size of 50 with 15 classes, you need decent memory/space. The gdc-rnaseq-tool (https://github.com/cpreid2/gdc-rnaseq-tool) will download (discussed in the introduction section) and merge the expression data.

Here is the template slurm script for the task. Before you use the script, please make sure the slurm script memory is optimal for your query.

```
#!/bin/bash
#SBATCH --time=10:00:00
#SBATCH --job-name="GDC50"
#SBATCH --cpus-per-task=4
#SBATCH --mem=10g

echo "Job Started at `date`"
# Make sure you have installed gdc-rnaseq-tool under /data/${USER}TC1 directory
cd /data/${USER}/TC1/gdc-rnaseq-tool-50-3stypes
module load python/3.5
python3 gdc-rnaseq-tool.py mgdc_manifest_20200309_162520_50.txt --hugo

echo "Job Ended  at `date`"
```

Bring the merged dataframe, **Merged_FPKM-UQ-50_3stypes.tsv** and continue the analysis/modeling. For your convenience, I have completed this step and transfered the merged RNASeq expression file. The file, Merged_FPKM-UP-50_3stypes.tsvl, is available under Data forlder

2.15 Read the merged expression file

[17]:
```
df_FPKM_UQ = pd.read_csv("Data/Merged_FPKM-UQ-50_3stypes.tsv",␣
 ↪low_memory=False, sep="\t")
print("Merged expression file")
df_FPKM_UQ.iloc[0:3, 0:6]
```

```
Merged expression file
```

[17]:

	Unnamed: 0	gene_name	TCGA-05-4249-01A-01R-1107-07	\
0	ENSG00000000003.13	TSPAN6	315981.544960	
1	ENSG00000000005.5	TNMD	0.000000	
2	ENSG00000000419.11	DPM1	542921.887819	

	TCGA-05-4389-01A-01R-1206-07	TCGA-05-4390-01A-02R-1755-07	\

```
0                  709336.737655                  356558.245525
1                     217.936145                       0.000000
2                  646521.025015                  771959.299629

   TCGA-05-4417-01A-22R-1858-07
0                  348652.528936
1                    1282.639158
2                  373545.187633
```

[18]:
```
df_FPKM_UQ.shape
```

[18]:
```
(60483, 152)
```

2.16 submitters_id mapping to project_id

The merged expression file contains submitters_id_list but doesnt include project_id. Submitters_id_list can be searched in GDC manually to find the relevant project_id. I have completed the steps and made the files available.

[19]:
```
cols = df_FPKM_UQ.columns[2:].values.tolist()
print("Length of cols variable: ",len(cols))
print("First few Submitters_ID", cols[1:4])

# DONT UNCOMMENT THE FOLLOWING LINES
# DONT DELETE THIS IS USED TO WRITE OUT THE FILE, submitters_id_list.txt
# this file was searched to find the project_id list from GDC website
# cols
# type(cols)
# with open('Data/submitters_id_list_50.txt', 'w') as f:
#     for item in cols:
#         f.write("%s\n" % item)
```

```
Length of cols variable:  150
First few Submitters_ID ['TCGA-05-4389-01A-01R-1206-07',
'TCGA-05-4390-01A-02R-1755-07', 'TCGA-05-4417-01A-22R-1858-07']
```

2.17 Map "submitters_ID" to "Project_ID"

This step was done using GDC website.

[20]:
```
submitters_id_to_project_id = pd.read_csv("Data/submitters_id_to_project_id_50.
 ↪tsv", low_memory=False, sep="\t")
print("Here is the Submitters_ID to Project_ID mapping list:")
print(submitters_id_to_project_id.iloc[0:4,0:6])
```

```
Here is the Submitters_ID to Project_ID mapping list:
            submittedAliquot ID  mappedCaseId mappedProject
0  TCGA-05-4249-01A-01R-1107-07  TCGA-05-4249     TCGA-LUAD
```

```
1  TCGA-05-4389-01A-01R-1206-07  TCGA-05-4389     TCGA-LUAD
2  TCGA-05-4390-01A-02R-1755-07  TCGA-05-4390     TCGA-LUAD
3  TCGA-05-4417-01A-22R-1858-07  TCGA-05-4417     TCGA-LUAD
```

[21]:
```
submitters_id_to_project_id.columns
submitters_id_to_project_id.mappedProject.value_counts()
```

[21]:
```
TCGA-LGG     50
TCGA-THCA    50
TCGA-LUAD    50
Name: mappedProject, dtype: int64
```

[22]:
```
# Transpose the data
dft_FPKM_UQ = df_FPKM_UQ.T
# remove the two two rows and save the output
dftm_FPKM_UQ = dft_FPKM_UQ.drop(dft_FPKM_UQ.index[0:2], axis=0)
```

[23]:
```
dftm_FPKM_UQ.shape
```

[23]:
```
(150, 60483)
```

Extract the Submitter ID from the index and attach it as a column also called Submitter ID

[24]:
```
dftm_FPKM_UQ['submitter_id'] = dftm_FPKM_UQ.index

# reset the index
dftm_FPKM_UQ = dftm_FPKM_UQ.reset_index(drop=True)
sid_list = submitters_id_to_project_id['submittedAliquot ID'].values.tolist()

dftm_FPKM_UQ['Project_id'] = ' '
for idx, val in dftm_FPKM_UQ['submitter_id'].items():
    temp_sid = sid_list.index(val)
    dftm_FPKM_UQ['Project_id'][idx] =␣
 ↪submitters_id_to_project_id['mappedProject'][temp_sid]

dftm_FPKM_UQ.drop(['submitter_id'], axis = 1,  inplace=True)
```

2.18 Final check before moving on

[25]:
```
print(dftm_FPKM_UQ.Project_id.value_counts())
dftm_FPKM_UQ.iloc[[0,49,123],[0,1,2,3,4,5,6,7,8,9,60483]]
```

```
TCGA-LGG     50
TCGA-THCA    50
TCGA-LUAD    50
Name: Project_id, dtype: int64
```

[25]:
```
           0        1       2        3        4        5        6       7  \
0     315982        0  542922  89170.7  28230.6   113983   109903  416956
49    309252        0  943647  50705.5  12560.9  87490.8   369044  616390
123   229996  936.194  320436  46994.1  14297.2    66556  35900.7  181326

            8       9 Project_id
0     60672.3  595059  TCGA-LUAD
49    78783.3  185087  TCGA-THCA
123    382598  264040   TCGA-LGG
```

2.19 Convert outcome into numerical quantity

[26]:
```
# multiple options for accomplishing this task
le = preprocessing.LabelEncoder()

# Create a label (category) encoder object
dftm_FPKM_UQ['Project_id'] = le.fit_transform(dftm_FPKM_UQ.Project_id.values)
```

2.19.1 These are the coded variables and their mappings

[27]:
```
num = np.arange(0,len(le.classes_),1)
print("codenum", num)

print("labels: ", le.inverse_transform(num))
```

```
codenum [0 1 2]
labels:  ['TCGA-LGG' 'TCGA-LUAD' 'TCGA-THCA']
```

2.20 Split the features and outcome variables

[28]:
```
# Use to_categorical on your labels
features = dftm_FPKM_UQ.drop(['Project_id'], axis=1)
outcome = dftm_FPKM_UQ.Project_id
outcome
print(outcome)
print(type(features))
```

```
0      1
1      1
2      1
3      1
4      1
      ..
145    0
146    0
147    0
148    0
```

```
149    0
Name: Project_id, Length: 150, dtype: int32
<class 'pandas.core.frame.DataFrame'>
```

2.21 Scaling procedure for the features

FPKM* (FPKM_UQ or FPKM) to TPM Mathematically it can be shown that either the input data is FPKM or FPKM_UQ, you can follow the same scaling to get to TPM.

[29]:
```
Image(filename='Img/scaling1.PNG')
```

[29]:

$$\text{temp}_i = \left(\frac{\text{FPKM}^*}{\sum_j \text{FPKM}^*_j} \right) \times 10^6$$

$$\text{TPM}_i = log_{10}(\text{temp}_i)$$

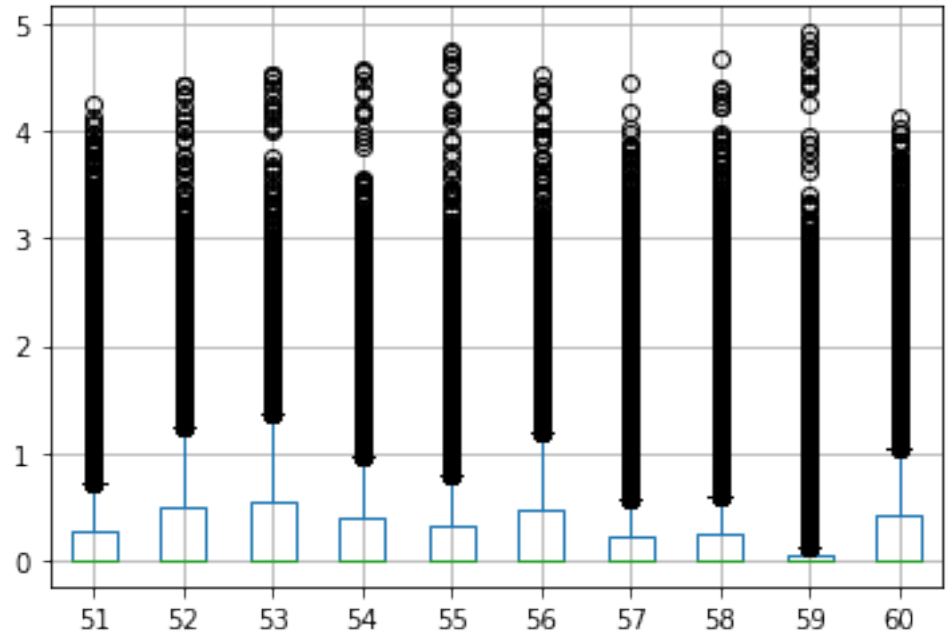
features: 150 x 60,483 sum(axis = 1): RowSum

[30]:
```
# TPM
sfeatures = features.div(features.sum(axis=1), axis=0)
sfeatures = sfeatures * 1000000

sfeatures1 = sfeatures.astype(np.float64).apply(np.log10)
sfeatures1[sfeatures1 < 0] = 0
```

2.22 Let us look at the distribution of few selected samples

[31]:
```
df_temp = sfeatures1.iloc[51:61, :]
df_temp.T.boxplot()
```

[31]: <matplotlib.axes._subplots.AxesSubplot at 0x212b3e11cc8>



2.23 Save the files for later use

[32]:
```
# sfeatures1.to_csv('Data/TC1-data3stypes.tsv', sep='\t', index=False)
# outcome.to_csv('Data/TC1-outcome-data3stypes.tsv', sep='\t', index=False,␣
↪header = False)
```

2.24 Part-II: Convolutional Neural Network

- Splitting the data
- Model preparation
- Training/Testing
- Exporting the Weights
- inference option

Check out the Jupyter Notebook (TC1-ConvNN.ipynb) for Part-II from https://github.com/ravichas/ML-TC1

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