Empirical Bayes Shrinkage and False Discovery Rate Estimation, Allowing For Unwanted Variation

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Abstract

We combine two important ideas in the analysis of large-scale genomics experiments (e.g. experiments that aim to identify genes that are differentially expressed between two conditions). The first is use of Empirical Bayes (EB) methods to handle the large number of potentially-sparse effects, and estimate false discovery rates and related quantities. The second is use of factor analysis methods to deal with sources of unwanted variation such as batch effects and unmeasured confounders. We describe a simple modular fitting procedure that combines key ideas from both these lines of research. This yields new, powerful EB methods for analyzing genomics experiments that account for both sparse effects and unwanted variation. In realistic simulations, these new methods provide significant gains in power and calibration over competing methods. In real data analysis we find that different methods, while often conceptually similar, can vary widely in their assessments of statistical significance. This highlights the need for care in both choice of methods and interpretation of results. All methods introduced in this paper are implemented in the R package vicar available at https://github.com/dcgerard/vicar.

1 Introduction

Many modern genomics experiments involve scanning the genome, or a list of genomic units (e.g. “genes”), to detect differences between groups of samples. For example, a simple “differential expression” experiment might measure the expression (activity level) of many genes in samples from two groups, and aim to identify at which genes these groups differ in their mean expression. The motivation is that identifying such genes may yield insights into the biological basis of differences between the groups.

Analyses of such experiments involve many issues, but two are particularly important and arise repeatedly. The first is that effects are often sparse — for example, in a differential expression experiment, many genes may show little difference in expression between two groups. The second is that genomic experiments are often plagued by “unwanted variation” such as batch effects and unmeasured confounders [Leek and Storey, 2007, 2008, Gagnon-Bartsch and Speed, 2012]. It is crucial to address both these issues during statistical analyses. The sparsity of effects requires careful handling of statistical significance thresholds to avoid large numbers of false discoveries. And unwanted variation, if unaccounted for, can obscure or confound signals of interest, and can create the appearance of signals where they do not exist.

Here we combine two ideas that have been used to address these issues. The first is the use of Empirical Bayes (EB) methods to assess the sparsity of effects, and estimate false discovery rates (FDRs) and related quantities [e.g Efron, 2004, 2008, Stephens, 2016]. The second is the use of factor analysis (FA) to deal with sources of unwanted variation such as batch effects and unmeasured confounders [e.g. Lucas et al., 2006, Leek and Storey, 2007, 2008, Gagnon-Bartsch and Speed, 2012, Sun et al., 2012, Gerard and Stephens, 2017].

Keywords and phrases: batch effects; empirical Bayes; RNA-seq; surrogate variable analysis; unobserved confounding; unwanted variation.
Wang et al., 2017]. By combining ideas from both these lines of research we provide powerful new analysis methods that simultaneously account for both sparse effects and unwanted variation.

Our work is not the first to combine sparsity of effects with FA for unwanted variation. Indeed “Fully Bayesian” approaches that do this were among the first work on dealing with unwanted variation [e.g. Lucas et al., 2006, Carvalho et al., 2008]. However, these methods are complex, computationally challenging, and have not been widely adopted, perhaps in part because of lack of easy-to-use software implementations. In comparison our EB methods are relatively simple, and we provide implementations in an R package. Also, our EB methods exploit recently-introduced semi-parametric prior distributions [Stephens, 2016] which assume that the distribution of effects is unimodal at zero. These priors are both computationally convenient, and more flexible than those used in previous Bayesian work.

More recently, Sun et al. [2012] and Wang et al. [2017] introduced (non-Bayesian) approaches that combine sparsity of effects and FA for unwanted variation. Indeed Wang et al. [2017] give theory that supports combining these two ideas: the estimation of the effects and the FA are intimately entwined, and assuming sparsity of effects helps identify the unwanted variation. To implement this idea Wang et al. [2017] — building directly on Sun et al. [2012] — jointly estimate the effects and the unwanted variation, using a penalty to induce sparsity on the effects. Our work here takes a similar approach, but replaces the penalty approach with EB methods to induce sparsity. The EB approach has several advantages over a penalized approach: it provides not only sparse point estimates, but also shrunken interval estimates, and estimates of FDRs and related quantities. And the semi-parametric prior distributions we use are considerably more flexible than the penalty approach (which often has only a single parameter to control sparsity and shrinkage).

Our methods based on assuming sparse (or, more precisely, unimodal) effects provide an attractive alternative to methods based on “control genes” [Gagnon-Bartsch and Speed, 2012], which are genes assumed a priori to have no effect. Like the sparsity assumption, the control gene assumption helps identify the unwanted variation [Gagnon-Bartsch and Speed, 2012, Wang et al., 2017]. However, while the sparsity assumption is almost universally adopted in genomic analyses (implicitly or explicitly), the control gene assumption brings a considerable additional burden: specifying a suitable set of controls is non-trivial and potentially error-prone. Furthermore, even when the controls are perfectly chosen, our methods can produce better results, particularly if the number of control genes is small (see Section 4). (It would be straightforward to incorporate control genes — as well as sparsity — into our method, but we do not pursue this here.)

One key feature of our method (also shared by several methods mentioned above) is its “modularity”. In particular we exploit a modular fitting procedure [e.g. Wang et al., 2017] that jointly estimates the effects and FA, while also separating out the FA from the rest of the method. Consequently, no particular approach to FA is “baked in” to our method; instead it can easily accommodate any approach to FA, including for example Bayesian approaches to FA [e.g. Hoff, 2007, Stegle et al., 2008, Engelhardt and Stephens, 2010, Stegle et al., 2010]. Similarly, the method can accommodate a range of possible pre-processing steps that are often necessary in genomic data analysis. The modular approach also simplifies computation, and eases both implementation and interpretation. Indeed our methods maintain much of the simple modular structure and logic of the simplest existing approaches to this problem. The benefits of modularity, while widely recognized in software design, are rarely explicitly acknowledged in statistical methods development, and we believe they merit greater recognition.

On notation: we generally denote matrices by uppercase boldface (A), vectors by lowercase boldface (a), scalars by lowercase non-boldface (a), and sets with calligraphic letters (A). There are exceptions when the context is clear. For example β is sometimes a matrix and sometimes a vector. Elements of a vector or matrix are denoted by their lowercase non-boldface versions. For example ai is the ith element of a and aij is the (i, j)th element of A. We let A_{n \times p} denote that the matrix A has dimension n \times p, i.e. A \in \mathbb{R}^{n \times p}. 

2
2 Background

2.1 Typical analysis pipeline

Genomics researchers often aim to identify which genomic features are associated with one or more biological factors of interest. For example, which genes have activity levels that differ, on average, between males and females? To assess this they would measure gene expression at many genes on samples of each sex, and then perform statistical analyses to identify which genes show significant differences in mean levels between the two groups.

There are many ways to perform such statistical analyses [e.g. Soneson and Delorenzi, 2013], but in outline a typical analysis might involve:

1. For each gene, \( j \), estimate an effect size \( \hat{\beta}_j \) and corresponding standard error \( \hat{s}_j \). (In our example \( \hat{\beta}_j \) would represent the estimated difference in mean gene expression between the two sexes.) For example, this might be achieved by applying a linear model to appropriately normalized and/or transformed expression data [e.g. Law et al., 2014], combined with methods to moderate (stabilize) variance estimates [e.g. Smyth, 2004].

2. For each gene, \( j \), use \( \hat{\beta}_j, \hat{s}_j \) to obtain a \( p \)-value, \( p_j \), testing the null hypothesis that gene \( j \) shows no effect. Then apply FDR methods [Benjamini and Hochberg, 1995, Storey, 2003] to the set of all \( p \) values to decide which genes are “significant”.

2.2 Adaptive shrinkage

Building on ideas of Efron [2004, 2008], Stephens [2016] suggests an alternative to Step 2 above, which he calls “adaptive shrinkage” or ASH. Specifically, Stephens [2016] suggests combining the “observations” \( (\hat{\beta}, \hat{s}) \) from Step 1 with a flexible but powerful assumption: that the true effects \( (\beta) \) come from a unimodal distribution with mode at 0. This assumption captures the expectation that many effects will be at or near 0, and is effectively an analogue of (or alternative to) the “sparsity assumption” often made in this context. Stephens [2016] provides methods to estimate this unimodal distribution, and to compute posterior distributions and measures of significance for each effect — the local FDR (lfdr; Efron [2008]), and local false sign rate (lfsr; Stephens [2016]) — analogous to the standard pipeline above. Stephens [2016] highlights several advantages of this approach: it better accounts for differences in measurement precision \( \hat{s}_j \) among genes; it can provide better (less conservative) estimates of the FDR, provided the unimodal assumption holds; and it provides calibrated interval estimates for each effect, which are otherwise difficult to obtain.

In more detail: ASH uses the normal means model [Stein, 1981] to relate the observations \( (\hat{\beta}, \hat{s}) \) to the effects \( \beta \):

\[
\hat{\beta} | \beta, \hat{s} \sim N_p(\beta, S),
\]

where \( N_p \) denotes the \( p \)-dimensional multivariate normal distribution and \( S := \text{diag}(\hat{s}_1^2, \ldots, \hat{s}_p^2) \). Thus the likelihood for \( \beta \) is

\[
L(\beta; \hat{\beta}, \hat{s}) = \prod_{j=1}^p N(\hat{\beta}_j | \beta_j, \hat{s}_j^2),
\]

where \( N(\cdot | a, b^2) \) denotes the normal density function with mean \( a \) and variance \( b^2 \). This likelihood is then combined with the unimodal assumption:

\[
\beta_1, \ldots, \beta_p \overset{iid}{\sim} g \in \mathcal{U},
\]

where \( \mathcal{U} \) denotes the space of unimodal distributions with mode at 0.

Stephens [2016] provides methods to fit the model (2)-(3) using a two-step EB approach:
1. Estimate $g$ by maximizing the marginal likelihood:

$$\hat{g} = \arg \max_{g \in U} p(\hat{\beta} | g, \hat{s}) = \arg \max_{g \in U} \prod_{j=1}^{p} \int_{\beta_j} N(\hat{\beta}_j | \hat{\beta}_j, \hat{s}_j^2) g(d\beta_j).$$

(4)

2. Compute posterior distributions $p(\beta_j | \hat{g}, \hat{\beta}, \hat{s})$, and return posterior summaries, including the lfdr and lfsr.

In practice, ASH approximates the optimization (4) by exploiting the fact that any unimodal distribution can be approximated arbitrarily well using a finite mixture of uniform distributions. Using this representation, (4) becomes a convex optimization problem over a finite (but large) set of mixture weights $\pi = (\pi_1, \ldots, \pi_M)$ (see equation (12) later). This can be solved efficiently using interior point methods [Boyd and Vandenberghe, 2004, Koenker and Mizera, 2014].

2.3 Removing Unwanted Variation

Unwanted variation can plague genomics experiments that aim to identify systematic differences in gene expression, or other genomics features, among groups of samples [Leek and Storey, 2008, 2007, Stegle et al., 2010, Leek et al., 2010, Gagnon-Bartsch and Speed, 2012, Sun et al., 2012, Appendix A.1 of the Supplementary Materials]. Unwanted variation may include measured variables such as batch, or sample covariates such as age or sex, but also — and most challengingly — unmeasured variables, such as aspects of sample preparation and handling that may be difficult to measure and control. Unwanted variation, if unaccounted for, can obscure or confound signals of interest, and can create the appearance of signals where they do not exist.

As the severity of the problems caused by unwanted variation has been increasingly recognized, many statistical methods have been developed to help ameliorate them [Lucas et al., 2006, Leek and Storey, 2007, Sun et al., 2012, Gagnon-Bartsch et al., 2013, Gerard and Stephens, 2017, Wang et al., 2017]. Most of these methods are based on a “factor-augmented regression model” [Leek and Storey, 2007, 2008]:

$$Y_{n \times p} = X_{n \times k} \beta_{k \times p} + Z_{n \times q} \alpha_{q \times p} + E_{n \times p},$$

(5)

where $y_{ij}$ is the normalized expression level of gene $j$ in sample $i$; $X$ is a matrix containing observed covariates, with $\beta$ a matrix of corresponding effects; $Z$ is a matrix of unobserved factors causing unwanted variation, with $\alpha$ a matrix of corresponding effects; and $E$ has independent (Gaussian) errors with means 0 and column-specific variances $\text{var}(e_{ij}) = \sigma_j^2$. In (5) only $Y$ and $X$ are known; other quantities are to be estimated.

Here we focus on the common setting where only one of the covariates in the columns of $X$ is of interest, and the other $k-1$ covariates are included to improve the model (e.g. to control for measured confounders, or as an intercept term). To further simplify notation we focus on the case $k = 1$, so $X$ is an $n$-vector, and $\beta$ is a $p$-vector of the effects of interest. However, our methods and software implementation allow $k > 1$. See Appendix A.2 of the Supplementary Materials for details. See also Appendix A.3 of the Supplementary Materials where we further discuss how to apply these methods when a single linear combination of the effects are of interest.

There are many approaches to fitting (5). Here we exploit a modular approach used by several previous methods, including RUV4 [Gagnon-Bartsch et al., 2013], LEAPP [Sun et al., 2012], and CATE [Wang et al., 2017]. In outline this involves:

1. For each gene $j$ ($j = 1, \ldots, p$) obtain an initial estimate $\hat{\beta}_j$ for $\beta_j$ ignoring unwanted variation by using ordinary least squares (OLS) regression of the $j$th column of $Y$ on $X$.

2. Form the matrix of residuals from these regressions, $\tilde{Y} := Y - X\hat{\beta}$, and perform a FA on these residuals. (Some methods, including CATE and the methods we present here, perform this step in practice by applying FA to a slightly different matrix. However, the end result is similar or identical, and we find it simpler and more intuitive to describe the methods in terms of the residual matrix. See
Appendix A.2 of the Supplementary Materials for details.) Performing an FA on $\tilde{Y}$ means fitting a model of the form:

$$\tilde{Y} = \tilde{Z}\tilde{\alpha} + \tilde{E}. \quad (6)$$

Most methods are flexible about exactly how FA is performed here, at least in principal if not in software. The resulting estimate $\hat{\alpha}$ of $\tilde{\alpha}$ in (6) can be viewed as an estimate of $\alpha$ in (5). This step also yields estimates $\hat{\sigma}_j^2$ of the residual variances $\sigma_j^2$ in (5).

3. Estimate $\beta$ by jointly estimating $(\beta, z)$ in the following “simplified model”:

$$\tilde{\beta} \sim N_p(\beta + \tilde{\alpha}^T z, S), \quad (7)$$

where $z \in \mathbb{R}^q$, $\tilde{\beta} \in \mathbb{R}^p$ are the OLS estimates from Step 1, and $S = \text{diag}(\hat{s}_1^2, \ldots, \hat{s}_p^2)$ where $\hat{s}_j$ is an estimated standard error of $\hat{\beta}_j$,

$$\hat{s}_j^2 = \hat{\sigma}_j^2 / (X^TX). \quad (8)$$

Model (7) has a simple interpretation: the OLS estimates $\hat{\beta}$ are equal to the true coefficients $(\beta)$ plus a bias term due to unwanted variation ($\tilde{\alpha}^T z$) plus some noise ($N_p(0, S)$). That is $z$ can be interpreted as capturing the effect of the unwanted variation on the OLS estimates.

This modular approach to fitting the model (5) is less ad hoc than it may first seem, and can be rigorously justified (Wang et al. [2017]; see Appendix A.2 of the Supplementary Materials for a detailed review).

A key way in which methods differ is the assumptions they make when fitting model (7). This model contains $p + q$ parameters but only $p$ observations, so additional assumptions are clearly necessary [Wang et al., 2017].

One type of method assumes that some genes are “control genes” [Gagnon-Bartsch et al., 2013]. That is, to assume that for some set $C \subseteq \{1, \ldots, p\}$, the effects $\beta_j = 0$ for all $j \in C$. For these control genes (7) becomes:

$$\hat{\beta}_C \sim N_p(\tilde{\alpha}_C^T z, S_C), \quad (9)$$

where $\hat{\beta}_C$ denotes the elements of $\tilde{\beta}$ that correspond to indices in $C$. Fitting this model yields an estimate for $z$, $\hat{z}$, say. Substituting this estimate into (7) then yields an estimate for $\beta$,

$$\hat{\beta}' = \hat{\beta} - \tilde{\alpha}^T \hat{z}. \quad (10)$$

This approach is used by both RUV4 and the negative controls version of CATE (CATEnc), with the difference being that RUV4 uses OLS when estimating $z$ whereas CATEnc uses generalized least squares (GLS).

An alternative approach, used by LEAPP [Sun et al., 2012] and the robust regression version of CATE (CATErr) [Wang et al., 2017], is to assume the effects $\beta$ are sparse. Both LEAPP and CATErr do this by introducing a penalty on $\beta$ when fitting (7). LEAPP returns the estimates of $\beta$ from this step (so these estimates are sparse and/or shrunk due to the sparsity-inducing penalty). CATErr, instead only keeps the estimates of $z$ and estimates $\beta$ by (10). Our methods here essentially involve replacing the sparsity-inducing penalty with the unimodal assumption from ASH.

3 MOUTHWASH

Here we combine the EB method from ASH with the modular fitting procedure for removing unwanted variation outlined above. This yields an analysis pipeline that combines the benefits of ASH (see above) while also removing unwanted variation. In brief, our new method involves replacing the likelihood (1) in ASH with the likelihood (7), which accounts for unwanted variation. We then modify the EB approach of ASH to optimize over both the unimodal prior distribution $g$ and the unwanted variation $z$. We call this
method MOUTHWASH (Maximizing Over Unobservables To Help With Adaptive SHrinkage).

In more detail, MOUTHWASH involves:

1. Estimate effects \( \hat{\beta}_j \) by OLS regression of the \( j \)th column of \( Y \) on \( X \).

2. Obtain \( \hat{\alpha} \) and \( \hat{\sigma}_j \) by applying a FA to the residual matrix \( \tilde{Y} \). (These first two steps are the same as RUV4, LEAPP and CATE, as outlined above.)

2b. Optionally, apply variance moderation [Smyth, 2004] to adjust the \( \hat{\sigma}_j \)'s [as in Gagnon-Bartsch et al., 2013]. We do this using \( n - k - q \) as the degrees of freedom.

3. Estimate the unimodal effects distribution \( g \) and the unwanted variation effects \( z \) by maximum (marginal) likelihood applied to (7):

\[
(\hat{g}, \hat{z}) := \arg \max_{(g, z) \in U \times \mathbb{R}^k} \prod_{j=1}^p \int_{\beta_j} N(\hat{\beta}_j | \beta_j + \hat{\alpha}_j z, \hat{s}^2_j) g(d\beta_j),
\]

where \( \hat{s}_j \) is defined in (8).

4. Compute posterior distributions \( p(\beta_j | \hat{g}, \hat{z}, \hat{\beta}, \hat{s}) \), and return posterior summaries.

The key new step is Step 3. As in Stephens [2016] we approximate this optimization by optimizing \( g \) over a set of finite mixture distributions indexed by mixing proportions \( \pi \):

\[
g(\beta_j | \pi) = \pi_0 \delta_0(\beta_j) + \sum_{m=1}^M \pi_m f_m(\beta_j),
\]

where the \( f_k \) are pre-specified component pdf's with one of the following forms:

i) \( f_m(\cdot) = N(0, \tau_m^2) \),

ii) \( f_m(\cdot) = U[-a_m, a_m] \),

iii) \( f_m(\cdot) = U[-a_m, 0] \) or \( U[0, a_m] \),

where \( U[a, b] \) denotes the uniform density with lower limit \( b \) and upper limit \( a \). These three different options correspond respectively to (approximately) optimizing \( g \) over i) all (zero-centered) scale mixtures of normals; ii) symmetric unimodal distributions with mode at 0; iii) all unimodal distributions with mode at 0.

With this mixture representation the integral in (11) can be computed analytically, and optimization can be performed using either an EM algorithm (Appendix A.4.1 of the Supplementary Materials) or a coordinate ascent algorithm (Appendix A.4.2 of the Supplementary Materials). Although this optimization problem is — in contrast to ASH — no longer convex, we have found that with appropriate initialization of \( \pi \) (initializing \( \pi_0 \) close to 1) these algorithms produce consistently reliable results (Supplementary Figure S2). Thus, for each simulated and real dataset we run MOUTHWASH once from this initialization.

Identifiability

In (5), as in any factor model, identifiability issues arise. Specifically, the following likelihoods are equivalent:

\[
p(Y | \beta, ZA, A^{-1} \alpha, \Sigma) = p(Y | \beta, Z, \alpha, \Sigma),
\]

for any non-singular \( A \in \mathbb{R}^{p \times q} \). The result of this non-identifiability is that (in the absence of prior information on \( \alpha \)) the estimate of \( \alpha \) from Step 2 above can be considered identified only up to its rowspace. It therefore seems desirable that the estimates obtained in Steps 3 and 4 of MOUTHWASH should depend on \( \hat{\alpha} \) only through its rowspace. Gagnon-Bartsch et al. [2013] proved that their estimator satisfied this property. We prove in Theorem 1 (Appendix A.5 of the Supplementary Materials) that our estimator also satisfies this property.
3.1 Errors in variance estimates

The performance of MOUTHWASH (and other related methods) depends on obtaining accurate variance estimates $\hat{\sigma}_j$ in Step 2. In practice this can be a major problem. See for example Section 3.9.4 of Gagnon-Bartsch et al. [2013], Section 6 of Gerard and Stephens [2017], and Perry and Pillai [2015] (who consider a similar model to (5) with the assumption that the unobserved factors are orthogonal to the observed covariates). Intuitively, the difficulty may arise either from mis-specifying the number of latent factors and thus attributing either too much or too little variation to the noise [Gagnon-Bartsch et al., 2013]; or it may arise because $\hat{\alpha}$ is assumed known but is in fact estimated and so the variance in the assumed model (7) is too small.

Both Gagnon-Bartsch et al. [2013] and Perry and Pillai [2015] address this issue by applying a multiplicative factor to the variance estimates. (Gagnon-Bartsch et al. [2013] selects this factor using control genes, whereas Perry and Pillai [2015] selects this factor via asymptotic arguments.) Here we deal with this issue in a similar way by including a multiplicative parameter, $\xi > 0$ in (7).

Specifically, we modify (7) to:

$$\hat{\beta} \sim N_p(\beta + \hat{\alpha}^Tz, \xi S), \quad (14)$$

and estimate $\xi$ along with $g$ and $z$. Thus, Step 3 becomes:

$$(\hat{g}, \hat{z}, \hat{\xi}) = \arg \max_{(g,z,\xi) \in U \times \mathbb{R}^k \times \mathbb{R}^+} \prod_{j=1}^p \int \beta_j \mathcal{N}(\hat{\beta}_j | \beta_j + \hat{\alpha}_j^T z, \xi \hat{s}_j^2) g(\beta_j) d\beta_j, \quad (15)$$

and the posterior distributions in Step 4 are computed conditional on $\hat{\xi}$. We have found that this modification can be vital for good performance of MOUTHWASH in practice.

3.2 Other Bells and Whistles

We have implemented several extensions to this approach in our software. These include i) allowing effects to depend on their standard errors; ii) extending (7) to a $t$ likelihood; iii) introducing a small regularization on the mixing proportions in $g$ to promote conservative behavior; and iv) reducing computational burden when $p$ is large by subsampling of genes. These are described in Appendix A.6 of the Supplementary Materials. (In our practical illustrations here we use the regularization iii), but not the other features.)

Additionally, to better account for the uncertainty in estimating $z$, we implemented a related procedure called BACKWASH (Bayesian Ajustment for Confounding Knitted W ith A daptive SHrinkage) that places a prior over $z$. See Appendix A.7 of the Supplementary Materials for details.

4 Empirical Evaluations

4.1 Simulations

To compare methods we generated simulated datasets from experimental data that contain real unwanted variation. Specifically, following Gerard and Stephens [2017], we simulated data by first randomly partitioning real RNA-seq data into two groups to produce “null” data, and then modifying it to spike in known amounts of signal. In brief, we modify the RNA-seq counts at a randomly selected subset of genes by “thinning” the RNA-seq data, reducing the RNA-seq counts in one group or the other to make each gene systematically less expressed in that group. See Appendix A.9 of the Supplementary Materials for details.

Because these simulations start by randomly assigning group labels to samples, they mimic a randomized experiment where unwanted variation is independent of treatment. In this sense they represent a “best-case” scenario, but with realistic, challenging, levels of unwanted variation. Although any simulation is inevitably a simplification, we believe that these simulations provide a substantially better guide to method performance in practice than simulating under an assumed (and undoubtedly imperfect) model.
We used these simulations to compare MOUTHWASH and BACKWASH with nine other estimation procedures that we follow with either qvalue [Storey, 2003] or ASH to estimate FDRs. (Although, based on Stephens [2016], we would advocate using the lfsr rather than FDR or lfdr, here we use FDR to allow comparison with methods that do not compute the lfsr.) These nine estimation methods are:

1. OLS: Ordinary Least Squares. This represents a naive method that does not account for unwanted variation.
2. SVA: The iteratively re-weighted least-squares version of Surrogate Variable Analysis [Leek and Storey, 2008], followed by the widely-used “voom-limma” pipeline [Law et al., 2014] to obtain effect estimates and standard errors controlling for the estimated surrogate variables.
3. CATErr: The robust regression version of CATE [Wang et al., 2017] (a variation on LEAPP [Sun et al., 2012]).
4. CATErr+MAD: CATErr, followed by median centering and median absolute deviation (MAD) scaling of the t-statistics [Sun et al., 2012, Wang et al., 2017]. At time of writing this was the default option in the cate package. (When applying ASH, we used the MAD as a multiplicative factor to adjust the variances [Gerard and Stephens, 2017], rather than scaling the t statistics.)
5. RUV2 [Gagnon-Bartsch and Speed, 2012].
6. RUV3 [Gerard and Stephens, 2017], with EB variance moderation [Smyth, 2004].
7. CATEnc: the negative controls version of CATE [Wang et al., 2017] (a variant on RUV4 [Gagnon-Bartsch et al., 2013]), which uses control genes to help estimation of confounders.
8. CATEnc+MAD: CATEnc followed by the same standardization used in CATErr+MAD.
9. CATEnc+Cal: CATEnc where a multiplicative factor, calculated using control genes [Gagnon-Bartsch et al., 2013], was used to adjust the variances.

The last five of these methods (RUV2, RUV3, CATEnc, CATEnc+MAD, CATEnc+Cal) require control genes, and we provided them a random subset of the actual null genes as controls, again representing a “best case” scenario for these methods. We did not adjust for library size in any method as library size can be considered another source of unwanted variation [Gerard and Stephens, 2017], which these methods are designed to account for.

We performed simulations with $p = 1000$ genes, varying the following parameters:
- The proportion of genes that are null $\pi_0 \in \{0.5, 0.9, 1\}$,
- The number of samples $n \in \{6, 10, 20, 40\}$,
- The number of control genes provided to methods that use control genes $m \in \{10, 100\}$.

We simulated 500 datasets for each combination of $\pi_0$, $n$, and $m$, and ran all methods on each dataset. We evaluated performances based on two criteria: first, the area under their receiver operating characteristic curve (AUC), a measure of their ability to distinguish null versus non-null genes; and second, accuracy of estimated proportion of null genes ($\hat{\pi}_0$), which is an important step in providing calibrated FDR estimates.

Figure 1 compares the AUCs of each method. MOUTHWASH and BACKWASH have almost identical performance, and the best AUCs in almost every scenario (SVA methods have better AUC in small sample sizes with $\pi_0 = 0.5$). This dominance is particularly pronounced when the number of control genes is small ($m = 10$), where methods that use control genes falter. With $m = 100$ high-quality control genes, methods that use control genes become competitive with MOUTHWASH and BACKWASH.

Figure 2 compares the estimates of $\hat{\pi}_0$ for each method when the true $\pi_0 = 0.9$ (results for $\pi_0 = 0.5$ and 1 are in Supplementary Figures S2 and S3). Many methods have median estimates of $\hat{\pi}_0$ very close to the true value of 0.9. However, the variances of these estimates are often high. In comparison, the estimates of $\pi_0$ from MOUTHWASH and BACKWASH are much less variable, and hence more accurate on average (particularly at higher sample sizes). CATErr+MAD+ASH and CATEnc+MAD+ASH work very well for larger sample sizes when $\pi_0$ is close to 1, but are anti-conservative for small sample sizes and highly conservative when $\pi_0 = 0.5$ (Supplementary Figure S2). Results from MOUTHWASH and BACKWASH are almost identical, suggesting that the additional complexity of BACKWASH is unnecessary in practice.
Figure 1: Comparison of mean AUCs among methods. Column facets vary \( m \), the numbers of control genes made available to methods that use control genes. Row facets vary \( \pi_0 \), the proportions of null genes. Different symbols represent different sample sizes \( n \). Horizontal lines indicate the highest mean AUC achieved by any method at a given combination of sample size, number of control genes, and proportion of null genes. The methods are ordered by their performance in the simulations with \( n = 40, m = 10, \pi_0 = 0.9 \).
Figure 2: Boxplots of estimates of \( \pi_0 \) for each method (true \( \pi_0 = 0.9 \)). Column facets vary \( m \), the numbers of control genes made available to methods that use control genes. Row facets vary \( n \), the sample size. The methods are ordered by the mean squared error of their estimates in the simulations with \( n = 40, m = 10, \pi_0 = 0.9 \). The dashed horizontal line shows \( y = 0.9 \).
4.2 Computation Time

Although our MOUTHWASH method is significantly slower than other existing methods (see Table S1 in the Supplementary Materials), it is nonetheless practical for realistic-sized data. For example, in tests with \( n = 100 \) and \( p = 10,000 \) MOUTHWASH had a median runtime of 140 seconds (on a 4.0 GHz quad-core PC running Linux with 32 GB of memory), and runtime is similar for other values of \( n \). Further speedups could be achieved if needed; see Appendix A.6 of the Supplementary Materials for discussion. BACKWASH requires a significantly longer runtime than MOUTHWASH, and given their similar performance we prefer MOUTHWASH in practice.

4.3 GTEx Data

To evaluate methods on real data, Gagnon-Bartsch and Speed [2012] used the idea of positive controls. A positive control is a gene that is \textit{a priori} thought likely to be associated with the covariate of interest. Gagnon-Bartsch and Speed [2012] used the example of sex and sex chromosomes: when the covariate of interest is the sex of an individual, then the genes on sex chromosomes are positive controls. The best confounder adjustment methods, then, are those that tend to have more positive controls among their most significant genes. This idea is also used in Gagnon-Bartsch et al. [2013] and Wang et al. [2017].

We applied this positive control method using RNA-seq datasets from 23 non-sex-specific tissues collected by the GTEx project [GTEx Consortium, 2015]. In each dataset we filtered out low-expressed genes (mean expression level < 10 reads), applied a log\(_2\) transformation to the gene expression count matrix (after adding a pseudo-count), and averaged results over technical replicates. We used a design matrix \( X \in \mathbb{R}^{n \times 2} \) with two columns: a column of 1’s (intercept), and a column of indicators for sex. We applied the same methods as in Section 4.1 to all 23 datasets. For methods that require negative controls we followed Gagnon-Bartsch and Speed [2012] in using housekeeping genes as negative controls (although opinions seem divided on the general appropriateness of this strategy; see Zhang et al. [2015] for a detailed discussion). Specifically, we used the list of housekeeping genes from Eisenberg and Levanon [2013], but excluding sex-chromosome genes. (A newer list of housekeeping genes was released by Lin et al. [2017] based on single cell sequencing results. We repeat our following analyses in Appendix A.10 of the Supplementary Materials using this newer list. The results of Appendix A.10 are similar to those obtained here.)

To compare methods we took the most significant 100 genes for each method on each tissue and counted how many of these genes are on a sex chromosome (\( s \)). We divided \( s \) for each method by the maximum \( s \) among all methods within a tissue. Figure 3 shows the results, with white indicating better performance (larger \( s \)). Methods are ordered from left to right by their median performance. Overall most methods performed comparably, CATEnc variants and SVA the notable exceptions, with SVA performing particularly poorly on a subset of the tissues. MOUTHWASH was among the best-performing methods of the ASH-variants, along with CATErr+ASH and CATErr+MAD+ASH.

Though many methods performed similarly in ranking the most significant genes, it would be wrong to think that they all produced the same results. In particular, the methods differ considerably in their assessments of significance and estimates of the proportion of null genes (\( \pi_0 \)). For example Table 1 shows median estimates of \( \pi_0 \) for each method across tissues. The estimates range from 0.28 to almost 1. Generally ASH-based methods produce smaller estimates of \( \pi_0 \) than qvalue-based methods, with the exceptions of MOUTHWASH, BACKWASH, and those methods whose variances were calibrated either using MAD or control genes. Though we do not know the true value of \( \pi_0 \) here, and it is possible that there are many non-sex chromosome genes with expression differences between the sexes, it is interesting that MOUTHWASH and BACKWASH, the best-performing methods in the simulations, estimate that most genes are null.

Another, perhaps still more striking, feature of the MOUTHWASH and BACKWASH results is shown in Figure 4 which shows the median \( \text{lfdr} \) for each ASH-based method as one moves down their list of the top 500 most significant genes. For both MOUTHWASH and BACKWASH the estimated \( \text{lfdr} \) sharply increase from 0 at around 50-100 genes. Furthermore, this sharp increase occurs just where the ranking starts to move away from genes on sex chromosomes (the shade moving from black, red in the online version, to light grey). Again, we do not know the truth here, but the behavior of MOUTHWASH/BACKWASH is consistent
Figure 3: Comparison of methods based on positive controls. For each method we computed the proportion $s$ of their most significant genes (testing for association with sex) that were on a sex chromosome. We then divided the $s$ for each method by the maximum $s$ among all methods. The tissues are ordered by sample size, and the methods are ordered by maximum median proportion. White indicates better performance than dark.
Table 1: Median estimate of $\pi_0$ for each method across tissues when testing for differences between sexes.

| Method                | $\pi_0$ |
|-----------------------|---------|
| SVA+ASH               | 0.28    |
| CATErr+ASH            | 0.33    |
| RUV3+ASH              | 0.38    |
| OLS+ASH               | 0.40    |
| RUV2+ASH              | 0.43    |
| CATEnc+ASH            | 0.55    |
| SVA+qvalue            | 0.70    |
| RUV3+qvalue           | 0.75    |
| CATErr+qvalue         | 0.76    |
| CATEnc+qvalue         | 0.78    |
| RUV2+qvalue           | 0.79    |
| OLS+qvalue            | 0.80    |
| CATEnc+Cal+ASH        | 0.89    |
| CATEnc+Cal+qvalue     | 0.90    |
| CATErr+MAD+ASH        | 0.91    |
| MOUTHWASH             | 0.99    |
| CATEnc+MAD+ASH        | 0.99    |
| BACKWASH              | 0.99    |
| CATEnc+MAD+qvalue     | 1.00    |
| CATErr+MAD+qvalue     | 1.00    |

with most of the true differences being at genes on sex chromosomes, and is strikingly different from most other methods. The MAD-calibrated methods also exhibit this behavior. However, in simulations with large sample sizes the MAD methods always estimated few genes to be significant, even when half of the genes were differentially expressed (Supplementary Figure S2), making it difficult to rely on their results. The increase in lfdr of CATEnc+Cal+ASH is not nearly as fast as that of MOUTHWASH and BACKWASH and much less consistent across tissues (Supplementary Figure S4).

5 Discussion

We have presented a simple modular approach to combining two key ideas for the analysis of genomic experiments: EB shrinkage to induce sparsity on effects, and FA to capture unwanted variation. Our results demonstrate that these new methods have competitive performance compared with a range of existing methods. They also highlight that even when methods agree closely in their rankings of genes (by strength of evidence against the null), they can vary widely in their assessments of significance (e.g. estimated FDRs). Indeed, even within a single “method”, significance assessments can be sensitive to details of how it is applied. For example, in our experience the way that variance estimates are dealt with can have a very dramatic effect on estimated FDRs and related quantities. In MOUTHWASH, the introduction of the variance inflation parameter $\xi$ has a substantial impact, and reduces the potential for anti-conservative (under-)estimates of FDR.

Although we have used the term “genomic experiments”, our methods are really aimed at a particular type of genomic experiment: where there is a single covariate which may be associated with many measured variables (e.g. a differential expression experiment, where treatment may affect the expression of many genes). One different type of genomic experiment that we do not address here is experiments to identify “expression Quantitative Trait Loci” (eQTLs), which are genetic variants associated with gene expression. The issues of sparse effects, and unwanted variation, certainly arise when attempting to identify eQTLs. And some methods to deal with these issues have been developed with a particular focus on eQTL studies.
Figure 4: Figure showing how median Ifdr changes through the list of 500 most significant genes. For each method we sorted the Ifdr’s across genes in each tissue, and took the median Ifdr across tissues at each rank. (Results for each tissue are in Supplementary Figure S4). The color of each point indicates the proportion of tissues that have a sex chromosome gene at that rank (red indicating higher proportion).
[e.g. Stegle et al., 2008, 2010, 2012, Fusi et al., 2012]. However, eQTL studies also differ in a crucial way from the studies considered here. Specifically, typical (population-based) eQTL studies involve many covariates (different genetic variants), each of which is typically associated with just one or a few genes (the strongest eQTLs are locally acting), rather than a single covariate associated with many genes. This difference is fundamental: when dealing with a single covariate that may affect many genes, it is both particularly important and particularly delicate to remove unwanted variation without also removing the effect of interest, whereas this issue is less pressing in eQTL studies. (Population substructure in the genotype data is a separate issue, which we do not discuss here.) Indeed, in eQTL studies, i) unwanted variation in expression data is rarely associated with the covariates of interest, and so usually decreases power rather than creating false positives; ii) when removing unwanted variation one need not be too concerned about accidentally removing signal of interest, and even very simple approaches such as using PCA on the expression matrix typically improve power [Pickrell et al., 2010]. Neither of these hold in the settings we focused on here.

One key feature of our approach is that, like many of the most popular current approaches, it is designed to be modular. In particular, although our results here are all based on using a simple FA (truncated PCA), our methods could easily accommodate other approaches to FA. For example, it could accommodate Bayesian methods such as SFA [Engelhardt and Stephens, 2010], or the FA implemented in the software PEER, which use a normal prior distribution on both the factors and loadings [Stegle et al., 2010]. In principle there could be objections to simply plugging these FAs into our approach: for example, the argument that the factor estimates are only identified up to their row-space does not always hold for Bayesian FA, so the property of MOUTHWASH that it depends on factor estimates only through their row-space might be considered suspect. Put another way, one could argue that when using prior distributions on the factors the modular approach to fitting (5) is suboptimal, and could be improved by a purpose-built joint fitting routine. However, the benefits of modular approaches are so great that it nonetheless seems worthwhile to explore these ideas.

Software

All methods introduced in this paper are implemented in the R package vicar available at https://github.com/dcgerard/vicar. Code to reproduce all results in this paper is available at https://github.com/dcgerard/mouthwash_sims (DOI: 10.5281/zenodo.1248856).

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A Supplementary Materials

A.1 Simple illustration

We present a simple example that illustrates the need to address unwanted variation. We took the top 10,000 expressed genes of an RNA-seq data on human muscle samples [GTEx Consortium, 2015] and randomly sampled six individuals, which we randomly split into two groups. All genes are thus theoretically “null” (unassociated with group assignment). However, when we apply the ASH method from Stephens [2016] to the OLS estimates of $\hat{\beta}$, $\hat{s}$ from these null data, it infers that almost all genes are non-null (estimated proportion of null genes, $\pi_0$, 0.0077), and indicates almost every gene is significant with lfdr close to 0 (Supplementary Figure S1, left panel). This behavior is not atypical (Supplementary Figure S3, top panels). Applying ASH with effects estimated using a more sophisticated RNA-seq data analysis pipeline instead of OLS [Law et al., 2014] slightly improved matters (Supplementary Figure S1, second panel from the left). In contrast applying MOUTHWASH and BACKWASH produced essentially no significant genes, with lfdrs clustering closer to 1 (Supplementary Figure S1, right panels).

A.2 Details of modular approach to fitting Factor-augmented Regression Model

Many methods (e.g. RUV4, LEAPP, and CATE) use a two-step approach to fitting the factor-augmented regression model (5). Wang et al. [2017] provide an elegant framing of this two-step approach as a rotation followed by estimation in two independent models. Since this plays a key role in our methods we review it here.

For convenience we repeat the factor-augmented regression model here:

$$Y_{n \times p} = X_{n \times k} \beta_{k \times p} + Z_{n \times q} \alpha_{q \times p} + E_{n \times p},$$

(16)

where we assume the number of samples $n$ is larger than the number of covariates $k$. As mentioned in the main text, we assume that only one covariate is of interest. Without loss of generality, we will assume that the “uninteresting” covariates are located in the first $k-1$ columns of $X$ and the “interesting” covariate is in the last column of $X$. Thus we can partition $\beta = (\beta_1 \beta_2^\top)$ so that $\beta_1 \in \mathbb{R}^{(k-1) \times p}$ contains the coefficients for the first $k-1$ covariates and $\beta_2 \in \mathbb{R}^p$ contains the coefficients for the covariate of interest.

Let $X = QR$ be the QR decomposition of $X$, where $Q \in \mathbb{R}^{n \times n}$ is an orthogonal matrix ($Q^\top Q = QQ^\top = I_n$) and $R_{n \times k} = (R_1 \ 0)$, where $R_1 \in \mathbb{R}^{k \times k}$ is an upper-triangular matrix. Pre-multiplying (16) by $Q^\top$ on both sides yields:

$$Q^\top Y = R \beta + Q^\top Z \alpha + Q^\top E,$$

(17)

which we write

$$\hat{Y} = R \beta + \hat{Z} \alpha + \hat{E}$$

(18)

where $\hat{Y} := Q^\top Y$, $\hat{Z} := Q^\top Z \hat{E} := Q^\top E$.

By exploiting the fact that $R_1$ is upper triangular, (18) can be rewritten as:

$$\hat{Y}_1 = R_{11} \beta_1 + r_{12} \beta_2^\top + \hat{Z}_1 \alpha + \hat{E}_1$$

(19)

$$\hat{y}_2^\top = r_{22} \beta_2 + \hat{z}_2 \alpha + \hat{e}_2^\top$$

(20)

$$\hat{Y}_3 = \hat{Z}_3 \alpha + \hat{E}_3.$$  

(21)

Here

$R_1 = \begin{pmatrix} R_{11} & r_{12} \\ 0 & r_{22} \end{pmatrix}$

(22)

and we have conformably partitioned each of $\hat{Y}$, $\hat{Z}$, $\hat{E}$ into i) their first $k-1$ rows; ii) their $k$th row; iii) the
remaining \( n - k \) rows, with for example
\[
\hat{Y} = \begin{pmatrix}
\hat{Y}_1 \\
\hat{Y}_2 \\
\hat{Y}_3
\end{pmatrix}.
\] (23)

We have used lower-case \( \tilde{y}_2, \tilde{z}_2, \tilde{e}_2 \) to indicate that these quantities are vectors.

The error terms in (19), (20), and (21) are independent, because \( \tilde{E} \) is equal in distribution to \( E \), which is matrix normal [Srivastava and Khatri, 1979, Dawid, 1981] with independent rows.

This rewriting suggests the following two-step estimation procedure, which in essence is the approach used by RUV4, LEAPP, and CATE:

1. Estimate \( \alpha \) and the \( \sigma_j \)'s using (21), specifically by applying some kind of FA to \( \tilde{Y}_3 \). Call these estimates \( \hat{\alpha} \) and \( \hat{\sigma}_j \).

2. Estimate \( \beta_2 \) and \( \tilde{z}_2 \) given \( \alpha \) and the \( \sigma_j \)'s using (20), which can be written:
\[
\tilde{y}_2 \sim N_p(r_{22}\beta_2 + \hat{\alpha}^T \tilde{z}_2, \hat{\Sigma}).
\] (24)

As equation (19) contains the nuisance parameters \( \beta_1 \), it is ignored.

In the main text we simplified the description by describing Step 1 as applying FA to the matrix of residuals obtained from regressing the columns of \( Y \) on \( X \) (6). As noted by Wang et al. [2017], for many choices of FA, applying FA to \( \tilde{Y}_3 \) is equivalent to applying FA to the residuals because \( \tilde{Y}_3 \) and the matrix of residuals have the same sample covariance matrix. (However, the mathematical derivation is clearer using \( \tilde{Y}_3 \), and our software implementation actually uses \( \tilde{Y}_3 \).)

Both MOUTHWASH and BACKWASH use this approach. Indeed, model (7) is the same as (24) with a simple change of notation:
\[
\hat{\beta} := y_2/r_{22}, \ \hat{\alpha} := \hat{\alpha}/r_{22}, \ S := \hat{\Sigma}/r_{22}^2, \ \text{and} \ z := \tilde{z}_2 \text{ and } \beta := \beta_2.
\] (25)

(26)

It is easy to show that \( \hat{\beta} = y_2/r_{22} \) are equal to the OLS estimates of \( \beta_2 \) obtained by regressing each column of \( Y \) on \( X \).

### A.3 Estimating linear combinations of the rows of \( \beta \)

Suppose a researcher is interested not in a single row of \( \beta \), but rather a single linear combination of \( \beta, c^T \beta \), for some \( c \in \mathbb{R}^k \). For example, if one were interested in a simple comparison of the effect of the first and second covariates, \( \beta_1 - \beta_2 \) (for all \( j = 1, \ldots, p \)), then \( c^T = (1, -1, 0, 0, \ldots, 0) \). As long as only one linear combination of the rows of \( \beta \) is of interest, MOUTHWASH and BACKWASH may be applied.

To do so, let the columns of \( L \in \mathbb{R}^{k \times (k - 1)} \) be any orthonormal basis of the orthogonal complement of the space spanned by \( c \) (e.g. take the columns of \( L \) to be the first \( k - 1 \) eigenvectors of \( \mathbf{I}_{k-1} - cc^T/\|c\|^2 \)). Then, assuming model (16), we have
\[
Y = X(c/\|c\|^2, L)(c_l^T \beta + Z\alpha + E),
\] (27)
since
\[
(c_l^T)^{-1} = (c/\|c\|^2, L).
\] (28)

Now let \( \bar{X} := (Xc/\|c\|^2, XL) \) and \( \bar{\beta} := (\beta^T c, \beta^T L)^T \). Then equation (27) is equal to
\[
Y = \bar{X}\bar{\beta} + Z\alpha + E,
\] (29)
where the first row of $\tilde{\beta}$ is equal to $c^T \beta$. We may now apply the modular approach used to fit MOUTHWASH and BACKWASH (as in Section A.2) using $\tilde{X}$ instead of $X$. Here, the first column of $\tilde{X}$ is the covariate of interest and its corresponding coefficients (the first row of $\tilde{\beta}$) represent the linear combination of the rows of $\beta$ that are of interest.

A.4 MOUTHWASH optimization details

A.4.1 EM algorithm for normal likelihood and normal mixtures

Here we describe the EM algorithm used for solving the optimization step (11) in MOUTHWASH when the mixture components in (12) are normal. (For the generalization to a $t_\nu$ likelihood and the case where the mixture components are uniform see the coordinate ascent updates in the next subsection).

The model is:

$$p(\hat{\beta}|z, \beta, \xi) = \prod_{j=1}^p N(\hat{\beta}_j|\beta_j + \hat{\alpha}_j^T z, \xi s_{jj}^2)$$

$$p(\beta) = \prod_{j=1}^p g(\beta_j|\pi)$$

$$g(\beta_j|\pi) = \pi_0 \delta_0(\beta_j) + \sum_{m=1}^M \pi_m N(\beta_j|0, \tau_m^2).$$

By integrating over $\beta$, we have

$$p(\hat{\beta}|z, \pi, \xi) = \prod_{j=1}^p p(\hat{\beta}_j|z, \pi, \xi)$$

$$p(\hat{\beta}_j|z, \pi, \xi) = \pi_0 N(\hat{\beta}_j|0, \xi s_{jj}^2) + \sum_{m=1}^M \pi_m N(\hat{\beta}_j|\hat{\alpha}_j^T z, \xi s_{jj}^2 + \tau_m^2).$$

Our goal is to maximize the likelihood (33) over $\pi, z,$ and $\xi$. In fact we consider the slightly more general problem of optimizing the penalized likelihood

$$p(\hat{\beta}|z, \pi, \xi) h(\pi|\lambda),$$

where $h(\pi|\lambda)$ is defined in (64).

To develop the EM algorithm, we use the usual approach for mixtures, introducing indicator variables that indicate which component of the mixture (32) gave rise to each $\beta_j$. Let $w_j = (w_{0j}, \ldots, w_{Mj})^T$ denote a one-of-$(M+1)$ indicator vector representing the mixture component that gave rise to $\beta_j$, so $\sum_{m=0}^M w_{mj} = 1$ and $p(w_{mj} = 1) = \pi_m$. Then the complete data likelihood is:

$$p(\hat{\beta}, W|z, \pi, \xi) h(\pi|\lambda)$$

$$= \left( \prod_{m=0}^M \pi_m^{\lambda_m - 1} \right) \prod_{j=1}^p \exp \left\{ \sum_{m=0}^M w_{mj} \log(\pi_m) - \left( \sum_{m=0}^M \frac{w_{mj}}{2(\xi s_{jj}^2 + \tau_m^2)} \right) (\hat{\beta}_j - \hat{\alpha}_j^T z)^2 \right\}$$

$$- \frac{1}{2} \sum_{m=0}^M w_{mj} \log(\xi s_{jj}^2 + \tau_m^2) - \frac{1}{2} \log(2\pi) \right\}.$$
And the complete data log-likelihood is:

\[
\ell_{\text{complete}}(z, \pi; \hat{\beta}, W) := \sum_{j=1}^{p} \left\{ \sum_{m=0}^{M} w_{mj} \log(\pi_m) - \left( \sum_{m=0}^{M} \frac{w_{mj}}{2(\xi^2_{s^2_{jj}} + \tau^2_m)} \right) (\hat{\beta}_j - \hat{\alpha}_j^T z)^2 - \frac{1}{2} M \sum_{m=0}^{M} \frac{w_{mj} \log(\xi^2_{s^2_{jj}} + \tau^2_m)}{2(\xi^2_{s^2_{jj}} + \tau^2_m)} + \sum_{m=0}^{M} (\lambda_m - 1) \log(\pi_m) \right\}
\]

(37)

Let \( \pi^{(\text{old})}, z^{(\text{old})} \), and \( \xi^{(\text{old})} \) be the current values of the parameters. Then

\[
p(w_{mj} = 1|\hat{\beta}_j, z^{(\text{old})}, \pi^{(\text{old})}, \xi^{(\text{old})}) = \frac{\pi_m^{(\text{old})} N(\hat{\beta}_j|\hat{\alpha}_j^T z^{(\text{old})}, \xi^{(\text{old})}/\xi_{s^2_{jj}}) + \tau^2_m)}{\sum_{i=0}^{M} \pi_i^{(\text{old})} N(\hat{\beta}_j|\hat{\alpha}_j^T z^{(\text{old})}, \xi^{(\text{old})}/\xi_{s^2_{jj}}) + \tau^2_m)} =: q_{mj}.
\]

(38)

The E-step of the EM algorithm involves forming the expected complete data log-likelihood, which simply involves replacing \( w_{kj} \) with \( q_{kj} \) in (37):

\[
\sum_{j=1}^{p} \left\{ \sum_{m=0}^{M} q_{mj} \log(\pi_m) - \left( \sum_{m=0}^{M} \frac{q_{mj}}{2(\xi^2_{s^2_{jj}} + \tau^2_m)} \right) (\hat{\beta}_j - \hat{\alpha}_j^T z)^2 - \frac{1}{2} M \sum_{m=0}^{M} \frac{q_{mj} \log(\xi^2_{s^2_{jj}} + \tau^2_m)}{2(\xi^2_{s^2_{jj}} + \tau^2_m)} + \sum_{m=0}^{M} (\lambda_m - 1) \log(\pi_m) \right\}
\]

(39)

The M-step then involves optimizing this over \( z, \pi, \) and \( \xi. \)

The update for \( \pi \) follows by recognizing the kernel of a multinomial likelihood

\[
\pi_m \leftarrow \frac{\sum_{j=1}^{p} q_{mj} + \lambda_m - 1}{\sum_{\ell=0}^{M} \left( \sum_{j=1}^{p} q_{\ell j} + \lambda_{\ell} - 1 \right)}
\]

(40)

\[
= \frac{\sum_{\ell=0}^{M} \sum_{j=1}^{p} q_{mj} + \lambda_m - 1}{\sum_{\ell=0}^{M} \sum_{j=1}^{p} q_{\ell j} + \sum_{\ell=0}^{M} \lambda_{\ell} - M}
\]

(41)

\[
= \frac{\sum_{j=1}^{p} q_{mj} + \lambda_m - 1}{p - M + \sum_{\ell=0}^{M} \lambda_{\ell}}.
\]

(42)

In the case when there is no penalty, \( \lambda_1 = \cdots = \lambda_M = 1, \) we have

\[
\pi_m \leftarrow \frac{1}{p} \sum_{j=1}^{p} q_{mj}.
\]

(43)

We then perform a few iterative updates on \( \xi \) and \( z. \) To update \( z \) given \( \xi, \) we note that optimizing (39) over \( z \) is the same as weighted linear regression with diagonal weight (precision) matrix \( \Theta_{\xi} \in \mathbb{R}^{p \times p} \) with diagonal elements \( \theta_{\xi,ij} = \sum_{m=0}^{M} \frac{q_{mj}}{\xi^2_{s^2_{ij}} + \tau^2_m}. \) We get

\[
z \leftarrow (\hat{\alpha} \Theta_{\xi} \hat{\alpha}^T)^{-1} \hat{\alpha} \Theta_{\xi} \hat{\beta}.
\]

(44)

To update \( \xi \) given \( z \) we can use some standard univariate optimizer, such as Brent’s method [Brent, 1971]. One step of this EM algorithm is presented in Algorithm 1. Iteratively performing the steps in Algorithm 1 is guaranteed to increase the likelihood toward a local maximum.
Algorithm 1 EM Algorithm for Normal Mixtures Prior and Normal Likelihood

1: Given the current values of the parameters in our model, \( \pi^{(\text{old})}, z^{(\text{old})} \), and \( \xi^{(\text{old})} \), let \( q_{mj} \) be defined as in (38).
2: Set \( \pi_m = \sum_{j=1}^{p} p_j q_{mj} + \lambda_m - 1 \).
3: repeat
   4: Let \( \Theta_\xi \) be a diagonal matrix with diagonal elements \( \theta_{\xi,jj} = \sum_{m=0}^{M} q_{mj} \xi_s^2_{jj} + \tau_m^2 \).
   5: Set \( z = (\hat{\alpha}_\Theta z^\top \hat{\alpha})^{-1} \hat{\alpha}_\Theta \hat{\beta} \).
6: Update \( \xi \) given \( z \) and \( \pi \) by maximizing (39) using Brent’s method.
until convergence

A.4.2 Coordinate Ascent for \( t_\nu \)-Uniform Problem

Here we describe the optimization steps used for the generalization of MOUTHWASH to a \( t_\nu \) likelihood (62) in the case where the mixture components are uniform. (This also applies to the normal likelihood with uniform components by setting \( \nu = \infty \)).

The model is:

\[
p(\hat{\beta}|z, \pi, \xi) = \prod_{j=1}^{p} t_\nu(\hat{\beta}_j|z, \pi, \xi_s^2_{jj})
\]

(45)

\[
p(\beta) \equiv \prod_{j=1}^{p} g(\beta_j|\pi)
\]

(46)

\[
g(\beta_j|\pi) = \pi_0 \delta_0(\beta_j) + \sum_{m=1}^{M} \pi_m U(\beta_j|a_m, b_m).
\]

(47)

By integrating over \( \beta \), we have

\[
p(\hat{\beta}|z, \pi, \xi) = \prod_{j=1}^{p} p(\hat{\beta}_j|z, \pi, \xi)
\]

(48)

\[
p(\hat{\beta}_j|z, \pi, \xi) = \pi_0 t_\nu(\hat{\beta}_j|\alpha^\top \hat{\alpha} z, \xi_s^2_{jj}) + \sum_{m=1}^{M} \pi_m \tilde{f}_m(\hat{\beta}_j|z, \xi),
\]

(49)

where

\[
\tilde{f}_m(\hat{\beta}_j|z, \xi) = T_\nu((\hat{\beta}_j - \alpha^\top \hat{\alpha} z - a_m)/(\xi^1/2 s_{jj})) - T_\nu((\hat{\beta}_j - \alpha^\top \hat{\alpha} z - b_m)/(\xi^1/2 s_{jj}))
\]

(50)

where \( T_\nu \) is the cdf of a standard \( t_\nu \) distribution. For ease of notation, we will also let \( \tilde{f}_0(\hat{\beta}_j|z, \xi) := t_\nu(\hat{\beta}_j|\alpha^\top \hat{\alpha} z, \xi s^2_{jj}) \).

To maximize the marginal likelihood (48), or rather the log-likelihood,

\[
\sum_{j=1}^{p} \log p(\hat{\beta}_j|z, \pi, \xi),
\]

(51)

we implemented a coordinate ascent algorithm to iteratively update \( z, \pi, \) and \( \xi \). To update \( \pi \) conditional on \( z \) and \( \xi \), we apply the same convex optimization procedure described in Stephens [2016] using the \texttt{ashr} package [Stephens et al., 2016]. To update \( \xi \) given \( \pi \) and \( z \), we use a standard univariate optimizer, Brent’s method [Brent, 1971].
To update $z$ given $\pi$ and $\xi$, we calculated the gradient of (51) with respect to $z$:

$$
\sum_{j=1}^{p} \hat{\alpha}_j \sum_{m=0}^{M} \frac{\pi_m \bar{f}_m(\hat{\beta}_j | z)}{p(\hat{\beta}_j | z, \pi, \xi)},
$$

where

$$
\bar{f}_0(\hat{\beta}_j | z) = \frac{(\nu + 1)(\hat{\beta}_j - \hat{\alpha}_j^T z)}{\nu \xi \sigma_{jj}^2} + (\hat{\beta}_j - \hat{\alpha}_j^T z)^2 t_\nu(\hat{\beta}_j|\hat{\alpha}_j^T z, \xi \sigma_{jj}^2), \quad \text{and}
$$

$$
\bar{f}_m(\hat{\beta}_j | z) = \left( \frac{1}{b_m - a_m} \right) \left( t_\nu(\hat{\beta}_j|\hat{\alpha}_j^T z - b_m, \xi \sigma_{jj}^2) - t_\nu(\hat{\beta}_j|\hat{\alpha}_j^T z - a_m, \xi \sigma_{jj}^2) \right).
$$

We then use a quasi-Newton approach to maximize (51) over $z$ using (52) (specifically we used the BFGS method).

## A.5 Identifiability

**Theorem 1.** For all non-singular $A \in \mathbb{R}^{q \times q}$, we have that

$$
\hat{g} = \arg \max_{g \in U} \max_{z \in \mathbb{R}^q} p(\hat{\beta}|g, z) = \arg \max_{g \in U} \max_{z \in \mathbb{R}^q} p(\hat{\beta}|g, z, \hat{\alpha}, S).
$$

**Proof.**

$$
\arg \max_{g \in U} \max_{z \in \mathbb{R}^q} p(\hat{\beta}|g, z, A\hat{\alpha}, S) = \arg \max_{g \in U} \max_{z \in \mathbb{R}^q} p(\hat{\beta}|g, A^T z, \hat{\alpha}, S)
$$

$$
= \arg \max_{g \in U} \max_{A^T z \in \mathbb{R}^q} p(\hat{\beta}|g, A^T z, \hat{\alpha}, S)
$$

$$
= \arg \max_{g \in U} \max_{z \in \mathbb{R}^q} p(\hat{\beta}|g, z, \hat{\alpha}, S),
$$

where (55) follows because $(A\hat{\alpha})^T z = \hat{\alpha}^T (A^T z)$, (56) follows because optimizing over $z$ is the same as optimizing over $A^T z$ for any non-singular $A$, and (57) follows from relabeling $A^T z$ to be $z$. 

## A.6 Mouthwash, additional Bells and Whistles

Here we describe additional features we have implemented in MOUTHWATCH (see also Section 3).

### A.6.1 Effects that depend on standard errors

Stephens [2016] modified (3) to allow the $\beta_j$’s to depend on the standard errors of the $\hat{\beta}_j$’s. This may make sense, for example, in gene expression studies if genes with higher variability tend to have larger effects. Specifically, Stephens [2016] set

$$
\frac{\hat{\beta}_j}{s_j} | s_j \sim g,
$$

where $\gamma \geq 0$ is specified. Estimating $g$ under (58) is straightforward except when both $\gamma = 1$ and we include the variance inflation parameter $\xi$ from (15). Under these conditions $g$ and $\xi$ become non-identifiable.

To see this, consider the simple case with no unwanted variation ($z = 0$), and write the normal term from (15) as

$$
\hat{\beta}_j / s_j \overset{d}{=} \frac{d}{s_j} + e_j, \quad \text{where } e_j \overset{iid}{\sim} N(0, \xi).
$$
So effectively $\beta_j/s_j + e_j$ are now \textit{iid} observations from a convolution of a distribution $g$ that is unimodal at 0 with a $N(0, \xi)$ distribution. This convolution is itself unimodal, and — whatever the true value of $\xi$ — could be fit perfectly using $\xi = 0$ and $g$ equal to the true $g$ convolved with the true $N(0, \xi)$. Thus it is impossible to guarantee accurate estimation of $\xi$ without making additional assumptions.

Although it is impossible to guarantee accurate estimation of $\xi$, it is possible to guarantee conservative (over-)estimates of $\xi$. This is formalized in the following lemma:

\textbf{Lemma 1.} For any distribution function, say $F$, unimodal about 0, there exists a maximal $\xi$ such that $F$ can be deconvolved into a $N(0, \xi)$ distribution function and another distribution function $G$ that is also unimodal about 0. That is, making $\xi$ any larger would result in a non-unimodal $G$.

See Appendix A.13 for proof.

Over-estimating $\xi$ is conservative in that it will over-shrink estimates of $\beta$ and over-estimate FDR. Motivated by Lemma 1 we can achieve this conservative behavior by introducing a small penalty term to encourage $\xi$ to be as large as possible. Specifically we maximize the penalized likelihood:

$$p(\hat{\beta}|\beta, z, S) f(\xi|\lambda\xi).$$

where

$$f(\xi|\lambda\xi) = \exp\{-\lambda\xi/\xi\},$$

and $\lambda\xi > 0$ is a penalty parameter that can be (in principle) arbitrarily small. Because $f(\xi|\lambda\xi)$ is increasing, the introduction of this term promotes $\xi$ to be as large as possible with $g$ unimodal.

\textbf{A.6.2 Generalizing normal likelihood to $t$ likelihood}

For small sample sizes the normality assumption in (7) might be better replaced with a $t$ distribution:

$$p(\hat{\beta}|\beta, z) = \prod_{j=1}^{p} t_{\nu}(\hat{\beta}_j|\beta_j + \alpha_j^T z, s_{jj}^2),$$

where $t_{\nu}(\cdot|a, b^2)$ denotes the density of a (generalized) $t$-distribution with degrees of freedom $\nu$, location parameter $a$, and scale parameter $b > 0$. That is,

$$t_{\nu}(\hat{\beta}|a, b^2) = \frac{\Gamma\left(\frac{\nu+1}{2}\right)}{\Gamma\left(\frac{\nu}{2}\right) \sqrt{\pi \nu b^2}} \left(1 + \frac{(\hat{\beta} - a)^2}{\nu b^2}\right)^{-\frac{\nu+1}{2}},$$

where $\Gamma(\cdot)$ is the gamma function. A similar generalization was implemented in [Stephens, 2016]. This replacement of a normal likelihood with a $t$ does not greatly complicate computations when the mixture components in (12) are uniform, and we have implemented this case (Appendix A.4.2). The normal case is more complex and not implemented.

\textbf{A.6.3 Penalty on $\pi_0$ to promote conservative behavior}

Stephens [2016] included the option of incorporating a penalty on the mixing proportions to promote conservative (over-) estimation of $\pi_0$. We also include this option here. Specifically we allow a penalty of the form

$$h(\pi|\lambda) = \prod_{m=0}^{M} \pi_m^{\lambda^{-1}}.$$  

\textbf{22}
and maximize the penalized likelihood

$$p(\hat{\beta}|\beta, z, S)h(\pi|\lambda),$$

(65)

where \(p(\hat{\beta}|\beta, z, S)\) is defined in either (7) or (62). We use the same default value for \(\lambda\) as Stephens [2016]: \(\lambda_0 = 10\) and \(\lambda_i = 1\) for \(i = 1, \ldots, m\). This encourages conservative (over-) estimation of \(\pi_0\), which is often considered desirable in FDR contexts.

### A.6.4 Reducing computation for large \(p\)

MOUTHWASH is computationally practical for typical gene-expression studies, where \(p \approx 20,000\) genes. However, in contexts where \(p\) exceeds 100,000 [e.g. ChIP-seq, Ward et al., 2018] the run time can become inconvenient. To reduce run-time in such cases we suggest estimating \(z\) from (7) using a random subset of variables. As \(z\) typically contains at most a few dozen parameters, a modest-sized subset should provide reasonable estimates.

Specifically, we implemented the following speed-up strategy for \(p\) very large. First estimate \(g, z\) using a random subset of variables. Second, fixing the estimate of \(z\) from the first step, re-estimate \(g\) by maximum likelihood over all \(p\) variables (which is a convex optimization problem that can be solved efficiently even for very large \(p\)).

### A.7 BACKWASH

MOUTHWASH maximizes over \(z\) in (11). We now describe an alternative that aims to better allow for uncertainty in \(z\) by placing a prior \(p(z)\) on \(z\) when optimizing over \(g\). Because of the introduction of a prior distribution on \(z\) we call this approach BACKWASH for Bayesian Adjustment for Confounding Knitted With Adaptive SHrinkage. Specifically, BACKWASH replaces Step 3 of MOUTHWASH with:

3. Estimate \(g\) by:

$$\hat{g} := \arg \max_{g \in \mathcal{U}} p(\hat{\beta}|g, \hat{\alpha}, S)$$

(66)

$$= \arg \max_{g \in \mathcal{U}} \prod_{j=1}^p \int_{\beta_j} \int_{z} N(\hat{\beta}_j|\beta_j + \hat{\alpha}^T z, s_{jj}^2)g(z) \, dz \, d\beta_j.$$  

(67)

To specify the prior \(p(z)\), we require that inference depends on \(\hat{\alpha}\) only through its rowspace (see Section 3). A prior that satisfies this requirement is the so-called “\(g\)-prior” [Zellner, 1986, Liang et al., 2008]:

$$z \mid \hat{\alpha} \sim N_q(0, \phi^2 (\hat{\alpha}\hat{\alpha}^T)^{-1}),$$

(68)

where \(\phi \in \mathbb{R}^+\) is a hyperparameter that we estimate by maximum marginal likelihood. With this prior the marginal likelihood is

$$\int_{\beta} N_p(\hat{\beta}|\beta, S + \phi^2 \hat{\alpha}^T (\hat{\alpha}\hat{\alpha}^T)^{-1}\hat{\alpha}) \prod_{j=1}^p g(\beta_j) \, d\beta_j,$$

(69)

which depends on \(\hat{\alpha}\) only through its rowspace.

When we include the estimation of the hyperparameter \(\phi\), and a variance scaling parameter \(\xi \in \mathbb{R}^+\) (Section 3.1) the full BACKWASH Step 3 becomes:

3. Let

$$(\hat{g}, \hat{\phi}, \hat{\xi}) := \arg \max_{(g, \phi, \xi) \in \mathcal{U} \times \mathbb{R}^+ \times \mathbb{R}^+} p(\hat{\beta}|g, \phi, \xi, \hat{\alpha}, S)$$

(70)
arg max_{(g,\phi,\xi) \in U \times \mathbb{R}^+ \times \mathbb{R}^+} \int_{\beta} N_p(\hat{\beta}|\beta S + \phi^2 \hat{\alpha}^T(\hat{\alpha}\hat{\alpha}^T)^{-1}\hat{\alpha}) \prod_{j=1}^p g(\beta_j) d\beta_j. \tag{71}

Maximizing (71) is difficult, and so we resort to a variational approximation [Blei et al., 2017] and instead maximize a lower bound for the marginal likelihood over \(g, \phi, \) and \(\xi\) (see Appendix A.8 for details).

### A.8 Variational EM Algorithm for BACKWASH

In this section, we present the Variational Expectation Maximization (VEM) algorithm that we developed for the BACKWASH procedure in Section A.7. For a good introduction to variational methods, see Bishop [2006]. The model in Section A.7 is

\[
[\hat{\beta}|\beta, \phi, \xi] \sim N_p(\beta|\xi S + \phi^2 \hat{\alpha}^T(\hat{\alpha}\hat{\alpha}^T)^{-1}\hat{\alpha}) \tag{72}
\]

\[
\beta_j \text{ i.i.d. s.t. } p(\beta_j) = \sum_{m=0}^M \pi_m N(\beta_j|0, \tau^2_m), \tag{73}
\]

where the \(\tau_m\)’s are known. Let

\[
A := \hat{\alpha}^T(\hat{\alpha}\hat{\alpha}^T)^{-1/2} \in \mathbb{R}^{p \times q}. \tag{74}
\]

We augment model (72)-(73) with a standard Gaussian vector \(v \in \mathbb{R}^q\) and 1-of-\(M\) binary vectors \(w_j \in \mathbb{R}^M, j = 1, \ldots, p\). Then (72)-(73) may be equivalently represented by

\[
\hat{\beta} \doteq \beta + \phi A v + e \tag{75}
\]

\[
v \sim N_q(0, I_q) \tag{76}
\]

\[
e \sim N_p(0, \xi S) \tag{77}
\]

\[
p(\beta_j, w_j) = \prod_{m=0}^M \left[ \pi_m N(\beta_j|0, \tau^2_m) \right]^{w_{jm}}. \tag{78}
\]

Our variational approach will be to maximize over \((f, \pi, \phi, \xi)\) the following lower-bound of the log-marginal likelihood

\[
\log p(\hat{\beta}|\pi, \phi, \xi) \geq \int f(\beta, W, v) \log \left( \frac{p(\hat{\beta}, \beta, W, v|\pi, \phi, \xi)}{f(\beta, W, v)} \right) d\beta dW dv, \tag{79}
\]

where \(f\) an element of some constrained class of densities and

\[
p(\hat{\beta}, \beta, W, v|\pi, \phi, \xi) = p(\hat{\beta}|\beta, v, \phi, \xi)p(\beta, W|\pi)p(v). \tag{80}
\]

We perform mean-field variational inference and constrain \(f\) to be factorized by

\[
f(\beta, W, v) = f(v) \prod_{j=1}^p f(\beta_j, w_j). \tag{81}
\]

This is the only assumption that we place on the form of the variational densities. Here, we are indexing the variational densities by their arguments. After maximizing (79) over \((f, \pi, \phi, \xi)\), we use the \(f(\beta_j, w_j)\)’s to provide posterior summaries for the \(\beta_j\)’s.

The variational updates for all parameters involved are presented in Algorithm 2. As the derivations are standard and tedious, we place the details in Appendix A.12, though we make a few comments here. First, the variational density of \(v\) is a multivariate normal which we parameterize with mean \(\mu_v\) and covariance
The variational densities of the $\beta_j$'s turn out to be mixtures of Gaussians which we parameterize with mixing means $\mu_{jm}$, mixing variances $\sigma_{jm}$, and mixing proportions $\gamma_{jm}$. Importantly, if the prior on the $\beta_j$'s contains a $\tau_m$ that is 0, representing a point mass at 0, then the variational densities of the $\beta_j$'s also must have a point mass at 0. This allows us to return local false discovery rates. The $\lambda_m$'s in Algorithm 2 are the same penalties as in Section 3.2.

Finally, we use the same initialization of the $\pi$'s as ASH. The choice of initialization of $\phi$ is not so clear, but we choose a default of 1. Finally, we use the same initialization of the $\tau_m$'s as ASH.

**Algorithm 2** Variational Expectation Maximization algorithm to fit BACKWASH.

1. Initialize parameters:
   - Initialize $\mu_\beta$ by the posterior means from fitting ASH on $(\hat{\beta}, S)$.
   - Initialize $\mu_v = (A^T S^{-1} A)^{-1} A^T S^{-1} (\beta - \mu_\beta)$.
   - Initialize $\xi = 1$.
   - Initialize $\phi = 1$.

2. repeat
   - Set $r = \hat{\beta} - \phi A \mu_v$.
   - for $j = 1, \ldots, p$ do
     - for $m = 0, \ldots, M$ do
       - Set $\sigma^2_{jm} = \left(\frac{1}{\xi_m^2} + \frac{1}{\xi_j^2}\right)^{-1}$.
       - Set $\mu_{jm} = r_j \sigma^2_{jm} / (\xi_s^2_{jj} + \xi_j^2 m)$.
       - Set $\gamma_{jm} = \frac{\pi_m N(r_j | 0, \xi_s^2_{jj} + \xi_j^2 m)}{\sum_{m=0}^M \pi_m N(r_j | 0, \xi_s^2_{jj} + \xi_j^2 m)}$.
     - end for
   - Set $\mu_{\beta j} = \sum_{m=0}^M \gamma_{jm} \mu_{jm}$.
   - end for
   - for $m = 0, \ldots, M$ do
     - Set $\pi_m = \frac{\sum_{j=1}^p \gamma_{jm} + \lambda_m - 1}{\sum_{j=1}^p \gamma_{jm} + \sum_{m=0}^M \lambda_m - (M+1)}$.
   - end for
   - Set $\Sigma_v = \left(\frac{s^2}{\xi} A^T S^{-1} A + I_q\right)^{-1}$.
   - Set $\mu_v = \frac{\phi}{\xi} \Sigma_v A^T S^{-1} (\hat{\beta} - \mu_\beta)$.
   - Set $\phi = \frac{\mu_v^T A^T S^{-1} A \mu_v + \text{tr}(A^T S^{-1} A \Sigma_v)}{\mu_v^T A^T S^{-1} A \mu_v}$.
   - Set
     \[
     \xi = \frac{1}{p} \left\{ \beta^T S^{-1} \beta + \sum_{j=1}^p \frac{1}{S_{jj}} \sum_{m=0}^M \gamma_{jm} (\mu_{jm}^2 + \sigma_{jm}^2) + \phi^2 \text{tr} \left(A^T S^{-1} A (\mu_v \mu_v^T + \Sigma_v) \right) \right\}
     \]
     \[ - 2\beta^T S^{-1} \mu_\beta - 2\phi \beta^T S^{-1} A \mu_v + 2\phi \mu_\beta^T S^{-1} A \mu_v \right\}.
   \]
   - Calculate the penalized ELBO (130).

until Convergence
A.9 Simulation details

We describe here how we simulated the data in Section 4.1. The procedure is the same as in Gerard and Stephens [2017].

First, we took the top $p$ expressed genes from the GTEx RNA-seq data [GTEx Consortium, 2015] and randomly sampled $n$ individuals, yielding an $n 	imes p$ count matrix $Z$. We then randomly assigned $n/2$ samples to one group and other $n/2$ samples to a second group. At this point, all gene expression levels are theoretically unassociated with the group label as group assignment was done independently of any gene expression. We used this as one scenario in our simulations (where $\pi_0 = 1$).

We then added signal to a proportion $(1 - \pi_0)$ of genes, randomly chosen from the set of genes represented in the null data, as follows. First, we sampled the effect sizes from a $N(0, 0.8^2)$, the variance being chosen as to make the AUC of all methods neither too close to 1 nor too close to 0.5. For $j \in \Omega$, the set of non-null genes, let

$$a_{j_1, \ldots, a_{j(1-\pi_0)p}} \sim N(0, 0.8^2),$$

be the effect sizes. For each $j \in \Omega$, we then drew new counts $w_{ij}$ from $z_{ij}$ by

$$w_{ij} | z_{ij} \sim \begin{cases} \text{Binomial}(z_{ij}, 2^{a_{ij} \cdot x_{ij}}) & \text{if } a_{ij} < 0 \text{ and } j \in \Omega, \\ \text{Binomial}(z_{ij}, 2^{-a_{ij} \cdot (1-x_{ij})}) & \text{if } a_{ij} > 0 \text{ and } j \in \Omega, \\ \delta(z_{ij}) & \text{if } j \notin \Omega, \end{cases}$$

Here, $\delta(a)$ is notation for a point-mass at $a$. We then used $W$ as our new response matrix of counts. To obtain the $Y$ in (5), we simply took a log$_2$ transformation of the elements of $W$.

The intuition behind this approach is that if the original counts $z_{ij}$ are Poisson distributed, then the new counts $w_{ij}$ are also Poisson distributed with $a_{ij}$ being the approximate log$_2$-effect between groups. That is, if $z_{ij} \sim \text{Poisson}(\lambda_j)$, then

$$[w_{ij}|a_j, a_j < 0, j \in \Omega] \sim \text{Poisson}(2^{a_{ij} \cdot x_{ij}} \lambda_j)$$

$$[w_{ij}|a_j, a_j > 0, j \in \Omega] \sim \text{Poisson}(2^{-a_{ij} \cdot (1-x_{ij})} \lambda_j).$$

Hence,

$$E[\log_2(w_{ij}) - \log_2(w_{kj})|a_j, a_j < 0, j \in \Omega] \approx a_j x_{ij} - a_j x_{kj} = a_j (x_{ij} - x_{kj}), \text{ and}$$

$$E[\log_2(w_{ij}) - \log_2(w_{kj})|a_j, a_j > 0, j \in \Omega] \approx -a_j (1-x_{ij}) + a_j (1-x_{kj}) = a_j (x_{ij} - x_{kj}).$$

See also Kvam et al. [2012], Reeb and Steibel [2013], Soneson and Delorenzi [2013], van de Wiel et al. [2014], Rocke et al. [2015] for similar simulation settings.

A.10 Analysis using the control genes of Lin et al. [2017]

We repeated the analysis of the GTEx data in Section 4.3 using the list of control genes collated by Lin et al. [2017]. This list was created using single cell sequencing data and contains only moderate overlap with the list developed by Eisenberg and Levanon [2013]. We observe:

1. The lfdr estimates for the control gene methods are mostly similar when using the two different lists. Compare Figures 4 and S6. Also compare Figures S4 and S7.

2. The estimates of the proportion of genes that are null are also mostly similar when using the two lists. Compare Tables 1 and S2.

3. RUV2 methods improved slightly in the positive control analysis when using the list from Lin et al. [2017]. Compare Figures 3 and S5. However, again, most of the methods performed similarly in ranking
the most significant genes.

The comparable performance of control gene methods when using the lists of Lin et al. [2017] and Eisenberg and Levanon [2013] does not indicate that these lists are of comparable quality. Recall that MOUTHWASH and BACKWASH both indicate that the vast majority of genes are null. Thus, it might be that many lists of “control genes” would give similar performance, because the vast majority of these “control genes” would indeed by null.

### A.11 t-likelihood Variance Inflated CATE

#### Algorithm 3

EM Algorithm for fitting a regression with $t$-errors

1: E-step: Set

$$w_j = \frac{\nu_j + 1}{(\hat{\beta}_C j - \hat{\alpha}_{C j}^\top z_{(old)})^2 / (\xi_{(old)} s_{C j}^2) + \nu_j} \quad (89)$$

2: M-step: Let $W := \text{diag}(w_1, \ldots, w_m)$. Set

$$z_{(new)} = (\hat{\alpha}_C W S_C^{-1} \hat{\alpha}_C) - 1 \hat{\alpha}_C W S_C^{-1} \hat{\beta}_C$$

$$\xi_{(new)} = \frac{1}{m} \sum_{j=1}^{m} \frac{w_j}{\sigma_j^2} (\hat{\beta}_j - \hat{\alpha}_{C j}^\top z_{(new)})^2 \quad (90)$$

To improve robustness to modeling assumptions, we explored modifying CATE to use a $t$-likelihood in its second step. This is akin to the ideas presented in Section 3.2. We replace (9) with

$$[\hat{\beta}_C j | \hat{\alpha}_C^\top z, \xi, s_{C j}^2] \sim t_{\nu_j} (\hat{\alpha}_C^\top z, \xi s_{C j}^2),$$

where $t_{\nu_j}(\cdot | a, b^2)$ is as defined in (63) and $\hat{\alpha}_{C j}$ is the $j$th column of $\hat{\alpha}_C$. The degrees of freedom ($\nu_j$’s) are assumed known. CATE uses (9) to estimate $z$ by maximum likelihood. Hence, we use (92) to estimate $z$ and $\xi$ by maximum likelihood. To do so, we apply an expectation-maximization (EM) algorithm that is similar to that discussed in Appendix A.2 of Lange et al. [1989]. The model (92) can be represented by including a latent variable $\tau_j$ for each observation

$$\hat{\beta}_C j | \tau_j \sim N(\hat{\alpha}_C^\top z, \tau_j \xi s_{C j}^2), \quad \tau_j \sim \text{Inverse-Gamma}(\nu_j/2, \nu_j/2), \quad (93)$$

Using (93), an EM algorithm to fit this model is easily obtained. One step of this algorithm is presented in Algorithm 3. Repeated applications of the step in Algorithm 3 is guaranteed to increase the likelihood at each iteration, converging to a local maximum.

### A.12 Derivation of VEM Algorithm

Here, we derive the updates for the variational EM algorithm presented in Section A.8. We begin by writing out all densities involved:

$$p(\hat{\beta}, \beta, W, v | \pi, \xi, \phi) = p(\hat{\beta}|\beta, v, \xi, \phi)p(\beta, W|\pi)p(v). \quad (94)$$

$$p(\hat{\beta}|\beta, v, \xi, \phi) = (2\pi)^{-p/2} \xi^{-p/2} \det(S)^{-1/2} \exp \left( -\frac{1}{2\xi} (\hat{\beta} - \beta - \phi Av)^\top S^{-1} (\hat{\beta} - \beta - \phi Av) \right), \quad (95)$$

$$p(\beta, W | \pi) = \prod_{j=1}^{p} \prod_{m=0}^{M} \left\{ \pi_m (2\pi \tau_m^2)^{-1/2} \exp \left( -\frac{1}{2\tau_m^2} \beta_j^2 \right) \right\}^{w_{jm}} \quad (96)$$
\[
p(v) = (2\pi)^{-q/2} \exp\left(-\frac{1}{2} v^T v\right), \tag{97}
\]
\[
h(\pi) = \prod_{m=0}^{M} \pi_m^{\lambda_m - 1}. \tag{98}
\]

**Update of \( f(\beta_j, w_j) \):** By a general result in mean-field variational inference [see Bishop, 2006, for example] we update \( f(\beta_j, w_j) \) by

\[
\log f(\beta_j, w_j) \propto E_{-(\beta_j, w_j)} \left[ \log p(\beta_j, v, \pi, \xi, \phi) \right], \tag{99}
\]

where “\( \propto \)” here denotes that the relationship holds up to an additive constant that does not depend on \( (\beta_j, w_j) \), and \( E_{-(\beta_j, w_j)}[\cdot] \) denotes that we take the expectation with respect to all variational densities except that of \( (\beta_j, w_j) \). Let \( r := \beta - \phi A E[v] \). Then we have

\[
(99) \propto E_{-(\beta_j, w_j)} \left[ \log p(\hat{\beta}|\beta, v, \pi, \xi, \phi) + \log p(\beta_j, w_j|\pi) \right] \tag{100}
\]
\[
\propto E_{-(\beta_j, w_j)} \left[ -\frac{1}{2\xi} (\hat{\beta} - \beta - \phi A v)^T S^{-1} (\hat{\beta} - \beta - \phi A v) \right] + \log p(\beta_j, w_j|\pi) \tag{101}
\]
\[
\propto -\frac{1}{2\xi s_{jj}} \left( \beta_j^2 - 2\beta_j r_j \right) + \log p(\beta_j, w_j|\pi) \tag{102}
\]
\[
\propto \log N(\beta_j|\beta_j, \xi s_{jj}^2) + \log p(\beta_j, w_j|\pi) \tag{103}
\]
\[
\propto \log N(r_j|\beta_j, \xi s_{jj}^2) + \sum_{m=0}^{M} w_{jm} \log \left( \pi_m N(\beta_j|0, \tau_m^2) \right) \tag{104}
\]
\[
\propto \sum_{m=0}^{M} w_{jm} \log \left( \pi_m N(r_j|0, \xi s_{jj}^2) N(\beta_j|0, \tau_m^2) \right) \tag{105}
\]
\[
\propto \sum_{m=0}^{M} w_{jm} \log \left( \pi_m N(r_j|0, \xi s_{jj}^2 + \tau_m^2) N(\beta_j|\mu_{jm}, \sigma_{jm}^2) \right), \tag{106}
\]

where

\[
\sigma_{jm}^2 := \left( \frac{1}{\tau_m^2} + \frac{1}{\xi s_{jj}^2} \right)^{-1}, \text{ and} \tag{107}
\]
\[
\mu_{jm} := r_j \sigma_{jm}^2 / (\xi s_{jj}^2). \tag{108}
\]

Equation (106) follows by standard Bayesian conjugacy arguments. Equation (106) is the log-kernel of a density of a mixture of normals with mixing means \( \mu_{jm} \) for \( m = 0, \ldots, M \) and mixing variances \( \sigma_{jm}^2 \) for \( m = 0, \ldots, M \). The mixing weights are proportional to \( \pi_m N(r_j|0, \xi s_{jj}^2 + \tau_m^2) \). Since the mixing weights must sum to unity we have that they are

\[
\gamma_{jm} := \frac{\pi_m N(r_j|0, \xi s_{jj}^2 + \tau_m^2)}{\sum_{m=0}^{M} \pi_m N(r_j|0, \xi s_{jj}^2 + \tau_m^2)}. \tag{109}
\]

**Update \( f(v) \):** Again, using a standard argument from mean-field variational inference, we update the variational density of \( v \) with

\[
\log f(v) \propto E_v \left[ \log p(\hat{\beta}, \beta, W, v|\pi, \xi, \phi) \right] \tag{110}
\]

28
\[ \alpha E_{\cdot v} \left[ \log p(\hat{\beta}|\beta, v, \xi, \phi) + \log p(v) \right] \]  
(111)

\[ \alpha E \left[ -\frac{1}{2\xi} (\hat{\beta} - \beta - \phi A v)^T S^{-1} (\hat{\beta} - \beta - \phi A v) \right] - \frac{1}{2} v^T v \]  
(112)

\[ \alpha - \frac{1}{2} \left( \phi^2 v^T A^T S^{-1} A v - 2 \phi \xi v^T A^T S (\hat{\beta} - E[\beta]) \right) - \frac{1}{2} v^T v \]  
(113)

\[ \alpha - \frac{1}{2} \left[ v^T \left( \phi^2 \xi A^T S^{-1} A + I_q \right) v - 2 \phi \xi v^T A^T S (\hat{\beta} - E[\beta]) \right] . \]  
(114)

Equation (114) is the log-kernel of a multivariate normal density with covariance matrix \( \Sigma_v \) and mean \( \mu_v \), where

\[ \Sigma_v := \left( \frac{\phi^2}{\xi} A^T S^{-1} A + I_q \right)^{-1}, \]  
(115)

\[ \mu_v := \frac{\phi}{\xi} \Sigma_v A^T S^{-1} (\hat{\beta} - E[\beta]). \]  
(116)

**Update \( \phi \):** We update \( \phi \) by finding

\[ \phi^{(\text{new})} = \arg \max_{\phi} E \left[ \log p(\hat{\beta}, \beta, W, v|\pi, \xi, \phi) \right] \]  
(117)

\[ = \arg \max_{\phi} E \left[ \log p(\beta|\beta, v, \xi, \phi) \right] \]  
(118)

\[ = \arg \max_{\phi} E \left[ -\frac{1}{2\xi} (\hat{\beta} - \beta - \phi A v)^T S^{-1} (\hat{\beta} - \beta - \phi A v) \right] \]  
(119)

\[ = \arg \min_{\phi} \left\{ \phi^2 \text{tr} \left( A^T S^{-1} A E[vv^T] \right) - 2\phi E[v^T A^T S^{-1} (\hat{\beta} - E[\beta])] \right\} \]  
(120)

\[ = \frac{E[v^T A^T S^{-1} (\hat{\beta} - E[\beta])]}{\text{tr} \left( A^T S^{-1} A E[vv^T] \right)} . \]  
(121)

**Update \( \xi \):** We update \( \xi \) by finding

\[ \xi^{(\text{new})} = \arg \max_{\xi} E \left[ \log p(\hat{\beta}, \beta, W, v|\pi, \xi, \phi) \right] \]  
(122)

\[ = \arg \max_{\xi} E \left[ \log p(\beta|\beta, v, \xi, \phi) \right] \]  
(123)

\[ = \arg \max_{\xi} \left\{ -\frac{p}{2} \log(\xi) - \frac{1}{2\xi} E \left[ (\hat{\beta} - \beta - \phi A v)^T S^{-1} (\hat{\beta} - \beta - \phi A v) \right] \right\} \]  
(124)

\[ = \frac{1}{p} E \left[ (\hat{\beta} - \beta - \phi A v)^T S^{-1} (\hat{\beta} - \beta - \phi A v) \right] . \]  
(125)

**Update \( \pi \):** Finally, we update \( \pi \) by

\[ \pi^{(\text{new})} = \arg \max_{\pi} E \left[ \log p(\hat{\beta}, \beta, W, v|\pi, \xi, \phi) \right] + \log(h(\pi)) \]  
(126)

\[ = \arg \max_{\pi} E \left[ \log p(W|\pi) \right] + \log(h(\pi)) \]  
(127)
\[
= \arg \max_{\pi} \sum_{m=0}^{M} \left( \sum_{j=1}^{p} E[w_{jm}] + \lambda_m - 1 \right) \log(\pi_m).
\] (128)

Hence, we have
\[
\pi_{m}^{(new)} = \frac{\sum_{j=1}^{p} E[w_{jm}] + \lambda_m - 1}{\sum_{m=0}^{M} \left( \sum_{j=1}^{p} E[w_{jm}] + \lambda_m - 1 \right)}.
\] (129)

All of the expectations in the above updates are tedious to compute but standard so we omit the details.

For our convergence criterion, we monitor the increase in the lower-bound of the log-marginal likelihood (79). It can be written in closed form as
\[
\int f(\beta, W, v) \log \left( \frac{p(\beta, W, v | \pi, \phi, \xi)}{f(\beta, W, v)} \right) d\beta dW dv
\]
\[
= -\frac{p}{2} \log(\xi) - \frac{1}{2\xi} \left\{ \beta^T S^{-1} \beta + \sum_{j=1}^{p} \sum_{m=0}^{M} \gamma_{jm}(\mu_{jm}^2 + \sigma_{jm}^2) + \phi^2 \text{tr} \left( A^T S^{-1} A \mu_v \mu_v^T + \Sigma_v \right) \right\}
\]
\[
- 2\beta^T S^{-1} \mu_\beta - 2\phi \beta^T S^{-1} A \mu_v + 2\phi \mu_\beta^T S^{-1} A \mu_v \right\}
\]
\[
+ \sum_{j=1}^{p} \left\{ \gamma_{j0} \log(\pi_0) + \sum_{m=1}^{M} \gamma_{jm} \left( \log(\pi_m) - \frac{1}{2} \log(2\pi) - \frac{1}{2} \log(\tau_m^2) - \frac{1}{2} \log(\tau_m^2 + \sigma_{jm}^2) \right) \right\}
\]
\[
- \frac{1}{2} \mu_v^T \mu_v - \frac{1}{2} \text{tr}(\Sigma_v) + \sum_{m=0}^{M} (\lambda_m - 1) \log(\pi_m)
\]
\[
+ \frac{1}{2} \log \det(\Sigma_v) - \sum_{j=1}^{p} \left\{ \gamma_{j0} \log(\gamma_{j0}) + \sum_{m=1}^{M} \gamma_{jm} \left( \log(\gamma_{jm}) - \frac{1}{2} \log(2\pi) - \frac{1}{2} \log(\sigma_{jm}^2) - \frac{1}{2} \right) \right\}
\]
\[
+ \text{constant},
\]
where “constant” indicates an additive constant that is independent of all parameters that we are optimizing over.

A.13 Proof of Lemma 1

We make use of the following results from Lukacs [1970].

**Theorem 2** (Theorem 2.1.1 from Lukacs [1970]). Let \( F(x) \) be a distribution function with characteristic function \( f(t) \). Then
1. \( f(0) = 1 \),
2. \( |f(t)| \leq 1 \),
where \(|\cdot|\) denotes the modulus.

**Theorem 3** (Theorem 4.5.1 from Lukacs [1970] due to Aleksandr Yakovlevich Khinchin). A distribution function is unimodal with vertex \( x = 0 \) if, and only if, its characteristic function \( f(t) \) can be represented as
\[
f(t) = \frac{1}{t} \int_{0}^{t} h(u) du \quad (-\infty \leq t \leq \infty),
\] (131)
where \( h(u) \) is a characteristic function.
Proof of Lemma 1. Let \( f(t) \) be the characteristic function of \( F \) and let \( g(t) \) be the characteristic function of \( G \). Recall that the characteristic function of a \( N(0, \xi) \) random variable is
\[
k(t) := e^{-\frac{1}{2} \xi t^2}.
\]
Since \( F \) is a convolution of \( G \) and a \( N(0, \xi) \) distribution function, we have
\[
f(t) = e^{-\frac{1}{2} \xi t^2} g(t) \Rightarrow g(t) = e^{\frac{1}{2} \xi t^2} f(t).
\]
Since \( f(t) \) is unimodal about 0, we use representation (131) and write
\[
g(t) = e^{\frac{1}{2} \xi t^2} \frac{1}{t} \int_0^t h(u) \, du,
\]
where \( h(t) \) is a characteristic function. Using integration by parts, we can write (134) as
\[
g(t) = \frac{1}{t} \int_0^t \left[ \xi u^2 e^{\frac{1}{2} \xi u^2} \frac{1}{u} \int_0^u h(v) \, dv + e^{\frac{1}{2} \xi u^2} h(u) \right] \, du.
\]
We now show that the integrand in (135) is not a characteristic function for sufficiently large \( \xi \). Using (131) and (132), we can write the integrand in (135) as
\[
\xi u^2 k(u) f(u) + k(u) h(u).
\]
Since
\[
|\xi u^2 k(u) f(u) + k(u) h(u)| = \xi |u^2 k(u) f(u) + k(u) h(u)/\xi|,
\]
it is now clear that for any fixed non-zero \( u \), the limit of (137) as \( \xi \to \infty \) is \( \infty \). Thus, for any fixed non-zero \( u \), we can make \( \xi \) large enough so that the modulus of (136) is larger than 1, violating property 2 of Theorem 2. Thus, for large enough \( \xi \), (136) is not a characteristic function.

It remains to note that the integrand in (135) is unique up to a set of Lebesgue measure 0. That is, if
\[
g(t) = \frac{1}{t} \int_0^t q(u) \, du \quad (-\infty \leq t \leq \infty),
\]
then
\[
q(u) = \xi u^2 e^{\frac{1}{2} \xi u^2} \frac{1}{u} \int_0^u h(v) \, dv + e^{\frac{1}{2} \xi u^2} h(u),
\]
except on a set of Lebesgue measure zero. Thus, \( q(u) \xrightarrow{\xi \to \infty} \infty \) almost everywhere, and so there is no choice of \( q(u) \) that is a characteristic function for all \( \xi \). Hence, for large enough \( \xi \), \( G \) is not unimodal. \( \square \)
A.14 Supplementary Figures

Figure S1: Histograms of lfdr for four methods applied to a single simulated null dataset. From left to right: OLS followed by ASH; a voom transformation followed by limma and hierarchical shrinkage of variances [Law et al., 2014] followed by ASH; MOUTHWASH; and BACKWASH.

Table S1: Computation time, in seconds, of the methods fit in Section 4.1 when \( n = 100 \) and \( p = 10,000 \). The “Time” column contains the 0.5, 0.025, and 0.975 quantiles of computation time over 100 replicates.

| Method       | Time (sec)         |
|--------------|--------------------|
| OLS          | 0.03 (0.03, 0.04)  |
| RUV2         | 0.08 (0.08, 0.11)  |
| CATErr       | 0.18 (0.17, 0.24)  |
| CATEnc       | 0.64 (0.62, 0.88)  |
| RUV3         | 1.13 (1.1, 1.35)   |
| SVA          | 2.09 (2.07, 2.76)  |
| MOUTHWASH    | 139.85 (134.57, 150.1) |
Figure S2: Boxplots of estimates of $\pi_0$ for all the methods when $\pi_0 = 0.5$. The rows are the sample sizes, the columns are the number of control genes used (for methods that use control genes). The methods are ordered by the their mean squared error in the case when there are 10 control genes and the sample size is 40. The dashed horizontal line has a y-intercept at 0.5.
Figure S3: Boxplots of estimates of $\pi_0$ for all the methods when $\pi_0 = 1$. The rows are the sample sizes, the columns are the number of control genes used (for methods that use control genes). The methods are ordered by the their mean squared error in the case when there are 10 control genes and the sample size is 40. The dashed horizontal line has a $y$-intercept at 1.
Figure S4: Smallest 500 lfdr’s versus rank for each method in each tissue from the GTEx data. Each facet is a different method and each line is a different tissue.
Figure S5: This is a repeat of Figure 3 except the control gene methods use the list from Lin et al. [2017]. See Figure 3 for a description.
Table S2: Median estimate of $\pi_0$ for each method across tissues when testing for differences between sexes. This is the same table as Table 1 except the control gene methods used the list from Lin et al. [2017].

| Method                  | $\pi_0$ |
|-------------------------|---------|
| SVA+ASH                 | 0.28    |
| CATErr+ASH              | 0.33    |
| RUV3+ASH                | 0.39    |
| OLS+ASH                 | 0.40    |
| RUV2+ASH                | 0.40    |
| CATEnc+ASH              | 0.49    |
| SVA+qvalue              | 0.70    |
| CATEnc+Cal+ASH          | 0.71    |
| CATErr+qvalue           | 0.76    |
| CATEnc+qvalue           | 0.77    |
| RUV2+qvalue             | 0.78    |
| RUV3+qvalue             | 0.78    |
| OLS+qvalue              | 0.80    |
| CATEnc+Cal+qvalue       | 0.87    |
| CATErr+MAD+ASH          | 0.91    |
| MOUTHWASH               | 0.99    |
| CATEnc+MAD+ASH          | 0.99    |
| BACKWASH                | 0.99    |
| CATEnc+MAD+qvalue       | 1.00    |
| CATErr+MAD+qvalue       | 1.00    |
Figure S6: This is a repeat of Figure 4 except the control gene methods use the list from Lin et al. [2017]. See Figure 4 for a description.
Figure S7: This is a repeat of Figure S4 except the control gene methods use the list from Lin et al. [2017]. See Figure S4 for a description.
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