our main point

1. section 1: introduction begins here (suggestions: try writting your introductions last).
   (a) what is my topic (question)?
   (b) why is it important?
   (c) how will I plan to proceed with my
2. previous comparative gene set enrichment analysis does not take....
3. we propose a method that allows DE within the test set as well as the background gene set.

Competitive gene set enrichment analysis for correlated expression data

Abstract

To be filled

1 Introduction

What is competitive gene set test

Competitive gene set test (Goeman and Bühlmann, 2007) is a gene expression analysis that compares differential expression (DE) for a pre-defined set of genes to that for all other genes. The pre-defined set of genes may represent biological pathways or network. Incorporating such prior knowledge of the grouping makes it easier for biologists to interpret the results of DE analysis. Most competitive gene set tests are two-stage procedures: on the first stage, a *local statistics* that measures the association between the response (e.g., the expression value) and the predictors (e.g., treatment condition) is calculated for each gene; on the second stage, a p-value is reported from some form of global statistics that use local statistics as observations and gene set information as predictor.

What are the existing methods

Independent gene set test

A number of gene set tests assume independence of gene expression profiles. They are parametric or rank-based procedures that assume the local statistics to be independent and identically distributed, or gene permutation procedures that generates the same approximate null. Examples include, among many others, z-test/t-test that compares the mean of the local statistics in the test set to the mean of those not in the test (e.g., Kim and Volsky, 2005), the 2×2 contingency-table-based tests that examine the over-representation of DE genes in the test set (see Huang et al., 2009 for a review), and permutation-based tests that evaluate p-values by permuting gene lables (e.g., sigPathway of Tian et al., 2005, "geneSetTest" in the limma package Smyth, 2004). However, tests assuming independence of genes may result in inflated false discovery rate (Efron and Tibshirani, 2007) and loss of power as genes in a gene set are often correlated and function together.

Tests that account for between-gene correlations

To accont for between-gene correlation in gene set tests, methods have been proposed. One attempt is to evaluate the p-value of the global test by permuting the biological samples (see, for example, Subramanian et al., 2005 and Efron and Tibshirani, 2007). Permuting the sample lables preserves, in contrast to permuting gene lables that ignores, the between-gene correlaiton structure and thus protects the test against such correlation. The very famous example of this kind is the *Gene Set Enrichment Analysis* (GSEA) proposed by Subramanian et al. (2005). However, this approach implicitly alters the null hypothesis being tested and therefore the null and the alternative hypotheses are difficult to characterize.

Another attempt is to conduct global test that works with the between-gene correlation structures. Wu and Smyth (2012) proposed Correlation Adjusted MEan RAnk (CAMERA) gene set test that estimates a variance inflation factor associated with correaltions between gene expression profiles, and then incorporates it into the parametric or rank-based test. However, as shown by (the paper to be finished) that the correlation of local test statistics can be over-estimated when a fraction of genes are DE. CAMERA, relying on the assumption that correlations between local statisitcs are euqal to

correlations between expression profiles, may be too conservative since the variance inflation factor is over-estimated. (This is a self-contained gene set test) Huang and Lin (2013) uses a multivariate linear regression model in which the between-gene correlations are explicitly modeled by a working covariance matrix.

What do we propose?

What is the plan of this paper?

2 Methods

Overview of our method (denoted as OurMethod, will be easily replaced when we have a better new name)

Different from CAMERAWu and Smyth (2012) or GSEA (Subramanian et al., 2005)

Our method is based on case-control

2.1 The general assumptions for expression data

In a treatment-control gene expression experiment, we denote by Y_{ijk} a random variable for the expression level of gene i from observational unit j in treatment group k, with i taking the values $1, \ldots, m$ (the number of genes), j taking the values $1, \ldots, n_k$ (the total number of biological samples), and k being either 1 for control or 2 for treatment. Correspondingly, Y^*_{ijk} represents the standardized expression levels (described in REF???) for gene i of sample j, with $Y^*_{ijk} \sim N(0,1)$ (??? Normal assumption necessary here???) if sample j comes from the control group, and $Y^*_{ijk} \sim N(\Delta_i, 1)$ if it comes from the treatment group. Here, Δ_i is a *DE effect*: compared to the control group, gene i is not DE if $\Delta_i = 0$, up-regulated if $\Delta_i > 0$ and down-regulated if $\Delta_i < 0$. In a gene expression experiment, the DE effect Δ_i consists of two parts: 1) the treatment which determines whether a gene is DE or not; and 2) the DE effect size or strength when the gene is DE. For 1), we let $\boldsymbol{Z} = (Z_1, \ldots, Z_m)$ be a vector of DE indicators, where $Z_i = 1$ if gene i is DE and $Z_i = 0$ otherwise, and (DO WE NEED TO ASSUME Z_is TO BE INDEPENDENT OF EACH OTHER?

$$Z_i \sim \text{Binom}(1, p_i) \tag{1}$$

For 2), we denote δ_i as the *DE effect size* for gene i and δ_i follows some distribution f_δ with mean and variance

$$E(\delta_i) = \mu_\delta, \quad \text{Var}(\delta_i) = \sigma^2_\delta \tag{2}$$

We further assume that the DE indicator Z_i is independent of the DE effect size δ_i for gene $i = 1, \ldots, m$. Therefore, the DE effect can be expressed as

$$\Delta_i = Z_i \delta_i, \tag{3}$$

It can be shown that (details in Appendix 6),

$$E(\Delta_i) = p_i \mu_\delta, \quad \text{Var}(\Delta_i) = p_i \sigma^2_\delta + p_i(1-p_i)\mu^2_\delta, \quad i = 1, \ldots, m. \tag{4}$$

We assume that conditioning on the DE effects, expression levels for different samples are independent, but expression levels for different genes of the same sample may be correlated. Denote $C_{m\times m}$ as the gene correlation matrix, with entry ρ_{i_1,i_2} being the correlation between genes i_1 and i_2. Note that the between-gene correlation ρ_{i_1,i_2} is a constant, regardless of whether the sample is from the treatment or from the control group.

2.2 Gene set test allowing for between-gene correlation

We denote by I_t and I_b the gene set being tested and background set (i.e., the genes not in the test set). Let $\boldsymbol{x} = (x_1, \ldots, x_m)$ be a indicator vector of whether or not gene i belongs to the test set and thus $I_t = \{i : x_i = 1\}$ and $I_b = \{i : x_i = 0\}$. We assume that the DE probability is p_t for genes in the test set and p_b for genes in the background set. For gene i, denote $U_i = \bar{Y}_{i.2} - \bar{Y}_{i.1}$ as the difference in mean expression levels between the treatment and the control group, where $\bar{Y}_{i.k} = \sum_{j=1}^{n_k} Y_{ijk}/n_k$. It follows from equation (4) that $\boldsymbol{U} = (U_1, \ldots, U_m)$ has mean

$$E(U_i) = \begin{cases} p_t \mu_\delta, & \text{if } i \in I_t \\ p_b \mu_\delta, & \text{if } i \in I_b \end{cases} \tag{5}$$

and covariance (see Appendix 6 for detail)

$$\text{Var}(\boldsymbol{U}) = \boldsymbol{D} + \sigma_2^2 \boldsymbol{C} \tag{6}$$

where $\boldsymbol{D} = \text{diag}(d_1, \ldots, d_m)$ with $d_i = p_t\sigma_\delta^2 + p_t(1-p_t)\mu_\delta^2$ if $i \in I_t$ and $d_i = p_b\sigma_\delta^2 + p_b(1-p_b)\mu_\delta^2$ if $i \in I_b$, $\sigma_2^2 = \frac{1}{n_1} + \frac{1}{n_2}$ and $\boldsymbol{C}$ is the between-gene correlation matrix .

(**The test**) The DE probability affects both the mean vector in equation (5) and the covariance in equation (6). Under this framework, the test set is not enriched only if the probability of DE in the test set is the same as that in the background set. Therefore, the hypothesis for enrichment testing can be statistically formulated as

$$H_0\text{: } p_t = p_b \overset{\text{def}}{=} p_0 \text{ Versus } H_1\text{: } p_t \neq p_b \tag{7}$$

We can combine equations (5) and (6) into the following linear model

$$\boldsymbol{U} = \beta_0 \mathbf{1}_m + \beta_1 \boldsymbol{x} + \boldsymbol{\epsilon}, \quad \text{Cov}(\boldsymbol{\epsilon}) = \boldsymbol{D} + \sigma_2^2 \boldsymbol{C} \tag{8}$$

with $\beta_0 = p_b\mu_\delta$, $\beta_1 = (p_t - p_b)\mu_\delta$ and $\mathbf{1}_m$ being a vector of ones. Now the hypothesis testing problem in (7) becomes

$$H_0\text{: } \beta_1 = 0 \text{ Versus } H_1\text{: } \beta_1 \neq 0. \tag{9}$$

Under the null of (9), we have $E(\boldsymbol{U}) = \beta_0 \mathbf{1}_m$ and $\text{Var}(\boldsymbol{U}) = \sigma_1^2 \boldsymbol{I}_m + \sigma_2^2 \boldsymbol{C}$ where $\boldsymbol{I}_m$ is an identity matrix and $\sigma_1^2 = p_0\sigma_\delta^2 + p_0(1-p_0)\mu_\delta^2$.

(**Estimating the parameters**) In practice, we need to estimate β_0, σ_1^2 and $\boldsymbol{C}$ in model 8 for enrichment test. Our strategy is to use *quasi-likelihood*, which requires only the mean and the variance of $\boldsymbol{U}$. The between-gene correlation matrix $\boldsymbol{C}$ is estimated by the residual sample correlations after the treatment differences have been nullified (the same as is done by Efron (2007) or Wu and Smyth (2012)), and is treated as known in estimating β_0 and σ_1^2. Denoting $\hat{\boldsymbol{C}}$ as the estimate of $\boldsymbol{C}$ and,

$$\boldsymbol{\Sigma} = \sigma_1^2 \boldsymbol{I}_m + \sigma_2^2 \hat{\boldsymbol{C}} \tag{10}$$

The score equations for β_0 and σ_1^2 are

$$\begin{aligned} (\boldsymbol{U} - \beta_0 \mathbf{1}_m)^T \boldsymbol{\Sigma}^{-1} \mathbf{1}_m &= 0 \\ (\boldsymbol{U} - \beta_0 \mathbf{1}_m)^T \boldsymbol{\Sigma}^{-1} \hat{\boldsymbol{C}} (\boldsymbol{U} - \beta_0 \mathbf{1}_m) &= \text{trace}(\boldsymbol{\Sigma}^{-1} \hat{\boldsymbol{C}}) \end{aligned} \tag{11}$$

.... something to catch up.....

The enrichment test statistic for the test set is

$$T = \frac{\left[\boldsymbol{x}^T (\boldsymbol{U} - \hat{\beta}_0 \mathbf{1}_m)\right]^2}{\left[\boldsymbol{x}^T (\boldsymbol{I} - \boldsymbol{H})\right] \boldsymbol{\Sigma} \left[\boldsymbol{x}^T (\boldsymbol{I} - \boldsymbol{H})\right]^T} \tag{12}$$

Under the null, $T \sim \chi^2(1)$.

2.3 Other competitive gene set tests

We will compare OurMethod to three existing gene set tests: GSEA (Subramanian et al., 2005) modified from the original R-GSEA script (`http://software.broadinstitute.org/gsea/index.jsp`) to accommodate single gene set test, two versions of the CAMERA procedure (Wu and Smyth, 2012), and two versions of the geneSetTest procedure in the limma package (Smyth, 2005). By "two versions" we mean, respectively, parametric and rank based. We will denote the two versions by geneSetTest-modt and geneSetTest-rank for geneSetTest, and by CAMERA-modt and CAMERA-rank for CAMERA. Because GSEA and OurMethod do not support linear models, the implementations are restricted to two-group comparisons.

All of the three tests use local genewise statistics as observations to conduct global tests comparing genes in the test set to those in the background set. They may differ, however, either in terms of the local statistics used to compare factors of interest (e.g. treatment vs. control) at the gene level, or in terms of the global statistics used to summarize the significance of the test set compared to the background set. For GSEA, the local statistics are the rankings of genes according to a ranking metric (e.g. signal-to-noise ratio, t-statistic), then based on the rankings an enrichment score for the test set is calculated, and the significance of the enrichment score is determined by randomly permuting the sample labels. For the parametric version, both CAMERA-modt and geneSetTest-modt use certain type of local statistics (e.g., the moderated t-statistics Smyth (2004)), and determine whether the means of the local statistics are significantly different for genes in the test set versus genes in the background set. The difference is how they evaluate the global statistics: CAMERA-modt uses a t-statistic that allows the local statistics in the test set to be correlated by first estimating a variance inflation factor, and then incorporating it into the t-statistic to adjust for between-gene correlation (see materials and methods section of Wu and Smyth (2012)); geneSetTest-modt evaluates p-values by comparing the observed mean of the local statistics in the test set, to those (??? is it clear?) obtained by randomly permuting the gene labels. For the rank-based test , CAMERA-rank and geneSetTest-rank conduct a Wilcoxon-Mann-Whitney rank sum test, and they amount to, respectively, CAMERA-modt and geneSetTest-modt in that they compare the rankings instead of the local statistics themselves for genes in the test set to those for genes in the background set.

(other methods such as sigPathway or PAGE will be mentioned in the introduction part.)

3 Examples and Numerical Results

3.1 Simulations

In this section, we present results from type I error and power simulations under a range of between-gene correlation structures.

The simulations run as follows: first, we simulate an entire gene set containing $m = 500$ genes, from which we sample $m_1 = 100$ genes to represent those in the test set, and the remaining $m_2 = 400$ genes those in the background set; second, for gene $i = 1, \ldots, m$, we simulate the DE effect Δ_i by first generating the DE size δ_i from $N(2, 1)$ and the DE indicator Z_i from $\text{Binom}(1, p_i)$, where $p_i = p_t$ if gene i belongs to the test set and $p_i = p_b$ otherwise, and then setting Δ_i to be the product of Z_i and δ_i; third, we set the "true" mean expression values $\mu_1 = \mathbf{0}_m$ and $\boldsymbol{\mu}_2 = \boldsymbol{\Delta}$, respectively, for the control and treatment groups; fourth, we simulate n_1 samples from $\text{MVN}(\boldsymbol{\mu}_1, \boldsymbol{\Sigma})$ for the control group and n_2 samples from $\text{MVN}(\boldsymbol{\mu}_2, \boldsymbol{\Sigma})$, where the covariance $\boldsymbol{\Sigma} = (\sigma_{i_1,i_2})_{m\times m}$ may take one of the following forms:

(a0): the genes are independent of each other (i.e., $\boldsymbol{\Sigma} = \boldsymbol{I}_m$).

(a): only the genes in the test set are correlated, with exchangeable correlation structure, that is, $\text{Cor}(Y_{i_1}, Y_{i_2}) = \sigma_{i_1,i_2} = \rho$ for $\forall i_1, i_2 \in I_t$ and $\text{Cor}(Y_{i_3}, Y_{i_4}) = \sigma_{i_3,i_4} = 0$ if at least one of i_3, i_4 does not belong to I_t.

(c): all genes are correlated, with exchangeable correlation structure, that is, $\text{Cor}(Y_{i_1}, Y_{i_2}) = \sigma_{i_1,i_2} = \rho$ for $\forall i_1, i_2 \in I$.

(e): genes are correlated within the test set and within the background set; but any two genes, one from the test set and the other from the background set, are independent. That is, the correlation structure is block diagonal, with $\text{Cor}(Y_{i_1}, Y_{i_2}) = \sigma_{i_1,i_2} = \rho_1$ for $i_1, i_2 \in I_t$, $\text{Cor}(Y_{i_3}, Y_{i_4}) = \sigma_{i_3,i_4} = \rho_2$ for $i_3, i_4 \in I_b$, and $\text{Cor}(Y_{i_5}, Y_{i_6}) = \sigma_{i_5,i_6} = 0$ for $\forall\ i_5 \in I_t, \forall\ i_6 \in I_b$.

(f): all genes are correlated, but the correlation between two genes depend on whether they belong to the test set or not. Specifically, $\text{Cor}(Y_{i_1}, Y_{i_2}) = \sigma_{i_1,i_2} = \rho_1$ for $i_1, i_2 \in I_t$, $\text{Cor}(Y_{i_3}, Y_{i_4}) = \sigma_{i_3,i_4} = \rho_2$, for $i_3, i_4 \in I_b$, and $\text{Cor}(Y_{i_5}, Y_{i_6}) = \sigma_{i_5,i_6} = \rho_3$ for $\forall\ i_5 \in I_t, \forall\ i_6 \in I_b$.

(g): genes are correlated in the same way as those from a real data.

3.1.1 Type I error simulations

In the above simulation setup, the test set is not enriched if DE probabilities are the same for the genes in the test set and for those in the background set (i.e., $p_t = p_b = p_0$). However, it is shown in (the paper to be finished) that the test statistics correlation between two genes is not equal to their sample correlation when at least one gene is truly DE (under two sample t-test???). Therefore, if there are true DE genes in the entire gene set, approaches assuming the same correlation between local statistics and between expression values may not perform well. To illustrate this point, we performed two groups of simulations for each of the correlation structures above: in group A_1, we simulated expression data with no DE genes (i.e., $p_t = p_b = 0$); and in group A_2, we simulated data sets with the same DE probabilities for all genes (i.e., DE prorabilities are the same for genes in the test set and for those in the background set with $p_t = p_b = 0.2$).

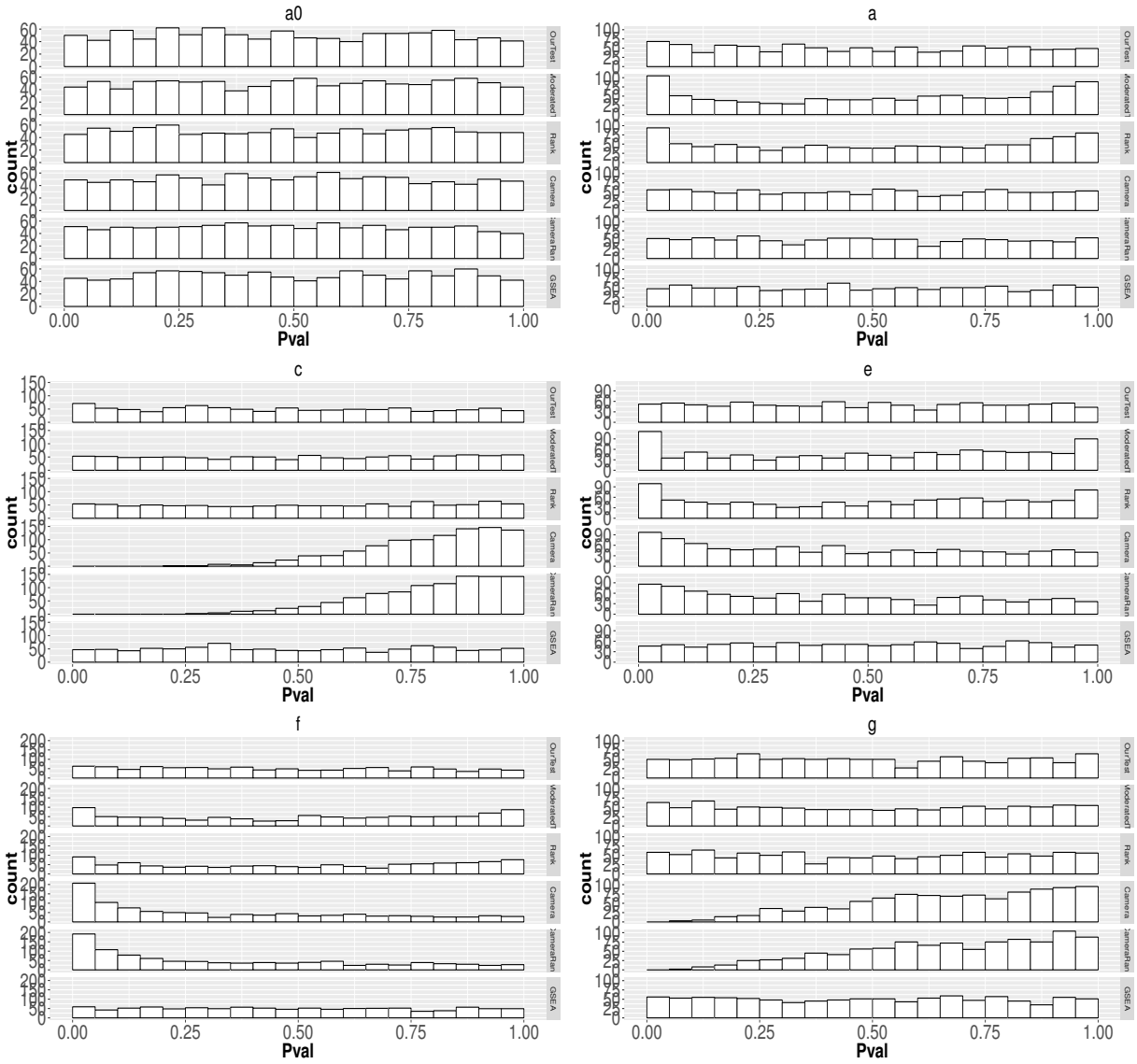
For group A_1, Figure 1 shows the histograms of type I error rates for the six approaches (OurMethod, geneSetTest-modt, geneSetTest-rank, CAMERA-modt, CAMERA-rank and GSEA) under the six correlation structures. OurMethod and GSEA hold the size of type I error rates correctly for all 6 correlation structures, with simulated p-values uniformly distributed on $[0, 1]$. The two version of CAMERA control type I errors correctly for correlation structures (a0) and (a). However, both are too conservative for the case of (c) and (g), and anti-conservative for correlation structures (e) and (f). geneSetTest procedures may be too liberal depending on the underlying correlation structures.

For the group A_2 simulation where DE probabilities are 0.2 across all genes, we summarize the results of the type I error rate simulation in Table 1. OurMethod continues to hold the size of type I error rates under all six correlation structures. However, GSEA is highly skewed towards small p-values and the two versions of CAMERA procedures are too conservative under all correlation structures, and the only exception is that CAMERA controls type I error rates correctly for (a0) where genes are simulated to be independent. The two versions of geneSetTest performs reasonably well.

Explain why this happens

Consistent accuracy is shown for OurMethod across all simulations, but the accuracy of the other three methods may be affected by two factors: the between-gene correlation structures, and DE probability of each gene. OurMethod controls the size of type I error rates well becaues it takes into account the between-gene correlation and works directly with the sample correlation between genes, and therefore is robust against the two factors. (rewrite from here, because this is only my understanding.) The GSEA evaluates the enrichment score of a test set by generating its null distribution from sample permutation, and therefore the between-gene correlation is preserved when there are no DE genes, but [explain when DE exists]... For CAMERA, the global statistics take into account only the between-gene correlation in the test set, and therefore does not work for cases where genes in the background set are also correlated in group A_1 simulations. More importantly, according to (the paper to be finished), the variance inflation factor of the local statistics (moderated t-test in Wu and Smyth (2012)) may be over-estimated when a fraction of genes are DE, and therefore the global test statistic is under-estimated, resulting in conservative p-values in group A_2 simulations. geneSetTest permutes the gene lables to examine the significance of the test set, and therefore it relies on independence between genes. The performances of both versions of geneSetTest are thus unpredictable in group A_1. In group A_2 where there are DE genes both in the test set and in the background set, the correlation between the local statistics are smaller (in absolute value) than the correlation between the genes. Since the genes

Figure 1: Type I error rates for gene set tests, p-value distribution for case (a0) - (g) from left to right, from top to bottom, NO gene is DE



are simulated to be slightly correlated ($\rho_1 = 0.1, \rho_2 = 0.05, \rho_3 = -0.05$), the correlation between the local statistics are almost negligible for geneSetTest procedure.

3.1.2 Power simulation

WHAT TO PRESENT

Table 1: Type I error rate of gene set tests for correlated expression values

Method	Norminal p-values				Norminal p-values			
	0.01	0.05	0.1	0.2	0.01	0.05	0.1	0.2
	(a0)				(a)			
OurTest	0.012	0.066	0.124	0.241	0.011	0.049	0.097	0.198
geneSetTest-modt	0.012	0.058	0.109	0.215	0.006	0.052	0.099	0.219
geneSetTest-rank	0.012	0.055	0.110	0.214	0.018	0.060	0.116	0.202
CAMERA	0.006	0.059	0.135	0.217	0.000	0.000	0.000	0.006
CAMERA-Rank	0.003	0.054	0.112	0.229	0.001	0.009	0.023	0.079
GSEA	0.989	0.995	0.997	0.997	0.228	0.608	0.794	0.927
	(c)				(e)			
OurTest	0.007	0.052	0.103	0.202	0.008	0.056	0.093	0.207
geneSetTest-modt	0.007	0.051	0.098	0.187	0.015	0.058	0.106	0.217
geneSetTest-rank	0.006	0.050	0.106	0.190	0.024	0.082	0.138	0.225
CAMERA	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.009
CAMERA-Rank	0.000	0.000	0.000	0.000	0.000	0.020	0.054	0.127
GSEA	0.942	0.984	0.992	0.995	0.108	0.469	0.731	0.899
	(f)				(g)			
OurTest	0.012	0.050	0.098	0.226	0.007	0.059	0.116	0.211
geneSetTest-modt	0.010	0.071	0.115	0.213	0.010	0.061	0.104	0.218
geneSetTest-rank	0.007	0.061	0.112	0.221	0.014	0.072	0.142	0.247
CAMERA	0.000	0.000	0.004	0.019	0.000	0.000	0.001	0.006
CAMERA-Rank	0.006	0.041	0.096	0.210	0.000	0.000	0.002	0.009
GSEA	0.015	0.188	0.434	0.770	0.943	0.975	0.983	0.992

3.2 Maybe real data analysis???

4 Conclusion

5 AcknowledgeMents

6 Appendix

First $E(\Delta_i) = E(Z_i\delta_i) = E(Z_i)E(\delta_i) = p_i\mu_\delta$. Next note that

$$\mathrm{Var}(\Delta_i) = E[(Z_i\delta_i)^2] - [E(Z_i\delta_i)]^2 = \mathrm{Var}(Z_i)[E(\delta_i)]^2 + \left[(EZ_i)^2 + \mathrm{Var}(Z_i)\right]\mathrm{Var}(\delta_i) = p_i\sigma_\delta^2 + p_i(1-p_i)\mu_\delta^2$$

Let $T_i = \bar{Y}_{i,2} - \bar{Y}_{i,1}$ be the difference in mean expression levels between the treatment group and the control group. We have

$$E(T_i) = E(\bar{Y}_{i,2}) - E(\bar{Y}_{i,1}) = E(\Delta_i) = E(Z_i\delta_i) = p_i\mu_\delta$$

The covariance between two genes i_1 and i_2 is given by (I HAVE CONCERNS HERE, IS IT VALID TO ASSUME THAT DE EFFECTS ARE INDEPENDENT BETWEEN GENES? WE SEE CO-EXPRESSION!! OR WE'VE ALREADY TAKEN THAT INTO ACCOUNT BY "CORRELATION BETWEEN GENES"),

$$\begin{aligned}
\mathrm{Cov}(T_{i_1}, T_{i_2}) &= E\left[\mathrm{Cov}(T_{i_1}, T_{i_2}|\Delta_{i_1}, \Delta_{i_2})\right] + \mathrm{Cov}\left[E(T_{i_1}|\Delta_{i_1}), E(T_{i_2}|\Delta_{i_2})\right] \\
&= E\left(\frac{1}{n_1}\rho_{i_1,i_2} + \frac{1}{n_2}\rho_{i_1,i_2}\right) + \mathrm{Cov}(\Delta_{i_1}, \Delta_{i_2}) \\
&= \left(\frac{1}{n_1} + \frac{1}{n_2}\right)\rho_{i_1,i_2}
\end{aligned} \tag{13}$$

For gene i, the variance $\mathrm{Var}(T_i) = \mathrm{Var}(\bar{Y}_{i,1}) + \mathrm{Var}(\bar{Y}_{i,2})$, with

$$\mathrm{Var}(\bar{Y}_{i,1}) = \frac{1}{n_1}$$

$$\begin{aligned}
\mathrm{Var}(\bar{Y}_{i,2}) &= \frac{1}{n_2^2}\left[\sum_{j=1}^{n_2}\mathrm{Var}(Y_{ij2}) + 2\sum_{1\le j_1<j_2\le n_2}\mathrm{Cov}(Y_{ij_1 2}, Y_{ij_2 2})\right] \\
&= \frac{1}{n_2}\mathrm{Var}(Y_{ij2}) + \frac{n_2-1}{n_2}\mathrm{Cov}(Y_{ij_1 2}, Y_{ij_2 2}) \\
&= \frac{1}{n_2}\left[E\left(\mathrm{Var}(Y_{ij2}|\Delta_i)\right) + \mathrm{Var}\left(E(Y_{ij2}|\Delta_i)\right)\right] \\
&+ \frac{n_2-1}{n_2}\left[E\left(\mathrm{Cov}(Y_{ij_1 2}, Y_{ij_2 2}|\Delta_i)\right) + \mathrm{Cov}\left(E(Y_{ij_1 2}|\Delta_i), E(Y_{ij_2 2}|\Delta_i)\right)\right] \\
&= \frac{1}{n_2} + \mathrm{Var}(\Delta_i)
\end{aligned} \tag{14}$$

Therefore $\mathrm{Var}(T_i) = \frac{1}{n_1} + \frac{1}{n_2} + \mathrm{Var}(\Delta_i)$, and it follows

$$\mathrm{Cov}(\boldsymbol{T}) = \boldsymbol{D} + \sigma_2^2 \boldsymbol{C} \tag{15}$$

where $\boldsymbol{D}$ is a diagonal matrix with $\mathrm{Var}(\Delta_i) = p_i\sigma_\delta^2 + p_i(1-p_i)\mu_\delta^2$ as its ith diagonal element, and $\sigma_2^2 = \left(\frac{1}{n_1} + \frac{1}{n_2}\right)$.

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