Effects of Clioquinol on Yeast

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Date: 04.17.2022

Analyze the microarray dataset made available by the following study: *"The metal chelating and chaperoning effects of clioquinol: insights from yeast studies"*.

The microarray data is available here.

- Show a hierarchical clustering of samples. (Just a hierarchical clustering (ie a dendrogram) of samples, not a heatmap of expression values).
- Show a clustergram (heatmap, combined with clustering of samples and clustering of genes) of expression values.
- Report the top 10 most different genes between the Clioquinol and control groups.
- Report the functional annotations (GO Biological Processes and KEGG Pathways) that are significantly different between the two groups.
- Discuss whether your results align with the findings reported in the paper.
- 5% of your grade is for optimizing download & parse functions.

In [1]:
```
%load_ext autoreload
```

In [2]:
```
# Imports
%autoreload 2
import matplotlib as mpl
import seaborn as sns
import pandas as pd
import numpy as np
import re, math, rich, tools
from statsmodels.stats import multitest
from scipy.cluster import hierarchy
from scipy.spatial import distance
from scipy import stats
```

In [3]:
```
# Download and parse GSE and GPL data
gse = tools.geodlparse('GSE17257')
gpl = tools.geodlparse(*gse.metadata['platform_id'])

# Prepare dataframe with expression values
gse_data = pd.concat(
    [ gsm.table.set_index('ID_REF')['VALUE'] for _,gsm in gse.gsms.items() ],
    axis=1
).set_axis([ x for x,_ in gse.gsms.items() ], axis=1, inplace=False)

# Merge gse data with genes from the GPL
gse_data = gse_data \
    .merge(gpl.table[['ID', 'Gene Symbol']], how='left',
           left_index=True, right_on='ID') \
    .rename({'ID_REF': 'ID', 'Gene Symbol': 'Gene'}, axis=1) \
    .set_index(['ID', 'Gene']) \
    .sort_index(axis=1)
```

```
# Label columns as 'Control' or 'Clioquinol'
columns = gse.phenotype_data['title'] \
    .apply(lambda x: re.search(r'DMSO|CQ', x)[0]) \
    .replace({'DMSO': 'Control', 'CQ': 'Clioquinol'}) \
    .sort_index().reset_index()
gse_data.columns = [columns['index'].values, columns['title'].values]

gse_data.head()
```

```
Loading cached data...
Loading cached data...
```

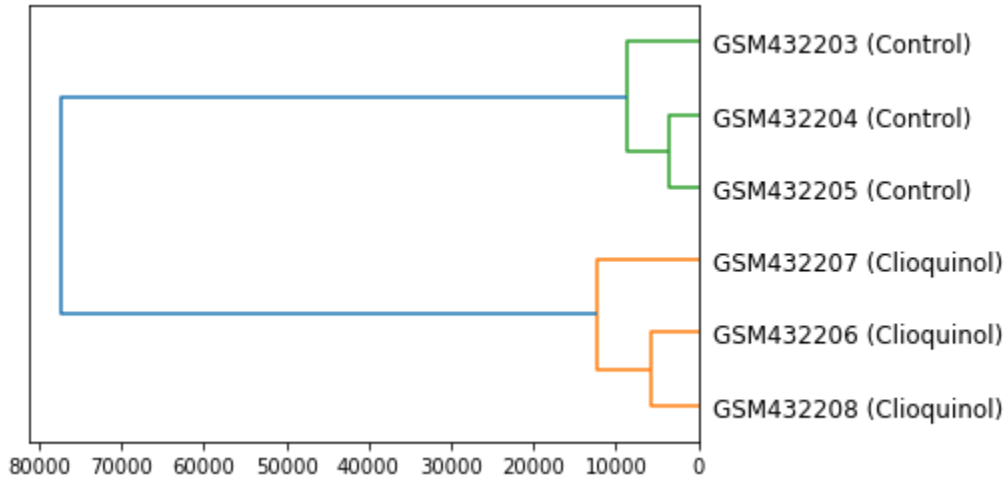
Out[3]:

		GSM432203	GSM432204	GSM432205	GSM432206	GSM432207	GSM432208
		Control	Control	Control	Clioquinol	Clioquinol	Clioquinol
ID	Gene						
AFFX-BioB-5_at	NaN	212.502	414.776	383.139	351.550	893.897	476.621
AFFX-BioB-M_at	NaN	237.005	469.306	473.493	407.539	1056.330	511.049
AFFX-BioB-3_at	NaN	216.365	463.476	466.453	385.204	1055.990	493.247
AFFX-BioC-5_at	NaN	336.581	731.790	716.848	647.458	1672.720	837.061
AFFX-BioC-3_at	NaN	575.513	1099.120	1117.000	1003.060	2141.200	1275.160

Hierarchical Clustering of Samples

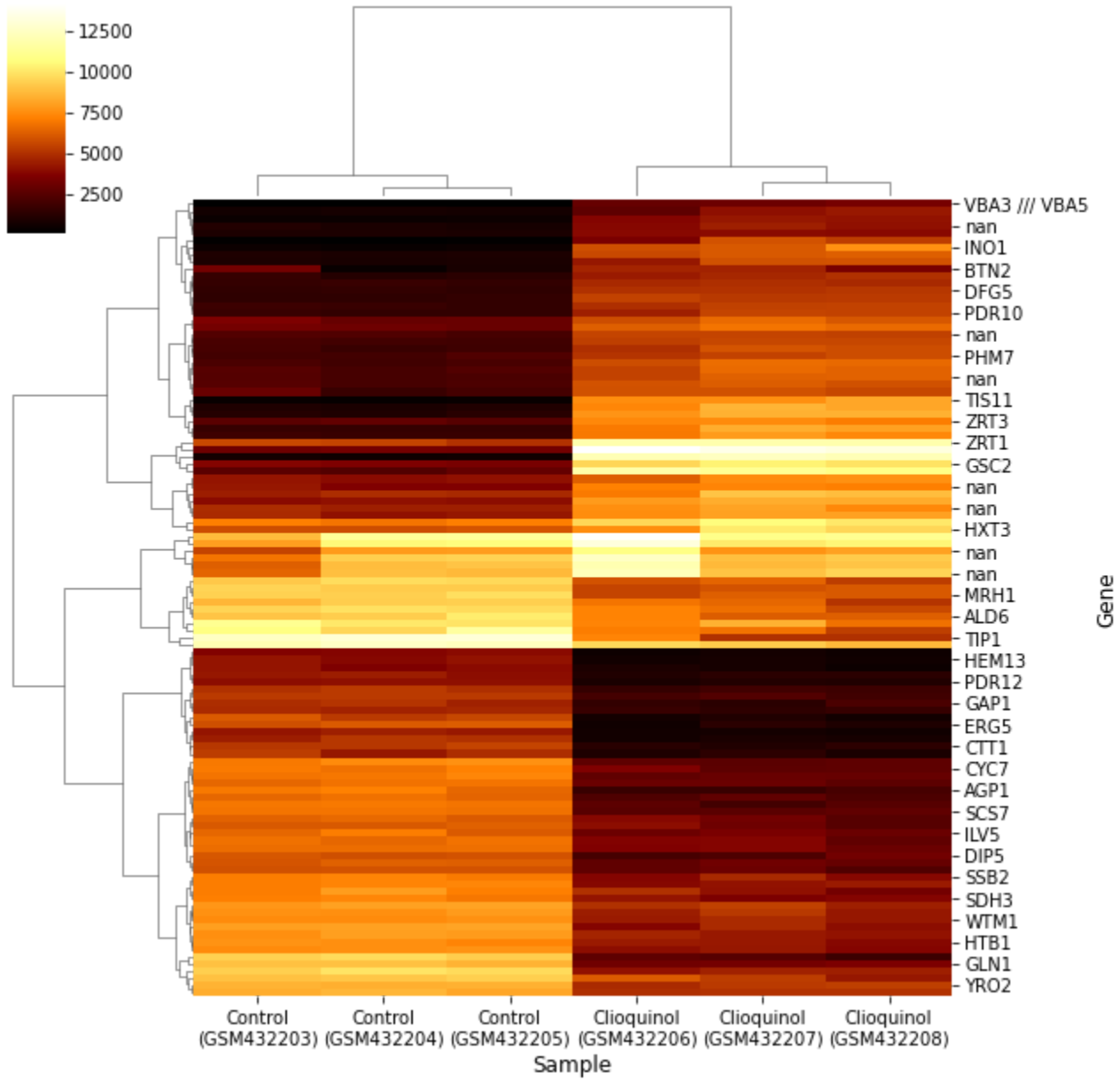
In [4]:
```
# Filter out genes based on variance (keep the 90% percentile)
# This data will be used for clustering to save time.
gse_vars = gse_data.var(axis=1)
sorted_vars = np.sort( gse_vars.values )
min_var = sorted_vars[ math.floor(sorted_vars.size * .99) ]
gse_filt = gse_data.drop( gse_data[ gse_vars < min_var ].index, axis=0 )
```

In [5]:
```
# Compute distance matrix
dist = distance.pdist(gse_filt.transpose(), metric='euclidean')
# Perform hierarchical clustering
phy = hierarchy.linkage(dist, method='ward', metric='euclidean')
# Plot dendrogram
with mpl.rc_context({'figure.figsize': (6, 4)}):
    hierarchy.dendrogram(
        phy, orientation='left', distance_sort='descending',
        labels=[f'{x} ({y})' for x,y in gse_filt.columns.to_flat_index()]
    )
```



Clustergram

In [6]:
```
# Create clustergram
fig = sns.clustermap(gse_filt.droplevel(0), method='ward', metric='euclidean',
                     cmap='afmhot', figsize=(9,10), dendrogram_ratio=.2)
## Set axis labels and tick labels
fig.ax_heatmap.set_xlabel('Sample', fontsize=12)
fig.ax_heatmap.set_ylabel('Gene', fontsize=12)
fig.ax_heatmap.set_xticklabels(
    [f'{y}\n({x})' for x,y in gse_filt.columns.to_flat_index()],
    rotation=0, fontsize=10
);
```



Differentially Expressed Genes

The top 10 different genes will be selected from genes with high fold changes (> 1.5, up or down-regulated), ordered by the adjusted p-values.

In [7]:

```
# Compute the (signed) fold change (Clioquinol / Control)
deg = gse_data.groupby(lambda x: x[1], axis=1).mean()
signed_FC = (deg['Clioquinol'] / deg['Control']).values
deg['signed_FC'] = np.where(signed_FC < 1, -1/signed_FC, signed_FC)

# Compute p-values
deg['pvalue'] = stats.ttest_ind(
    gse_data.loc[:, gse_data.columns.get_level_values(1) == 'Clioquinol'].values,
    gse_data.loc[:, gse_data.columns.get_level_values(1) == 'Control'].values,
    axis=1
).pvalue
# Benjamini-Hochberg FDR Correction
deg['adj_pvalue'] = multitest.fdrcorrection(deg['pvalue'])[1]

# Remove any probes with no Gene symbols mapped
deg = deg.iloc[:, 2:].reset_index().dropna(subset='Gene')
```

```
# Select most different genes
deg = deg[ (deg['adj_pvalue'] < .01) & (abs(deg['signed_FC']) > 1.5) ]

# Sort genes by pvalues and  Fold Change
deg = deg \
    .sort_values('signed_FC', key=abs, ascending=False) \
    .sort_values('adj_pvalue') \
    .reset_index(drop=True)

# Report the top 10 most different genes between the Clioquinol and control groups
rich.print(f"[bold]Top 10 Most Different Genes Between Clioquinol and Control Groups[/bold]")
deg.iloc[:10, 1:]
```

Top 10 Most Different Genes Between Clioquinol and Control Groups

Out[7]:

	Gene	signed_FC	pvalue	adj_pvalue
0	GDE1	4.518178	1.851874e-08	0.000141
1	ZPS1	22.450493	5.477797e-08	0.000150
2	NHA1	3.510948	5.420390e-08	0.000150
3	KIN3	-2.904634	1.082083e-07	0.000227
4	STT4	2.612336	1.773988e-07	0.000277
5	SOG2	2.778441	2.520131e-07	0.000344
6	RPC40	-3.450987	2.987672e-07	0.000363
7	SDP1	-2.735229	4.745902e-07	0.000422
8	TUL1	2.056774	4.739991e-07	0.000422
9	DFG5	3.548155	4.738792e-07	0.000422

Functional Annotations of Differentially Expressed Genes

Functional Annotation analysis was performed using *DAVID Functional Annotation Tool*.
The results were downloaded to the following files: `'DAVID_BP.txt'`, `'DAVID_KEGG.txt'`.
The top results returned by DAVID are shown below.

Gene Ontology Biological Processes

Sublist	Category	Term	RT	Genes	Count	%	P-Value	Benjamini
	GOTERM_BP_DIRECT	ribosome biogenesis	RT		74	6.8	5.4E-15	8.9E-12
	GOTERM_BP_DIRECT	rRNA processing	RT		76	6.9	2.1E-11	1.8E-8
	GOTERM_BP_DIRECT	ion transport	RT		37	3.4	9.2E-9	5.1E-6
	GOTERM_BP_DIRECT	rRNA methylation	RT		26	2.4	6.7E-8	2.8E-5
	GOTERM_BP_DIRECT	fungal-type cell wall organization	RT		40	3.7	6.2E-7	2.1E-4
	GOTERM_BP_DIRECT	cell wall organization	RT		32	2.9	8.6E-7	2.4E-4
	GOTERM_BP_DIRECT	ribosomal large subunit biogenesis	RT		26	2.4	2.0E-6	4.5E-4
	GOTERM_BP_DIRECT	cellular amino acid biosynthetic process	RT		29	2.7	2.2E-6	4.5E-4
	GOTERM_BP_DIRECT	transmembrane transport	RT		64	5.9	3.3E-6	6.2E-4
	GOTERM_BP_DIRECT	cell cycle	RT		74	6.8	7.8E-6	1.3E-3
	GOTERM_BP_DIRECT	lipid metabolic process	RT		39	3.6	1.0E-5	1.3E-3
	GOTERM_BP_DIRECT	cellular iron ion homeostasis	RT		19	1.7	1.0E-5	1.3E-3
	GOTERM_BP_DIRECT	translational initiation	RT		19	1.7	1.0E-5	1.3E-3
	GOTERM_BP_DIRECT	translation	RT		59	5.4	1.1E-5	1.3E-3
	GOTERM_BP_DIRECT	cadmium ion transmembrane transport	RT		7	0.6	1.3E-5	1.4E-3
	GOTERM_BP_DIRECT	maturation of LSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	RT		19	1.7	1.4E-5	1.5E-3
	GOTERM_BP_DIRECT	iron ion homeostasis	RT		13	1.2	3.3E-5	3.3E-3
	GOTERM_BP_DIRECT	cellular response to DNA damage stimulus	RT		50	4.6	4.1E-5	3.8E-3
	GOTERM_BP_DIRECT	maturation of 5.8S rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	RT		12	1.1	5.6E-5	4.9E-3
	GOTERM_BP_DIRECT	DNA duplex unwinding	RT		16	1.5	7.2E-5	6.0E-3
	GOTERM_BP_DIRECT	metal ion transport	RT		9	0.8	8.0E-5	6.3E-3
	GOTERM_BP_DIRECT	maturation of SSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	RT		25	2.3	1.0E-4	7.7E-3
	GOTERM_BP_DIRECT	siderophore transport	RT		7	0.6	1.3E-4	9.1E-3
	GOTERM_BP_DIRECT	endonucleolytic cleavage in 5'-ETS of tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	RT		13	1.2	3.8E-4	2.6E-2
	GOTERM_BP_DIRECT	regulation of catalytic activity	RT		31	2.8	5.4E-4	3.6E-2
	GOTERM_BP_DIRECT	endonucleolytic cleavage to generate mature 5'-end of SSU-rRNA from (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	RT		13	1.2	6.6E-4	4.2E-2
	GOTERM_BP_DIRECT	carbohydrate metabolic process	RT		26	2.4	8.8E-4	5.4E-2
	GOTERM_BP_DIRECT	endonucleolytic cleavage in ITS1 to separate SSU-rRNA from 5.8S rRNA and LSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	RT		17	1.6	9.1E-4	5.4E-2
	GOTERM_BP_DIRECT	phosphorylation	RT		41	3.7	1.2E-3	7.1E-2
	GOTERM_BP_DIRECT	DNA replication	RT		22	2.0	1.3E-3	7.4E-2
	GOTERM_BP_DIRECT	iron ion transport	RT		7	0.6	1.7E-3	9.3E-2
	GOTERM_BP_DIRECT	translational frameshifting	RT		5	0.5	2.0E-3	1.0E-1
	GOTERM_BP_DIRECT	invasive growth in response to glucose limitation	RT		13	1.2	2.2E-3	1.1E-1
	GOTERM_BP_DIRECT	hydrogen ion transmembrane transport	RT		22	2.0	2.3E-3	1.1E-1
	GOTERM_BP_DIRECT	protein ubiquitination	RT		30	2.7	3.4E-3	1.6E-1

KEGG Pathways

Sublist	Category	Term	RT	Genes	Count	%	P-Value	Benjamini
☐	KEGG_PATHWAY	Metabolic pathways	RT		187	17.1	2.9E-25	4.1E-23
☐	KEGG_PATHWAY	Biosynthesis of secondary metabolites	RT		87	8.0	1.7E-11	1.2E-9
☐	KEGG_PATHWAY	Cell cycle - yeast	RT		43	3.9	1.1E-9	5.1E-8
☐	KEGG_PATHWAY	Ribosome biogenesis in eukaryotes	RT		29	2.7	1.9E-8	6.5E-7
☐	KEGG_PATHWAY	Biosynthesis of amino acids	RT		38	3.5	7.5E-8	2.1E-6
☐	KEGG_PATHWAY	Meiosis - yeast	RT		39	3.6	1.5E-7	3.5E-6
☐	KEGG_PATHWAY	MAPK signaling pathway - yeast	RT		34	3.1	1.6E-6	3.1E-5
☐	KEGG_PATHWAY	Pyrimidine metabolism	RT		13	1.2	1.9E-4	3.3E-3
☐	KEGG_PATHWAY	Ubiquitin mediated proteolysis	RT		17	1.6	2.3E-4	3.6E-3
☐	KEGG_PATHWAY	Alanine, aspartate and glutamate metabolism	RT		12	1.1	4.4E-4	6.0E-3
☐	KEGG_PATHWAY	Biosynthesis of unsaturated fatty acids	RT		7	0.6	5.0E-4	6.3E-3
☐	KEGG_PATHWAY	2-Oxocarboxylic acid metabolism	RT		13	1.2	7.0E-4	8.1E-3
☐	KEGG_PATHWAY	ABC transporters	RT		9	0.8	7.9E-4	8.4E-3
☐	KEGG_PATHWAY	Fatty acid metabolism	RT		10	0.9	1.1E-3	1.1E-2
☐	KEGG_PATHWAY	Fatty acid elongation	RT		6	0.5	1.2E-3	1.1E-2
☐	KEGG_PATHWAY	Valine, leucine and isoleucine biosynthesis	RT		7	0.6	1.8E-3	1.5E-2
☐	KEGG_PATHWAY	Aminoacyl-tRNA biosynthesis	RT		12	1.1	8.1E-3	6.6E-2
☐	KEGG_PATHWAY	Phenylalanine, tyrosine and tryptophan biosynthesis	RT		7	0.6	9.9E-3	7.6E-2
☐	KEGG_PATHWAY	Lysine biosynthesis	RT		6	0.5	1.1E-2	8.2E-2
☐	KEGG_PATHWAY	Carbon metabolism	RT		23	2.1	1.4E-2	9.8E-2
☐	KEGG_PATHWAY	Pentose phosphate pathway	RT		9	0.8	1.8E-2	1.1E-1
☐	KEGG_PATHWAY	Arginine and proline metabolism	RT		8	0.7	1.9E-2	1.1E-1
☐	KEGG_PATHWAY	Pantothenate and CoA biosynthesis	RT		8	0.7	1.9E-2	1.1E-1
☐	KEGG_PATHWAY	Amino sugar and nucleotide sugar metabolism	RT		10	0.9	2.1E-2	1.2E-1
☐	KEGG_PATHWAY	N-Glycan biosynthesis	RT		9	0.8	2.7E-2	1.5E-1
☐	KEGG_PATHWAY	Non-homologous end-joining	RT		5	0.5	2.7E-2	1.5E-1
☐	KEGG_PATHWAY	Oxidative phosphorylation	RT		15	1.4	3.1E-2	1.5E-1
☐	KEGG_PATHWAY	Tryptophan metabolism	RT		7	0.6	3.1E-2	1.5E-1
☐	KEGG_PATHWAY	RNA polymerase	RT		9	0.8	3.3E-2	1.5E-1
☐	KEGG_PATHWAY	DNA replication	RT		9	0.8	3.3E-2	1.5E-1
☐	KEGG_PATHWAY	Biosynthesis of cofactors	RT		24	2.2	3.7E-2	1.6E-1
☐	KEGG_PATHWAY	Inositol phosphate metabolism	RT		7	0.6	3.9E-2	1.6E-1
☐	KEGG_PATHWAY	Glycerolipid metabolism	RT		9	0.8	3.9E-2	1.6E-1
☐	KEGG_PATHWAY	Lysine degradation	RT		6	0.5	4.0E-2	1.7E-1
☐	KEGG_PATHWAY	Phenylalanine metabolism	RT		4	0.4	4.6E-2	1.8E-1
☐	KEGG_PATHWAY	Arginine biosynthesis	RT		6	0.5	5.2E-2	2.0E-1
☐	KEGG_PATHWAY	Peroxisome	RT		10	0.9	5.4E-2	2.0E-1

Comparison with Results from *Li, C., Wang, J., & Zhou, B. (2010)*

- The study found 448 downregulated genes and 600 upregulated genes (2-fold change).
- In my analysis, I found 569 significantly downregulated genes and 527 upregulated genes.

In [8]:
```
print(f"{(deg['signed_FC'] < 0).sum()} Downregulated Genes",
      f"{(deg['signed_FC'] > 0).sum()} Upregulated Genes", sep='\n')
```

```
569 Downregulated Genes
527 Upregulated Genes
```

- The upregulation of 19 transporters (or corresponding regulators) of transition metal ions in the Clioquinol treated samples was of particular interest in the study.
- In my analysis, 12 of the 19 genes reported were found to be significantly (pvalue < 0.01) upregulated.
  - The fold-change values I computed were within an acceptable margin of error of the values reported in the study.
  - The output of the following code block shows the transporter genes found and their Fold changes

In [9]:
```
study_genes = ['CTR1', 'CTR2', 'FRE1', 'FRE2', 'FRE3', 'FRE5', 'FRE7',
               'FTR1', 'FET3', 'ENB1', 'ARN1', 'VEL1', 'ZAP1', 'ZPS1',
               'ZRT1', 'ZRT3', 'PCA1', 'SMF1', 'SMF3']
deg[deg.Gene.isin(study_genes)].reset_index(drop=True)[['Gene', 'signed_FC']]
```

Out[9]:

	Gene	signed_FC
0	ZPS1	22.450493
1	ZRT1	2.215286
2	ZRT3	2.791968
3	FET3	4.370772
4	PCA1	3.215591
5	SMF3	1.963703
6	ENB1	3.073379
7	ZAP1	6.882041
8	SMF1	1.974717
9	FRE3	2.413438
10	CTR2	1.898063
11	ARN1	6.454200

- The differences between the findings reported in the paper and the my results are likely due to the difference in the amount of genes included in the analyses.
  - In the study, only genes with positive signals (significant detection p-values) were used. As such, only 2729 genes (probes) were included in their analysis.
  - However, in my analysis, all 10928 genes (probes) on the microarray were included.

The inclusion of potentially less reliable signals (high detection p-values) is the most likely cause of the discrepancies between our results.