Gene Set Enrichment Analysis

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1. **Simple GSEA**

2. **GSEA using Linear Models**

3. **Hypergeometric Testing Used for GSEA**

Outline

1. Simple GSEA
2. GSEA using Linear Models
3. Hypergeometric Testing Used for GSEA

Gene set enrichment analysis

- Unlike per-gene analysis ...
- Search for categories where the constituent genes show changes in expression level over the experimental conditions.
- Use predefined gene set such as KEGG pathways, GO classifications, chromosome bands, and protein complexes.
- No need to make a cutoff between genes that are differentially expressed and those that are not.
- Provided in the *GESABase*, *Category*, *GOstats* and *topGO*.

Simple GSEA

Consider two group comparison

- Start with data quality assessment.
- Compute per-gene t-statistics: t_k for each gene k.
- Null hypothesis: no difference in mean expression

$$H_o : Z_K = 0$$

$$Z_K = \frac{1}{\sqrt{|K|}} \sum_{k \in K} t_k \sim \mathcal{N}(0,1),$$

  where K denotes the gene sets, and $|K|$ the number of genes in the gene set.

- Alternative approach: use permutation test to assess which gene sets have an unusually large absolute value of z_K.

Data preparation

ALLfill_bcrneg

```
> library(ALL)
> library(hgu95av2.db)
> data(ALL)
> bcell <- grep("^B", as.character(ALL$BT))
> types <- c("NEG", "BCR/ABL")
> moltyp <- which(as.character(ALL$mol.biol) %in% types)
> # subsetting
> ALL_bcrneg <- ALL[, intersect(bcell, moltyp)]
> ALL_bcrneg$BT <- factor(ALL_bcrneg$BT)
> ALL_bcrneg$mol.biol <- factor(ALL_bcrneg$mol.biol)
> # nonspecific filter: remove genes that does not
> ## show much variation across samples
> library(genefilter)
> filt_bcrneg <- nsFilter(ALL_bcrneg,
+                         var.cutoff=0.5)
> ALLfilt_bcrneg <- filt_bcrneg$eset
```

Using KEGG

- Data representation: create an incidence matrix `Am` where $a_{ij} = 1$ if gene j is in gene set i and $a_{ij} = 0$ otherwise.

```
> library(KEGG.db)
> library(GSEABase)
> gsc <- GeneSetCollection(ALLfilt_bcrneg,
+                                  setType=KEGGCollection())
> Am <- incidence(gsc)
```

- `ExpressionSet` object retains only those features that are in the incidence matrix `Am`.

```
> nsF <- ALLfilt_bcrneg[colnames(Am), ]
```

Using KEGG

Exercise

1. How many gene sets and how many genes are represented by the incidence matrix `Am`?
2. How many gene sets have fewer than ten genes in them?
3. What is the largest number of gene sets in which a gene can be found?
4. What is the name of this gene set? (use KEGGPATHID2NAME)

Using KEGG

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Code

```
> dim(nsF)
> dim(Am)
> nGene <- rowSums(Am)
> rownames(Am)[nGene < 10]
> sort(nGene, decreasing=TRUE)[1]
> KEGGPATHID2NAME[["05200"]]
```

Using KEGG

- Compute the per-gene test statistics using the rowttests function.

```
> rtt <- rowttests(nsF, "mol.biol")
> names(rtt)

[1] "statistic" "dm"        "p.value"

> rttStats <- rtt$statistic
```

Using KEGG

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> names(rtt)
```

```
[1] "statistic" "dm"        "p.value"
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```
> rttStats <- rtt$statistic
```

- Reduce the incidence matrix by removing all gene sets that have fewer than ten genes in them.

```
> selectedRows <- (rowSums(Am) > 10)
> Am2 <- Am[selectedRows, ]
```

Using KEGG

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> rtt <- rowttests(nsF, "mol.biol")
> names(rtt)
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[1] "statistic" "dm"          "p.value"
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> rttStats <- rtt$statistic
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- Reduce the incidence matrix by removing all gene sets that have fewer than ten genes in them.

```
> selectedRows <- (rowSums(Am) > 10)
> Am2 <- Am[selectedRows, ]
```

- Compute z_k for each pathway: $z_K = \frac{1}{\sqrt{|K|}} \sum_{k \in K} t_k$.

```
> tA <- as.vector(Am2 %*% rttStats)
> tAadj <- tA /sqrt(rowSums(Am2))
> names(tAadj) <- rownames(Am2)
```

Using KEGG

Exercise

1. Which pathways have remarkably low (< 5) and high aggregate statistics (> 5)?
2. What is the name the pathway that has the lowest z_k score?
3. Use KEGG2heatmap to plot a heatmap for the genes in this pathway.

Using KEGG

Exercise

1. Which pathways have remarkably low (< 5) and high aggregate statistics (> 5)?
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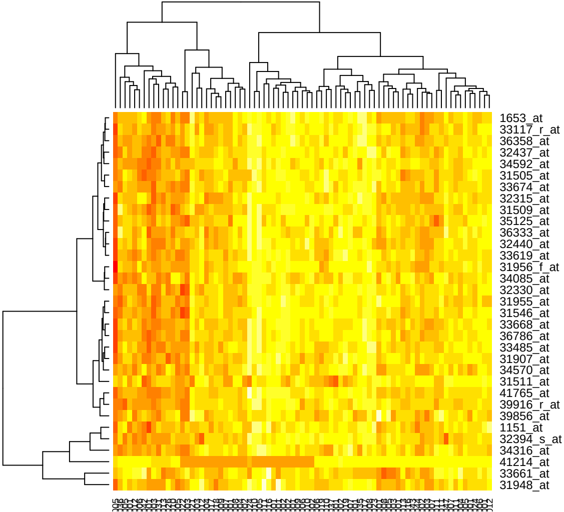
Code

```
> smPW <- tAadj[tAadj < -5]
> mget(names(smPW),KEGGPATHID2NAME)
> lgPW <- tAadj[tAadj > 5]
> mget(names(lgPW), KEGGPATHID2NAME)
```

KEGG2heatmap

```
> KEGG2heatmap("03010", nsF, "hgu95av2")
```



Permutation testing

- Assess the significant gene sets with respect to a reference distribution build by a number of permutations.
- gseattperm: permute the sample labels.
- Return *p*-value w.r.t. to a reference distribution:
  - `Lower`: proportion of permutation *t*-statistics that were smaller than the observed *t*-statistics
  - `Upper`: proportion of permutation *t*-statistics that were larger than the observed *t*-statistics

Code: using gseattperm

```
> library(Category)
> set.seed(123)
> pvals <- gseattperm(nsF, nsF$mol.biol, Am2, 1000)
> pvalCut <- 0.05
> lowC <- rownames(pvals)[pvals[, 1] <= pvalCut]
> unlist(getPathNames(lowC), use.names=FALSE)

[1] "Glycerophospholipid metabolism"
[2] "Ribosome"
```

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Chromosome bands

- Use the mapping of genes to chromosome bands.
- To answer whether there are anomalies in the pattern of gene expression that related to chromosome bands.
- Use GSEA linear models.

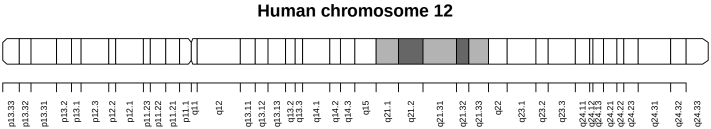

Figure: Ideogram for human chromosome 12. The shaded bands together represent 12q21. Notice that the chromosome bands are hierarchically nested, and they almost form a partition. (D. Sarker et. al. 2007)

Reference

"Using Categories defined by Chromosome Bands" by D. Sarker et. al.

Data preparation

- Consider the comparison of BCR/ABL and NEG groups.
- Use `ALL_bcrneg` object.
- Use nsFilter to remove probes with no Entrez Gene ID and no mapping to a chromosome band. Ensure that each Entrez Gene ID maps to exactly one probeset which has the highest IQR. Also remove probes with lack of variation (var < 0.5).

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Code: nonspecific filtering

```
> ALLfilt <- nsFilter(ALL_bcrneg, require.entez=TRUE,
+                     remove.dupEntrez=TRUE,
+                     require.CytoBand=TRUE,
+                     var.func=IQR,
+                     var.cutoff=0.5)$eset
```

Data preparation

- Compute per-gene t-statistics using limma.

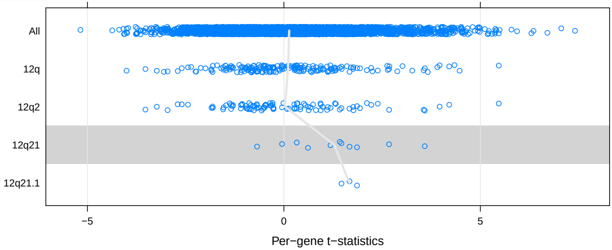
Data preparation

- Compute per-gene *t*-statistics using limma.

Code: moderate *t*-statistics

```
> library(limma)
> design <- model.matrix(~0 + ALLfilt$mol.biol)
> colnames(design) <- c("BCR/ABL", "NEG")
> contr <- c(1, -1)
> fit1 <- lmFit(ALLfilt, design)
> fit2 <- contrasts.fit(fit1, contr)
> fit3 <- eBayes(fit2)
> tlimma <- topTable(fit3, number=nrow(fit3),
+                    adjust.method="none")
> ## annotation
> entrezUniverse <- unlist(mget(tlimma$ID,
+                               hgu95av2ENTREZID))
> tstats <- tlimma$t
> names(tstats) <- entrezUniverse
```

Linear models



- Fitting linear model with per-gene t-statistics: for each category j,

$$y_i = \beta_0 + \beta_1 a_{ij} + \varepsilon_i,$$

where $a_{ij} = 1$ if gene i is associated with category j, and 0 otherwise. The index i may range over from universal genes to a subset of genes.

- $\beta_1 \sim \mathcal{N}(0, 1)$

Linear models

- Create a ChrMapLinearMParams object.

Code: instance of class ChrMapLinearMParams

```
> library(Category)
> params <- new("ChrMapLinearMParams",
+               conditional=FALSE,
+               testDirection="up",
+               universeGeneIds=entrezUniverse,
+               geneStats=tstats,
+               annotation="hgu95av2",
+               pvalueCutoff=0.01,
+               minSize=4L)
```

Calling the `linearMTest` function

- linearMTest: compute the *p*-values for detecting up- or down-regulation of predefined gene sets.

Code: linearMTest

```
> lman <- linearMTest(params)
> lman
> summary(lman)
```

Exercise

1. Get familiar with the structure of `ChrMapLinearMParams` class? ChrMapLinearMParams or help("ChrMapLinearMParams-class")
2. Perform conditional GSEA linear models to find interesting chromosome bands that are up-regulated.
3. Summarize the result of the conditional test using summary.

Exercise

1. Get familiar with the structure of `ChrMapLinearMParams` class? ChrMapLinearMParams or help("ChrMapLinearMParams-class")
2. Perform conditional GSEA linear models to find interesting chromosome bands that are up-regulated.
3. Summarize the result of the conditional test using summary.

Code: conditional test

```
> slotNames(params)
> paramsCond <- params
> paramsCond@conditional <- TRUE
> lmanCond <- linearMTest(paramsCond)
> summary(lmanCond)
```

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Hypergeometric testing

- Basic concept: Suppose there are N balls in an urn, n are white and m are black. Drawing k balls out of the urn without replacement, how many black balls do we expect to get? What is the probability of getting x black balls?
- Hypergeometric testing of under- and over-representation of GO terms
  1. gene universe
  2. GO categories (categorize genes by GO terms)
  3. a list of interesting genes (differentially expressed genes identified by limma or just simply t-test by rowttests)

Hypergeometric testing

	Interesting (Black)	Not (White)	
In GO term	n_{11}	n_{12}	K
Not in GO term	n_{21}	n_{22}	$N-K$
	l	$N-l$	N

Suppose there are j interesting genes in the GO term ($n_{11} = j$), compute the probability of seeing j or more black balls in K draws.

Data preparation

- Define gene universe (a vector of Entrez Gene IDs).
- Select a list of interesting genes (a vector of Entrez Gene ID).

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Code: gene selection via *t*-test

```
> ttests <- rowttests(ALLfilt_bcrneg, "mol.biol")
> smPV <- ttests[ttests$p.value < 0.005, ]
> selectedEntrezIds <- unlist(mget(rownames(smPV),
+                                  hgu95av2ENTREZID))
> entrezUniverse=unlist(mget(featureNames(ALLfilt_bcrneg),
+   hgu95av2ENTREZID))
```

Hypergeometric testing

- Create GOHyperGParams object.

Code: GOHyperGParams

```
> library(GOstats)
> hgCutoff <- 0.001
> GOparams <- new("GOHyperGParams",
+                 geneIds=selectedEntrezIds,
+                 universeGeneIds=entrezUniverse,
+                 annotation="hgu95av2.db",
+                 ontology="BP",
+                 pvalueCutoff=0.001,
+                 conditional=TRUE,
+                 testDirection="over")
```

- Outputs and summary.

Code: hyperGTest

```
> hgOver <- hyperGTest(GOparams)
> class(hgOver)
> summary(hgOver)
```

- Outputs and summary.

Code: hyperGTest

```
> hgOver <- hyperGTest(GOparams)
> class(hgOver)
> summary(hgOver)
```

- Exercise: get results in details using termGraphs and htmlReort.

```
> showMethods("htmlReport")
> htmlReport(hgOver, file="hgResult.html")
```

Summary

1. Basic idea behind GSEA.
2. Simple GSEA: *t*-tests and permutation.
3. Using KEGG categories.
4. Linear models and chromosome band categories.
5. Hypergeometric testings on GO BP terms.

Reference

- Assaf P. Oron et. al., Gene set enrichment analysis using linear models and diagnostics, *Bioinformatics*, vol. 24 no. 22, pp. 2566-2591, 2008.
- Florian Hahne et. al., *Bioconductor Case Studies*, chapter 13-14, Springer, 2008.
- Deepayan Sarker et. al., *Using Categories defined chromosome bands*, Bioconductor Category package vignette.
- D. Sarker et.al., Modeling gene expression data via chromosome bands, *Bioinformatics*, 2007.