Introduction

In this markdown, we re-analyze data from a published study on response of lung cancer cells to the tyrosine kinase inhibitor, erlotinib (PMID: 25404012). Erlotinib is used as a therapeutic agent in lung cancer patients who carry mutations in the epidermal growth factor receptor (EGFR). Patients initially respond well to the drug, but inevitably develop resistance. We focus on 243 phosphorylation sites in 194 proteins that were significantly upregulated by treatment with the EGFR ligand, epidermal growth factor (EGF), and downregulated by erlotinib. These sites are likely to be targets of EGFR-regulated pathways that are inhibited by drug treatment. We retrieve kinases for these sites from iPTMnet using iptmnetr and then compute some basic statistics on the results.

Retrieving Kinase Information

In this part, we retrieve kinases from iPTMnet for the EGFR/erlotinib-regulated sites using pyiptmnet, and write the table of kinase-site relationships to a file. The sites are listed in the file egfr_sites_formatted.txt. The input file has three tab-delmited columns: UniProtAC of the phosphorylated protein, amino acid residue of the phosphorylated site, and position of the phosphorylated site (e.g., P12345 S 100).

In [7]:
```
import pyiptmnet.api as api
```

In [17]:
```
kinase_info =  api.get_ptm_enzymes_from_file("Supplementary Data 1.txt")
kinase_info = kinase_info.sort_values(by="enz_name")
kinase_info.head()
```

Out[17]:

	enz_name	enz_id	sub_name	sub_id	ptm_type	site	site_position	score	
238	ABL1	P00519	EGFR	P00533	Phosphorylation	Y1197	1197	3	neXtP
277	AKT1	P31749	EIF4B	P23588	Phosphorylation	S422	422	3	n
280	AKT1	P31749	PDCD4	Q53EL6	Phosphorylation	S457	457	3	neXtP
279	AKT1	P31749	FLNC	Q14315	Phosphorylation	S2233	2233	3	
278	AKT1	P31749	IRS1	P35568	Phosphorylation	S629	629	3	

In [18]:
```
kinase_info.to_csv("Supplementary Data 2.txt",sep='\t')
```

In [19]:
```
#Find number of kinase-site pairs
num_kinase_site_pairs = len(kinase_info)
```

Out[19]: 337

Basic Statistics

Next, we compute:

- Number of kinase-site pairs
- Number of sites with at least one kinase
- Number of kinases
- Number of sites per kinase
- Number of kinases that phosphorylate three or more sites

In [25]:
```
#Find number of sites with at least one kinase
kinase_info["full_site"] = kinase_info["sub_id"] + " " + kinase_info["site"]
unique_sites = kinase_info.drop_duplicates(subset="full_site")\
len(unique_sites)
```

In [28]:
```
#Find number of unique kinases
unique_kinases = kinase_info.drop_duplicates(subset="enz_id")
len(unique_kinases)
```

Out[28]: 53

In [32]:
```
#Find number of sites per kinase
kinase_tally_sorted  = kinase_info["enz_name"].value_counts()
kinase_tally_sorted
```

Out[32]:

```
PRKCA       6
RPS6KB1     6
PRKCD       5
MAPK1       5
RPS6KA1     5
PRKACA      4
AKT1        4
MAP2K2      4
EGFR        4
PLK1        4
CHEK1       4
MAPK3       3
AURKB       3
PRKCE       3
PRKD1       3
RPS6KA3     3
MAP2K1      3
SRC         2
JAK2        2
PDPK1       2
BRAF        2
MAP3K8      2
RPS6KA5     2
PRKCH       2
PAK2        2
CSNK2A1     2
PRKCZ       2
ROCK1       2
CAMK2A      2
IKBKB       2
PRKD3       2
ABL1        1
SGK1        1
DAPK1       1
PTK6        1
HCK         1
MAPKAPK5    1
MTOR        1
MKNK1       1
PAK1        1
ROCK2       1
EEF2K       1
MAPK8       1
RPS6KA4     1
PASK        1
MAPK13      1
PKD1        1
RET         1
AKT2        1
MAPK14      1
INSR        1
LCK         1
Name: enz_name, dtype: int64
```

In [49]:
```
#Find number of kinases that phosphorylate three or more sites
high_freq_kinases = kinase_tally_sorted[kinase_tally_sorted >= 3]
num_high_freq_kinases = len(high_freq_kinases)
num_high_freq_kinases
```

Out[49]: 17

iptmnetr_use_case

Introduction

In this markdown, we re-analyze data from a published study on response of lung cancer cells to the tyrosine kinase inhibitor, erlotinib (PMID: 25404012). Erlotinib is used as a therapeutic agent in lung cancer patients who carry mutations in the epidermal growth factor receptor (EGFR). Patients initially respond well to the drug, but inevitably develop resistance. We focus on 243 phosphorylation sites in 194 proteins that were significantly upregulated by treatment with the EGFR ligand, epidermal growth factor (EGF), and downregulated by erlotinib. These sites are likely to be targets of EGFR-regulated pathways that are inhibited by drug treatment. We retrieve kinases for these sites from iPTMnet using iptmnetr and then compute some basic statistics on the results.

Retrieving Kinase Information

In this part, we retrieve kinases from iPTMnet for the EGFR/erlotinib-regulated sites using iptmnetr, and write the table of kinase-site relationships to a file. The sites are listed in the file Supplementary Data 1.txt. The input file has three tab-delmited columns: UniProtAC of the phosphorylated protein, amino acid residue of the phosphorylated site, and position of the phosphorylated site (e.g., P12345 S 100).

```
setwd("~/Documents/Bioinformatics/PIR/EGFR_phosphoproteomics/phosphoproteomics_workflow")
library("iptmnetr", lib.loc="/Library/Frameworks/R.framework/Versions/3.4/Resources/library")
```

```
## Loading required package: httr
```

```
## Loading required package: jsonlite
```

```
set_host_url("http://34.233.45.77")
kinase_info <- get_ptm_enzymes_from_file("Supplementary Data 1.txt")
```

```
## No encoding supplied: defaulting to UTF-8.
```

```
write.table(kinase_info, file="Supplementary Data 2.txt", sep = '\t', quote = FALSE, col.names = NA)
head(kinase_info)
```

```
##   enz_name enz_id sub_name sub_id        ptm_type site site_position score
## 1     SGK1 O00141    NDRG1 Q92597 Phosphorylation S330           330     4
## 2    EEF2K O00418    EEF2K O00418 Phosphorylation T353           353     4
## 3    CHEK1 O14757   PRRC2B Q5JSZ5 Phosphorylation S166           166     1
## 4    CHEK1 O14757  PROSER2 Q86WR7 Phosphorylation  S43            43     1
## 5    CHEK1 O14757  RACGAP1 Q9H0H5 Phosphorylation S203           203     1
## 6    CHEK1 O14757    SENP2 Q9HC62 Phosphorylation S344           344     1
##                        source                              pmids
## 1 neXtProt,PSP,Signor,UniProt          15461589,20416281,16964243
## 2        neXtProt,PSP,UniProt 22669845,22329831,22216903,23186163
## 3                         PSP                             21851590
## 4                         PSP                             21851590
## 5                         PSP                             21851590
## 6                         PSP                             21851590
```

Basic Statistics

Next, we compute:

• Number of kinase-site pairs

• Number of sites with at least one kinase

• Number of kinases

• Number of sites per kinase

• Number of kinases that phosphorylate three or more sites

```
#Find number of kinase-site pairs
num_kinase_site_pairs <- nrow(kinase_info)
num_kinase_site_pairs
```

```
## [1] 118
```

```
#Find number of sites with at least one kinase
kinase_info$full_site <- paste(kinase_info$sub_id, kinase_info$site, sep = " ")
dup_sites <- duplicated(kinase_info$full_site)
num_unique_sites <- nrow(kinase_info[!dup_sites,])
num_unique_sites
```

```
## [1] 50
```

```
#Find number of unique kinases
num_unique_kinases <- nrow(kinase_info[!duplicated(kinase_info$enz_id),])
num_unique_kinases
```

```
## [1] 52
```

```
#Find number of sites per kinase
library("plyr", lib.loc="/Library/Frameworks/R.framework/Versions/3.4/Resources/library")
kinase_tally <- count(kinase_info, "enz_name")
kinase_tally_sorted <- kinase_tally[order(-kinase_tally$freq),]
kinase_tally_sorted
```

```
##    enz_name freq
## 35    PRKCA    6
## 50  RPS6KB1    6
## 20    MAPK1    5
## 36    PRKCD    5
## 46  RPS6KA1    5
## 2      AKT1    4
## 7     CHEK1    4
## 11     EGFR    4
## 18   MAP2K2    4
## 33     PLK1    4
## 34   PRKACA    4
## 4     AURKB    3
## 17   MAP2K1    3
## 23    MAPK3    3
## 37    PRKCE    3
## 40    PRKD1    3
## 47  RPS6KA3    3
## 5      BRAF    2
## 6    CAMK2A    2
## 8   CSNK2A1    2
## 13    IKBKB    2
## 15     JAK2    2
## 19   MAP3K8    2
## 29     PAK2    2
## 31    PDPK1    2
## 38    PRKCH    2
## 39    PRKCZ    2
## 41    PRKD3    2
## 44    ROCK1    2
## 49  RPS6KA5    2
## 52      SRC    2
## 1      ABL1    1
## 3      AKT2    1
## 9     DAPK1    1
## 10    EEF2K    1
## 12      HCK    1
## 14     INSR    1
## 16      LCK    1
## 21   MAPK13    1
## 22   MAPK14    1
## 24    MAPK8    1
## 25 MAPKAPK5    1
## 26    MKNK1    1
## 27     MTOR    1
## 28     PAK1    1
## 30     PASK    1
## 32     PKD1    1
## 42     PTK6    1
## 43      RET    1
## 45    ROCK2    1
## 48  RPS6KA4    1
## 51     SGK1    1
```

```
#Find number of kinases that phosphorylate three or more sites
high_freq_kinases <- kinase_tally_sorted[ which(kinase_tally_sorted$freq >= 3),]
num_high_freq_kinases <- nrow(high_freq_kinases)
num_high_freq_kinases
```

```
## [1] 17
```