ROBUST ESTIMATION OF ISOFORM EXPRESSION WITH RNA-SEQ DATA

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ABSTRACT. Qualifying gene and isoform expression is one of the primary tasks for RNA-Seq experiments. Given a sequence of counts representing numbers of reads mapped to different positions (exons and junctions) of isoforms, methods based on Poisson generalized linear models (GLM) with the identity link function have been proposed to estimate isoform expression levels from these counts. These Poisson based models have very limited ability in handling the overdispersion in the counts brought by various sources, and some of them are not robust to outliers. We propose a negative binomial based GLM with identity link, and use a set of robustified quasi-likelihood equations to make it resistant to outliers. An efficient and reliable numeric algorithm has been identified to solve these equations. In simulations, we find that our approach seems to outperform existing approaches. We also find evidence supporting this conclusion in real RNA-Seq data.

1. INTRODUCTION

Through a regulated process called alternative splicing, most genes in eukaryotes code for multiple types of mRNAs, which finally turn into different proteins called “isoforms”. As isoforms from the same gene function differently, dysregulation of alternative splicing can contribute to disease [López-Bigas et al., 2005, e.g.], and thus it is of great importance and interest for biologists to study gene expression at isoform level.

Before the appearance of the ultra-high-throughput sequencing (also called the next-generation sequencing) technologies, genome-wide measurements of gene expression mainly rely on microarrays, which have very limited ability in discovering new isoforms and measuring isoform expression as the design of microarrays relies on the reference genome/transcriptome. In recent years, the ultra-high-throughput sequencing of transcriptomes (RNA-Seq) is gradually taking place of microarrays and becoming arguably the first choice in studying transcriptomes. A main advantage of RNA-Seq is its ability in efficiently discovering new isoforms and studying gene expression at isoform level. To quantitatively measure the expression levels of isoforms, the number of reads (short sequences generated by sequencing) mapped to each position of exons and junctions is counted. In the ideal case, this number of reads can be modeled by a Poisson distribution with mean being a linear combination of isoform expression [Jiang and Wong, 2009]. By maximizing the likelihood, algorithms have been developed to estimate the isoform expression [Jiang and Wong, 2009, Trapnell et al., 2010, Li and Dewey, 2011]. See Pachter...
for a detailed review of the methods for transcription quantification using RNA-Seq.

Although these methods have been quite successful, there are still many challenges that seriously limit their reliability and accuracy. Some of the challenges are recently pointed out by Jiang and Salzman [2013]: systematic biases are often introduced during sequencing and mapping processes, and the incompleteness in transcript annotation databases also introduces additional uncertainty. To eliminate these effects, they propose to add an L1-penalty term to the likelihood function of the Poisson distribution. Their method has been shown to be able to correct some of the biases in a robust manner.

In this paper, we argue that using Poisson distribution practically limits the ability of the model to handle usually overdispersed read counts, as well as various biases and uncertainties. We propose a generalized linear model (GLM) based on negative binomial distributions to efficiently handle these extra variation. Although negative binomial based models with the log link function have been very popular for the identification of differentially expressed genes based on RNA-Seq data, such as edgeR [Robinson et al., 2010], DESeq [Anders and Huber, 2010], ShrinkBayes [Van De Wiel et al., 2012], and baySeq [Hardcastle and Kelly, 2010], they have not been used for isoform expression estimation, and an important reason is that isoform expression requires GLM with identity link, which automatically brings in constraints in the parameter space, making it difficult to solve the likelihood function. To circumvent this problem, previous isoform expression estimation algorithms, which are based on Poisson GLM with identity link, often uses Expectation-Maximization (EM) instead of Newton-Raphson to give the estimate of parameters. However, such a simple EM algorithm is not available for negative binomial distributions.

We get the solution of our model by solving a set of quasi-likehood equations. These equations use Huber-like penalties, so their solutions are robust to outliers. Moreover, a simple (one-dimensional) primitive function can be found for these functions, making the constraint optimization convenient. On both simulated and real data, our method is able to give more accurate and reliable estimate of isoform expression than existing methods. To the best of our knowledge, this is the first successful example of a robust negative binomial based GLM with the identity link function.

2. Robust quasi-likelihood equations for a negative-binomial regression model

2.1. A negative-binomial regression model. In Salzman et al. [2011], a Poisson regression model is provided to model both single-end and paired-end RNA-Seq data for isoform expression. We adopt their notations and extend it to a negative-binomial regression model.

Suppose a gene has I annotated distinct transcript isoforms and J possible distinct read types. Simply put, a read type is a group of reads mapped to the same position of an exon or a junction. For example, suppose a gene has only two isoforms; the first isoform has only one exon of length 100 nt, and the second isoform is composed of the exon and another exon of length 200 nt. Suppose each read is single-end and of length 50 nt, then this gene has 251 possible distinct read types: 51 types from exon 1 (positions 1 to 50 of exon 1, positions 2 to 51 of exon 1, . . . ,
and positions 51 to 100 of exon 1), 151 types from exon 2, and 49 types from the junction (positions 52 to 100 of exon 1 + position 1 of exon 2, positions 53 to 100 of exon 1 + positions 1 to 2 of exon 2, ..., and position 100 of exon 1 + positions 1 to 49 of exon 2). We let \( \theta \) be the \( I \times 1 \) vector representing the abundance of the isoforms in the sample, and \( n \) be a \( J \times 1 \) read count vector, where \( n_j \) denotes the number of reads of type \( j \).

Previous methods assume the following Poisson distribution based model

\[
(2.1) \quad n_j | \theta \sim \text{Poisson} \left( \sum_{i=1}^{I} \theta_i a_{ij} \right).
\]

On the above, \( A = (a_{ij}) \) is an \( I \times J \) “sampling rate” matrix with its \((i,j)\)-th element \( a_{ij} \) denoting the rate that read type \( j \) is sampled from isoform \( i \). Matrix \( A \) describes the compositions of the isoforms. In our previous example, \( A \) will be a \( 2 \times 251 \) matrix, with the first 51 columns be \([1, 1]\) and the other 200 columns be \([0, 1]\), meaning that the first 51 types of reads can come from both isoforms and thus has a larger Poisson mean \( \theta_1 + \theta_2 \), while the other 200 types of reads can only come from isoform 2 and thus has a smaller Poisson mean \( \theta_2 \).

In the example, all elements of matrix \( A \) are either 0 or 1, showing whether a read type can be generated from an isoform. In real data, people have found that different read types, even from the same exon/junction, can have quite different rates in sequencing [Li et al., 2010, Hansen et al., 2010]. For example, for read types 250 and 251 in our previous example, although they are from the same exon and both come from isoform 2 for certain, they still have means \( a_{2,250} \theta_2 \) and \( a_{2,251} \theta_2 \), with \( a_{2,250} \neq a_{2,251} \). These rates often depend on the nucleotide composition of and around the reads, and can be partly modeled [Li et al., 2010, Roberts et al., 2011, Wu et al., 2011, e.g..]. However, accurate estimation of the rates is very difficult, especially for paired-end data, and this inaccuracy brings extra variation that needs to be included in the model. Therefore, we propose to use the following negative binomial model,

\[
(2.2) \quad n_j | \theta \sim \text{NB} \left( \sum_{i=1}^{I} \theta_i a_{ij}, \phi \right),
\]

where NB is short for “negative binomial”, the first term in parentheses is the mean of the distribution, and \( \phi \) is the dispersion parameter so that \( \text{var}(n_j) = \text{mean}(n_j) + \phi \cdot \text{mean}(n_j)^2 \). Actually, since the estimation of rates are often difficult and cumbersome, people tend to skip this step and use 1 or 0 for \( a_{ij} \)'s. In this case, the counts are very heavily over-dispersed, and using a negative binomial regression instead of Poisson is pressing.

This negative binomial regression model also helps take into account of other biases and variations such as uncertainties in isoform annotations. De novo assembly of transcriptomes often results in many isoforms that have very low expression levels. Including all these transcriptomes greatly increase the computational load and can cause non-identifiability problems, and excluding them brings extra variations in the model. De novo assembly as well as reference transcriptome can also have mis-specified boundaries of some exons, which also brings extra variations that need to be handled by the model. Using a negative binomial model helps incorporating these biases/extra variations.
Recent years, negative binomial based generalized linear models (GLMs) are extensively used for modeling RNA-Seq count data in the literatures of identification of differentially expressed genes. Many state of art methods, such as edgeR [Robinson et al., 2010], DESeq [Anders and Huber, 2010], ShrinkBayes [Van De Wiel et al., 2012], and baySeq [Hardcastle and Kelly, 2010], have been proposed to estimate the coefficients and the dispersion parameters, and they have had great success. These methods all use GLMs with log link, while both Model 2.1 and 2.2 use identity link, which is required by the nature of isoform expression: the expression of a gene or a part of a gene is the sum, not the product, of isoforms. Unlike log link, identity link requires additional constraints on the coefficients to make the mean of the Poisson distribution or negative binomial distribution nonnegative, and thus brings difficulties in estimating the coefficients.

### 2.2. A robust quasi-likelihood estimator.

Model 2.2 is a negative-binomial regression model with identity link and constraints \( \theta_i \geq 0, \ i = 1, \ldots, I \). We will discuss the estimation of the dispersion parameter \( \phi \) in section 2.4. Here we assume \( \phi \) is known and the only parameters needs to be optimized is \( \theta \). This optimization is often done by maximizing the log-likelihood, which, however, gives estimate that is very sensitive to outliers [Pregibon, 1982, Stefanski et al., 1986, Künsch et al., 1989, Morgenthaler, 1992, Ruckstuhl and Welsh, 2001, e.g.]. Outliers are generated by various reasons and are often common in sequencing data [AC’t Hoen et al., 2013, Li and Tibshirani, 2011]. It is worth noting here the difference between “biases/extra variations” and “outliers”. The former are systematic uncertainties that affect a significant proportion of counts (for example, inaccuracy in estimating \( a_{ij} \) affects every \( n_j \)), and the latter are scattered and unpredictable “errors” that often affect only a small proportion of counts. The dispersion parameter in negative binomial distribution is able to efficiently taken into account the former but not the latter.

An efficient way to deal with outliers is to use robust estimators. The theories of robust estimation is very well studied and widely applied for ordinarily least squares, but less for generalized linear models [Hampel et al., 2011, Huber and Ronchetti, 2009, Maronna et al., 2006, e.g.]. In a recent publication, Zhou et al. [2014], a robustified version of the adjusted profile likelihood is proposed for negative-binomial-based GLMs for identification of differentially expressed genes, but this solution does not apply to identity link. We propose an approach based on Cantoni and Ronchetti [2001], where the authors proposed a set of M-estimators of Mallow’s type that work on a large group of generalized linear models, especially on binomial models and Poisson models. When used on our negative binomial model, the estimator is given by the solution to the following set of \( I \) equations:

\[
\sum_{j=1}^{J} \nu(n_{j}, \mu_{j})w_{j}a_{ij} - \sum_{j=1}^{J} \mathbb{E}[\nu(n_{j}, \mu_{j})]w_{j}a_{ij} = 0, \text{ for } i = 1, \ldots, I.
\]

In the equations,

- \( \mu_{j} = \sum_{i=1}^{I} \theta_{i}a_{ij} \) is the expectation of \( n_{j} \).
- \( w_{j} \) is a pre-specified weight for the \( j \)-th row of matrix \( A \). In the literature of robust regression, \( w_{j} \) is often set to be a robust version of the Mahalanobis distances from the overall mean of the rows of matrix \( A \), or set to be all 1’s. In this work, we use the latter choice for simplicity.
• $\nu(n_j, \mu_j) = \frac{1}{\sqrt{V_j}} \cdot h \left( \frac{n_j - \mu_j}{\sqrt{V_j}} \right)$, where $V_j = \mu_j + \phi \mu_j^2$ is the variance of $n_j$, and $h$ is the first derivative of the Huber loss function,

$$h \left( \frac{n_j - \mu_j}{\sqrt{V_j}} \right) = \begin{cases} \frac{n_j - \mu_j}{\sqrt{V_j}}, & \text{if } \left| \frac{n_j - \mu_j}{\sqrt{V_j}} \right| \leq c; \\ c \cdot \text{sign}(n_j - \mu_j), & \text{otherwise.} \end{cases}$$

Here $c$ is a positive constant, and $c = 2.5$ is usually a reasonable value [Rousseeuw and Leroy, 2005].

• $\mathbb{E}[\nu(n_j, \mu_j)]$ is the expectation of $\nu(n_j, \mu_j)$. This term ensures the Fisher consistency of the estimator. Cantoni and Ronchetti have shown that $\mathbb{E}[\nu(n_j, \mu_j)]$ has a closed form in the case of binomial models and Poisson models [Cantoni and Ronchetti, 2001]. We have further shown that the following closed form also exists for negative binomial distributions (See Appendix for details): $\mathbb{E}[\nu(n_j, \mu_j)] = \frac{c}{\sqrt{V_j}} (\Pr(Y_j \geq k_{j2} + 1) - \Pr(Y_j \leq k_{j1})) + \frac{\mu_j}{V_j} [\Pr(k_{j1} \leq \tilde{Y}_j \leq k_{j2} - 1) - \Pr(k_{j1} + 1 \leq Y_j \leq k_{j2})]$, where $k_{j1} = \lfloor \mu_j - c\sqrt{V_j} \rfloor$, $k_{j2} = \lceil \mu_j + c\sqrt{V_j} \rceil$, $Y_j \sim \text{NB}(\mu_j, \phi)$, and $\tilde{Y}_j \sim \text{NB}(1 + \phi)\mu_j, \frac{\phi}{\sigma + 1})$. Here $\lfloor \cdot \rfloor$ means “floor”, the largest integer no greater than $\cdot$.

2.3. An algorithm to solve the quasi-likelihood equation. Usually, the solution to GLMs are obtained by using the iterative re-weighted least squares algorithm (IRLS). IRLS often works for log link, but often fails for identity link with boundary constraints. For Poisson GLMs with identity link (Model 2.1), people have proposed to view the source of each reads as latent variables and then an EM algorithm can be applied to find the maximum likelihood estimation efficiently. However, such a simple EM algorithm is not available for negative binomial distributions.

The solution of our model can be obtained by solving 2.3, a set of $I$ equations. However, with the constraints $\theta_i \geq 0$, there may not always be a solution satisfying the set of estimating equations 2.3. A better way that we have found is to use the primitive function, if we view the left hand side of 2.3 as the first derivative to $\theta_i$, as below:

$$Q = \sum_{j=1}^{J} \int_{n_j}^{\mu_j} [\nu(n_j, t) - \mathbb{E}[\nu(n_j, t)]] \, dt$$

Solving 2.3 is equivalent to minimizing 2.4 with constraints $\theta_i \geq 0$. This primitive function includes $J$ one dimensional integrations that can be easily done numerically. We have found providing both the primitive function 2.4 and the vector of first derivatives (the left hand sides of equations 2.3), R function optim can find the solution quickly and reliably by using the L-BFGS-B algorithm [Byrd et al., 1995, Zhu et al., 1997]. optim also requires a starting value of $\theta_i$, and we have found using the regular maximum likelihood solution of Poisson model, which can be easily achieved using the EM algorithm, works nicely. We have successfully tested our optimization approach in thousands of simulations with different parameter settings and random seeds, as well as in a real RNA-Seq dataset with thousands of genes of different structures and expression levels.
2.4. Estimation of the dispersion parameter. Recent years, the estimation of the dispersion parameter for negative binomial distribution has been studied extensively and state-of-art methods have been proposed, especially for the problem of differential expression identification [Robinson et al., 2010, Anders and Huber, 2010, e.g.,]. They are, unfortunately, usually not robust to outliers. Robust estimation of dispersion in negative binomial regression models is generally a very difficult problem, and we do not attempt to give a general solution. Instead, we assume that the dispersion parameter \( \phi \) is the same for all genes, and we propose a method that works for genes with a unique isoform. We then use the estimate of \( \theta \) given by one-isoform genes for all genes.

For a gene with only one isoform, our model becomes

\[
 n_j \mid \theta \sim \text{NB} (a_j \theta, \phi).
\]

We first try to get a robust estimate of \( \theta \) regardless of the value of \( \phi \). Let \( m_j = \frac{n_j}{a_j} \), then \( \mathbb{E}(m_j) = \theta \), which is the same for all \( j \)'s. We sort \( m_1, \ldots, m_J \) from smallest to largest, then outliers, if exist, are likely to appear on the two ends. To exclude them, we let \( S_\alpha \) be the set of \( j \)'s that \( m_j \) is between the \( \alpha \)'th quantile and \((1 - \alpha)\)'th quantile of \( m_1, \ldots, m_J \), with \( \alpha \) being a pre-specified constant \( \in [0, 0.25] \).

Then we estimate \( \theta \) by \( \hat{\theta} = \frac{\sum_{j \in S_\alpha} n_j}{\sum_{j \in S_\alpha} a_j} \). Simulations have shown that \( \hat{\theta} \) is a very robust estimation of \( \theta \) given that the proportion of outliers does not exceed \( \alpha \).

Given \( \hat{\theta} \), we use a moment estimator to estimate \( \phi \). Since \( \mathbb{E}n_j = a_j \theta \) and \( V(n_j) = a_j \theta + \phi a_j^2 \theta^2 \), we can estimate \( \phi \) by \( \frac{\sum_{j=1}^{J} (n_j - a_j \hat{\theta})^2 - \sum_{j=1}^{J} a_j \hat{\theta}^2}{\sum_{j=1}^{J} a_j^2 \hat{\theta}^2} \). To robustify this estimator, we let \( \beta \) be a pre-specified constant, and \( S_\beta \) be the set of \( j \)'s that \( m_j \) is between the \( \alpha \)'th quantile and \((1 - \alpha)\)'th quantile of \( m_1, \ldots, m_J \).

Then

\[
 \hat{\phi} = \frac{\sum_{j \in S_\beta} (n_j - a_j \hat{\theta})^2 - \sum_{j \in S_\beta} a_j \hat{\theta}}{\sum_{j \in S_\beta} a_j^2 \hat{\theta}^2}.
\]

This estimator turns out to underestimate \( \theta \) as \( S_\beta \) excludes \( m_j \)'s that are most diverse (even when there are actually no outliers). To eliminate this bias, we calculate \( \hat{\theta} \) under a series of \( \beta \) that is no larger than \( \alpha \), and then fit a natural cubic spline on the relationship between \( \hat{\theta} \) and \( \beta \), and predict the value of \( \hat{\theta} \) at the point \( \beta = 0 \). Simulations (Section 3.3) have shown that the resulted \( \hat{\theta} \) robustly estimates \( \theta \) with relatively small bias.

In practice, we use the above method to estimate \( \hat{\theta} \) for every gene with only one isoform, and then use their average as the estimated \( \theta \) that will be used for all genes.

If one needs to use different dispersion parameters for different genes, our methods works in one case: when the \( A \) matrix composes with 0’s and 1’s. As we have discussed in Section 2.1, this is the case when the users assign the same rate to all read types. In this case, many columns of the \( A \) matrix will be the same, and we can use our method for \( n_j \)'s whose corresponding columns in \( A \) matrix are the same to estimate \( \phi \), as these \( n_j \)'s have the same mean.

In the general case that \( a_{ij} \) are different from each other, our method only works for gene with one isoform, but \( \phi \) estimated by them may be generalized to other genes if further assumptions are made. For example, if one assume that \( \phi \)
is a smooth function of gene expression, which is a common assumption in the literature of differential expression identification [Anders and Huber, 2010], then we can estimate this smooth function using genes with one isoform. We leave these to future research.

3. Simulation results

3.1. Simulating data. In this section, we assess the performance of our method and compare it with other methods on simulation data with different gene structures, sequencing depths, and levels of overdispersion. Data are simulated according to 2.2 with different values of dispersion $\phi$, isoform expression $\theta = (\theta_1, \ldots, \theta_I)$, and sampling rate matrix $A = \{a_{ij}\}$. Three values of $\phi$ (0, 0.4, and 1) are used to represent no dispersion, moderate dispersion, and strong dispersion. $A$ and $\theta$ are simulated using the following four schemes:

1. genes with only one isoform. We let $\theta = 1$ and $A=bA'$. Here $A' = (A_1, \ldots, A_{50})$, where $A_1, \ldots, A_{50}$ are independently generated from Uniform(0.1, 2). $b$ is a constant equal to 10, 100 or 1000, corresponding to genes with small read counts, moderate read counts, and large read counts. Note that the read counts depends on the gene expression level and the sequencing depth. After $(n_1, \ldots, n_I)$ are generated according to 2.2, outliers are added: we let $n_1$ be 20 times of its expectation, representing a very large value and we call it “outlier to the right”, or 0, representing a very small value and we call it “outlier to the left”.

2. genes with two isoforms and both isoforms are expressed. We let $\theta = (\theta_1, \theta_2) = (0.8, 0.2)$ and $A=bA'$. Here $A'$ is a $2 \times 50$ matrix with all elements in the first row and the first 25 elements in the second row independently generated from Uniform(0.1, 2), and the last 25 elements in the second row being 0. Again, $b$ is a constant equal to 10, 100 or 1000. We let $n_1$ be 20 times of its expectation or 0 to represent outliers.

3. genes with two isoforms and only one isoform is expressed. We use Scheme 2 to simulate data except letting $\theta = (\theta_1, \theta_2) = (1, 0)$. This represents the case when the solution is on the boundary of the feasible region of our optimization problem 2.4.

4. genes with five isoforms. We generate $\vartheta_1, \ldots, \vartheta_5$ from Uniform(0, 1) independently, and then set one of them equals 0. Then we let $\theta_i = \vartheta_i / \sum_{k=1}^{5} \vartheta_k$, and $\theta = (\theta_1, \ldots, \theta_5)$. $A=bA'$, where $b$ equals 10, 100, or 1000. $A'$ is a $5 \times 100$ matrix, with its elements independently generated from Uniform(0.1, 2) with a half chance, or equals 0 otherwise. To add outliers, we let $n_1$ and $n_2$ be 20 times of their expectations or 0’s.

3.2. Comparison of performance of different methods. We ran our algorithm, as well as three other algorithms on the simulated data:

1. “MLE”: proposed by Jiang and Wong [2009], this algorithm gives the maximum likelihood estimate based on Poisson model 2.1. It does not take outliers into account.

2. “Lasso1”: proposed by Jiang and Salzman [2013], this algorithm maximizes an L1-penalized log-likelihood function of Poisson model 2.1. The L1-penalty identifies suspected outliers and reduces their influence on the estimate.
“Lasso2”: also proposed by Jiang and Salzman [2013], this algorithm discards all counts that are detected as outliers by Lasso1 and then calculates the maximum likelihood estimate of the other counts. Lasso2 fails in some simulations when the dispersion parameter is large, as in this case all counts are detected as outliers by Lasso1 and then discarded by Lasso2. We output the estimate of Lasso1 for Lasso2 in this case.

To measure the performance, we use RMSE = \( \sqrt{\frac{1}{I} \sum_{i=1}^{I} (\hat{\theta}_i - \theta_i)^2} \), where \( \theta_i \) and \( \hat{\theta}_i \) are the true and estimated value of the expression of the \( i \)th isoform. Since in our simulation we always let \( \sum_{i=1}^{I} \theta_i = 1 \), this RMSE can be viewed as the root mean squared error relative to the total expression of all isoforms.

Tables 1 to 4 give the RMSE of all methods under each of the four schemes. We did 100 simulations for each simulation scheme, and report the mean and the standard error of the mean of the 100 simulations. For short, we call our program “R-QLE”, which stands for robust quasi-likelihood estimate. The smallest RMSE in each simulation scheme is marked as bold. The first impression is that while MLE often gives the largest RMSE, there is no single method that always gives the smallest RMSE. However, it is clear that our method has the best overall performance.

Our method gives the smallest RMSE in 48 out of 72 (67%) simulations. Importantly, although in some simulations our method gives comparable or a bit larger RMSE than Lasso1 or Lasso2, we haven’t observed in any of our simulations that our method gives an RMSE that is > 30% larger than the best method. Only in 2 out of 72 (3%) simulations is our RMSE > 20% larger than the best method, and 4 out of 72 (6%) simulations is our RMSE > 10% larger than the best method. This means that the performance of our method is very reliable. Lasso1 and Lasso2 can give substantially larger RMSE than our method. In 17 and 5 out of 72 (24% and 7%) of simulations, Lasso1 gives RMSE that is > 50% and > 100% larger than our method, respectively. These two numbers are 27 and 12 out of 72 (38% and 17%) for Lasso2. Especially, Lasso1 and Lasso2 tend to give much larger RMSE when the dispersion parameter is median (0.4) or high (1), or \( b \) is median (100) to large (1000).

Additionally, we find that comparing the case of “two isoforms, both express” and “two isoforms, only one expresses”, the advantage of our method is even larger in the latter case, indicating that our method’s reliability on the margin of feasible regions.

3.3. Influence of the estimation of dispersion parameter. In all the above simulations, we assume that the dispersion parameter is known. For real data, as we have discussed in Section 2.4, we estimate the dispersion for each single-isoform gene, and use the mean of estimated dispersion for all genes. We check the performance of this strategy on simulation data. We simulate data according to Scheme 1, and let 10% of counts to be outliers. With a half chance, these outliers are 20 times of the expected value, and 0 otherwise. We simulate 100 genes as a group, and use the average of the estimated dispersions as the final estimate of dispersion. Table 5 gives the mean and standard error of the mean based on 100 groups. We see that the bias of the estimation is acceptably small.
### Table 1. RMSE on simulation data: One isoform

|          | outliers to the left | outliers to the right |
|----------|----------------------|-----------------------|
|          | R-QLE | MLE | Lasso1 | Lasso2 | R-QLE | MLE | Lasso1 | Lasso2 |
| $b = 10$ |       |     |        |        |       |     |        |        |
| $\phi = 0$ | .0373 | .0393 | .0383 | .0476 | .0349 | .3909 | .0391 | .0351 |
|          | (.0026) | (.0028) | (.0026) | (.0035) | (.0029) | (.00199) | (.0032) | (.0027) |
| $\phi = 0.4$ | .0871 | .0935 | .1286 | .1667 | .0929 | .3920 | .0891 | .1218 |
|          | (.0059) | (.0067) | (.0081) | (.0101) | (.0067) | (.0237) | (.0067) | (.0084) |
| $\phi = 1$ | .1200 | .1317 | .2671 | .3730 | .1083 | .3052 | .1733 | .3049 |
|          | (.0084) | (.0092) | (.0110) | (.0134) | (.0096) | (.0222) | (.0092) | (.0121) |
| $b = 100$ |       |     |        |        |       |     |        |        |
| $\phi = 0$ | .0133 | .0237 | .0135 | .0160 | .0116 | .3568 | .0127 | .0124 |
|          | (.0011) | (.0017) | (.0011) | (.0011) | (.0010) | (.0184) | (.0010) | (.0010) |
| $\phi = 0.4$ | .0736 | .0840 | .1470 | .1675 | .0780 | .3289 | .1068 | .1448 |
|          | (.0053) | (.0053) | (.0082) | (.0089) | (.0058) | (.0212) | (.0072) | (.0084) |
| $\phi = 1$ | .1102 | .1082 | .2892 | .3232 | .1260 | .3567 | .2273 | .2808 |
|          | (.0079) | (.0083) | (.0125) | (.0138) | (.0087) | (.0220) | (.0120) | (.0136) |
| $b = 1000$ |       |     |        |        |       |     |        |        |
| $\phi = 0$ | .0034 | .0205 | .0036 | .0044 | .0033 | .3855 | .0040 | .0033 |
|          | (.0003) | (.0011) | (.0003) | (.0003) | (.0003) | (.0192) | (.0003) | (.0003) |
| $\phi = 0.4$ | .0679 | .0759 | .1348 | .1422 | .0808 | .4050 | .1080 | .1156 |
|          | (.0055) | (.0062) | (.0088) | (.0095) | (.0060) | (.0226) | (.0075) | (.0084) |
| $\phi = 1$ | .1163 | .1193 | .2984 | .3091 | .1497 | .4180 | .2473 | .2619 |
|          | (.0081) | (.0084) | (.0124) | (.0129) | (.0103) | (.0241) | (.0121) | (.0126) |

### Table 2. RMSE on simulation data: Two isoforms, both expressed

|          | outliers to the left | outliers to the right |
|----------|----------------------|-----------------------|
|          | R-QLE | MLE | Lasso1 | Lasso2 | R-QLE | MLE | Lasso1 | Lasso2 |
| $b = 10$ |       |     |        |        |       |     |        |        |
| $\phi = 0$ | .0481 | .0488 | .0490 | .0575 | .0529 | .4628 | .0605 | .0465 |
|          | (.0028) | (.0028) | (.0030) | (.0036) | (.0034) | (.0201) | (.0038) | (.0027) |
| $\phi = 0.4$ | .0910 | .0959 | .1031 | .1309 | .1145 | .4410 | .1051 | .1079 |
|          | (.0052) | (.0050) | (.0048) | (.0059) | (.0073) | (.0207) | (.0059) | (.0052) |
| $\phi = 1$ | .1453 | .1421 | .1765 | .2289 | .1887 | .4870 | .1561 | .1951 |
|          | (.0081) | (.0091) | (.0065) | (.0075) | (.0130) | (.0251) | (.0080) | (.0080) |
| $b = 100$ |       |     |        |        |       |     |        |        |
| $\phi = 0$ | .0169 | .0286 | .0166 | .0179 | .0163 | .4295 | .0183 | .0154 |
|          | (.0008) | (.0014) | (.0009) | (.0009) | (.0009) | (.0184) | (.0011) | (.0008) |
| $\phi = 0.4$ | .0906 | .0951 | .1141 | .1294 | .1100 | .4415 | .1026 | .1240 |
|          | (.0043) | (.0050) | (.0050) | (.0058) | (.0071) | (.0203) | (.0060) | (.0067) |
| $\phi = 1$ | .1346 | .1382 | .2036 | .2273 | .1808 | .4333 | .1796 | .2148 |
|          | (.0068) | (.0073) | (.0071) | (.0083) | (.0100) | (.0193) | (.0075) | (.0088) |
| $b = 1000$ |       |     |        |        |       |     |        |        |
| $\phi = 0$ | .0055 | .0245 | .0057 | .0065 | .0051 | .4319 | .0058 | .0049 |
|          | (.0003) | (.0012) | (.0003) | (.0004) | (.0003) | (.0202) | (.0004) | (.0003) |
| $\phi = 0.4$ | .0880 | .0974 | .1157 | .1211 | .1085 | .4838 | .1114 | .1254 |
|          | (.0044) | (.0049) | (.0051) | (.0053) | (.0065) | (.0225) | (.0059) | (.0064) |
| $\phi = 1$ | .1327 | .1323 | .2053 | .2195 | .1626 | .4592 | .1857 | .2026 |
|          | (.0073) | (.0080) | (.0073) | (.0087) | (.0110) | (.0184) | (.0079) | (.0085) |
Table 3. RMSE on simulation data: Two isoforms, only one expressed

| $b$  | $\theta$ | outliers to the left | outliers to the right |
|------|----------|----------------------|-----------------------|
|      |          | R-QLE    | MLE     | Lasso1  | Lasso2  | R-QLE    | MLE     | Lasso1  | Lasso2  |
| 10   | 0        | 0.0342   | 0.0361  | 0.0351  | 0.0453  | 0.0402   | 0.4163  | 0.0468  | 0.0354  |
|      |          | (0.0029) | (0.0030) | (0.0030) | (0.0036) | (0.0030) | (0.0175) | (0.0033) | (0.0030) |
|      | 0.4      | 0.0754   | 0.0847  | 0.1095  | 0.1485  | 0.0993   | 0.4319  | 0.1036  | 0.1270  |
|      |          | (0.0056) | (0.0058) | (0.0070) | (0.0079) | (0.0065) | (0.0299) | (0.0067) | (0.0077) |
| 100  | 0        | 0.1245   | 0.1368  | 0.2092  | 0.2718  | 0.1475   | 0.4164  | 0.1619  | 0.2395  |
|      |          | (0.0088) | (0.0105) | (0.0104) | (0.0117) | (0.0122) | (0.0188) | (0.0113) | (0.0110) |
|      | 0.4      | 0.0101   | 0.0177  | 0.0102  | 0.0121  | 0.0114   | 0.4037  | 0.0133  | 0.0104  |
|      |          | (0.0006) | (0.0011) | (0.0006) | (0.0008) | (0.0070) | (0.0186) | (0.0009) | (0.0007) |
| 1000 | 0        | 0.0038   | 0.0162  | 0.0039  | 0.0047  | 0.0040   | 0.4094  | 0.0048  | 0.0037  |
|      |          | (0.0003) | (0.0009) | (0.0003) | (0.0003) | (0.0003) | (0.0191) | (0.0004) | (0.0003) |
|      | 0.4      | 0.0610   | 0.0745  | 0.1179  | 0.1283  | 0.0742   | 0.4391  | 0.1009  | 0.1139  |
|      |          | (0.0045) | (0.0055) | (0.0061) | (0.0066) | (0.0053) | (0.0186) | (0.0063) | (0.0070) |
|      | 1        | 0.1215   | 0.1322  | 0.2499  | 0.2731  | 0.1524   | 0.4701  | 0.2247  | 0.2697  |
|      |          | (0.0081) | (0.0092) | (0.0102) | (0.0108) | (0.0114) | (0.0274) | (0.0103) | (0.0115) |
|      |          |          |         |         |         |          |         |         |         |
|      |          |          |         |         |         |          |         |         |         |

Table 4. RMSE on simulation data: Five isoforms

| $b$  | $\phi$  | outliers to the left | outliers to the right |
|------|---------|----------------------|-----------------------|
|      |         | R-QLE    | MLE     | Lasso1  | Lasso2  | R-QLE    | MLE     | Lasso1  | Lasso2  |
| 10   | 0       | 0.0288   | 0.287   | 0.289   | 0.0330  | 0.0305   | 0.1646  | 0.0360  | 0.0279  |
|      |         | (0.0013) | (0.0019) | (0.0014) | (0.0015) | (0.0012) | (0.0066) | (0.0014) | (0.0013) |
|      | 0.4     | 0.0443   | 0.461   | 0.488   | 0.0633  | 0.0535   | 0.1766  | 0.0506  | 0.0487  |
|      |         | (0.0021) | (0.0020) | (0.0022) | (0.0023) | (0.0023) | (0.0076) | (0.0020) | (0.0022) |
| 100  | 0       | 0.0086   | 0.0124  | 0.0087  | 0.0094  | 0.0087   | 0.1588  | 0.0112  | 0.0080  |
|      |         | (0.0003) | (0.0005) | (0.0003) | (0.0004) | (0.0003) | (0.0064) | (0.0004) | (0.0003) |
|      | 0.4     | 0.0400   | 0.0464  | 0.0506  | 0.0561  | 0.0442   | 0.1642  | 0.0442  | 0.0531  |
|      |         | (0.0016) | (0.0019) | (0.0018) | (0.0019) | (0.0020) | (0.0063) | (0.0017) | (0.0018) |
| 1000 | 0       | 0.0597   | 0.0678  | 0.0856  | 0.0967  | 0.0796   | 0.1979  | 0.0743  | 0.0928  |
|      |         | (0.0027) | (0.0028) | (0.0029) | (0.0031) | (0.0036) | (0.0075) | (0.0029) | (0.0033) |
|      |         |          |         |         |         |          |         |         |         |
|      |         |          |         |         |         |          |         |         |         |
|      | 0.4     | 0.0403   | 0.0453  | 0.0545  | 0.0581  | 0.0442   | 0.1709  | 0.0492  | 0.0545  |
|      |         | (0.0016) | (0.0019) | (0.0019) | (0.0019) | (0.0019) | (0.0067) | (0.0019) | (0.0020) |
| 1000 | 0       | 0.0532   | 0.0606  | 0.0907  | 0.0947  | 0.0638   | 0.1731  | 0.0802  | 0.0880  |
|      |         | (0.0026) | (0.0024) | (0.0027) | (0.0028) | (0.0032) | (0.0060) | (0.0026) | (0.0028) |
Table 5. Estimation of dispersion parameters

\[
\begin{array}{cccc}
\phi = 0.2 & 0.2048 (0.0117) & 0.1714 (0.0059) & 0.1678 (0.0059) \\
0.4 & 0.4070 (0.0164) & 0.3482 (0.0131) & 0.3427 (0.0132) \\
0.6 & 0.6155 (0.0252) & 0.5207 (0.0230) & 0.5207 (0.0200) \\
\end{array}
\]

We assume that all genes have the same dispersion parameter. This assumption can be strong for real data. So we study the performance of our method when an inaccurate dispersion parameter is used. We simulate data under Scheme 4 (five isoforms) using three different dispersions 0, 0.4, and 1, and estimate the dispersion using an inaccurate estimation of dispersion, 0.3. Table 6 gives the mean and the standard error of mean under 100 simulations.

Comparing Table 6 with Table 4, of course the performance of MLE, Lasso1, and Lasso2 do not change, as they do not use the dispersion. The RMSE of our method increases significantly in the cases when the true dispersion is 0 and \( b = 100 \) or 1000. This is easy to understand, as in this case, the true outliers will not be regarded as outliers when one assumes the dispersion is 0.4, a much larger value than the true value. Nevertheless, the RMSE is still small comparing with the RMSEs under simulation data with larger dispersions. When the data is simulated under \( \phi = 0.4 \) or 1, the RMSE of our method does not increase significantly, and it still outperforms other methods in many cases. The comparisons under the other three simulation schemes give similar conclusions.

Table 6. RMSE on simulation data (using \( \phi = 0.3 \) for estimation): Five isoforms

\[
\begin{array}{cccc|cccc}
\text{outliers to the left} & \text{R-QLE} & \text{MLE} & \text{Lasso1} & \text{Lasso2} & \text{outliers to the right} & \text{R-QLE} & \text{MLE} & \text{Lasso1} & \text{Lasso2} \\
\hline
\text{b = 10} & \phi = 0 & 0.0296 & \textbf{0.0287} & 0.0289 & 0.0330 & 0.0368 & \textbf{0.1646} & 0.0360 & \textbf{0.0279} \\
& & (0.0014) & (0.0013) & (0.0014) & (0.0015) & (0.0015) & (0.0066) & (0.0014) & (0.0013) \\
& \phi = 0.4 & \textbf{0.0442} & 0.0461 & 0.0488 & 0.0633 & 0.0514 & \textbf{0.1766} & 0.0506 & \textbf{0.0487} \\
& & (0.0021) & (0.0020) & (0.0022) & (0.0023) & (0.0022) & (0.0076) & (0.0020) & (0.0022) \\
& \phi = 1 & \textbf{0.0643} & 0.0701 & 0.0728 & 0.1016 & 0.0664 & 0.1959 & \textbf{0.0638} & 0.0811 \\
& & (0.0025) & (0.0028) & (0.0022) & (0.0028) & (0.0031) & (0.0071) & (0.0027) & (0.0025) \\
\text{b = 100} & \phi = 0 & 0.0125 & 0.0124 & \textbf{0.0087} & 0.0094 & 0.0188 & 0.1588 & 0.0112 & \textbf{0.0080} \\
& & (0.0005) & (0.0005) & (0.0003) & (0.0004) & (0.0006) & (0.0064) & (0.0004) & (0.0003) \\
& \phi = 0.4 & \textbf{0.0402} & 0.0464 & 0.0506 & 0.0561 & \textbf{0.0427} & 0.1642 & 0.0442 & 0.0531 \\
& & (0.0016) & (0.0019) & (0.0018) & (0.0019) & (0.0019) & (0.0063) & (0.0017) & (0.0018) \\
& \phi = 1 & \textbf{0.0639} & 0.0678 & 0.0865 & 0.0967 & \textbf{0.0662} & 0.1979 & 0.0743 & 0.0928 \\
& & (0.0026) & (0.0028) & (0.0029) & (0.0031) & (0.0029) & (0.0075) & (0.0029) & (0.0033) \\
\text{b = 1000} & \phi = 0 & 0.0087 & 0.0101 & \textbf{0.0028} & 0.0031 & 0.0158 & 0.1617 & 0.0038 & \textbf{0.0027} \\
& & (0.0004) & (0.0004) & (0.0001) & (0.0001) & (0.0006) & (0.0066) & (0.0002) & (0.0001) \\
& \phi = 0.4 & \textbf{0.0407} & 0.0453 & 0.0545 & 0.0581 & \textbf{0.0432} & 0.1709 & 0.0492 & 0.0545 \\
& & (0.0016) & (0.0019) & (0.0019) & (0.0019) & (0.0019) & (0.0067) & (0.0019) & (0.0020) \\
& \phi = 1 & \textbf{0.0586} & 0.0606 & 0.0907 & 0.0947 & \textbf{0.0549} & 0.1731 & 0.0802 & 0.0880 \\
& & (0.0023) & (0.0024) & (0.0027) & (0.0028) & (0.0027) & (0.0060) & (0.0026) & (0.0028) \\
\end{array}
\]
4. Real data analysis

For real data analysis, we use RNA-Seq data from the H1 human embryonic stem cell line generated by the Cold Spring Harbor Laboratory in the ENCODE project [Consortium et al., 2004]. A total of 78 million single-end reads of 75 bp mapped to the RefSeq human annotation database [Pruitt et al., 2009] are used in the analysis. We apply the same four algorithms as in the simulated data analysis: R-QLE, MLE, Lasso1 and Lasso2.

For each of the 20,297 annotated genes, based on its annotated isoforms, we count the number of reads mapped to each exons or junctions, which we define as read types. We then apply our algorithm for estimating the dispersion parameter to genes with only one isoform, at least 20 read types and a median read count across all read types of at least 10. A total of 1,751 genes are used for the estimation. The mean of estimated dispersion parameters is 0.304, which is used as the dispersion parameter in R-QLE for later analysis.

We estimate the isoform expression values using the four algorithms for a total of 13,272 genes having at least 100 mapped reads. We then estimate the gene expression value as the sum of expression values of all its isoforms and use the gene expression value as the basis for comparisons across different methods. Overall, all the four algorithms give quite concordant results. For example, the Spearman correlation coefficients between the estimates given by R-QLE and three other algorithms (MLE, Lasso1 and Lasso2) are 0.922, 0.981 and 0.938, respectively. Only 292 genes have a change larger than 2 folds between their estimates given by R-QLE and MLE.

To compare the robustness of the four algorithms, for each gene, we remove a quarter of the observed data, by removing the first quarter of elements in N and the first quarter of columns in A correspondingly, and re-estimate isoform expression values using the four algorithms. The rationale is that a more robust method should be less affected when part of the observations are removed. The Spearman correlation coefficients between the estimates based on the complete data and partial data, using the four algorithms, are 0.978 (R-QLE) > 0.965 (Lasso1) > 0.951 (MLE) > 0.941 (Lasso2). We can see that R-QLE clearly outperforms all three other methods. The difference becomes even larger when we only focus on the 292 genes with a change larger than 2 folds: 0.978 (R-QLE) > 0.955 (Lasso1) > 0.924 (MLE) > 0.923 (Lasso2).

5. Conclusion

The most commonly considered GLMs for counts data are Poisson distribution based and with log link, which is numerically easy to deal with. However, the nature of isoform expression based on RNA-Seq data requires a GLM based on negative binomial distribution, with identity link, and robust to outliers. There has not been any successful example of such GLM models, and one reason can be the difficulty in optimizing the coefficients. We have identified a numeric algorithm that appeared to be both efficient and reliable. Simulation results show that the estimate of isoform expression from our method is more accurate and reliable than existing methods, and the reliability of our method is also shown in real data.
6. Appendix section

In this appendix, we give the closed form for $\mathbb{E}[\nu(n_j, \mu_j)]$. We would like to find the expectation of

$$
\nu(n_j, \mu_j) = \begin{cases} 
\frac{n_j - \mu_j}{V_j}, & \text{if } \left| \frac{n_j - \mu_j}{\sqrt{V_j}} \right| \leq c; \\
\frac{c}{\sqrt{V_j}} \text{sign}(n_j - \mu_j), & \text{otherwise}.
\end{cases}
$$

Here $n_j \sim \text{NB}(\mu_j, \phi)$. To simplify the notation, we write $n_j$ as $n$, $\mu_j$ as $\mu$, and $V_j$ as $V$. Let $k_1 = \left\lfloor \mu - c\sqrt{V} \right\rfloor$ and $k_2 = \left\lceil \mu + c\sqrt{V} \right\rceil$, and $Y$ be a random variable that follows $\text{NB}(\mu, \phi)$ distribution, then

$$
\mathbb{E}[\nu(n, \mu)] = \sum_{s=k_1+1}^{k_2} \frac{s - \mu}{V} \Pr(Y = s) - \frac{c}{\sqrt{V}} \sum_{s=0}^{k_1} \Pr(Y = s) + \frac{c}{\sqrt{V}} \sum_{s=k_2+1}^{+\infty} \Pr(Y = s)
$$

$$
= \frac{1}{V} \sum_{s=k_1+1}^{k_2} s \Pr(Y = s) - \frac{\mu}{V} \Pr(k_1 + 1 \leq Y \leq k_2)
$$

$$
+ \frac{c}{\sqrt{V}} (\Pr(Y \geq k_2 + 1) - \Pr(Y \leq k_1))
$$

We want to find a simple form for $M \triangleq \sum_{s=k_1+1}^{k_2} s \Pr(Y = s)$. Plugging the probability density function of the negative binomial distribution, we have

$$
M = \frac{1}{\Gamma(\phi^{-1})} \left( \frac{\phi^{-1}}{\mu + \phi^{-1}} \right)^{\phi^{-1}} \sum_{s=k_1+1}^{k_2} \frac{s}{s} \frac{\Gamma(s + \phi^{-1})}{\Gamma(s + 1)} \left( \frac{\mu}{\mu + \phi^{-1}} \right)^{s}
$$

$$
= \frac{1}{\Gamma(\phi^{-1})} \left( \frac{\phi^{-1}}{\mu + \phi^{-1}} \right)^{\phi^{-1}} \sum_{s=k_1+1}^{k_2} \frac{\Gamma(s + \phi^{-1})}{\Gamma(s)} \frac{\mu'}{\mu' + \phi^{-1}}
$$

Let $\phi' = 1 + \phi^{-1}$ and $\frac{\mu'}{\mu' + \phi^{-1}} = \frac{\mu}{\mu' + \phi} = \frac{\phi}{\phi + 1}$, that is, $\phi' = \frac{\phi}{\phi + 1}$ and $\mu' = (1 + \phi)\mu$. Then

$$
M = \frac{1}{\Gamma(\phi^{-1})} \left( \frac{\phi^{-1}}{\mu + \phi^{-1}} \right)^{\phi^{-1}} \sum_{s=k_1+1}^{k_2} \frac{\Gamma(s + 1 - \phi^{-1})}{\Gamma(s + 1)} \left( \frac{\mu'}{\mu' + \phi'^{-1}} \right)^{s-1+1}
$$

$$
= \frac{1}{\Gamma(\phi^{-1})} \left( \frac{\phi^{-1}}{\mu + \phi^{-1}} \right)^{\phi^{-1}} \left( \frac{\mu'}{\mu' + \phi'^{-1}} \right) \sum_{s=k_1}^{k_2-1} \frac{\Gamma(s + \phi'^{-1})}{\Gamma(s + 1)} \left( \frac{\mu'}{\mu' + \phi'^{-1}} \right)^{s}
$$

$$
= \frac{\Gamma(\phi'^{-1})}{\Gamma(\phi^{-1})} \left( \frac{\phi^{-1}}{\mu + \phi^{-1}} \right)^{\phi^{-1}} \left( \frac{\mu'}{\mu' + \phi'^{-1}} \right) \left( \frac{\phi'^{-1}}{\mu' + \phi'^{-1}} \right) \left( \frac{\mu'}{\mu' + \phi'^{-1}} \right)^{-\phi'^{-1}}
$$

$$
\sum_{s=k_1}^{k_2-1} \frac{\Gamma(s + \phi'^{-1})}{\Gamma(s + 1)} \left( \frac{\phi'^{-1}}{\mu' + \phi'^{-1}} \right) \left( \frac{\mu'}{\mu' + \phi'^{-1}} \right)^{s}
$$

The elements in the sum of the second pair of brackets is the probability density function of $\text{NB}((1 + \phi)\mu, \frac{\phi}{\phi + 1})$. Thus, the sum in the second pair of brackets equals $\Pr(k_1 \leq \tilde{Y} \leq k_2 - 1)$, where $\tilde{Y} \sim \text{NB}((1 + \phi)\mu, \frac{\phi}{\phi + 1})$. It is also easy to show that the
part in the first pair of brackets can be simplified to $\mu$. Therefore, $M = \mu \Pr(k_1 \leq \tilde{Y} \leq k_2 - 1)$, and

$$
E[\nu(n, \mu)] = \frac{\mu}{V} \left[\Pr(k_1 \leq \tilde{Y} \leq k_2 - 1) - \Pr(k_1 + 1 \leq Y \leq k_2)\right] + c \sqrt{\frac{V}{V}} \left(\Pr(Y \geq k_2 + 1) - \Pr(Y \leq k_1)\right).
$$

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