Source of Data: https://www.nature.com/articles/sdata201861#Sec8m Unifying cancer and normal RNA sequencing data from different sources

Goal of notebook:

Take different subtypes of cancer and cluster them to better understand differences in expression between them.

Additionally do differential gene expression analysis on healthly vs tumor, and see the effect on PCA to clustering

Types of cancer: Lung: LUAD, LUSC Colon: Sigmoid, transverse Kidney cortex: KIRC,KIRP,KICH

GTEX: Database of different tissues completly from non-cancer donors TCGA: From the cancer genome atlas program data matched for a patient from tumor and surrounding "normal" tissue

TABLE 1: List of cancer data avalible

GTEx tissue / TCGA cancer type	GTEx	TCGA normal	TCGA tumor	Total
bladder / blca	11	19	411	441
breast / brca	218	114	1112	1444
cervix / cesc	11	3	304	318
uterus / ucec	90	24	180	294
uterus / ucs		0	57	57
colon-sigmoid / read	173	10	94	277
colon-transverse / coad	203	41	295	539
liver / lihc	136	50	371	557
salivary gland / hnsc	70	44	520	634
esophageal / esca	790	11	185	986
prostate / prad	119	52	497	668
stomach / stad	204	35	415	654
thyroid / thca	355	59	505	919
lung / luad	374	59	528	961
lung / lusc		51	504	555
kidney cortex / kirc	36	72	541	649
kidney cortex / kirp		32	290	322
kidney cortex / kich		25	66	91
Total	2790	701	6875	10366

In [3]:
```
import pandas as pd
import os
import matplotlib.pyplot as plt
import numpy as np
```

In [2]:
```
#Using RSEMcounts because DEseq2 does differential gene analysis through count data
!ls | grep "rsemcount" > file_names.txt
```

In [1]:
```
f = open("file_names.txt", "r")
list_files = f.read().split('\n')

list_files
```

Out[1]:
```
['luadrsemcounttcgat.txt',
 'luadrsemcounttcga.txt',
 'lungrsemcountgtex.txt',
 'luscrsemcounttcgat.txt',
```

```
 'luscrsemcounttcga.txt',
 '']
```

In [4]:
```
#Loading in data
lung_luad_TCGA_norm = pd.read_csv(list_files[1],sep='\t')
lung_luad_TCGA_tumor = pd.read_csv(list_files[0],sep='\t')
```

In [6]:
```
#Create subsets of TCGA norm and tumor where the IDS are found in both datasets: Same Pa
lung_luad_norm = sorted(lung_luad_TCGA_norm)
lung_luad_tumor = sorted(lung_luad_TCGA_tumor)
```

In [7]:
```
matching_IDs_norm = []

#find the IDs in lung_luad_norm that are the same in the tumor
#in order to match patients tumor and "normal" tissue

for ID in lung_luad_norm[2:]:
    print("-".join(ID.split('-')[0:3]))
    #only the first three sections of ID are found in both tumor and normal tissue
    ID_3 = "-".join(ID.split('-')[0:3])

    if any(ID_3 in ID_t for ID_t in lung_luad_tumor[2:]):
        matching_IDs_norm.append(ID)

matching_IDs_norm
```

```
TCGA-38-4625
TCGA-38-4626
TCGA-38-4627
TCGA-38-4632
TCGA-44-2655
TCGA-44-2657
TCGA-44-2661
TCGA-44-2662
TCGA-44-2665
TCGA-44-2668
TCGA-44-3396
TCGA-44-3398
TCGA-44-5645
TCGA-44-6144
TCGA-44-6145
TCGA-44-6146
TCGA-44-6147
TCGA-44-6148
TCGA-44-6776
TCGA-44-6777
TCGA-44-6778
TCGA-49-4490
TCGA-49-4512
TCGA-49-6742
TCGA-49-6743
TCGA-49-6744
TCGA-49-6745
TCGA-49-6761
TCGA-50-5930
TCGA-50-5931
TCGA-50-5932
TCGA-50-5933
TCGA-50-5935
TCGA-50-5936
TCGA-50-5939
TCGA-50-6595
TCGA-55-6968
TCGA-55-6969
TCGA-55-6970
```

```
TCGA-55-6971
TCGA-55-6972
TCGA-55-6975
TCGA-55-6978
TCGA-55-6979
TCGA-55-6980
TCGA-55-6981
TCGA-55-6982
TCGA-55-6983
TCGA-55-6984
TCGA-55-6985
TCGA-55-6986
TCGA-73-4676
TCGA-91-6828
TCGA-91-6829
TCGA-91-6831
TCGA-91-6835
TCGA-91-6836
TCGA-91-6847
TCGA-91-6849
```

Out[7]:

```
['TCGA-38-4625-11A-01R-1758-07',
 'TCGA-38-4626-11A-01R-1758-07',
 'TCGA-38-4627-11A-01R-1758-07',
 'TCGA-38-4632-11A-01R-1755-07',
 'TCGA-44-2655-11A-01R-1758-07',
 'TCGA-44-2657-11A-01R-1758-07',
 'TCGA-44-2661-11A-01R-1758-07',
 'TCGA-44-2662-11A-01R-1758-07',
 'TCGA-44-2665-11A-01R-1758-07',
 'TCGA-44-2668-11A-01R-1758-07',
 'TCGA-44-3396-11A-01R-1758-07',
 'TCGA-44-3398-11B-01R-1758-07',
 'TCGA-44-5645-11A-01R-1628-07',
 'TCGA-44-6145-11A-01R-1858-07',
 'TCGA-44-6146-11A-01R-1858-07',
 'TCGA-44-6147-11A-01R-1858-07',
 'TCGA-44-6148-11A-01R-1858-07',
 'TCGA-44-6776-11A-01R-1858-07',
 'TCGA-44-6777-11A-01R-1858-07',
 'TCGA-44-6778-11A-01R-1858-07',
 'TCGA-49-4490-11A-01R-1858-07',
 'TCGA-49-6742-11A-01R-1858-07',
 'TCGA-49-6743-11A-01R-1858-07',
 'TCGA-49-6744-11A-01R-1858-07',
 'TCGA-49-6745-11A-01R-1858-07',
 'TCGA-49-6761-11A-01R-1949-07',
 'TCGA-50-5930-11A-01R-1755-07',
 'TCGA-50-5931-11A-01R-1858-07',
 'TCGA-50-5932-11A-01R-1755-07',
 'TCGA-50-5933-11A-01R-1755-07',
 'TCGA-50-5935-11A-01R-1858-07',
 'TCGA-50-5936-11A-01R-1628-07',
 'TCGA-50-5939-11A-01R-1628-07',
 'TCGA-50-6595-11A-01R-1858-07',
 'TCGA-55-6968-11A-01R-1949-07',
 'TCGA-55-6969-11A-01R-1949-07',
 'TCGA-55-6970-11A-01R-1949-07',
 'TCGA-55-6971-11A-01R-1949-07',
 'TCGA-55-6972-11A-01R-1949-07',
 'TCGA-55-6975-11A-01R-1949-07',
 'TCGA-55-6978-11A-01R-1949-07',
 'TCGA-55-6979-11A-01R-1949-07',
 'TCGA-55-6980-11A-01R-1949-07',
 'TCGA-55-6981-11A-01R-1949-07',
 'TCGA-55-6982-11A-01R-1949-07',
```

```
'TCGA-55-6983-11A-01R-1949-07',
'TCGA-55-6984-11A-01R-1949-07',
'TCGA-55-6985-11A-01R-1949-07',
'TCGA-55-6986-11A-01R-1949-07',
'TCGA-73-4676-11A-01R-1755-07',
'TCGA-91-6828-11A-01R-1858-07',
'TCGA-91-6829-11A-01R-1858-07',
'TCGA-91-6831-11A-02R-1858-07',
'TCGA-91-6835-11A-01R-1858-07',
'TCGA-91-6836-11A-01R-1858-07',
'TCGA-91-6847-11A-01R-1949-07',
'TCGA-91-6849-11A-01R-1949-07']
```

In [8]:
```
#Take the correct subset from TCGA from whole dataset
matching_subset_norm = matching_IDs_norm.copy()[0:11]

tumor_IDs = []

for ID in matching_subset_norm:
    ID_3 = "-".join(ID.split('-')[0:3])
    tumor_IDs = tumor_IDs + [x for x in lung_luad_tumor if ID_3 in x]

lung_luad_TCGA_norm_subset = lung_luad_TCGA_norm.copy()[lung_luad_TCGA_norm.columns[lung
lung_luad_TCGA_tumor_subset = lung_luad_TCGA_tumor.copy()[lung_luad_TCGA_tumor.columns[l
```

In [9]:
```
lung_luad_TCGA_norm_subset['Hugo_Symbol'] = lung_luad_TCGA_norm['Hugo_Symbol']
lung_luad_TCGA_tumor_subset['Hugo_Symbol'] = lung_luad_TCGA_tumor['Hugo_Symbol']
```

In [11]:
```
norm_TCGA = lung_luad_TCGA_norm_subset.set_index('Hugo_Symbol')#.to_csv('lung_luad_TCGA_

tumor_TCGA = lung_luad_TCGA_tumor_subset.set_index('Hugo_Symbol')#.to_csv('lung_luad_TCG


result = pd.concat([tumor_TCGA,norm_TCGA],axis=1)

result_columns = sorted(result)

result[result_columns] = result[result_columns].apply(np.int64)


result
```

Out[11]:

Hugo_Symbol	TCGA-38-4625-01A-01R-1206-07	TCGA-44-3396-01A-01R-1206-07	TCGA-38-4627-01A-01R-1206-07	TCGA-38-4626-01A-01R-1206-07	TCGA-44-2665-01A-01R-A278-07	TCGA-44-2661-01A-01R-1107-07	TCGA-44-2657-01A-01R-1107-07	TCGA-44-2668-01A-01R-0946-07	TCGA-44-2668-01A-01R-A278-07	TCGA-44-2662-01A-01R-0946-07	...	TCGA-38-4627-11A-01R-1758-07	TCG
OR11G2	0	0	0	0	0	0	0	0	0	0	...	0	
DEFB123	0	0	0	0	0	0	0	0	0	0	...	0	
SBNO2	0	17399	4915	16662	2887	3285	2717	19786	8347	10144	...	2599	68
HSD3B1	0	0	0	2	0	0	0	0	0	0	...	0	
EIF4E	5	5587	2950	4971	3629	3891	2055	4812	1934	4747	...	2452	22
...	...	...	...	...	...	...	...	...	...	...	...	...	
ZBTB48	0	1767	671	1940	878	915	1058	979	344	960	...	486	11
RAB19	0	164	37	70	48	57	31	52	62	54	...	15	
KLHL21	0	3140	2979	5001	958	2151	1941	2601	1118	3714	...	1270	28

LYG1	0	32	6	25	50	72	28	26	7	15	...	8	
ZNF711	0	118	200	285	738	63	256	73	35	138	...	79	1

20242 rows × 26 columns

In [12]:
```
#Outputing the processed subset of data to load into DESeq2
result.to_csv('lung_luad_TCGA_tumor_norm_subset_3.csv',index=True)
```

In [22]:
```
sample_info = pd.read_csv("Luad_TCGA_sampleinfo.csv",sep=',')

sample_info = sample_info.copy().set_index('Unnamed: 0')
sample_info
```

Out[22]:

Unnamed: 0	Cancer_or_healthy
TCGA-38-4625-01A-01R-1206-07	Cancer
TCGA-44-3396-01A-01R-1206-07	Cancer
TCGA-38-4627-01A-01R-1206-07	Cancer
TCGA-38-4626-01A-01R-1206-07	Cancer
TCGA-44-2665-01A-01R-A278-07	Cancer
TCGA-44-2661-01A-01R-1107-07	Cancer
TCGA-44-2657-01A-01R-1107-07	Cancer
TCGA-44-2668-01A-01R-0946-07	Cancer
TCGA-44-2668-01A-01R-A278-07	Cancer
TCGA-44-2662-01A-01R-0946-07	Cancer
TCGA-44-2655-01A-01R-0946-07	Cancer
TCGA-38-4632-01A-01R-1755-07	Cancer
TCGA-44-2665-01A-01R-0946-07	Cancer
TCGA-44-2662-01A-01R-A278-07	Cancer
TCGA-38-4625-01A-01R-1206-07.1	Cancer
TCGA-38-4632-11A-01R-1755-07	Healthy
TCGA-38-4627-11A-01R-1758-07	Healthy
TCGA-44-2665-11A-01R-1758-07	Healthy
TCGA-44-2662-11A-01R-1758-07	Healthy
TCGA-44-2661-11A-01R-1758-07	Healthy
TCGA-44-2668-11A-01R-1758-07	Healthy
TCGA-38-4625-11A-01R-1758-07	Healthy
TCGA-44-3396-11A-01R-1758-07	Healthy
TCGA-44-2657-11A-01R-1758-07	Healthy
TCGA-38-4626-11A-01R-1758-07	Healthy
TCGA-44-2655-11A-01R-1758-07	Healthy

In [45]:
```
sample_info.to_csv('lung_luad_sampleinfo_indexed.csv',index=True)
```

In [58]:
```
#Comparing PCA between healthy and cancer lung, all genes vs top 200 most differentially
```

```
import numpy as np
import pandas as pd
import matplotlib.pyplot as plt
```

In [ ]:

In [96]:
```
#Lists of IDS
matching_subset_norm
tumor_IDs
```

Out[96]:
```
['TCGA-38-4625-01A-01R-1206-07',
 'TCGA-38-4625-01A-01R-1206-07.1',
 'TCGA-38-4626-01A-01R-1206-07',
 'TCGA-38-4627-01A-01R-1206-07',
 'TCGA-38-4632-01A-01R-1755-07',
 'TCGA-44-2655-01A-01R-0946-07',
 'TCGA-44-2657-01A-01R-1107-07',
 'TCGA-44-2661-01A-01R-1107-07',
 'TCGA-44-2662-01A-01R-0946-07',
 'TCGA-44-2662-01A-01R-A278-07',
 'TCGA-44-2665-01A-01R-0946-07',
 'TCGA-44-2665-01A-01R-A278-07',
 'TCGA-44-2668-01A-01R-0946-07',
 'TCGA-44-2668-01A-01R-A278-07',
 'TCGA-44-3396-01A-01R-1206-07']
```

In [13]:
```
#convert FPKM to TPM for the right files
lung_luad_TCGA_norm_FPKM = pd.read_csv("luadrsemfpkmtcga_healthy.txt",sep='\t')
lung_luad_TCGA_tumor_FPKM = pd.read_csv("luadrsemfpkmtcga_tumor.txt",sep='\t')
```

In [14]:
```
lung_luad_TCGA_norm_FPKM_subset = lung_luad_TCGA_norm_FPKM.copy()[lung_luad_TCGA_norm_FP

lung_luad_TCGA_tumor_FPKM_subset = lung_luad_TCGA_tumor_FPKM.copy()[lung_luad_TCGA_tumor
```

In [15]:
```
#convert FPKM to TPM --> fraction of total expression
lung_luad_TCGA_norm_TPM_subset = lung_luad_TCGA_norm_FPKM_subset.copy().div(lung_luad_TC

lung_luad_TCGA_tumor_TPM_subset = lung_luad_TCGA_tumor_FPKM_subset.copy().div(lung_luad_
```

In [4]:
```
def normalize_data(gene_exp_table):
    #Find fold change relative to the mean
    gene_means = np.mean(gene_exp_table,axis=0)
    gene_exp_table_divide = np.divide(gene_exp_table,gene_means)
    gene_exp_array_log = np.log2(gene_exp_table_divide)

    #Zero mean the data
    gene_means = gene_exp_array_log.mean(axis=0)
    gene_exp_final = gene_exp_array_log - gene_means

    return gene_exp_final
```

In [17]:
```
norm_TPM_subset = lung_luad_TCGA_norm_TPM_subset.T
norm_TPM_subset_np = norm_TPM_subset.copy().to_numpy()

tumor_TPM_subset = lung_luad_TCGA_tumor_TPM_subset.T
tumor_TPM_subset_np = tumor_TPM_subset.copy().to_numpy()

#replace dropout events with very small expression
norm_TPM_subset_np[norm_TPM_subset_np == 0] = 0.01
tumor_TPM_subset_np[tumor_TPM_subset_np == 0] = 0.01

print(np.all(norm_TPM_subset_np))
```

```
True
```

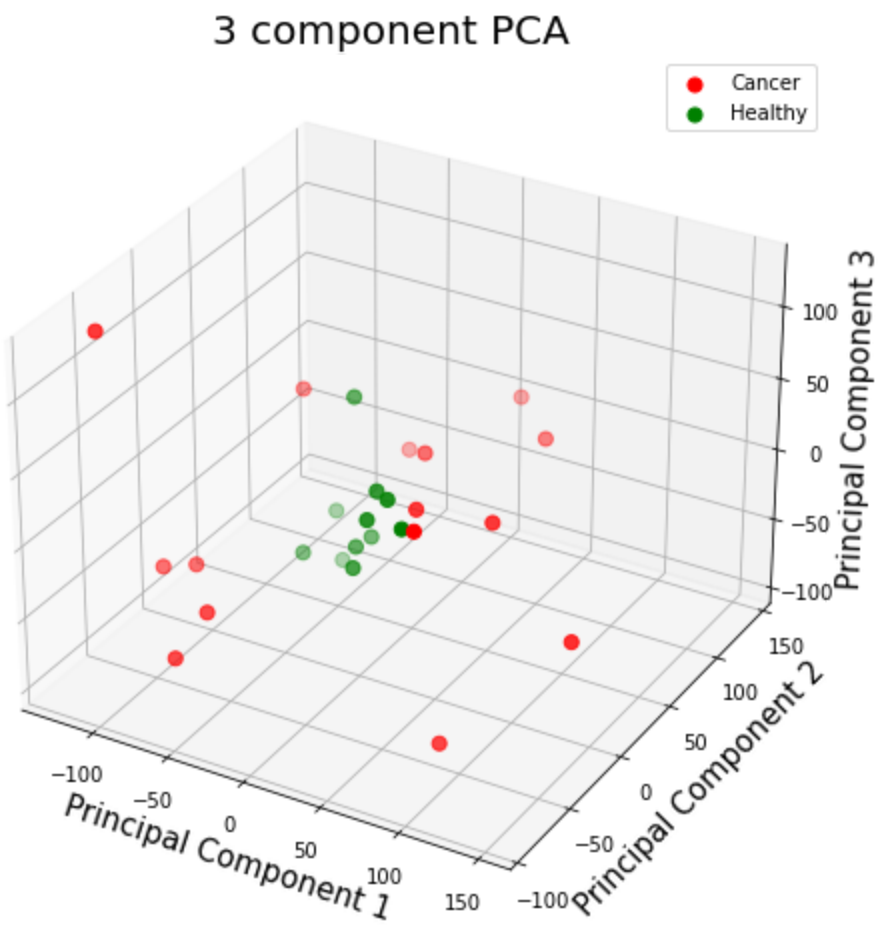
In [18]:
```
#apply normalization
norm_TPM_subset_np = normalize_data(norm_TPM_subset_np.copy())
tumor_TPM_subset_np = normalize_data(tumor_TPM_subset_np.copy())

tumor_norm = np.concatenate((tumor_TPM_subset_np, norm_TPM_subset_np), axis=0)
```

In [20]:
```
#PCA on whole data
from sklearn.decomposition import PCA
pca = PCA(n_components=2)
principalComponents = pca.fit_transform(tumor_norm)
principalDf = pd.DataFrame(data = principalComponents
             , columns = ['principal component 1', 'principal component 2'])
```

In [29]:
```
finalDf = pd.concat([principalDf, sample_info.reset_index()[['Cancer_or_healthy']]], axi
finalDF_output_norm = finalDf
```

In [55]:
```
def PCA_2d(title,target1,target2,column_of_targets,data):

    fig = plt.figure(figsize = (8,8))
    ax = fig.add_subplot(1,1,1)
    ax.set_xlabel('Principal Component 1', fontsize = 15)
    ax.set_ylabel('Principal Component 2', fontsize = 15)
    ax.set_title(title, fontsize = 20)
    targets = [target1, target2]
    colors = ['r', 'g']
    for target, color in zip(targets,colors):
        indicesToKeep = data[column_of_targets] == target
        ax.scatter(data.loc[indicesToKeep, 'principal component 1']
                   , data.loc[indicesToKeep, 'principal component 2']
                   , c = color
                   , s = 50)
    ax.legend(targets)
    ax.grid()

    return 1


#PCA_2d('All genes: Tumor vs Healthy, 2 components','Cancer', 'Healthy','Cancer_or_healt
```

In [130…
```
finalDf = pd.concat([principalDf, sample_info.reset_index()[['Cancer_or_healthy']]], axi

fig = plt.figure(figsize = (8,8))
ax = fig.add_subplot(projection='3d')
ax.set_xlabel('Principal Component 1', fontsize = 15)
ax.set_ylabel('Principal Component 2', fontsize = 15)
ax.set_zlabel('Principal Component 3', fontsize = 15)
ax.set_title('3 component PCA', fontsize = 20)
targets = ['Cancer', 'Healthy']
colors = ['r', 'g']
for target, color in zip(targets,colors):
    indicesToKeep = finalDf['Cancer_or_healthy'] == target
    ax.scatter(finalDf.loc[indicesToKeep, 'principal component 1']
               , finalDf.loc[indicesToKeep, 'principal component 2']
               , finalDf.loc[indicesToKeep, 'principal component 3']
               , c = color
               , s = 50)
ax.legend(targets)
ax.grid()
```



In [128…

```
pca = PCA(n_components=3)
principalComponents = pca.fit_transform(tumor_norm)
principalDf = pd.DataFrame(data = principalComponents
             , columns = ['principal component 1', 'principal component 2','principal co
principalDf
```

Out[128]:

	principal component 1	principal component 2	principal component 3
0	89.502554	-4.718252	28.799292
1	11.616791	141.247883	0.715372
2	-47.661454	-71.911132	-32.389854
3	86.465360	-49.097728	-95.406365
4	10.560884	47.406744	19.147794
5	-55.929943	-89.382962	-55.290808
6	56.057704	98.145493	10.944242
7	50.970306	-19.516960	35.457194
8	-127.928305	-57.652731	126.500102
9	149.717690	-19.638993	-23.793081
10	-101.710069	-39.585559	-39.391066
11	-81.638886	62.773739	27.337266
12	-40.924931	106.752076	-31.510079
13	-87.236195	-29.314596	-40.112388
14	88.138493	-75.507023	68.992377
15	-0.284425	12.080964	-20.353952
16	-37.411641	31.478458	-26.104923

17	10.279607	-19.935403	-17.598351
18	16.380644	-6.445260	28.851327
19	29.772478	-15.681145	33.078778
20	-0.400380	-2.223010	-17.960108
21	-35.928507	-1.672410	-33.650633
22	-28.741691	24.950919	-54.330749
23	-23.375751	29.250624	59.454342
24	50.020221	-31.623644	29.705095
25	19.689446	-20.180094	18.909175

In [31]:
```
#Take subset of data, the top 200 most differntially expressed genes
DE_genes = pd.read_csv('DE_Genes.csv',sep=',')

DE_genes['Gene'] = DE_genes['Unnamed: 0']
```

In [32]:
```
lung_luad_TCGA_norm_FPKM

de_genes = sorted(DE_genes['Gene'])

select_genes = lung_luad_TCGA_norm_FPKM[lung_luad_TCGA_norm_FPKM['Hugo_Symbol'].isin(de_

select_genes
```

Out[32]:

	Hugo_Symbol	Entrez_Gene_Id	TCGA-55-6980-11A-01R-1949-07	TCGA-55-6984-11A-01R-1949-07	TCGA-50-5936-11A-01R-1628-07	TCGA-91-6849-11A-01R-1949-07	TCGA-55-6968-11A-01R-1949-07	TCGA-50-5930-11A-01R-1755-07	TCGA-49-6745-11A-01R-1858-07	TCG 59 1 0 18
214	CES1	1066	6207.38	10659.59	11267.44	8840.04	12076.21	13306.94	5633.22	4210
395	SPAG4	6676	158.79	63.00	87.03	175.07	166.73	115.16	124.37	57
450	C19orf59	199675	2004.85	3257.52	3168.41	1617.00	5147.73	3901.01	4575.41	1175
668	ACVRL1	94	2384.37	1088.92	3589.58	3716.20	1697.45	2502.97	2090.03	3212
696	CYP24A1	1591	13.72	8.25	3.76	28.24	2.07	3.50	12.36	1
...	...	...	...	...	...	...	...	...	...	...
19506	VIPR1	7433	2271.40	447.82	3515.68	6426.31	753.83	2384.37	854.13	5403
19513	EPHX3	79852	59.55	115.97	54.72	73.03	62.56	90.77	62.12	65
19552	GOLM1	51280	417.77	543.96	356.05	743.43	463.65	457.25	540.19	285
19635	B4GALNT4	338707	14.03	14.24	7.34	19.53	18.29	1.95	3.66	6
19640	UBE2T	29089	62.12	72.52	42.41	70.51	149.12	62.12	165.57	29

200 rows × 61 columns

In [33]:
```
select_genes_norm_TPM_subset = lung_luad_TCGA_norm_TPM_subset[lung_luad_TCGA_norm_TPM_su

select_genes_tumor_TPM_subset = lung_luad_TCGA_tumor_TPM_subset[lung_luad_TCGA_tumor_TPM


tumor_norm_select = pd.concat((select_genes_tumor_TPM_subset, select_genes_norm_TPM_subs
```

```
tumor_norm_select
```

Out[33]:

	TCGA-93-7348-01A-21R-2039-07	TCGA-91-8496-01A-11R-2403-07	TCGA-05-4397-01A-01R-1206-07	TCGA-69-7761-01A-11R-2170-07	TCGA-55-7281-01A-11R-2039-07	TCGA-50-6590-01A-12R-1858-07	TCGA-97-A4M1-01A-11R-A24X-07	TCGA-05-4402-01A-01R-1206-07	TCGA-44-7667-01A-31R-2060-0
214	54.459321	117.374789	650.642659	27.814737	20.745286	14.301084	297.361657	27.665667	11.59082
395	39.855011	6.812443	18.789152	58.858061	9.458059	64.524769	7.353438	53.121564	10.15496
450	0.901826	114.164402	0.358448	2.153244	8.699970	0.727360	34.886835	6.110185	1.52420
668	15.937595	20.720938	10.400745	10.290675	13.769257	13.433222	44.168964	12.791456	11.51072
696	10.500266	2.824854	0.072842	13.130135	4.066566	22.467875	0.014904	33.138372	11.19444
...	...	...	...	...	...	...	...	...	
19506	10.796407	12.830981	1.085300	7.625071	2.352226	0.680526	50.043657	6.067847	5.81303
19513	14.259748	2.353079	2.318904	12.078313	23.022624	15.545529	7.353438	9.221568	37.50094
19552	161.777504	32.534399	84.152911	186.111658	85.433986	95.149399	22.843793	118.763234	48.81485
19635	19.495182	0.496318	1.745074	3.432100	0.611003	20.104193	4.770570	1.070966	47.15058
19640	18.441611	11.804257	204.430946	39.906856	10.498415	46.895607	11.166107	25.453973	87.41752

200 rows × 26 columns

In [35]:
```
#transpose
tumor_norm_select_np = tumor_norm_select.T.to_numpy()

#replace dropout events with very small expression
tumor_norm_select_np[tumor_norm_select_np == 0] = 0.01

#normalize
tumor_norm_select_np = normalize_data(tumor_norm_select_np.copy())
```

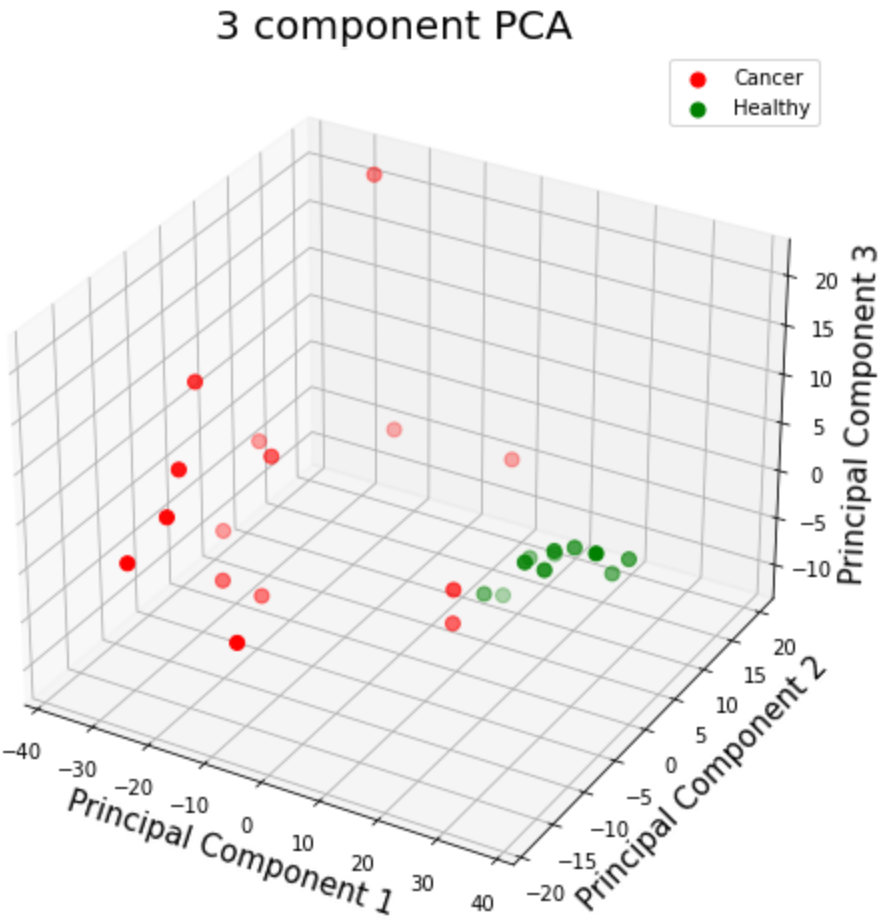
In [37]:
```
from sklearn.decomposition import PCA
pca = PCA(n_components=2)
principalComponents = pca.fit_transform(tumor_norm_select_np)
principalDf1 = pd.DataFrame(data = principalComponents
             , columns = ['principal component 1', 'principal component 2'])
```

In [38]:
```
finalDf = pd.concat([principalDf1, sample_info.reset_index()[['Cancer_or_healthy']]], ax

select_finalDF_output = finalDf
```

In [195…
```
pca = PCA(n_components=3)
principalComponents = pca.fit_transform(tumor_norm_select_np)
principalDf = pd.DataFrame(data = principalComponents
             , columns = ['principal component 1', 'principal component 2','principal co
```

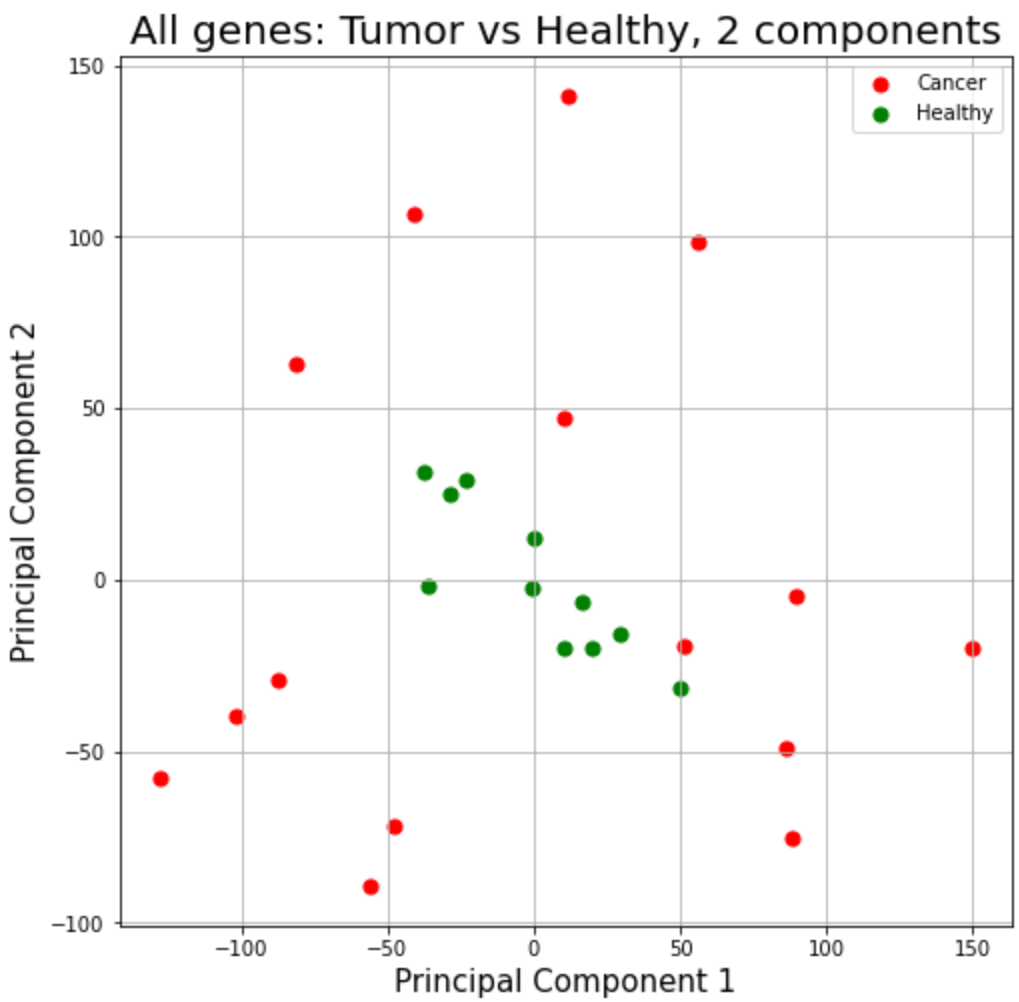
In [196…
```
finalDf = pd.concat([principalDf, sample_info.reset_index()[['Cancer_or_healthy']]], axi

fig = plt.figure(figsize = (8,8))
ax = fig.add_subplot(projection='3d')
ax.set_xlabel('Principal Component 1', fontsize = 15)
ax.set_ylabel('Principal Component 2', fontsize = 15)
ax.set_zlabel('Principal Component 3', fontsize = 15)
ax.set_title('3 component PCA', fontsize = 20)
targets = ['Cancer', 'Healthy']
colors = ['r', 'g']
for target, color in zip(targets,colors):
```

```
    indicesToKeep = finalDf['Cancer_or_healthy'] == target
    ax.scatter(finalDf.loc[indicesToKeep, 'principal component 1']
               , finalDf.loc[indicesToKeep, 'principal component 2']
               , finalDf.loc[indicesToKeep, 'principal component 3']
               , c = color
               , s = 50)
ax.legend(targets)
ax.grid()
```



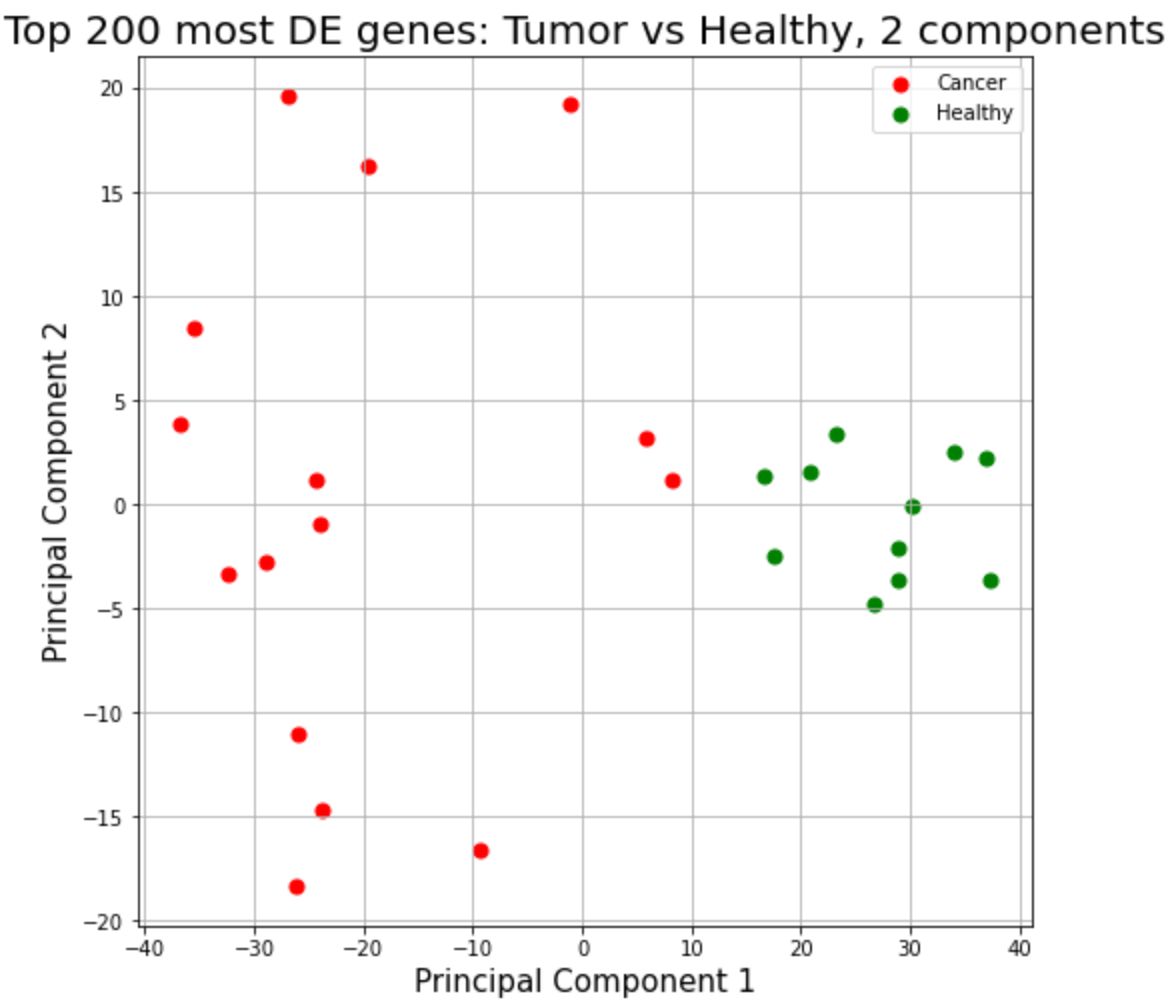
In [39]:
```
PCA_2d('All genes: Tumor vs Healthy, 2 components','Cancer', 'Healthy','Cancer_or_health
```

Out[39]:
```
1
```



In [40]:
```
PCA_2d('Top 200 most DE genes: Tumor vs Healthy, 2 components','Cancer', 'Healthy','Canc
```

Out[40]:
```
1
```



Compare the clustering of different cancer and their subtypes

In [7]:
```
import pandas as pd
import os
import matplotlib.pyplot as plt
import numpy as np

luad = pd.read_csv('luadrsemfpkmtcga_tumor.txt',sep='\t')

lusc = pd.read_csv('luscrsemfpkmtcga_tumor.txt',sep='\t')
```

In [8]:
```
#take sample of 50 from each dataset
luad_sub = luad.copy().iloc[:,2:52]
lusc_sub = lusc.copy().iloc[:,2:52]

luad=luad_sub
lusc=lusc_sub

luad_tpm = luad_sub.copy().div(luad_sub.sum(axis=0), axis=1)*1000000
lusc_tpm = lusc_sub.copy().div(lusc_sub.sum(axis=0), axis=1)*1000000
```

In [9]:
```
#join the two dataset together
luad_lusc = pd.concat((luad_tpm, lusc_tpm), axis=1)

#transpose
luad_lusc_np = luad_lusc.T.to_numpy()

#replace dropout events with very small expression
luad_lusc_np[luad_lusc_np == 0] = 0.01
```

```
#normalize
luad_lusc_np = normalize_data(luad_lusc_np.copy())
```

In [10]:
```
#PCA
from sklearn.decomposition import PCA
pca = PCA(n_components=2)
principalComponents = pca.fit_transform(luad_lusc_np)
principalDf = pd.DataFrame(data = principalComponents
             , columns = ['principal component 1', 'principal component 2'])
```

In [11]:
```
#create sample_info
#first 50 are LUAD secound 50 are LUSC

luad_or_lusc = []

for i in range(0,100):
    if i < 50:
        luad_or_lusc.append('LUAD')
    else:
        luad_or_lusc.append('LUSC')

luad_lusc.columns.tolist()

sample_info = pd.DataFrame(list(zip(luad_lusc.columns.tolist(), luad_or_lusc)),columns =



finalDf_luad_lusc = pd.concat([principalDf, sample_info.reset_index()[['LUAD_or_LUSC']]]
```

In [71]:
```
coad = pd.read_csv('coadrsemfpkmtcgat.txt',sep='\t')

read = pd.read_csv('readrsemfpkmtcgat.txt',sep='\t')


#take sample of 50 from each dataset
coad_sub = coad.copy().iloc[:,2:52]

read_sub = read.copy().iloc[:,2:52]


#convert from FPKM to TPM
coad_tpm = coad_sub.copy().div(coad_sub.sum(axis=0), axis=1)*1000000
read_tpm = read_sub.copy().div(read_sub.sum(axis=0), axis=1)*1000000



#join the two dataset together
coad_read = pd.concat((coad_tpm, read_tpm), axis=1)

#transpose
coad_read_np = coad_read.T.to_numpy()

#replace dropout events with very small expression
coad_read_np[coad_read_np == 0] = 0.02

#normalize
coad_read_np = normalize_data(coad_read_np.copy())
```

In [ ]:
```

```

In [73]:
```
#PCA
from sklearn.decomposition import PCA
pca = PCA(n_components=2)
```

```
principalComponents = pca.fit_transform(coad_read_np)
principalDf = pd.DataFrame(data = principalComponents
             , columns = ['principal component 1', 'principal component 2'])

coad_or_read = []

for i in range(0,100):
    if i < 50:
        coad_or_read.append('COAD')
    else:
        coad_or_read.append('READ')



sample_info = pd.DataFrame(list(zip(coad_read.columns.tolist(), coad_or_read)),columns =

finalDf_colon = pd.concat([principalDf, sample_info.reset_index()[['coad_or_read']], ax
```

In [58]:

```
kich = pd.read_csv('kichrsemfpkmtcgat.txt',sep='\t')

kirc = pd.read_csv('kircrsemfpkmtcgat.txt',sep='\t')

kirp = pd.read_csv('kirprsemfpkmtcgat.txt',sep='\t')



#take sample of 50 from each dataset
kich_sub = kich.copy().iloc[:,2:52]

kirc_sub = kirc.copy().iloc[:,2:52]

kirp_sub = kirp.copy().iloc[:,2:52]


kich =kich_sub
kirc =kirc_sub
kirp = kirp_sub

#convert from FPKM to TPM
kich_tpm = kich_sub.copy().div(kich_sub.sum(axis=0), axis=1)*1000000
kirc_tpm = kirc_sub.copy().div(kirc_sub.sum(axis=0), axis=1)*1000000
kirp_tpm = kirp_sub.copy().div(kirp_sub.sum(axis=0), axis=1)*1000000



#join the two dataset together
kich_kirc = pd.concat((kich_tpm, kirc_tpm), axis=1)

kich_kirc_kirp = pd.concat((kich_kirc, kirp_tpm), axis=1)


#transpose
kich_kirc_kirp_np = kich_kirc_kirp.T.to_numpy()


#replace dropout events with very small expression
kich_kirc_kirp_np[kich_kirc_kirp_np == 0] = 0.02


#normalize
kich_kirc_kirp_np = normalize_data(kich_kirc_kirp_np.copy())
```

In [60]:

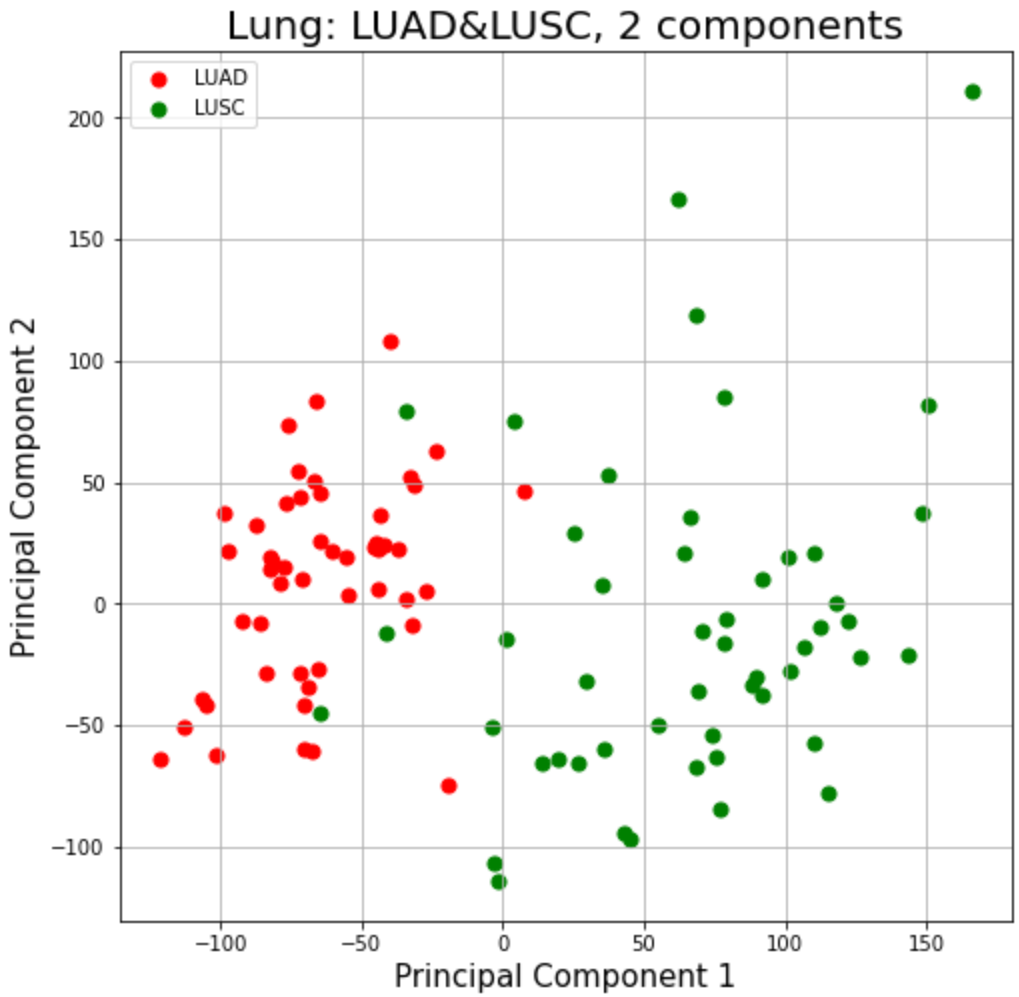
```
from sklearn.decomposition import PCA
pca = PCA(n_components=2)
principalComponents = pca.fit_transform(kich_kirc_kirp_np)
```

```
principalDf = pd.DataFrame(data = principalComponents
             , columns = ['principal component 1', 'principal component 2'])

kich_kirc_kirp_l = []

for i in range(0,150):
    if i < 50:
        kich_kirc_kirp_l.append('KICH')
    elif i<100:
        kich_kirc_kirp_l.append('KIRC')
    else:
        kich_kirc_kirp_l.append('KIRP')

kich_kirc_kirp.columns.tolist()

sample_info = pd.DataFrame(list(zip(kich_kirc_kirp.columns.tolist(), kich_kirc_kirp_l)),

finalDf = pd.concat([principalDf, sample_info.reset_index()[['kich_kirc_kirp']]], axis =
```

In [ ]:

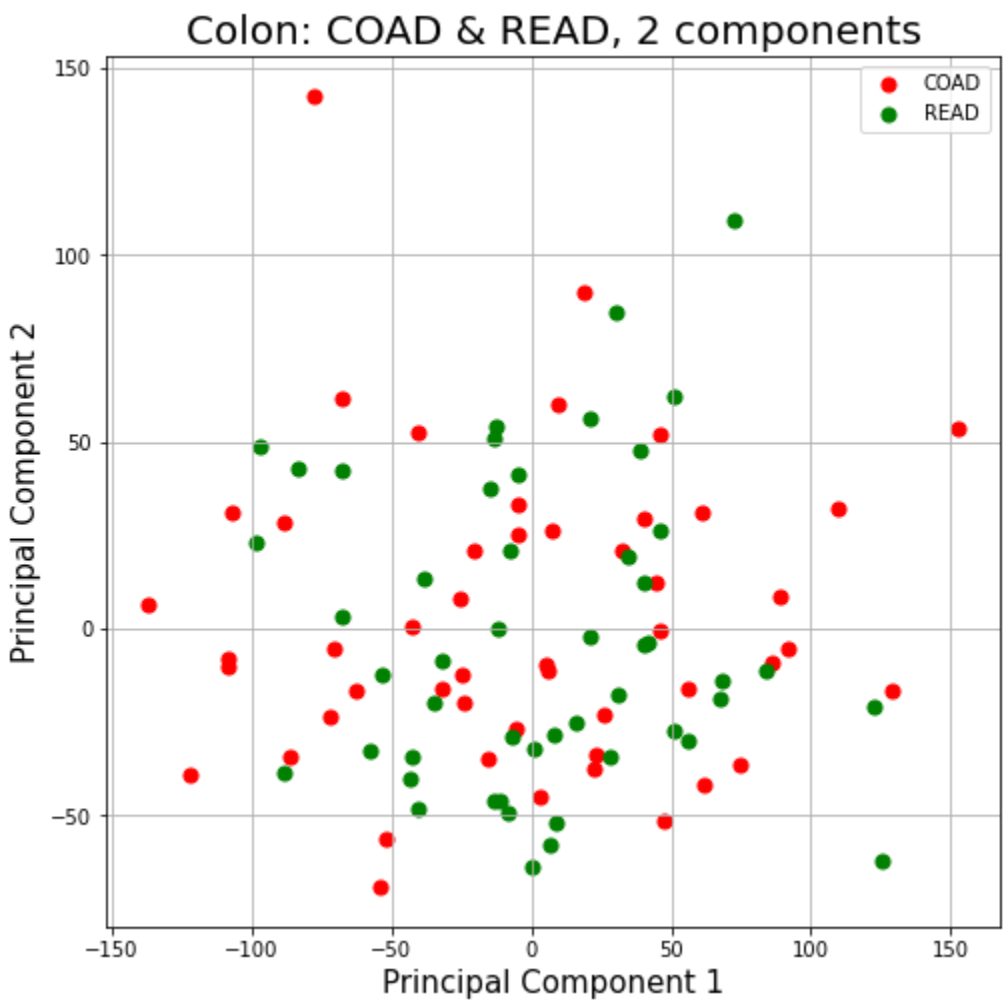
In [56]:
```
PCA_2d('Lung: LUAD&LUSC, 2 components','LUAD', 'LUSC',
       'LUAD_or_LUSC',finalDf_luad_lusc)
```

Out[56]: 1



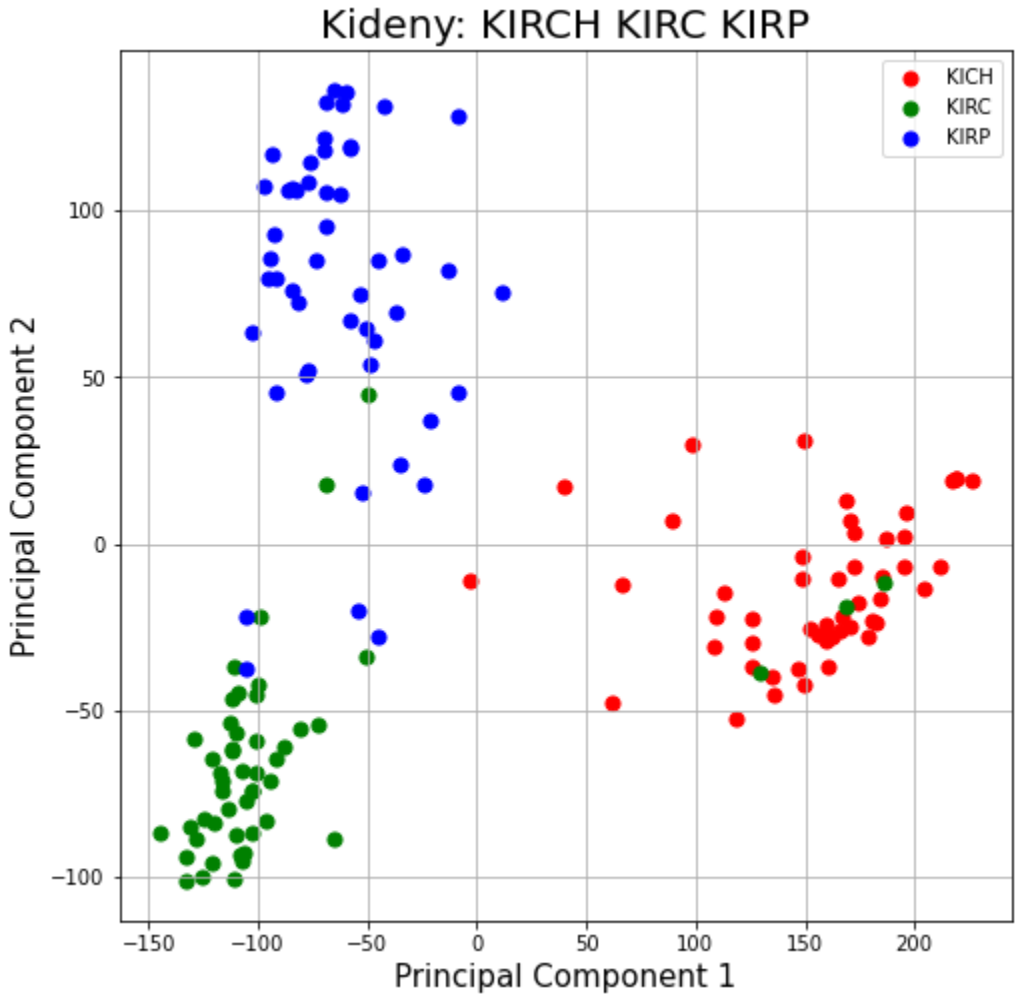
In [74]:
```
PCA_2d('Colon: COAD & READ, 2 components','COAD', 'READ',
       'coad_or_read',finalDf_colon)
```

Out[74]: 1



In [62]:

```
fig = plt.figure(figsize = (8,8))
ax = fig.add_subplot(1,1,1)
ax.set_xlabel('Principal Component 1', fontsize = 15)
ax.set_ylabel('Principal Component 2', fontsize = 15)
ax.set_title('Kideny: KIRCH KIRC KIRP', fontsize = 20)
targets = ['KICH', 'KIRC','KIRP']
colors = ['r', 'g','b']

for target, color in zip(targets,colors):
    indicesToKeep = finalDf['kich_kirc_kirp'] == target
    ax.scatter(finalDf.loc[indicesToKeep, 'principal component 1']
               , finalDf.loc[indicesToKeep, 'principal component 2']
               , c = color
               , s = 50)
ax.legend(targets)
ax.grid()
```



Looking at the three cancer we can see that lung is generally clusters well togther, colon cancer does not seperate into distinct clusters and kidney has clear clusters for each subtype of cancer. Interestingly the two colon cancers COAD and READ corresspound to the upper and lower tracts of the colon. Although being from different parts of the colon appear on first look to not have distinctly different gene expression. This could point to similarities in the treatment profiles for each 'type' even though distinctions between the two may not be useful.

Further work would be to do a k-means clustering algorithm itself and metrics for the clustering such as mean distance from centriod. This metric would allow for a quantitative number to the tightness of each cluster

Looking closly at the kidney clustering there are three KIRC datapoints found in the center of the KICH cluster, distinctly far away from the other KIRC. Further investigation could be done to see if those points were mislabeled or if the diagnosis for KIRC was incorrect in those patient cases.

The goal of this analysis was to do initial characterization different cancers and their subtypes. Looking beyond this basic analysis, another question would be to identify marker genes between different subtypes based on the clustering. This initial investigation aids in finding marker genes, because it clarifies which cancers are easier to find differences in types, kidney or lung compared to colon.

Looking even deeper into the futher, I aim to use this dataset alongside scRNA-seq data to do deconvolution of single cell counts from bulkRNA. Many papers have been written about converting bulkRNA to scRNA, and the since this dataset has already been cleaned and processed as specified in the paper. Converting this large dataset into single cell counts could be useful in identifing trends of single cells in a variety of cancers.