Accounting for Nuisance Covariates when Using RNA-Seq Data to Identify Differentially Expressed Genes

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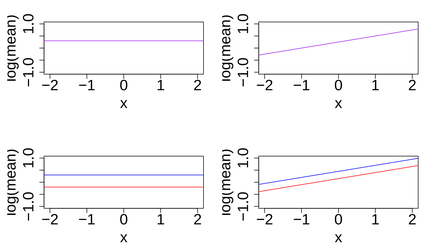
Prototypical Dataset

	Treatment 1				Treatment 2			
	u_{11}	u_{12}	$\cdots$	u_{1n}	u_{21}	u_{22}	$\cdots$	u_{2n}
x	0.5	0.95	...	-1.42	-.45	.89	...	1.2
gene 1	56	2014	$\cdots$	28	31	975	$\cdots$	3289
gene 2	0	2	$\cdots$	1	0	0	$\cdots$	1
gene 3	1	3	$\cdots$	0	0	0	$\cdots$	0
⋮	⋮	⋮		⋮	⋮	⋮		⋮
gene J	1701	264	$\cdots$	345	14	234	$\cdots$	34

Analysis Strategies

- Ignore the covariate.
- Include the covariate in a model for each gene.
- Assume the covariate is associated with transcript abundance of some subset of genes and consider model selection criteria or model averaging strategies when performing inference for treatment effects.

Scenarios Considered



Model for Simulated Data

$i = 1, 2;\ j = 1, \cdots, J;\ k = 1, \cdots, n$

$$y_{ijk} \sim \text{NegBin}(\mu_{ijk}, \omega_j), \quad \log(\mu_{ijk}) = \tau_{ij} + \beta_j x_k,$$

where

- $n = 5$ or $n = 20$
- $x_k \sim N(0, 1)$.

Parameters Used for Simulation

- $\tau_{1j} - \tau_{2j}$ represents the log fold change for gene j.
- ω_j is the negative binomial dispersion parameter.
- Values for $\tau_{1j} - \tau_{2j}$ and ω_j were simulated to match values estimated from real data.
- In all simulations, 80% of the $\tau_{1j} - \tau_{2j}$ values were set to zero among $J = 1000$ genes.

Simulated Slope Coefficients

- $\beta_j \sim \frac{\nu}{2} \times \text{Unif}(L, U) + \frac{\nu}{2} \times (-\text{Unif}(L, U)) + (1\text{-}\nu) \times \delta_{\{0\}}$
- $\nu \in \{0, 0.25, 0.50, 0.75\}$
- $(L, U) \in \{(0.1, 0.5), (1, 1.5)\}$

Testing for Trt and Cov Effects

- The `QuasiSeq` R package (Lund et al., 2012) was used to obtain a p-value for each test.
- $p_{j\tau}$ is the p-value for the test of $H_{0j} : \tau_{1j} = \tau_{2j}$ for the no-covariate model, $\log(\mu_{ijk}) = \tau_{ij}$.
- $p_{j\tau|\beta}$ is the p-value for the test of $H_{0j} : \tau_{1j} = \tau_{2j}$ for the covariate model, $\log(\mu_{ijk}) = \tau_{ij} + \beta_j x_k$.
- $p_{j\beta|\tau}$ is the p-value for the test of $H_{0j} : \beta_j = 0$ for the covariate model, $\log(\mu_{ijk}) = \tau_{ij} + \beta_j x_k$.

Methods for Identification of DEG

1. nocov: Convert $p_{j\tau}$ $(j = 1, \cdots, J)$ to q-values.
2. cov: Convert $p_{j\tau|\beta}$ $(j = 1, \cdots, J)$ to q-values.
3. ebp: Convert
   $p_{j\tau} I[\text{EBP}(p_{j\beta|\tau}) > 0.5] + p_{j\tau|\beta} I[\text{EBP}(p_{j\beta|\tau}) \leq 0.5]$
   $(j = 1, \ldots, J)$ to q-values.
4. aic: Convert
   $p_{j\tau} I[\text{AIC}_{j\tau} < \text{AIC}_{j\tau\beta}] + p_{j\tau|\beta} I[\text{AIC}_{j\tau} \geq \text{AIC}_{j\tau\beta}]$
   $(j = 1, \ldots, J)$ to q-values.
5. aaa: Compute
   $$\text{EBP}_j = \text{EBP}(p_{j\tau})\text{EBP}(p_{j\beta|\tau}) + \text{EBP}(p_{j\tau|\beta})[1 - \text{EBP}(p_{j\beta|\tau})]$$
   $(j = 1, \ldots, J)$.

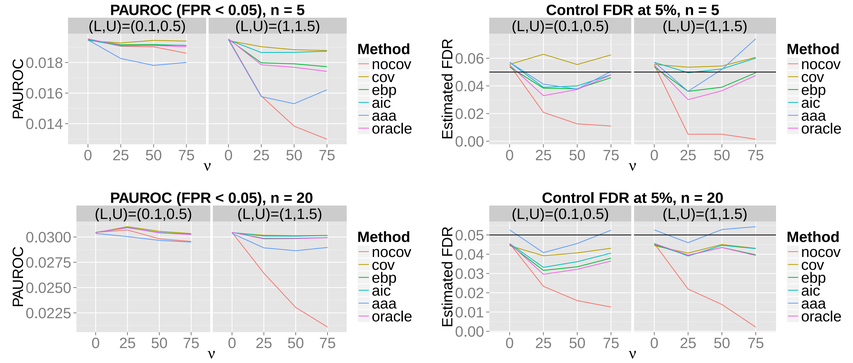
Computing EBP

- Suppose p_j is a p-value for testing a null hypothesis H_{0j} for $j = 1, \cdots, J$.
- $\text{EBP}(p_j) := \hat{\text{P}}(H_{0j}|p_j) = \frac{\hat{\pi}_0}{\hat{f}(p_j)}$, where $\hat{f}$ is the Grenander estimator of the pdf of p-values, which is the nonparametric MLE of decreasing pdf of p-values, and $\hat{\pi}_0 = \hat{f}(1)$.

Evaluation of Methods

- For 100 replications of each simulation setting, we compute
  - Average Area Under the Receiver Operating Characteristic Curve
  - Estimated FDR when FDR is nominally controlled at 0.05
- We use Storey's FDR control procedure based on q-values for Method 1, 2, 3, and 4, and average accummulative EBP for Method 5.

Simulation Results



Comments on the Results and Conclusions

- For the simulation settings we considered, the cov method based on $p_{j\tau|\beta}$ performed best overall.
- The price paid for including an irrelevant covariate in the model was far less than the cost of excluding an important covariate.
- All other methods relied on $p_{j\tau}$ to some extent and performed poorly whenever a substantial portion of genes were associated with the covariate.
- When $\beta_j \neq 0$, $p_{j\tau}$ is likely to be inflated because variation in the response unexplained by the model pushes the test of $H_{0j} : \tau_{1j} = \tau_{2j}$ toward nonsignificance.
- Even when $\beta_j = 0$, $p_{j\tau}$ is likely to be inflated if $\beta_{j^*} \neq 0$ for a substantial portion of genes $j^* \neq j$.
- Borrowing information across genes to estimate gene-specific dispersions can lead to overestimation of dispersions when $\beta_{j^*} \neq 0$ for many genes and the covariate is excluded from the model for each gene.
- When using existing software for RNA-seq analysis that requires the model for each gene to have the same design matrix, it may be best to favor flexibility over simplicity.
- More sophisticated empirical Bayes of fully Bayesian strategies for combining model selection and inference are needed.

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