PGSEA Example Workflow Using Expression Data from GEO

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1 Get Your Data

To test for enrichment of gene sets using our package, you only need two basic items: a gene expression data set and a list of gene sets. Your expression data must either already be in ratio form, or include reference samples by which to generate a ratio on the fly. As you will see later in this example, the "reference" argument will be used because the data is not already in the form of a ratio ("experiment"/"reference").

Online gene expression data repositories represent a great wealth of knowledge and this example workflow will help guide your own analysis by using simple Bioconductor tools to exploit data from such a source, like GEO. To start off we must load the GEOquery library, and then download and parse the data with the getGEO command.

```
> library(PGSEA)
> library(GEOquery)
> library(GSEABase)
> gse <- getGEO("GSE7023", GSEMatrix = TRUE)
```

Next we process the raw data from GEO to generate the phenoData for the ExpressionSet, an object that holds gene expression data within Bioconductor. See the GEOquery vignette for more information.

```
> subtype <- gsub("\\.", "_", gsub("subtype: ", "", phenoData(gse[[1]])$characteristics_ch1)
> pheno <- new("AnnotatedDataFrame", data = data.frame(subtype),
+     varMetadata = data.frame(labelDescription = "subtype"))
> rownames(pheno@data) <- colnames(exprs(gse[[1]]))
> eset <- new("ExpressionSet", exprs = exprs(gse[[1]]), phenoData = pheno)
```

Next we must load the gene sets to be used, in this case we will use some included example gene sets that we were able to curate from various publications.

This list of gene sets is by no means an extensive or comprehensive list; it is merely included for example purposes. Information on the creation of each gene set has not been included, but may be available upon request.

```
> data(VAIgsc)
> details(VAIgsc[[1]])
```

```
setName: CMYC.1 down
geneIds: 27, 101, ..., 377582 (total: 89)
geneIdType: EntrezId
collectionType: ExpressionSet
setIdentifier: 185b7a75-038d-4a5e-c498-d25297d24c9d
description:
organism:
pubMedIds: 16273092
urls:
contributor: Karl Dykema <karl.dykema@vai.org>
setVersion: 0.0.1
creationDate: Fri Sep 14 12:00:24 2007
```

2 Run PGSEA

Finally, we are to the point where we can run PGSEA.

```
> pg <- PGSEA(eset, VAIgsc, ref = which(subtype == "NO"))
```

The results come back in the form of a matrix. Here is a look at a portion of it. A result of "NA" means that the test did not pass the significance threshold.

```
> pg[5:8, 5:8]
```

```
            GSM162152 GSM162153 GSM162154 GSM162155
HRAS.1 down        NA        NA        NA        NA
HRAS.1 up          NA  3.707952  2.960228        NA
SRC.1 down         NA        NA        NA        NA
SRC.1 up           NA        NA        NA        NA
```

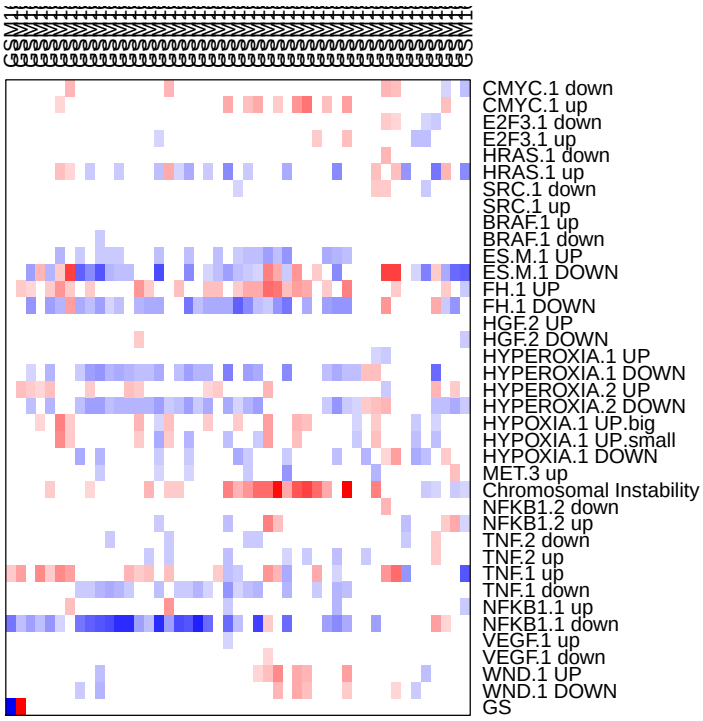
3 Visualize Results

Next we will want to create an attractive plot so that we can visually interpret our results. Looking at the range of the results helps us pick a reasonable scale for the color gradient.

```
> range(pg, finite = TRUE)
```

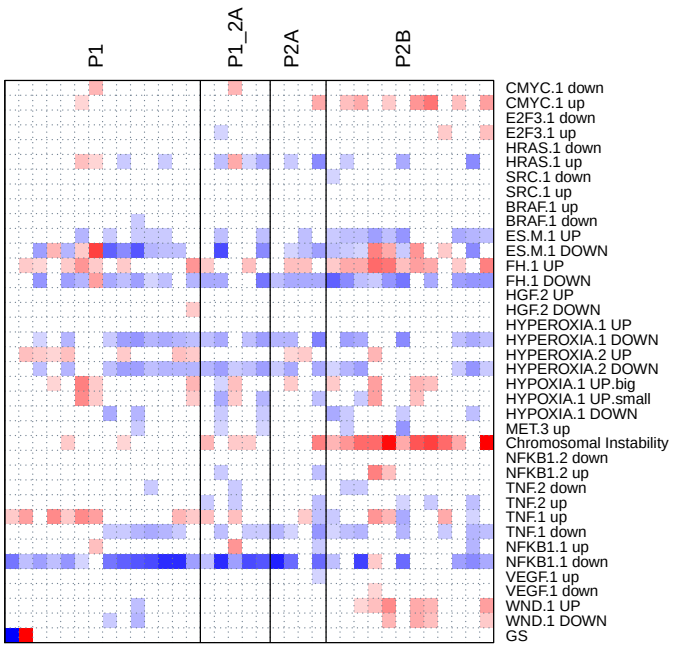
```
[1] -12.64043  17.62942
```

```
> smcPlot(pg, col = .rwb, scale = c(-15, 15))
```



Next with the addition of a few different arguments we can make this plot look much nicer.

```
> smcPlot(pg, factor(subtype), col = .rwb, scale = c(-15, 15),
+     margins = c(1, 1, 6, 9), show.grid = TRUE, r.cex = 0.75)
```



4 Analyze Your Results

One might visually attempt to determine significant differences between our subtypes, but a computational approach is more robust. One simple way to further mine these results is to return an unfiltered matrix from PGSEA and then use a linear modeling approach. In this example we will highlight the differences between Papillary RCC Type 2b and normal renal tissue.

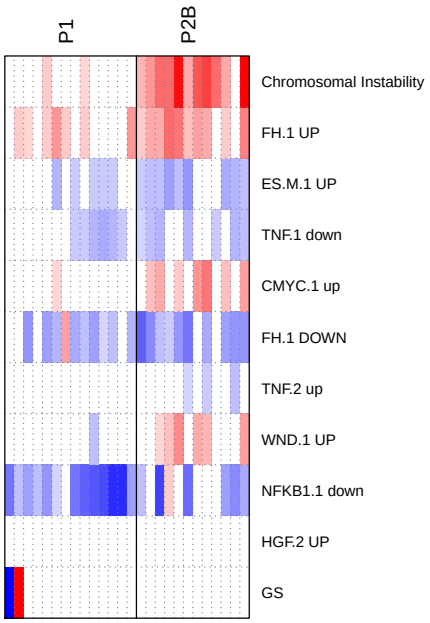
```
> pgNF <- PGSEA(eset, VAIgsc, ref = which(subtype == "NO"), p.value = NA)
> library(limma)
> design <- model.matrix(~-1 + factor(subtype))
> colnames(design) <- names(table(subtype))
> fit <- lmFit(pgNF, design)
> contrast.matrix <- makeContrasts(P2B - NO, levels = design)
> fit <- contrasts.fit(fit, contrast.matrix)
> fit <- eBayes(fit)
> topTable(fit, n = 10)[, c("logFC", "t", "adj.P.Val")]
```

```
       logFC         t    adj.P.Val
25  9.107348  7.795340 2.179072e-08
13  5.111695  6.434137 1.176181e-06
11 -3.417913 -6.169907 1.953786e-06
```

```
31 -2.783328 -4.845456 1.165604e-04
2   3.539790  4.818909 1.165604e-04
14 -5.053123 -4.352914 4.470032e-04
29 -2.179557 -3.578635 4.265204e-03
36  2.695910  3.473171 5.101648e-03
33 -3.784797 -2.670381 3.858354e-02
15  1.087091  2.660417 3.858354e-02
```

Now that we have figured out the significantly different gene sets, we can grab the rownames from "topTable" and insert it into our plotting function.

```
> smcPlot(pg[as.numeric(rownames(topTable(fit, n = 10))), ], factor(subtype,
+     levels = c("P1", "P2B")), col = .rwb, scale = c(-15, 15),
+     margins = c(1, 1, 6, 19), show.grid = TRUE, r.cex = 0.75)
```



Biological interpretation of these results is tricky, and can only end up as "good" as the gene sets and gene expression data used. We have some confidence in most of these signatures, and in this case, a few of the results are worth of note.

The "FH.1" gene sets were created from gene expression data generated from fumarate-hydratase (gene symbol: "FH") deficient uterine fibroids (PMID: 16319128). Using these signatures in PGSEA attempted to detect the same

pattern of altered expression as was seen in the fibroids. "FH" is known to be involved with aggressive renal papillary cancers (PMIDs: 17895761, 17392716ÿ 17270241, etc.) so this result is expected. While the involvement of "FH" and renal cancer was already well-established, the association with "c-MYC" was unreported, so it was an interesting research topic for us. For more information see: "Detection of DNA copy number changes and oncogenic signaling abnormalities from gene expression data reveals MYC activation in high-grade papillary renal cell carcinoma." (Cancer Res. 2007 Apr 1;67(7):3171-6. PMID: 17409424)

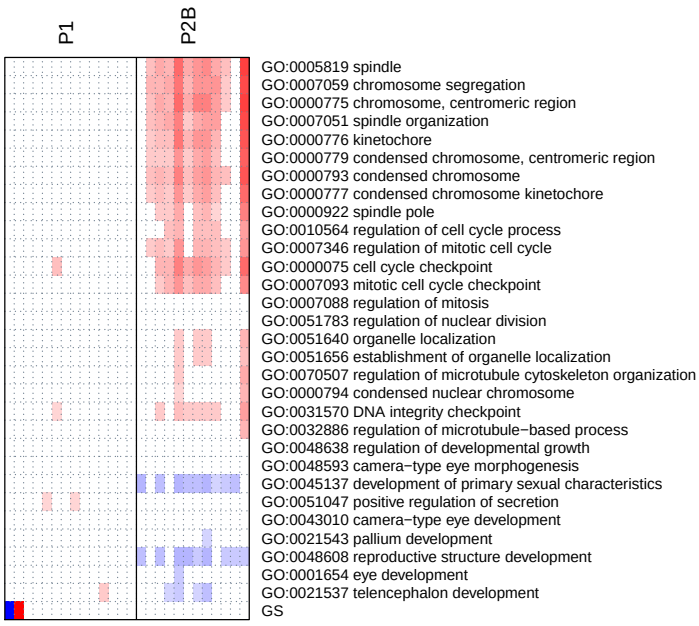
5 Using Large Databases of GeneSets with PGSEA

Instead of using our included example gene sets, one may wish to go another route and use a large database such as "GO". We have included a helper function to quickly generate gene sets from this database.

```
> gos <- go2smc()
> pg <- PGSEA(eset, gos, ref = which(subtype == "NO"))
> pgNF <- PGSEA(eset, gos, ref = which(subtype == "NO"), p.value = NA)
> design <- model.matrix(~-1 + factor(subtype))
> colnames(design) <- names(table(subtype))
> fit <- lmFit(pgNF, design)
> contrast.matrix <- makeContrasts(P2B - P1, levels = design)
> fit <- contrasts.fit(fit, contrast.matrix)
> fit <- eBayes(fit)
```

The linear model approach above will highlight the differences between subtypes P2B and P1 for all go terms. Below, we plot the results and restrict the subtypes shown to those two that we are currently interested in.

```
> smcPlot(pg[as.numeric(rownames(topTable(fit, n = 30, resort.by = "logFC"))),
+     ], factor(subtype, levels = c("P1", "P2B")), col = .rwb,
+     scale = c(-15, 15), margins = c(1, 1, 6, 19), show.grid = TRUE,
+     r.cex = 0.75)
```


P1
P2B
GO:0005819 spindle
GO:0007059 chromosome segregation
GO:0000775 chromosome, centromeric region
GO:0007051 spindle organization
GO:0000776 kinetochore
GO:0000779 condensed chromosome, centromeric region
GO:0000793 condensed chromosome
GO:0000777 condensed chromosome kinetochore
GO:0000922 spindle pole
GO:0010564 regulation of cell cycle process
GO:0007346 regulation of mitotic cell cycle
GO:0000075 cell cycle checkpoint
GO:0007093 mitotic cell cycle checkpoint
GO:0007088 regulation of mitosis
GO:0051783 regulation of nuclear division
GO:0051640 organelle localization
GO:0051656 establishment of organelle localization
GO:0070507 regulation of microtubule cytoskeleton organization
GO:0000794 condensed nuclear chromosome
GO:0031570 DNA integrity checkpoint
GO:0032886 regulation of microtubule−based process
GO:0048638 regulation of developmental growth
GO:0048593 camera−type eye morphogenesis
GO:0045137 development of primary sexual characteristics
GO:0051047 positive regulation of secretion
GO:0043010 camera−type eye development
GO:0021543 pallium development
GO:0048608 reproductive structure development
GO:0001654 eye development
GO:0021537 telencephalon development
GS