iLINCS API R Notebook

Analyzing TCGA breast cancer proteomics RPPA dataset

Searching for the dataset in the TCGA collection (ie portal)

```
portal<-"TCGA"
searchTerm<-"BRCA"
dataType<-"Proteomics"</pre>
apiUrl <- paste0("http://www.ilincs.org/api/PublicDatasets/findDatasets?term=",searchTerm,"&portal=",po
req <- GET(apiUrl)</pre>
json <- httr::content(req, as = "text")</pre>
breastProteomics <- fromJSON(json)</pre>
breastProteomics[,c("experiment", "assay", "nsamples", "description")]
              experiment assay nsamples
## 1 TCGA_BRCA_RPPA_2019 RPPA
                                      937
       TCGA OV RPPA 2019 RPPA
                                      443
##
         937 RPPA breast invasive carcinoma (BRCA) samples from TCGA project. Data were downloaded via
## 2 443 RPPA ovarian serous cystadenocarcinoma (OV) samples from TCGA project. Data were downloaded vi
```

Getting metadata for the TCGA breast cancer proteomics RPPA dataset (TCGA_BRCA_RPPA_2019)

```
experiment <- "TCGA_BRCA_RPPA_2019"

apiUrl <- paste("http://www.ilincs.org/api/ilincsR/getSamples?id=",experiment,sep="")
req <- GET(apiUrl)
json <- httr::content(req, as = "text")
sampleMeta <- fromJSON(json)
sampleMetaData <- data.frame(sampleMeta$data$rows)
head(sampleMetaData)</pre>
```

```
##
                                                       sample_type primary_diagnosis morphology gender
                 MeasurementName age_quartile
## 1 TCGA-3C-AALI-01A-21-A43F-20
                                        49-57 Primary solid Tumor
                                                                               C50.9
                                                                                         8500/3 female b
## 2 TCGA-3C-AALK-01A-21-A43F-20
                                        49-57 Primary solid Tumor
                                                                               C50.9
                                                                                         8500/3 female b
                                                                               C50.9
## 3 TCGA-4H-AAAK-01A-21-A43F-20
                                        49-57 Primary solid Tumor
                                                                                         8520/3 female
                                                                               C50.9
## 4 TCGA-5L-AAT1-01A-21-A43F-20
                                        58-66 Primary solid Tumor
                                                                                         8520/3 female
## 5 TCGA-5T-A9QA-01A-21-A43F-20
                                        49-57 Primary solid Tumor
                                                                               C50.9
                                                                                         8523/3 female b
                                                                               C50.9
## 6 TCGA-A1-A0SF-01A-21-A17I-20
                                        49-57 Primary solid Tumor
                                                                                         8500/3 female
     RPPA_Clusters neoplasm_cancer_status
                                                        histological_type
## 1
              < NA >
                               TUMOR FREE Infiltrating Ductal Carcinoma
                                                                                     Post (prior bilater
## 2
              <NA>
                               TUMOR FREE Infiltrating Ductal Carcinoma
## 3
              <NA>
                               TUMOR FREE Infiltrating Lobular Carcinoma
                                                                                     Post (prior bilater
## 4
              <NA>
                               WITH TUMOR Infiltrating Lobular Carcinoma
                                                                                     Post (prior bilater
## 5
              <NA>
                                     <NA>
                                                           Other, specify
## 6
              < NA >
                               TUMOR FREE Infiltrating Ductal Carcinoma Pre (<6 months since LMP AND n
```

```
PR_status PR_level_cell_percentage HER2_level HER2_status HER2_FISH margin_status distant_metastas
                                                                                                                            Positive
## 1 Positive
                                                                                 <10%
                                                                                                          <NA>
                                                                                                                                                              <NA>
                                                                                                                                                                                     Negative
                                                                                                                                                                                                                                          < N
           Positive
                                                                            80-89%
                                                                                                           <NA>
## 2
                                                                                                                            Positive
                                                                                                                                                              < NA >
                                                                                                                                                                                            Close
                                                                                                                                                                                                                                          < N
## 3 Positive
                                                                            70-79%
                                                                                                                                                              <NA>
                                                                                                                                                                                                                                          < N.
                                                                                                               2+
                                                                                                                          Equivocal
                                                                                                                                                                                     Negative
## 4
             Positive
                                                                            10-19%
                                                                                                               2+
                                                                                                                          Equivocal
                                                                                                                                                              <NA>
                                                                                                                                                                                     Negative
                                                                                                                                                                                                                                          < N.
## 5
            Negative
                                                                                 <NA>
                                                                                                               2+
                                                                                                                          Equivocal Negative
                                                                                                                                                                                              <NA>
                                                                                                                                                                                                                                          < N.
            Positive
                                                                            90-99%
                                                                                                           <NA>
                                                                                                                                                                                    Negative
                                                                                                                            Negative
                                                                                                                                                              < NA >
           mut_impact_BRCA1 mut_deleterious_BRCA1 mut_impact_CASP8 mut_deleterious_CASP8 mut_impact_CBFB mut_
## 1
                                            NO
                                                                                               NO
                                                                                                                                                                                         NO
                                                                                                                                                                                                                              NO
## 2
                                            NO
                                                                                               NO
                                                                                                                                      NO
                                                                                                                                                                                         NO
                                                                                                                                                                                                                              NO
## 3
                                           NO
                                                                                               NO
                                                                                                                                      NO
                                                                                                                                                                                         NO
                                                                                                                                                                                                                              NO
                                           NO
                                                                                              NO
                                                                                                                        MODERATE
                                                                                                                                                                                         NO
## 4
                                                                                                                                                                                                                              NO
## 5
                                       <NA>
                                                                                          <NA>
                                                                                                                                  <NA>
                                                                                                                                                                                     <NA>
                                                                                                                                                                                                                          <NA>
                                            NO
                                                                                               NO
                                                                                                                                      NO
                                                                                                                                                                                         NO
## 6
                                                                                                                                                                                                                              NO
           mut_impact_CHD4 mut_deleterious_CHD4 mut_impact_CTCF mut_deleterious_CTCF mut_impact_ERBB2 mut_del
## 1
                                         NO
                                                                                          NO
                                                                                                                 MODERATE
                                                                                                                                                                                NO
                                                                                                                                                                                                                        NO
## 2
                                         NO
                                                                                          NO
                                                                                                                               NO
                                                                                                                                                                                NO
                                                                                                                                                                                                                       NO
## 3
                                         NO
                                                                                          NO
                                                                                                                               NO
                                                                                                                                                                                NO
                                                                                                                                                                                                          MODERATE
## 4
                           MODERATE
                                                                                          NO
                                                                                                                               NO
                                                                                                                                                                                NO
                                                                                                                                                                                                                       NO
## 5
                                     <NA>
                                                                                      <NA>
                                                                                                                           <NA>
                                                                                                                                                                            <NA>
                                                                                                                                                                                                                    <NA>
## 6
                                         NO
                                                                                          NO
                                                                                                                               NO
                                                                                                                                                                                NO
                                                                                                                                                                                                                       NO
           mut_impact_GATA3 mut_deleterious_GATA3 mut_impact_GPS2 mut_deleterious_GPS2 mut_impact_KMT2C mut_impact_start mut_impact_GPS2 mut_impact_KMT2C mut_impact_start mut_impact_start
## 1
                                           NO
                                                                                                                                    NO
                                                                                                                                                                                                              MODERATE
                                                                                               NO
                                                                                                                                                                                    NO
## 2
                                            NO
                                                                                               NO
                                                                                                                                    NO
                                                                                                                                                                                    NO
                                                                                                                                                                                                                            NO
## 3
                                            NO
                                                                                              NO
                                                                                                                                    NO
                                                                                                                                                                                    NO
                                                                                                                                                                                                                            NO
## 4
                             MODERATE
                                                                                              NO
                                                                                                                      MODERATE
                                                                                                                                                                                    NO
                                                                                                                                                                                                                            NO
## 5
                                       <NA>
                                                                                          <NA>
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                                                                                              NO
                                                                                                                                    NO
                                                                                                                                                                                     NO
                                            NO
                                                                                                                                                                                                              MODERATE
           mut_deleterious_MAP3K1 mut_impact_NCOR1 mut_deleterious_NCOR1 mut_impact_NF1 mut_deleterious_NF1 m
## 1
                                                         NO
                                                                                                 NO
                                                                                                                                                                                       NO
                                                                                                                                                                                                                                     NO
## 2
                                                         NO
                                                                                   MODERATE
                                                                                                                                                    NO
                                                                                                                                                                                       NO
                                                                                                                                                                                                                                      NO
## 3
                                                         NO
                                                                                                 NO
                                                                                                                                                    NO
                                                                                                                                                                                      NO
                                                                                                                                                                                                                                     NO
                                                         NO
                                                                                                 NO
                                                                                                                                                    NO
                                                                                                                                                                                                                                     NO
## 4
                                                                                                                                                                                  HIGH
## 5
                                                     <NA>
                                                                                             <NA>
                                                                                                                                                <NA>
                                                                                                                                                                                   <NA>
                                                                                                                                                                                                                                 <NA>
## 6
                                                         NO
                                                                                                 NO
                                                                                                                                                    NO
                                                                                                                                                                                       NO
                                                                                                                                                                                                                                      NO
##
           mut_deleterious_PTEN mut_impact_PTPRD mut_deleterious_PTPRD mut_impact_RB1 mut_deleterious_RB1 mut
## 1
                                                                                                                                                                                  NO
                                                                                                                                                                                                                                 NO
## 2
                                                     NO
                                                                                            NO
                                                                                                                                                NO
                                                                                                                                                                                  NO
                                                                                                                                                                                                                                 NO
## 3
                                                     NO
                                                                                            NO
                                                                                                                                                NO
                                                                                                                                                                                  NO
                                                                                                                                                                                                                                 NO
                                                                                            NO
                                                                                                                                                                                                                                NO
## 4
                                                     NO
                                                                                                                                               NO
                                                                                                                                                                                  NO
## 5
                                                 <NA>
                                                                                        <NA>
                                                                                                                                           <NA>
                                                                                                                                                                              <NA>
                                                                                                                                                                                                                             <NA>
## 6
                                                     NO
                                                                                          LOW
                                                                                                                                                NO
                                                                                                                                                                                  NO
                                                                                                                                                                                                                                 NO
           mut_deleterious_TBX3 mut_impact_TP53 mut_deleterious_TP53
##
## 1
                                                     NO
                                                                                     HIGH
                                                                                                                                           NO
## 2
                                                     NO
                                                                                          NO
                                                                                                                                           NO
                                                                                          NO
                                                                                                                                           NO
## 3
                                                     NO
                                                                                                                                           NO
## 4
                                                     NO
                                                                                          NO
## 5
                                                 <NA>
                                                                                                                                      <NA>
                                                                                      <NA>
## 6
                                                     NO
                                                                                          NO
                                                                                                                                           NO
```

Summary of the PAM50 mRNA factor

table(sampleMetaData\$PAM50_mRNA)

Creating signature by comparing "Luminal A" and "HER2-enriched"

```
filter<-"PAM50_mRNA:Luminal A,,,PAM50_mRNA:HER2-enriched"
property <- "PAM50_mRNA"</pre>
treatment <- "Luminal A"</pre>
baseline <- "HER2-enriched"
apiUrl <- "http://www.ilincs.org/api/ilincsR/LincsDataAnalysis"
req <- POST(apiUrl, body = list(exp =experiment,prop = property,treatment=treatment,baseline=baseline,
createdProteomicSignaturesSessionID <- httr::content(req)$sessionID</pre>
proteinSignatureFileUrl=paste0("http://www.ilincs.org/tmp/completeSig_",createdProteomicSignaturesSessi
diffProteinExpSignature<-read.table(proteinSignatureFileUrl,header=T,sep="\t",stringsAsFactors = F)[,c(
head(diffProteinExpSignature)
##
           PROBE ID_geneid Name_GeneSymbol Value_LogDiffExp Significance_pvalue
## 1
                      2099
                                       ESR1
                                                                     1.320409e-25
        ER-alpha
                                                    2.835721
## 2 HER2 pY1248
                      2064
                                      ERBB2
                                                    -1.300406
                                                                     3.561584e-23
## 3
            HER2
                      2064
                                      ERBB2
                                                                     2.861979e-22
                                                    -1.598668
```

1.255441

2.887453

-1.152552

2.495637e-20

8.200612e-20

2.058972e-19

Retrieving Top 12 Differentially expressed proteins (p-value<1e-10) (results in Fig 3A)

BCL2

EGFR

PGR

top12Proteins<-diffProteinExpSignature[diffProteinExpSignature\$Significance_pvalue<1e-10,]
top12Proteins</pre>

```
PROBE ID_geneid Name_GeneSymbol Value_LogDiffExp Significance_pvalue
##
## 1
         ER-alpha
                        2099
                                        ESR1
                                                     2.8357212
                                                                       1.320409e-25
## 2 HER2_pY1248
                        2064
                                       ERBB2
                                                    -1.3004059
                                                                       3.561584e-23
                        2064
                                       ERBB2
## 3
             HER2
                                                    -1.5986681
                                                                       2.861979e-22
## 4
            Bc1-2
                         596
                                         BCL2
                                                     1.2554406
                                                                       2.495637e-20
                                         PGR
## 5
               PR
                        5241
                                                     2.8874526
                                                                       8.200612e-20
## 6
      EGFR_pY1068
                        1956
                                         EGFR
                                                    -1.1525515
                                                                       2.058972e-19
## 7
             ASNS
                         440
                                         ASNS
                                                    -0.6198126
                                                                       1.002036e-16
                        2305
                                       FOXM1
## 8
            FoxM1
                                                    -0.5444826
                                                                       8.617266e-16
## 9
        Cyclin_B1
                         891
                                       CCNB1
                                                                       5.824394e-15
                                                    -0.9719865
## 10
            GATA3
                        2625
                                       GATA3
                                                     0.9602510
                                                                       4.811066e-12
             G6PD
                        2539
                                        G6PD
                                                    -0.5373067
                                                                       1.266588e-11
## 11
## 12 4E-BP1_pS65
                        1978
                                    EIF4EBP1
                                                    -0.2793193
                                                                       9.949816e-11
```

Heatmap of protein to top 12 proteins (Fig 3A)

4

5

6 EGFR_pY1068

Bc1-2

PR.

596

5241

1956

```
f1 = colorRamp2(c(0,1), c("green", "red"))
f2 = colorRamp2(c(-1, 0,1), c("blue", "black", "yellow"), space = "RGB")
```

```
load(url(paste("http://www.ilincs.org/tmp/",experiment,".RData",sep="")),verbose=T)
## Loading objects:
     eset
##
proteinExpressionEset<-get("eset")</pre>
proteinExpressionEset<-proteinExpressionEset[fData(proteinExpressionEset)$PROBE %in% top12Proteins$PROB
proteinExpressionEset<-proteinExpressionEset[,order(pData(proteinExpressionEset)$PAM50_mRNA)]
meanPE<-apply(exprs(proteinExpressionEset),1,mean)</pre>
proteinExpressionDataTable<-sweep(exprs(proteinExpressionEset),1,meanPE,"-")</pre>
pam50ColumnAnnotation = HeatmapAnnotation(PAM50_mRNA=pData(proteinExpressionEset) $PAM50_mRNA,col = list
  Heatmap(proteinExpressionDataTable, col = f2, cluster_columns = F, cluster_rows=T, show_column_names
 PAM50_mRNA
                                            ER-alpha
                                           Bcl-2
                                           GATA3
                                           PR
                                                             Log2
                                                                        PAM50_mRNA
                                           Cyclin_B1
                                                             Differential
                                                                           HER2-enriched
                                                             Expression
                                                                           Luminal A
                                            ASNS
                                                               1
                                                               0.5
                                           FoxM1
                                                               0
                                                                -0.5
                                           4E-BP1_pS65
                                           G6PD
                                           EGFR_pY1068
                                           HER2_pY1248
                                            HER2
```

Analyzing TCGA breast cancer transcriptom RNA-seq dataset

Searching for the dataset in the TCGA collection (ie portal)

```
portal<-"TCGA"
searchTerm<-"BRCA"
assay<-"RNA-seq"
apiUrl <- paste0("http://www.ilincs.org/api/PublicDatasets/findDatasets?term=",searchTerm,"&portal=",portal=",portal=")</pre>
```

```
req <- GET(apiUrl)</pre>
json <- httr::content(req, as = "text")</pre>
breastTranscriptomics <- fromJSON(json)</pre>
breastTranscriptomics[breastTranscriptomics$dataType=="Gene Expression",c("experiment","assay","nsample
##
                  experiment
                                assay nsamples
## 5
          TCGA_BRCA_RNASeqV2 RNA-seq
                                           919
## 6 TCGA_BRCA_RNASeqV2_2019 RNA-seq
                                          1215
       TCGA_OV_RNASeqV2_2019 RNA-seq
                                           309
##
## 5
                                                          919 RNA-seq breast invasive carcinoma (BRCA) sa
## 6
        1215 RNA-seq breast invasive carcinoma (BRCA) samples from TCGA project. Data were downloaded v
## 9 309 RNA-seq ovarian serous cystadenocarcinoma (OV) samples from TCGA project. Data were downloaded
```

Getting metadata for the newest TCGA breast cancer transcriptomics RNA-seq dataset (TCGA_BRCA_RNASeqV2_2019)

```
experiment <- "TCGA_BRCA_RNASeqV2_2019"

apiUrl <- paste("http://www.ilincs.org/api/ilincsR/getSamples?id=",experiment,sep="")
req <- GET(apiUrl)
json <- httr::content(req, as = "text")
sampleMeta <- fromJSON(json)
sampleMetaData <- data.frame(sampleMeta$data$rows)
head(sampleMetaData)</pre>
```

##		Mea	asurementName age_quart	ile	sample_type	primary_diag	nosis mor	ohology	gender
##	1	TCGA-3C-AAAU-01A	A-11R-A41B-07 49	9-57 Primar	ry solid Tumor		C50.9	8520/3	_
##	2	TCGA-3C-AALI-01A	A-11R-A41B-07 49	9-57 Primar	ry solid Tumor		C50.9	8500/3	female 1
##	3	TCGA-3C-AALJ-01A	A-31R-A41B-07 58	3-66 Primar	y solid Tumor		C50.9	8500/3	female 1
##	4	TCGA-3C-AALK-01A	A-11R-A41B-07 49	9-57 Primar	ry solid Tumor		C50.9	8500/3	female 1
##	5	TCGA-4H-AAAK-01A	A-12R-A41B-07 49	9-57 Primar	ry solid Tumor		C50.9	8520/3	female
##	6	TCGA-5L-AATO-01A	A-12R-A41B-07 26	3-48 Primar	ry solid Tumor		C50.9	8520/3	female
##		RPPA_Clusters ne	eoplasm_cancer_status		histologica	l_type			
##	1	<na></na>	WITH TUMOR 1	Infiltratir	ng Lobular Car	cinoma Pre (<	6 months s	since LN	IP AND no
##	2	<na></na>	TUMOR FREE	Infiltrati	ng Ductal Car	cinoma	Post	(prior	bilater
##	3	<na></na>	TUMOR FREE	Infiltrati	ng Ductal Car	cinoma	Post	(prior	bilatera
##	4	<na></na>	TUMOR FREE	Infiltrati	ng Ductal Car	cinoma			
##	5	<na></na>			ng Lobular Car		Post	(prior	bilatera
##	6	<na></na>	TUMOR FREE 1	Infiltratir	ng Lobular Car	cinoma	Post	(prior	bilatera
##		PR_status PR_lev	v el_cell_percentage <code>HEF</code>			ER2_FISH marg	gin_status	distant	_metasta
##	1	Positive	50-59%	<na></na>	Negative	<na></na>	Negative		
##	2	Positive	<10%	<na></na>	Positive	<na></na>	Negative		
##	3	Positive	30-39%	<na> Ir</na>	determinate	<na></na>	Negative		
##	4	Positive	80-89%	<na></na>	Positive	<na></na>	Close		
##	5	Positive	70-79%	2+	Equivocal	<na></na>	Negative		
##	6		50-59%	1+	Negative	<na></na>	Positive		
##		=	${ t l}$ mut_deleterious_BRCA1	_				_	
##		<na></na>			<na></na>	<	:NA>		JA>
##		NC			NO		NO		NO
##		<na></na>			<na></na>	<	:NA>		JA>
##		NC		-	NO		NO		NO
##	5	NC) NO)	NO		NO		NO

```
## 6
                                                        NO
                                                                                                                        NO
                                                                                                                                                                           NO
                                                                                                                                                                                                                                           NO
                                                                                                                                                                                                                                                                                          NO
              mut_impact_CHD4 mut_deleterious_CHD4 mut_impact_CTCF mut_deleterious_CTCF mut_impact_ERBB2 mut_del
## 1
                                               <NA>
                                                                                                            <NA>
                                                                                                                                                            <NA>
                                                                                                                                                                                                                          <NA>
## 2
                                                                                                                  NO
                                                                                                                                                MODERATE
                                                    NO
                                                                                                                                                                                                                               NO
                                                                                                                                                                                                                                                                                 NO
## 3
                                               <NA>
                                                                                                             <NA>
                                                                                                                                                            <NA>
                                                                                                                                                                                                                          <NA>
                                                                                                                                                                                                                                                                            <NA>
## 4
                                                    NO
                                                                                                                  NO
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                                                                                                                                                                                                                                                                                 NO
## 5
                                                    NO
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                                                                                                                                                                                                                                                               MODERATE
## 6
                                                                                                                  NO
                                                                                                                                                                                                                               NO
                                                    NO
                                                                                                                                                                 NO
              mut_impact_GATA3 mut_deleterious_GATA3 mut_impact_GPS2 mut_deleterious_GPS2 mut_impact_KMT2C mut_impact_state mut_impact_stat
## 1
                                                  <NA>
                                                                                                                  <NA>
                                                                                                                                                                  <NA>
                                                                                                                                                                                                                               <NA>
                                                                                                                                                                                                                                                                                  <NA>
## 2
                                                       NO
                                                                                                                        NO
                                                                                                                                                                       NO
                                                                                                                                                                                                                                     NO
                                                                                                                                                                                                                                                                     MODERATE
## 3
                                                  <NA>
                                                                                                                   <NA>
                                                                                                                                                                  <NA>
                                                                                                                                                                                                                               <NA>
                                                                                                                                                                                                                                                                                  <NA>
## 4
                                                        NO
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                                                                                                                                                                       NO
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                                                                                                                                                                                                                                                                                       NO
## 5
                                                        NO
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                                                                                                                                                                       NO
                                                                                                                                                                                                                                     NO
                                                                                                                                                                                                                                                                                       NO
## 6
                                                        NO
                                                                                                                        NO
                                                                                                                                                                       NO
                                                                                                                                                                                                                                     NO
                                                                                                                                                                                                                                                                                       NO
              mut_deleterious_MAP3K1 mut_impact_NCOR1 mut_deleterious_NCOR1 mut_impact_NF1 mut_deleterious_NF1 m
## 1
                                                                                                                      <NA>
                                                                                                                                                                                      <NA>
                                                                                                                                                                                                                                   <NA>
                                                                                                                                                                                                                                                                                             <NA>
                                                                   <NA>
## 2
                                                                         NO
                                                                                                                           NO
                                                                                                                                                                                           NO
                                                                                                                                                                                                                                       NO
                                                                                                                                                                                                                                                                                                   NO
## 3
                                                                   <NA>
                                                                                                                      <NA>
                                                                                                                                                                                      <NA>
                                                                                                                                                                                                                                   <NA>
                                                                                                                                                                                                                                                                                             <NA>
## 4
                                                                         NO
                                                                                                         MODERATE
                                                                                                                                                                                            NO
                                                                                                                                                                                                                                        NO
                                                                                                                                                                                                                                                                                                   NO
## 5
                                                                         NO
                                                                                                                           NO
                                                                                                                                                                                            NO
                                                                                                                                                                                                                                        NO
                                                                                                                                                                                                                                                                                                   NO
## 6
                                                                         NO
                                                                                                                           NO
                                                                                                                                                                                            NO
                                                                                                                                                                                                                                        NO
                                                                                                                                                                                                                                                                                                   NO
              mut_deleterious_PTEN mut_impact_PTPRD mut_deleterious_PTPRD mut_impact_RB1 mut_deleterious_RB1 mut
##
## 1
                                                              <NA>
                                                                                                                <NA>
                                                                                                                                                                                <NA>
                                                                                                                                                                                                                             <NA>
                                                                                                                                                                                                                                                                                        <NA>
## 2
                                                                                                               HIGH
                                                                   NO
                                                                                                                                                                                      NO
                                                                                                                                                                                                                                  NO
                                                                                                                                                                                                                                                                                             NO
## 3
                                                              <NA>
                                                                                                                <NA>
                                                                                                                                                                                 <NA>
                                                                                                                                                                                                                             <NA>
                                                                                                                                                                                                                                                                                        <NA>
## 4
                                                                   NO
                                                                                                                     NO
                                                                                                                                                                                      NO
                                                                                                                                                                                                                                  NO
                                                                                                                                                                                                                                                                                             NO
## 5
                                                                   NO
                                                                                                                      NO
                                                                                                                                                                                      NO
                                                                                                                                                                                                                                  NO
                                                                                                                                                                                                                                                                                             NO
## 6
                                                                   NO
                                                                                                                     NO
                                                                                                                                                                                      NO
                                                                                                                                                                                                                                  NO
                                                                                                                                                                                                                                                                                             NO
              mut_deleterious_TBX3 mut_impact_TP53 mut_deleterious_TP53
## 1
                                                              <NA>
                                                                                                            <NA>
                                                                                                                                                                           <NA>
## 2
                                                                   NO
                                                                                                            HIGH
                                                                                                                                                                                NO
## 3
                                                                                                             <NA>
                                                              <NA>
                                                                                                                                                                           <NA>
## 4
                                                                   NO
                                                                                                                  NO
                                                                                                                                                                                NO
## 5
                                                                   NO
                                                                                                                  NO
                                                                                                                                                                                NO
## 6
                                                                   NO
                                                                                                                  NO
                                                                                                                                                                                NO
```

Summary of the PAM50 mRNA factor

Creating signature by comparing "Luminal A" and "HER2-enriched"

```
filter<-"PAM50_mRNA:Luminal A,,,PAM50_mRNA:HER2-enriched"
property <- "PAM50_mRNA"
treatment <- "Luminal A"
baseline <- "HER2-enriched"
apiUrl <- "http://www.ilincs.org/api/ilincsR/LincsDataAnalysis"</pre>
```

```
req <- POST(apiUrl, body = list(exp =experiment,prop = property,treatment=treatment,baseline=baseline,
createdSignaturesSessionID <- httr::content(req)$sessionID</pre>
signatureFileUrl=paste0("http://www.ilincs.org/tmp/completeSig_",createdSignaturesSessionID,".xls")
diffGeneExpSignature<-read.table(signatureFileUrl,header=T,sep="\t",stringsAsFactors = F)[,c("ID_geneid
head(diffGeneExpSignature)
##
     ID_geneid Name_GeneSymbol Value_LogDiffExp Significance_pvalue
## 1
          2099
                          ESR1
                                       3.966901
                                                        1.246425e-43
## 2
          2886
                          GRB7
                                      -2.680763
                                                        7.551647e-43
## 3
         10948
                        STARD3
                                      -2.124142
                                                        8.181878e-43
## 4
                          BCL2
                                                        2.223119e-38
           596
                                       2.378674
## 5
         80129
                       CCDC170
                                       2.612447
                                                        1.178032e-37
## 6
          2064
                         ERBB2
                                      -2.487254
                                                        1.457141e-37
```

Retrieving results for top 12 Differentially expressed proteins (results in Fig 3B)

diffGeneExpTop12Proteins<-diffGeneExpSignature[which(diffGeneExpSignature\$Name_GeneSymbol %in% top12Proteins

##		ID_geneid	Name_GeneSymbol	<pre>Value_LogDiffExp</pre>	Significance_pvalue
##	1	2099	ESR1	3.9669005	1.246425e-43
##	4	596	BCL2	2.3786737	2.223119e-38
##	6	2064	ERBB2	-2.4872541	1.457141e-37
##	12	5241	PGR	4.7020572	2.802427e-34
##	101	2539	G6PD	-1.1821240	2.614818e-24
##	105	2305	FOXM1	-1.5630961	2.822134e-24
##	146	2625	GATA3	1.4899675	2.436816e-22
##	194	891	CCNB1	-1.1241082	3.479925e-21
##	1812	440	ASNS	-0.6974389	2.196442e-09
##	3956	1978	EIF4EBP1	-0.6832938	8.336803e-06
##	11192	1956	EGFR	-0.4563731	6.009345e-02

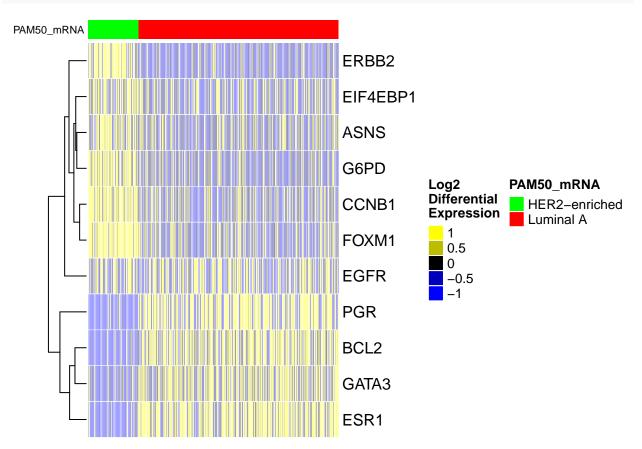
Heatmap of gene expression data for top 12 proteins (Fig 3B)

```
f1 = colorRamp2(c(0,1), c("green", "red"))
f2 = colorRamp2(c(-1, 0,1), c("blue", "black", "yellow"), space = "RGB")
load(url(paste("http://www.ilincs.org/tmp/",experiment,".RData",sep="")),verbose=T)

## Loading objects:
## eset
geneExpressionEset<-get("eset")
geneExpressionEset<-geneExpressionEset[fData(geneExpressionEset)$ID_geneid %in% diffGeneExpTop12Protein
geneExpressionEset<-geneExpressionEset[,order(pData(geneExpressionEset)$PAM50_mRNA)]

meanPE<-apply(exprs(geneExpressionEset),1,mean)
geneExpressionDataTable<-sweep(exprs(geneExpressionEset),1,meanPE,"-")

pam50ColumnAnnotation = HeatmapAnnotation(PAM50_mRNA=pData(geneExpressionEset)$PAM50_mRNA,col = list(PAM50_mRNA)</pre>
```



Connectivity analysis of the transcriptional "Luminal A" vs "HER2-enriched" signature

Upload the signature file that was previousl downloaded

```
sigFilename<-paste0("sigFileForUpload_",createdSignaturesSessionID,".tsv")
write.table(diffGeneExpSignature,file=sigFilename,col.names = T,row.names = F,sep="\t",quote = F)
apiUrl<-"http://www.ilincs.org/api/SignatureMeta/upload"
req <- POST(apiUrl, body=list(file=upload_file(sigFilename)))
uploadedFileName <- httr::content(req)$status$fileName[[1]]
uploadedFileName</pre>
```

[1] "processedSig_Sat_May_7_22_14_25_2022_7940542.xls"

Find connected CP signatures

```
apiUrl <- "http://www.ilincs.org/api/ilincsR/findConcordances"
req <- (POST(apiUrl, body = list(file=uploadedFileName, lib="LIB_5"), encode = "form"))
connectedCpSignatures <- data.table::rbindlist(httr::content(req)$concordanceTable, use.names = TRUE, f
head(connectedCpSignatures)</pre>
```

similarity pValue nGenes compound lincsPertID GeneTargets concentration time

```
## 1: 0.5366747 1.123590e-73
                               973
                                       KPT-330
                                                 LSM-45842
                                                                      XPO1
                                                                                 0.37uM 24h LINCSCP
## 2: 0.5323634 2.597747e-72
                               973 Palbociclib
                                                  LSM-1071 CCND1|CDK4|CDK6
                                                                                         24h LINCSCP
                                                                                   10uM
## 3: 0.5223302 3.263468e-69
                               973 BMS-536924
                                                  LSM-1210
                                                                     IGF1R
                                                                                   10uM
                                                                                         24h LINCSC
## 4: 0.5179384 6.885138e-68
                               973 Palbociclib
                                                  LSM-1071 CCND1|CDK4|CDK6
                                                                                 3.33uM 24h LINCSCP
## 5: 0.5160682 2.488524e-67
                               973
                                       WZ 3146
                                                  LSM-5809
                                                                     EGFR
                                                                                   10uM
                                                                                         24h LINCSC
## 6: 0.5143386 8.108895e-67
                               973 Palbociclib
                                                  LSM-1071 CCND1|CDK4|CDK6
                                                                                 1.11uM 24h LINCSCP
```

Group analysis of top 100 most connected signatures with signature of interest

```
signatureGroup <-connectedCpSignatures$signatureid[1:100]
apiUrl<-"http://www.ilincs.org/api/ilincsR/GroupLincsAnalysis"
req<-POST(apiUrl, body = list(idList = signatureGroup,noOfGenes = 50), encode = "json")
groupAnalysisSessionID <- httr::content(req)$data[[2]]
groupAnalysisSessionID</pre>
## [1] "Sat_May__22_14_50_7_7185586"
```

Load r ExpressionSet from the signature group analysis

```
load(url(paste("http://www.ilincs.org/tmp/filteredeset_",groupAnalysisSessionID,".RData",sep="")),verbo
## Loading objects:
     filteredeset_Sat_May__22_14_50_7_7185586
groupAnalysisEset<-get(paste("filteredeset_",groupAnalysisSessionID,sep=""))</pre>
groupAnalysisEset
## ExpressionSet (storageMode: lockedEnvironment)
## assayData: 559 features, 100 samples
    element names: exprs
## protocolData: none
## phenoData
     sampleNames: LINCSCP_135677 LINCSCP_139907 ... LINCSCP_66347 (100 total)
##
     varLabels: signatureID compound ... treatment (6 total)
##
     varMetadata: labelDescription
##
## featureData
    featureNames: 7153::TOP2A::DNA topoisomerase II alpha 9961::MVP::major vault protein ... 80349::WD
##
    fvarLabels: ID_geneid Name_GeneSymbol DESCRIPTION
     fvarMetadata: labelDescription
## experimentData: use 'experimentData(object)'
## Annotation:
```

Creating heatmap of top 100 connected signatures (Fig 3C)

```
f2 = colorRamp2(c(-1, 0,1), c("blue", "black", "yellow"), space = "RGB")

Heatmap(exprs(groupAnalysisEset), col = f2, cluster_columns = T, cluster_rows=T, column_names_gp = gp
```

