BADER: Bayesian analysis of differential expression in RNA sequencing data

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Abstract

Identifying differentially expressed genes from RNA sequencing data remains a challenging task because of the considerable uncertainties in parameter estimation and the small sample sizes in typical applications. Here we introduce Bayesian Analysis of Differential Expression in RNA-sequencing data (BADER). Due to our choice of data and prior distributions, full posterior inference for BADER can be carried out efficiently. The method appropriately takes uncertainty in gene variance into account, leading to higher power than existing methods in detecting differentially expressed genes. Moreover, we show that the posterior samples can be naturally integrated into downstream gene set enrichment analyses, with excellent performance in detecting enriched sets. An open-source R package (BADER) that provides a user-friendly interface to a C++ back-end is available on Bioconductor.

1 Introduction

Transcription profiling by deep sequencing (RNA-seq) has become the technology of choice for quantifying gene expression, because of its high throughput, its large dynamic range, and the possibility to identify novel transcripts. However, noise in RNA-seq experiments is not yet well understood. Hence, appropriate statistical models to quantify gene expression and assess statistical significance of differential expression (DE) are needed and are the object of intense research (e.g., Garber et al. [2011]).

Due to biological variation, gene-level RNA-seq count data are overdispersed relative to the Poisson distribution. Most methods assume that the read counts come from negative binomial distributions and differ in their treatment of the (over-)dispersion parameters. The

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edgeR method (Robinson and Smyth, 2007; Robinson et al., 2010) estimates dispersion parameters of individual genes robustly by shrinking them toward a genome-wide parametric function relating dispersion to mean, whereas DEseq (Anders and Huber, 2010) models this relation nonparametrically. Lund et al. (2012) noticed the importance of taking uncertainty in the estimation of the dispersion parameter into account and were able to integrate it into their statistical test. More recently, Wu et al. (2013) observed systematic over- and under-shrinkage of the gene-specific dispersion parameters in edgeR and DEseq, respectively. To circumvent this issue, Wu et al. (2013) stayed within a frequentist paradigm but placed a prior on dispersion parameters and thus were able to obtain better DE detection in their analyses.

Moreover, calling DE genes is typically the starting point for further analyses, most prominently gene set enrichment analyses. However, the power for calling a gene DE typically increases with its average read counts. Frequentist approaches that do not take this variation in power into account are bound to mischaracterize uncertainty regarding whether sets are enriched. To cope with the difference in testing power among genes, dedicated gene set enrichment methods have been investigated (Young et al., 2010; Mi et al., 2012).

Altogether, the limitations met by frequentist approaches — for example, properly quantifying uncertainty for identifying DE genes and for downstream analyses — call for a fully Bayesian treatment. Hardcastle and Kelly (2010) followed an empirical Bayesian approach called baySeq. However, the prior parameters are estimated using quasi-likelihood methods, which ignores uncertainty in the parameter estimation. Two Bayesian approaches, MMseq (Turro et al., 2011), which focuses on Poisson noise, and the more recent Bitseq (Glaus et al., 2012), which accounts for overdispersed data, were developed to tackle the more complicated problem of estimating expression levels of transcript isoforms originating from the same locus, leading to highly coupled estimates. In order to address this difficult task, BitSeq required some shortcuts in the inference procedure. Moreover, the approach does not return explicit posterior probabilities for genes to be differentially expressed. Instead, the authors advise to use the ranking of the most probable up- and down-regulated genes.

Here, we propose a fully Bayesian analysis of differential expression in RNA sequencing data (BADER). Because we perform full posterior inference, our approach takes account of all uncertainties in a natural way and is theoretically justified.

BADER allows the dispersion parameters to be gene-specific, yet it is robust even for datasets with very small sample sizes due to borrowing of information across genes through a common hyperprior. We model the log fold change of a gene (i.e., the log ratio of the mean expression levels between two groups) by a continuous random variable with a point mass at zero. This provides a probabilistic indicator whether a gene exhibits true DE, or if the difference in expression is caused by random variability. It also yields a density estimate for the magnitude of the log fold change in the case of DE. Using simulated data, we show that BADER exhibits excellent performance in terms of DE detection.

Finally, we demonstrate how posterior samples of the DE indicators can be integrated into downstream analyses in the case of gene set enrichment. Our results show that this approach has more power to detect enriched gene sets than a frequentist counterpart.
2 Methods

2.1 Model Description

Assume that from an RNA-seq experiment we have a set of read counts of the form:

\[ \{ k_{ij}^T : i = 1, \ldots, n_T; j = 1, \ldots, m; T = A, B \} \]  

where \( i \) denotes the library or sample, \( j \) denotes the genomic region, and \( T \) denotes the experimental condition (treatment). “Genomic region” here could be a gene, an exon, or a set of exon, but henceforth we will refer to it as a gene for simplicity.

In many recent publications (e.g., Robinson and Smyth [2007], Anders and Huber [2010]), the negative-binomial distribution is the overdispersed-Poisson data model of choice for such data. This distribution can be written as the marginal distribution of a Poisson random variable whose rate parameter follows a gamma distribution. Here we assume that the Poisson rate parameter follows a lognormal distribution, leading to the following two-stage data model (see Appendix B.1 for more details):

\[ \begin{align*}
  k_{ij}^T & \sim \text{Pois}(s_i^T e^{\lambda_{ij}^T}); \quad \text{for all } i, j, T, \\
  \lambda_{ij}^T & \sim \text{N}(\mu_j^T, e^{\alpha_j^T}); \quad \text{for all } i, j, T,
\end{align*} \]

where \( s_i^T \) denotes the sampling depth of sample or library \( i \) in group \( T \), which we estimate using the median ratio estimator of Anders and Huber (2010). The data model (2) was chosen for computational reasons. While inference from the gamma and lognormal distribution typically leads to the same conclusions (Atkinson [1982], McCullagh and Nelder [1989, pp. 286/293]), the latter allows us to obtain many MCMC parameter updates in closed form (see Section 2.2 below), which results in more efficient MCMC sampling (see Appendix B.2).

The log mean rates for the two groups \( A \) and \( B \) are noted by \( \mu_j^A \) and \( \mu_j^B \), respectively. For \( \mu_j^A \), we assume a noninformative prior with density \( [ \mu_j^A ] \propto 1 \), independently for \( j = 1, \ldots, m \).

A crucial component of our model is the log-fold-change parameter, \( \gamma_j := \mu_j^B - \mu_j^A \), whose prior distribution is assumed to be a mixture distribution of a point mass at zero — indicating no differential expression (DE) for gene \( j \) — and a normal distribution:

\[ \gamma_j | I_j, \sigma_\gamma^2 \sim \begin{cases} 
  0, & I_j = 0 \\
  N(0, \sigma_\gamma^2), & I_j = 1; 
\end{cases} \quad j = 1, \ldots, m, \]

where \( I_j \) iid \( \sim B(\pi), j = 1, \ldots, m \), are Bernoulli DE indicators.

The parameters \( \alpha_j^T \) in (2) determine the degree of overdispersion (for \( \alpha_j^T \to -\infty \), the data model for \( k_{ij}^T \) in (2) is exactly Poisson). We assume

\[ \alpha_j^T \sim N(\psi, \tau^2); \quad j = 1, \ldots, m; T = A, B. \]

For all hyperparameters in (3) and (4), we assume independent noninformative priors: \( \pi \sim U(0, 1) \), \( [\psi] \propto 1 \), \( [\sigma_\gamma^2] \propto 1/\sigma_\gamma^2 \), and \( [\tau^2] \propto 1/\tau^2 \). The latter two priors are the Jeffreys prior for the variance parameter of a normal distribution.
2.2 Posterior Inference

Given a set of RNA-seq counts, \( \{k_{ij}^T\} \), as in [1], the primary interest is in the posterior distribution of the log-fold-change parameters, \( \{\gamma_j\} \). Once we have obtained this (joint) distribution, it is trivial to derive quantities of interest, like the individual posterior DE probabilities, \( p_j := P(I_j = 1|\{k_{ij}^T\}); \ j = 1, \ldots, m \). A great advantage of Bayesian inference is that we obtain \textit{joint} posterior distributions, and so we do not have to account for multiple testing explicitly (see, e.g., [Scott and Berger, 2006]). We can also use the results for further downstream analyses (e.g., for gene set enrichment; see Section 3.3 below).

Because there is no closed-form expression for the joint posterior of all unknown variables in the model, we resort to sampling-based inference via a Markov chain Monte Carlo (MCMC) algorithm. We use a Gibbs sampler ([Geman and Geman, 1984]), which cycles through all unknowns and updates each parameter based on its so-called full conditional distribution (FCD). For a generic parameter (or set of parameters) \( \theta \), the FCD is defined as the conditional distribution of \( \theta \) given the data and all other variables in the model, and we denote the FCD as \( [\theta] \).

The FCDs of the rate and dispersion parameters are not available in closed form,

\[
[\lambda_{ij}^T] \propto \text{Poi}(k_{ij}^T|s_i^T e^{\lambda_{ij}^T} \gamma_j I(T = B), \exp(\alpha_j^T)),
\]

\[
[\alpha_j^T] \propto (\prod_i N(\lambda_{ij}^T|\mu_j^A + \gamma_j I(T = B), \exp(\alpha_j^T))) N(\alpha_j^T|\psi_0, \tau^2),
\]

and so we use adaptive Metropolis-Hastings steps ([Haario et al. 2001]) to update these parameters for all \( i, j, T \).

The updates of the hyperparameters are given by:

\[
\pi| \cdot \sim \text{Beta}(1 + \sum_j I_j, 1 + \sum_j (1 - I_j)),
\]

\[
\sigma_j^2| \cdot \sim \text{InvGamma}(\sum_j I_j/2, \sum_j \gamma_j^2 I_j/2),
\]

\[
\psi_0| \cdot \sim N(\sum I_j \alpha_j^T/2m, \tau^2/2m),
\]

\[
\tau^2| \cdot \sim \text{InvGamma}(m, \sum I_j (\alpha_j^T - \psi_0)^2/2).
\]

Initial tests showed heavy posterior dependence between \( I_j, \mu_j^A \) and \( \gamma_j \). Fortunately, our choice of the lognormal distribution in [2] allows updating these parameters jointly from \( [\gamma_j, \mu_j^A, I_j] \) by integrating both \( \mu_j^A \) and \( \gamma_j \) out when updating \( I_j \) and by integrating \( \gamma_j \) out when updating \( \mu_j^A \). Let \( \Omega \) denote the set of all parameters, and define \( \overline{\lambda_j^T} := \sum_i \lambda_{ij}^T/n_T \), \( v_0 := \exp(\alpha_j^B) \) and \( v_1 := \exp(\alpha_j^B) + n_T \sigma_j^2 \). The updates are as follows:

\[
I_j|\Omega \setminus \{\mu_j^A, \gamma_j, I_j\} \overset{\text{ind.}}{\sim} B(\pi N_1/(\pi N_1 + (1 - \pi) N_0)), \text{ where } N_l := N(\overline{\lambda_j^T}, (\exp(\alpha_j^A) + v_l)/n_T);
\]

\[
\mu_j^A|\Omega \setminus \{\mu_j^A, \gamma_j\} \overset{\text{ind.}}{\sim} N\left(\overline{\lambda_j^T} v_j + \overline{\lambda_j^T} \exp(\alpha_j^A) v_j, \frac{\exp(\alpha_j^A) v_j}{n_T (\exp(\alpha_j^A) + v_j)}\right);
\]

\[
\gamma_j|\Omega \setminus \{\gamma_j\} \overset{\text{ind.}}{\sim} \begin{cases} 0, & I_j = 0, \\ N\left(\frac{\sigma_j^2 \sum (\lambda_{ij}^T - \mu_j^A)}{v_1}, \frac{\sigma_j^2 \exp(\alpha_j^B)}{v_1}\right), & I_j = 1, \end{cases}
\]

for \( j = 1, \ldots, m \). Further details and proofs can be found in Appendix A.
Typically, we run the MCMC algorithm for 20,000 iterations, discarding the first 10,000 as burn-in steps and saving every 10th remaining step. In a setup with 10,000 genes and 2 samples in each group, one iteration takes around 0.13 seconds on a 2 GHz 64-bit processor with the BADER software. Computation times should change approximately linearly for other numbers of genes or samples, as the required number of computations for posterior inference is $O(mn)$.

3 Results

3.1 Inference on the Dispersion Parameter

To assess inference on dispersion parameters in a realistic case, we used a large dataset published by Pickrell et al. (2010), available from http://bowtie-bio.sourceforge.net/recount/ [Frazee et al., 2011]. Of the 69 samples in the dataset, we took subsamples of $n \in \{2, 5, 10, 20\}$, corresponding to typical sample sizes in RNA-seq datasets. Genes where the sum of the remaining counts for all samples were less or equal to 5 were dropped from the analysis. We assumed that the posterior medians of $\mu_j$ and $\alpha_j$ from the full dataset were the “true” values, and calculated the empirical coverage of central 80% posterior credible intervals for these parameters derived from the subsamples. For both parameters and for all (sub-)sample sizes, the empirical coverage of the “true” values was between about 70% and 90%, indicating accurate calibrations of the posterior distributions of the gene-specific dispersion parameters.

3.2 Determining Differential Expression

We ran BADER on a dataset from Katz et al. (2010), where we dropped all genes for which the sum of all counts were less or equal to 5. Figure 1 shows a scatterplot of the posterior means of the log fold change parameters against the normalized mean counts, with red stars indicating the genes with a posterior probability of DE higher than 0.9. Interestingly, we find some genes with high absolute value of the log fold change parameter but smaller posterior probability of DE than 0.9, due to the heterogeneous gene-specific posterior uncertainty allowed by our flexible model.

Because we do not know the ground truth, these results do not tell us whether BADER improves upon existing methods in terms of DE detection. Hence, we then compared methods on simulated datasets where we do know the underlying truth. We generated 100 datasets, each consisting of $n_A = n_B = 2$ samples for $m = 5000$ “genes,” divided into 250 sets of 20 genes each. Of these groups, 90% were assumed to be not enriched and we chose a small probability of DE (0.1) for a gene in these groups. The remaining 10% of groups were assumed to be enriched and consequently, genes in these sets were DE with a high probability of 0.75. The individual gene expression counts were simulated according to model (2) with hyperparameters $\psi_0 = -3$, $\tau = 0.8$, and $\sigma_\gamma = \log_2(1.5)$, which were chosen to be as realistic as possible. The mean log expression level for gene set $l$ was chosen as $\mu_l^A \sim N(5, 1)$ for $l = 1, \ldots, 250$, with individual gene log expression levels in set $l$ simulated as $\mu_j^A | \mu_l^A \sim N(\mu_l^A, 0.5^2)$. In this subsection, we will focus on determining DE for individual
1.4.0), applying the methods from the packages’ vignettes. We compared results averaged

Figure 1: Scatterplot of the posterior mean of the log fold change parameter from BADER against
the normalized mean count for genes in the dataset from [Katz et al., 2010]. The red stars indicate
genes with a posterior probability of DE higher than 0.9. The plot inserts show the posterior
distribution of the log fold change parameter of a gene found to be DE with a low mean count, and
of a gene with a high absolute log fold change parameter but posterior probability of DE smaller
than 0.9.

genes, and we will consider inference on enriched gene sets in Section 3.3.

Using these 100 simulated datasets, we compared BADER (version 0.99.2) to edgeR
[Robinson et al., 2010] (version 2.6.10), DESeq [Anders and Huber, 2010] (version 1.12.1),
baySeq [Hardcastle and Kelly, 2010] (version 1.14.1), and DSS [Wu et al., 2013] (version
1.4.0), applying the methods from the packages’ vignettes. We compared results averaged
over all 100 datasets using a receiver operating characteristic (ROC) curve, which plots the
true positive rate versus the false positive rate when varying over a threshold parameter $q$
(Figure 2). In the case of edgeR, DESeq, and DSS, we take all genes to be DE for which the
p-value is lower than $q$. In the case of baySeq and BADER, we take genes to be DE if the
corresponding posterior probability is larger than $1 - q$. This analysis showed that BADER
outperformed all other approaches in calling genes differentially expressed.

It is important to note again that, while BADER performs well in terms of DE detection,
the output from our algorithm is much richer than just posterior DE probabilities for
individual genes. Among other things, we obtain the joint posterior distribution of the log-
fold-change parameters $\gamma_j$ (see Equation (3)), which, for example, also contains the posterior
distribution of the magnitude of the fold change for DE genes. In addition, this output allows
for further downstream analyses, an example of which is given in the next section.

### 3.3 Gene Set Enrichment

Once we have obtained samples from the posterior distribution as described in Section 2.2, inference on sets or groups of genes ("gene set enrichment") can proceed without much difficulty. For example, one could use the posterior distributions of DE indicators as an input to the algorithm of Bauer et al. (2010), for which groups of genes are defined to be enriched if all genes in that group are DE.

Here, for each set of genes $S \subset \{1, \ldots, m\}$, we test the "competitive null hypothesis" (Goeman and Bühlmann, 2007) that the genes in $S$ are at most as often differentially expressed as the remaining genes by considering the posterior probability of the event

$$\sum_{j \in S} I_j / |S| > \sum_{j \in S^c} I_j / |S^c|,$$

where the $I_j$ are the DE indicators introduced in (3).

A problem with gene set enrichment has been that for genes with low counts, the power for DE detection is low. Hence, in testing for category enrichment, categories or groups of genes with low counts are rarely determined to be enriched. Young et al. (2010) and Mi et al. (2012) attempt to remedy this problem by accounting for differences in gene length. However, we believe that different gene lengths are not the only cause of bias in enrichment analysis, and it is necessary to account for differences in gene-specific DE uncertainty directly.
We considered the same 100 simulated datasets as described in Section 3.2. For BADER, we obtained the posterior probability for a gene set to be enriched by estimating the posterior probability of $\hat{\pi}_j$. We compared our results to a frequentist approach (henceforth referred to as FF) that consisted of testing for DE for each gene using DESeq (Anders and Huber, 2010) with a significance level of 0.05, followed by calculating the p-value of Fisher’s exact test for over-representation of DE genes in the set under consideration. Figure 3 shows the ROC curves for the two methods. BADER considerably outperforms the FF approach. The difference in performance is especially large when focusing on gene sets with low mean expression level (e.g., $\bar{\mu}_A < 3$; figure not shown).

4 Discussion

We developed a fully Bayesian model for the analysis of RNA-seq count data (BADER). We described proper MCMC-based posterior inference, taking into account all uncertainty without shortcuts. BADER is distributed open-source on Bioconductor as an R package with an efficient C++ back-end.

We demonstrated the value of posterior samples of our log-fold-change parameters with a point mass at zero. Their use in DE detection is highly competitive and natural, avoiding explicit adjustments for multiple testing. Moreover, their integration with downstream analyses is not only conceptually easier, but also suffers less from biases than many frequentist approaches. We demonstrated this point by using BADER posterior samples for gene set
enrichment analysis, showing more power to detect enriched gene sets.

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A Some details on posterior inference

Posterior inference is summarized in Section 2.2 of the main article. Here we derive the updating steps that allow joint sampling from the distribution \([\gamma_j, \mu_j^A, I_j].\) This is achieved by integrating out both \(\mu_j^A\) and \(\gamma_j\) when updating \(I_j,\) and integrating out \(\gamma_j\) when updating \(\mu_j^A.\)

Again, let \(\Omega\) denote the set of all parameters, \(\lambda^*_j := \sum_i \lambda^*_i/n_T,\) and \(P_{\mu_j^A}\) and \(P_{\gamma_j}\) are the measures corresponding to the prior distributions of \(\mu_j^A\) and \(\gamma_j,\) respectively. We begin by proving the following proposition:

**Proposition 1.**

\[ N(x|\mu_1, \sigma_1^2)N(x|\mu_2, \sigma_2^2) = N\left(x \middle| \frac{\mu_1 \sigma_1^2 + \mu_2 \sigma_2^2}{\sigma_1^2 + \sigma_2^2}, \frac{\sigma_1^2 \sigma_2^2}{\sigma_1^2 + \sigma_2^2} \right) \]

**Proof.**

\[
\begin{align*}
N(x|\mu_1, \sigma_1^2)N(x|\mu_2, \sigma_2^2) & = \frac{1}{\sqrt{2\pi\sigma_1^2}} \exp\left(\frac{(x - \mu_1)^2}{2\sigma_1^2}\right) \cdot \frac{1}{\sqrt{2\pi\sigma_2^2}} \exp\left(\frac{(x - \mu_2)^2}{2\sigma_2^2}\right) \\
& = \frac{1}{2\pi\sigma_1\sigma_2} \exp\left(\frac{(x - \mu_1 \sigma_1^2 + \mu_2 \sigma_2^2)^2}{2\sigma_1^2 \sigma_2^2}ight) \times \exp\left(\frac{\sigma_1^2 + \sigma_2^2}{2\sigma_1^2 \sigma_2^2} \left(\frac{\mu_1 \sigma_1^2 + \mu_2 \sigma_2^2}{\sigma_1^2 + \sigma_2^2} - \left(\frac{\mu_1 \sigma_1^2 + \mu_2 \sigma_2^2}{\sigma_1^2 + \sigma_2^2}\right)^2\right)\right) \\
& = N\left(x \middle| \frac{\mu_1 \sigma_1^2 + \mu_2 \sigma_2^2}{\sigma_1^2 + \sigma_2^2}, \frac{\sigma_1^2 \sigma_2^2}{\sigma_1^2 + \sigma_2^2} \right) \times \frac{1}{\sqrt{2\pi\sqrt{\sigma_1^2 + \sigma_2^2}}} \exp\left(\frac{\mu_1 \sigma_1^2 \sigma_2^2 + 2\mu_1 \mu_2 \sigma_1^2 \sigma_2^2 + \mu_2 \sigma_2^4 \sigma_2^2}{2\sigma_1^2 \sigma_2^2 (\sigma_1^2 + \sigma_2^2)}\right) \\
& = N\left(x \middle| \frac{\mu_1 \sigma_1^2 + \mu_2 \sigma_2^2}{\sigma_1^2 + \sigma_2^2}, \frac{\sigma_1^2 \sigma_2^2}{\sigma_1^2 + \sigma_2^2} \right) N(\mu_1, \sigma_1^2 + \sigma_2^2).
\end{align*}
\]

Then we have

\[
[I_j|\Omega \setminus \{\mu_j^A, \gamma_j, I_j\}] = \int [I_j|\Omega \setminus I_j] dP_{\mu_j^A} dP_{\gamma_j}
\]

\[
\propto \int \pi^I_j (1 - \pi)^{1 - I_j} \prod_i N(\lambda_{ij}|\mu_j^A, e^{\alpha_j^A})N(\lambda_j^B|\mu_j^A + \gamma_j, e^{\alpha_j^B}) d\mu_j^A d\gamma_j
\]

\[
\propto \int \pi^I_j (1 - \pi)^{1 - I_j} N\left(\mu_j^A \middle| \lambda_j^X, \frac{\exp(\alpha_j^A)}{n_T}\right)N\left(\mu_j^A \middle| \lambda_j^B - \gamma_j, \frac{\exp(\alpha_j^B)}{n_T}\right) d\mu_j^A d\gamma_j
\]

\[
\approx \int \pi^I_j (1 - \pi)^{1 - I_j} N\left(\lambda_j^X \middle| \lambda_j^A, \frac{\exp(\alpha_j^A)}{n_T}\right)N\left(\lambda_j^B \middle| \lambda_j^B - \gamma_j, \frac{\exp(\alpha_j^B)}{n_T}\right) d\gamma_j.
\]
Applying Proposition [1] again yields
\[
P(I_j = 1|\Omega \setminus \{\mu_j^A, \gamma_j, I_j\}) \propto \pi N(\lambda_j^A|\lambda_j^B, (e^{\alpha_j^A} + e^{\alpha_j^B})/n_T + \sigma_j^2),
\]
\[
P(I_j = 0|\Omega \setminus \{\mu_j^A, \gamma_j, I_j\}) \propto (1 - \pi) N(\lambda_j^A|\lambda_j^B, (e^{\alpha_j^A} + e^{\alpha_j^B})/n_T),
\]
which is a Bernoulli distribution with parameter \( \frac{\pi N_1}{\pi N_1 + (1 - \pi) N_0} \), where
\[
N_0 := N(\lambda_j^A|\lambda_j^B, (e^{\alpha_j^A} + e^{\alpha_j^B})/n_T),
\]
\[
N_1 := N(\lambda_j^A|\lambda_j^B, (e^{\alpha_j^A} + e^{\alpha_j^B})/n_T + \sigma_j^2).
\]
To obtain \([\mu_j^A|\Omega \setminus \{\mu_j^A, \gamma_j\}])\), note that
\[
[\mu_j^A|\Omega \setminus \{\mu_j^A\}] \propto \prod_{i,T} N(\lambda_{ij}^T|\mu_j^A + \gamma_j I(T = B), e^{\alpha_j^T})
\]
and
\[
\gamma_j \sim \begin{cases} 
 0, & I_j = 0, \\
 N(0, \sigma_j^2), & I_j = 1,
\end{cases}
\]
and so, applying Proposition [1] again, we have
\[
[\mu_j^A|\Omega \setminus \{\mu_j^A, \gamma_j\}] \propto \begin{cases} 
 C_0 N \left( \mu_j^A \left| \frac{\lambda_j^A e^{\alpha_j^B} + \lambda_j^B e^{\alpha_j^A}}{e^{\alpha_j^j} + e^{\alpha_j^B}} \right., \frac{e^{\alpha_j^A} e^{\alpha_j^B}}{n_T(e^{\alpha_j^j} + e^{\alpha_j^B})} \right), & I_j = 0, \\
 C_1 N \left( \mu_j^A \left| \frac{\lambda_j^A e^{\alpha_j^B} + \lambda_j^B e^{\alpha_j^A}}{e^{\alpha_j^j} + e^{\alpha_j^B} + n_T \sigma_j^2} \right., \frac{e^{\alpha_j^A} e^{\alpha_j^B}}{n_T(e^{\alpha_j^j} + e^{\alpha_j^B} + n_T \sigma_j^2)} \right), & I_j = 1,
\end{cases}
\]
where
\[
C_0 := \pi N(\lambda_j^A|\lambda_j^B, (e^{\alpha_j^A} + e^{\alpha_j^B})/n_T),
\]
\[
C_1 := (1 - \pi) N(\lambda_j^A|\lambda_j^B, (e^{\alpha_j^A} + e^{\alpha_j^B})/n_T + \sigma_j^2).
\]
As \(C_0\) and \(C_1\) do not contain \(\mu_j^A\), we can write this as,
\[
\mu_j^A|\Omega \setminus \{\mu_j^A, \gamma_j\} \overset{\text{ind.}}{\sim} \begin{cases} 
 N \left( \frac{\lambda_j^A e^{\alpha_j^B} + \lambda_j^B e^{\alpha_j^A}}{e^{\alpha_j^j} + e^{\alpha_j^B}} \right., \frac{e^{\alpha_j^A} e^{\alpha_j^B}}{n_T(e^{\alpha_j^j} + e^{\alpha_j^B})} \right), & I_j = 0, \\
 N \left( \frac{\lambda_j^A e^{\alpha_j^B} + \lambda_j^B e^{\alpha_j^A}}{e^{\alpha_j^j} + e^{\alpha_j^B} + n_T \sigma_j^2} \right., \frac{e^{\alpha_j^A} e^{\alpha_j^B}}{n_T(e^{\alpha_j^j} + e^{\alpha_j^B} + n_T \sigma_j^2)} \right), & I_j = 1.
\end{cases}
\]
Finally, we have
\[
[\gamma_j|\Omega \setminus \{\gamma_j\}] \propto \begin{cases} 
 \prod_i N(\lambda_{ij}^B|\mu_j^A + \gamma_j, e^{\alpha_j^B}) N(\gamma_j|0, \sigma_j^2), & I_j = 0, \\
 N(\gamma_j|0, \sigma_j^2) & I_j = 1,
\end{cases}
\]
which can be shown to be a mixture mixture of a point mass at zero and a normal distribution:
\[
\gamma_j|\Omega \setminus \{\gamma_j\} \overset{\text{ind.}}{\sim} \begin{cases} 
 0, & I_j = 0, \\
 N \left( \frac{\sigma_j^2 \sum_{i} (\lambda_{ij}^B - \mu_j^A)}{\sigma_j^2 + e^{\alpha_j^B}}, \frac{\sigma_j^2 e^{\alpha_j^B}}{\sigma_j^2 + e^{\alpha_j^B} + n_T \sigma_j^2} \right), & I_j = 1.
\end{cases}
\]
B Comparison between our Poisson-lognormal data model and a negative-binomial data model

B.1 Analytic comparison

For the analysis of RNAseq counts, the data model is often (e.g., Robinson and Smyth [2007]; Anders and Huber [2010]) assumed to be of the form,

\[ k_{ij}^T | \mu_j^T, \alpha_j^T \sim \text{NegBinom}(s_i^T e^{\mu_j^T}, e^{\alpha_j^T}); \quad i = 1, \ldots, n_T; \quad j = 1, \ldots, m; \quad T = A, B, \]  

instead of the two-stage Poisson-lognormal described in Section 2.1 of the main article. However, the two models are closely related. Model (6) can also be written in two stages (e.g., Anders and Huber, 2010):

\[ k_{ij}^T | \lambda_{ij}^T \sim \text{Poi}(s_i^T e^{\lambda_{ij}^T}); \quad i = 1, \ldots, n_T; \quad j = 1, \ldots, m; \quad T = A, B, \]  

\[ e^{\lambda_{ij}^T} | \mu_j^T, \alpha_j^T \sim \text{Gam}(e^{\mu_j^T}, e^{\alpha_j^T}); \quad i = 1, \ldots, n_T; \quad j = 1, \ldots, m; \quad T = A, B, \]  

where \( \text{Gam}(a, b) \) denotes a gamma distribution with mean \( a \), squared coefficient of variation \( b \), and variance \( a^2 b \). Clearly, like the Poisson-lognormal model in Section 2.1 of the main article, this negative-binomial data model is also a type of overdispersed Poisson model with overdispersion parameter \( \alpha^T_j \). The difference between the two data models is the type of distribution describing the overdispersion (lognormal versus gamma). These two distributions are fairly similar. In fact, it has been shown that inference using the lognormal distribution will typically produce the same conclusions as inference using the gamma distribution [Atkinson, 1982; McCullagh and Nelder, 1989, pp. 286/293].

B.2 Numerical Comparison

In Section A we have seen that the Poisson-lognormal data model allows closed-form updating of the heavily dependent parameters \( \gamma_j, \mu_j^A, \) and \( I_j \) in the MCMC sampler. When implementing an MCMC sampler for the negative-binomial model (6), this is not possible, and we need to resort to Metropolis-Hastings (MH) updates for these three parameters. We update the parameters jointly, using an extension of the adaptive MH sampler of [Haario et al., 2001], to account for that fact that \( I_j \) follows a discrete distribution: After an initial burn-in phase, the proposed values for the three parameters at the \( (l+1) \)-th MCMC iteration are drawn from,

\[ I_j^{(l)} \sim B(I_{l-1}), \]  

\[ \left( \begin{array}{c} \gamma_j^{(l)} \\ \mu_j^{A(l)} \end{array} \right) \mid I_j^{(l)} \sim \left\{ \begin{array}{ll} \delta_0, N((\mu_j^{A(l_0)}, \Sigma_0^{(l_1)}))', & I_j^{(l)} = 0, \\ N((\gamma_j^{(l_1)}, \mu_j^{A(l_1)}), \Sigma_1^{(l_1)}), & I_j^{(l)} = 1, \end{array} \right. \]

where the superscript \( (l) \) denotes parameter values at the previous iteration \( l \), \( l_0 = l_0(l) \) and \( l_1 = l_1(l) \) denote the maximum of all \( q \leq l \) for which \( I_j^{(q)} = 0 \) and \( I_j^{(q)} = 1 \), respectively, and \( \Sigma_0^{(l)} \) and \( \Sigma_1^{(l)} \) are calculated following [Haario et al., 2001].

To compare the two data models numerically regarding mixing, speed, and accuracy, we simulated data separately from each model using the following setup: \( m = 1000, n \in \{2, 5, 10, 20\}, \) \( \pi_0 = 0.5, \psi_0 = -3, \sigma_\gamma = 0.8, \) and \( \tau = 0.8. \)
In initial tests, the negative binomial model did not converge. With the noninformative prior on \( \tau \) (see the end of Section 2.1 in the main article), this parameter drifted off to \(+\infty\) as some \( \alpha_j^T \) tended to \(-\infty\) (indicating no overdispersion). Hence, we had to leave \( \tau \) fixed for the negative-binomial model. To ensure comparability, we did so for the lognormal model in this simulation study as well.

We simulated a dataset from this setup for each of the models, and fitted the models for each dataset using 5,000 burn-in steps and 10,000 subsequent MCMC iterations, of which we saved every 10th step for inference. As a measure of yield per time unit, we took the effective sample size (ESS) per minute of \( \mu_j^T \) and \( \alpha_j^T \). The ESS estimates the number of independent samples that would have the equivalent amount of information as the actual MCMC sample (in our case, of size 1,000), by correcting for autocorrelation (we use the implementation in the R package CODA; see Plummer et al., 2006). As measure of accuracy, we used the average (over all genes) continuous ranked probability score (CRPS; see Gneiting and Raftery, 2007).

Results of the simulation study are shown in Table 1. The yield of effective samples of \( \alpha_j^T \) and \( \mu_j^T \) per minute is much higher for the lognormal than for the negative-binomial model. Even without taking run time into account, the posterior distributions from the former model yield lower average CRPS, indicating higher accuracy. (But since the parameters in the two models are not exactly the same, the CRPS might not be directly comparable.)

We also tried applying the negative-binomial model to the dataset of Pickrell et al. (2010), holding \( \tau \) fixed at a point estimate from a large dataset. However, we encountered even more severe mixing problems than for the simulated data. The traceplots of the dispersion parameters, \( \alpha_j^T \), did not indicate convergence even after 30,000 MCMC iterations.
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